IN THE PENNSYLVANIA OFFICE OF OPEN RECORDS

IN THE MATTER OF:

CAITRIONA FITZGERALD,
Complainant,

vs. **Docket No.: AP 2015-2380**

ALLEGHENY COUNTY,
Respondent.

AFFIDAVIT

I, ROBERT HUSTON, do hereby state the following:

1. My name is Robert Huston. Presently, I am the Director of the Forensic Science Branch at the Allegheny County Office of the Medical Examiner. My duties include, but are not limited to, overseeing the daily operations that occur within the Forensic Science Branch at the Office of the Medical Examiner.

2. I was informed that a Right-to-Know Law request was received seeking the source code for the software program TrueAllele.

3. After a thorough search, I was unable to find the source code for the software program TrueAllele and notified the appropriate parties that the request should be denied.

4. Later, after that denial was appealed, I became aware that the original request sought more than just the source code for the software program TrueAllele. Based on this clarification, I conducted a thorough search and investigation and identified the enclosed records as responsive to the request. These records include purchase and service contracts between Allegheny County and Cybergenetics, as well as technical specifications and user manuals for the TrueAllele software program.

5. My office contacted the Complainant to explain the miscommunication that led to the initial denial and attempted to resolve the issue outside of the appeal process.

Robert Huston 11/5/15

Sworn to and subscribed before me

this 5th day of November, 2015.

Annie Marbury
Notary Public

COMMONWEALTH OF PENNSYLVANIA
Notarial Seal
Annie Marbury, Notary Public
City of Pittsburgh, Allegheny County
My Commission Expires Oct. 15, 2016
MEMBER, PENNSYLVANIA ASSOCIATION OF NOTARIES

EXHIBIT D

MODIFICATION TO CONTRACT NUMBER 73948

THIS MODIFICATION TO CONTRACT NUMBER 73948 in accordance with Allegheny County Executive Action Number 7051-08 dated December 18, 2008 is entered into as of this 19th of February, 2009 by and between CyberGenetics ("SUPPLIER") and the COUNTY OF ALLEGHENY ("COUNTY").

RECITALS

WHEREAS, SUPPLIER and the COUNTY entered into a contract as of the approval of Executive Action Number 5559-07 dated May 11, 2007, whereby SUPPLIER agreed to provide certain products and related services; and

WHEREAS, SUPPLIER and the COUNTY desire to ratify and confirm the terms of CONTRACT NUMBER 73948 as modified hereby on behalf of SUPPLIER; and

NOW, THEREFORE, SUPPLIER and the COUNTY, intending to be legally bound, hereby agree as follows:

Modification. CONTRACT NUMBER 73948 is modified as follows:

The expiration date of contract number 73948 has been extended through May31, 2009.

Total cost of this Contract Modification not to exceed $67,775

IN WITNESS WHEREOF, the parties hereto through their duly authorized officers have executed this Agreement as of the date first written above.

CyberGenetics

By:_____ Date 19 Feb 2009
Name: MARK W. PERLIN
Title: CEO

COUNTY OF ALLEGHENY

By:_____ Date_____
James M. Flynn, Jr.
County Manager

CONTRACT APPROVED FOR ADMINISTRATIVE AND FISCAL COMPLETENESS:

3/9/09
CONTROLLER / DATE

EXHIBIT
tabbies
E

By: _____ Date 2/24/09
Michael Wojcik
County Solicitor

By: _____ Date 2.24.9
Allan J. Opsitnick
Assistant County Solicitor

By: _____ Date 2/24/09
John Deighan
Chief Purchasing Officer

Approved as to form

PURCHASE AGREEMENT

This Purchase Agreement dated as of June 12, 2009, in accordance with Allegheny County Executive Action Number 5956-09 as approved June 12, 2009, is by and between the County of Allegheny ("the County"), a political subdivision of the Commonwealth of Pennsylvania, and Cybergenetics Corporation.

RECITALS

WHEREAS, the Supplier is in the business of selling certain products and related services, as further described herein; and

WHEREAS, the Supplier desires to sell and the County desires to purchase certain products and related services all upon and subject to the terms and conditions set forth herein.

WHEREAS, County has determined, after appropriate investigation, that Supplier is the sole source of the subject matter of this Agreement.

NOW, THEREFORE, Supplier and County, intending to be legally bound, hereby agree as follows:

ARTICLE 1 - CERTAIN DEFINITIONS

1.1 "Agreement" shall mean this Purchase Agreement, including the main body of this Agreement, Attachment A, Attachment B, and all other attachments and exhibits attached hereto.

1.2 "Applicable Law(s)" shall mean all applicable federal, state and local laws, statutes, ordinances, codes, rules, regulations, standards, orders and other governmental requirements of any kind, including, but not limited to, those relating to (i) affirmative action and equal employment opportunity, (ii) nondiscrimination based on race, color, creed, religion, sex, age, ethnic origin or existence of a disability, (iii) wages and hours, (iv) workers' compensation and unemployment insurance, (v) labor and employment conditions, (vi) occupational safety and health and (vii) the environment and the use and handling and disposal of toxic and/or hazardous substances and materials.

1.3 "Employee Taxes" shall mean all taxes, assessments, charges and other amounts whatsoever payable in respect of, and measured by the wages of, the Supplier's employees (or subcontractors), as required by the Federal Social Security Act and all amendments thereto and/or any other applicable federal, state or local law.

1.4 "County's Destination" shall mean such delivery location(s) or destination(s) as County may prescribe from time to time.

1.5 "Products" shall mean the products and related services to be sold by Supplier hereunder as identified and described on Attachment A hereto and incorporated herein.

1.6 "Purchase Order" shall mean any authorized written, electronic, telephone or fax order sent or made by County pursuant hereto, including, but not limited to, written purchase orders, requisitions sent by fax machine, and orders in such other form and/or mode of transmission as County and Supplier may from time to time agree. Each Purchase Order will specify items such as: specific Products requested, quantity, delivery schedule, destination, and total price of the Purchase Order. Each Purchase Order issued under this Agreement shall be made part of, and be incorporated into this Agreement, and shall reference this Agreement on the face of each Purchase Order. Should any Purchase Order not conform to or satisfy the terms of this Agreement, Supplier shall have five (5) days after receipt to reject the Purchase Order. By not rejecting the Purchase Order within five (5) days, Supplier will have accepted the Purchase Order. Acceptance by Supplier is limited to the provisions of this Agreement and the Purchase Order. No additional or different provisions proposed by Supplier shall apply. In addition, the parties agree that this Agreement and issued Purchase Orders constitute a contract for the sale of goods and/or services and satisfy all statutory and legal formalities of a contract.

1.7 "Services" shall mean any services or other duties to be performed by Supplier hereunder including, without limitation, all services and duties described in Section 2.4.

1.8 "Unemployment Insurance" shall mean the contribution required of Supplier, as an employer, in respect of, and measured by, the wages of its employees (or Subcontractors) as required by any applicable federal, state or local unemployment insurance law or regulation.

ARTICLE 2 - AGREEMENT TO SELL

2.1 Supplier hereby agrees to (i) sell to County as County may from time to time designate, such Products as County may order by Purchase Order (or by any other means) and (ii) provide to County the Services, all in accordance with and subject to the terms, covenants and conditions of this Agreement. County agrees to purchase those Products ordered by County by Purchase Order (or by any other means) in accordance with and subject to the terms, covenants and conditions of this Agreement.

2.2 All Purchase Orders issued by County to Supplier for Products during the Term (as hereinafter defined) of this Agreement are subject to the provisions of this Agreement as though fully set forth in such Purchase Order. In the event that the provisions of this Agreement conflict with any Purchase Order issued by County to Supplier, the provisions of this Agreement shall govern. No other terms and conditions including, but not limited to, those contained in Supplier's standard printed terms and conditions, on Supplier's order acknowledgment, invoices or otherwise, shall have any application to or effect upon or be deemed to constitute an amendment to or to be incorporated into this Agreement, any Purchase Order, or any transactions occurring pursuant hereto or thereto, unless this Agreement shall be specifically amended to adopt such other terms and conditions in writing by the parties.

2.3 Notwithstanding any other provision of this Agreement to the contrary, County shall have no obligation to order or purchase any Products hereunder and the placement of any Purchase Order shall be in the sole discretion of County. Without limiting the generality of the foregoing, the actual quantity of Products to be purchased hereunder shall be determined by County in its sole discretion. This Agreement is not exclusive. Supplier expressly acknowledges

and agrees that County may purchase at its sole discretion, products which are identical or similar to the Products described in this Agreement from any third party.

2.4 In addition to the sale of Products as provided above, Supplier shall provide the following services at no cost or charge to County:

(i) During the Term of this Agreement, Supplier shall provide County One year warranty on instruments listed on Attachment A. The warranty will consist of 90 day free plus nine months with payment a part of the total price. Details specified in Attachment A

ARTICLE 3 - TERM AND TERMINATION

3.1 The term of this Agreement shall commence on July 6, 2009 and shall expire on July 5, 2010, subject to any earlier termination as provided herein. Notwithstanding the foregoing, County may extend the term of this Agreement for an additional period of up to 90 days (3) months (the "Extension Term") by giving Supplier written notice specifying the length of such extension no less than thirty (30) days prior to the expiration of the original term. (The original term together with any extension thereof as provided herein is hereafter referred to as the "Term.")

3.2 Notwithstanding anything to the contrary contained in this Agreement, County may terminate this Agreement at any time with or without cause by providing to Supplier no less than thirty (30) days prior written notice of termination.

3.3 Either party may terminate this Agreement by written notice to the other party if the other party breaches any of its obligations hereunder and fails to remedy the breach within sixty (60) days after receiving written notice of such breach from the non-breaching party.

ARTICLE 4 - PRICING, INVOICES AND PAYMENT

4.1 County shall pay to Supplier for all Products ordered and delivered in compliance with the terms and conditions of this Agreement (i) the price or prices specified for each such Product on Attachment A attached hereto and made a part hereof. Unless Attachment A expressly provides otherwise, the prices for Products set forth on Attachment A hereto shall remain fixed during the entire Term of this Agreement and shall not be increased as a result of the quantity of Products ordered, the delivery time within which such Products are required to be delivered to County or for any other reason. Unless otherwise directed by County, Supplier shall utilize such common carrier for the delivery of Products as Supplier may select provided, however, that Supplier shall obtain delivery services hereunder at rates and terms not less favorable than those paid by Supplier for its own account or for the account of any other customer of Supplier.

4.2 Supplier shall submit original invoices to County in form and substance and format acceptable to County. All invoices must reference the County's Purchase Order number, contain an itemization of amounts for Products purchased during the applicable invoice period and any other information requested by County, and must otherwise comply with the provisions of this Agreement and such reasonable requirements as may be prescribed by County from time to time. Invoices shall be addressed as directed by County.

4.3 The prices specified on Attachment A, include (i) all taxes and duties of any kind which Supplier is required to pay with respect to the sale of Products covered by this Agreement and (ii) all charges for packing, packaging and loading.

4.4 Notwithstanding any other agreement of the parties as to the payment of shipping/delivery costs, all purchases hereunder shall be F.O.B. County's Destination. Supplier shall bear all risk of loss during transit.

4.5 Except as specifically set forth on Attachment A hereto, County shall not be responsible for any additional costs or expenses of any nature incurred by Supplier in connection with the provision of the Products or Services, including without limitation travel expenses, clerical or administrative personnel, long distance telephone charges, etc. ("Incidental Expenses"). To the extent that Attachment A expressly requires County to reimburse Supplier for Incidental Expenses, and notwithstanding anything else set forth in this Agreement, including Attachment A hereto, County shall not be responsible for any such reimbursement unless the expenses to be reimbursed are (i) approved, in each instance, in advance by County; and (ii) substantiated by appropriate receipts and related documentation. It is acknowledged and agreed that County may, as a condition of its approval of any such Incidental Expense reimbursement, require in each instance Supplier to utilize suppliers or service providers prescribed by County, which may include suppliers or service providers which are affiliated with County.

4.6 Supplier represents, warrants and covenants that the prices, charges and/or fees for Products and/or Services set forth in this Agreement are at least as favorable as the prices, charges and/or fees Supplier charges to other of its customers or clients for products and services similar to the Products and Services and under similar circumstances and conditions. If Supplier agrees or contracts with other clients or customers similarly situated during the Term of this Agreement, and offers or agrees to financial terms more favorable than those set forth herein, Supplier hereby agrees that it will reduce the prices, charges and/or fees charged to County in respect of the Products and/or Services hereunder to the most favorable rates received by those other clients or customers.

ARTICLE 5 - INDEMNIFICATION

Supplier agrees that it shall indemnify, defend and hold harmless County and its respective officials, directors, employees and agents (collectively, the "Indemnities"), from and against any and all damages, claims, losses, expenses, costs, obligations and liabilities (including without limitation reasonable attorney's fees), suffered directly or indirectly by any of the Indemnities by reason of, or arising out of, (i) any breach of any covenant, representation or warranty made by Supplier in or pursuant to this Agreement, (ii) any failure by Supplier to perform or fulfill any of its obligations, covenants or agreements set forth in this Agreement, (iii) the negligence or intentional misconduct of Supplier, any subcontractor of Supplier, or any of their respective employees, agents or contractors, (iv) any failure of Supplier, its subcontractors, or their respective employees to comply with any Applicable Law, (v) any litigation, proceeding or claim by any third party relating in any way to the obligations of Supplier under this Agreement or Supplier's performance under this Agreement, (vi) any Employee Taxes or Unemployment Insurance; or (vii) any claim alleging that the Products, the Services or any part thereof infringe any patent, copyright, trademark, trade secret or other intellectual property interest in any country. Such obligation to indemnify shall not apply where the damage, claim,

loss, expense, cost, obligation or liability is due to the negligence or willful misconduct of County or its officials, directors, employees, agents or contractors. The provisions of this Article shall survive the expiration or termination of this Agreement.

ARTICLE 6 - WARRANTIES

Supplier covenants, guaranties and warrants that all Products (including all replacement Products which Supplier furnishes) (i) shall be new, free from defects in material and workmanship (including damage due to unsatisfactory packaging by Supplier), (ii) shall be in strict accordance with the Supplier's specifications, drawings, samples or other descriptions and with any specifications, drawings, samples and other descriptions approved or adopted by County, and (iii) shall comply with all Applicable Laws. Supplier warrants and represents that all Products furnished hereunder shall be merchantable, suitable for their intended use, and free from defects in design. Supplier further warrants that all Products furnished hereunder will be free of any claim of any nature by any third person and that Supplier will convey clear title thereto to County. In addition to, and not in limitation of, the foregoing, Supplier makes all of the warranties, guarantees and representations set forth at Attachment B attached hereto and made a part hereof. All of the warranties and guarantees provided by Supplier herein shall remain in full force and effect and shall not be diminished as a result of any utilization by County of Products in accordance with their intended use. Any attempt by Supplier to limit, disclaim, or restrict any of the above warranties, or any remedy of County, by acknowledgment or otherwise, in accepting or performing any Purchase Order, shall be null and void and ineffective without County's express written consent.

ARTICLE 7 - INSPECTION AND REJECTION

7.1 County shall have the right to inspect and test Products at any time prior to shipment, and within a reasonable time after arrival at the ultimate delivery destination. Products shall not be deemed accepted until after final inspection by County. The making or failure to make any inspection of or payment for or acceptance of Products shall in no way impair the right of County to reject nonconforming Products, or to avail itself of any other remedies to which it may be entitled, notwithstanding its knowledge of the nonconformity or defect, the substantiality of the nonconformity or defect or the ease with which the nonconformity or defect could have been discovered.

7.2 If any of the Products are found at any time to be defective in material or workmanship, damaged, or otherwise not in conformity with the requirements of this Agreement or any applicable Purchase Order, including without limitation any applicable drawings and specifications, County may, in addition to any other rights or remedies which it may have under this Agreement, or under law or equity, at its option and at Supplier's sole cost and expense, (i) correct or have corrected the damage, non-conformity or defect, or (ii) return any damaged, non-conforming or defective Products to Supplier for correction or replacement, or (iii) require Supplier to inspect the Products and remove or replace damaged, non-conforming or defective Products with conforming Products. If County elects option (iii) in the preceding sentence and Supplier fails promptly to make the necessary inspection, removal and replacement, County, at its option, may inspect and sort the Products and Supplier shall bear the cost thereof. Payment by County of any invoice shall not constitute acceptance of the Products covered by such invoice, and acceptance by County shall not relieve Supplier of its warranties or other obligations under this Agreement.

7.3 The provisions of this Article shall survive the expiration or termination of this Agreement.

ARTICLE 8 - SUBSTITUTIONS

Supplier may not make any substitutions of Products, or any portion thereof, of any kind without the prior written consent of County.

ARTICLE 9 - COMPLIANCE WITH LAWS

Supplier agrees to comply with all Applicable Laws. Without limitation of the foregoing sentence, Supplier shall comply with all applicable equal employment opportunity, affirmative action, and all other contract clauses required by Applicable Law and shall, at Supplier's expense, secure and maintain in full force during the Term of this Agreement, any and all licenses, permits, approvals, authorizations, registrations and certificates, if any, required by Applicable Law in connection with the performance of the Services. At County's request, Supplier shall provide to County copies of any or all such licenses, permits, approvals, authorizations, registration and certificates.

ARTICLE 10 – PUBLICITY / CONFIDENTIALITY

10.1 No news releases, public announcements, advertising materials, or confirmation of same, concerning any part of this Agreement or any Purchase Order issued hereunder shall be issued or made without the prior written approval of County. Supplier shall not in any advertising, sales materials or in any other way use any of the names or logos of County without the prior written approval of County.

10.2 Any knowledge or information which Supplier or any of its affiliates shall have disclosed or may hereafter disclose to County, and which in any way relates to the Products or Services covered by this Agreement shall not, unless otherwise specifically agreed to in writing by County, be deemed to be confidential or proprietary information, and shall be acquired by County, free from any restrictions, as part of the consideration for this Agreement.

ARTICLE 11 - EXAMINATION OF FINANCIAL RECORDS

Supplier shall maintain books, program and financial records, documents and other evidence pertaining to costs and expenses related to this Agreement in such detail as will properly reflect all costs of labor, materials, equipment, supplies, services and other costs and expenses of whatever nature for which County funding has been provided under the provisions of this Agreement. The Supplier shall maintain such books, records, documents and other materials in accordance with Generally Accepted Accounting Principles, where applicable. The Supplier shall provide access, during normal business hours, to such books, program and financial records, documents and other evidence upon request of the County Manager, the County Controller or their designees upon receipt of reasonable advance notice, either oral or written. Supplier's books, records, program and financial records, documents and other evidence pertaining to services provided under this Agreement shall be preserved and made available for a period of three (3) years following the termination of this Agreement. The County Manager, the County Controller or their designees may audit, examine, review, photocopy, and/or make

excerpts or transcripts of any of Supplier's books, records, program and financial records, documents and other evidence. Any deficiencies noted in any audit reports or otherwise must be fully resolved by the Supplier, to the County's sole satisfaction, within thirty (30) days after the Supplier's receipt of written notice of such deficiencies. Failure of the Supplier to comply with the provisions set forth in this paragraph may constitute a violation of this Agreement and, at the County's sole discretion, may result in the County withholding future payments.

ARTICLE 12 - DELIVERY REQUIREMENTS

TIME IS OF THE ESSENCE WITH RESPECT TO EACH PURCHASE ORDER ISSUED HEREUNDER. If Supplier for any reason anticipates difficulty in complying with the required delivery date, or in meeting any of the other requirements hereunder or under any Purchase Order, Supplier shall promptly notify County in writing. If Supplier does not comply with the applicable delivery schedule, in addition to any other remedies it may have, County may require delivery by fastest method available and charges or costs resulting from such method (including, but not limited to overtime or premium wages, premium shipping rates, etc.), if any, must be fully prepaid and/or absorbed by Supplier without additional cost to County. It is Supplier's responsibility to comply with the delivery schedule applicable to each Purchase Order, but not to anticipate County's requirements.

ARTICLE 13 - RISK OF LOSS AND PASSAGE OF TITLE

Supplier shall have the risk of loss of or damage to any Products until passage of title to County. County shall have the risk of loss of or damage to the Products after title has passed to County. Title to Products shall not transfer until the Products have been received by County at County's Destination.

ARTICLE 14 - REMEDIES

Any right or remedy of Supplier or County set forth in this Agreement shall not be exclusive, and, in addition thereto, Supplier and County shall have all rights and remedies under applicable law, including without limitation, equitable relief. The provisions of this Article shall survive the expiration or termination of this Agreement.

ARTICLE 15 - RELATIONSHIP OF PARTIES

Supplier is an independent contractor and is not an agent, servant, employee, legal representative, partner or joint venturer of County. Nothing herein shall be deemed or construed as creating a joint venture or partnership between Supplier and County. Neither party has the power or authority to bind or commit the other.

ARTICLE 16 - NOTICES

All notices, required or permitted to be given or made in this Agreement shall be in writing. Such notice(s) shall be deemed to be duly given or made if delivered by hand, by certified or registered mail or by nationally recognized overnight courier to the address specified below:

If to County:

John Deighan
County of Allegheny
Division of Purchasing and Supplies
436 Grant Street
Room 206 Courthouse
Pittsburgh, PA 15219

If to Supplier:

Ria David
Cyber genetics
160 N. Craig Street
Suite 210
Pittsburgh, PA 15213

Either party may change its notice address by giving the other party written notice of such change in the manner specified above.

ARTICLE 17 - FORCE MAJEURE

Delay in performance or non-performance of any obligation contained herein shall be excused to the extent such failure or non-performance is caused by force majeure. For purposes of this Agreement, "force majeure" shall mean any cause or agency preventing performance of an obligation which is beyond the reasonable control of either party hereto, including without limitation, fire, flood, sabotage, shipwreck, embargo, strike, explosion, labor trouble, accident, riot, acts of governmental authority (including, without limitation, acts based on laws or regulations now in existence as well as those enacted in the future), acts of God, and delays or failure in obtaining raw materials or transportation. A party affected by force majeure shall promptly provide notice to the other, explaining the nature and expected duration thereof, and shall act diligently to remedy the interruption or delay if it is reasonably capable of being remedied. In the event of a force majeure situation, deliveries or acceptance of deliveries that have been suspended shall not be required to be made up on the resumption of performance.

ARTICLE 18 - WAIVER

No delay or failure by either party to exercise any right, remedy or power herein shall impair such party's right to exercise such right, remedy or power or be construed to be a waiver of any default or an acquiescence therein; and any single or partial exercise of any such right, remedy or power shall not preclude any other or further exercise thereof or the exercise of any other right, remedy or power. No waiver hereunder shall be valid unless set forth in writing executed by the waiving party and then only to the extent expressly set forth in such writing.

ARTICLE 19 - PARTIES BOUND; ASSIGNMENT

This Agreement shall inure to the benefit of and shall be binding upon the respective successors and assigns of the parties hereto, but it may not be assigned in whole or in part by Supplier without the prior written consent of County. Supplier shall not delegate its duties under

this Agreement nor assign monies due or to become due to it hereunder without prior written consent of County. County may freely assign this Agreement.

ARTICLE 20 - SEVERABILITY

To the extent possible, each provision of this Agreement and any Purchase Order shall be interpreted in such a manner as to be effective and valid under applicable law. If any provision of this Agreement or any Purchase Order issued in accordance with this Agreement is declared invalid or unenforceable, by judicial determination or otherwise, such provision shall not invalidate or render unenforceable the entire Agreement or Purchase Order, but rather the entire Agreement or Purchase Order shall be construed as if not containing the particular invalid or unenforceable provision or provisions and the rights and obligations of the parties shall be construed and enforced accordingly.

ARTICLE 21 - INCORPORATION; ENTIRE AGREEMENT

21.1 All the provisions of Attachments A are hereby incorporated herein and made a part of this Agreement. In the event of any apparent conflict between any provision set forth in the main body of this Agreement and any provision set forth in Attachment A or B, the provisions shall be interpreted, to the extent possible, as if they do not conflict. In the event that such an interpretation is not possible, the provisions set forth in the main body of this Agreement shall control.

21.2 This Agreement (including Attachments hereto) constitutes the entire agreement of the parties relating to the subject matter hereof and supersedes any and all prior written and oral agreements or understandings relating to such subject matter.

ARTICLE 22 - HEADINGS

Headings used in this Agreement are for convenience of reference only and shall in no way be used to construe or limit the provisions set forth in this Agreement.

ARTICLE 23 - MODIFICATIONS

Except as may be expressly provided otherwise herein, this Agreement may be modified or amended only by a writing executed by both parties hereto.

ARTICLE 24 - GOVERNING LAW

This Agreement shall be governed by and interpreted in accordance with the laws of Pennsylvania, without regard to its choice of law provisions.

IN WITNESS WHEREOF, the parties have executed this Agreement as of the day and year first written above.

ALLEGHENY COUNTY:

By:_____ Date_____
James M. Flynn, Jr.
County Manager

By:_____ Date 6/25/09
John Deighan
Chief Purchasing Officer

Approved as to Form:

By:_____ Date 6/26/09
Michael Wojcik
County Solicitor

By:_____ Date 6.25.9
Allan J. Opsitnick
Assistant County Solicitor

SUPPLIER:

By: Ria David Date 6/18/09
Name: RIA DAVID
Title: President

ATTACHMENT A
to Purchase Agreement dated as of June 12, 2009 by and between County of Allegheny, and Cyber genetics Corporation

PRODUCTS, SERVICES, SPECIFICATIONS AND PRICES
Cyber genetics Corp. quote AC – 5A-2009 dated May 19, 2009 which is attached to this sheet

Cybergenetics

Quote

Date	Quote #
19-May-09	AC-5A-2009

Valid
90 days

Quote To:
Allegheny County Crime Laboratory Forensic Biology Section 542 Fourth Avenue Pittsburgh, PA 15219 Attn: Robert Askew

Contact:
Cybergenetics 160 N. Craig Street Suite 210 Pittsburgh, PA 15213

Description:	Amount:
TrueAllele® Casework Technology	
TrueAllele Casework ViewStation 4 @ $10,000 each	$40,000
Customer responsible for all shipping and delivery charges	
Questions? Contact Ria David Phone: 412.683.3004 Email: ria@cybgen.com	
Total	$40,000

Item # 28308

SERVICES AGREEMENT

This Services Agreement dated as of September 30, 2009 in accordance with Allegheny County Executive Action Number 6595-09 as approved on September 22, 2009, is by and between the County of Allegheny ("the County"), a political subdivision of the Commonwealth of Pennsylvania, and Cybergenetics Corporation ("Supplier").

RECITALS

WHEREAS, the Supplier is in the business of providing certain services as further described herein; and

WHEREAS, the Supplier desires to provide and the County desires and deems it necessary in the public interest to use certain services all upon and subject to the terms and conditions set forth herein.

NOW, THEREFORE, Supplier and the County, intending to be legally bound, hereby agree as follows:

ARTICLE 1 - *CERTAIN DEFINITIONS*

1.1 "Agreement" shall mean this Services Agreement, including the main body of this Agreement and Attachment A.

1.2 "Applicable Law(s)" shall mean all applicable federal, state and local laws, statutes, ordinances, codes, rules, regulations, standards, orders and other governmental requirements of any kind, including, but not limited to, those relating to (i) affirmative action and equal employment opportunity, (ii) nondiscrimination based on race, color, creed, religion, sex, age, ethnic origin or existence of a disability, (iii) wages and hours, (iv) workers' compensation and unemployment insurance, (v) labor and employment conditions, (vi) occupational safety and health and (vii) the environment and the use and handling and disposal of toxic and/or hazardous substances and materials.

1.3 "Employee Taxes" shall mean all taxes, assessments, charges and other amounts whatsoever payable in respect of, and measured by the wages of, the Supplier's employees (or subcontractors), as required by the Federal Social Security Act and all amendments thereto and/or any other applicable federal, state or local law.

1.4 "Services" shall mean any services or other duties to be performed by Supplier hereunder *including, without limitation, all services and duties described in* Section 2 and Attachment A.

1.5 "Unemployment Insurance" shall mean the contribution required of Supplier, as

an employer, in respect of, and measured by, the wages of its employees (or Subcontractors) as required by any applicable federal, state or local unemployment insurance law or regulation.

ARTICLE 2 - AGREEMENT TO SELL

2.1 Supplier hereby agrees to provide the County as the County may from time to time designate, such Services as the County may require and provide to the County the Services, all in accordance with and subject to the terms, covenants and conditions of this Agreement. The County agrees to use these Services in accordance with and subject to the terms, covenants and conditions of this Agreement.

2.2 Notwithstanding any other provision of this Agreement to the contrary, the County shall have no obligation to order or purchase any Services hereunder. Without limiting the generality of the foregoing, the actual quantity of Services to be used hereunder shall be determined by the County in its sole discretion. This Agreement is not exclusive. Supplier expressly acknowledges and agrees that the County may use or purchase at its sole discretion, services which are identical or similar to the Services described in this Agreement from any third party.

2.3 During the Term of this Agreement, Supplier shall provide the County services as described in Attachment A.

ARTICLE 3 - TERM AND TERMINATION

3.1 The term of this Agreement shall commence on August 31, 2009 and shall expire on August 30, 2010 and subject to any earlier termination as provided herein.

3.2 Notwithstanding anything to the contrary contained in this Agreement, the County may terminate this Agreement at any time with or without cause by providing to Supplier no less than thirty (30) days prior written notice of termination.

3.3 Either party may terminate this Agreement by written notice to the other party if the other party breaches any of its obligations hereunder and fails to remedy the breach within fifteen (15) days after receiving written notice of such breach from the non-breaching party.

ARTICLE 4 - PAYMENT

4.1 Prices are as stated on Attachment A unless Attachment A expressly provides otherwise, the prices for Services shall remain fixed during the entire Term of this Agreement and shall not be increased as a result of the quantity for Services provided, or for any other reason.

ARTICLE 5 - COMPLIANCE WITH LAWS

5.1 Supplier agrees to comply with all Applicable Laws. Without limitation of the foregoing sentence, Supplier shall comply with all applicable equal employment opportunity, affirmative action, and all other contract clauses required by Applicable Law and shall, at Supplier's expense, secure and maintain in full force during the Term of this Agreement, any and all licenses, permits, approvals, authorizations, registrations and certificates, if any, required by Applicable Law in connection with the performance of the Services. At the County's request, Supplier shall provide to the County copies of any or all such licenses, permits, approvals, authorizations, registration and certificates.

ARTICLE 6 - DELIVERY REQUIREMENTS

6.1 TIME IS OF THE ESSENCE WITH RESPECT TO THE SERVICES PROVIDED. If Supplier for any reason anticipates difficulty in complying with the required services, or in meeting any of the other requirements hereunder, Supplier shall promptly notify the County in writing.

ARTICLE 7 REMEDIES

7.1 Any right or remedy of Supplier or the County set forth in this Agreement shall not be exclusive, and, in addition thereto, Supplier and the County shall have all rights and remedies under applicable law, including without limitation, equitable relief. The provisions of this Article shall survive the expiration or termination of this Agreement.

ARTICLE 8 EXAMINATION OF FINANCIAL RECORDS

8.1 Supplier shall maintain books, program and financial records, documents and other evidence pertaining to costs and expenses related to this Agreement in such detail as will properly reflect all costs of labor, materials, equipment, supplies, services and other costs and expenses of whatever nature for which County funding has been provided under the provisions of this Agreement. The Supplier shall maintain such books, records, documents and other materials in accordance with Generally Accepted Accounting Principles, where applicable. The Supplier shall provide access, during normal business hours, to such books, program and financial records, documents and other evidence upon request of the County Manager, the County Controller or their designees upon receipt of reasonable advance notice, either oral or written. Supplier's books, records, program and financial records, documents and other evidence pertaining to services provided under this Agreement shall be preserved and made available for a period of three (3) years following the termination

of this Agreement. The County Manager, the County Controller or their designees may audit, examine, review, photocopy, and/or make excerpts or transcripts of any of Supplier's books, records, program and financial records, documents and other evidence. Any deficiencies noted in any audit reports or otherwise must be fully resolved by the Supplier, to the County's sole satisfaction, within thirty (30) days after the Supplier's receipt of written notice of such deficiencies. Failure of the Supplier to comply with the provisions set forth in this paragraph may constitute a violation of this Agreement and, at the County's sole discretion, may result in the County withholding future payments.

ARTICLE 9 - NOTICES

9.1 All notices, required or permitted to be given or made in this Agreement shall be in writing. Such notice(s) shall be deemed to be duly given or made if delivered by hand, by certified or registered mail or by nationally recognized overnight courier to the address specified below:

If to the County:

Chief Purchasing Officer
County of Allegheny
Division of Purchasing and Supplies
436 Grant Street
Room 206, Courthouse
Pittsburgh, PA 15219

If to Supplier:

Cyber genetics
160 N. Craig Street
Suite 210
Pittsburgh, PA 15213

Either party may change its notice address by giving the other party written notice of such change in the manner specified above.

ARTICLE 10 - FORCE MAJEURE

10.1 Delay in performance or non-performance of any obligation contained herein shall be excused to the extent such failure or non-performance is caused by force majeure. For purposes of this Agreement, "force majeure" shall mean any cause or agency preventing performance of an obligation which is beyond the reasonable control of either party hereto, including without

limitation, fire, flood, sabotage, shipwreck, embargo, strike, explosion, labor trouble, accident, riot, acts of governmental authority (including, without limitation, acts based on laws or regulations now in existence as well as those enacted in the future), acts of God, and delays or failure in obtaining raw materials or transportation. A party affected by force majeure shall promptly provide notice to the other, explaining the nature and expected duration thereof, and shall act diligently to remedy the interruption or delay if it is reasonably capable of being remedied. In the event of a force majeure situation, deliveries or acceptance of deliveries, which have been suspended, shall not be required to be made up on the resumption of performance.

ARTICLE 11 - WAIVER

11.1 No delay or failure by either party to exercise any right, remedy or power herein shall impair such party's right to exercise such right, remedy or power or be construed to be a waiver of any default or an acquiescence therein; and any single or partial exercise of any such right, remedy or power shall not preclude any other or further exercise thereof or the exercise of any other right, remedy or power. No waiver hereunder shall be valid unless set forth in writing executed by the waiving party and then only to the extent expressly set forth in such writing.

ARTICLE 12 - PARTIES BOUND; ASSIGNMENT

12.1 This Agreement shall inure to the benefit of and shall be binding upon the respective successors and assigns of the parties hereto, but it may not be assigned in whole or in part by Supplier without the prior written consent of the County. Supplier shall not delegate its duties under this Agreement nor assign monies due or to become due to it hereunder without prior written consent of the County.

ARTICLE 13 - SEVERABILITY

13.1 To the extent possible, each provision of this Agreement shall be interpreted in such a manner as to be effective and valid under applicable law. If any provision of this Agreement is declared invalid or unenforceable, by judicial determination or otherwise, such provision shall not invalidate or render unenforceable the entire Agreement, but rather the entire Agreement shall be construed as if not containing the particular invalid or unenforceable provision or provisions and the rights and obligations of the parties shall be construed and enforced accordingly.

ARTICLE 14 - INCORPORATION; ENTIRE AGREEMENT

14.1 All the provisions of Attachment A are hereby incorporated herein and made a part of this Agreement. In the event of any apparent conflict between any provision set forth in the main body of this Agreement and any provision set forth in Attachment A, the provisions shall be interpreted, to the extent possible, as if they do not conflict. In the event that such an interpretation is not possible, the provisions set forth in the main body of this Agreement shall control.

14.2 This Agreement (including Attachment A hereto) constitutes the entire agreement of the parties relating to the subject matter hereof and supersedes any and all prior written and oral agreements or understandings relating to such subject matter.

ARTICLE 15 - HEADINGS

15.1 Headings used in this Agreement are for convenience of reference only and shall in no way be used to construe or limit the provisions set forth in this Agreement.

ARTICLE 16 - MODIFICATIONS

16.1 Except as may be expressly provided otherwise herein, this Agreement may be modified or amended only by a writing executed by both parties hereto.

ARTICLE 17 GOVERNING LAW

17.1 This Agreement shall be governed by and interpreted in accordance with the laws of Pennsylvania without regard to its choice of law provisions.

17.2

IN WITNESS WHEREOF, the parties have executed this Agreement as of the day and year first written above.

THE COUNTY:

By: _____ Date _____
James M. Flynn, Jr.
County Manager

By: _____ Date 10-8-09
John Deighan
Chief Purchasing Officer

Approved as to Form:

By: _____ Date _____
Michael Wojcik
County Solicitor

By: _____ Date 10.8.9
Allan J. Opsitnick
Assistant County Solicitor

SUPPLIER:

By: Ria David Date 10/5/09
Name: Ria David
Title: President

Attachment A

Pricing and Description of Services
Per attached quote AC-8B-2009, AC-9B-2009, AC-9A-2009 covering IMac Computers, Support,, LIMS Integration and Process and productivity customization

Cybergenetics

Quote

Date	Quote #
4-Aug-09	AC-8B-2009

Valid
120 days

Quote To:
Allegheny County Crime Laboratory Forensic Biology Section 542 Fourth Avenue Pittsburgh, PA 15219 Attn: Robert Askew

Contact:
Cybergenetics 160 N. Craig Street Suite 210 Pittsburgh, PA 15213

Description:	Amount:
TrueAllele® Casework Technology	
Additional TrueAllele Casework Training 3 students @ $2,000 each	$6,000
Advanced laboratory	$3,000
Customer responsible for all shipping and delivery charges	
Questions? Contact Ria David Phone: 412.683.3004 Email: ria@cybgen.com	
Total	$9,000

Cybergenetics

Date	Invoice #
15-Sep-09	AC-9B-2009

PO Number	Terms
	Net 30 days

Bill To:

Allegheny County Crime Laboratory
Forensic Biology Section
542 Fourth Avenue
Pittsburgh, PA 15219

Attn: Robert Askew

Remit To:

Cybergenetics
160 N. Craig Street
Suite 210
Pittsburgh, PA 15213

Attn: Donna Scheuble

Description:	Amount:
TrueAllele® Casework Technology	
Y-STR support Cybergenetics is incorporating Y-STR processing into its TrueAllele® Technology in order to facilitate the Allegheny County workflow. Cybergenetics will therefore provide support in order to maintain this Y-STR process.	$15,000
LIMS integration The DNA laboratory requested that Cybergenetics provide expertise in integrating its TrueAllele system with STaCS LIMS computer system. Cybergenetics is supplying this database integration support.	$15,000
Process and productivity customization Allegheny County has an NIJ-funded grant with specific process and productivity requirements. Cybergenetics is adapting its TrueAllele technology, servers and workstations to comply with these customization requests.	$10,000
Customer responsible for all shipping and delivery charges	
Questions? Contact Ria David Phone: 412.683.3004 Email: ria@cybgen.com	
Total	$40,000

Cybergenetics

Date	Invoice #
15-Sep-09	AC-9A-2009

PO Number	Terms
	Net 30 days

Bill To:

Allegheny County Crime Laboratory
Forensic Biology Section
542 Fourth Avenue
Pittsburgh, PA 15219

Attn: Robert Askew

Remit To:

Cybergenetics
160 N. Craig Street
Suite 210
Pittsburgh, PA 15213

Attn: Donna Scheuble

Description:	Amount:
TrueAllele® Casework Technology	
TrueAllele Casework ViewStation 4 @ $10,000 each	$40,000
Each ViewStation Includes iMac 24" 2.93 GHz Intel Core Duo TrueAllele VUier client software VMware Fusion software 1 year Cybergenetics service and support 3 year manufacturer's warranty	
Customer responsible for all shipping and delivery charges	
Questions? Contact Ria David Phone: 412.683.3004 Email: ria@cybgen.com	
Total	$40,000

PURCHASE AGREEMENT

This Purchase Agreement dated as of Jan 19, 2010, in accordance with Allegheny County Executive Action Number 5069-10 as approved on Jan. 12, 2010, is by and between the County of Allegheny ("the County"), a political subdivision of the Commonwealth of Pennsylvania, and Cybergenetics ("Supplier").

RECITALS

WHEREAS, the Supplier is in the business of selling certain products and related services, as further described herein; and

WHEREAS, the Supplier desires to sell and the County desires to purchase certain products and related services all upon and subject to the terms and conditions set forth herein.

NOW, THEREFORE, Supplier and County, intending to be legally bound, hereby agree as follows:

ARTICLE 1 - CERTAIN DEFINITIONS

1.1 "Agreement" shall mean this Purchase Agreement, including the main body of this Agreement, Attachment A, Attachment B, and all other attachments and exhibits attached hereto.

1.2 "Applicable Law(s)" shall mean all applicable federal, state and local laws, statutes, ordinances, codes, rules, regulations, standards, orders and other governmental requirements of any kind, including, but not limited to, those relating to (i) affirmative action and equal employment opportunity, (ii) nondiscrimination based on race, color, creed, religion, sex, age, ethnic origin or existence of a disability, (iii) wages and hours, (iv) workers' compensation and unemployment insurance, (v) labor and employment conditions, (vi) occupational safety and health and (vii) the environment and the use and handling and disposal of toxic and/or hazardous substances and materials.

1.3 "Employee Taxes" shall mean all taxes, assessments, charges and other amounts whatsoever payable in respect of, and measured by the wages of, the Supplier's employees (or subcontractors), as required by the Federal Social Security Act and all amendments thereto and/or any other applicable federal, state or local law.

1.4 "County's Destination" shall mean such delivery location(s) or destination(s) as County may prescribe from time to time.

1.5 "Products" shall mean the products and related services to be sold by Supplier hereunder as identified and described on Attachment A hereto and incorporated herein.

1.6 "Purchase Order" shall mean any authorized written, electronic, telephone or fax order sent or made by County pursuant hereto, including, but not limited to, written purchase orders, requisitions sent by fax machine, and orders in such other form and/or mode of transmission as County and Supplier may from time to time agree. Each Purchase Order will specify items such as: specific Products requested, quantity, delivery schedule, destination, and total price of the Purchase Order. Each Purchase Order issued under this Agreement shall be made part of, and be incorporated into this Agreement, and shall reference this Agreement on the face of each Purchase Order. Should any Purchase Order not conform to or satisfy the terms of this Agreement, Supplier shall have five (5) days after receipt to reject the Purchase Order. By not rejecting the Purchase Order within five (5) days, Supplier will have accepted the Purchase Order. Acceptance by Supplier is limited to the provisions of this Agreement and the Purchase Order. No additional or different provisions proposed by Supplier shall apply. In addition, the parties agree that this Agreement and issued Purchase Orders constitute a contract for the sale of goods and/or services and satisfy all statutory and legal formalities of a contract.

1.7 "Services" shall mean any services or other duties to be performed by Supplier hereunder including, without limitation, all services and duties described in Section 2.4.

1.8 "Unemployment Insurance" shall mean the contribution required of Supplier, as an employer, in respect of, and measured by, the wages of its employees (or Subcontractors) as required by any applicable federal, state or local unemployment insurance law or regulation.

ARTICLE 2 - AGREEMENT TO SELL

2.1 Supplier hereby agrees to (i) sell to County as County may from time to time designate, such Products as County may order by Purchase Order (or by any other means) and (ii) provide to County the Services, all in accordance with and subject to the terms, covenants and conditions of this Agreement. County agrees to purchase those Products ordered by County by Purchase Order (or by any other means) in accordance with and subject to the terms, covenants and conditions of this Agreement.

2.2 All Purchase Orders issued by County to Supplier for Products during the Term (as hereinafter defined) of this Agreement are subject to the provisions of this Agreement as though fully set forth in such Purchase Order. In the event that the provisions of this Agreement conflict with any Purchase Order issued by County to Supplier, the provisions of this Agreement shall govern. No other terms and conditions including, but not limited to, those contained in Supplier's standard printed terms and conditions, on Supplier's order acknowledgment, invoices or otherwise, shall have any application to or effect upon or be deemed to constitute an amendment to or to be incorporated into this Agreement, any Purchase Order, or any transactions occurring pursuant hereto or thereto, unless this Agreement shall be specifically amended to adopt such other terms and conditions in writing by the parties.

2.3 Notwithstanding any other provision of this Agreement to the contrary, County shall have no obligation to order or purchase any Products hereunder and the placement of any Purchase Order shall be in the sole discretion of County. Without limiting the generality of the foregoing, the actual quantity of Products to be purchased hereunder shall be determined by County in its sole discretion. This Agreement is not exclusive. Supplier expressly acknowledges

and agrees that County may purchase at its sole discretion, products which are identical or similar to the Products described in this Agreement from any third party.

2.4 In addition to the sale of Products as provided above, Supplier shall provide the following services at no cost or charge to County:

ARTICLE 3 - TERM AND TERMINATION

3.1 The term of this Agreement shall commence on February 1, 2010 and shall expire on January 31, 2011, subject to any earlier termination as provided herein. Notwithstanding the foregoing, County may extend the term of this Agreement for an additional period of up to 90 days (3) months (the "Extension Term") by giving Supplier written notice specifying the length of such extension no less than thirty (30) days prior to the expiration of the original term. (The original term together with any extension thereof as provided herein is hereafter referred to as the "Term.")

3.2 Notwithstanding anything to the contrary contained in this Agreement, County may terminate this Agreement at any time with or without cause by providing to Supplier no less than thirty (30) days prior written notice of termination.

3.3 Either party may terminate this Agreement by written notice to the other party if the other party breaches any of its obligations hereunder and fails to remedy the breach within sixty (60) days after receiving written notice of such breach from the non-breaching party.

ARTICLE 4 - PRICING, INVOICES AND PAYMENT

4.1 County shall pay to Supplier for all Products ordered and delivered in compliance with the terms and conditions of this Agreement (i) the price or prices specified for each such Product on Attachment A attached hereto and made a part hereof. Unless Attachment A expressly provides otherwise, the prices for Products set forth on Attachment A hereto shall remain fixed during the entire Term of this Agreement and shall not be increased as a result of the quantity of Products ordered, the delivery time within which such Products are required to be delivered to County or for any other reason. Unless otherwise directed by County, Supplier shall utilize such common carrier for the delivery of Products as Supplier may select provided, however, that Supplier shall obtain delivery services hereunder at rates and terms not less favorable than those paid by Supplier for its own account or for the account of any other customer of Supplier.

4.2 Supplier shall submit original invoices to County in form and substance and format acceptable to County. All invoices must reference the County's Purchase Order number, contain an itemization of amounts for Products purchased during the applicable invoice period and any other information requested by County, and must otherwise comply with the provisions of this Agreement and such reasonable requirements as may be prescribed by County from time to time. Invoices shall be addressed as directed by County.

4.3 The prices specified on Attachment A, include (i) all taxes and duties of any kind which Supplier is required to pay with respect to the sale of Products covered by this Agreement and (ii) all charges for packing, packaging and loading.

4.4 Notwithstanding any other agreement of the parties as to the payment of shipping/delivery costs, all purchases hereunder shall be F.O.B. County's Destination. Supplier shall bear all risk of loss during transit.

4.5 Except as specifically set forth on Attachment A hereto, County shall not be responsible for any additional costs or expenses of any nature incurred by Supplier in connection with the provision of the Products or Services, including without limitation travel expenses, clerical or administrative personnel, long distance telephone charges, etc. ("Incidental Expenses"). To the extent that Attachment A expressly requires County to reimburse Supplier for Incidental Expenses, and notwithstanding anything else set forth in this Agreement, including Attachment A hereto, County shall not be responsible for any such reimbursement unless the expenses to be reimbursed are (i) approved, in each instance, in advance by County; and (ii) substantiated by appropriate receipts and related documentation. It is acknowledged and agreed that County may, as a condition of its approval of any such Incidental Expense reimbursement, require in each instance Supplier to utilize suppliers or service providers prescribed by County, which may include suppliers or service providers which are affiliated with County.

4.6 Supplier represents, warrants and covenants that the prices, charges and/or fees for Products and/or Services set forth in this Agreement are at least as favorable as the prices, charges and/or fees Supplier charges to other of its customers or clients for products and services similar to the Products and Services and under similar circumstances and conditions. If Supplier agrees or contracts with other clients or customers similarly situated during the Term of this Agreement, and offers or agrees to financial terms more favorable than those set forth herein, Supplier hereby agrees that it will reduce the prices, charges and/or fees charged to County in respect of the Products and/or Services hereunder to the most favorable rates received by those other clients or customers.

ARTICLE 5 - INDEMNIFICATION

Supplier agrees that it shall indemnify, defend and hold harmless County and its respective officials, directors, employees and agents (collectively, the "Indemnities"), from and against any and all damages, claims, losses, expenses, costs, obligations and liabilities (including without limitation reasonable attorney's fees), suffered directly or indirectly by any of the Indemnities by reason of, or arising out of, (i) any breach of any covenant, representation or warranty made by Supplier in or pursuant to this Agreement, (ii) any failure by Supplier to perform or fulfill any of its obligations, covenants or agreements set forth in this Agreement, (iii) the negligence or intentional misconduct of Supplier, any subcontractor of Supplier, or any of their respective employees, agents or contractors, (iv) any failure of Supplier, its subcontractors, or their respective employees to comply with any Applicable Law, (v) any litigation, proceeding or claim by any third party relating in any way to the obligations of Supplier under this Agreement or Supplier's performance under this Agreement, (vi) any Employee Taxes or Unemployment Insurance; or (vii) any claim alleging that the Products, the Services or any part thereof infringe any patent, copyright, trademark, trade secret or other intellectual property interest in any country. Such obligation to indemnify shall not apply where the damage, claim,

loss, expense, cost, obligation or liability is due to the negligence or willful misconduct of County or its officials, directors, employees, agents or contractors. The provisions of this Article shall survive the expiration or termination of this Agreement.

ARTICLE 6 - WARRANTIES

Supplier covenants, guaranties and warrants that all Products (including all replacement Products which Supplier furnishes) (i) shall be new, free from defects in material and workmanship (including damage due to unsatisfactory packaging by Supplier), (ii) shall be in strict accordance with the Supplier's specifications, drawings, samples or other descriptions and with any specifications, drawings, samples and other descriptions approved or adopted by County, and (iii) shall comply with all Applicable Laws. Supplier warrants and represents that all Products furnished hereunder shall be merchantable, suitable for their intended use, and free from defects in design. Supplier further warrants that all Products furnished hereunder will be free of any claim of any nature by any third person and that Supplier will convey clear title thereto to County. In addition to, and not in limitation of, the foregoing, Supplier makes all of the warranties, guarantees and representations set forth at Attachment B attached hereto and made a part hereof. All of the warranties and guarantees provided by Supplier herein shall remain in full force and effect and shall not be diminished as a result of any utilization by County of Products in accordance with their intended use. Any attempt by Supplier to limit, disclaim, or restrict any of the above warranties, or any remedy of County, by acknowledgment or otherwise, in accepting or performing any Purchase Order, shall be null and void and ineffective without County's express written consent.

ARTICLE 7 - INSPECTION AND REJECTION

7.1 County shall have the right to inspect and test Products at any time prior to shipment, and within a reasonable time after arrival at the ultimate delivery destination. Products shall not be deemed accepted until after final inspection by County. The making or failure to make any inspection of or payment for or acceptance of Products shall in no way impair the right of County to reject nonconforming Products, or to avail itself of any other remedies to which it may be entitled, notwithstanding its knowledge of the nonconformity or defect, the substantiality of the nonconformity or defect or the ease with which the nonconformity or defect could have been discovered.

7.2 If any of the Products are found at any time to be defective in material or workmanship, damaged, or otherwise not in conformity with the requirements of this Agreement or any applicable Purchase Order, including without limitation any applicable drawings and specifications, County may, in addition to any other rights or remedies which it may have under this Agreement, or under law or equity, at its option and at Supplier's sole cost and expense, (i) correct or have corrected the damage, non-conformity or defect, or (ii) return any damaged, non-conforming or defective Products to Supplier for correction or replacement, or (iii) require Supplier to inspect the Products and remove or replace damaged, non-conforming or defective Products with conforming Products. If County elects option (iii) in the preceding sentence and Supplier fails promptly to make the necessary inspection, removal and replacement, County, at its option, may inspect and sort the Products and Supplier shall bear the cost thereof. Payment by County of any invoice shall not constitute acceptance of the Products covered by such

invoice, and acceptance by County shall not relieve Supplier of its warranties or other obligations under this Agreement.

7.3 The provisions of this Article shall survive the expiration or termination of this Agreement.

ARTICLE 8 - SUBSTITUTIONS

Supplier may not make any substitutions of Products, or any portion thereof, of any kind without the prior written consent of County.

ARTICLE 9 - COMPLIANCE WITH LAWS

Supplier agrees to comply with all Applicable Laws. Without limitation of the foregoing sentence, Supplier shall comply with all applicable equal employment opportunity, affirmative action, and all other contract clauses required by Applicable Law and shall, at Supplier's expense, secure and maintain in full force during the Term of this Agreement, any and all licenses, permits, approvals, authorizations, registrations and certificates, if any, required by Applicable Law in connection with the performance of the Services. At County's request, Supplier shall provide to County copies of any or all such licenses, permits, approvals, authorizations, registration and certificates.

ARTICLE 10 – PUBLICITY / CONFIDENTIALITY

10.1 No news releases, public announcements, advertising materials, or confirmation of same, concerning any part of this Agreement or any Purchase Order issued hereunder shall be issued or made without the prior written approval of County. Supplier shall not in any advertising, sales materials or in any other way use any of the names or logos of County without the prior written approval of County.

10.2 Any knowledge or information which Supplier or any of its affiliates shall have disclosed or may hereafter disclose to County, and which in any way relates to the Products or Services covered by this Agreement shall not, unless otherwise specifically agreed to in writing by County, be deemed to be confidential or proprietary information, and shall be acquired by County, free from any restrictions, as part of the consideration for this Agreement.

ARTICLE 11 - EXAMINATION OF FINANCIAL RECORDS

Supplier shall maintain books, program and financial records, documents and other evidence pertaining to costs and expenses related to this Agreement in such detail as will properly reflect all costs of labor, materials, equipment, supplies, services and other costs and expenses of whatever nature for which County funding has been provided under the provisions of this Agreement. The Supplier shall maintain such books, records, documents and other materials in accordance with Generally Accepted Accounting Principles, where applicable. The Supplier shall provide access, during normal business hours, to such books, program and financial records, documents and other evidence upon request of the County Manager, the County Controller or their designees upon receipt of reasonable advance notice, either oral or written. Supplier's books, records, program and financial records, documents and other evidence

pertaining to services provided under this Agreement shall be preserved and made available for a period of three (3) years following the termination of this Agreement. The County Manager, the County Controller or their designees may audit, examine, review, photocopy, and/or make excerpts or transcripts of any of Supplier's books, records, program and financial records, documents and other evidence. Any deficiencies noted in any audit reports or otherwise must be fully resolved by the Supplier, to the County's sole satisfaction, within thirty (30) days after the Supplier's receipt of written notice of such deficiencies. Failure of the Supplier to comply with the provisions set forth in this paragraph may constitute a violation of this Agreement and, at the County's sole discretion, may result in the County withholding future payments.

ARTICLE 12 - DELIVERY REQUIREMENTS

TIME IS OF THE ESSENCE WITH RESPECT TO EACH PURCHASE ORDER ISSUED HEREUNDER. If Supplier for any reason anticipates difficulty in complying with the required delivery date, or in meeting any of the other requirements hereunder or under any Purchase Order, Supplier shall promptly notify County in writing. If Supplier does not comply with the applicable delivery schedule, in addition to any other remedies it may have, County may require delivery by fastest method available and charges or costs resulting from such method (including, but not limited to overtime or premium wages, premium shipping rates, etc.), if any, must be fully prepaid and/or absorbed by Supplier without additional cost to County. It is Supplier's responsibility to comply with the delivery schedule applicable to each Purchase Order, but not to anticipate County's requirements.

ARTICLE 13 - RISK OF LOSS AND PASSAGE OF TITLE

Supplier shall have the risk of loss of or damage to any Products until passage of title to County. County shall have the risk of loss of or damage to the Products after title has passed to County. Title to Products shall not transfer until the Products have been received by County at County's Destination.

ARTICLE 14 - REMEDIES

Any right or remedy of Supplier or County set forth in this Agreement shall not be exclusive, and, in addition thereto, Supplier and County shall have all rights and remedies under applicable law, including without limitation, equitable relief. The provisions of this Article shall survive the expiration or termination of this Agreement.

ARTICLE 15 - RELATIONSHIP OF PARTIES

Supplier is an independent contractor and is not an agent, servant, employee, legal representative, partner or joint venturer of County. Nothing herein shall be deemed or construed as creating a joint venture or partnership between Supplier and County. Neither party has the power or authority to bind or commit the other.

ARTICLE 16 - NOTICES

All notices, required or permitted to be given or made in this Agreement shall be in writing. Such notice(s) shall be deemed to be duly given or made if delivered by hand, by

certified or registered mail or by nationally recognized overnight courier to the address specified below:

If to County:

Chief Purchasing Officer
County of Allegheny
Division of Purchasing and Supplies
436 Grant Street
Room 206 Courthouse
Pittsburgh, PA 15219

If to Supplier:

Cyber genetics
160 N. Craig Street
Suite 210
Pittsburgh, PA 15213
Attention Ria David

Either party may change its notice address by giving the other party written notice of such change in the manner specified above.

ARTICLE 17 - FORCE MAJEURE

Delay in performance or non-performance of any obligation contained herein shall be excused to the extent such failure or non-performance is caused by force majeure. For purposes of this Agreement, "force majeure" shall mean any cause or agency preventing performance of an obligation which is beyond the reasonable control of either party hereto, including without limitation, fire, flood, sabotage, shipwreck, embargo, strike, explosion, labor trouble, accident, riot, acts of governmental authority (including, without limitation, acts based on laws or regulations now in existence as well as those enacted in the future), acts of God, and delays or failure in obtaining raw materials or transportation. A party affected by force majeure shall promptly provide notice to the other, explaining the nature and expected duration thereof, and shall act diligently to remedy the interruption or delay if it is reasonably capable of being remedied. In the event of a force majeure situation, deliveries or acceptance of deliveries that have been suspended shall not be required to be made up on the resumption of performance.

ARTICLE 18 - WAIVER

No delay or failure by either party to exercise any right, remedy or power herein shall impair such party's right to exercise such right, remedy or power or be construed to be a waiver of any default or an acquiescence therein; and any single or partial exercise of any such right, remedy or power shall not preclude any other or further exercise thereof or the exercise of any other right, remedy or power. No waiver hereunder shall be valid unless set forth in writing executed by the waiving party and then only to the extent expressly set forth in such writing.

ARTICLE 19 - PARTIES BOUND; ASSIGNMENT

This Agreement shall inure to the benefit of and shall be binding upon the respective successors and assigns of the parties hereto, but it may not be assigned in whole or in part by Supplier without the prior written consent of County. Supplier shall not delegate its duties under this Agreement nor assign monies due or to become due to it hereunder without prior written consent of County. County may freely assign this Agreement.

ARTICLE 20 - SEVERABILITY

To the extent possible, each provision of this Agreement and any Purchase Order shall be interpreted in such a manner as to be effective and valid under applicable law. If any provision of this Agreement or any Purchase Order issued in accordance with this Agreement is declared invalid or unenforceable, by judicial determination or otherwise, such provision shall not invalidate or render unenforceable the entire Agreement or Purchase Order, but rather the entire Agreement or Purchase Order shall be construed as if not containing the particular invalid or unenforceable provision or provisions and the rights and obligations of the parties shall be construed and enforced accordingly.

ARTICLE 21 - INCORPORATION; ENTIRE AGREEMENT

21.1 All the provisions of Attachments A and B are hereby incorporated herein and made a part of this Agreement. In the event of any apparent conflict between any provision set forth in the main body of this Agreement and any provision set forth in Attachment A or B, the provisions shall be interpreted, to the extent possible, as if they do not conflict. In the event that such an interpretation is not possible, the provisions set forth in the main body of this Agreement shall control.

21.2 This Agreement (including Attachments hereto) constitutes the entire agreement of the parties relating to the subject matter hereof and supersedes any and all prior written and oral agreements or understandings relating to such subject matter.

ARTICLE 22 - HEADINGS

Headings used in this Agreement are for convenience of reference only and shall in no way be used to construe or limit the provisions set forth in this Agreement.

ARTICLE 23 - MODIFICATIONS

Except as may be expressly provided otherwise herein, this Agreement may be modified or amended only by a writing executed by both parties hereto.

ARTICLE 24 - GOVERNING LAW

This Agreement shall be governed by and interpreted in accordance with the laws of Pennsylvania, without regard to its choice of law provisions.

IN WITNESS WHEREOF, the parties have executed this Agreement as of the day and year first written above.

THE COUNTY:

By: _____ Date _____
James M. Flynn, Jr.
County Manager

By: _____ Date 1/28/10
John Deighan
Chief Purchasing Officer

Approved as to Form:

By: _____ Date _____
Michael Wojcik
County Solicitor

By: _____ Date 1.28.0
Allan J. Opsitnick
Assistant County Solicitor

SUPPLIER: Cybergenetics

By: _____ Date 1/25/10
Name: Ria David
Title: President

ATTACHMENT A
to Purchase Agreement dated as of January 19, 2010 by and between County Allegheny, and Cybergenetics

PRODUCTS, SERVICES, SPECIFICATIONS AND PRICES

4 True Allele Casework View Station($10,000 ea)	$40,000

Each View Station Includes
iMac 27' 2.93 GHz Intel Core Duo
True Allele VUler client software
VMware Fusion software allows iMacs
To function as both Mac and PC machines
1 year Cyber genetics Service and Support
Includes IT integration with True Allele system
3 year manufacturer's warranty

Reference Quote AC-1A-2010 Jan. 6, 2010 per Ria David

2008 Unit Efficiency Grant
Progress Report: January 1, 2011 through March 31, 2011

1. The paper regarding the validation of the Sperm Detection Microscope continues to await approval for publication. An update to the SDM computer (in October 2010) caused malfunctions that still remain unresolved as of March 31, 2011. The vendor has been working diligently to solve this problem, as all training has been suspended since October 2010.
2. Additional Y STR samples have yet to be run in order to complete the validation. Supplies have been purchased to facilitate this additional work. The completion date has been moved to the Fall of 2011.
3. The Janus robot software must be studied and a method must be written to incorporate the Janus into the robotics workflow (i.e., at the normalization stage). The Janus will then go through the approval process for implementation into casework analysis. A completion date of October/November 2011 is anticipated.
4. TrueAllele software must still be assessed so that a validation of the software and its implementation into the workflow can be executed. It is anticipated that this will not be completed by the end of 2011.
5. STaCS customization is complete but complicated programming problems still exist that render the software still unusable to its full capacity at this time. No completion date can be given at this time. Consideration is being given to severing the agreement with STaCS due to this long-standing, unresolvable issue.

Allegheny County Process Outline 30 Aug 2010

Initial contact

Define data

Receive data

Plan approach

Analyze data
- Analyze
  - Create DataDisk
  - Image Call → Cap View
  - Marker Call → Control Check
  - Allele Call
- Data
  - Upload gel(s)

Ask questions
- Request
  - Lane
  - Item
  - Request

Review results
- Review
  - Profile
    - Allele export
  - Data
  - Mixture
  - Match

Extract information

Report results
- Report
  - Table

Final contact

Walter Lorenz
Allegheny County Office of the Medical Examiner, forensic Laboratory
00Lab3726_4wt contributor 2 vs. 00Lab3726_7A
01-Sep-2010

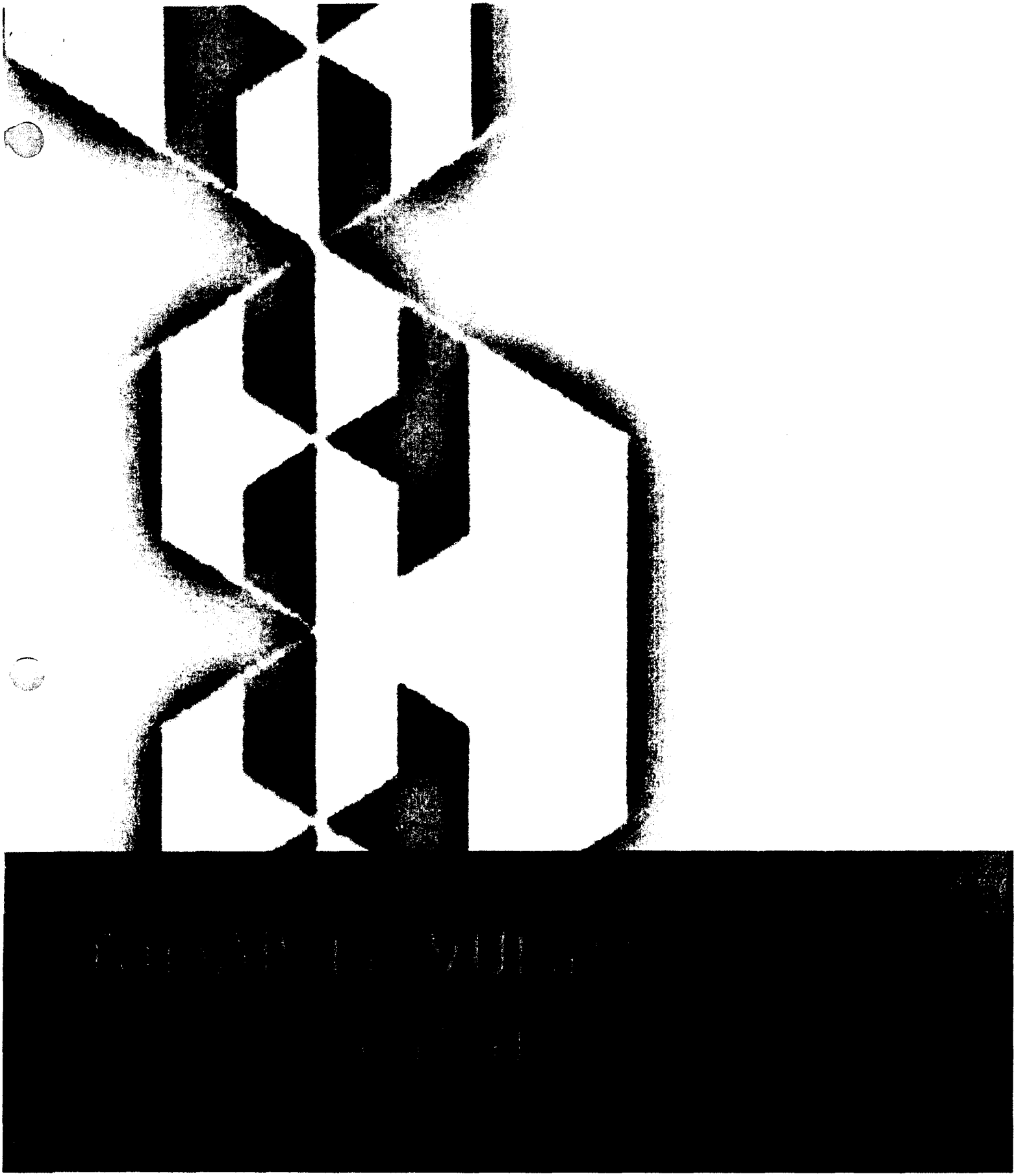
The LR calculation assumes two unknown contributors in the evidence relative to a African-American human population.
The match rarity between the evidence and suspect is 129 trillion.

The joint LR is approximately 129 trillion.
The log(LR) information is 14.11.

			Genotype Probability Distribution					
	Weighted allele pair	Likelihood	Likelihood		Likelihood Ratio Questioned		Reference	
Suspect locus	Numerator x	l(x)	Denominator q(x)	r(x)	LR s(x)	log(LR) l(x)*s(x)		l(x)*r(x)
CSF1PO	13, 13	0.985	0.852	0.0022			0.00219	
	12, 13	0.011	0.103	0.0287	1	0.01107	0.00032	
	11, 13	0.004	0.031	0.0206			0.00008	
	11, 12	0.000	0.011	0.1325			0.00003	
						0.01107	0.00263	4.210
0.624								
D13S317	12, 13	0.549	0.477	0.1503	1	0.54934	0.08257	
	11, 12	0.201	0.359	0.2424			0.04860	
	11, 13	0.097	0.070	0.1051			0.01019	
	12, 12	0.065	0.069	0.1733			0.01120	
	10, 12	0.065	0.020	0.0163			0.00106	
						0.54934	0.15383	3.571
0.553								
D16S539	9, 11	0.915	0.941	0.1292	1	0.91539	0.11826	
	9, 12	0.031	0.027	0.0861			0.00266	
	11, 12	0.013	0.020	0.1105			0.00144	
	9, 9	0.041	0.012	0.0504			0.00205	
						0.91539	0.12441	7.357
0.867								
D18S51	15, 18	0.710	0.737	0.0283	1	0.71020	0.02012	
	13, 15	0.072	0.077	0.0167			0.00120	
	13, 18	0.102	0.064	0.0081			0.00082	
	16, 18	0.049	0.046	0.0332			0.00162	

	15, 16	0.021	0.034	0.0683			0.00146	
	13, 16	0.025	0.024	0.0195			0.00049	
	12, 18	0.006	0.004	0.0143			0.00009	
	12, 15	0.003	0.003	0.0294			0.00008	
						0.71020	0.02611	27.199
1.435								
D21S11	28, 30	0.562	0.678	0.0771	1	0.56205	0.04336	
	30, 30	0.438	0.322	0.0275			0.01205	
						0.56205	0.05541	10.143
1.006								
D3S1358	16, 16	0.426	0.502	0.0791	1	0.42642	0.03375	
	14, 16	0.573	0.497	0.0576			0.03305	
						0.42642	0.06685	6.378
0.805								
D5S818	11, 12	0.773	0.870	0.1694	1	0.77278	0.13092	
	12, 12	0.151	0.099	0.1327			0.02010	
	11, 11	0.029	0.014	0.0541			0.00154	
	12, 13	0.007	0.005	0.1777			0.00123	
	11, 13	0.008	0.005	0.1135			0.00089	
						0.77278	0.15570	4.963
0.696								
D7S820	10, 11	0.848	0.901	0.1203	1	0.84763	0.10194	
	10, 10	0.043	0.032	0.1016			0.00434	
	8, 10	0.026	0.023	0.1234			0.00320	
	10, 12	0.021	0.014	0.0912			0.00193	
	8, 11	0.020	0.013	0.0730			0.00147	
	11, 12	0.015	0.007	0.0540			0.00082	
						0.84763	0.11490	7.377
0.868								
D8S1179	13, 14	0.915	0.967	0.1407	1	0.91472	0.12869	
	13, 15	0.017	0.010	0.0992			0.00167	
	14, 15	0.015	0.009	0.1347			0.00204	
	10, 14	0.019	0.005	0.0161			0.00031	
						0.91472	0.13358	6.847
0.836								
FGA	22, 23	0.608	0.799	0.0516	1	0.60777	0.03134	
	22, 25	0.091	0.073	0.0359			0.00327	
	23, 25	0.071	0.049	0.0311			0.00221	

	22, 22	0.053	0.041	0.0297			0.00158		
	23, 23	0.052	0.030	0.0224			0.00117		
						0.60777	0.03987	15.244	
1.183									
Penta_D	9, 9	0.856	0.841	0.0141			0.01206		
	9, 13	0.066	0.074	0.0201			0.00132		
	9, 11	0.059	0.070	0.0348	1	0.05920	0.00206		
	11, 13	0.006	0.004	0.0248			0.00015		
	11, 12	0.005	0.004	0.0390			0.00020		
						0.05920	0.01598	3.704	
0.569									
Penta_E	5, 7	0.702	0.659	0.0210	1	0.70238	0.01475		
	7, 12	0.116	0.198	0.0267			0.00308		
	7, 7	0.125	0.096	0.0105			0.00131		
	5, 12	0.042	0.044	0.0267			0.00113		
						0.70238	0.02035	34.523	
1.538									
TH01	8 , 8	0.880	0.779	0.0533	1	0.88030	0.04694		
	8 , 9	0.086	0.144	0.0699			0.00598		
	7 , 9.3		0.004	0.018	0.0829			0.00034	
	6 , 9.3		0.006	0.015	0.0288			0.00016	
	6 , 6	0.011	0.014	0.0165			0.00018		
	6 , 7	0.003	0.012	0.0949			0.00029		
	6 , 8	0.004	0.008	0.0593			0.00022		
	9.3, 9.3		0.004	0.006	0.0126			0.00005	
						0.88030	0.05431	16.209	
1.210									
TPOX	8, 8	1	1	0.1058	1	1	0.10578	9.453	
0.976									
vWA	16, 17	0.792	0.883	0.0967	1	0.79186	0.07660		
	16, 16	0.139	0.072	0.0635			0.00882		
	17, 17	0.049	0.029	0.0368			0.00179		
	15, 17	0.012	0.009	0.0886			0.00107		
						0.79186	0.08929	8.868	
0.948									



Cybergenetics

Table of Contents

1 Overview

We should review some initial information to help us begin our TrueAllele process. In the Getting Started Manual, we cover installing and upgrading the TrueAllele Casework software. The basics of starting the program, connecting to a database, and a glossary of common terms are also available in this manual.

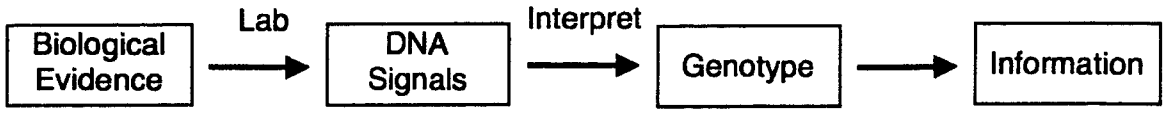


Figure 1. *DNA Interpretation Process*

The DNA interpretation process can be broken down into two transformations. The goal of these transformations is to preserve as much identification information as possible. The first step is where a laboratory transforms the biological evidence samples into DNA signals. The second transformation involves the interpretation of the DNA signals into genotypes. The TrueAllele Casework software quantitatively interprets DNA signals using probabilistic modeling. Mathematical formulas communicate the genotypes as identification information.

2 Hardware Requirements

The minimum system requirements for the VUIer software are:

OS: Windows XP or Mac OS X 10.5 or 10.6 (Intel)

CPU: Pentium 4 processor

RAM: 512 MB RAM (1024 MB recommended)

Storage: 700MB of free hard drive space

Display: screen resolution of 1024x768 (higher resolutions recommended)

3 Install

Now we will walk through installing the TrueAllele Casework software. A complete TrueAllele Casework package installation includes the MCR Engine, the Analysis Module of the VUIer software and the VUIer software. We can obtain the entire package from Cybergenetics.

3.1 MCR Engine

The MCR Engine is an underlying program that powers the graphics and statistical calculations of the VUIer software. We never have to independently start the MCR Engine, but the program must be preinstalled for proper operation of the VUIer software.

We start by double clicking the zip file MCR_Engine_Installer_v711.pkg.zip, which makes the package file MCR_Engine_Installer_v711.pkg available. We then double click the package file to begin the installation process.

On the introduction screen, we select 'Continue' to advance to the next screen.

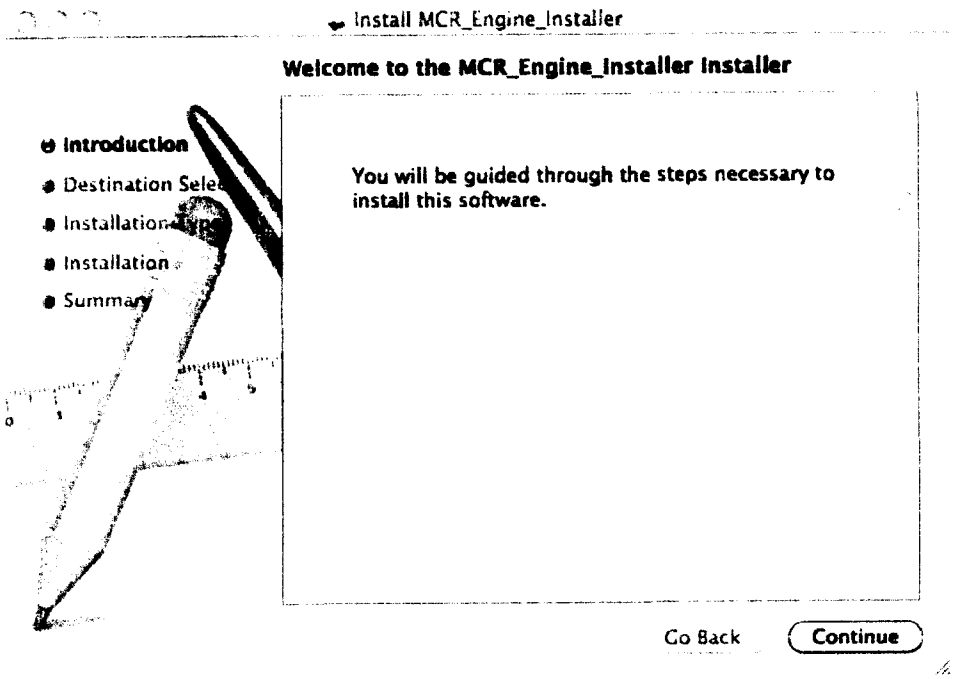


Figure 2. *Introduction Screen.*

In the Installation Type window, we select 'Install' to begin the installation process. When prompted, we enter the administrator user name and password.

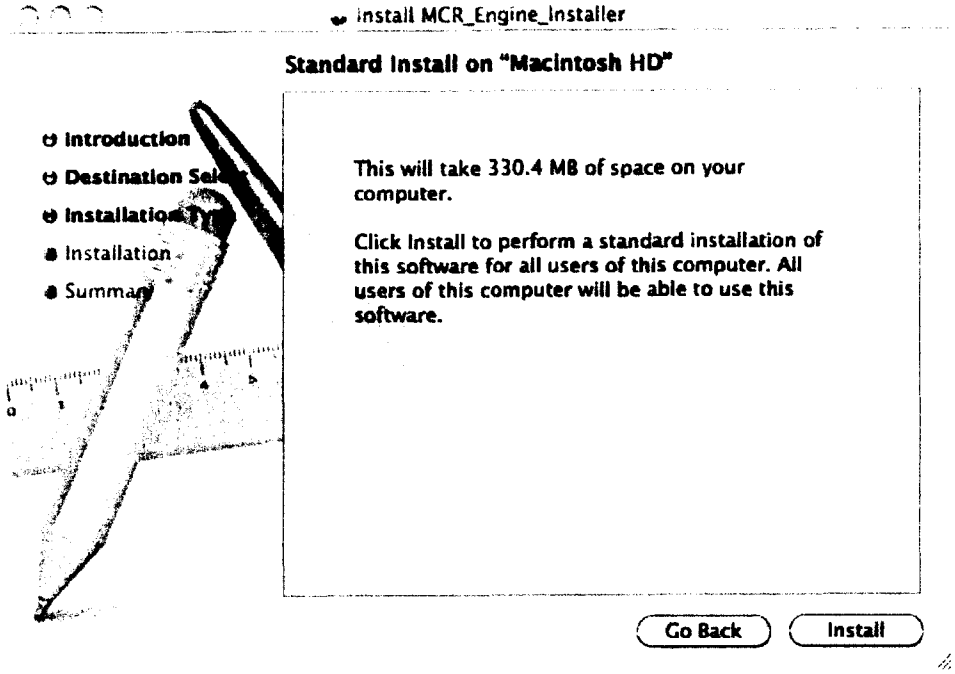


Figure 3. *Standard Install*

Once installation is complete, we select 'Close' to exit the installer.

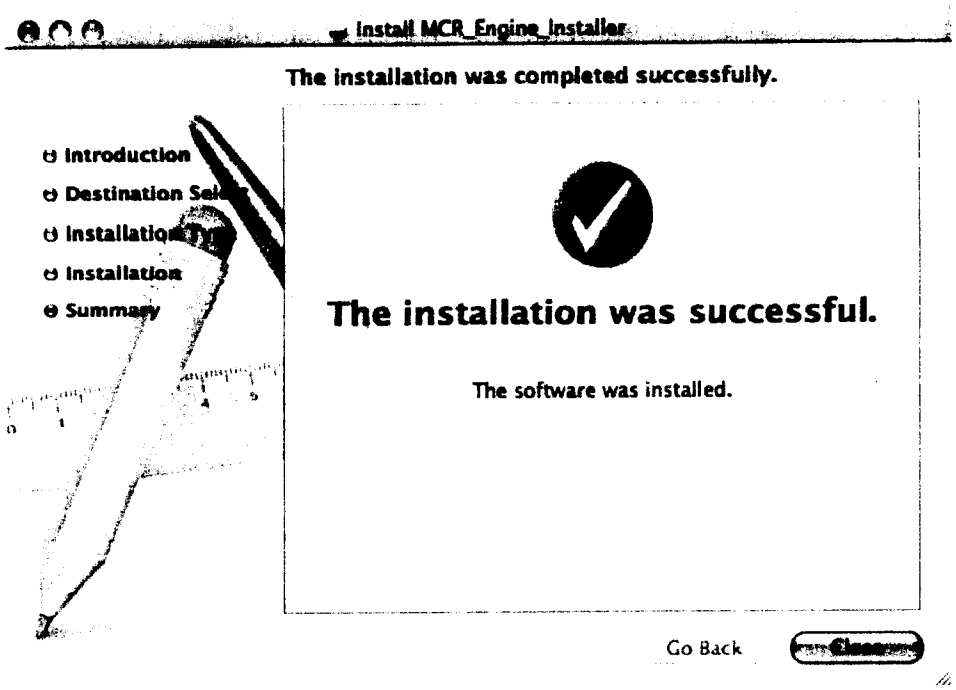


Figure 4. *Installation complete.*

3.2 Analysis

The Analysis Module transforms our raw sequencer files into quality checked peak data for upload to the TrueAllele Server. We install the Analysis software in two steps. First, we install the program itself and then create the user environment.

Program Installation

We start by double clicking on the zip file Analysis_app.pkg.zip, which makes the package file Analysis_app.pkg available. We then double click the package file to begin the installation process.

On the introduction screen, we select 'Continue' to advance to the next screen.

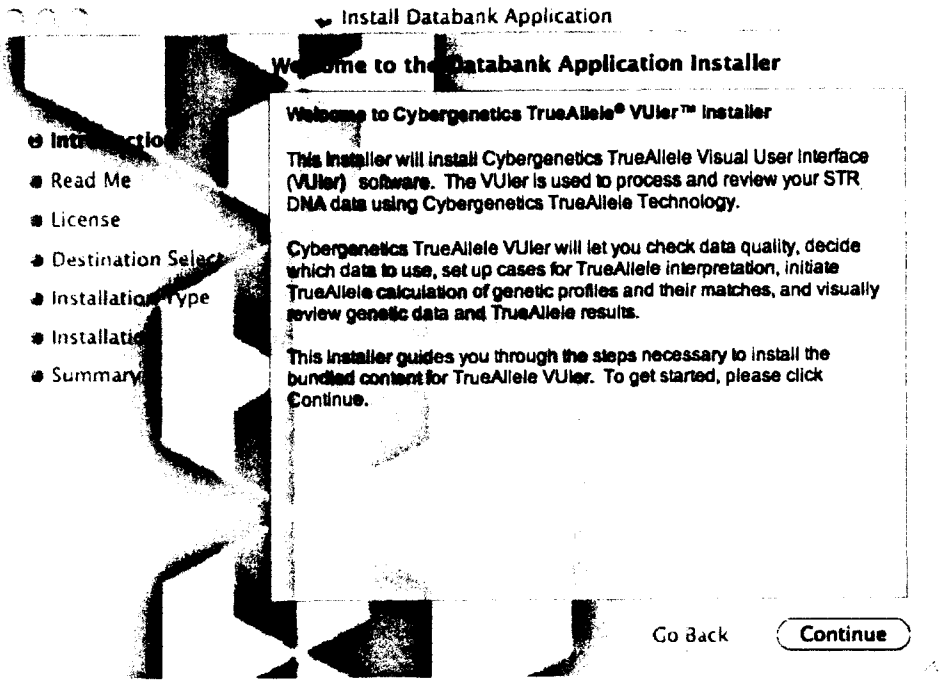


Figure 5. *Introduction Screen.*

The next screen contains a Read Me file for review. After reading the file, we select 'Continue' to advance to the next screen.

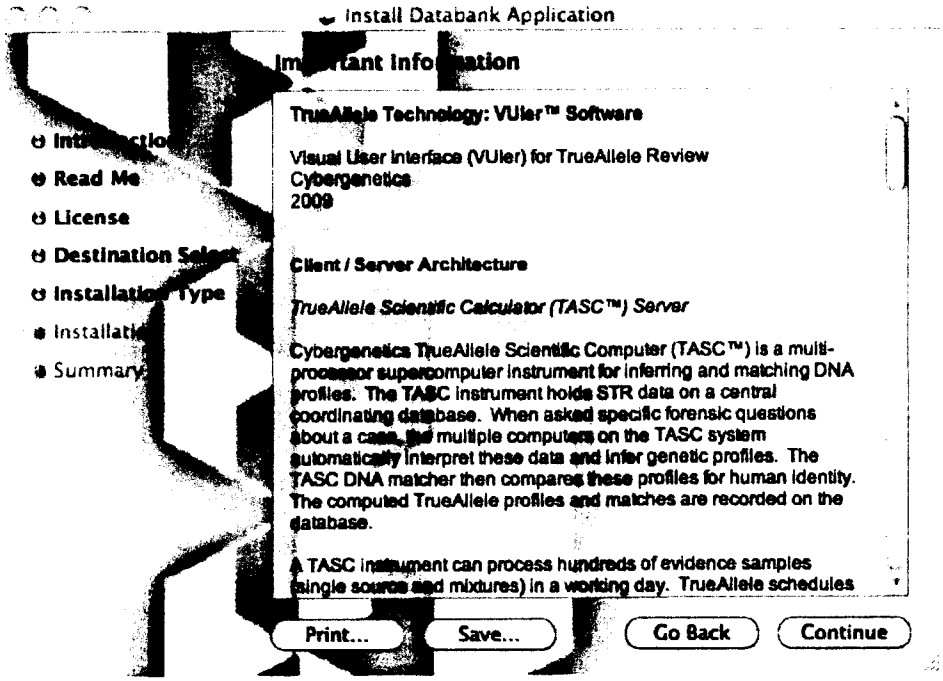


Figure 6. *Read Me.*

Next is the license agreement. After selecting 'Continue', a prompt appears to confirm our agreement with the license. We proceed by selecting 'Agree'.

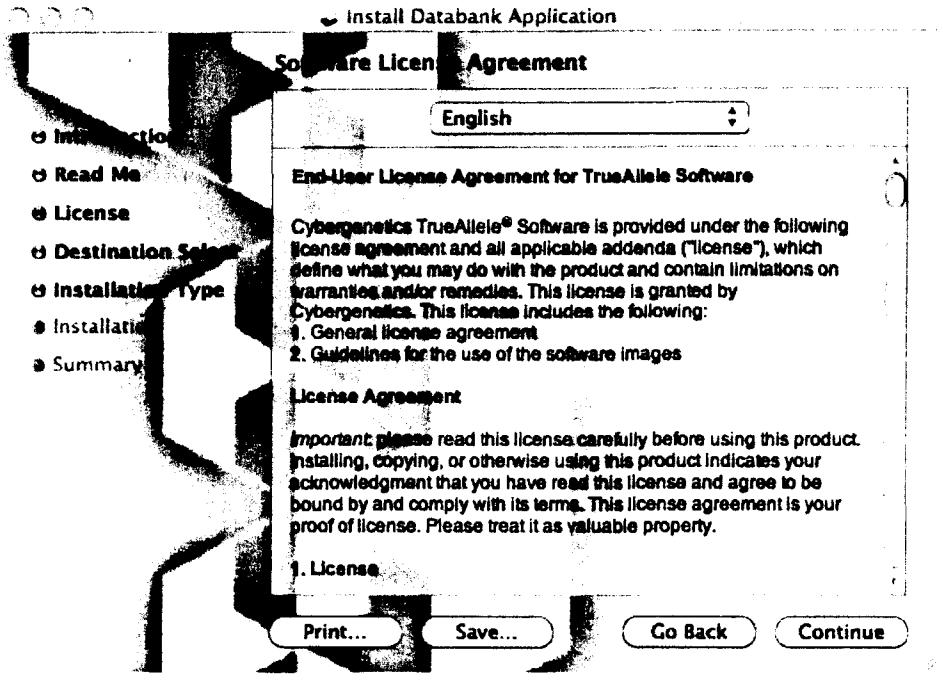


Figure 7. *License Agreement.*

A standard install is automatically available for us. We begin installation by selecting 'Install'. When prompted, we enter the administrator user name and password.

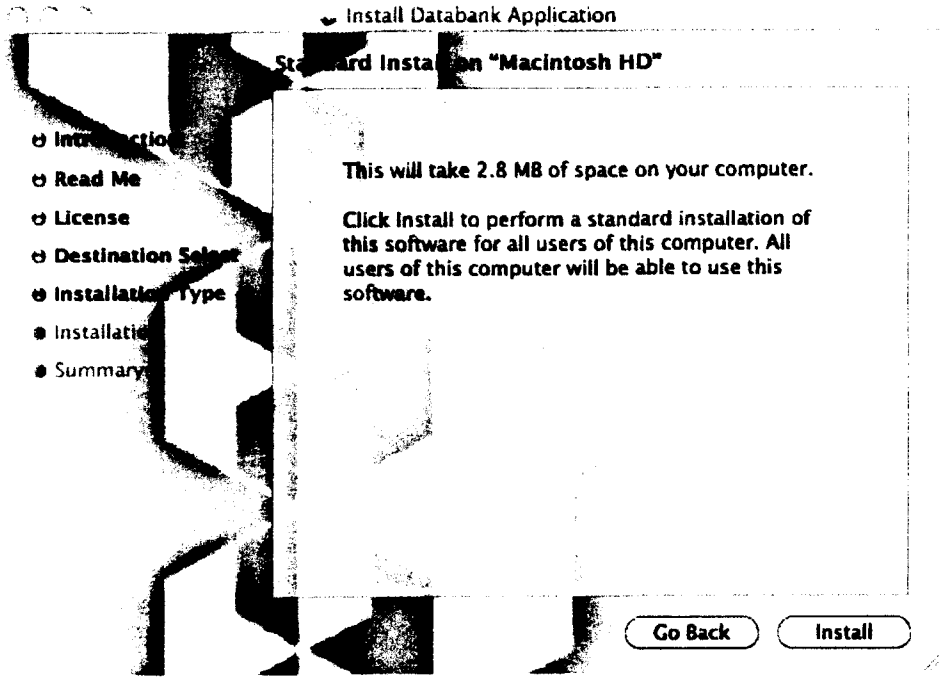


Figure 8. *Standard Install.*

After the installation is complete, we select 'Close' to finish the process.

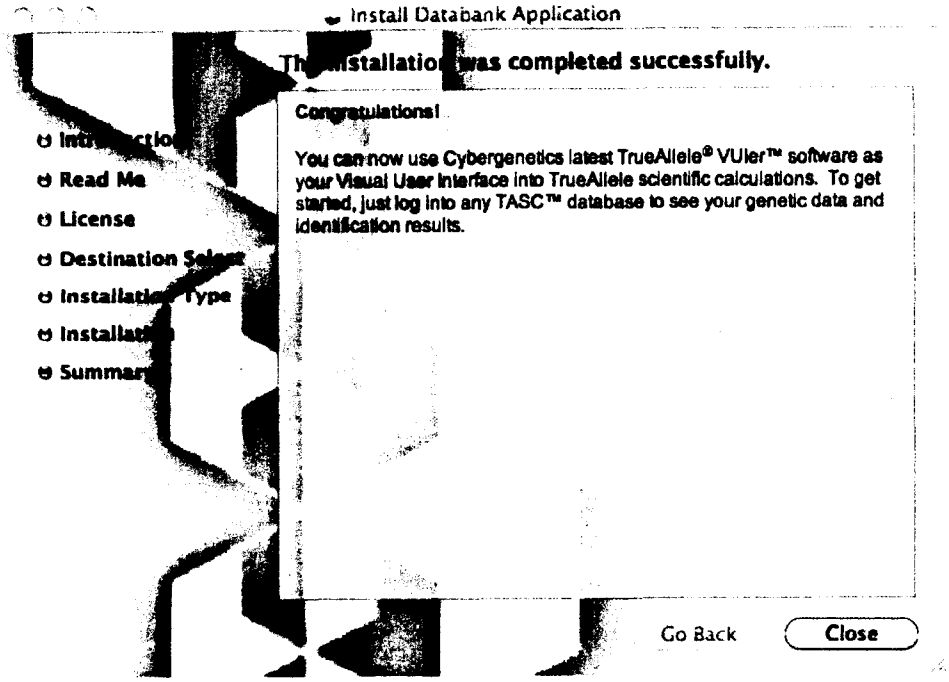


Figure 9. *Installation complete.*

This completes our installation of the Analysis software. Next, we proceed to the creation of the user environment.

User Environment

The user environment includes all necessary templates and settings for processing our data and is contained in a 'trueallele' folder. After installation, the 'trueallele' folder appears in the Documents folder of the account we are working under. For example, if we are logged in as 'DNA', the trueallele folder will be created under DNA's documents folder.

We start by double clicking the zip file Analysis_user.pkg.zip, which makes the package file Analysis_user.pkg available. We then double click the package file to begin the installation process.

On the introduction screen, we select 'Continue' to advance to the next screen.

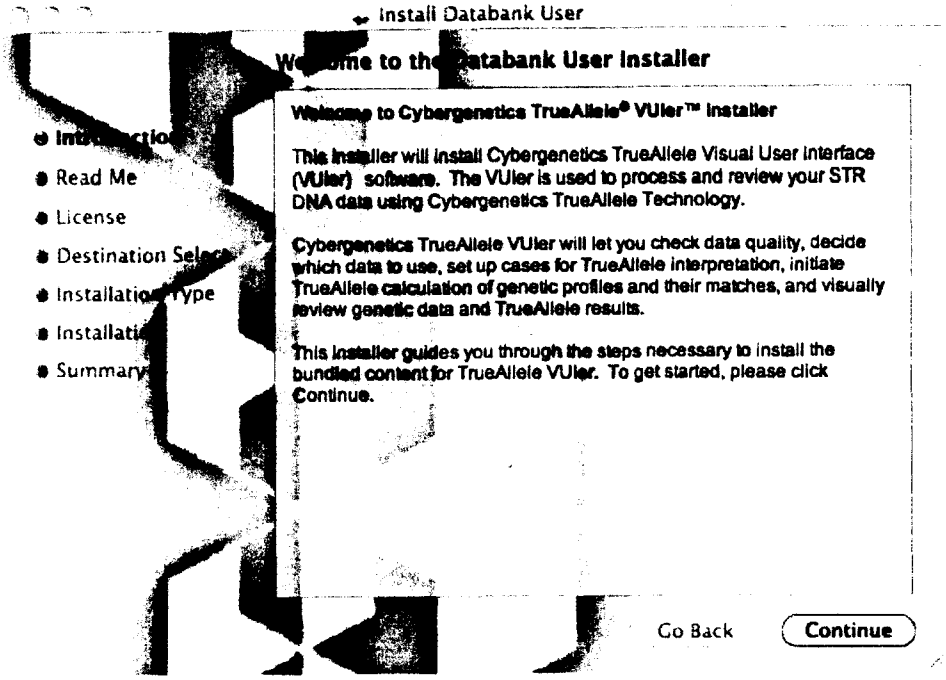


Figure 10. *Introduction Screen.*

The next screen contains a Read Me file for review. After reading the file, we select 'Continue' to advance to the next screen.

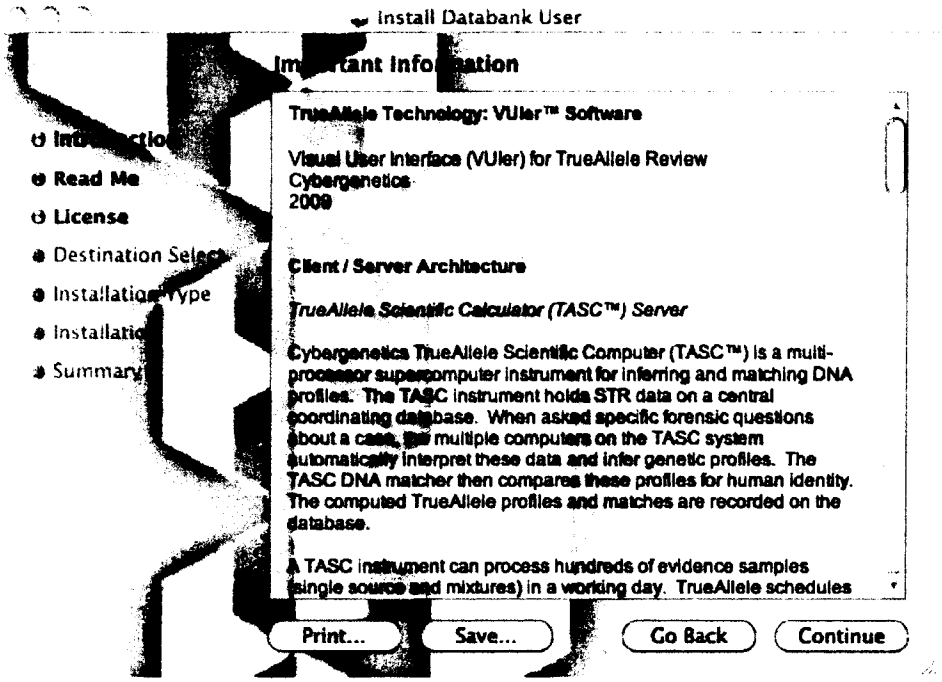


Figure 11. *Read Me.*

Next is the license agreement. After selecting 'Continue', a prompt appears to confirm our agreement with the license. We proceed by selecting 'Agree'.

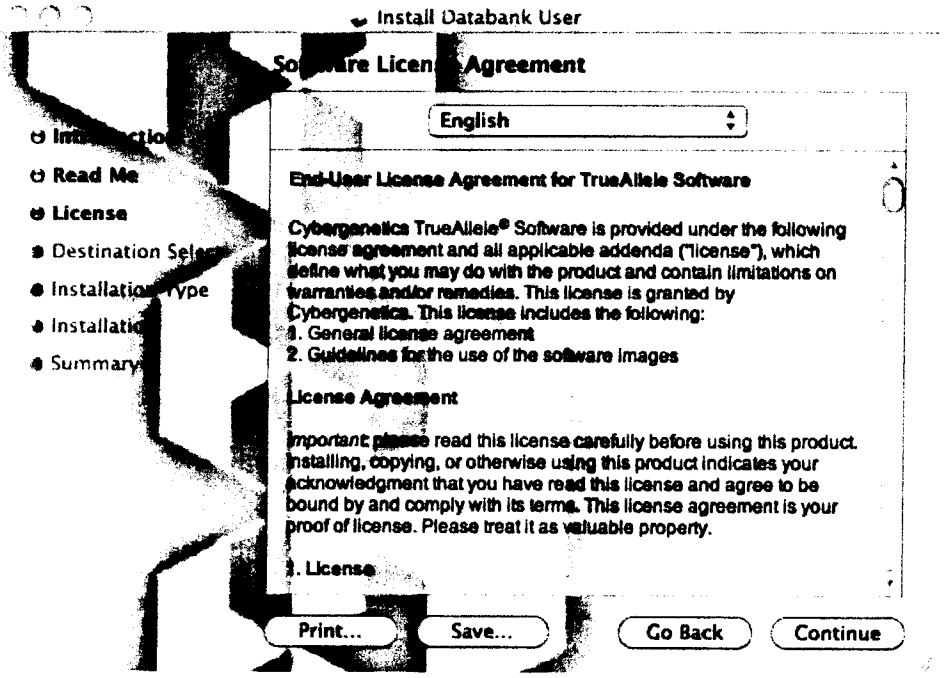


Figure 12. *License Agreement.*

A standard install is automatically available for us. We begin installation by selecting 'Install'. When prompted, we enter the administrator user name and password.

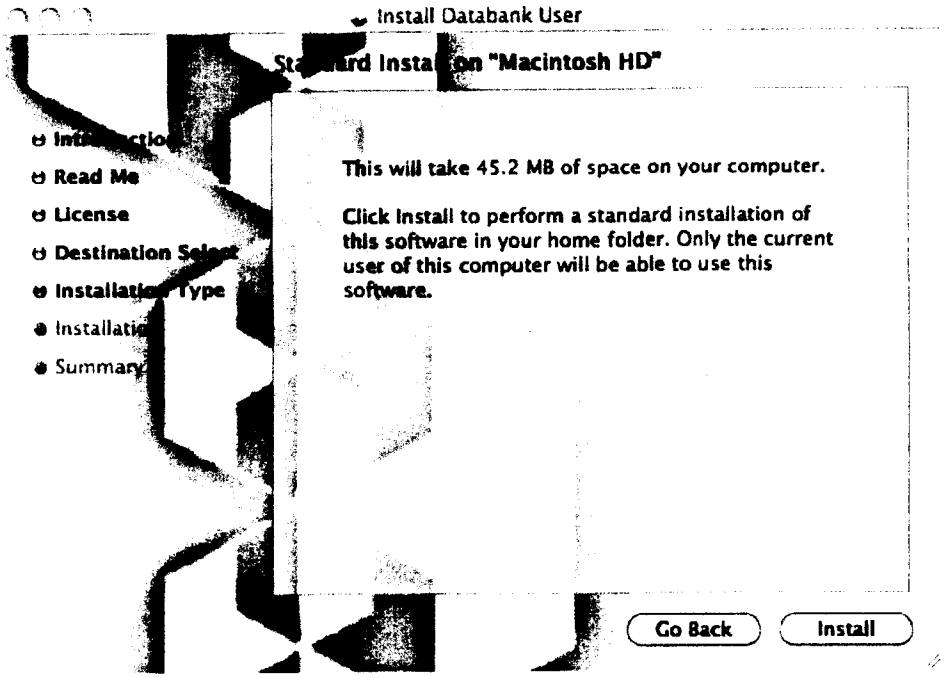


Figure 13. *Standard Install.*

After the installation is complete, we select 'Close' to finish the process.

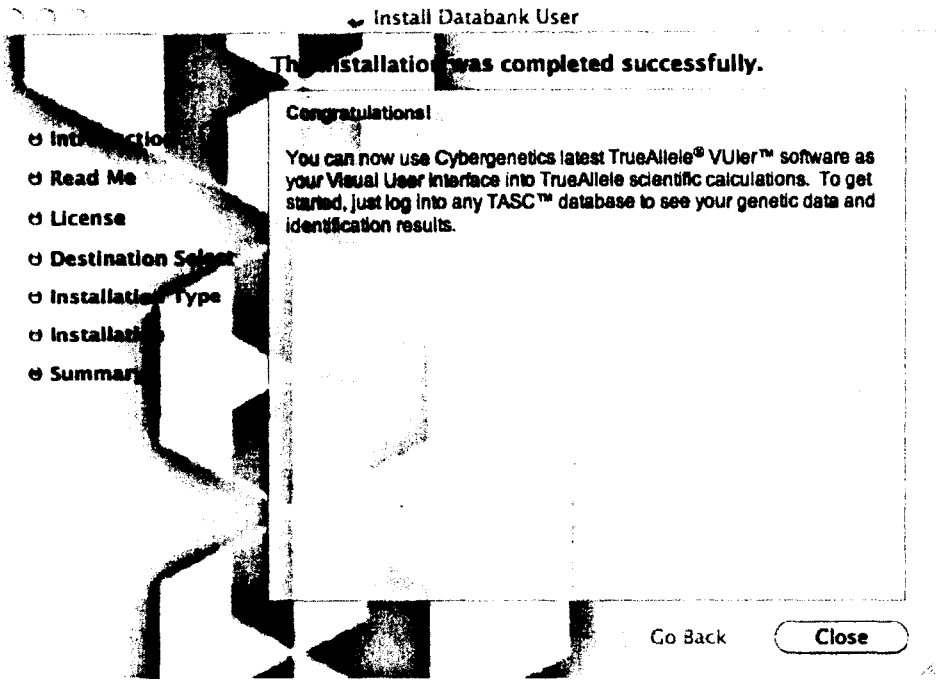


Figure 14. *Installation complete.*

3.3 VUIer

The VUIer software allows us to upload and ask questions of our case data, then review the answers produced by the genetic calculator.

We start by double clicking the zip file VUIer_installer.pkg.zip, which makes the package file VUIer_installer.pkg available. We then double click the package file to begin installation.

On the introduction screen, we select 'Continue' to advance to the next screen.

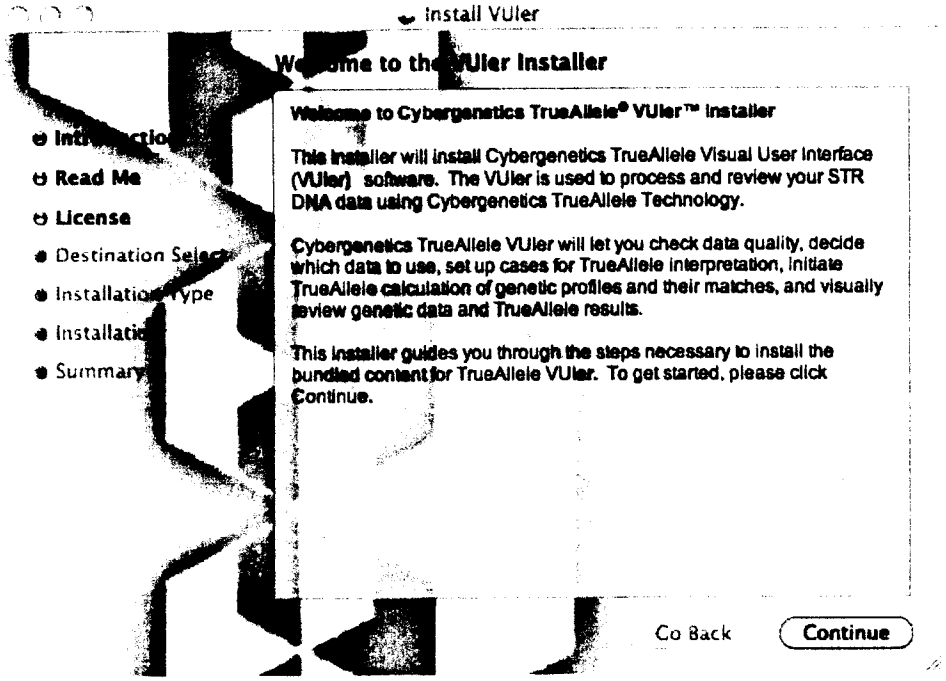


Figure 15. *Introduction Screen.*

The next screen contains a Read Me file for review. After reading the file, we select 'Continue' to advance to the next screen.

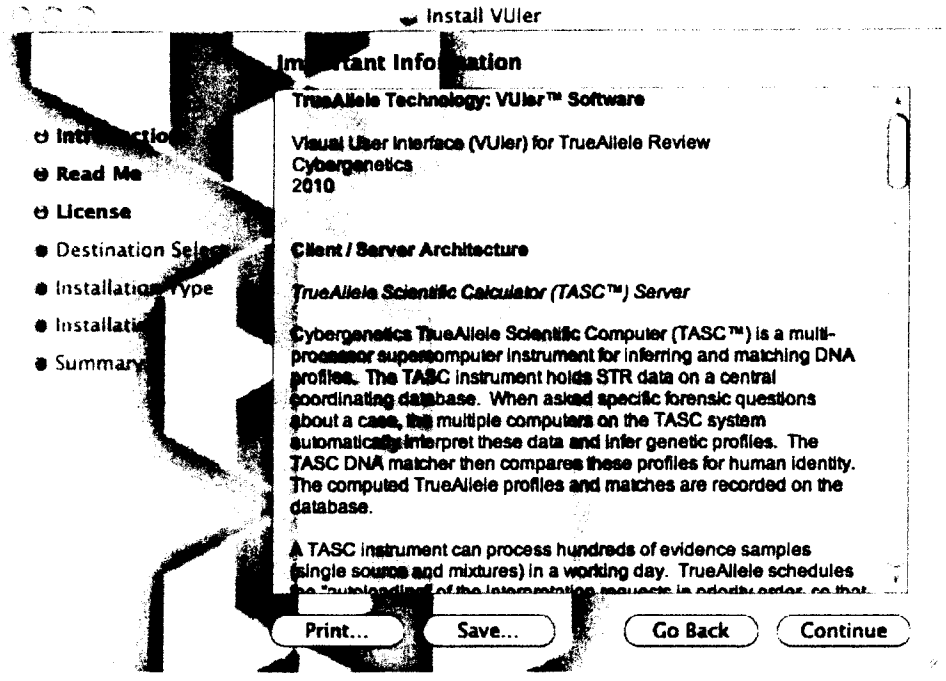


Figure 16. *Read Me.*

Next is the license agreement. After selecting 'Continue', a prompt appears to confirm our agreement with the license. We proceed by selecting 'Agree'.

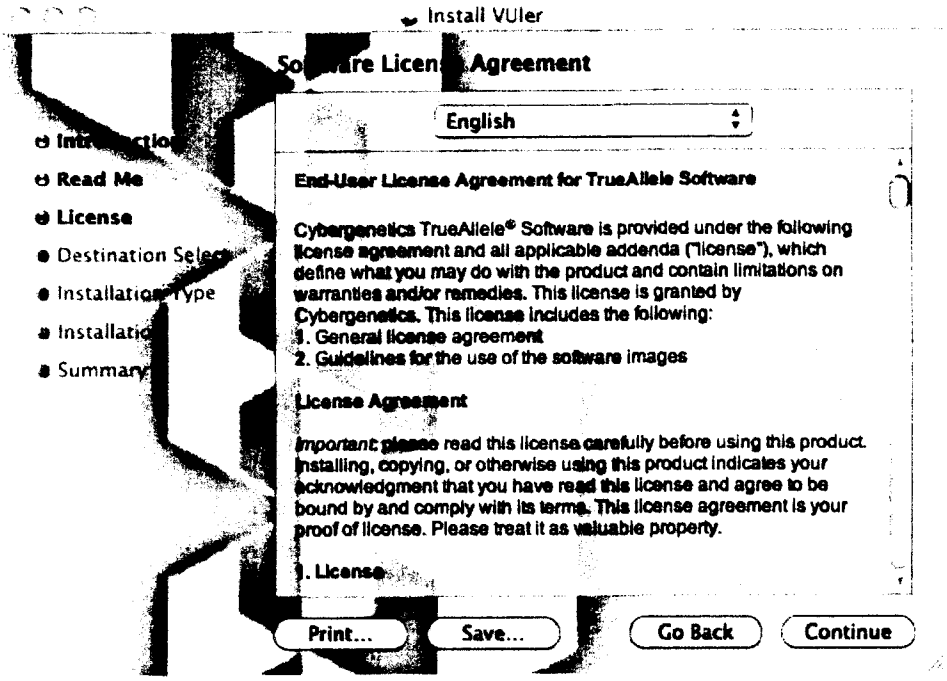


Figure 17. *License Agreement.*

We must perform a custom installation the first time we install the VUIer software on a computer (Note: this is not required for an upgrade as described in the Upgrade section). In the Installation Type window, we select 'Customize' to bring up the Custom Install options. We want to check the box next to TrueAllele. This will install some required user files for us.

We begin the installation by selecting 'Install'. When prompted, we enter the administrator user name and password.

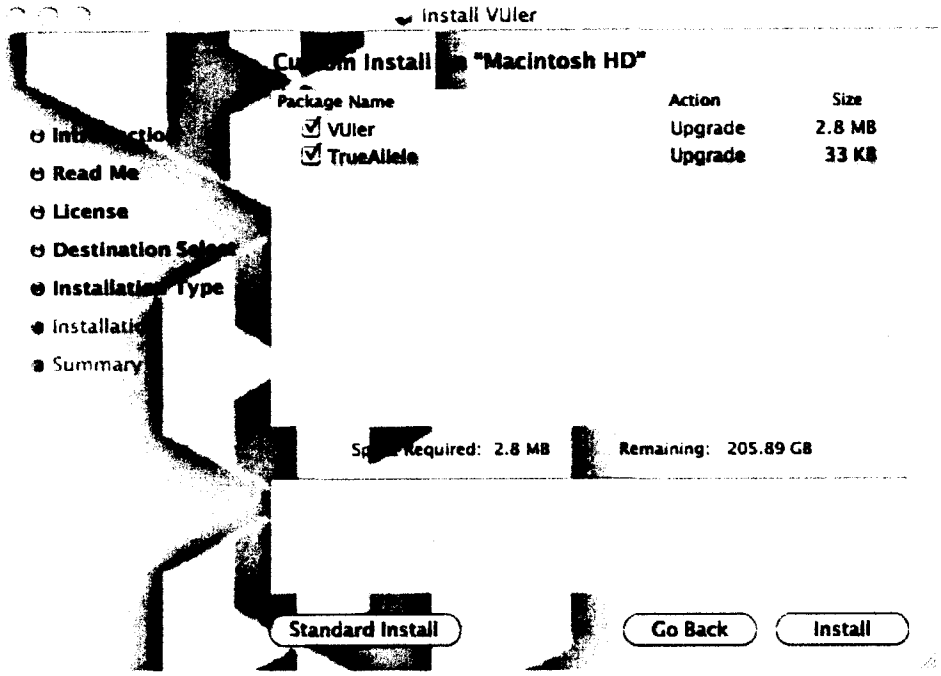


Figure 18. *Custom Install.*

After the installation is complete, we select 'Close' to finish the process. The VUIer software is now installed and available for use.

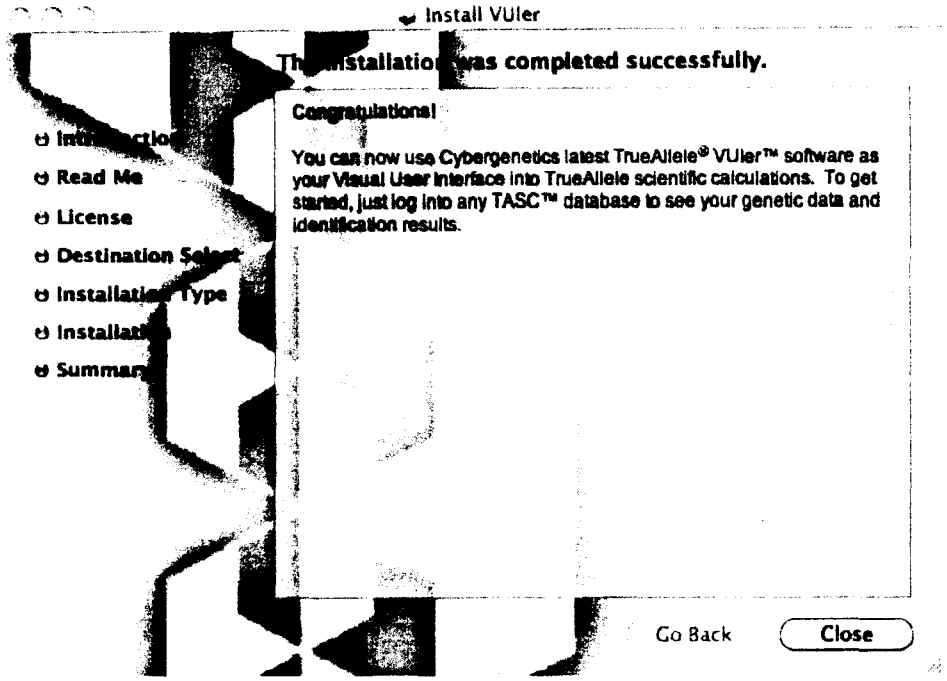


Figure 19. *Installation complete.*

3.4 Update

We follow the same process when updating the VUIer software as described in section 3.3. However, a custom installation is not necessary. In the Installation Type window, we select 'Install'. When prompted, we enter the administrator user name and password.

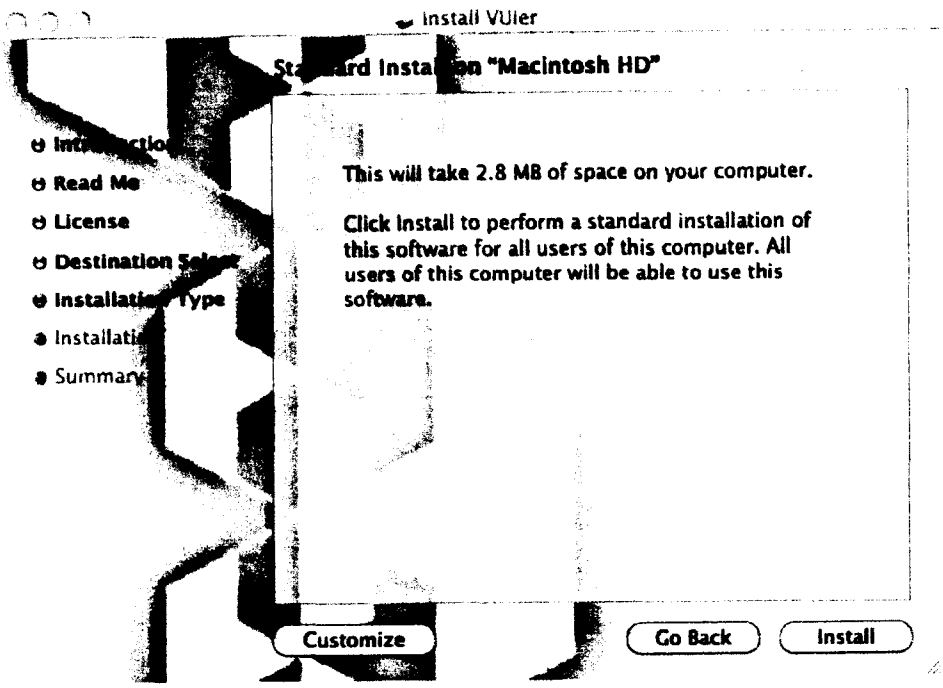


Figure 20. *Standard Install.*

After the installation is complete, we select 'Close' to finish the process. The VUIer software is now updated and available for use.

4 Open

Now we can review the typical method of opening the VUIer program. An alternative method for starting the program is covered in the **Appendix ▸ Diagnostic Support**.

4.1 Starting the VUIer program

We start the VUIer program by clicking its icon in the Dock (typically located at the bottom of the screen). On some computers, the Dock may automatically hide but placing our mouse cursor at the bottom of the screen will make it appear.

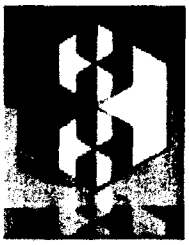


Figure 21. *VUIer icon.*

If the VUIer icon is not already in the Dock, we can place it there in a few simple steps. First, we select the Finder icon in the dock.

Figure 22. *Finder icon.*

The Finder window opens, displaying the computer's folders and files. From the left side bar, we select Applications. In the Applications folder, we locate the VUIer icon, then click and drag the icon down to the Dock. This will place VUIer in the Dock.

5 Connect

In the VUIer program, the *Connect window* gives us the ability to connect to a database or to switch to a different database at any time during the TrueAllele process. We open the Connect window by selecting the **Database ▸ Connect** menu option in any VUIer Module.

To connect, we select the desired database from the dropdown menu (this action fills in the *System* field as well). We enter a username and password under the *Authenticate* heading and click *Verify*. A blue check mark appears, confirming verification and indicating that the connection process is complete.

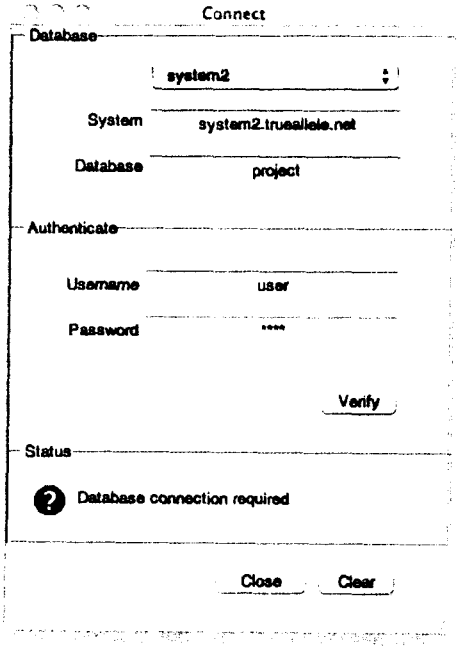


Figure 23. *The Connect window.*

To clear all of the information entered in the above fields, we can use the 'Clear' button at the bottom right of the Connect window. When we are finished with the connection process, the 'OK' button will close the window.

6 Closing

The TrueAllele Sever accepts a limited number of concurrent user connections. We must properly end our user sessions so the connection is open for another user. There are two main methods of ending a user session.

When we are done using the VUIer software, we can close the program by selecting **VUIer ▸ Quit**. This will close the program and automatically log out of any current connections.

If we do not want to close the VUier program but wish to end our current session, we can logout of a TrueAllele World. From within any module, we select **Database ▸ Logout.** This selection logs us out of our current database, making the connection available for other users.

To close a window within a module, we can select **File ▸ Close**. To close a module and go back to the module chooser, we can select **File ▸ Exit Module.** This selection brings up a dialog box asking if we are sure we want to exit the particular module and choose a different one. Here we can select "Yes" or "Cancel" to remain in the current module.

7 Appendix

This appendix is designed to provide specific information that may help us in our TrueAllele processing. A glossary of common terms is also provided.

7.1 Spinning Wait Cursor


Spinning Wait Cursor

The spinning wait cursor is equivalent to the hourglass icon on a PC. This icon indicates that TrueAllele is retrieving data from the database. This cursor is only active when it is held over a VUIer window; if the mouse is moved off of the window (for example, to the desktop), the cursor will go back to the normal pointer.

7.2 Diagnostic Support

To aid in diagnostic support of the VUIer program, an alternate means of starting the program is available. This method is not routinely used, but is available to aid in support calls to Cybergenetics.

First, we open a Terminal window by selecting the Terminal icon from the Dock.

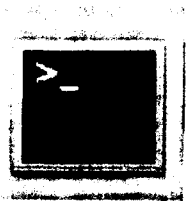


Figure 24. *Terminal icon.*

A blank Terminal window opens, displaying the computer's name and a command prompt.

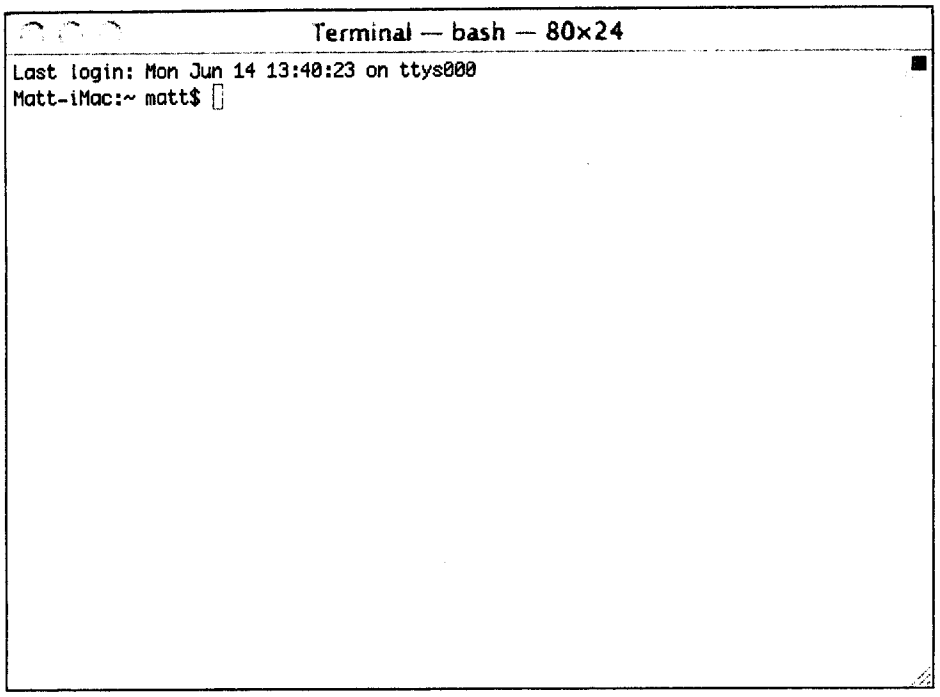


Figure 25. *A blank Terminal window.*

In the Terminal window, copy and paste the path

`/Applications/VUIer.app/Contents/MacOS/run_VUIer.sh`

and then press the enter key.

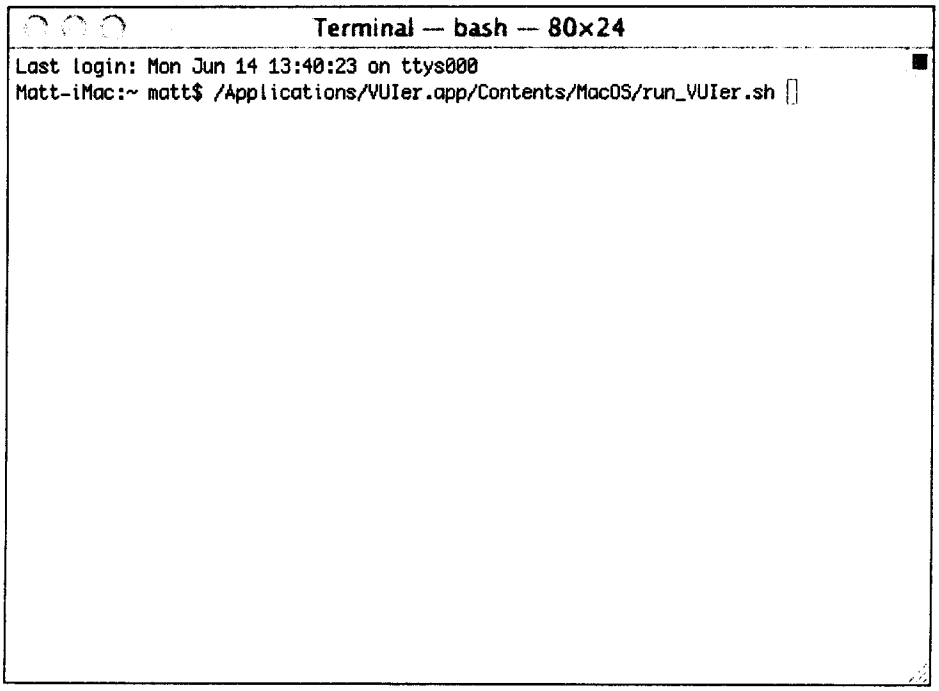


Figure 26. *A Terminal window with the path filled in.*

By starting the VUIer program this way, we allow any diagnostic messages that are displayed during software operation to appear in the Terminal window. We can copy and paste these messages into an email to Cybergenetics if support is required.

7.3 Glossary

This glossary presents some commonly used terms. The term of interest is italicized.

Evidence *data* comprise STR experiments on a set of samples.

An observed *genotype* is a probability distribution over a set of possible allele pairs at a locus.

The identification *hypothesis* is that a suspect contributed their DNA to an evidence sample.

Identification *information* is the logarithm of the identification LR.

A *likelihood function* determines the fit between data and model. (More formally, it is the probability of the data given a model.)

An identification *likelihood ratio* (LR) is the weight of evidence in favor of an identification hypothesis. The LR can be calculated as a ratio of two match probabilities.

A *Markov chain* is a visual history of the search that the system performs in order to find the best fit model for the data.

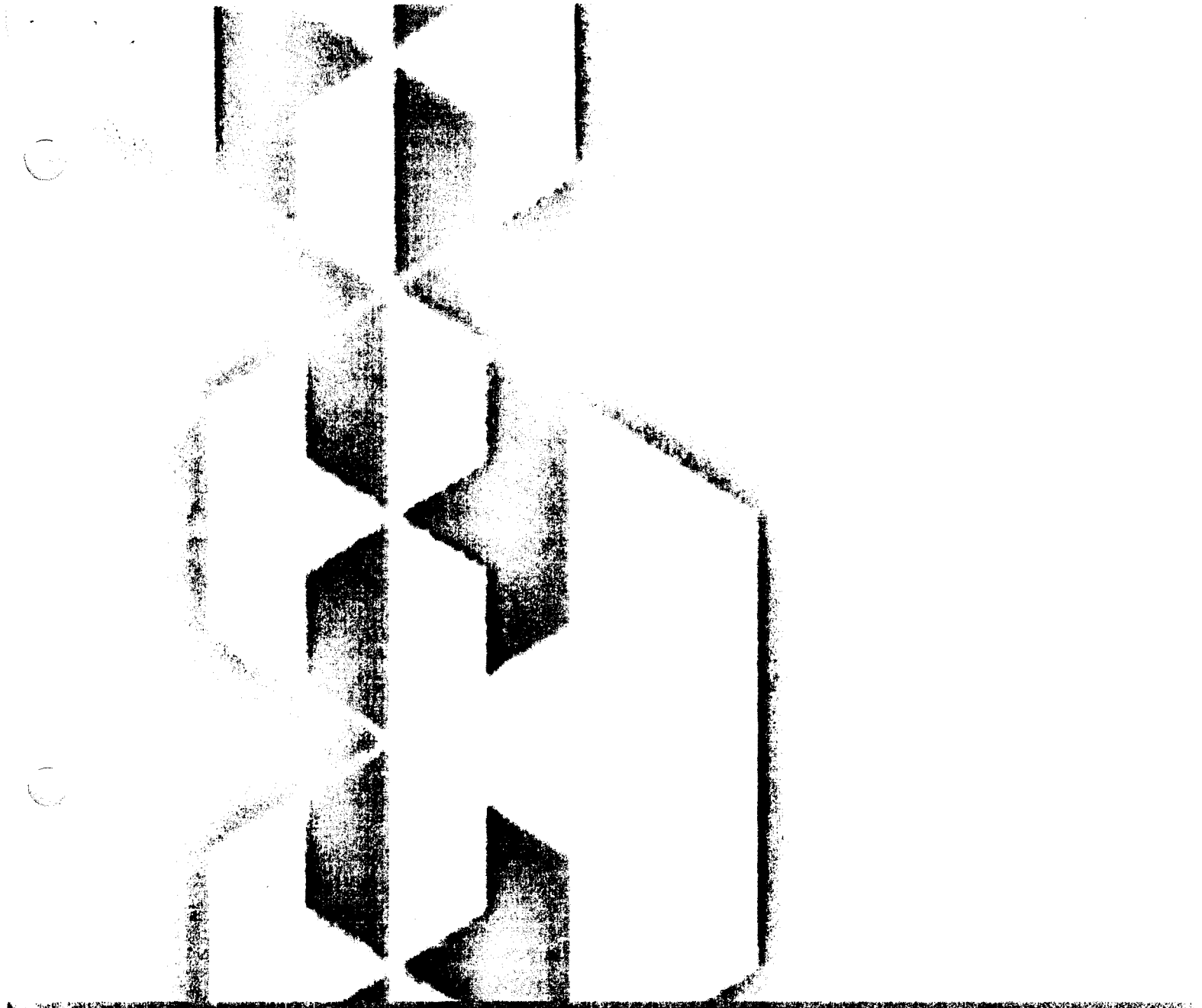
A *match* occurs when two genotypes are equal.

A *match probability* is the probability of a genotype match.

A *mixture* is a DNA sample that contains two or more individual contributors.

A probability *model* is a mathematical formulation that accounts for observed data.

A *validation* study assesses the efficacy and reproducibility of an interpretation method for extracting identification information from DNA data.



TrueAllele® Casework Workflow

Introduction

Cybergenetics

Table of Contents

1 Overview

Cybergenetics TrueAllele technology for automated interpretation and reporting of DNA evidence is based on biology, mathematics and computation. This document describes the TrueAllele workflow from a system and user perspective.

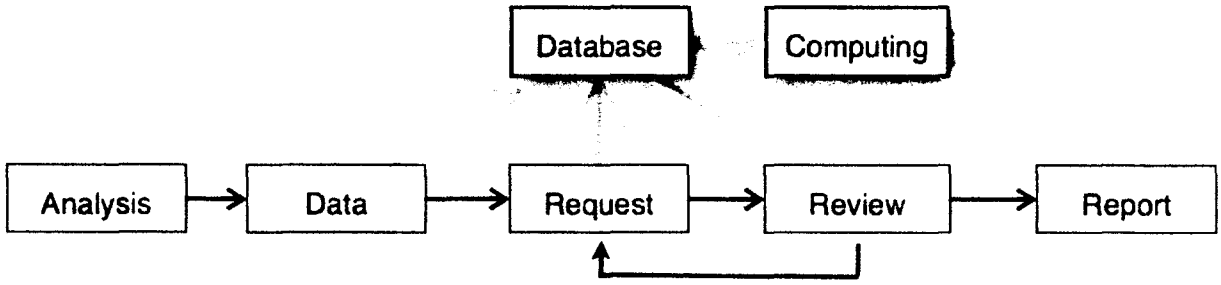


Figure 1. *Workflow.*

2 Workflow

2.1 Analysis

The DNA interpretation process requires quality-checked quantitative data. The TrueAllele Analysis computer starts with the laboratory's original electronic DNA files, and works with the user to check and quantify these raw data signals, in order to produce interpretation-ready data. For each 96-well plate of DNA samples and controls, Analysis applies multiple rules to the signals to ensure that good data move forward on to interpretation. The computer gives the lab feedback about any data issues that it finds. The process is fast, taking a few minutes of user time for a typical DNA plate.

To assess the DNA data signals in Analysis, a user opens a folder of electronic DNA sequencer files. He then asks the TrueAllele computer to check the DNA sizing calibration data (Figure 2), and looks for any problems with these (and other) control samples. Man-machine communication is exchanged visually, with the user pointing his mouse at the screen to explore an issue, and the computer responding by rendering a data image or figure that focuses on the user's question. After the computer has processed the peak events in the DNA data signals, the user has a data file of quality-checked quantified peaks ready for the database.

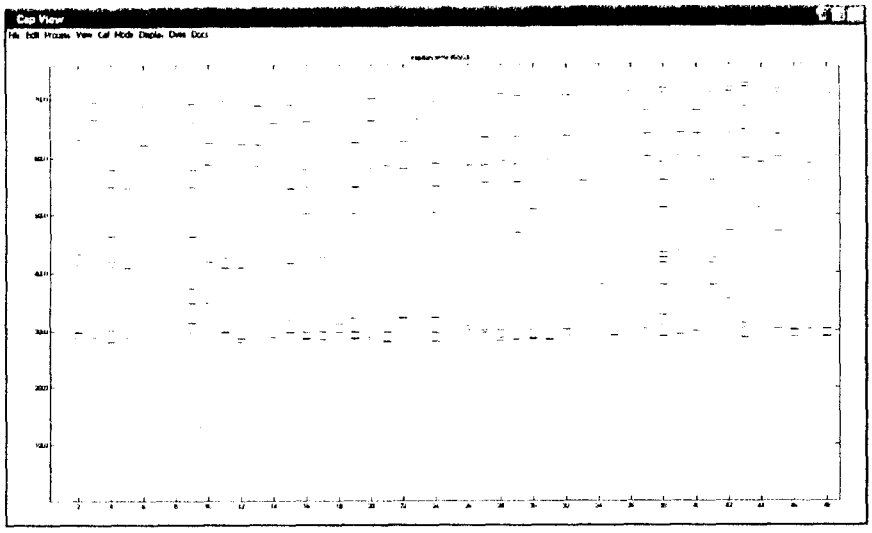


Figure 2. *Cap View.* The Capillary View (Cap View) allows a user to evaluate the quality of the data. A user visually determines if there are any sizing issues in the data.

2.2 Data

After peaks are quality checked in the Analysis phase, we can view them in the TrueAllele Data interface. This gives the user another opportunity to review the peaks before interpretation. The TrueAllele computer can signal the presence of any possible artifacts in the data, so that the user can evaluate the peak and take action upon it if necessary. Once the quality-checked peaks have been reassessed, they are ready for upload to a TrueAllele database, and then used in TrueAllele interpretation.

To upload quality-checked quantified peaks into a database, the user opens a "Visual User Interface for easy review" (VUIer™) Data window. He first connects to a TrueAllele database that will store the data. After opening the file created in the Analysis phase, the data peaks appear on the screen as intuitive visually rendered signals (Figure 3). Each data injection is shown within its own track. The user can ask the computer to show possible lingering data artifacts, along with pertinent data information. This annotating information is stored with the peak file on the database.

When the data review is complete, the user uploads the peak data to the database, making it available for creating TrueAllele interpretation requests.

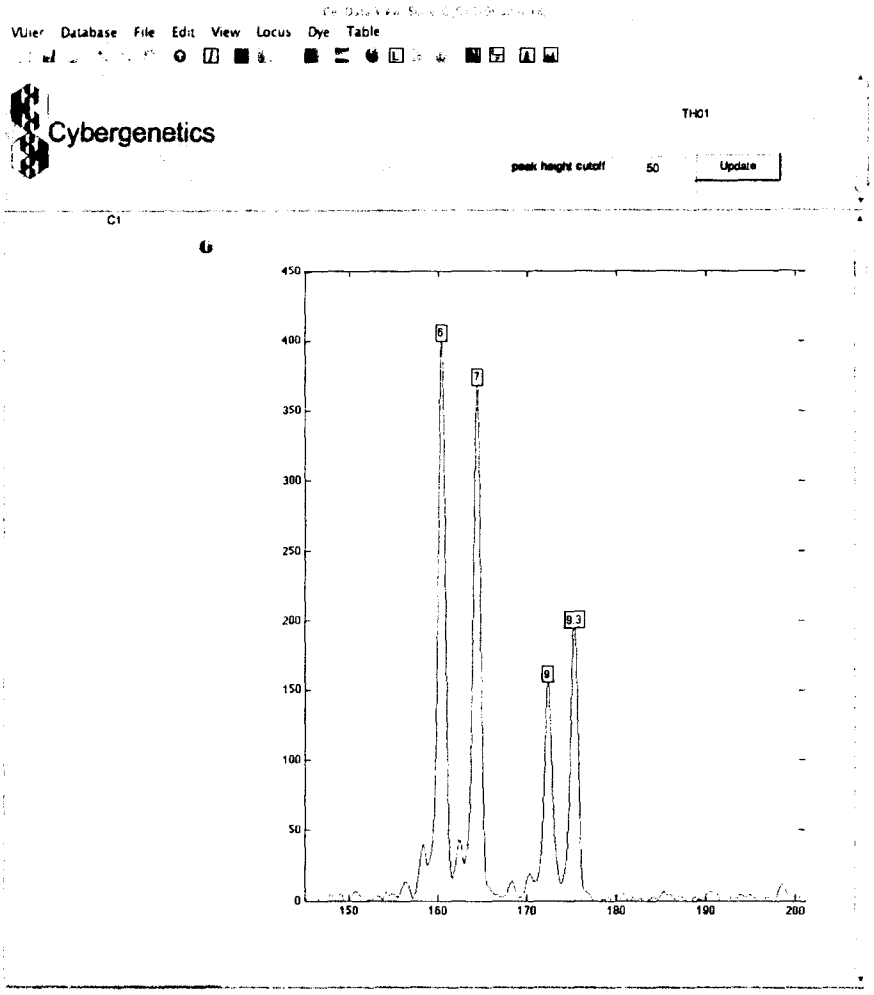


Figure 3. *Gel Data View.* Data window zoomed into TH01 locus of a two person mixture. In the data signal we see four allele peaks. The two tall peaks are from the major contributor, and the two smaller peaks are from the minor contributor.

2.3 Database

The uploaded DNA data reside on a TrueAllele PostgreSQL relational database. The database is like an electronic filing cabinet that permits information retrieval simultaneously from multiple file folders. The database provides persistent and secure storage for all the information needed by the TrueAllele user and system. The (over seventy five) database tables provide quantitative DNA data, TrueAllele interpretation questions, the computed results, and supporting information, such as

population frequencies. The database also helps administer system activities, and supports the monitoring expert system that coordinates the system.

The user logs on to a TrueAllele database to initiate processing or to review results. The user's interactions are mediated through the TrueAllele VUIer software installed on their computer. The VUIer database client exchanges DNA case data with the database, and presents information visually on the computer screen. All the user modules (e.g., Data, Request, Review, Report) automatically generate database queries and DNA visualizations through the VUIer (Figure 4). Typical displayed case information includes DNA data, genotype probability, mixture weight distribution and match rarity likelihood ratio values.

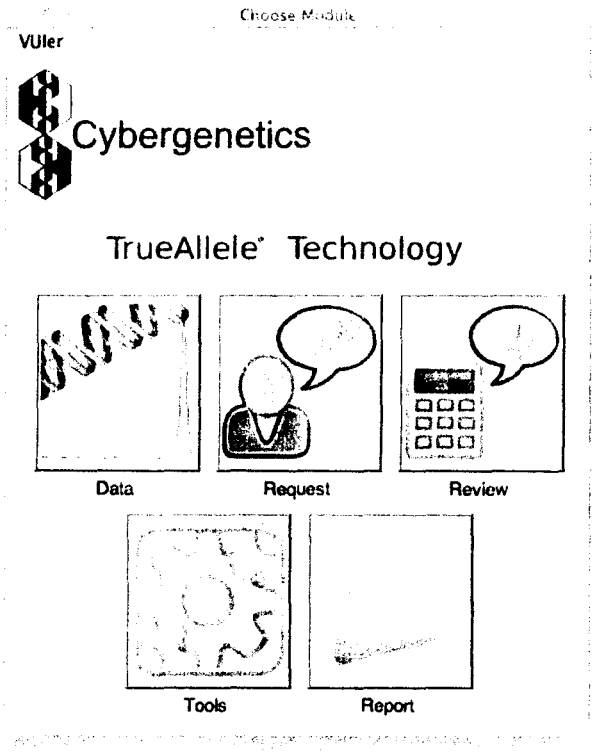


Figure 4. *Module Chooser.* The TrueAllele VUIer modules are entry points into the database. The Data, Request, Review and Report modules follow the flow of STR data through the interpretation process. The Tools module helps the user to admister a database.

2.4 Request

Once the data are on the database, we can ask DNA interpretation questions that the TrueAllele computer can solve for us. Each question involves one or more DNA evidence items, and can be run under different problem solving conditions. (Example conditions are how many unknown contributor genotypes to find, how much computer time to use, or whether to account for degraded DNA.) While a victim reference may be optionally included in a question, for total objectivity a suspect genotype is never used. Questions can be asked one at time, in duplicate for reproducibility, or in batches of a hundred or more. Regardless, once a question has been posed, the statistical calculating is done entirely by computer.

To ask interpretation questions in a case, the user opens a VUIer Request window. After connecting to his evidence database, he selects the DNA data that he wants to use. These data images appear visually in the interface, with each signal in its own track. He then forms visual DNA items (each corresponding to an evidence sample) from the track signals. Finally, he makes each case interpretation request by indicating one or more DNA items (Figure 5), and setting optional problem solving parameters. Once he is satisfied with his questions, the user uploads his interpretation requests to the TrueAllele database for computer processing.

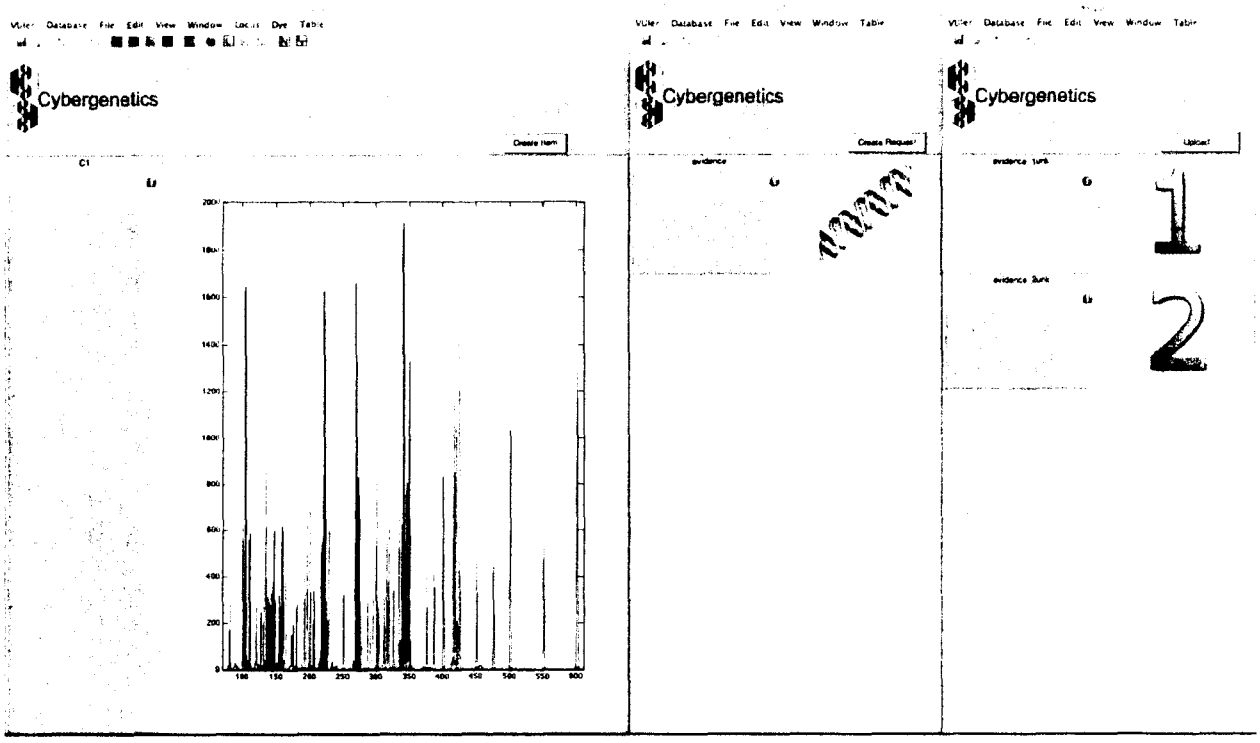


Figure 5. *Request Window.* When asking interpretation questions of the STR data, a user creates DNA items. Before uploading to the TrueAllele Database for processing, the user can visually see the data supporting each request.

2.5 Computing

After the user has posed DNA interpretation questions, a TrueAllele server interpretation computer automatically retrieves a request and its data from the database. TrueAllele interpretation uses all the data to infer a genotype distribution and mixture weight for each DNA contributor. To infer a genotype distribution, the computer explores various peak patterns to statistically model the data. Throughout this modeling process the computer considers many different variables, such as genotype, mixture weight, stutter and preferential amplification. As a result, the reported genotype distribution reflects how well a set of proposed patterns fit the data. Patterns that closely fit the data receive higher probabilities, and patterns that do not receive lower probabilities. A separate TrueAllele server computer then matches the inferred genotype distribution against provided references, and calculates a likelihood ratio statistic.

The TrueAllele parallel compute servers can process multiple requests at the same time. For example, solving a DNA interpretation question in duplicate creates two independent calculations, establishing statistical reproducibility. We routinely run 24 parallel TrueAllele processes on our system, each one working on a different case (figure 6). A typical DNA mixture takes about an hour or so to solve, so the overall throughput can be quite high (e.g., over 300 cases a day). When the problem solving is done, the computer stores its results (inferred genotype distributions, mixture weights, likelihood ratios, etc.) on the database for downstream review.

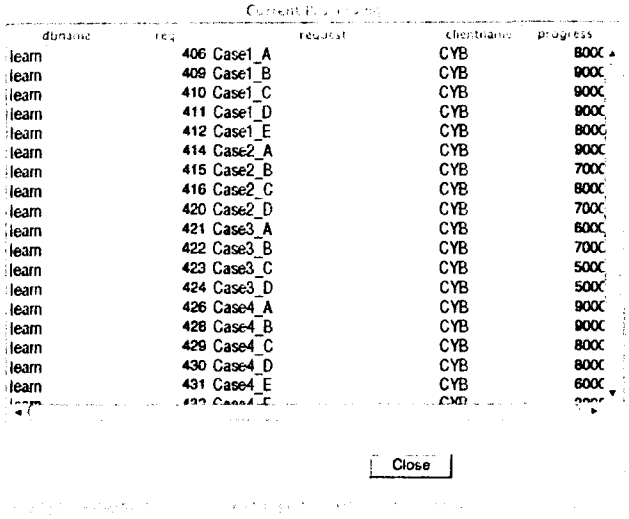


Figure 6. *Current Processing.* Multiple evidence interpretations can be processed at the same time on the TrueAllele system. In the Tools module, a user can monitor the current status of an interpretation process.

2.6 Review

Once the requests have finished processing, we can review the computer interpretation results. During this review process, we can see several aspects of the DNA case. For example, we can examine a contributor's genotype probability distribution, either visually or in a table. It is this key genotype variable, and its probability uncertainty, that establishes genetic identity. With multiple DNA contributors, we can visually review mixture information with informative pictures of

mixture weight probability. The quantitative match information can be seen visually at the different genetic loci.

The user first opens a VUIer Review window, and selects a request from the database. A Profile window appears, visually displaying computed genotype probability distributions. From here, the user can navigate to other windows, including ones for the original Data and the Mixture separation (Figure 7). When TrueAllele finds a match between an evidence contributor and a suspect, the Match window and tables show quantitative LR match information. An Explain window visually explains the computer's reasoning. A user can always ask more questions by exiting Review and returning to the Request module, where he can create new TrueAllele interpretation questions.

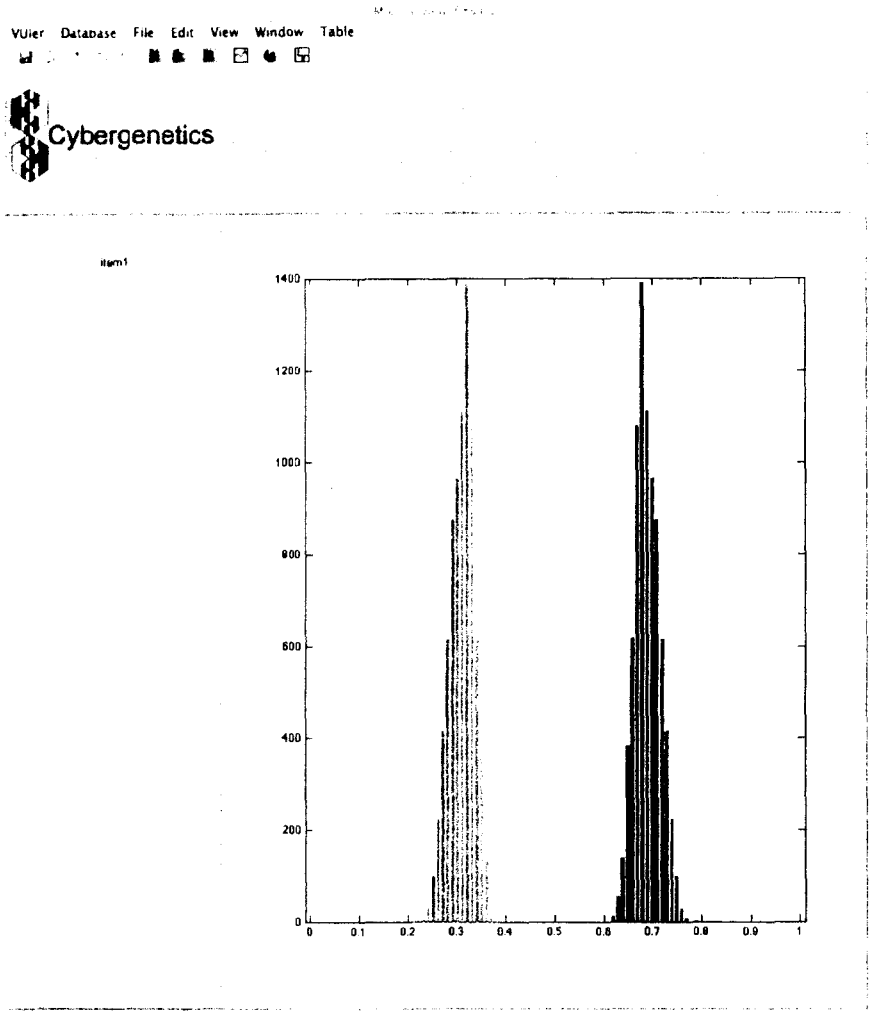
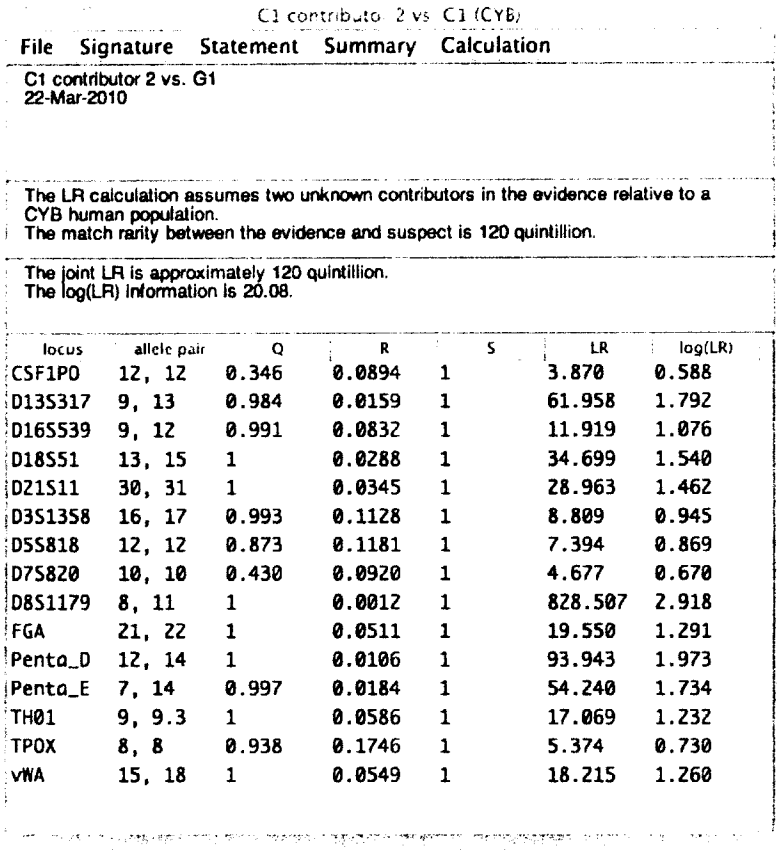


Figure 7. *Mixture Weight.* The Mixture window shows a histogram of the inferred mixture weight for each contributor in a DNA mixture. The sharper the bell curve, the more confidence the computer has in its answer.

2.7 Report

After the interpretation requests have been processed by the computer and reviewed by the analyst, we are ready to generate reports for court presentation. TrueAllele generates the customizable report automatically based on user selected options. A typical report consists of an evidence interpretation summary, lab information, a match rarity statement and detailed locus results. The reported match statistic incorporates appropriate population allele frequencies, and can apply a coancestry coefficient (theta) for a statistic with population substructure.

For automatic report generation, the user opens the VUIer Report window. After connecting to a TrueAllele database, the user downloads genotypes of interest: evidence contributors, suspect references, and population frequencies. The probability distributions of each genotype are displayed together visually in the VUIer Report window. The user can review different matches of evidence contributors to suspect references, and generate a report for any match (Figure 8). He can export his report from VUIer as a text document, and import it into a spreadsheet program.



C1 contributo 2 vs C1 (CYB)

File Signature Statement Summary Calculation

C1 contributor 2 vs. G1
22-Mar-2010

The LR calculation assumes two unknown contributors in the evidence relative to a CYB human population.
The match rarity between the evidence and suspect is 120 quintillion.

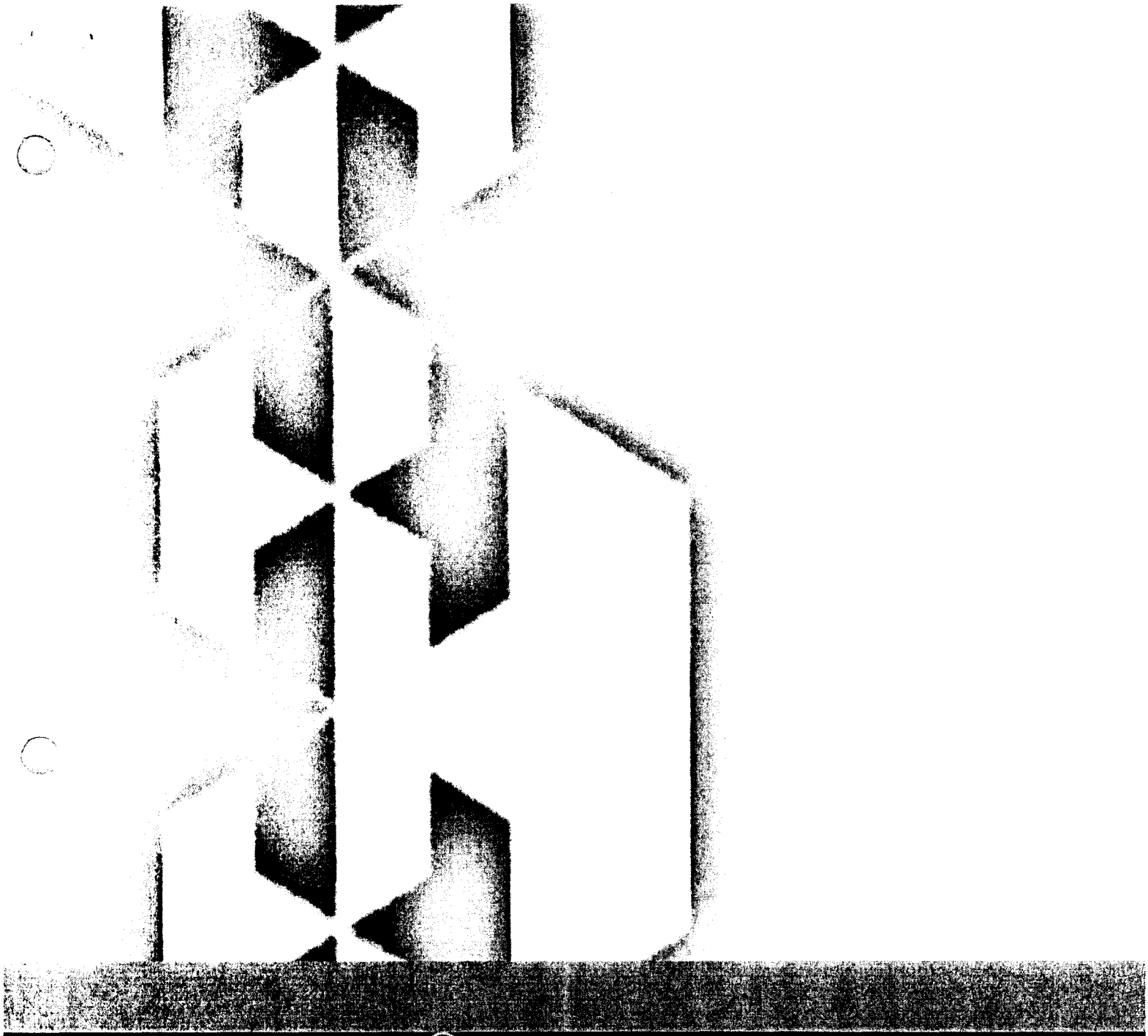
The joint LR is approximately 120 quintillion.
The log(LR) information is 20.08.

locus	allele pair	Q	R	S	LR	log(LR)
CSF1PO	12, 12	0.346	0.0894	1	3.870	0.588
D13S317	9, 13	0.984	0.0159	1	61.958	1.792
D16S539	9, 12	0.991	0.0832	1	11.919	1.076
D18S51	13, 15	1	0.0288	1	34.699	1.540
D21S11	30, 31	1	0.0345	1	28.963	1.462
D3S1358	16, 17	0.993	0.1128	1	8.809	0.945
D5S818	12, 12	0.873	0.1181	1	7.394	0.869
D7S820	10, 10	0.430	0.0920	1	4.677	0.670
D8S1179	8, 11	1	0.0012	1	828.507	2.918
FGA	21, 22	1	0.0511	1	19.550	1.291
Penta_D	12, 14	1	0.0106	1	93.943	1.973
Penta_E	7, 14	0.997	0.0184	1	54.240	1.734
TH01	9, 9.3	1	0.0586	1	17.069	1.232
TPOX	8, 8	0.938	0.1746	1	5.374	0.730
vWA	15, 18	1	0.0549	1	18.215	1.260

Figure 8. *Simple Report.* The TrueAllele likelihood calculation is displayed for case documentation and testifying. The likelihood ratio is a comparison of the genotype probability distributions before and after processing the STR data. The strength of information gain from the evidence is reported in a match rarity statement.

3 User Manual

Each User Manual will guide us through the TrueAllele Casework process. The tour begins with Analysis, where the goal is to quality check STR data. Once the data has passed through Analysis, the data is uploaded into to a TrueAllele World. This process is described in the Data Module Manual. The concept of creating and connecting to TrueAllele Worlds is detailed in the Database Manual. Once the data is on a database, a user can then begin asking DNA interpretation questions by following the Request Manual. How an analyst reviews interpretation results is detailed in the Review Manual. Finally, the Report Manual shows how to generate case documentation and match statements.



TrueAllele® Technology

Analyze Module

Quality Assurance

Cybergenetics

Table of Contents

1 Overview

The purpose of the Analyze Module is to transform raw sequencer data into quality-checked quantitated peaks. The output of the Analyze process is a gel file containing all signal and peak information from the original raw data. We can upload a gel file in the Data Module, making the data available for interpretation.

The Analyze Module uses DataDisks to organize the data as it proceeds through the process. A DataDisk is a folder that contains all the original electropherogram data, auditing information and output. A DataDisk is self-contained and structures the data for the software.

The general flow of processing requires the computer to perform a quality check on the data, and then we review the computer's quality check. The computer will direct us to any potential data issues as part of the review process.

2 Getting Started

To open the Analyze Module, we select the Analyze icon in the *Module Chooser* window (Figure 1).

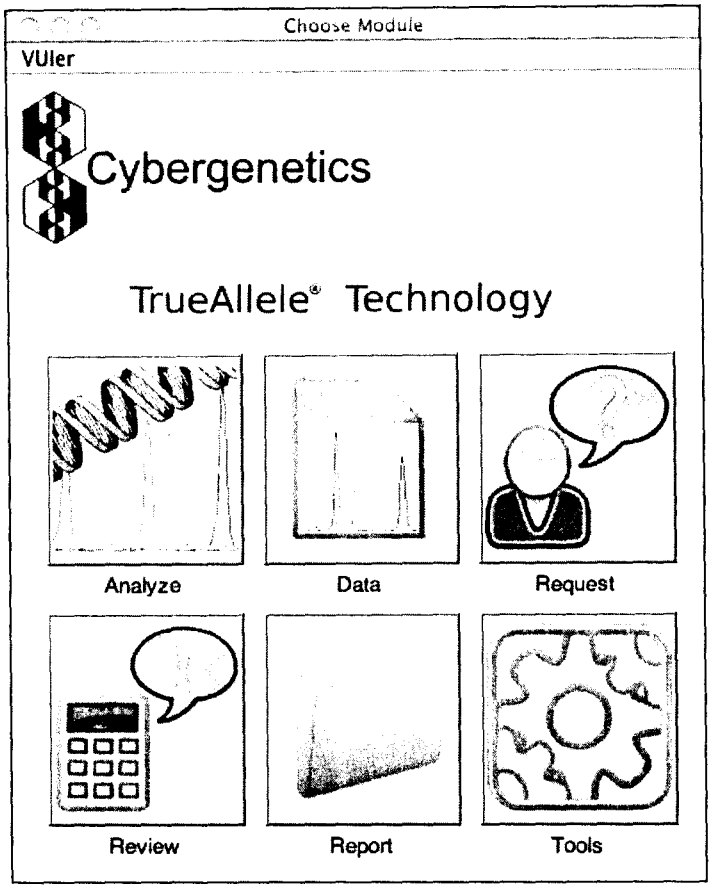


Figure 1. *Module Chooser window.*

This action opens the *Command window* of the program. The *Command window* is the starting point of Analyze and where each computer step, followed by human review, is driven (Figure 2).

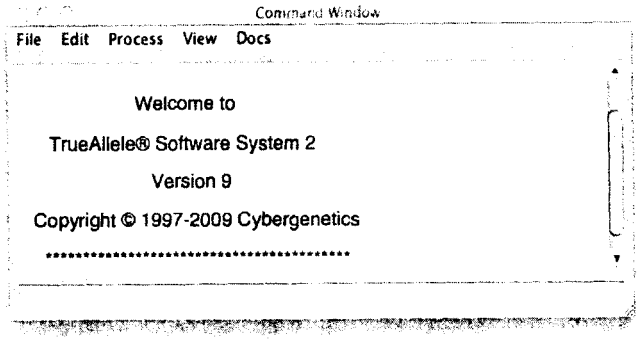


Figure 2. *Command Window.*

2.1 Initial Preferences

In order for the data to be read correctly, the initial preferences must be set for the specific type of sequencer data. The preferences tell the computer information about the type of data being used, such as size standard labels, kits/panels, and sequencer format. If these preferences have not been set up or the type of data has changed, we can review the **Appendix ▸ Initial Settings** or contact Cybergenetics for assistance. These settings can be viewed under **Edit ▸ Preferences ▸ Init**. Once confirmed, they will be retained between sessions.

After the computer has been told what type of data to expect, a *DataDisk* can be created. A DataDisk contains all the information necessary for the program to process the data.

2.2 Creating a DataDisk

The DataDisk is used to proceed through the Analyze process, quality checking the STR data prior to upload to a TrueAllele Database.

From the Command window, we can direct the computer to the location of a STR data folder. Selecting **File ▸ New** from the toolbar allows us to point to the folder on

the computer where the STR sequencer files are saved. Information on the layout of the folder is detailed in the **Appendix ▸ Sequencer Format**.

Upon opening a folder of STR data, a DataDisk is automatically created using the raw data and the template selected. Each DataDisk folder stores all of the information generated during the Analyze process. This folder is designated by a "_DD" and automatically saved in the same directory as the original data folder. Once a DataDisk has been created for a specific set of data, the DataDisk can be reopened in Analyze by selecting **File ▸ Open**.

3 Sizing

In the first step of the Analyze process, the computer tracks the size standards in Image Call. We can review this tracking in Cap View.

3.1 Image Call

The first computer process is Image Call, where the computer looks at the size standards. Tracking the size standard peaks takes about 1 minute per plate. This computer step is initiated by selecting **Process ▸ Image Call**.

3.2 Cap View

The first human review step is Cap View. Typically, we should spend about 1-2 minutes per plate in this interface.

To start the review process, select **View ▸ Cap View**. When first entering an interface, we are prompted to enter our initials. A default login of "user" is available.

In Cap View, we are presented with a "virtual gel" view showing the size standard dye (Figure 3). In this view, each injection is a single column. The size standard peaks are shown as bands. This provides a global overview of the sizing, allowing for efficient review.

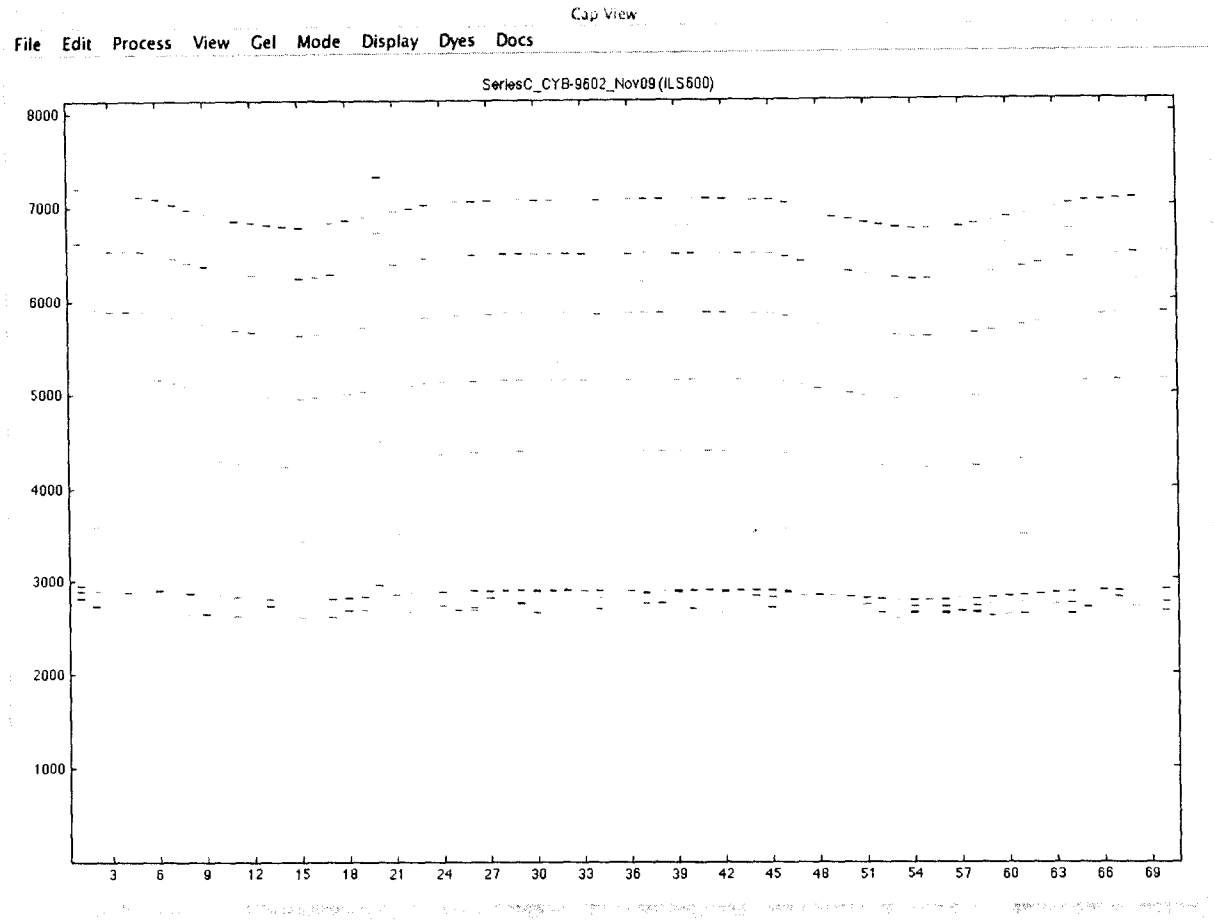


Figure 3. *Cap View.*

From the Cap View window, select **Display ▸ Grid**. This displays the computer's tracking results. The computer's tracking is displayed as blue x's. We visually scan across the injections, looking for deviations from the standard pattern.

Modify Mode

If we see an injection where the sizing pattern appears to be only slightly shifted or the incorrect band is labeled, we can edit the tracking manually. From the Cap View window, we select **Mode ▸ Modify**, opening an AutoEdit Sizing window (Figure 4).

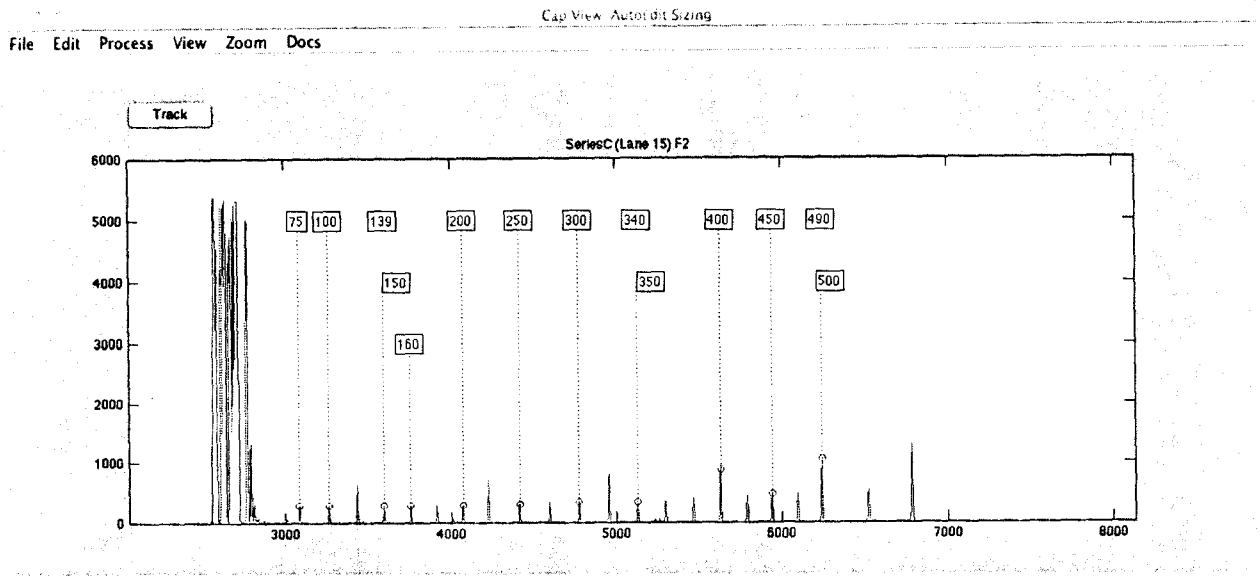


Figure 4. *AutoEdit Sizing window.*

The AutoEdit Sizing window allows us to modify the tracking of a size standard lane. We see the size standard dye displayed in the window; the x-axis displays the pixel coordinates and the y-axis displays the rfus. The labels across top of the window show the size standard labels and the blue circles indicate the tracked peaks.

We modify the tracking working from left to right along the electropherogram. We begin by ensuring that the size standard peaks are tracked and other non-standard peaks are not tracked. For example, we may notice that a non-standard spike peak has a blue circle, indicating it is being tracked. We can click the blue circle to remove the peak from consideration. We can also click on a peak to add a blue tracking circle. The software will automatically place the blue circle at the top of the peak.

Once, we have ensured the peaks are correctly tracked, we then consider the size standard labels. Selecting a white label will turn it gray, indicating that label will not be used. Selecting a gray label will reactive it, turning the label white. We want to ensure there is an equal number of tracked peaks and labels.

We tell the system to automatically re-track the lane by clicking the 'Track' button. Each label is assigned to a tracked peak, indicated by the dotted lines. Once we have re-tracked the injection, we can close the window. We'll see that the blue x's in the main interface are now updated as well.

Select Mode

If we see an injection where the sizing pattern appears to be largely incorrect, we can remove it from further processing. To do this, we select **Display ▸ Select**, which places us in Select mode. From here, we can click on the injections we wish to remove (Figure 5). We will be prompted by a dialog box to provide a reason for removing the lane.

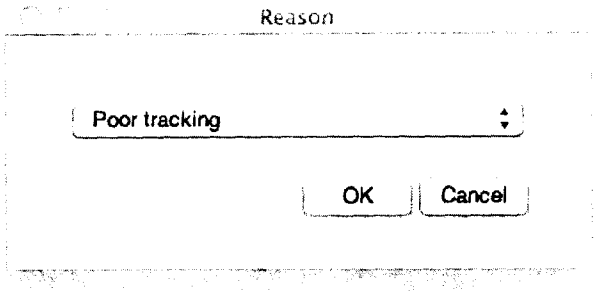


Figure 5. *Reason window.*

In the typical process, we scan the injections and remove any injections with sizing issues. Additional information on troubleshooting for problematic data is found in the **Appendix ▸ Troubleshooting – Sizing.**

4 Controls

The next step of the Analyze process involves quality checking our controls and allelic ladders. The computer does this in Marker Call. We can then review the results of these checks in Control Check.

4.1 Marker Call

Marker Call is where the program tracks the allelic ladders and verifies the quality of the allelic ladders, positive and negative controls. The computer also looks for other possible data issues. We initiate this step by selecting **Process ▸ Marker Call**. Review of the quality checks are organized by Analyze rules, which are described in detail in the **Appendix ▸ Analyze Rules.**

4.2 Control Check

In Control Check, we can review the results of Marker Call. This process typically takes about 30 seconds per plate, because the review is directed by Analyze rules. If no rules fired, then no further action is required. We begin the review by selecting **View ▸ Control Check**. Again, we will be prompted to log in with our initials or the default entry of "user."

Upon entering Control Check, we see a main window and a smaller 'Rules' window (Figure 6). The main window displays a 'virtual gel' view of a specific locus. The Rules window displays a list of the Analyze rules and check boxes. In the typical process, if none of the check boxes are checked, we are assured of the data quality and no further action is required. We can then leave this interface and proceed to Allele Call.

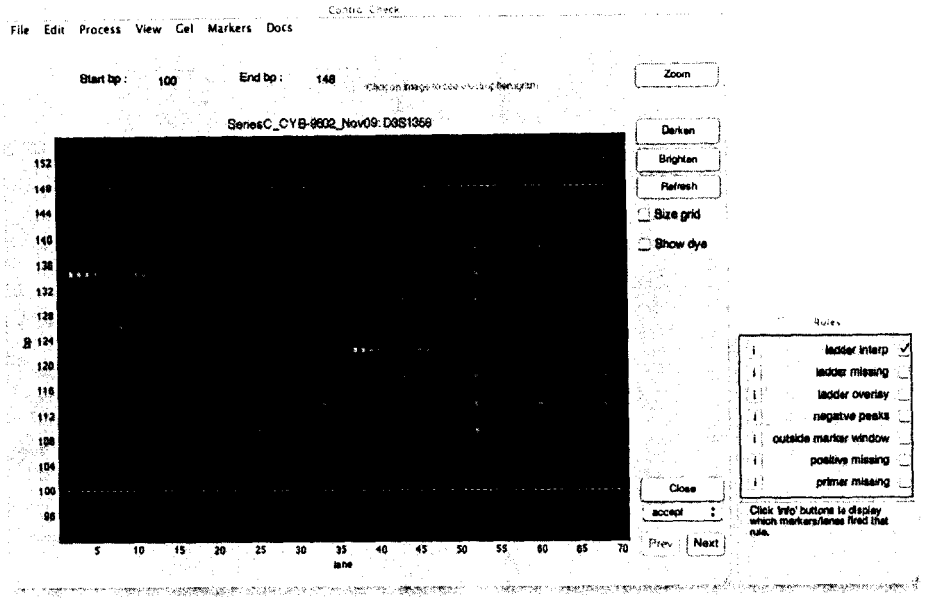


Figure 6. *Control Check.* Control Check window and Rules window.

If a rule has fired, Analyze directs us to review the possible issue. Each rule has associated interfaces to diagnosis these issues. A brief example is provided here, while the full details of the diagnostic interfaces are provided in the **Appendix ▸ Analyze Rules** and **Appendix ▸ Troubleshooting - Controls**.

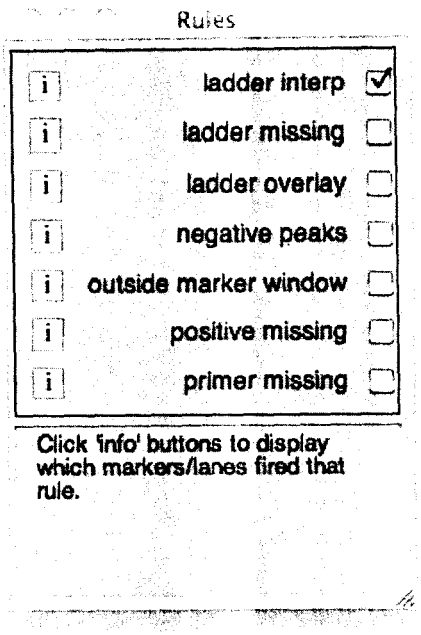


Figure 7. *Rules window.*

Let us assume the 'ladder interp' rule has fired (Figure 7). The ladder interp rule indicates that an allelic ladder peak was interpolated, likely due to low peak height. Selecting the 'i' box next to the rule will provide us with some additional information. In this example, we are directed to TH01. Ladder Check is the interface associated with this rule, so we select **View ▸ Ladder Check**.

We begin with a view showing all of the allelic ladders for our run overlaid on top of each other (Figure 8). We can switch to the TH01 marker by selecting **Markers ▸ TH01**. Vertical lines show the ladder peaks that Analyze has designated.

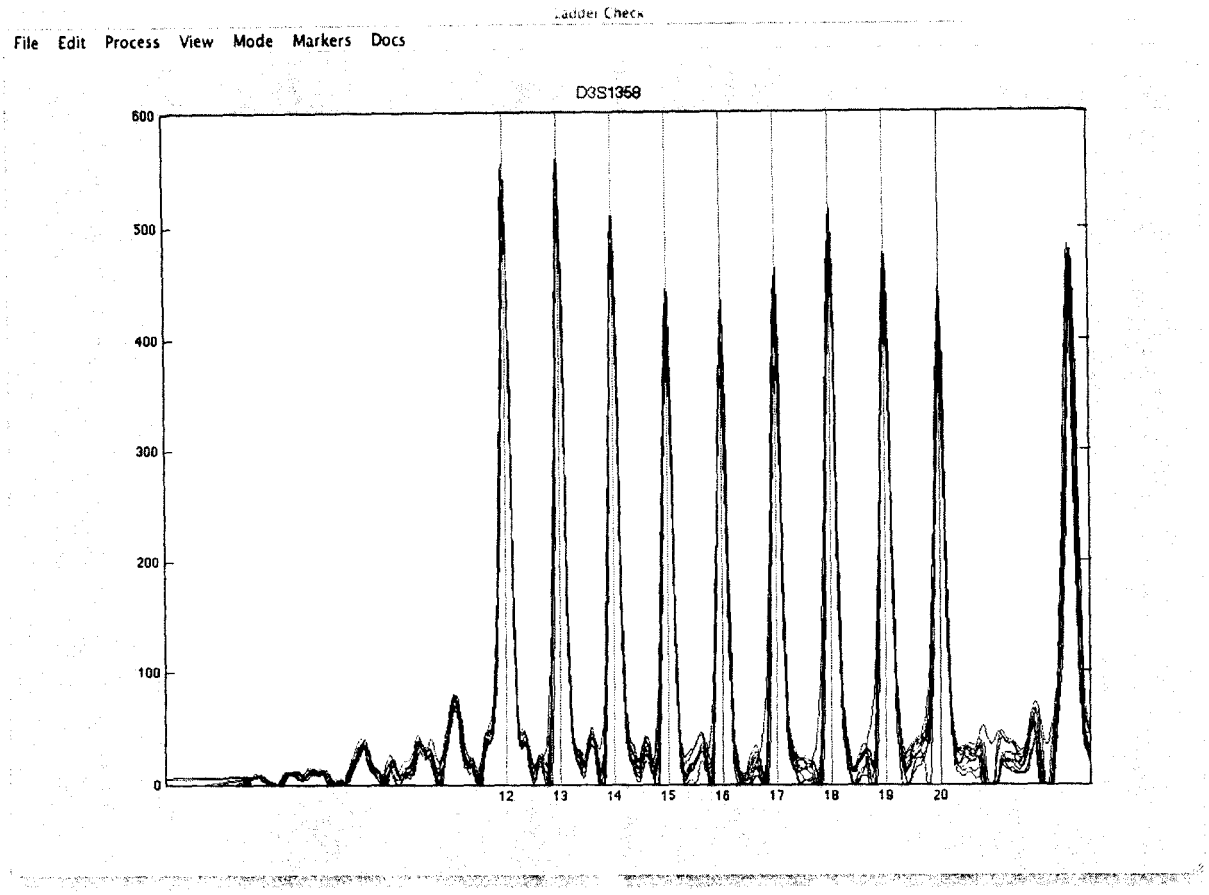


Figure 8. *Ladder Check.*

Clicking on the electropherogram will separate each ladder injection into its own row (Figure 9). We see in the fourth row that the vertical designation line is not through the center of the peak designated 13.3. We can click this lane to reject this specific ladder lane. All data dependent on this ladder lane will be automatically reassigned to a new allelic ladder.

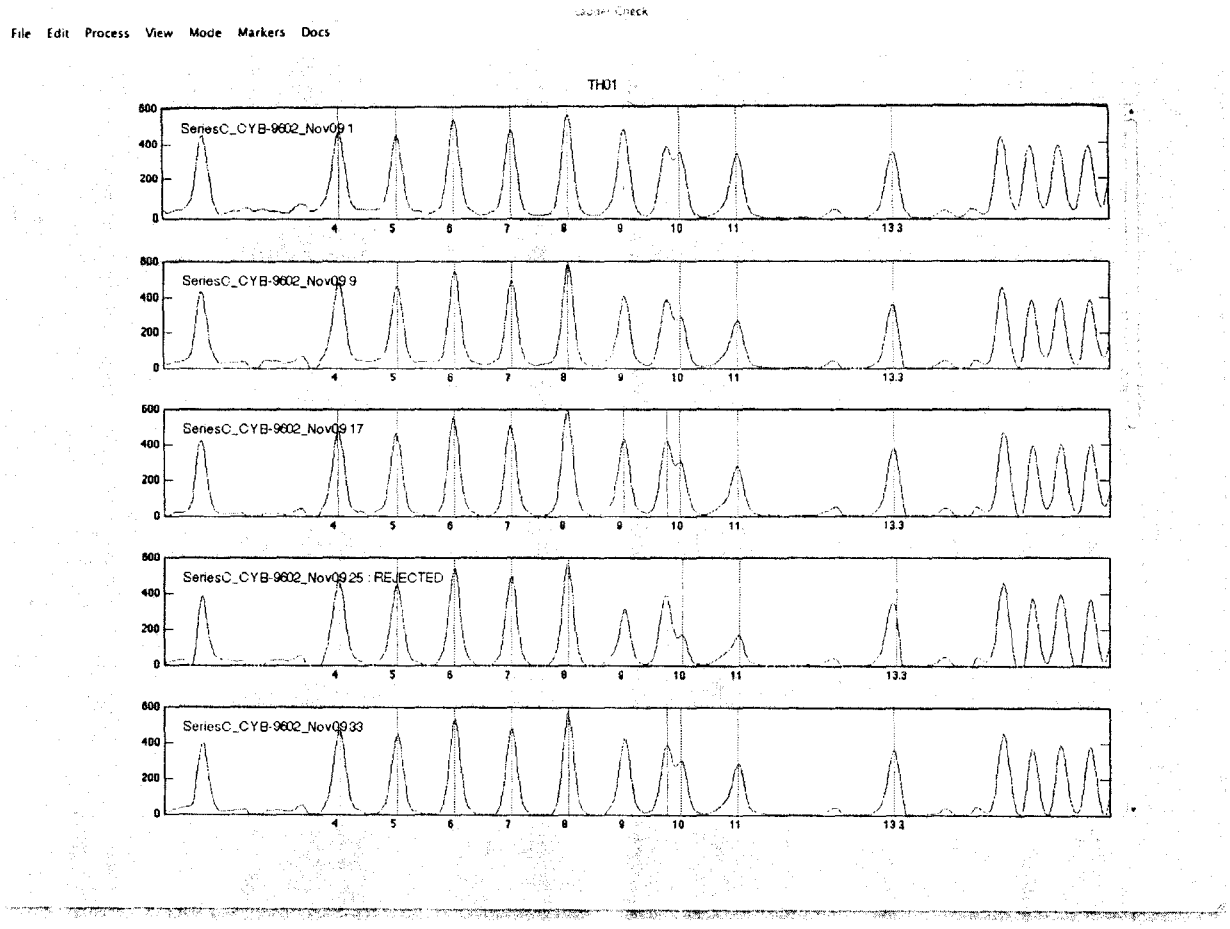


Figure 9. *Ladder Check showing a rejected ladder.*

Having addressed the rule firing, we are now assured of our run quality. We can proceed to the last step of the Analyze Module.

5 Output

Now that we have verified the run quality, the Analyze process is nearly complete. In Allele Call, the computer will perform peak quantitation and produce our gel file. There is no further human review necessary in the Analyze Module.

5.1 Allele Call

In Allele Call, the computer completes the process of transforming the raw electropherogram data into quality-checked peaks. This involves quantitation of each data peak. Allele Call takes about a minute of processing time per locus per plate. For example, a full 96-injection run of Identifiler data takes roughly 16 minutes to complete.

5.2 Gel File

The final output of the Analyze process is a gel file. This file contains the original data signals and all the quality-checked peak information.

The gel file is located in the DataDisk folder inside the data folder of each individual run (Figure 10). If more than one run was processed in the DataDisk, each run will have its own folder and gel file.

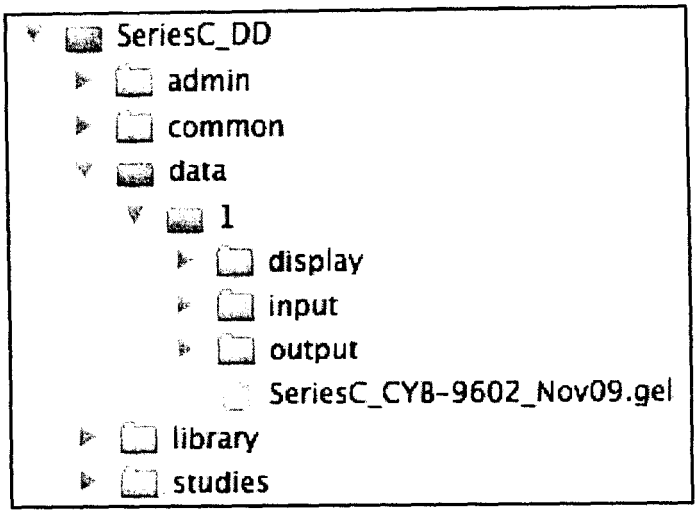


Figure 10. *Gel File Location.*

The gel file is used in the Data Module to allow for data upload and batch processing. This is discussed in the Data Manual.

6 Appendix

This appendix contains further details on DataDisk setup, rules descriptions and troubleshooting. The details covered here are intended for diagnosing specific issues and are not describing steps that are routinely performed in the typical process.

6.1 Initial Settings

In this section, we will see a brief overview of the initial settings. We access these settings by selecting **Edit ▸ Preferences ▸ Init...** from the Command window.

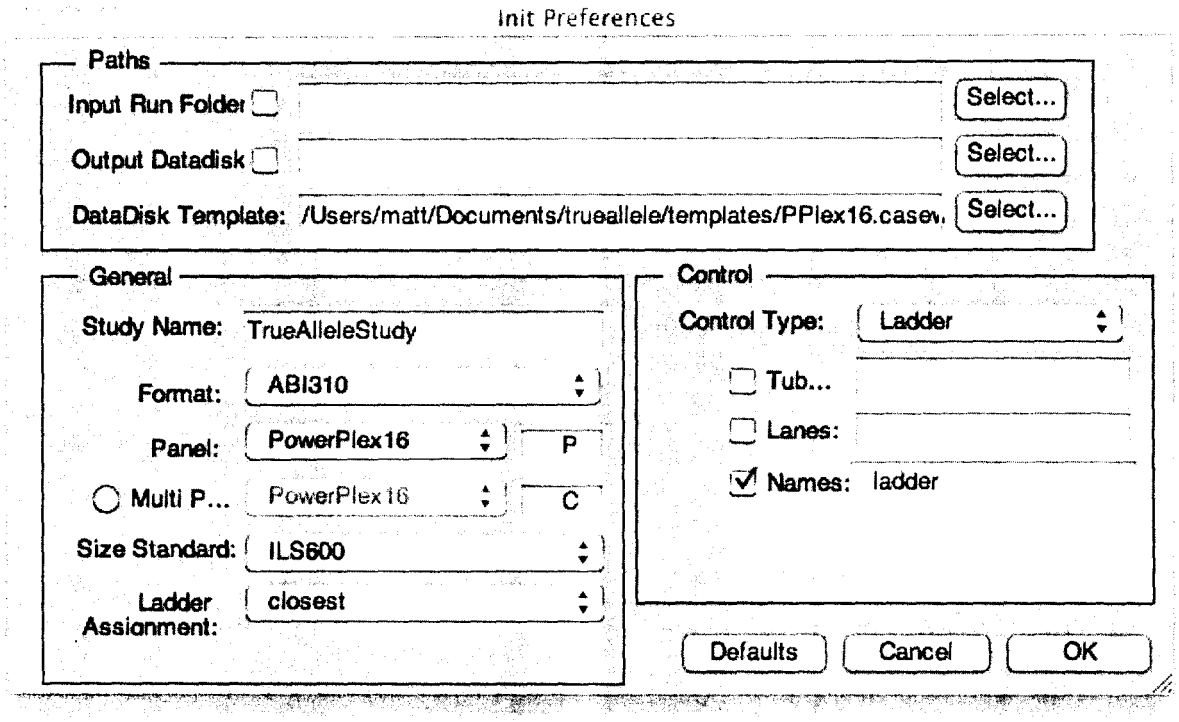


Figure 11. *Initial Preferences.*

Paths

The Paths section indicates to the program where to find the data, where to create the DataDisk and the type of data to expect. By default, the Input and Output are left blank, allowing us to specify a different location each time we create a DataDisk.

Input Run Folder: This setting allows us to specify the location of the data input. We typically leave this blank, as this will allow the program to prompt us upon DataDisk creation.

Output DataDisk: This setting allows us to specify a location for the newly created DataDisks. By default, leaving this blank will create the DataDisk in the same location as the input folder.

DataDisk Template: The DataDisk template provides the Analyze Module with the information required to run different types of data, such as different kits. We want to make sure the DataDisk template selected corresponds to the type of data we are processing. Casework templates for the commonly used kits are provided. If an additional kit is used for which a template is not provided, please contact Cybergenetics for assistance.

General

The General section is where we provide Analyze with some background information on the type of data to expect.

Study Name: The study name is used to name a batch of runs. The default name for the study is *TrueAlleleStudy*, and this can be changed as desired.

Format: This preference indicates the sequencer format. See additional notes on supported sequencer formats in the **Appendix ▸ Sequencer Formats.**

Panel: This preference indicates the panel used on the data. Note that the text box following this entry is only necessary if the Multi Panel feature is being used.

Multi Panel: For data where multiple panels are processed on one run, Analyze can determine the panel using flags in the sample name. Checking the Multi Panel button will enable this feature. The text boxes following the Panel and Multi Panel entries indicate the flags in the sample names used to differentiate the different panels.

Size Standard: This preference indicates the size standard used with the data.

Ladder Assignment: This preference sets the ladder assignment that is used with the data. By default, the setting is 'closest' for all capillary data. Staggered sample loading in gel data may require the 'loading' setting.

Control

The Control section allows us to set the position and name of the allelic ladders, as well as the negative and positive controls.

Control Type: This menu indicates the control we are currently addressing.

Tubes: The Tubes entry specifies the chosen control's location by tube number.

Lanes: The Lanes entry specifies the chosen control's location by lane / injection number.

Names: The string name match feature allows us to indicate a text string in order to find and designate the controls. For instance, if we had three allelic ladders in the

run with the names LADDER, Ladder-Co and Ladder-555 respectively, we could designate each as an allelic ladder by indicating 'ladder' in the Names entry.

6.2 Sequencer Formats

Available formats

The Analyze Module supports AutoSetup of capillary data from the following sources:
ABI 310 (PC)
ABI 3100 (standard and plate format)
ABI 3130
ABI 3700

Format specifications

ABI 310

To create a DataDisk with ABI 310 data, place the capillary run folder inside another folder. Additional run folders can be added as desired. Set the initial preferences and point to the capillary data folder as the input run folder. AutoSetup will then automatically create the DataDisk.

ABI 3100/3130

TrueAllele software supports three different formats for the ABI 3100: standard, plate and DC. The standard format has a set of 96 capillary files in each separate run, whereas the plate format has six sets of 16 capillary files grouped together in one run. Both formats result in one 'run' of 96 capillaries.

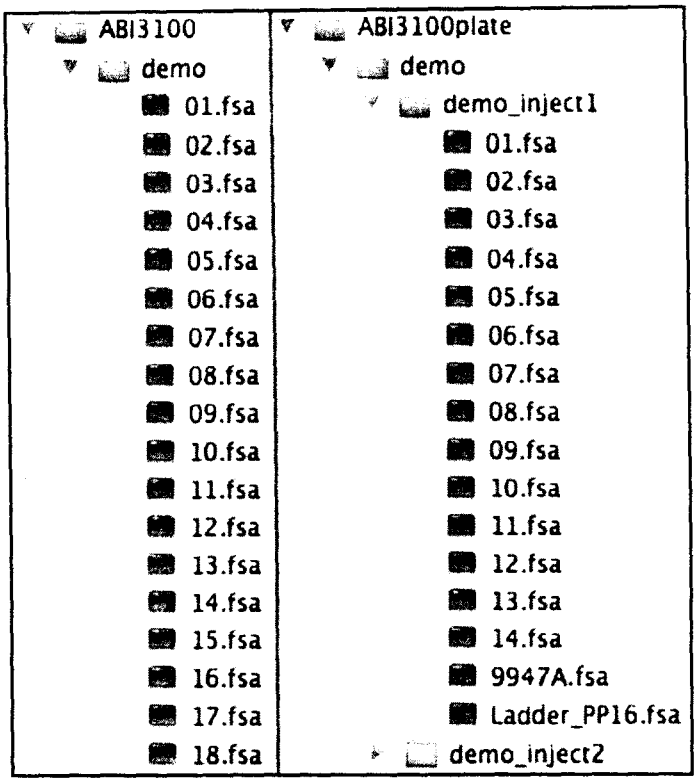


Figure 12. *ABI3100 – Standard format (left) and Plate format (right).*

The DC format acquires ABI 3100/3130 data using ABI data collection software versions 2 and 3. Note that the 3100dc format reads the panel directly from the capillary file. If we have not specified the panel during data collection, use the ABI3100 format instead (Figure 12).

Note that, depending on the collection software and protocol, 3130 data followed either the 3100 or 3100dc format specifications.

ABI 3700

ABI 3700 format follows the ABI 310 specification.

6.3 Troubleshooting – Sizing

This section provides some additional details and the actions to perform for a range of possible sizing issues. These actions all relate to the human review performed in Cap View.

The labels presented in Cap View do not appear to correspond to the size standard peaks that are observed.

When we see that the computer tracking is drastically different from the expected sizing, we want to confirm the size standard selected in Initial Preferences. It is possible that an incorrect size standard was used during DataDisk creation. If that is the case, we want to adjust the size standard setting. Then we will recreate the DataDisk and run the Image Call process again.

An injection is missing some or all labels

In some cases, a data issue may result in some or all labels missing for an injection. Sometimes, we can adjust the tracking in the AutoEdit Sizing window (discussed in the Modify Mode section). However, if no peaks were tracked and the labels are not present in the AutoEdit Sizing window, they cannot be manually entered. In this case, the peak heights of the size standards did not meet our cut off value and the sample must be rejected.

6.4 Analyze Rules

This section provides detailed descriptions of the Analyze rules that are used in Control Check. Rule descriptions and corresponding actions to perform when rules fire are presented here. A summary table is provided in the **Appendix ▸ Troubleshooting – Controls.**

Analyze Rule Descriptions

The *Control Check: Rules window* displays the results of TrueAllele gel quality assurance checks (Figure 13). Open the window by selecting **View ▸ Rules** in the Control Check window. Rule firings are indicated by a checked box to the right of the rule name in the Rules window. For additional information, such as which loci or lane(s) fired that particular rule, click on the 'i' button to the left of the rule name.

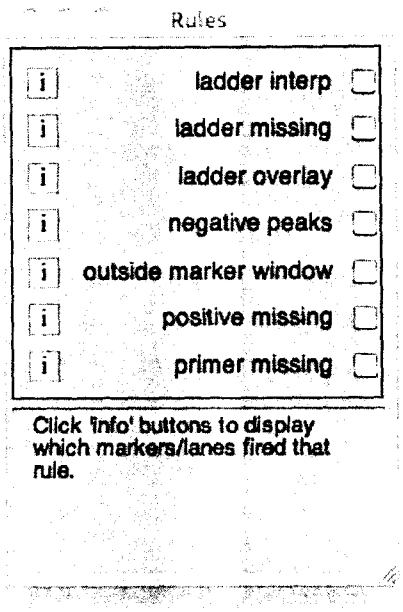


Figure 13. *Analyze Rules Window.*

Ladder Interp/Interpolated

This rule fires when the Analyze Module interpolate at least one peak in an allelic ladder. Firing tells us that the software could not find all of the expected allelic ladder peaks for the indicated loci. This may or may not be problematic; ladder tracking for the indicated locus should be verified in the Ladder Check interface found by selecting **View ▸ Ladder Check**.

Ladder Missing

Allelic ladders should be found in the designated lanes. When the Ladder Missing rule fires, allelic ladders for the indicated loci could not be found. We must check to ensure that the allelic ladder lanes were indicated correctly in the initial setup of the DataDisk, that the tracked lanes correspond to the layout file, and that allelic ladders were included in the run.

To see which allelic ladder(s) are missing for each locus of a particular run, click on the 'i' button to the left of the 'ladder missing' rule name. In the text box at the bottom of the Analyze Rule interface, the loci that have missing ladders will appear.

To see the missing (failed) ladders visually, go to **View ▸ Ladder Failures** in the menu bar. The Ladder Failures window will appear (Figure 14). In this particular example, there are three allelic ladders in lanes 1, 9 and 17. These allelic ladders passed for all loci.

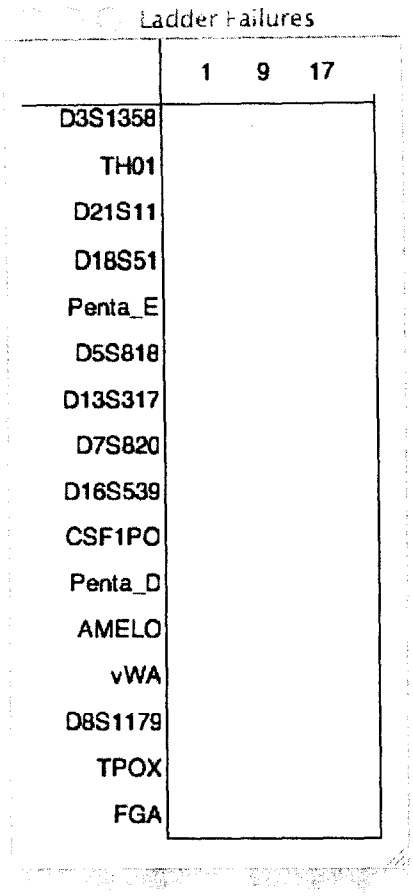


Figure 14. *Ladder Failures.*

If an allelic ladder fails, the Ladder Failures window indicates the failed allelic ladder with an 'X' next to the locus in the specific allelic ladder column.

Ladder Overlay

Allelic ladder peaks should not vary in length between lanes. For the indicated loci, the lengths of at least one of the allelic ladder peaks in two different lanes differ by at least 0.5 bp. Therefore, one (or more) of the allelic ladders may be sized incorrectly. We can view ladder overlay problems by selecting **View ▸ Ladder Check** in the menu bar for the specific marker (refer to Figures 8 & 9).

Negative Peaks

The designated negative control lanes should not contain any peaks. Firing indicates that peaks were found in the negative control lanes in the indicated marker regions. These peaks could indicate contamination of some type. If the rule fires, click on the 'i' button to see which negative control lane is flagged. Clicking on the lane opens the Electropherogram view for further review.

Outside marker window

True allelic peaks may be observed outside the user-defined marker ranges. When this rule fires, one or more peaks greater than the user-defined threshold were found. The specific lane, dye and size are indicated for each peak.

We indicate the range within which the Analyze Module should look for peaks falling outside the marker windows. For example, a peak could be found at 170 bp, but is not inside the D3S1358 and D16S539 Cofiler marker windows. When the peak is found, the specific lane, the dye, base pair size and height are displayed in the text box when the 'i' button is selected. If an allelic peak appears outside a marker window, the window can be adjusted from the main Control Check interface by altering the marker window 'start bp' and 'end bp.'

Positive Missing

The user-designated positive control lanes should contain a specific trace pattern for each dye plane. When the software cannot find adequate peaks or the correct pattern for one or more positive control lanes, it fires this rule. This could indicate either failure of the positive control(s) or the entire gel. If the rule fires, click on the 'i' button to see which positive control lane(s) fired the rule.

To see this visually, select **View ▸ Positive Control Check**. This interface shows all of the designated positive controls in the gel, the lane numbers and whether the controls passed or failed the rigorous quality assurance check (Figure 15).

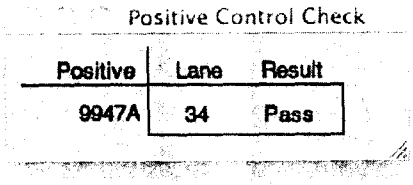


Figure 15. *Positive Control Check.*

The Positive Control Check interface also flags lanes that appear to be positive controls (indicated by the string 'Check'), which can be useful for diagnosing misloading and misdesignation problems.

Primer missing

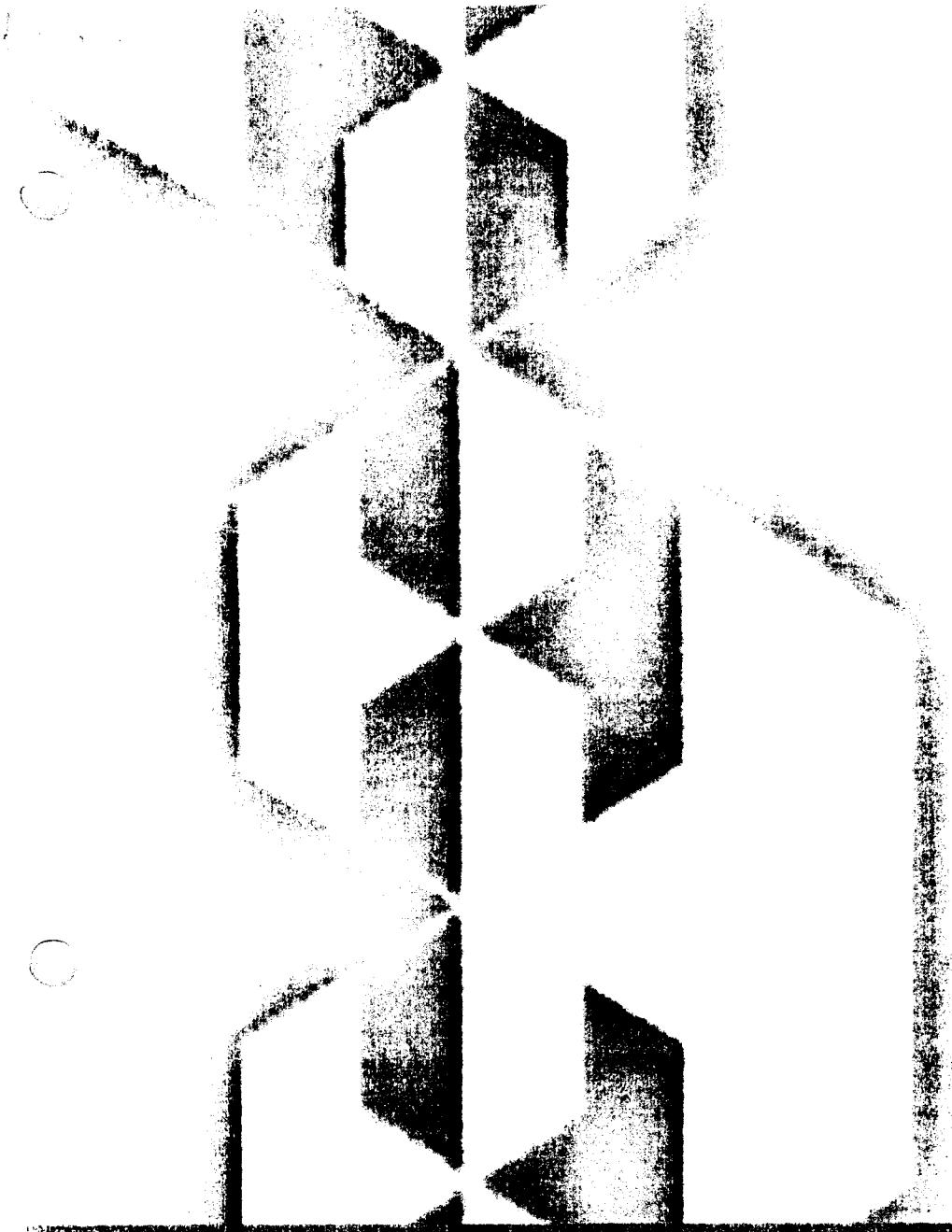
A true negative control lane should contain primer peaks. The primer missing rule reviews the primer region for each negative control on the gel. Lanes with insignificant or missing primer peaks fire the rule and can be viewed in Cap View.

6.5 Troubleshooting – Controls

This section summarizes the detailed information presented in the Analyze Rules section. For each rule, associated interfaces and actions are listed in the summary table.

Summary Table

Rule	Interface	Action
Ladder Interp	Ladder Check	Verify tracking, reject if necessary
Ladder Missing	Ladder Failures	Confirm at least one ladder is present
Ladder Overlay	Ladder Overlay	Confirm ladder sizing
Negative Peaks	Electropherogram	Verify absence of contamination
Outside Marker Window	Electropherogram	Adjust marker windows if necessary
Positive Missing	Positive Control Check	Verify positive controls
Primer Missing	Cap View	Verify primer presence in negative



TrueAllele® VUler™ Data Module

Uploading Data

Cybergenetics

Table of Contents

1 Overview

Once the sequencer data has been quality checked in TrueAllele® Analysis, it needs to be uploaded to a TrueAllele Server so it is available for interpretation. The focus of the Data module is to upload sequencer data to the TrueAllele Server.

In addition to sequencer data, the Data module allows for the upload of other data types such as profiles and populations. To allow for high volume processing, the Data module can also create a batch of requests automatically from a .gel file.

2 Database Connection

We select the database to which the data will be transferred by connecting to a TrueAllele World. The connect window is opened by selecting **Database ▸ Connect** (Figure 1). Connecting to a database is further described in the Getting Started Manual. If we haven't yet created a database, we can reference the TrueAllele World section of the Tools Manual where that process is described.

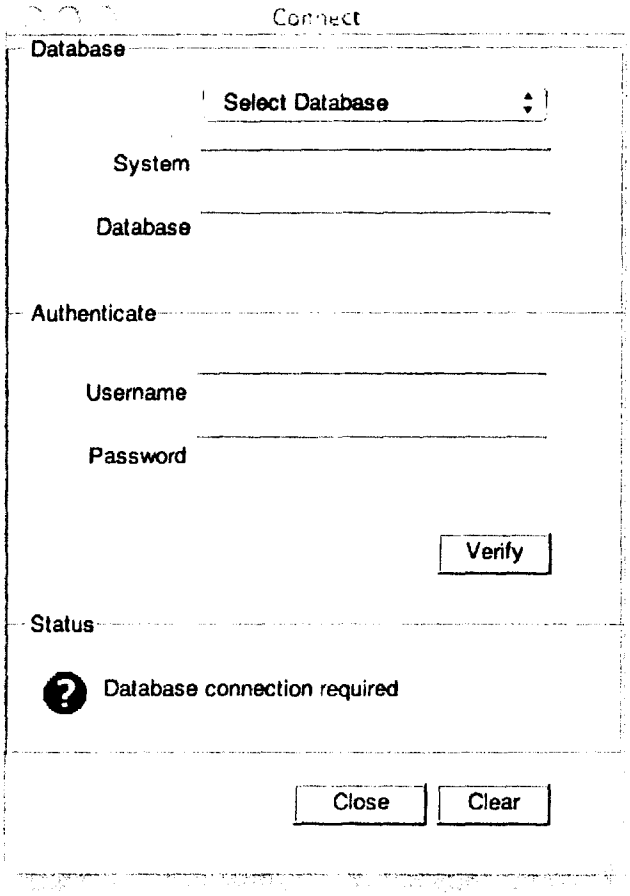


Figure 1. *Connect window.*

3 Sequencer data

Applicable icons:

Open Gel File

A key task of the Data module is to upload the quality checked peak data produced in the Analysis module. This process allows for a quick look at the data and an additional quality check of the peaks.

We begin by choosing a gel file. Selecting **File ▸ Open** or the Open Gel File icon in the toolbar will provide us with a dialog box. From here, we can navigate to and select a gel file.

Once we've selected the gel file, the sequencer data will be displayed visually in the Gel Data view (Figure 2).

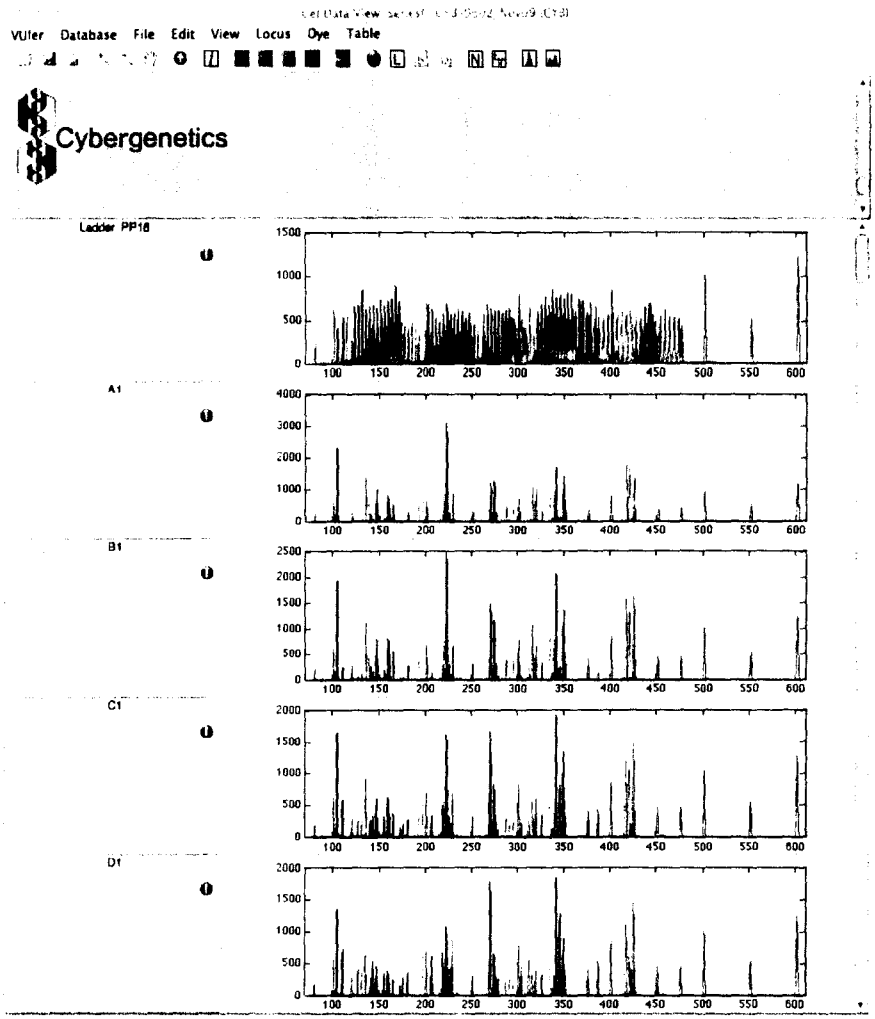


Figure 2. *Gel Data view.* Each track corresponds to one injection.

We see several rows, or *tracks,* of data. Each row is one injection from the selected gel file, containing a ladder, control or sample. We can see more information about each lane, including the size standard, well, and panel, by selecting the blue information button (i).

4 View Data

We can review the original data using a variety of tools. These tools will be shared in *Data Views* throughout the various modules in the system. At this point, only a quick review of the data will be necessary, because the key task of this module is to upload the data to a database. There are other points that we may review the STR data further, including during the formation of interpretation questions and review of our results.

The Request Manual section "Reviewing the Data" describes the Data view options in more detail. The following information explains some of the common features we will use in the upcoming modules.

Locus-by-Locus Viewing

Throughout the modules, clicking on an individual lane allows us to focus on a particular injection. We can then zoom to a specific locus using the **Locus** menu option (Figure 3). The drop down allows us to view the different sections of data in detail.

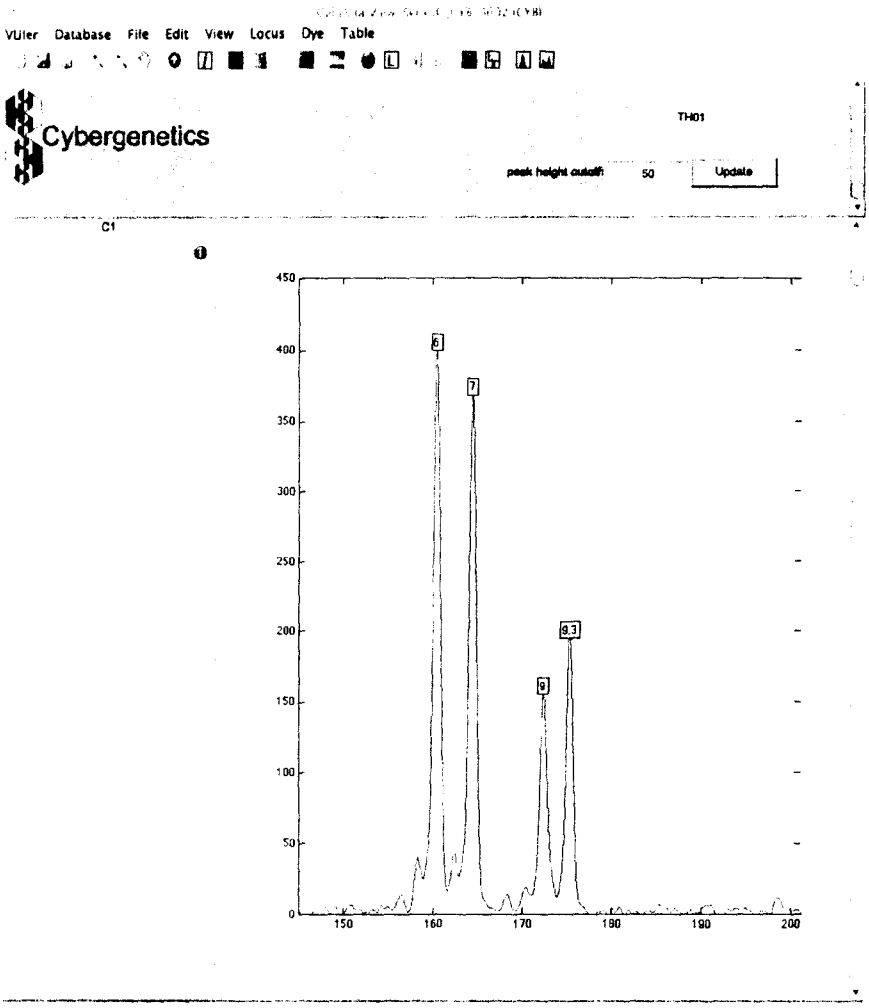


Figure 3. *Data window.* Here we see the TH01 locus in the C1 mixture sample. This data appears to be a typical 2:1 mixture and will be discussed in detail in the Request and Review manuals.

Labeling

Applicable Icons:

N Label Peaks

Label Axes

We can label axes of the various displays by selecting the "Label Axes" icon (located to the right of the N icon).

To label the peaks with their allele calls, we click the "Label Peaks" (N icon) in the tool bar. The length and height of a peak are available by right-clicking on a specific peak label. This information is also in the peak table, found under the **Table ▸ Peak** option in the toolbar (Figure 4).

Data Peak SeriesC_CY3-3602_Nov09 (CY8)

	track	name	locus	desig	length	height	area
20	4	C1	D16S539	7.3	274.70	26	12
21	4	C1	D16S539	8.1	277.40	19	9
22	4	C1	D16S539	9	280.00	127	60
23	4	C1	D16S539	9.2	282.20	21	10
24	4	C1	D16S539	10	283.90	32	15
25	4	C1	D16S539	11	287.90	298	141
26	4	C1	D16S539	12	292.00	213	101
27	4	C1	D16S539	13	296.00	322	152
28	4	C1	D18S51	12	305.79	21	11
29	4	C1	D18S51	12.2	307.80	18	10
30	4	C1	D18S51	13	310.00	266	162
31	4	C1	D18S51	14	313.89	28	17
32	4	C1	D18S51	14.2	316.12	23	15
33	4	C1	D18S51	15	318.00	305	189
34	4	C1	D18S51	16	321.90	539	322
35	4	C1	D18S51	17	325.90	601	379
36	4	C1	D18S51	18	329.80	13	9
37	4	C1	D18S51	24.3	356.98	11	7
38	4	C1	D21S11	24	202.90	12	6
39	4	C1	D21S11	28	219.00	49	23
40	4	C1	D21S11	29	223.00	542	275

Figure 4. *Peak table.*

For visual purposes, a display peak height cutoff is available, labeling only the peaks above a certain RFU. By default, the display cut off is set to 10 RFU. We can change the limit by entering a number in the peak height cutoff field and clicking "Update." This action will only update the labels in the Data view and peak table. It will not cause the peaks below the display cutoff to be ignored during the interpretation process.

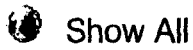
Zoom and Pan

Applicable Icons:

Zoom In

Zoom Out

Pan



If we are interested in getting a closer look at the data, we can zoom in or out using the magnifying glasses in the toolbar. To zoom, we click and drag the + magnifying glass over a section of data. The hand icon then allows us to pan through the electropherogram. To exit a zoom mode, we can click on the same magnifying glass a second time. This action does not reset to the original view.

To view the entire electropherogram again, we can zoom out or select *All* from the top of the **Locus** menu. We can also right click on the electropherogram and select "reset to original view". The globe icon can take us back to view all the injection lanes.

5 Gel Information

Applicable Icon:

Show Gel Information

Annotating information is provided along with the sequencer data, and we want to review it before uploading the gel. Selecting the *Show Gel Information* icon from the toolbar will open an information window (Figure 5). This information includes the lab name, sequencer, gel name, date of creation and the user name of the analyst who will be uploading the gel. Once we have confirmed this information is accurate, we can close the window by clicking on the *Apply* button.

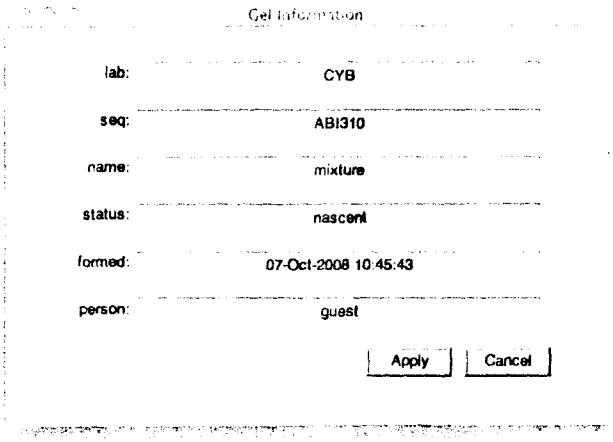


Figure 5. *Gel Information.*

After verifying the annotating information, we can perform an additional quality check on the data peaks if we choose. To see how we can check for spikes or peak morphology, refer to the **Appendix ▸ Spike and Peak Morphology**.

6 Upload Data

Applicable Icon:

 Upload Gel

Once we have confirmed the gel information, our last step is to upload the data to a TrueAllele Database. Having already established the database connection, we can upload the sequencer data by selecting the *Upload Gel* icon in the toolbar or by selecting **Database ▸ Upload ▸ Gel**. After verifying upload, a dialog displaying the status of the transfer appears. Once the transfer is complete, the sequencer data is available for use in forming individual case questions as described in the Request Manual.

We have just completed the upload of a single gel file. From here, we will be able to ask individual interpretation questions, or requests, in the next module. However, there may be times that setting up an entire batch of requests would be better for our workflow. The next section will walk us through this process.

7 Batch Request

If we have a large set of data to work with, we may want to automate our process. For example, if we are considering a volume crime process, we may wish to produce similar questions for over 60 lanes of data.

Uploading a batch of requests allows us to carry out this automated process. We must first upload the data as mentioned previously. We then select **Database ▸ Upload ▸ Batch**. This action will prompt us to select the gel file from which requests will be produced. We see a dialog box, allowing us to choose the questions we wish to ask about the data (Figure 6).

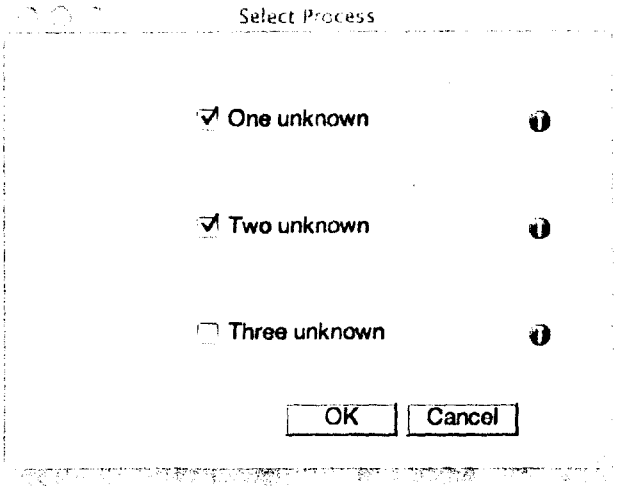


Figure 6. *Select Batch process.*

After we choose how many unknown contributors to infer, we set the naming of the requests and the parameters by selecting the blue information (i) button for each process (Figure 7). The Request manual describes each tab in further detail, as this window is the same window used when setting up requests individually. The only difference for a batch of requests is that the naming is automatically generated. In

the example below, for each of the one unknown requests, the wildcard (*) will be replaced with each sample's name followed by a "_1" to denote the request's one unknown processing.

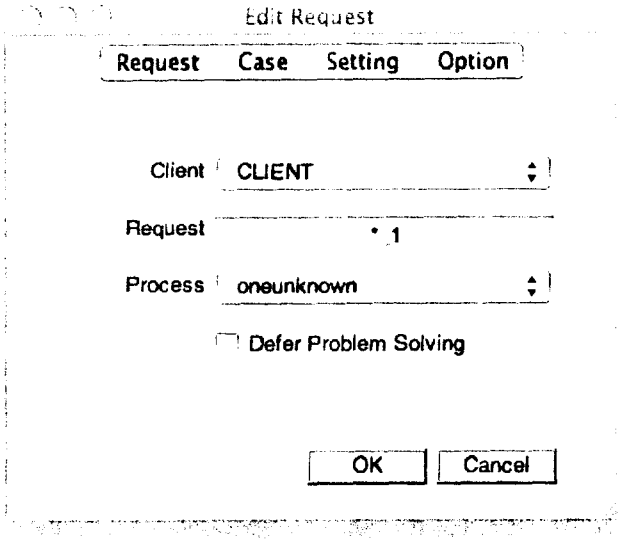


Figure 7. *Edit Request.*

After entering this information, we receive a list of requests and can remove any unwanted requests before upload (Figure 8). After confirming the requests, they will be uploaded to the database and become available for interpretation.

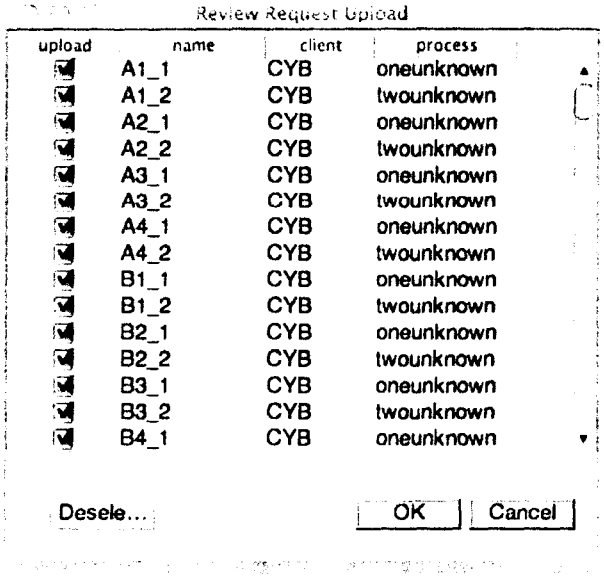


Figure 8. *A Batch of Requests.*

8 Reference Genotype

If we have a known profile we wish to use as a reference, a text file containing its genotype information can be uploaded in the Data module. This information is useful for matching purposes or to aid in interpretation by providing a victim reference.

To upload the profiles, we select either **Database ▸ Upload ▸ Genotype** or **Database ▸ Upload ▸ Likelihood** depending on the file type. A unique profile can be uploaded as either a Genotype (Figure 9) or Likelihood. The specifications on structuring and formatting a Genotype and Likelihood text file are provided in the **Appendix ▸ Specifications**. After the files are created, we can select a folder containing the known profiles, and then upload them to a database.

CYBSC.txt

locus	desig1	desig2	prob
D3S1358	16	17	1
TH01	9	9.3	1
D21S11	30	31	1
D18S51	13	15	1
D5S818	12	12	1
D13S317	9	13	1
D7S820	10	10	1
D16S539	9	12	1
CSF1PO	12	12	1
AMELO	1	2	1
vWA	15	18	1
D8S1179	8	11	1
TPOX	8	8	1
FGA	21	22	1
Penta_D	12	14	1
Penta_E	7	14	1

Figure 9. *Genotype Text File.*

9 Population Database

The VUIer™ can produce a match statistic based on any population provided in a TrueAllele World. To do this, we first must upload a population file to our database. This operation only needs to be performed once but must be done for each TrueAllele World individually.

By selecting **Database ▸ Upload ▸ Population,** we can choose the population file (Figure 10). The population will then be uploaded to the world and available for producing match statistics. More details on the structure and information contained in the population file can be found in the **Appendix ▸ Specifications**.

CAU.txt

sample	locus	desig	count
CAU	D3S1358	12	0
CAU	D3S1358	13	1
CAU	D3S1358	14	57
CAU	D3S1358	15	100
CAU	D3S1358	15.2	0
CAU	D3S1358	16	94
CAU	D3S1358	17	86
CAU	D3S1358	18	66
CAU	D3S1358	19	2
CAU	vWA	11	0
CAU	vWA	13	2
CAU	vWA	14	40
CAU	vWA	15	44
CAU	vWA	16	79
CAU	vWA	17	103
CAU	vWA	18	87
CAU	vWA	19	33
CAU	vWA	20	4
CAU	vWA	21	0

Figure 10. *Population Text File.*

10 TrueAllele Worlds

A TrueAllele World (TAW) is a specific database where all the STR data and interpretation request information is stored. In some instances, such as archival or reproducibility studies, it is useful to download or upload saved copies of a TrueAllele World.

Download

Similar to copying a world in the Tools Module, we have the option to download and save a copy of the current state of a database in the Upload Module. To download and save a copy of a world, we select **Database ▸ Download ▸ World** from the toolbar. This action will open a window where we will tell the computer the location and name of the world to copy and what to name the .taw file (Figure 11). This file can then serve as an archive of a database.

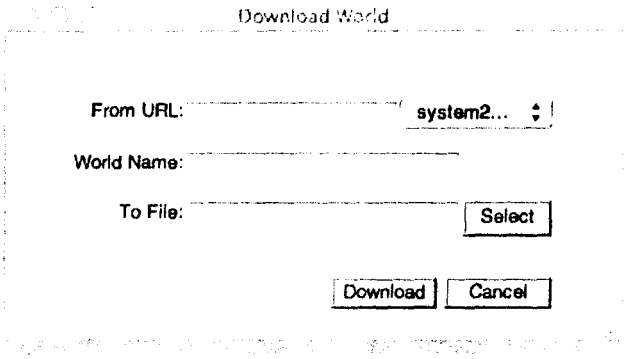


Figure 11. *Downloading a World.*

Upload

An archived copy of a TrueAllele World (.taw file) can be uploaded to a new database. To upload a world, we select **Database ▸ Upload ▸ World** from the toolbar. This action opens a window we use to indicate where to upload the World,

the location of the saved .taw file and the name of the new world to which we are uploading the database (Figure 12).

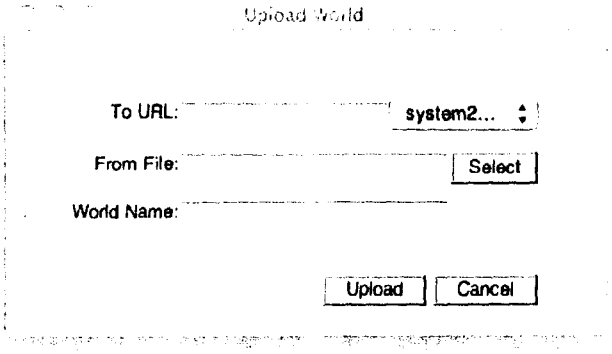


Figure 12. *Uploading a World.*

If we want to reprocess the requests on a database, the Tools Manual describes how to globally reset requests.

11 Appendix

11.1 Spike and Peak Morphology

Applicable Icons:

 Show Spikes

 Show Peak Morphology

By selecting the *Show Spikes* icon in the toolbar, the window will display only the rows with potential spike issues. The peak label corresponds to the dye color; for example, if the peak label is blue, the indicated peak is in the blue dye. Clicking the peak label will deactivate that peak, which is indicated by the peak label turning gray.

Once we have reviewed the possible spikes, we can then review peak morphology by selecting the *Show Peak Morphology* icon in the toolbar. Potential issues will again be displayed with colored peak labels and can be deactivated (Figure 13).

Selecting either icon a second time will return us to the original data view.

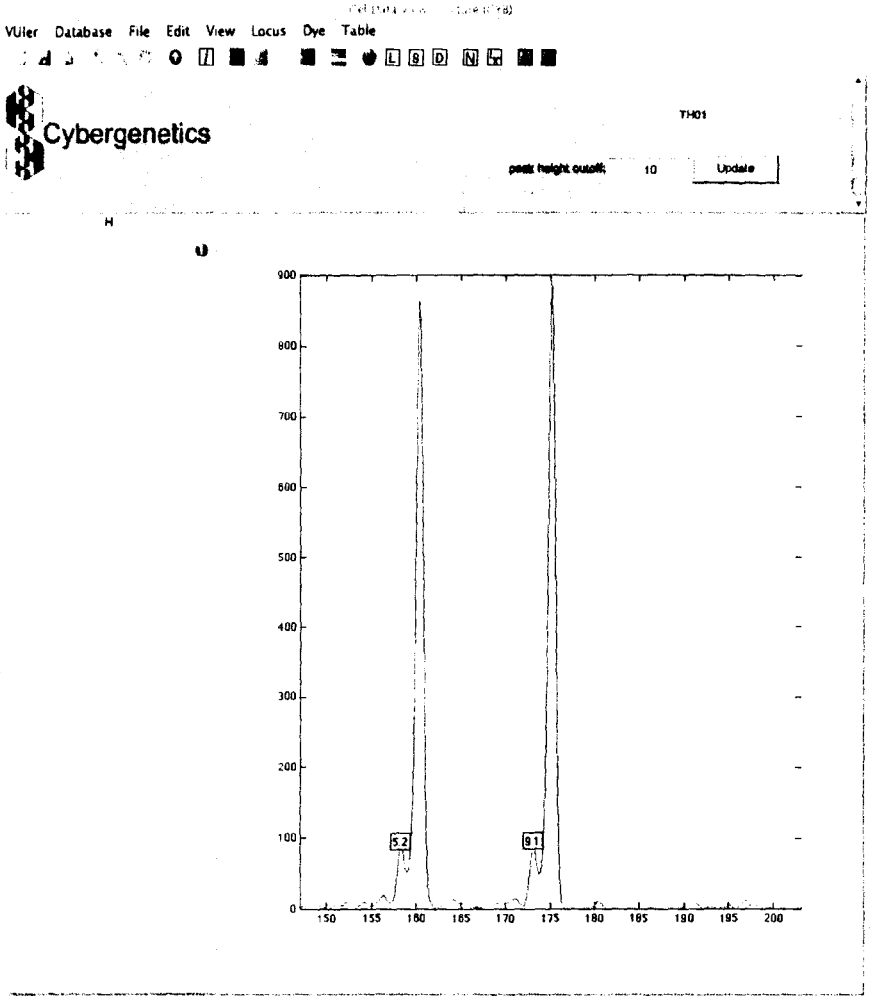


Figure 13. *Peak Morphology.*

11.2 Specifications

Likelihood or Genotype

In order to upload a profile, a text file must be created. It is easiest to create this file using a spreadsheet program and saving it as a .txt (text – tab delimited) file. The file should be named specifically as *Client$requestname*. The client must be a valid client on the database to which we are uploading the file. The request name should be the name we want to use when the profile is part of a case folder.

The text file should have four columns: locus, desig1, desig2 and prob. The locus column should list the exact name of every locus included in the profile being uploaded. An example of this layout is pictured below.

locus	desig1	desig2	prob
AMELO			
D18S51			
D3S1358			
D8S1179			
FGA			
TH01			
vWA			
D5S818			
D13S317			
D7S820			
TPOX			
CSF1PO			
Penta_D			
Penta_E			

In each "desig" column, we place the individual alleles comprising each allele pair. For example, in the Amelo row for a male genotype, we would place a 1 in the desig1 column and a 2 in the desig2 column.

The only difference between the likelihood and genotype files is in the "prob" (probability) column. For a genotype, the value in this column will be 1 for every locus. The value 1 denotes a definite allele pair with no uncertainty. The genotype form should be used for known reference profiles without data.

A likelihood file can be uploaded when there is uncertainty at one or more loci. In this case, we would have multiple rows for the uncertain locus, each row having a corresponding probability. For example, if we were unsure whether a profile for upload is male or female, we would denote that as follows.

locus	desig1	desig2	prob
AMELO	1	1	.50
AMELO	1	2	.50

This setup indicates to the system that each allele pair has a corresponding probability of ½. When viewed in the Review interface, these probabilities will be reflected in the Profile View like any other sample with an uncertain genotype.

Note that either type of file, once uploaded, can be placed into an existing study (case folder). This process is further described in the Request Manual.

Population

Population files need to be uploaded for matching purposes. These files provide the system with allele frequencies that are used to calculate probabilities. Much like the likelihood and genotype files, the population files are created as a spreadsheet and saved as tab-delimited text. The text file should be created with the following columns: sample, locus, desig, and count.

Sample refers to the name you will be giving the population; for example, Caucasian can be referred to as CAU. The sample name will be the same the entire way down the column. The files should also be saved as the sample name; after saving as tab-delimited text, the file name would be CAU.txt. Locus refers to each locus in the population we are uploading. Because there are numerous alleles possible at each locus, there will be multiple rows for the same locus. Each allele that is possible at a

locus is listed in the desig column. This includes microvariants that have an allele frequency within a population. The count column is the number of times that allele was seen during the sampling. This number is typically found in the original publication of the population information. Based on this information, the system is then able to calculate allele frequencies. An example locus for an African-American population may look like this:

sample	locus	desig	count
BLK	D3S1358	12	1
BLK	D3S1358	13	5
BLK	D3S1358	14	51
BLK	D3S1358	15	122
BLK	D3S1358	15.2	0
BLK	D3S1358	16	129
BLK	D3S1358	17	84
BLK	D3S1358	18	23
BLK	D3S1358	19	2

If there are multiple populations that need to be uploaded (i.e. Identifiler and Pro/Co), we must ensure that the overlapping ethnic groups are named differently. This allows for easy differentiation when choosing populations with which to report in the Report Module.

The uploading of a population database is only necessary once for a specific TrueAllele World. If we have multiple TrueAllele worlds, we will need to upload the population database to each world independently.

11.3 Troubleshooting

Overwriting Gel Files

If changes need to be made to a .fsa file after upload to the Data Module, we can create a new .gel file to replace the existing file. To overwrite the existing .gel file, name and upload the new .gel file exactly as the first.

Reuploading will reset any requests that use the data lanes from the gel and will automatically begin reprocessing. If it is necessary, inactivate any requests that do not require reprocessing prior to uploading the gel. Inactivating a World or request is described in the Tools Manual.

Upload Not Complete

When uploading a file, we may sometimes receive an "Upload Not Complete" message. This error could be caused by a number of different scenarios.

- Check to ensure that the Lab name entered in the Gel Information window is a valid lab name for that database. This can be done by going to the World Information menu of the Tools Module and selecting Lab from the drop down menu. If the necessary lab is not included, it should be added to the World. For information on adding a lab, refer to the Tools Manual.

- If the message appears when attempting to upload a likelihood or genotype file, check to ensure the file is named properly. Upload will not occur unless the file is in the *Client$requestname* format.

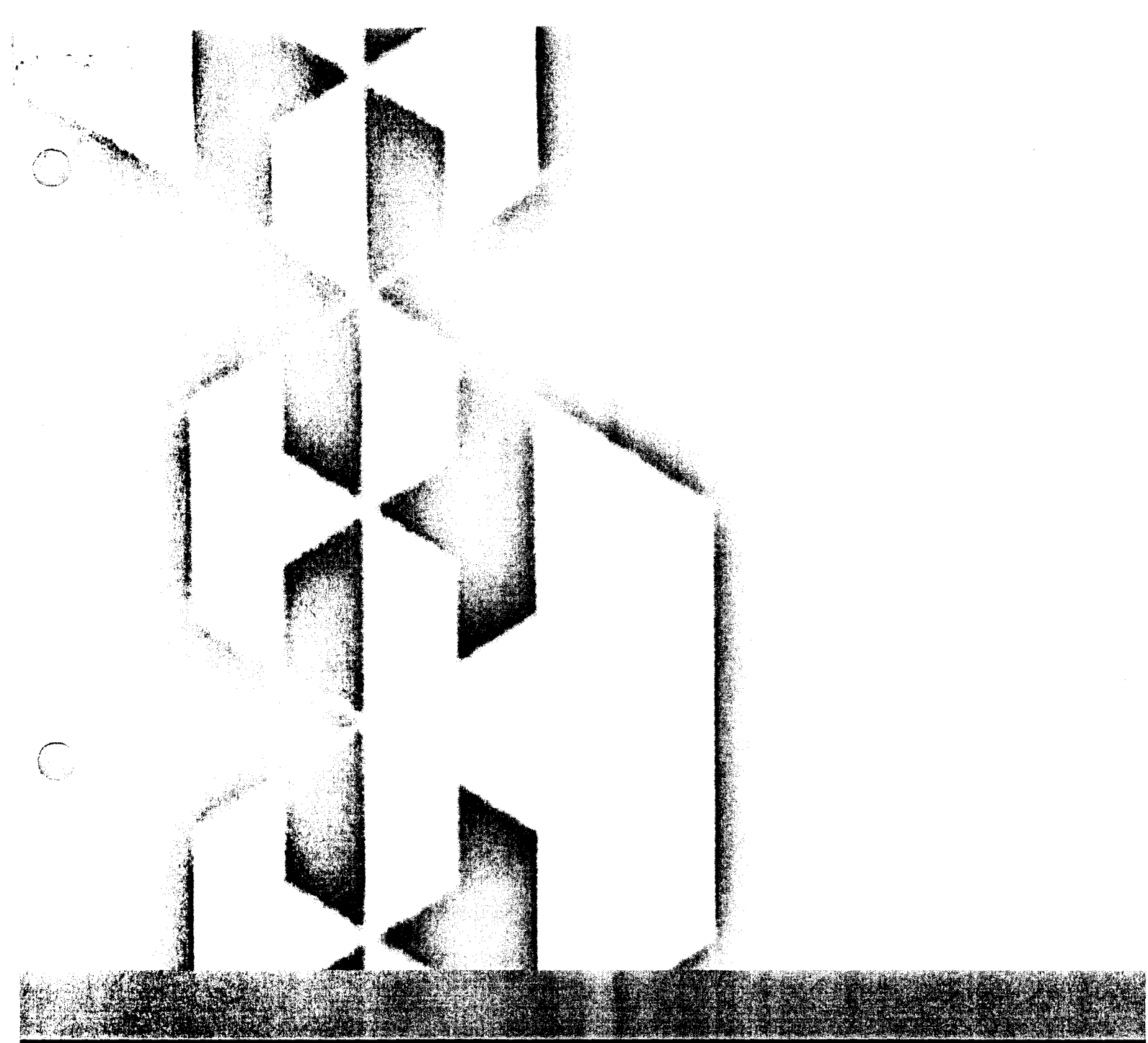
- Make sure the client being used for the likelihood or genotype file is a valid client on the database. This can be done by going to the World Information menu of the Tools Module and selecting Client from the drop down menu. If that client is not included on the list, it should be added to the World. For information on adding a client, refer to the Tools Manual.

- Incorrect file formats for the likelihood, genotype and population files can cause an upload not complete message to occur. Make sure that the specified formats are followed exactly as shown in section 11.2.

- Following from the incorrect file formats, likelihood and genotype files should use 1 and 2 to denote X and Y for the Amelogenin locus, respectively. Using X and Y will not allow upload to occur.

Continuous Upload

If we are attempting to upload data and the upload process is taking exceptionally long, it is possible that the network connection timed out during upload. In this case, it is best to close the program and restart. In most cases, this issue will resolve on the second attempt.



TrueAllele® VUIer™ Request Module

Asking Questions

Cybergenetics

Table of Contents

1 Overview

In order to obtain answers in a case, we must first ask questions. Once we decide what questions to ask, we can have the computer solve them for us. These questions, called interpretation *requests*, are formed based on information we know about the case and what we want to find out. After learning how to use the various features, we will set up an example case using the TrueAllele® VUIer™ Request Module.

Clicking the Request icon in the *Module Chooser* window accesses the Request Module, opening the home window (Figure 1).

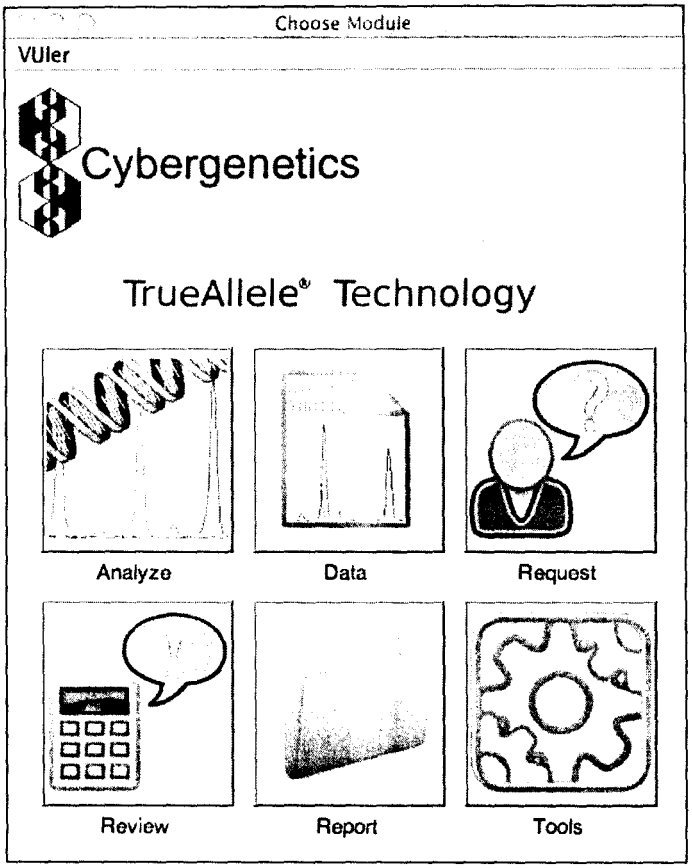


Figure 1. *Module Chooser window.*

2 Problem Solving

Before we begin to make requests, we should review our data and create a request plan to follow. This plan includes determining what questions we should be asking of our data. The most frequently asked questions are: Is the victim a contributor to the evidence? Is the suspect a contributor to the evidence? If so, to what extent does each contribute?

2.1 Determining the Problem

To determine what questions to pose to the system, we should know what data we are using. Connecting to the appropriate database allows access to data that was uploaded following Analysis. For tips on connecting, please see the "Connecting to a Database" section of the Getting Started manual.

Searching for STR Data

To search for data, we first choose **Database ▸ Find ▸ Data**. This action opens the *Find Lane from Data* window, which has multiple fields we can use to narrow our search (Figure 2). If all of the desired data is in a single .gel file, the most efficient way to search is via the "Gel/Run" field. When we fill in this field and click OK, all lanes of data on that gel will then be displayed in a *Select* window. Other search options are available in the *Find Lane from Data* window. These options are detailed in the **Appendix ▸ Advanced Search Options.**

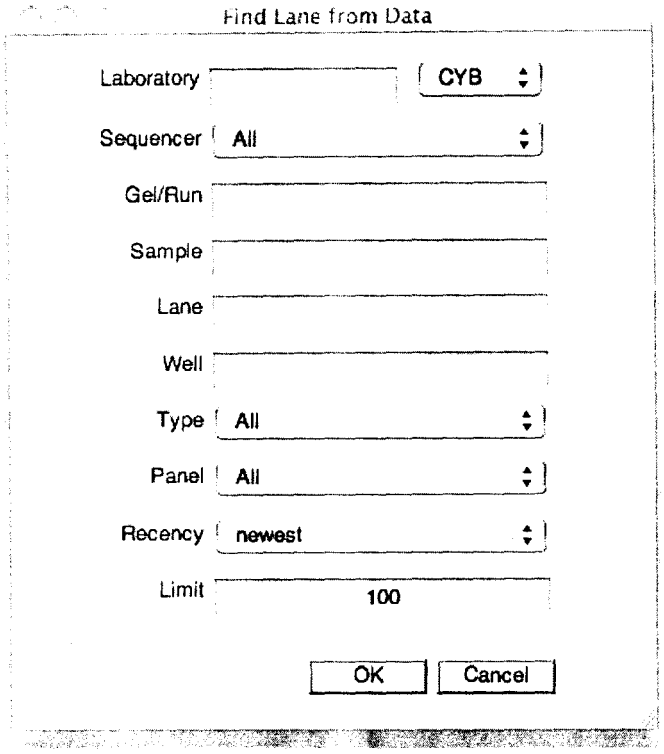


Figure 2. *Find Lane from Data window.*

The *Select* window displays several columns of information: the label of each lane, the lab under which the data was uploaded, the sequencer type, the gel name, and the lane number (Figure 3). Each row of information has a checkbox to the left; each lane of data that is checked will be displayed on screen in the *Lane* window after clicking OK.

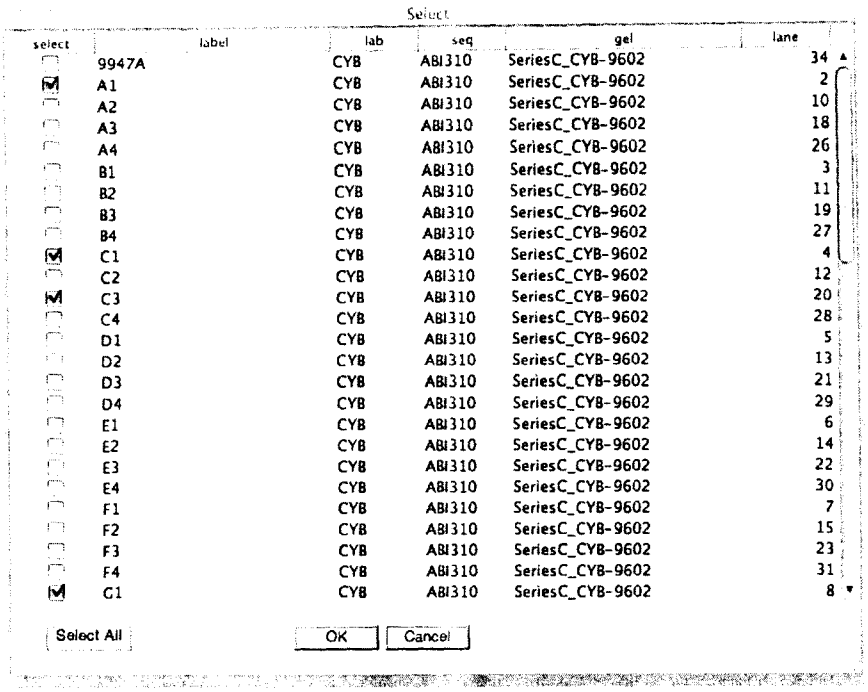


Figure 3. *Select window.*

After we select the data, the *Lane* window opens, displaying the electropherograms for the injections we chose (Figure 4). Each injection is displayed on screen as a track; the labels for the lanes are shown above the green boxes on the left, and the electropherograms are displayed on the right. The electropherograms display base pair size as the x-axis and RFU as the y-axis.

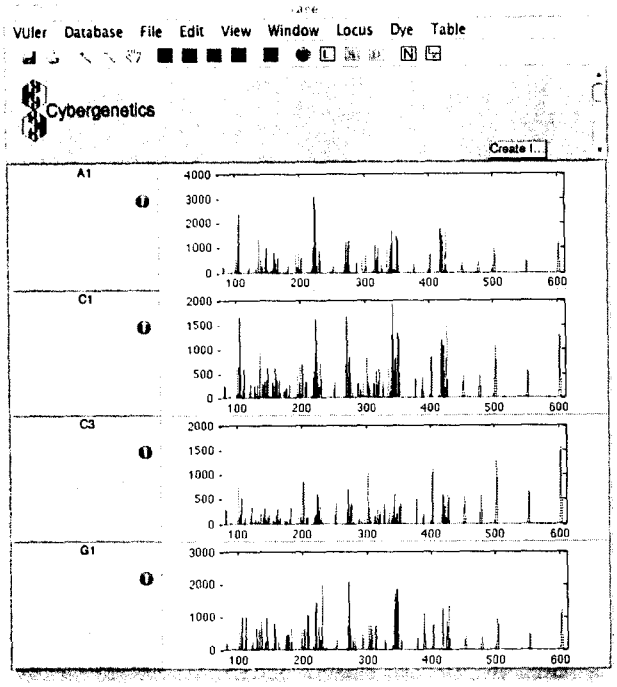


Figure 4. *Lane window.* Lane window showing data signals.

Information Button

In the green box for each track, we see an "i" button. Clicking this button opens an information window containing details about the lane, such as the well number and panel. For more information on this window, see the **Appendix ▸ Data Options**.

Reconstructing Requests

We can also search for STR data from previously made requests. STR data from previously made requests can be useful if we want to make a new request from the same data. We may do this, for example, to test a different number of contributors.

To find the STR data from a previous request, select **Database ▸ Find ▸ Request**. This action brings up the *Find Lane from Request* window, which is identical to the search window used in the Review Module (for more information on searching with this window, see the "Request Retrieval" section of the Review Module manual).

2.2 Reviewing the data

In order to determine what questions to ask, we have to review the data to determine a possible number of contributors. We can do this by looking at each locus and determining how many peaks are present. By default, all of the loci are displayed when the *Lane* window opens. To view the data locus-by-locus, we select **Locus** from the menu bar, which allows us to choose from any of the loci in that panel. After we select a locus, the locus name is displayed in the purple region at the top of the window. Here, it is helpful to go through each locus and note how many contributors the number of peaks indicate; this will assist us when creating the questions we will pose to the system.

Other ways to view the data

As in the Data Module, there are multiple ways to view our data. While viewing locus-by-locus is most common, we have several options.

Suppose we want to check for microvariants or an off ladder run. We can select the "N" button in the toolbar to turn on the peak labels. Clicking the "N" button a second time will turn off the labels.

We can check for spikes in our data by turning individual dyes on and off. Selecting one or more colored boxes in the toolbar does this for us. Each colored box represents one of the dyes in the kit. To turn on all of the dyes, we can select the rainbow button in the toolbar. Clicking a colored box a second time can turn any of the dyes off.

We can view the dyes for a lane of data separately by selecting the green area of the lane so that it is highlighted, and clicking the "D" button in the toolbar. This action opens a view that shows each dye as a track with the corresponding peak signals (Figure 5).

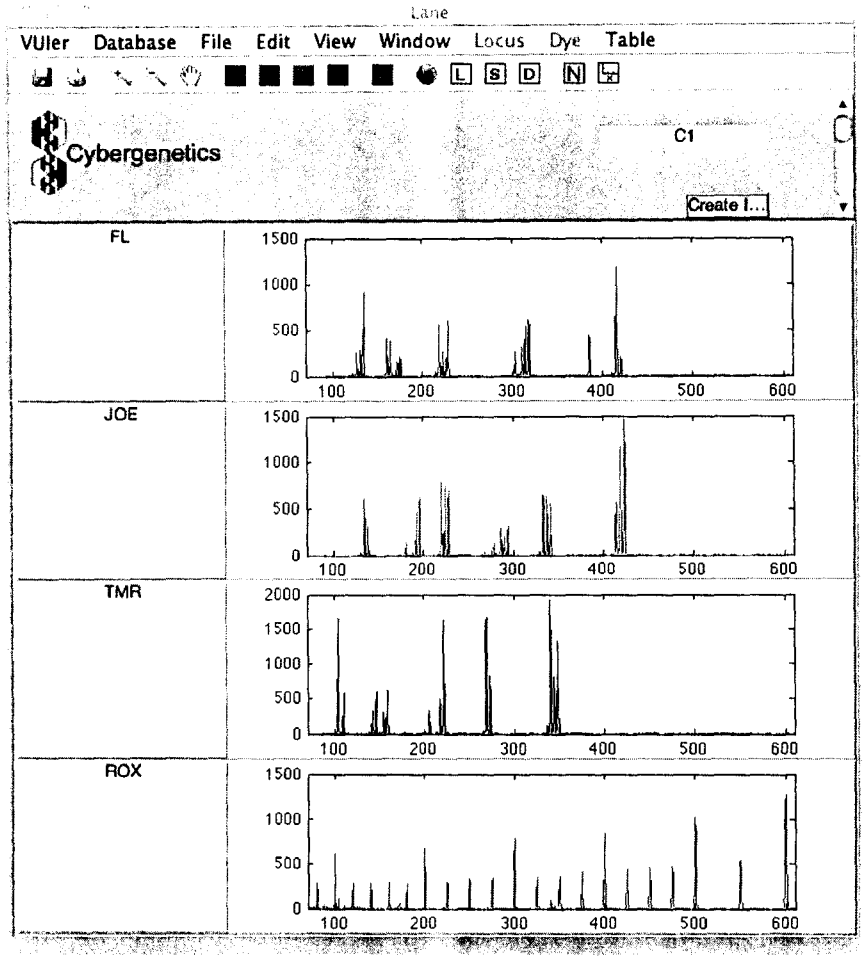


Figure 5. *Dye view in the Lane window.*

Another view for an individual lane of data is the sample view, which is accessed by selecting the green area of the lane so that it is highlighted and clicking the "S" button in the toolbar (Figure 6). Sample view shows us a locus-by-locus breakdown for each dye.

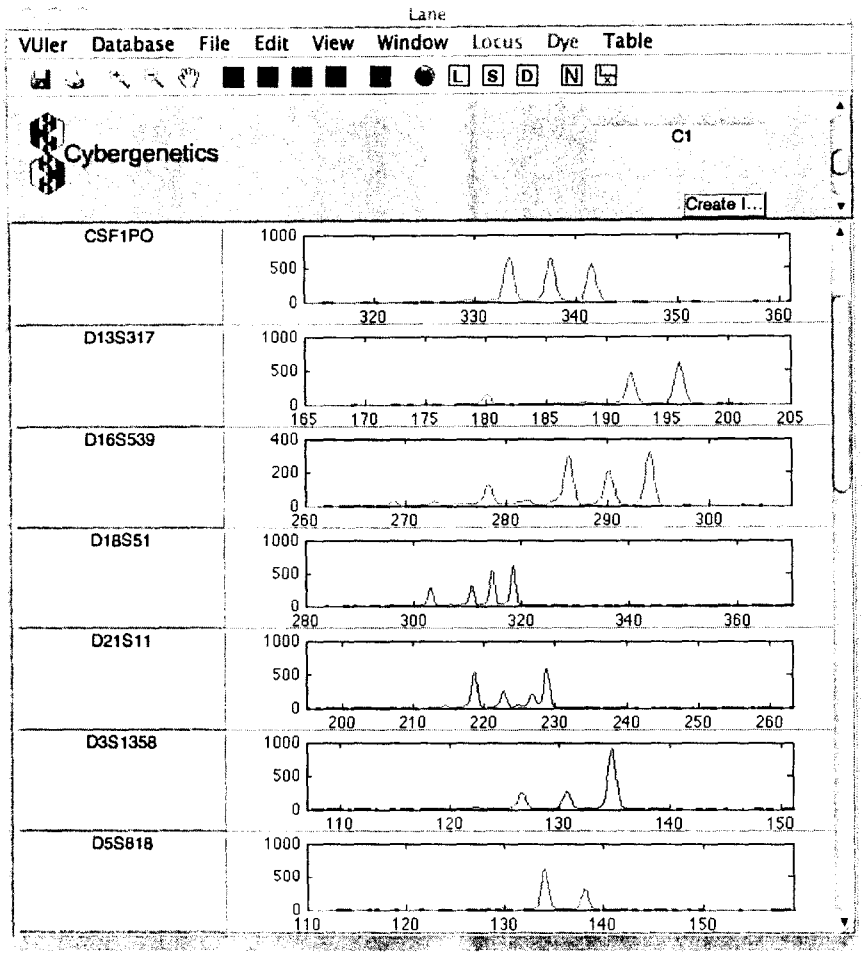


Figure 6. *Sample view in the Lane window* showing individual loci.

If we wish to exit sample or dye view, we can select the "L" button, which takes us back to the original Lane view. This action will take us back to only the lane of data we have been viewing. To view all of the lanes of data again, we can select the globe button in the toolbar.

3 DNA Items

Once we have acquired and reviewed the data for a case, we can begin to ask our questions by setting up requests. A request is generated in two general steps: creating a DNA item, and forming a question from that item.

3.1 Creating DNA items

A DNA item represents a unique PCR template. To create an item, we select the green area of the desired lane and click the "Create Item" button in the purple header region. We can select multiple lanes by right-clicking the green areas. This is useful in cases where two lanes originated from the same template, such as ProfilerPlus and Cofiler data.

Common Types and Roles

The *Create DNA Item* window opens with several fields to be filled in (Figure 7). The specimen name can be any name we wish to give to the item; it does not need to be the same as the request name. In the Type dropdown box, we can choose from Individual or DNA Template. Choosing "Individual" in the type field indicates to the system that the data is a known profile. When "individual" is selected as the type, the roles available from the Role dropdown box are Reference, Victim, Suspect and Elimination. When DNA Template is chosen in the Type dropdown, the available roles are Evidence and Quality Control. When creating any type of evidence request, Evidence should always be the selected role.

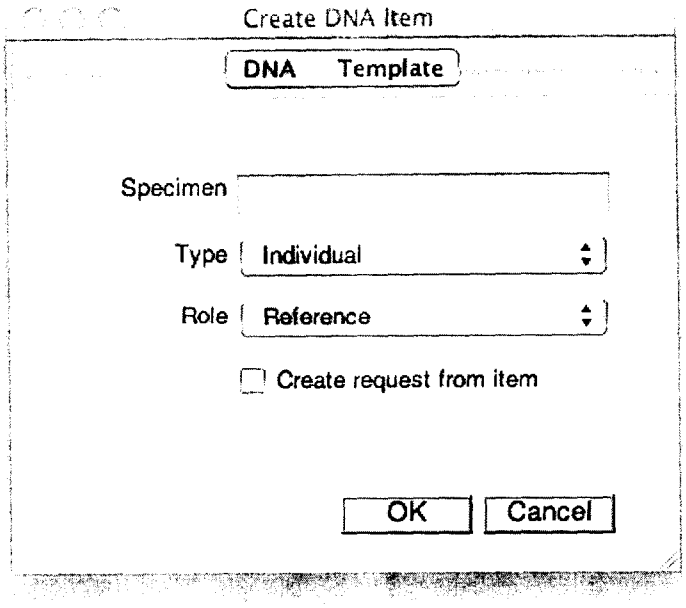


Figure 7. *Create DNA Item window.*

Template Options

A "Template" tab is also in the *Create DNA Item* window. This tab is needed when using extraction methods that differ from the norm or for using multiple amplifications of the same sample as a single template. More information on the Template tab can be found in the **Appendix ▸ DNA Item Option.**

DNA Window

After clicking OK and creating our DNA item, the *DNA* window opens. All of the DNA items that have been created are displayed in this window. Each item is shown with the specimen name above a purple select region and a corresponding icon to the right (Figure 8).

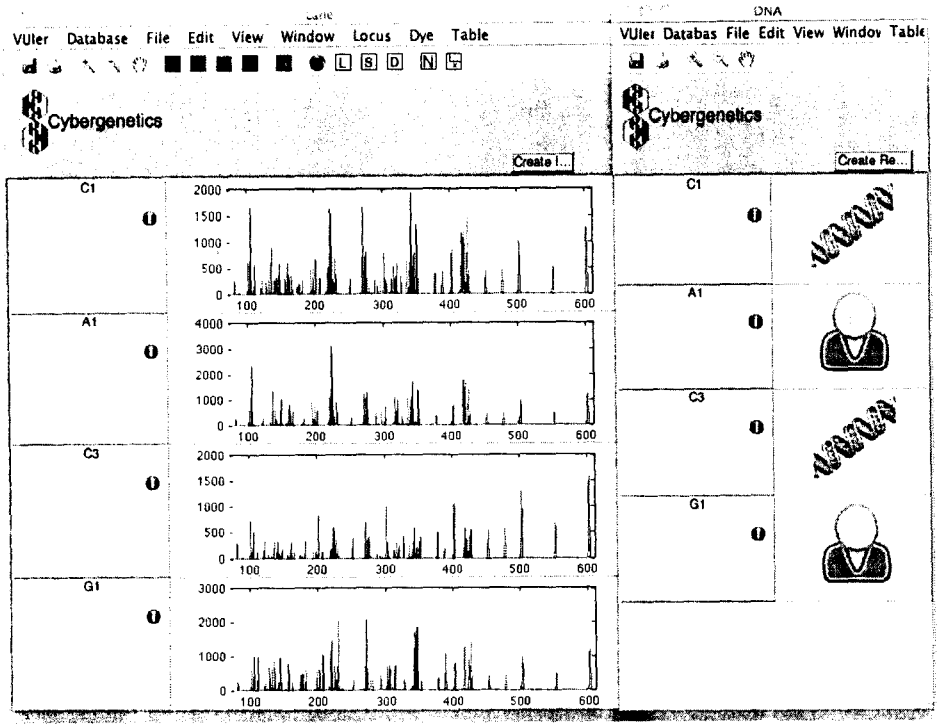


Figure 8. *DNA window*, in addition to the Lane window.

When DNA template is chosen as the type, the icon is a DNA strand. When Individual is chosen as the type, the icon is a person. If we need to edit any of the information about the DNA item, clicking the "i" button in the purple select region opens the *Create DNA Item* window, allowing us to make changes.

3.2 Create Request Shortcut

If we are creating a single request directly from a single item, we can jump straight to the *Create Request* window. We do this by checking the "Create Request from Item" checkbox in the *Create DNA Item* window.

4 Interpretation Requests

From the DNA items we have created, we can make requests, or questions to pose to the system. To make a request, we select the DNA item of interest and click the "Create Request" button in the purple header region. This action opens the *Create Request* window.

4.1 Request

The request tab is the first of 4 tabs in the *Create Request* window (Figure 9). Typically, the first thing to do is fill in the Request field. This window is where we designate the naming of our request. Naming can be done by any standard lab-set protocol; if there is no set protocol, using Case_item is an effective naming scheme.

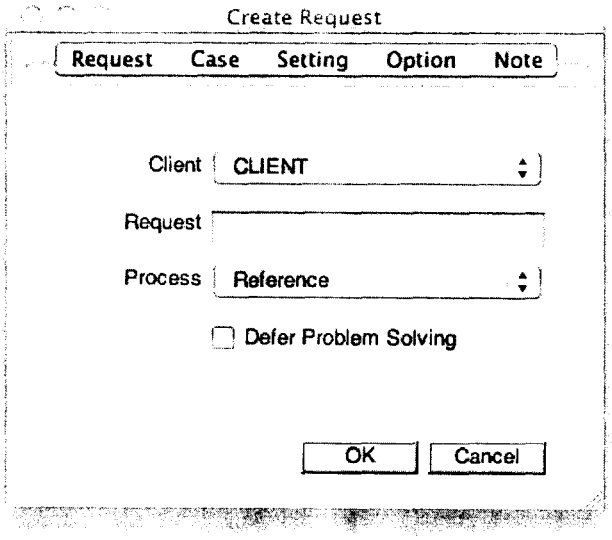


Figure 9. *Request tab* in Create Request window.

The Client field is a dropdown menu that lists all of the client names that are on the current database. The appropriate client name is required for a request to upload; if the correct client is not listed, please see the Tools Manual for help with adding a client to a database.

The Process dropdown menu is where we ask the system our question by telling it how to process the data. For a known sample, we choose the "Reference" process. For unknown samples, we choose the process based on the proposed unknown number of contributors.

When necessary, we can also use the "Defer Problem Solving" checkbox that allows us to set up a request with inactive status. This means that the computer will not pick up this request to solve until its status is changed to active. For more information on changing a request status to active, see the Tools Manual.

4.2 Case

In the Case tab, we designate what case folder the information should be placed in (Figure 10). For the first request, we have to type in the case name in the Case field. For all additional requests, this name will be available from the Case dropdown menu.

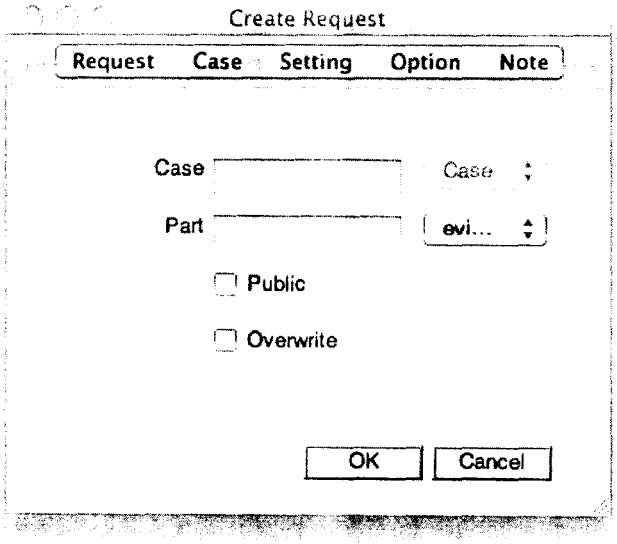


Figure 10. *Case tab* in Create Request window.

In the Part field, we tell the computer the role of each request. A dropdown menu lists evidence, victim and suspect as some options. We can also fill in the text field if we need other terms such as reference or elimination. All DNA template items must be listed as evidence; for matching purposes, any phrase that starts with "evi" will be matched against everything else.

Below the Part field is the Public checkbox. Checking the Public box will allow the request to be viewed by any user account on that TAW, including those with limited access. The second checkbox is the Overwrite checkbox. Overwrite will allow for a case name to be overwritten entirely. All information within the original case with that name will be deleted.

4.3 Setting

The Setting tab is where we allocate how many cycles the computer should go through when solving the questions (Figure 11).

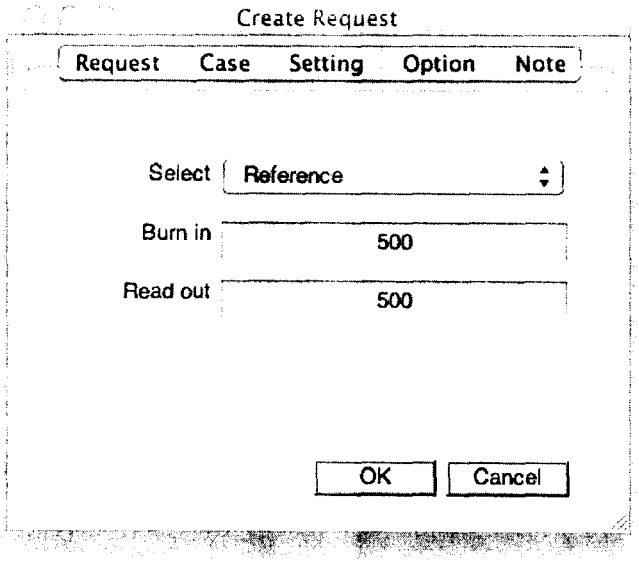


Figure 11. *Setting tab* in Create Request window.

The first number is the burn in, or how much time the system gets to search for the right region. The second number is the read out, or how long the system gets to

sample within that region. More information can be extracted from difficult data when there is more time for the system to sample.

A drop down menu lists six options: Reference, Screen, Regular, Thorough, Exhaustive and Custom. For any known reference items, the setting should be reference; this is a short cycle time useful for clean profiles. For simple mixture samples, the Screen setting should be used, as this is the minimum number of cycles at which an evidence sample should be run. This setting is often useful for pre-screening complex samples.

For a typical case sample, the Regular setting should be used. At 10,000 burn in and read out, this setting will usually solve average mixture samples and allow ample time for convergence to occur.

For challenging mixture samples, like degraded or three contributor mixtures, the Thorough or Exhaustive settings should be used. These settings allow for greater sampling of the data. While these settings will take more time for the cycles to complete, we will have more information extracted from the data than by using fewer cycles. Exhaustive is a useful setting for studies, as it ensures that requests have had more than sufficient time to sample. A Custom option also exists if we wish to set our own burn in and read out times.

4.4 Option

The Option tab offers settings that can be useful when solving complex mixture problems (Figure 12). The two most frequently used options, off ladder and degraded mixture, are described here; for information on the other options, please refer to the **Appendix ▸ Request Options**.

The Off Ladder option can be set to small, medium, or large. By default, every request is solved with a small off ladder setting to account for slight shifts that may

exist in data. If data appears to be severely shifted, the off ladder setting should be set to medium or large.

The Degraded mixture option is off by default. If we notice a pattern of decay in the data, the degraded mixture option can be turned on to account for DNA degradation.

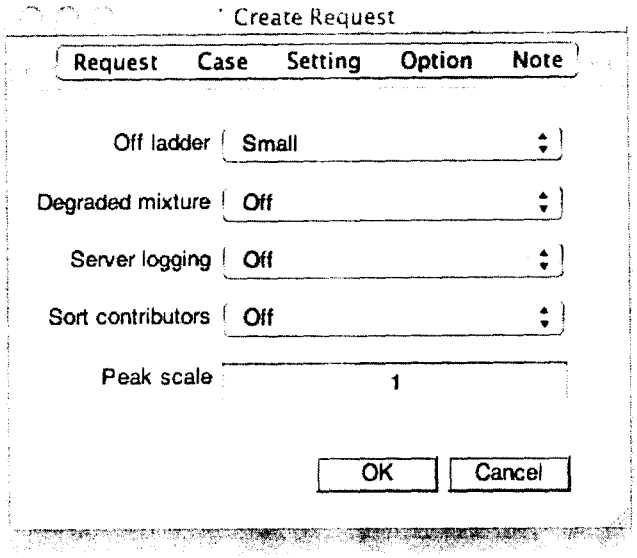


Figure 12. *Option tab* in Create Request window.

4.5 Note

When we are creating our request, we have the ability to make notes that will be saved and appended to the request (Figure 13). These notes can be useful when we are reviewing the request at a later time, or if a second person is performing a review.

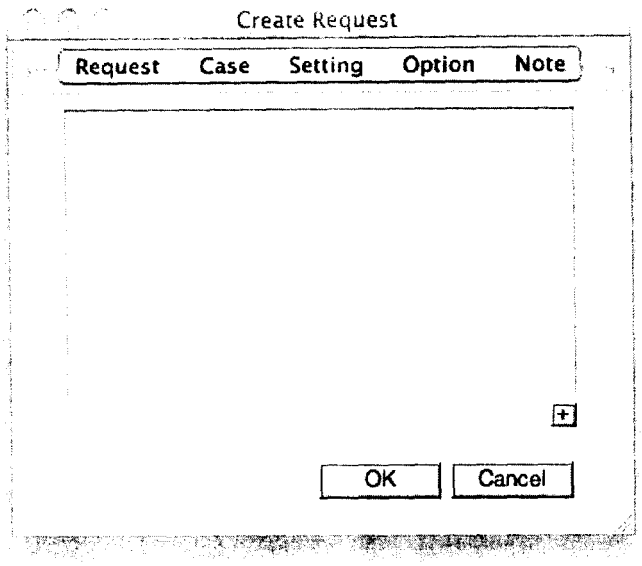


Figure 13. *Note tab* in Create Request window.

To add a note to a request, we click the "+" button in the lower right corner of the window. A *Note Editor* window opens, where we are able to type up to 10,000 characters in our note. The text will wrap, meaning that it is not necessary to hit return between lines. Using the return key will prevent the note from uploading. When we are finished, we click "Apply" and the note will appear in the tab of the *Create Request* window. Additional notes can be applied at any time, and there is no limit to the number of notes that can be applied.

4.6 Request Window

After all of the fields in the *Create Request* window have been filled in, we click OK. This action opens the *Request* window (Figure 14). The *Request* window is laid out similarly to the *DNA* window. Like a DNA item, the request name is displayed above a purple select region with a designation icon to the right. The icon denotes the process at which the request is running; a 1 is displayed for a one unknown process, a V is displayed for a victim reference, etc.

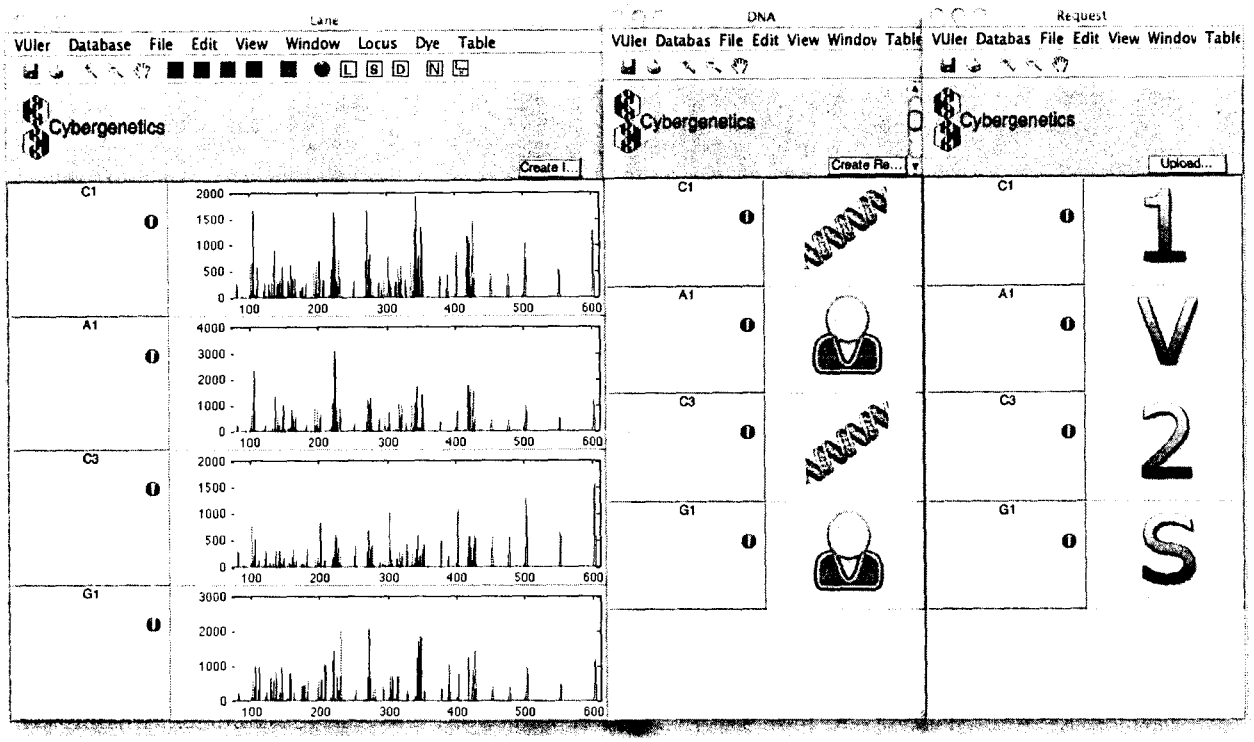


Figure 14. *Request window*, in addition to the DNA and Lane windows.

If we need to edit any of the information from the *Create Request* window, we can do this by selecting the "i" information button.

If we want to save our requests before upload, we can use the Import/Export function from the File menu. More details on importing and exporting requests can be found in the **Appendix ▸ Request Options**.

5 Final Check

The final step in posing questions to the system is uploading our requests. Before we upload, we should always do a final check of our work. We can do this by clicking the purple select region under the request name in the *Request* window. Clicking this area will highlight the associated item and data lanes to ensure that the proper data was used to make the requests.

5.1 Quality Check Table

After finishing our check, we can move forward with uploading our requests. To do this, we select the "Upload" button from the *Request* window header region. A *Review* window opens, showing the various settings for each request that was created (Figure 15). This window can be thought of as a quality check table, verifying that all of the requests with appropriate settings are in the upload list. It is also good to verify that all requests have a "no" in the overwrite column, unless there is a request that we wish to intentionally overwrite.

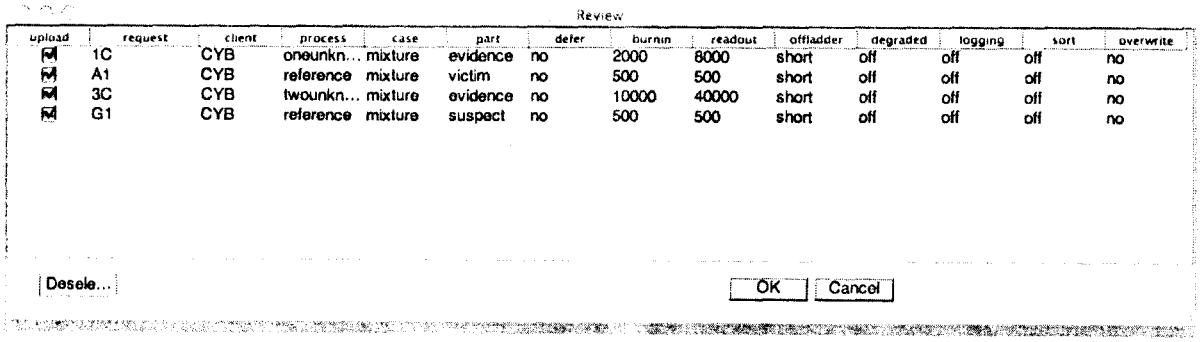


Figure 15. *Review window.* This acts as a quality check table.

5.2 Upload Complete

To finalize the upload, we can click OK and confirm by selecting "Yes" when the dialog appears asking if we are sure we want to upload these requests. When the requests have successfully uploaded to the server, a dialog box reading "Upload Complete" appears.

6 Known Genotypes

When there is a genotype that is already known, it can be used within a case as a reference without the need for re-interpretation of its STR data. To do this, we use a previously uploaded profile.

6.1 Finding a Profile

Finding an uploaded profile is used when we have uploaded a genotype or likelihood in the Data Module. We can find this profile and assign it a request name and case folder. To find an uploaded profile, we select **Database ▸ Find ▸ Profile**, which opens the *Find Profile* window (Figure 16).

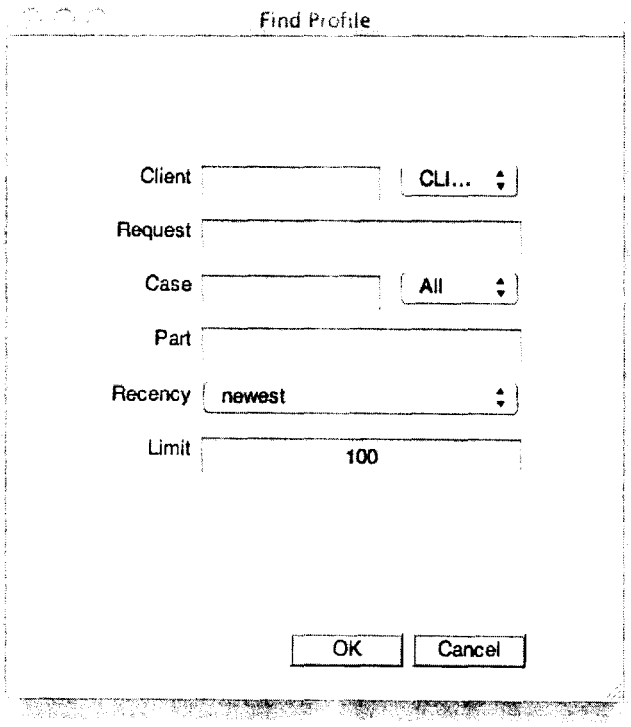


Figure 16. *Find Profile window.*

This window has five searchable fields; Client, Request, Case, Part and Recency. If a profile has not yet been designated to a case, searching by Client will bring up all of the profiles that were uploaded using that client name. After entering our search criteria, a *Select* window opens similar to the previous *Select* windows. However, we notice that there is no data information attached to the profile, which is expected since the genotype was uploaded manually.

After we select the profiles we want to use, both the DNA and Request windows open. The profiles are already designated as individual reference profiles, and only need to have a case and case part assigned. These windows and tasks are described further in section 4.2 of this manual.

7 Case Example

Now we will go through a simple case example in order to show the process that is used when in the Request Module.

We begin with known information about the case. We have three DNA samples: a victim reference sample, a suspect reference sample, and an evidence sample.

Upon reviewing the evidence data, we can conclude that the sample is a two-person mixture. Now that we know what type of samples are available, we can decide what information we hope to gain and formulate questions that will extract that information from the evidence.

7.1 Important Questions

For this case, we have two forensically important questions to ask about the data:

- Did the victim contribute to the DNA evidence?
- Did the suspect contribute to the DNA evidence?

In order to obtain answers, we will pose these questions to the TrueAllele system. We will incorporate the known case information and STR sequencer data into case requests that the system can interpret for us.

7.2 Did the victim contribute to the DNA evidence?

This question is forensically important because it will provide useful information to help interpret the DNA mixture evidence. If the victim is found to be a contributor, we can use the victim profile to help interpret the mixture and possibly get a more unique profile for the unknown contributor. If the victim is not found to be a

contributor, we know that we are looking for two unknown contributors and possibly two perpetrators.

Retrieving case data

The first step in our example case is to retrieve our sequencer data. We open a search window by selecting **Find ▸ Data** from the database menu. From here, we can select, search for, and open our case data (Figure 17).

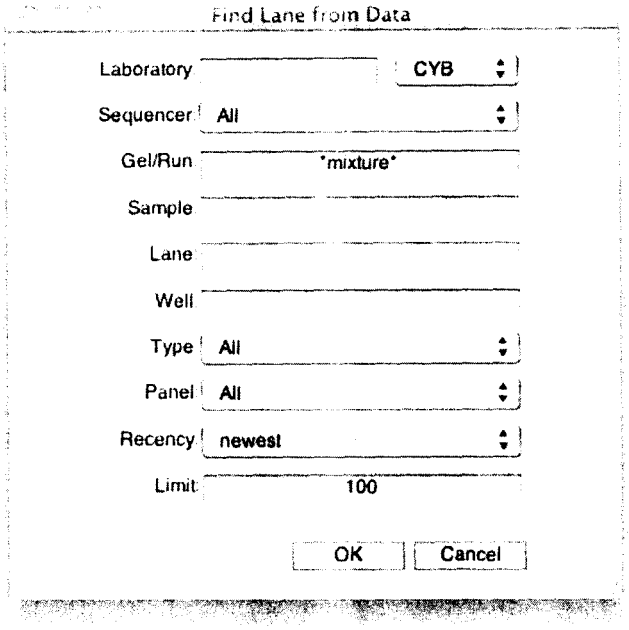


Figure 17. *Find Lane from Data window.*

When we open our data (Figure 18), we see the electropherograms for each sample in the Lane window. Here, we can briefly review our data.

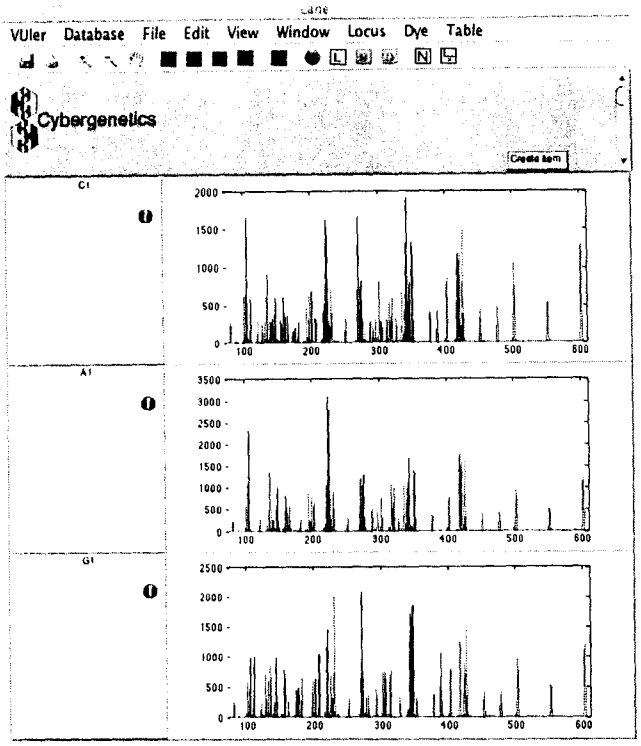


Figure 18. *Lane window.* Lane window showing data signals.

Zooming in on TH01 (Figure 19), we can see that there are four clearly visible peaks in the C1 evidence sample. After viewing this and other loci, we can verify that our evidence sample is most likely a two-person mixture.

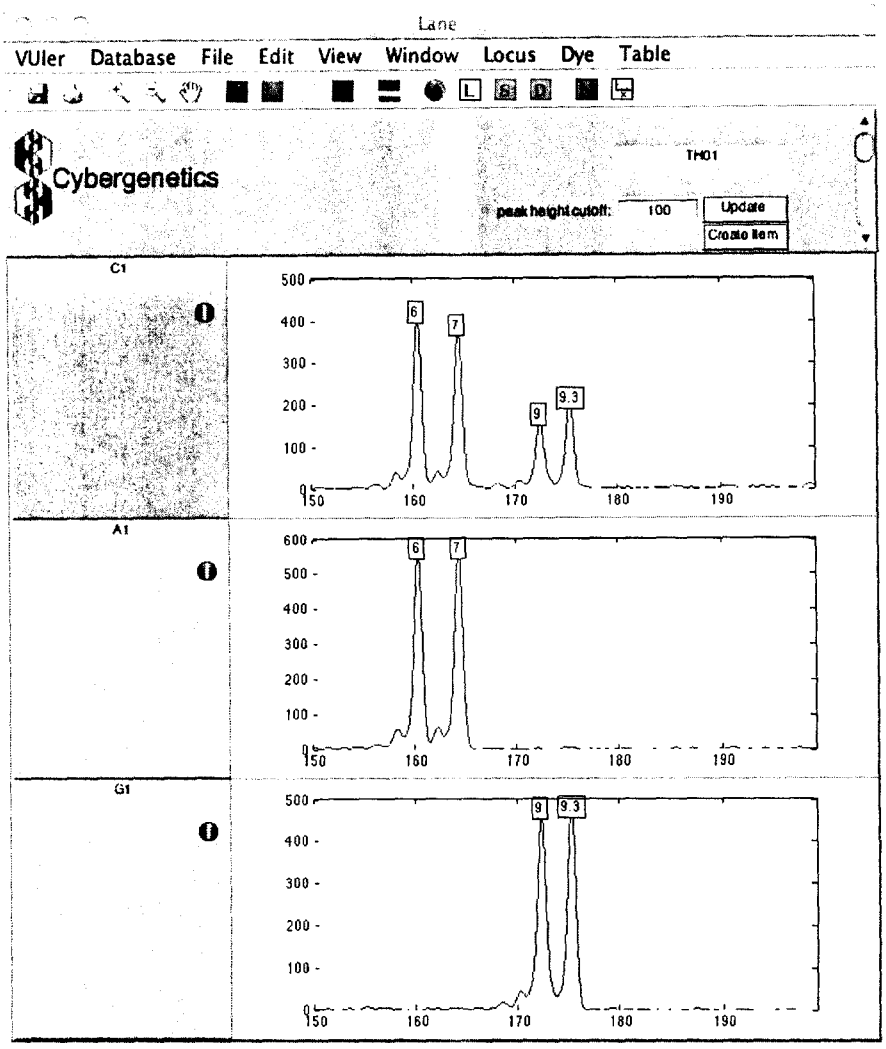


Figure 19. *Lane window* showing the TH01 locus.

DNA Items

Now that we have the sequencer data available, we can turn it into a DNA item. When we create the evidence item (Figure 20), we must specify the type of DNA (DNA template), as well as its role in the case (Evidence).

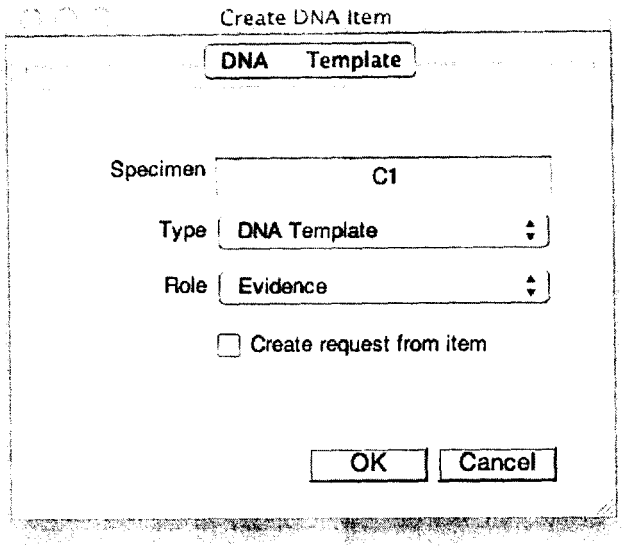


Figure 20. *Create DNA Item window.*

When we have finished creating the evidence DNA item, we see it appear in the DNA window to the right of the Lane window (Figure 21).

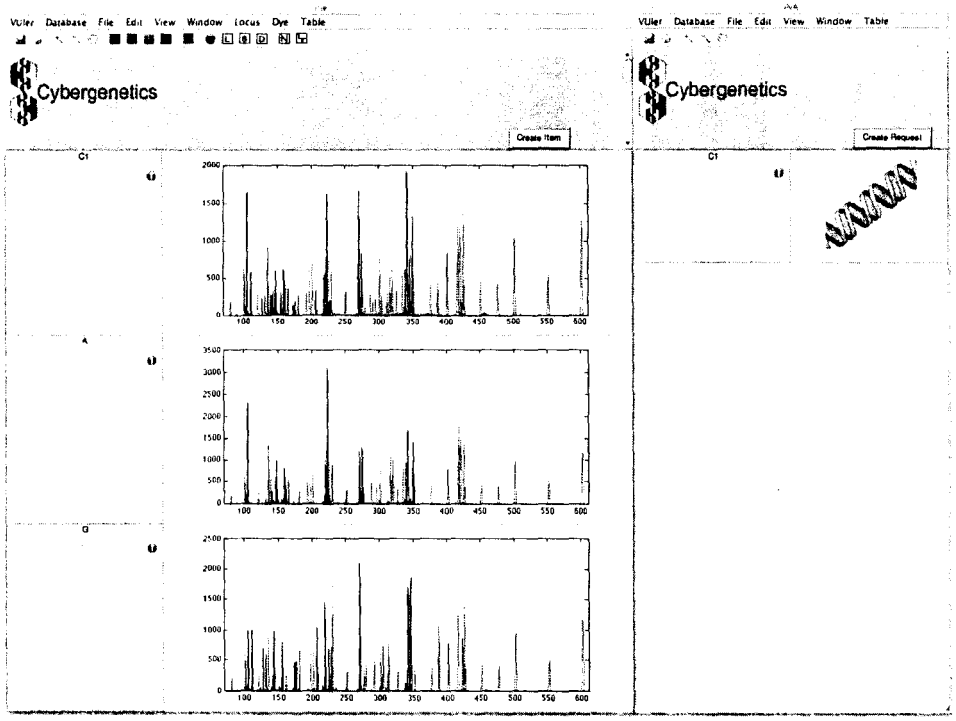


Figure 21. The *C1 evidence item* is now shown.

Requests

Once we have created the DNA item, we can begin to form our request. We specify how to process the sample (Figure 22). Processing as a two unknown indicates that we believe there are two contributors to this mixture, both of unknown identity.

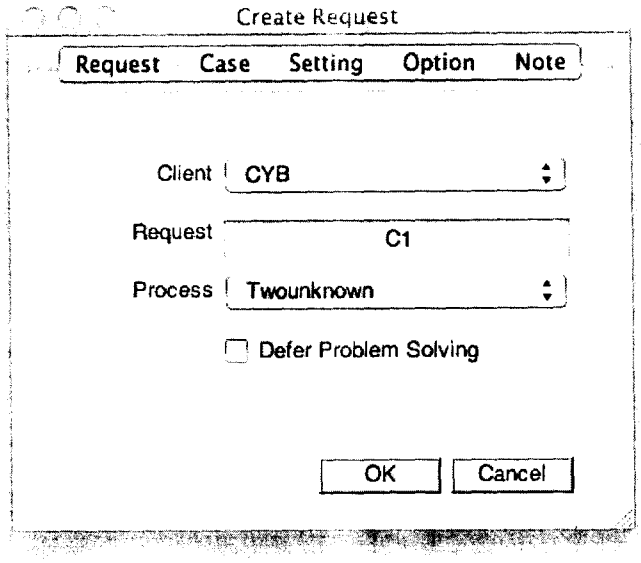


Figure 22. *Create Request window.*

Once the evidence request is created, it appears in the Request window (Figure 23).

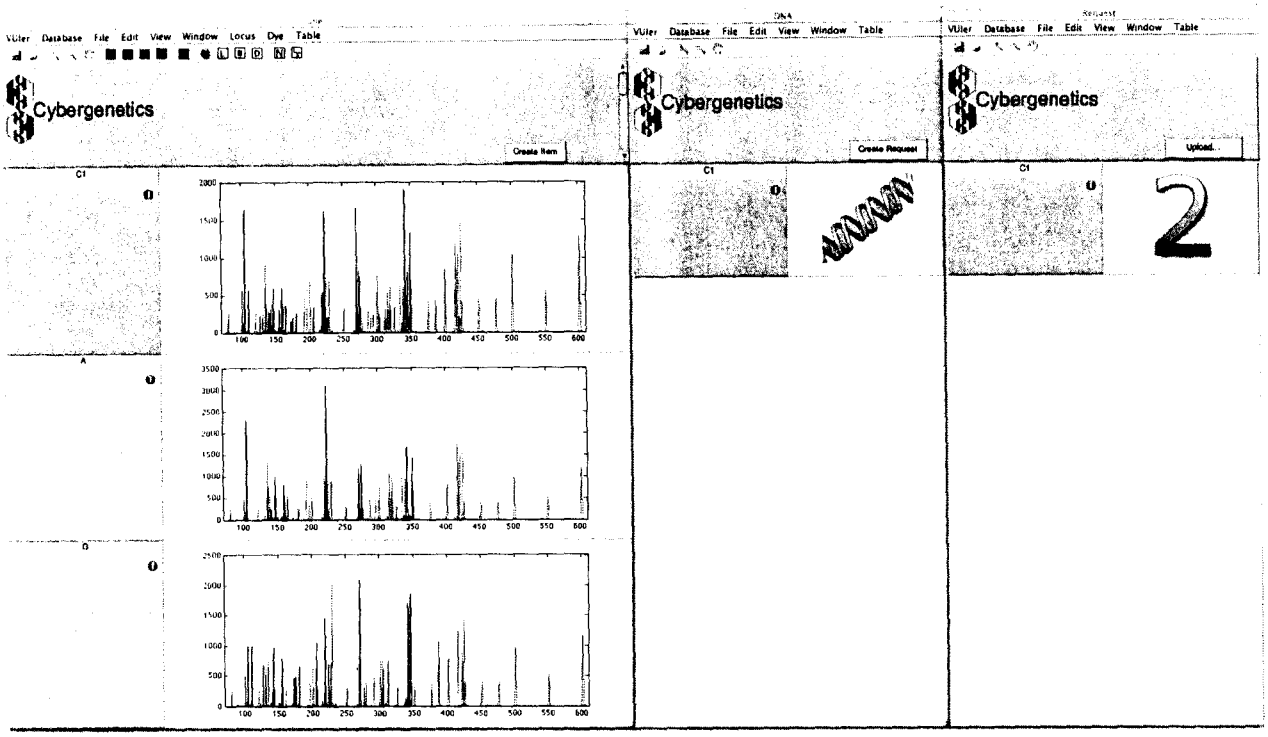


Figure 23. The *C1 evidence request* is now shown.

Comparing to the Victim

In looking at the mixture evidence and the victim reference sample, we concluded that the victim looks like a possible contributor to the mixture evidence. This observation leads us to our first question: did the victim contribute to the mixture evidence?

The TrueAllele system can help us answer that question by interpreting the request that we just made and inferring genotype probability distributions for each contributor of the mixture. We can then compare the genotype probability distributions of the mixture contributors to that of the victim, to see if our observation is correct. Now we must make a victim reference request following the same steps that we did for the evidence request (Figure 24).

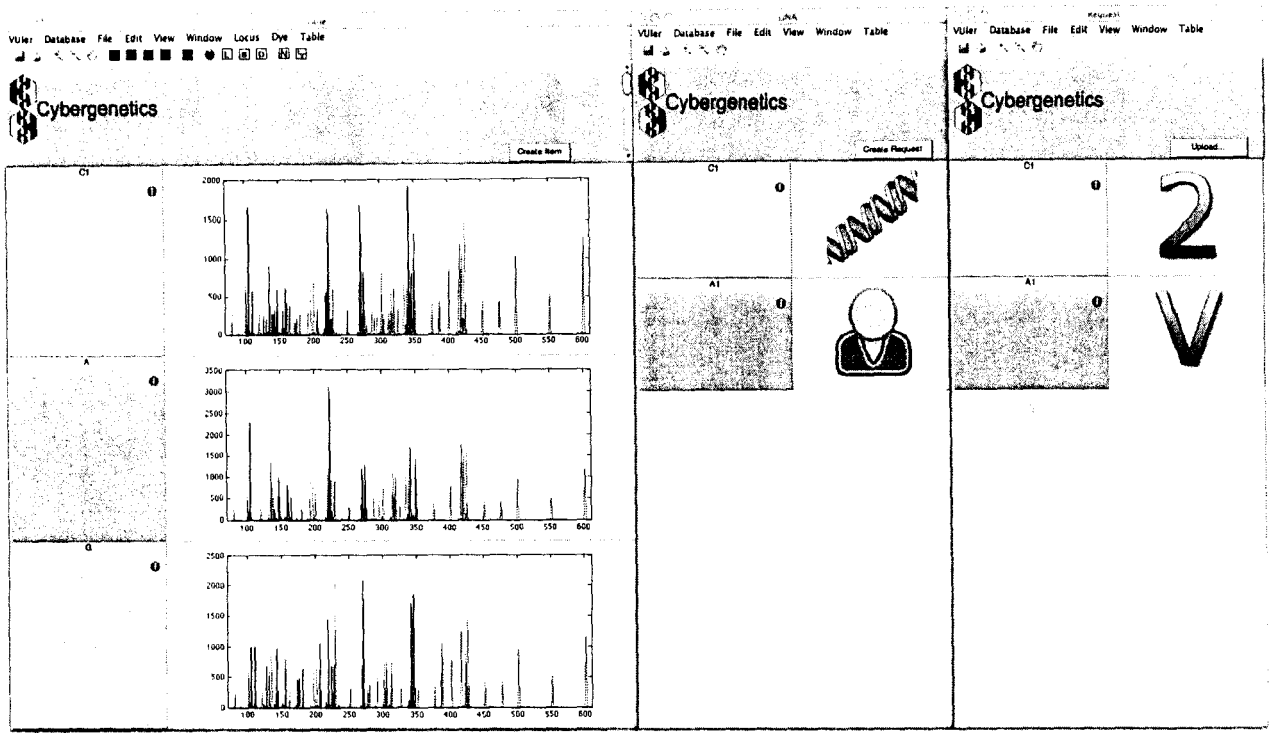


Figure 24. The *victim request* is shown with its associated item and data highlighted.

7.3 Did the Suspect Contribute to the DNA Evidence?

This is the most important question to answer, because it will either implicate or clear the suspect as the unknown contributor to this mixture evidence.

Using the Victim Reference

After reviewing our results (see the case example in the Review Manual), we see that our evidence request indicates that the victim is one of the contributors to the evidence. Now that the victim is a confirmed contributor, we will process this request as a one unknown sample. It is a two person mixture and we are providing the identity of one person; therefore, it is only a one unknown sample (Figure 25).

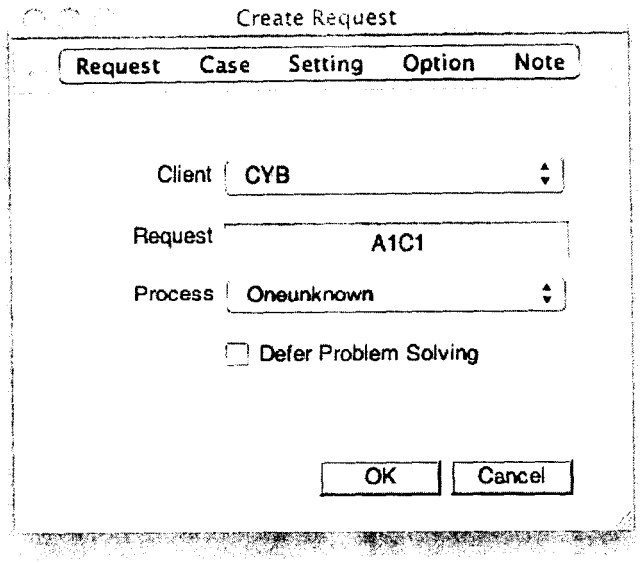


Figure 25. *Creating the A1C1 request.*

When we have finished creating the evidence request, we see it appear in the Request window under our victim request (Figure 26).

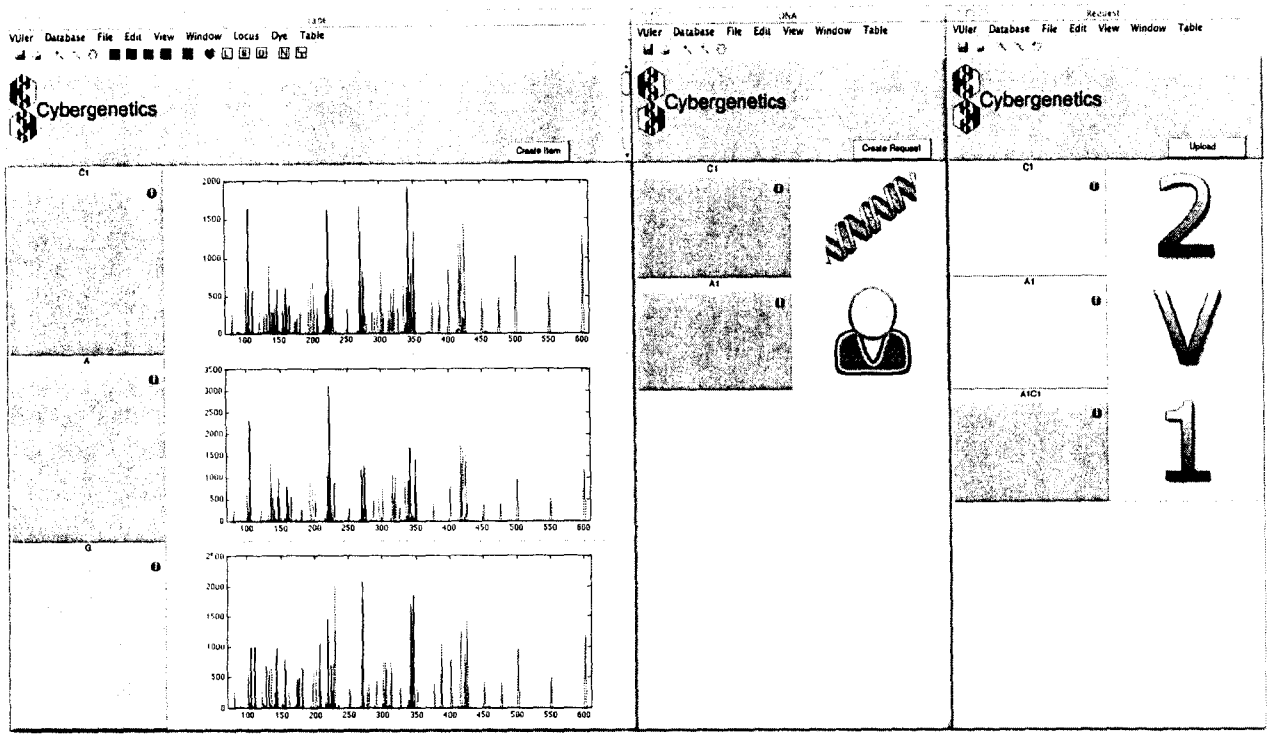


Figure 26. The *A1C1 request* is shown with its associated item and data highlighted.

Comparing to the Suspect

We are now concerned with answering our second question: did the suspect contribute to the DNA evidence? Therefore, we have one more request to make: the suspect reference (Figure 27).

We can perform this comparison in the same way that we did for the victim, keeping in mind that this question might have already been answered when we reviewed the first request. However, by adding more information to this request, we may see a sharper answer and a stronger likelihood ratio supporting a match between the suspect and the unknown contributor in the evidence.

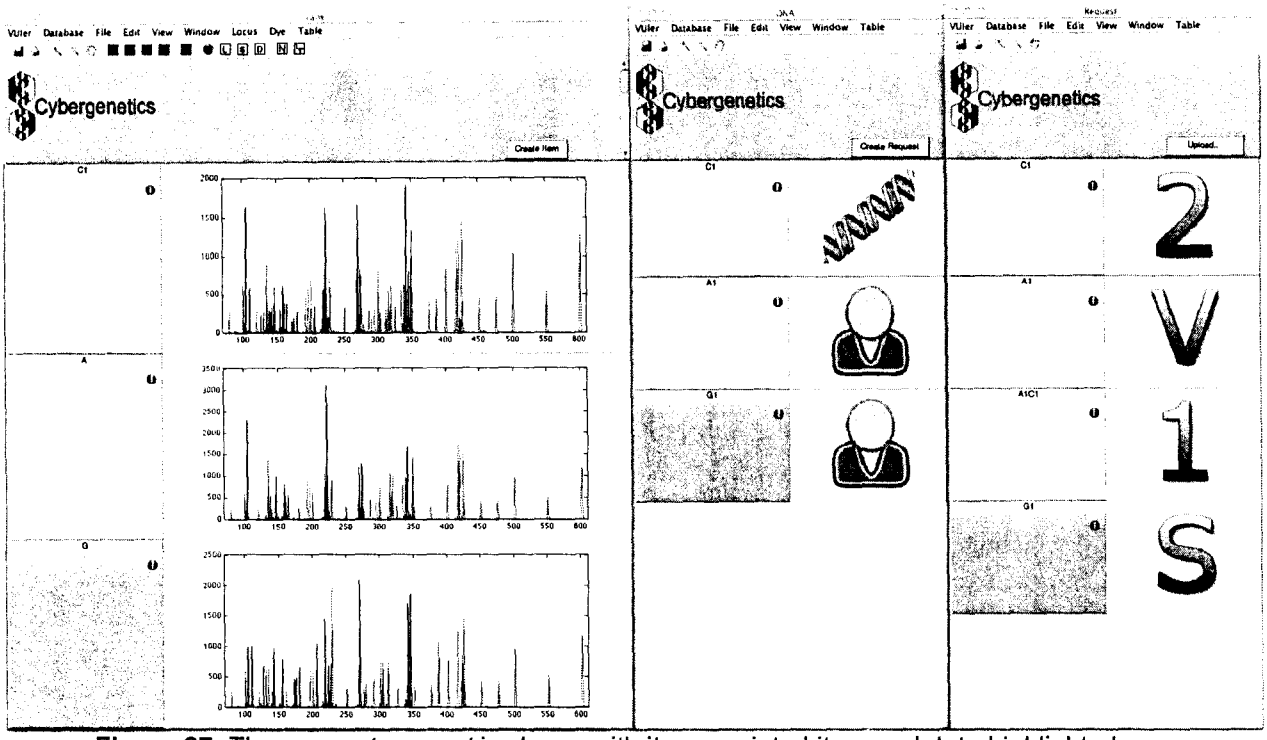


Figure 27. The *suspect request* is shown with its associated item and data highlighted.

Final Check

Once we are satisfied with the requests we have made, we can upload them to the database for solving. Clicking the upload button opens the Review window (Figure 28) and, after a final check, clicking OK begins the upload process.

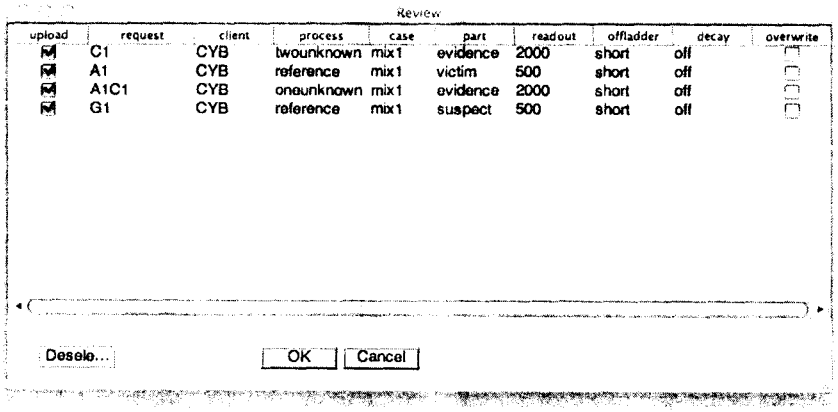


Figure 28. *Review window.* Reviewing requests before upload.

8 Appendix

This appendix is designed to discuss in detail specific features that may help us in our TrueAllele processing.

8.1 Advanced Searching Options

There are multiple ways to search for data when creating a request. For all of the following search fields, the wild card character can be used. The wild card * can be used to match any characters before or after the word we enter in a field.

Laboratory – The lab name can be chosen from the drop down menu or manually typed

Sequencer – The sequencer name can be chosen from the drop down menu

Sample – The sample name from the specific injection can be typed

Lane – The lane number can be manually typed

Well – The well number (ex: A4) can be manually typed

Type – The type of sample (Sample, Ladder, Positive, Negative) can be chose from the drop down menu

Panel – The marker panel used to process the data can be chosen from a drop down menu.

Recency – Data can be searched by the oldest or newest data uploaded to the TrueAllele World.

Limit – This field limits how many search results will appear. The default value is 100, but any number can be typed in the field.

8.2 Data Options

Data Tools

Right clicking peaks – When peak labels are turned on, we have the ability to right click the peak label box for more information. Right clicking shows us

the peak designation, peak length, peak height and the ability to turn the peak off.

Peak Table – Selecting **Table ▸ Peak** from the Lane window opens the *Data Peak* table. For each lane of data, the table lists the sample name, locus, peak designation, peak length, peak height, and peak area.

Deactivating non-data peaks – If we have non-data or artifact peaks that may affect how the system would interpret a genotype distribution, we can turn these peaks off. Turning the peak off removes the non-data peak so that each time that lane is viewed, the peak will always be off. We turn a peak off by right clicking the peak label and choosing "Peak off." The peak label will then turn gray.

Deleting a Lane of Data

If we see a lane of data that should not be used, we can ensure that the lane is not available. We can do this by selecting the lane of data and choosing **Edit ▸ Delete** from the Lane window. This action will effectively turn the lane off, rendering it unavailable for use when creating items. Turning a lane off can also be done by right clicking in the electropherogram area of the lane and selecting "Lane off." After a lane has been turned off, it becomes gray and moves to the bottom of the lane window. We can undo this action by right clicking in the electropherogram region and selecting "Lane on."

Information button

The information button for a lane of data opens to the Lane window. This window has three tabs: Lane, Product and Note. The Lane tab has six rows of information: Gel name, lab (as identified in the Data Module), sequencer, sample name, well number and panel. This information is not editable.

The Product tab contains editable information about the PCR process. The first drop down menu is for *PCR lab*. This menu provides the ability for a user to distinguish between the lab that extracted the biological specimen and the lab that performed the amplification, in the rare cases where these are separate entities. The *Amplification* drop down allows us to differentiate between varying PCR that may have been used. For instances where multiple amplifications of the same specimen were run, we can change the *Aliquot* drop down menu. The aliquot menu allows for up to 10 amplifications of the same sample to be designated. Unless the amplifications are to be combined for TrueAllele interpretation, it is not necessary to change the aliquot number. This change will indicate to the system that the injections come from the same DNA template, altering how a pattern is matched to the data.

The Note tab allows us to create notes that are attached to a particular lane of data (Figure 29). By clicking the + button in the Note tab, the *Note Editor* window opens. We can add up to 10,000 characters of text. Applying the note will permanently attach it to the lane and the note is then visible in the Note tab. If more than 10,000 characters are required, we can add as many additional notes as we wish. These notes can then be viewed or added to in the Review Module, which is described further in the Review manual. It should also be noted that the *Note Editor* window text wraps; it is not necessary to hit the return button.

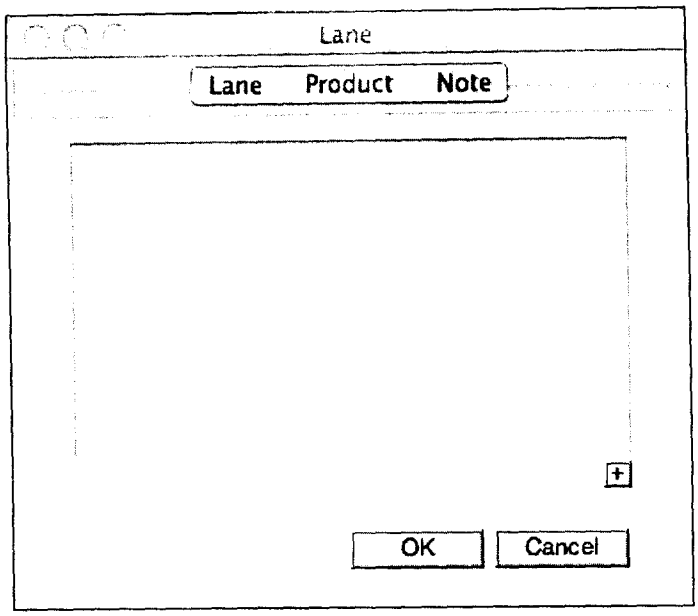


Figure 29. *Note tab. The Note tab allows the user to attach notes to a lane of data, which are viewable in the Review Module.*

8.3 DNA Item Options

Template Options

The template tab in the Create DNA Item window is only used when there are alterations to the normal extraction process. There are several fields that can be changed in this tab.

Specimen – The specimen name is the name given to the item in the DNA tab

DNA Lab – The DNA lab drop down menu denotes the lab that performed the extraction process on the biological sample.

Cutting – The cutting number allows us to designate different cuttings from the same biological sample.

Extraction – The extraction drop down menu allows us to choose what DNA extraction method was used. The default setting is "standard;" this setting should only be changed if a different extraction method was used.

Aliquot – The aliquot drop down refers to the amplification number. This number should be changed when you have multiple amplifications of the same DNA template. Aliquot number can be changed to numbers 1 through 10. If we have multiple amplifications, we can combine them as one item so that they system understand that they comprise a single DNA template.

8.4 Request Options

Delete

An item or a request can be deleted prior to upload. We may need to delete an item if we used an incorrect lane of data, or delete a request if we used the wrong item. To delete an item, we choose **Edit ▸ Delete** from the DNA window. To delete a request, we perform the same action in the Request window.

Duplicate

When we are looking for reproducibility statistics, it can be helpful to create a duplicate request on demand. We can do this in the Request window prior to upload. To duplicate a request, we select the individual request and choose **Edit ▸ Duplicate** from the menu bar (we can also use the command + D keyboard shortcut). This action brings up an *Edit Request* window identical to the one used when creating a request. Because we are creating a duplicate, all of the fields will be filled in exactly like the request we are duplicating. The Request field will have the name of the original request with "_copy" appended to the end. This name can be changed if desired. After double-checking all of the parameters and clicking OK, a new request icon will appear in the Request window.

Option Tab

Server Logging – This is a setting where a request, while processing, will save diagnostic information. This takes up space and memory on the server during processing, and so is typically only used in rare cases. The default setting is off; this feature is only used when receiving diagnostic support from Cybergenetics.

Sort Contributors – This setting places restrictions on the Markov chain when solving mixture problems. The default setting is off; this feature is used when receiving diagnostic support from Cybergenetics.

Peak scale – The peak scale number accounts for variation in data when peaks are artificially inflated or deflated due to different PCR run parameters from normal conditions. Examples of differing run parameters include different injection times, post-PCR processing, and cycling conditions.

A suggested way of choosing a peak scale factor is comparing the height of the artificially inflated peaks to normal data. The height ratio value can be entered as the peak scale value. However, this is a starting point; some experimentation with values may be necessary to determine the appropriate number.

Importing/Exporting Request

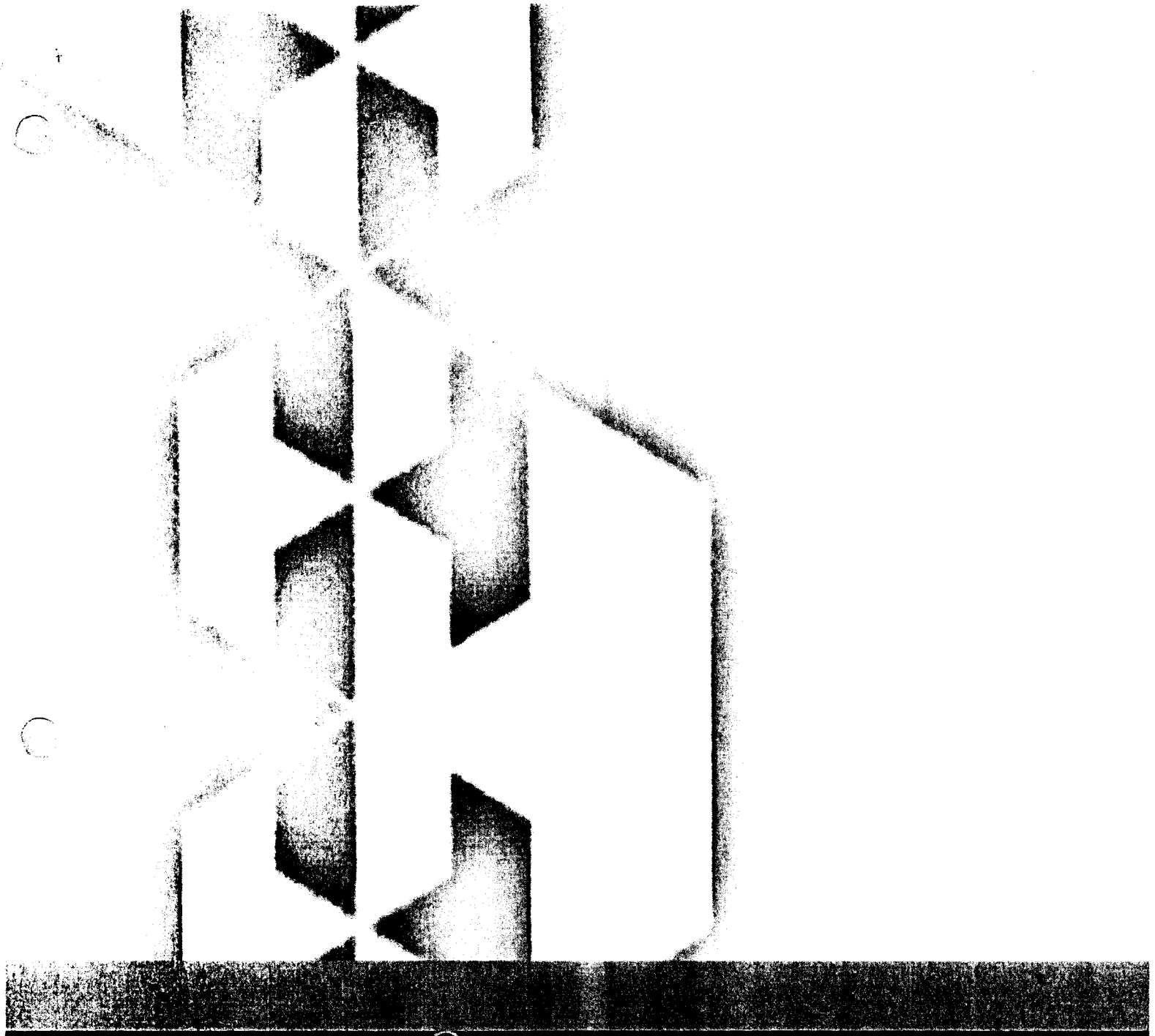
If, for any reason, we should have to leave the Request Module prior to upload, we can save our work at any point and come back to it. Selecting **File ▸ Export** from the Lane, DNA or Request windows can save the request state. This action opens the *Save request file as* window. Here we name the request file and choose the location where we want to save it. These files are saved as .req files, meaning that they can only be used to import or export a request state.

To open one of these saved request files, we choose **File ▸ Import** from any menu bar in the Request Module. The *Select a request file* window opens and we find the .req file that was previously saved and click Open. The state of our previous request session is then restored and can be continued. This feature can be useful if a technical review is required before upload, or if we want to set up a similar series of requests with the same data and using different parameters.

Creating a Joint Request

Many times we can gain more information from individual pieces of data if we run them as a joint request. This means that the system would use multiple pieces of data and fit genotype patterns to each of those pieces at the same time. By having more data to use in its model, the system can become more certain of its answers.

To create a joint request, we select multiple DNA items by right clicking in the select areas. After choosing the items, we proceed with creating the request in the same way that we would a typical request. The system will analyze both DNA templates and use them to fit a probability distribution for a genotype.



TrueAllele® VUIer™ Review Module

Getting Answers

Cybergenetics

Table of Contents

1 Overview

In the Request Module, we asked DNA interpretation questions of the evidence. After the computer cycled through the STR data and statistically modeled all possible data patterns, an inferred genotype from the evidence was determined. After the genotype was inferred, it was then compared to the provided references.

Clicking the Review icon in the *Module Chooser* window accesses the Review Module, opening the home window (Figure 1).

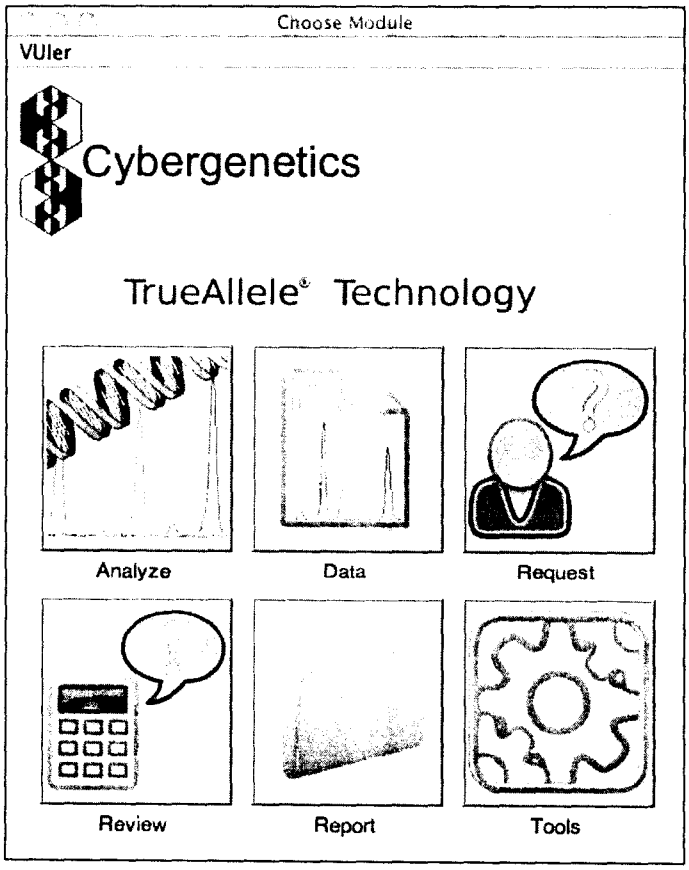


Figure 1. *Module Chooser window.* The Review icon opens the module.

In the Review Module, we can view the computer's answers to our questions. If our references match, we will know who contributed to the DNA evidence and to what extent. The computer's results are presented visually as pictures and tables in several interactive user interfaces. These windows provide the user access to the original electropherogram, the inferred genotype and mixture weight, and match scores. The Review Module also provides an opportunity to question and understand the inferred results.

2 Assess Solution

The Review Module offers an open workflow so that we may explore and quality check the computer's processing. Depending upon the data and the questions we ask of it, the Review process may take a few seconds or the request may require more close attention.

We will be exploring the review windows in this module. These windows can be viewed in any order and in any combination. However, it is important to remember that all of the windows may not be applicable to a specific request. After examining the computer's answer, we may conclude that the computer has had sufficient time to model the data and has produced a reliable answer. However, we may also decide that a longer processing time or parameter adjustment may be necessary to extract all the identification information.

3 Request Retrieval

To view our answers, we have to access our requests by connecting to the database. This action is described in the Getting Started Manual.

Once connected, to review an inferred genotype for a specific question, we use the **Database ▸ Find** menu option to open the *Find Request* window (Figure 2).

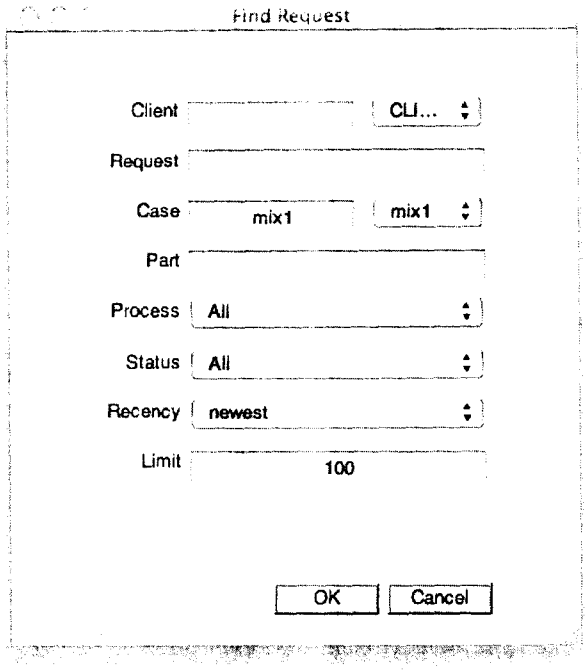


Figure 2. *Find Request window.* Using Find Request to search the database.

It is often helpful to limit our search results to a specific case. For example, we can select a case from the *Case* drop down menu to limit our search results to requests included in that specific case folder only. A more detailed description of the search fields is located in the **Appendix ▸ Advanced Searching**.

A list of the requests that meet our search criteria appears in the *Select* window (Figure 3). From here, we can choose a specific request to review by checking its box and clicking OK. Our search results are saved in the *Select* window until we conduct another search and will be available later to select a different request from the same query. To access the search results, we can use the **Database ▸ Select** menu option.

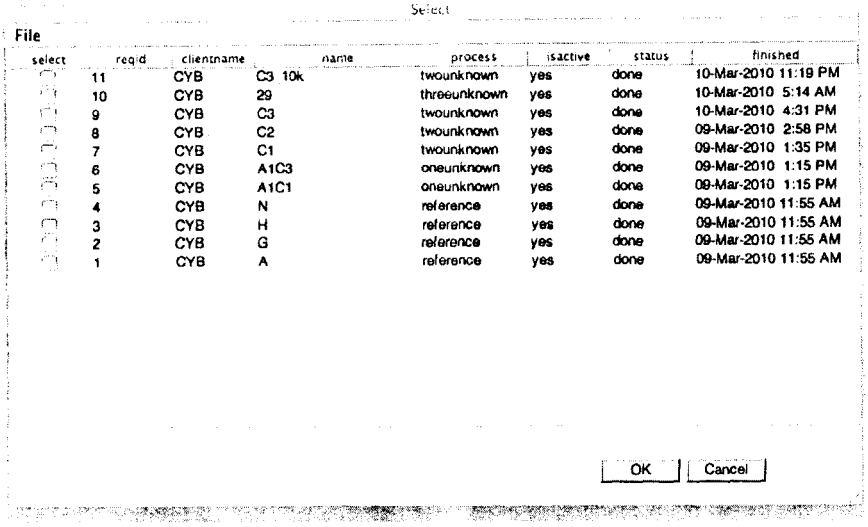


Figure 3. *Select window.* The Select window shows the list of requests.

4 Profile View

After selecting a request, the *Profile* window opens, showing the inferred genotypes and their associated probabilities as bar charts for each locus. The number of inferred profiles depends upon the number of contributors specified in the request processing.

4.1 Probability Distribution

In the *Profile* window, each contributor's genotype probability distribution is displayed as a different set of colored bars. One or more genotypes ("allele pairs") are shown at each locus. The bars represent how likely that genotype is, given the DNA evidence. The genotype probability distribution is calculated by modeling peak events and other effecting variables.

To review the inferred genotypes, we can view the probability distribution by individual loci and by contributor. For a particular locus of interest, we can zoom in by clicking the purple space below the locus label or by selecting from the **Locus** menu in the toolbar (Figure 4). When we are finished, the globe icon will return us to the complete profile.

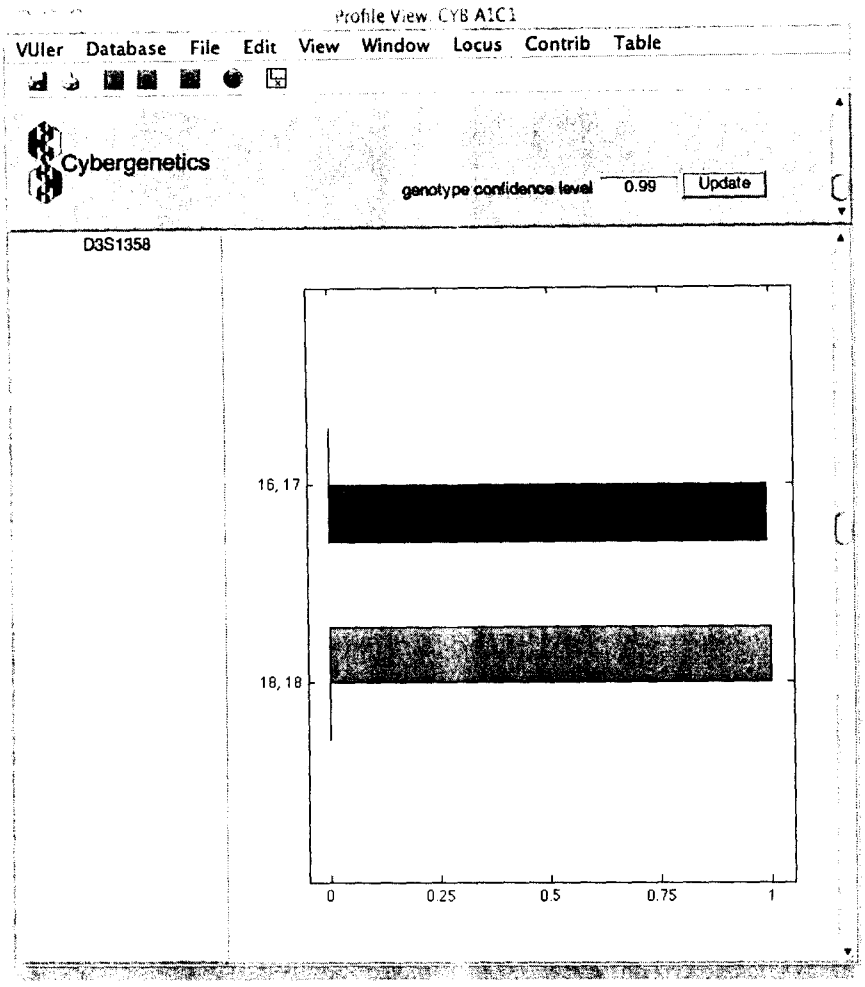


Figure 4. *Profile window.* Zoom in to D3S1358 using the Locus menu.

If we only want to view a single contributor, we can deselect or turn off the other inferred contributors by clicking their contributor number in the toolbar (Figure 5).

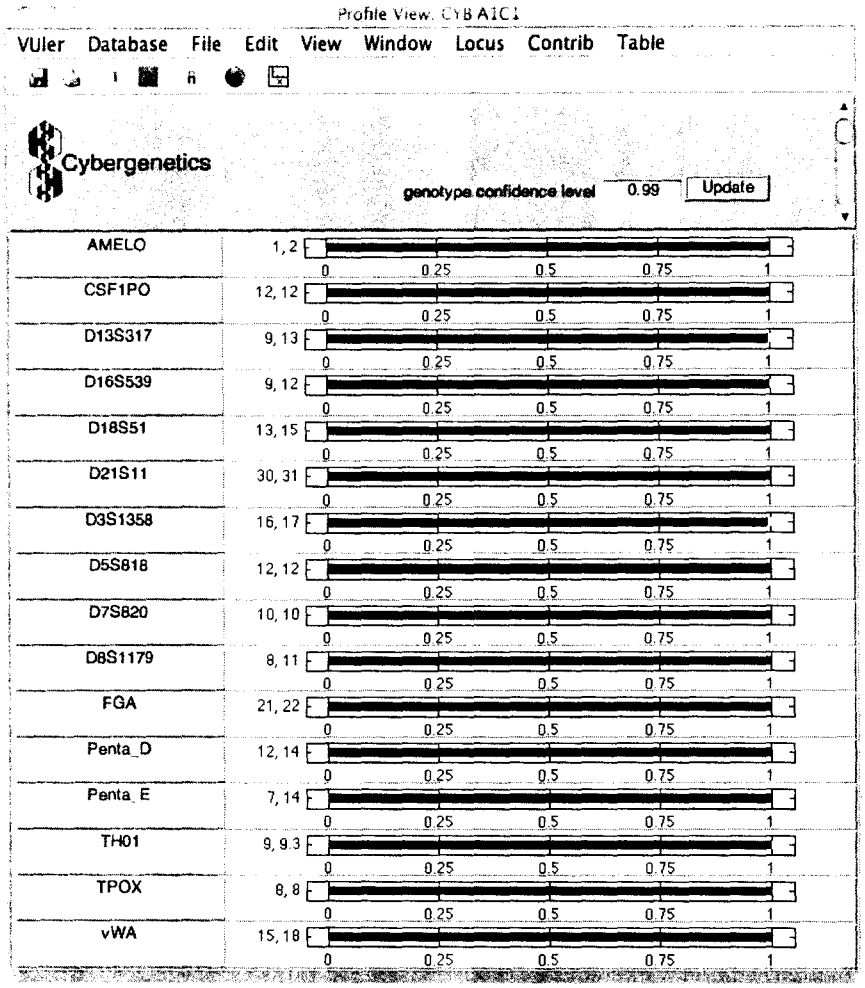


Figure 5. *Profile window.* The profile of one of the inferred contributors.

4.2 Tables

To view or document the genotype probability distribution as a table, we can select **Table ▸ Profile** from the toolbar. This action opens a table containing a list of the genotypes with their inferred probabilities (Figure 6).

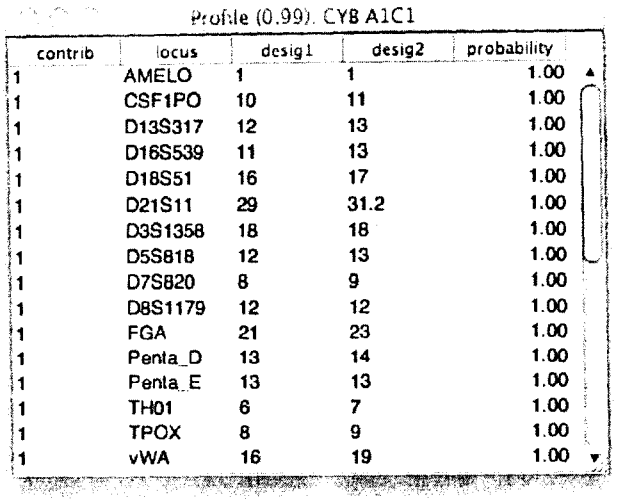


Figure 6. *Profile Table.* Shows the contributor, locus, allele pair, and probability.

Similarly, we can view an allele table by selecting **Table ▸ Allele** (Figure 7). We can set the confidence level for these tables by updating the "genotype confidence level" field in the upper right corner of the Profile window. When the table opens, we can mark the obligate alleles by selecting **Obligate ▸ On.** In an uncertain profile, this action will designate the obligate alleles with a "+" sign.

The Allele table can be exported as a text file or in the Common Message Format (CMF) for CODIS. More information on CODIS exports can be found in the **Appendix ▸ Tables**.

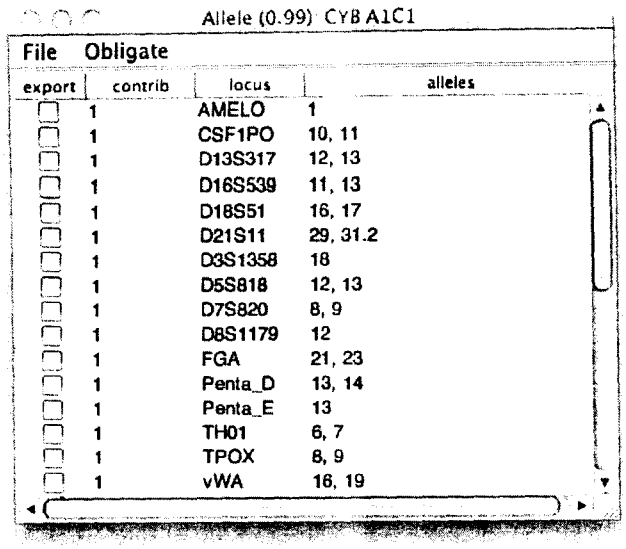


Figure 7. *Allele Table.* Contains the contributor, locus, and alleles and an option to export alleles.

Tables can be copied and pasted into a spreadsheet program. Additional information for documenting and printing tables is located in the **Appendix ▸ Tables**.

5 Mixture View

The mixture weight is a property of the DNA template, so it is shared across all locus PCR experiments for a sample. We can examine the inferred mixture weight by choosing **Window ▸ Mixture**. In the *Mixture* window, the weight of each contributor is expressed as a probability distribution, or histogram (Figure 8).

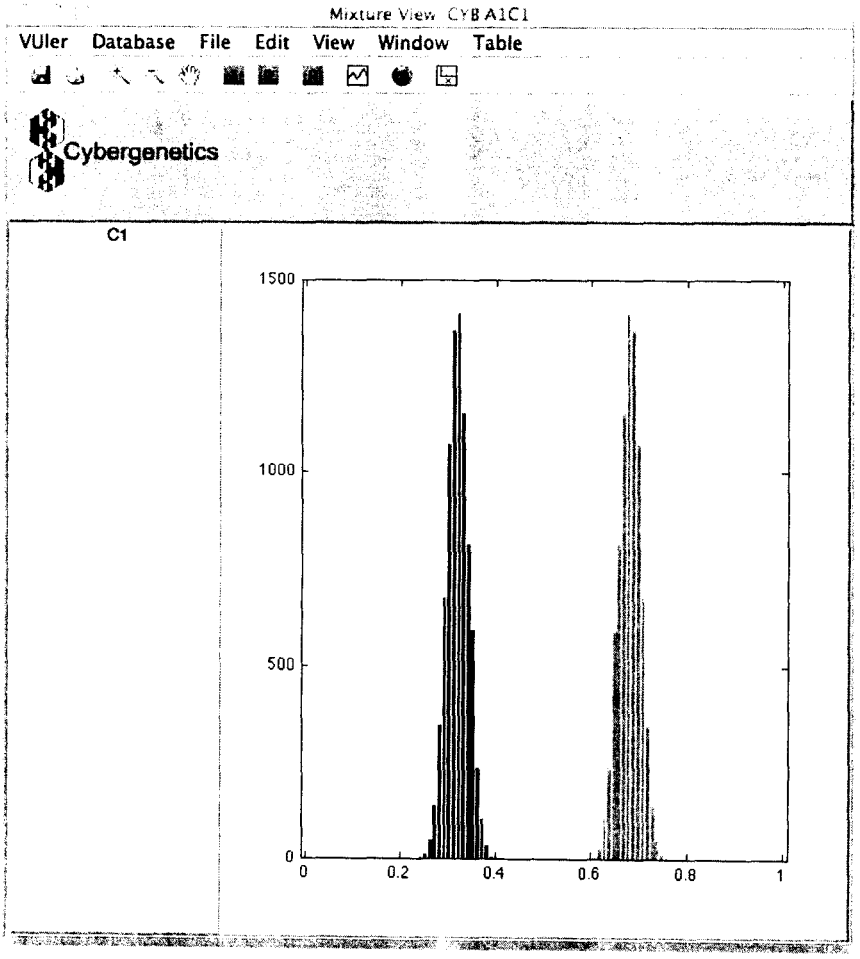


Figure 8. *Mixture window*. Histogram view.

5.1 Weight Histogram

The mixture window displays the mixture weight distribution for each contributor. The shape of the histogram gives us information about the inferred mixture weight. An ideal histogram should have a bell shape.

5.2 History View

To help assess the quality of the computer's variable sampling, we can view the graphed *History* of the computer's sampling of mixture weights, known as a Markov chain (Figure 9). By clicking on the graph icon, we can switch between the *Histogram* and *History* view.

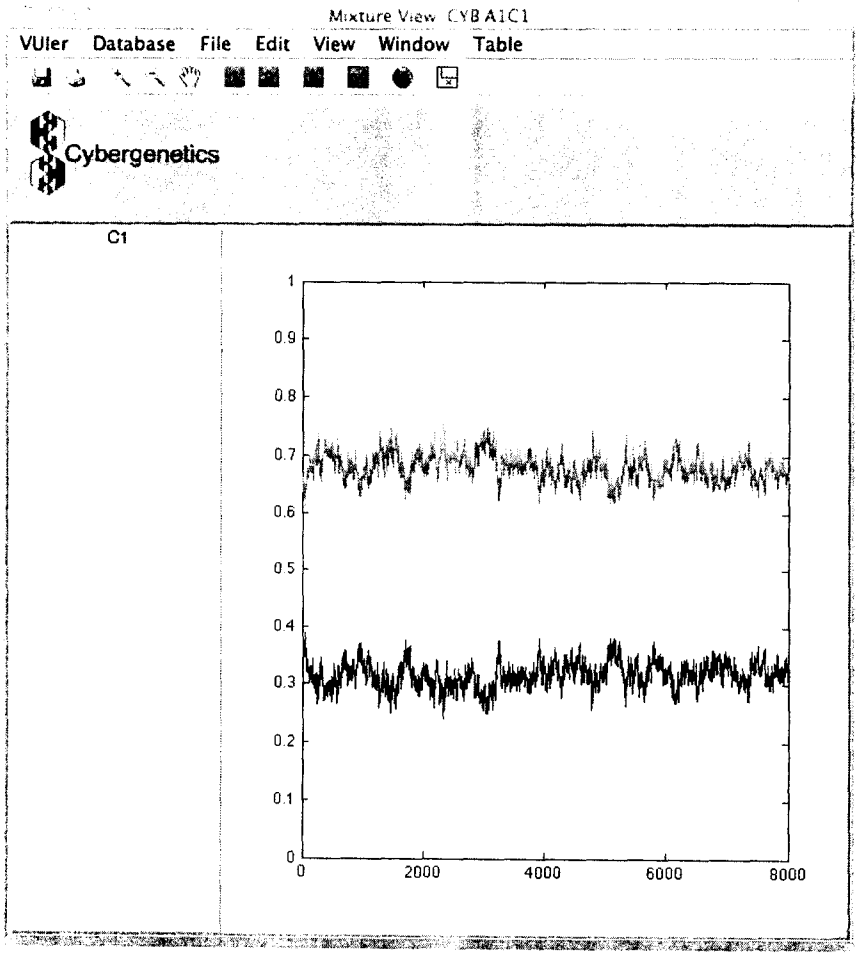


Figure 9. *Mixture window.* History view.

The weights modeled for each contributor are recorded and should settle on an answer by the end of the cycling process. This settling, or convergence, is represented by a *Convergence* statistic, which is described in the section below. If the chains have not settled by the end of cycling, have not moved at any point, or the convergence is over 1.20, then the computer has not sufficiently sampled the data to produce a reliable statistic. In these cases, it is necessary to reset the request with longer or advanced Cycle Settings. The settings are described in detail in the Request Manual. Ways to reset requests are described in the **Appendix ▸ Troubleshooting** section of this manual.

5.3 Tables

Several tables that correspond with the visual information within the Mixture window are available, giving us access to the numerical data. Any of the tables in the Mixture window can be copied and saved in a spreadsheet program. The **Appendix ▸ Tables** contains more information on documenting and printing tables.

Weight and Variance

The *Weight* and *Variance* tables can be accessed from the **Table** menu in the toolbar. The *Weight* table displays the mean of the histogram, which represents the inferred mixture weight per contributor. The *Variance* table shows additional information such as the standard deviation, which represents the variation across the observed locus experiments. A low standard deviation means that the mixture weight is roughly the same across the loci.

Convergence

The convergence statistic is documented under the **Table** menu in the toolbar. Ideally, a convergence score should be less than 1.20. More information on convergence is provided in the **Appendix ▸ Troubleshooting**.

6 Match View

The comparison of a reference to an inferred genotype is the last step in the computer processing. A comparison of two genotypes determines the possible association between them. When a match is present, its strength is described using a likelihood ratio (LR), characterizing the rarity of the match relative to a relevant reference population. The commonly used DNA statistics (random match probability, CPI, CLR) are all likelihood ratios.

The match strength between the inferred genotypes and the reference is the gain in information we see after examining the evidence data. If the comparison of the known profiles to an inferred genotype did not produce a positive likelihood ratio, the Match window will not be available for review.

6.1 Strength View

The results of comparisons with positive LRs are presented in the *Match* window, which is located in **Window ▸ Match**. The pop-out menu lists each contributor and any matching references. We can select a match (displayed as the log(LR) value, the evidence item, and the reference it matches) to open the Match window. The match we are viewing is indicated at the top of the window (Figure 10).

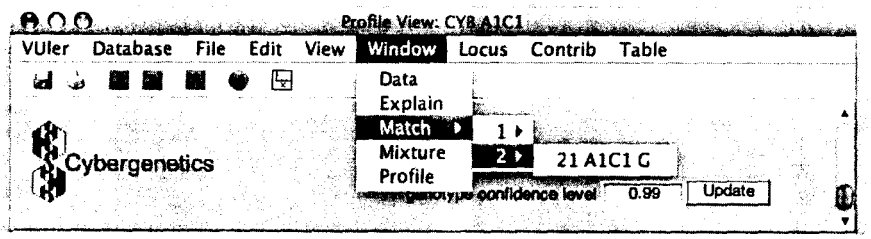


Figure 10. *Match window.* Selecting a match for review.

The Match window opens in Strength view, showing the match strength contributed by each locus (Figure 11). The match strength is displayed in log units, represented here as black bars. Greater identification information produces a larger LR. A LR greater than 1 at an individual locus, indicated by a black bar to the right, contributes positively to the overall match strength. A LR less than 1 produces a leftward red bar and subtracts from the overall match strength. The LR calculations in the Match View are performed using the CYB multiethnic population database. This population is for investigative purposes only; for LR calculations using specific ethic groups and population substructure, use the Report Module.

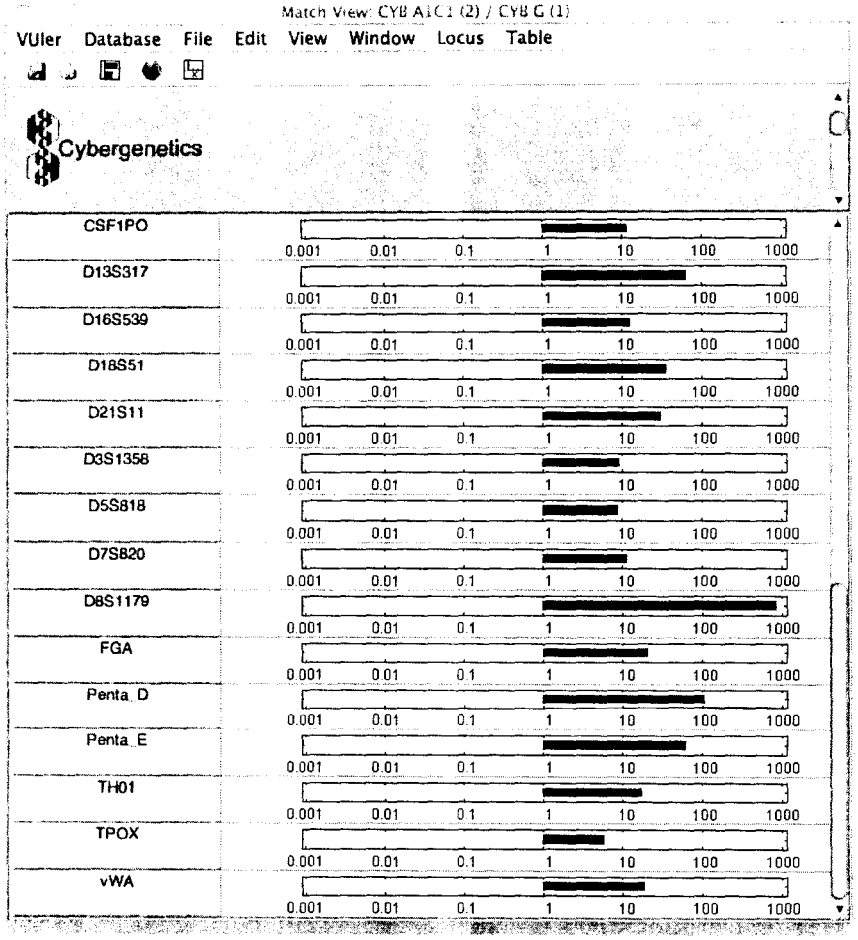


Figure 11. *Match window.* Strength view.

6.2 Genotype View

We can switch to Genotype view to see a direct comparison of the known reference genotype to the evidence's matching genotype probabilities by clicking the bar graph icon in the upper left corner (Figure 12). The black bars represent the probability of the known reference genotype and is usually at 100%. We can compare the evidence's matching genotype probabilities (gray bars) to the known reference genotype.

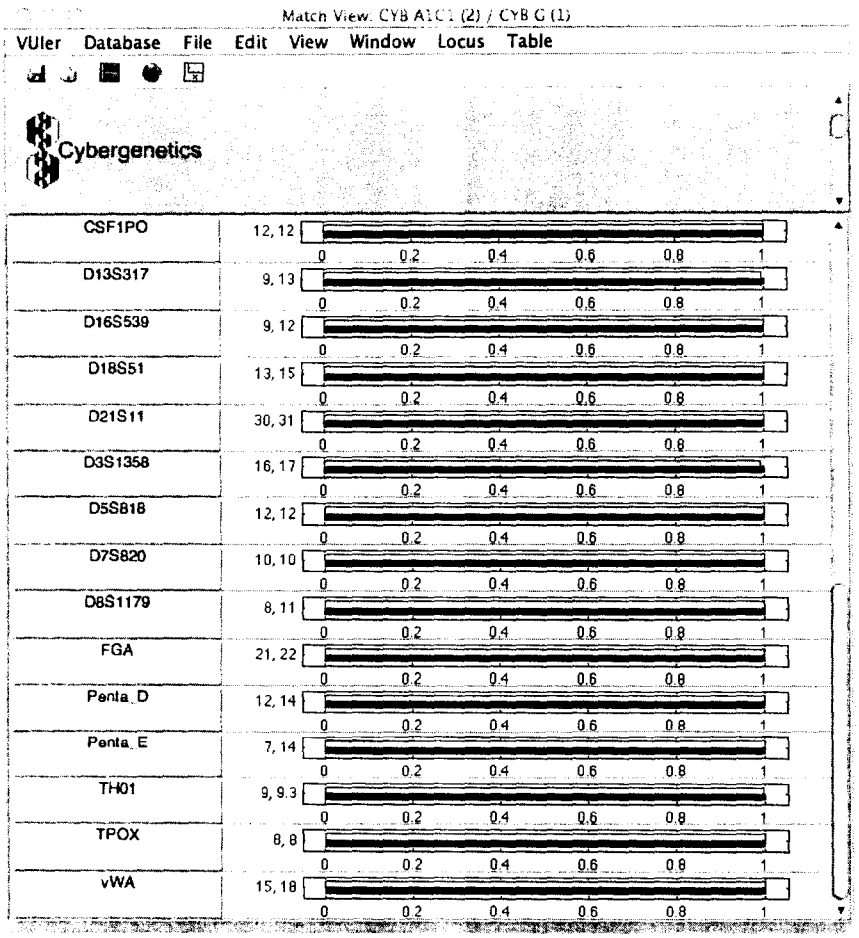


Figure 12. *Match window.* Genotype view.

6.3 Tables

Several tables that correspond with the visual information within the Match window are available, giving us access to the numerical data. Any of the tables in the Match window can be copied and saved in a spreadsheet program. The **Appendix ▸ Tables** contains more information on documenting and printing tables.

Strength

The strength per locus we saw visually when first opening the match window is represented in a table located under **Table ▸ Strength** (Figure 13). The "likelihood ratio" column shows the exact amount of strength contributed by each locus. We can multiply LRs for the individual loci to obtain the combined match strength. The "information" column contains the log of the likelihood ratio. This column can be summed to obtain the overall log(LR).

Strength: CYB A1C1 (2) / CYB G (1)

locus	likelihood ratio	information
CSF1PO	11.35	1.06
D13S317	63.24	1.80
D16S539	12.16	1.09
D18S51	35.40	1.55
D21S11	29.44	1.47
D3S1358	8.93	0.95
D5S818	8.61	0.94
D7S820	11.02	1.04
D8S1179	831.76	2.92
FGA	20.18	1.31
Penta_D	103.75	2.02
Penta_E	60.39	1.78
TH01	17.22	1.24
TPOX	5.79	0.76
vWA	18.49	1.27

Figure 13. *Strength Table.* Contains the likelihood ratio and information.

Match

To see the exact match strength, we can view the match table by selecting **Table ▸ Match** from the menu bar (Figure 14). The joint likelihood ratio for each contributor is listed in this table. The number of loci where the inferred genotype and the known profile match is recorded as a hit and the number of loci without a match, a miss.

Match. CYB A1C1 (2) / CYB G (1)

clientA	reqA	contribA	clientB	reqB	contribB	likelihood ratio	hit	miss
CYB	A1C1	1 CYB		A		1 10^(20.58)	15	0
CYB	A1C1	2 CYB		G		1 10^(21.18)	15	0

Figure 14. *Match Table.* Summary of the match results.

Profile

A complete breakdown of the matching genotype probabilities for the inferred genotype and the known genotype are listed in the Profile table. We can open this table by selecting **Table ▸ Profile** from the toolbar (Figure 15). This table contains information that we saw visually in the Genotype view. A genotype frequency using the Cybergenetics population database is reported here as a reference.

Profile: CYB A1C1 (2) / CYB G (1)

locus	desig1	desig2	probA	probB	probAB	freq
AMELO	1	2	1	1	1	0.49
CSF1PO	12	12	1	1	1	0.09
D13S317	9	13	0.9940	1	0.9941	0.02
D16S539	9	12	0.9980	1	0.9980	0.08
D18S51	13	15	1	1	1	0.03
D21S11	30	31	1	1	1	0.03
D3S1358	16	17	0.9910	1	0.9914	0.11
D5S818	12	12	0.9990	1	0.9989	0.12
D7S820	10	10	1	1	1	0.09
D8S1179	8	11	1	1	1	0.00
FGA	21	22	1	1	1	0.05
Penta_D	12	14	1	1	1	0.01
Penta_E	7	14	0.9990	1	0.9990	0.02
TH01	9	9.3	0.9970	1	0.9969	0.06
TPOX	8	8	1	1	1	0.17
vWA	15	18	1	1	1	0.05

Figure 15. *Profile Table.* Contains the matching allele pairs and corresponding information.

7 Data View

After reviewing the other windows in the Review Module, we may find a particular locus of interest and want to examine the original electropherogram. At any point in the Review process, the STR data is available under the **Window ▸ Data** option.

7.1 Electropherogram View

The format of the *Data* window is similar to the data views in the Data and Request modules (Figure 16). The navigation of this window is described fully in the Request manual. An info button under the sample name allows us to view the *Lane window* (as described in the Data manual) and view or add notes about the data.

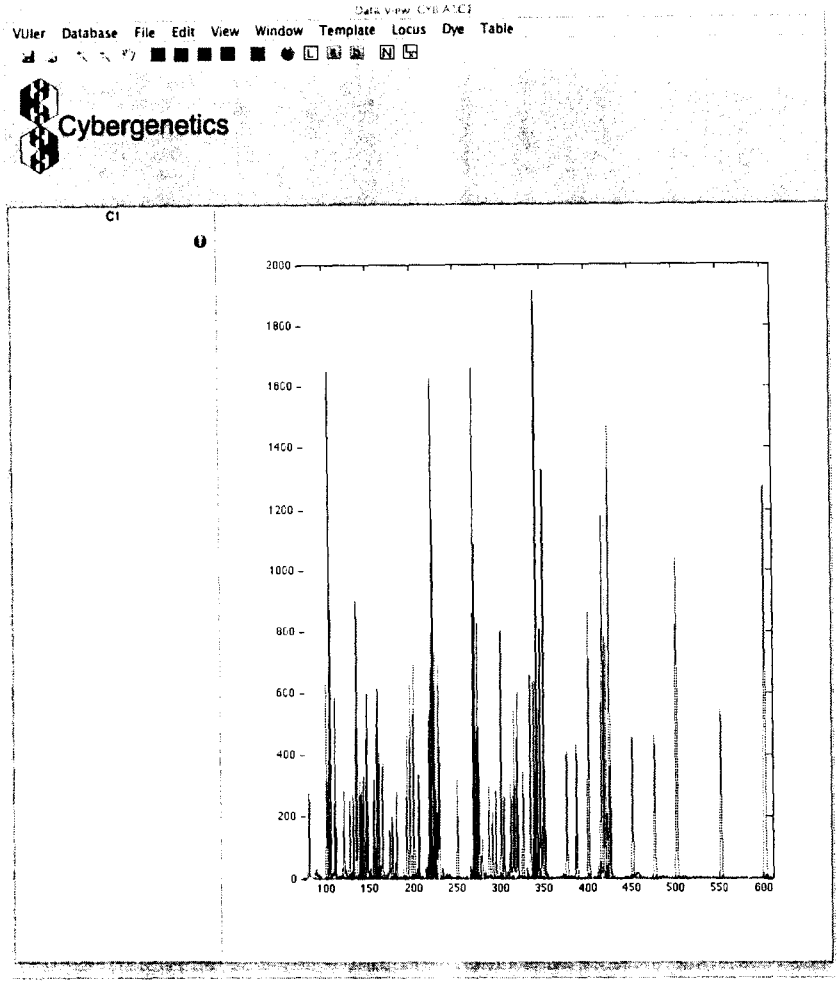


Figure 16. *Data window*. Electropherogram data.

The Data view is useful in assessing the quality of the computer's inference. Here, we can review the data to see if we asked the appropriate interpretation requests. The data can usually explain any sources of ambiguity. After reviewing the data, we may want to request reprocessing with a different number of contributors, a victim reference, or consider setting degradation, off ladder, or longer cycling parameters.

Before determining if resetting a request is necessary, it may be helpful to challenge the inferred genotype in the Explain window (see Explain View). Options for resetting requests are described in the **Appendix ▸ Troubleshooting**.

8 Explain View

The *Explain* window is a tool that allows us to challenge and assess the computer's inferred genotype probability distribution and mixture weight. The Explain window shows us how the computer models different genotype and mixture weight combinations. The fit of the model against the original peak data determines the probability of the combination, or how likely it is based on the data.

To open the *Explain* window, we select **Window ▸ Explain** from the toolbar.

8.1 Viewing Options

Applicable Icons:

 Display Genotype

 Display Pattern

 Display Deviation

 Composite View

In this window, we are given several viewing options. We can navigate between views by clicking the *Display Genotype*, *Display Pattern* and *Display Deviation* icons in the tool bar. To display the three views together, we can select the *Display Composite*. Just as in other windows, we can concentrate on a single locus by using the **Locus** menu.

Genotype

When we first open the *Explain* window, the *Genotype* view is displayed (Figure 17). The Genotype view visually shows the contribution from each DNA donor. The bars show how much each contributor donated to the peak height observed in the data. Each contributor is color-coded, corresponding to the Profile and Mixture windows.

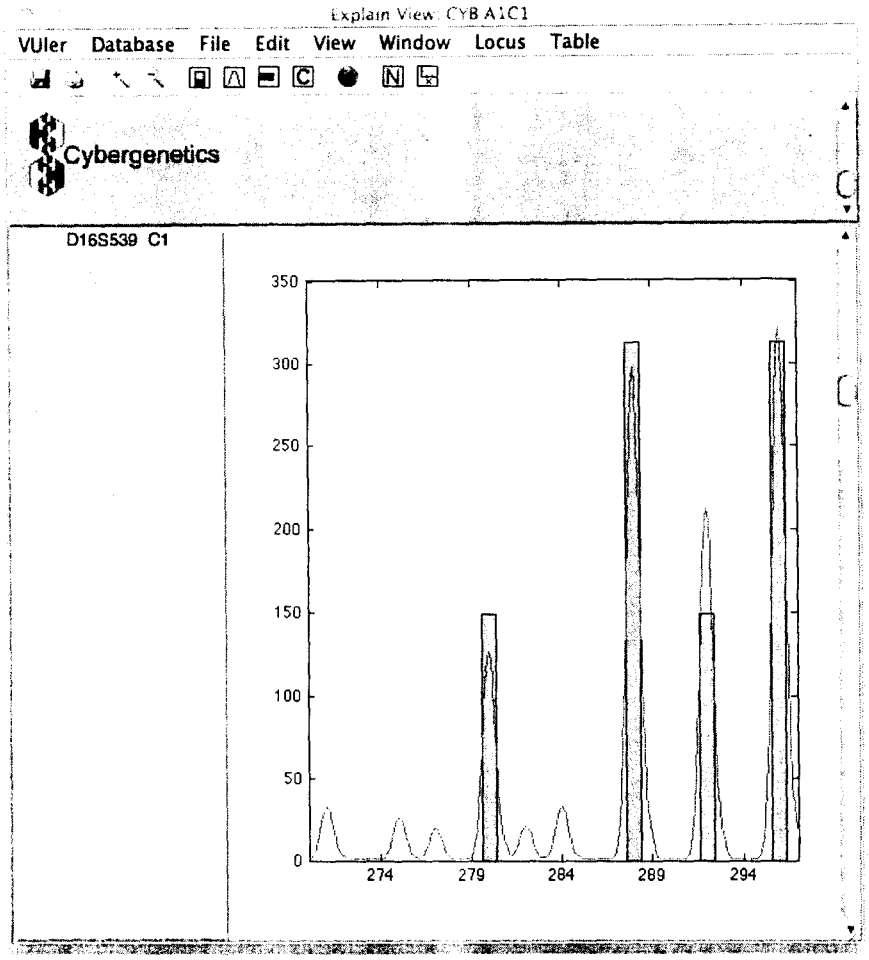


Figure 17. *Genotype view.* Relative DNA donor contribution.

Pattern

The Pattern display provides a model of a specific mixture weight and genotype combination (Figure 18). The gray peaks represent how the computer would expect the peak data to look based on the combination. This model is compared to the actual peak data to determine the genotype probability.

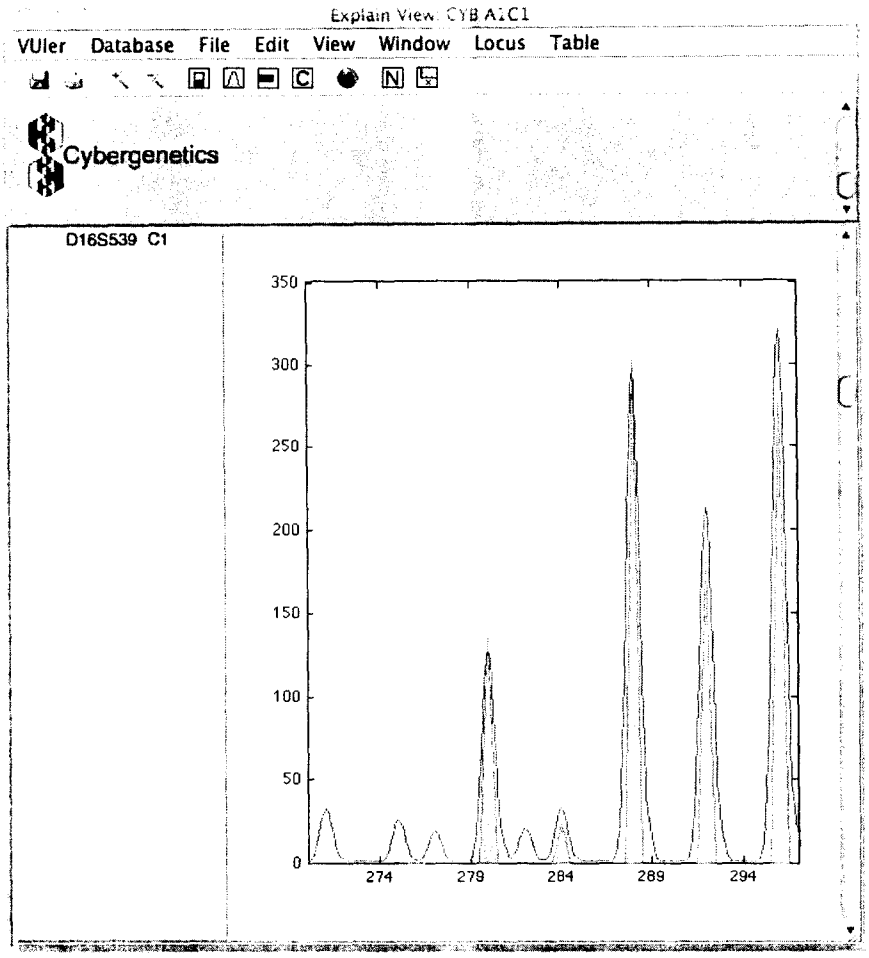


Figure 18. *Pattern view.* The modeled pattern against STR data.

Deviation

The Deviation display shows how well the gray-peak model fits the actual peak data (Figure 19). The black bar represents the model's deviation. A model that fits the peak data well will have a small deviation, indicated by a small black bar relative to the scale, and a higher genotype probability. A model that fits the data poorly will have a lower genotype probability and be represented by a larger black bar. Note that the scale on the x-axis will vary as the deviation greatly increases or decreases. The better a model fits the data, the higher the probability that specific genotype and mixture weight combination is given. We are looking for the model with the lowest overall deviation because it best fits the data.

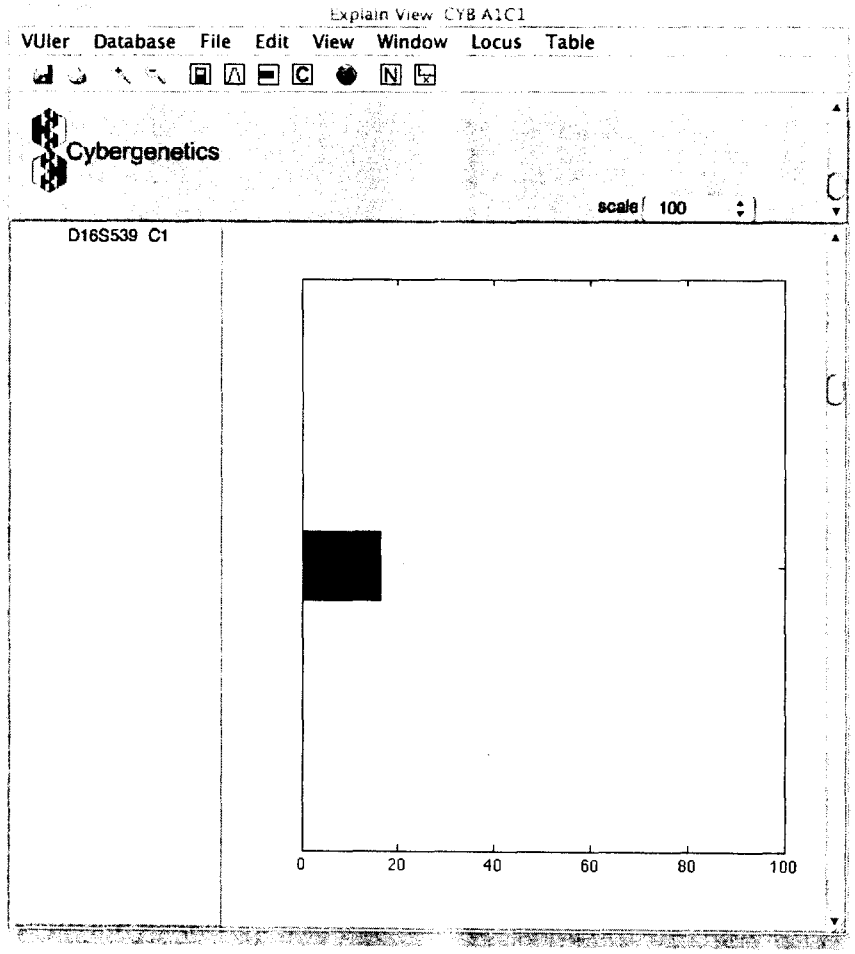


Figure 19. *Deviation view.* Displays the deviation between the model and data.

Composite

The Composite display assembles the Genotype, Pattern, and Deviation views into one screen (Figure 20). As we explore different genotype and mixture weight combinations, we can see how the change affects the model and how this new model affects the deviation.

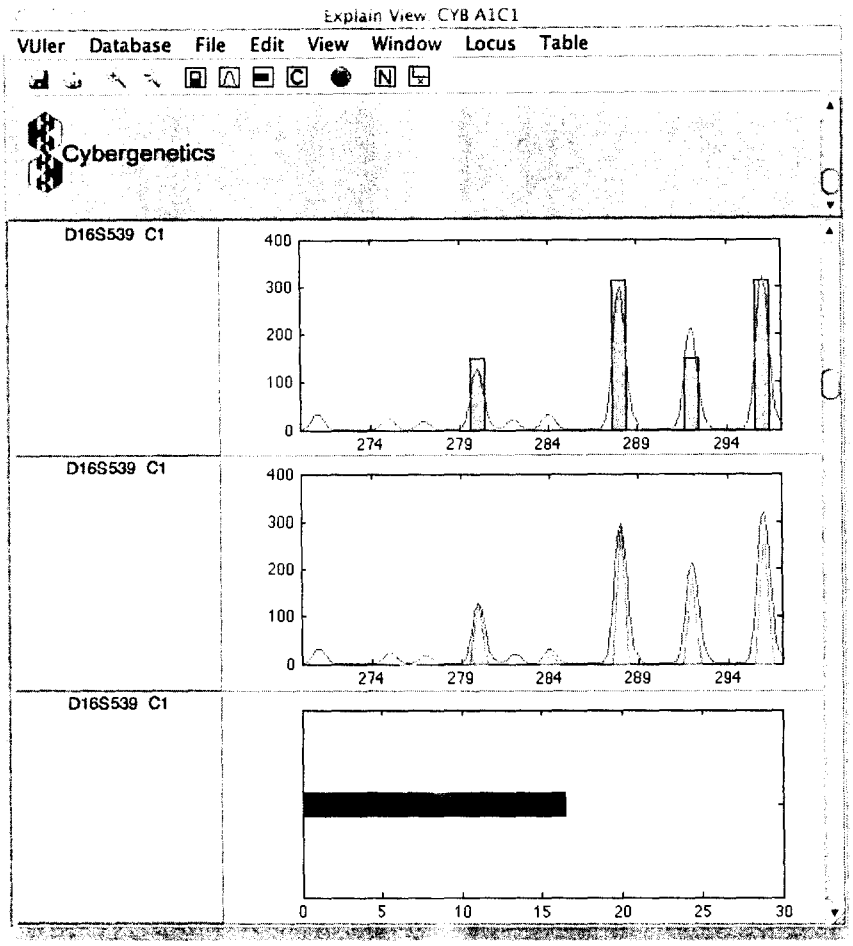


Figure 20. *Composite view.* Displays Genotype, Pattern, and Deviation in single window.

8.2 Exploring Combinations

In looking at the data, we may see a locus we wish to investigate further. Maybe we see another possible allele pair that we feel may fit the data as well or better than

those indicated by the computer. The Explain window gives us the opportunity to explore different combinations of genotype and mixture weight possibilities and see how a model formed with those values fits the data.

To change what genotype or mixture weight is displayed, we go to **Window ▸ Genotype** or **Window ▸ Weight** in the Explain window (Figure 21). This action will open a window where we can adjust the genotypes or mixture weights that are currently being modeled. After we have selected a new combination to model, we select **Update.**

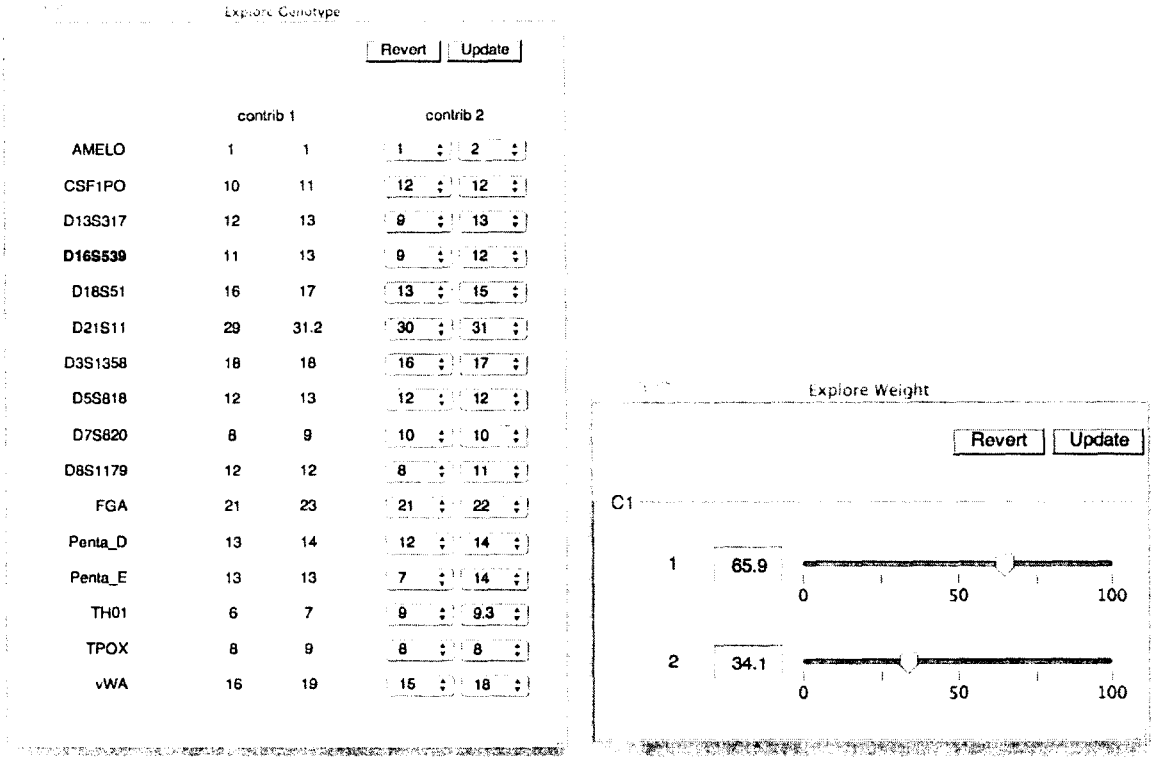


Figure 21. *Explore Dialogs.* Choosing possible genotypes (left) and mixture weights (right).

The **Revert** option returns us to the initial combination. It is important to note that this genotype and mixture weight is the last combination the computer sampled. It is not necessarily the matching or most probable combination.

It is often useful to try some of the genotype possibilities listed in the Profile view for the inferred profile. We can use this tool to understand why the computer gave some genotypes higher probabilities than others.

9 Request Information

While assessing the results from an interpretation request, we can make notes or view the processing parameters. This information is located in the toolbar under **File ▸ Get Info**.

9.1 Setting

The Setting tab includes the Burn in and Read out parameters. When the Request Information window opens, this is the first tab we see (Figure 22). It is useful to refer to this menu when determining if a request requires additional processing.

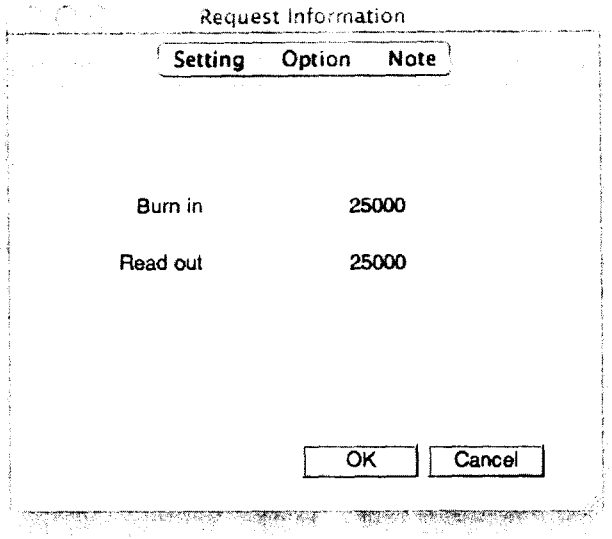


Figure 22. *Setting tab.* Parameter information for the request.

9.2 Option

The Option tab displays all of the complex mixture options that were selected in Request. These options include off ladder, degraded mixture, server logging, sort

contributors and peak scale. This information can be useful to note in our formal documentation, as well as determining the need for any additional processing.

9.3 Notes

In addition to viewing any notes that were made in the Request module, notes from our assessment of the results can be recorded in the Notes tab (Figure 23).

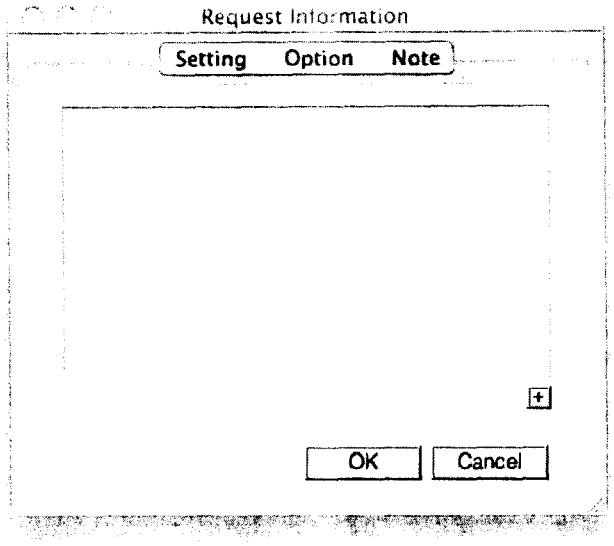


Figure 23. *Note tab.* The note tab allows us to record our observations from the Review process.

To add a note, we can click the + button in Note tab, the *Note Editor* window opens. Up to 10,000 characters of text can be added in a single note. Clicking "Apply" in the *Note Editor* window will permanently attach the note to the request. Like adding notes in Request, we can add as many additional notes as we wish.

The request results will become part of our case reports, to be ultimately used in court. We use the next module, Report, to make more formal documentation about our results. These reports will summarize the information we've looked at in the

Review Module, and also include specific population information and case assumptions.

10 Case Example

To continue with our case example from the Request Manual, we will go through a typical review of results.

The computer answers our interpretation questions by inferring genotypes and matching those genotypes to the references we provided. In Review, we will determine if the computer has had sufficient time to sample the request. Then, if our references matched, we will see who contributed to the DNA evidence.

It is important to remember that the more complex the question, the more time a review of the computer's solution may take.

10.1 Did the victim contribute to the DNA evidence?

We asked our first question of the DNA evidence assuming two unknown contributors in the mixture. For this request, the computer inferred the two contributors from the data, and compared the genotypes to the references to see if matches existed.

Two unknown request

To view the answers, we must first retrieve the request from the database. We use the **Database ▸ Find** menu option to open the Find Request window (Figure 24). To limit our search results to this specific case, we can select "mix1" from the *Case* drop down menu.

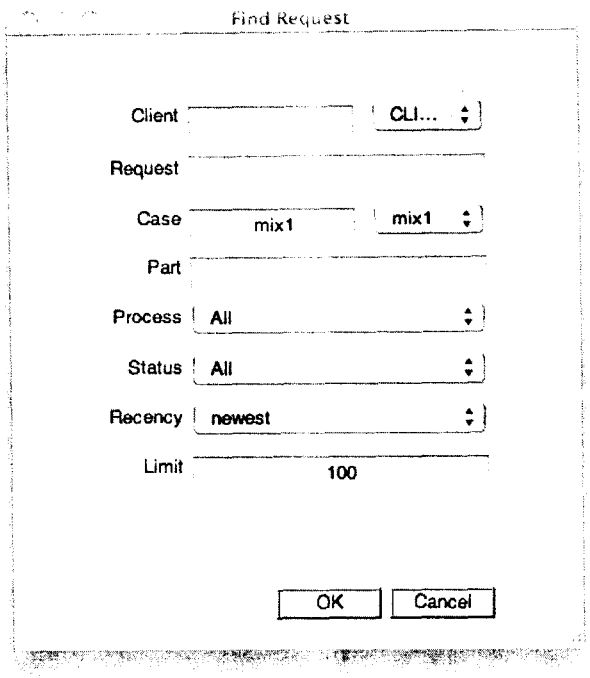


Figure 24. *Find Request window.* Using Find Request to search the database.

The list of questions we asked in the Request Module appears in the Select window (Figure 25). We choose C1 to view the two unknown contributors request results.

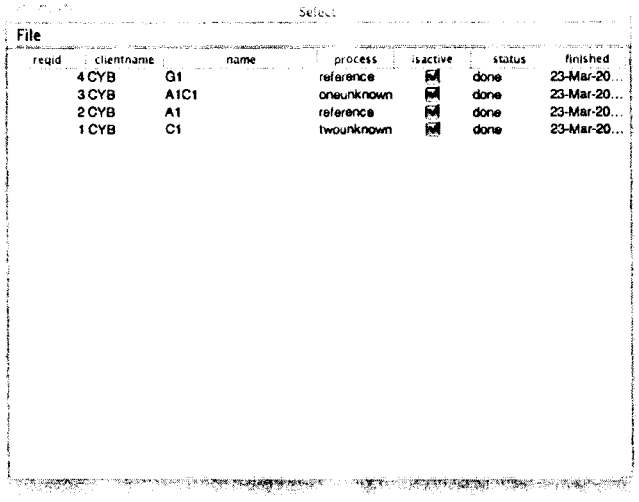


Figure 25. *Select window.* The Select window shows the list of requests.

Major contributor profile

The Profile window opens, showing the inferred genotypes and their probabilities as bar charts for each locus. One inferred genotype is given in blue, the other in orange. We will examine both contributors individually.

The computer inferred the profile of the first contributor as the major contributor (as we will confirm later) based on the STR data. We can hide the minor contributor's genotype and view only the major contributor by pressing the orange "2" in the toolbar. Now we can see only the blue genotype bars (Figure 26).

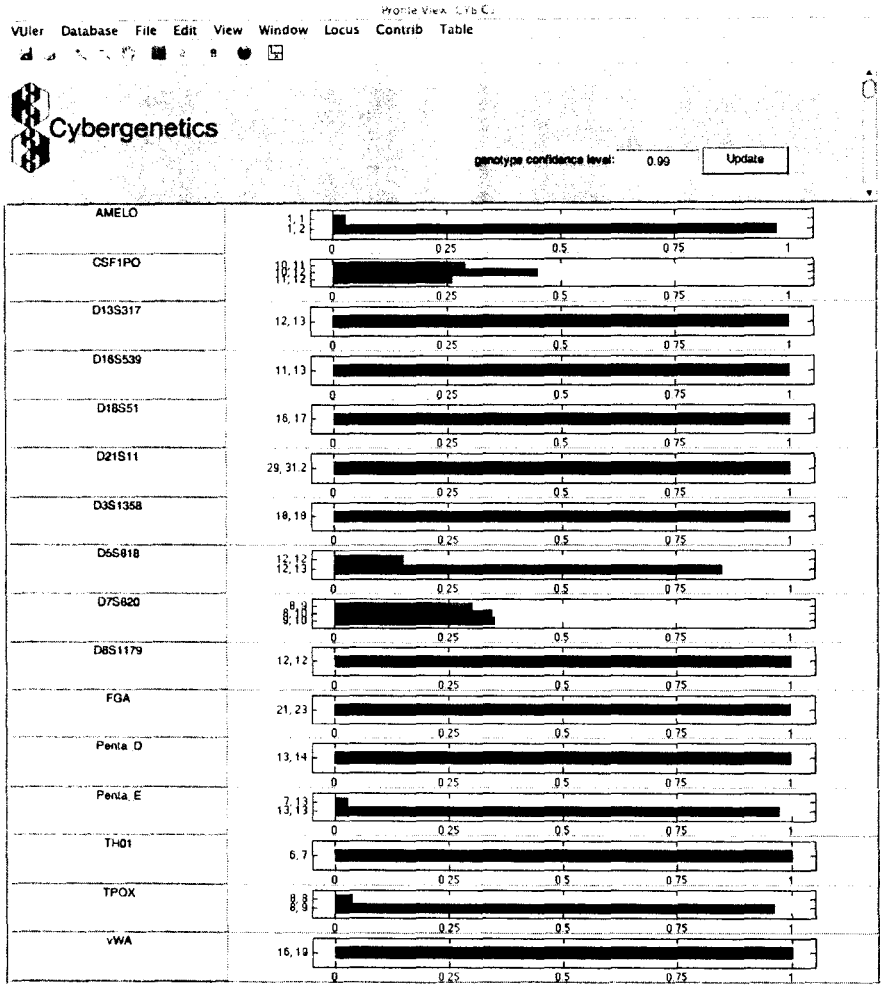


Figure 26. *Profile window.* The major contributor genotype in Profile.

There is ambiguity in this major contributor profile due to uncertainty present in the DNA evidence data. For example, locus CSF1PO has three genotypes possibilities, all with different probabilities. Although a single, unique profile was not obtained, the inferred genotype is highly informative.

Major contributor mixture weight

We can examine the mixture weight for this piece of DNA evidence by choosing **Window ▸ Mixture**. This histogram is narrow, indicating that the mixture weight is predominately consistent across all of the loci (Figure 27). The first contributor's probability distribution (blue) peak falls around 0.70, indicating its average mixture weight for the template to be 70%.

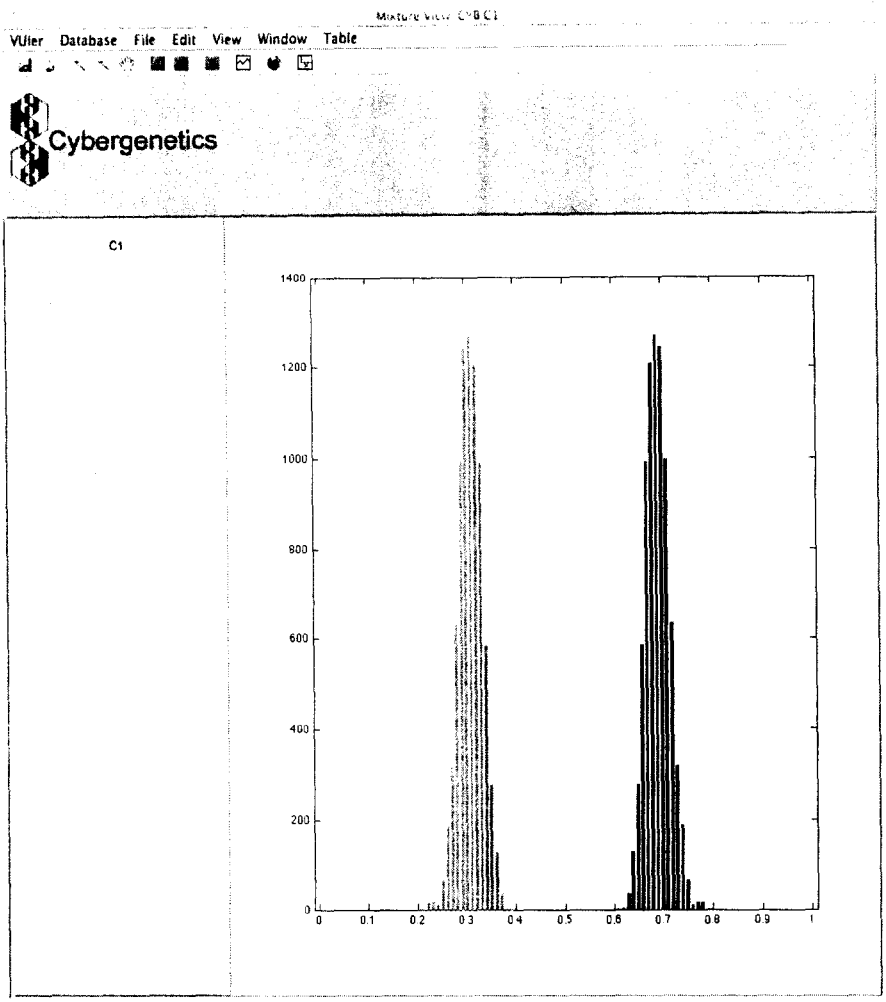


Figure 27. *Mixture window.* The mixture weights displayed as histograms.

We can confirm this finding by looking at the Data window, which is viewable by selecting **Window ▸ Data.** The data shows four peaks (two for each contributor) that fit with what we would expect from a 70:30 mixture (Figure 28).

The exact mixture weight of each contributor is in the Variance table, which we can view by selecting **Table ▸ Variance** in the Mixture window (Figure 29). The narrow histogram shows a small standard deviation value of 2.2%.

The Mixture window confirms that contributor 1 (blue) is the major contributor. Next we will see if the major contributor matches any references.

Major contributor match

We can see the match to contributor 1's genotype by choosing **Window ▸ Match ▸ 1 ▸ 19 C1 A1**. This indicates that contributor 1 of the evidence (item C1) matches the reference (item A1) with a LR of 19. We open the Match window, and see the likelihood ratio for each locus where the inferred major contributor matches victim reference A1 (Figure 30).

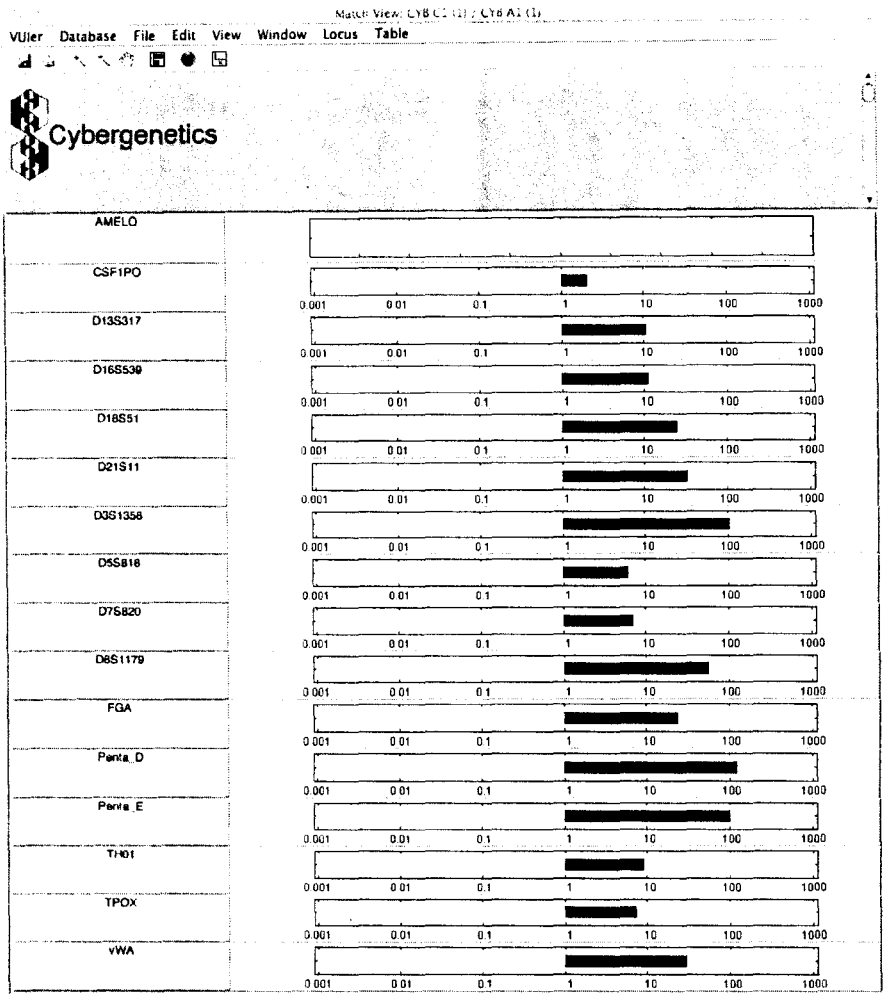


Figure 30. *The Match window.* The Match window showing the likelihood ratios.

To see the exact match strength, we can view the match table by selecting **Table ▸ Match** from the menu bar. Contributor 1 of evidence C1 matches the victim reference with a match strength of $10^{19.42}$, or 26.30 quintillion, relative to our CYB multi-ethnic population database. From this match information, we can conclude that the victim did contribute to the DNA evidence.

10.2 Did the suspect contribute to the DNA evidence?

Now that we have examined the major contributor, we will look at the minor contributor. The minor contributor in a case contributes less DNA to an evidence sample. The resulting STR information contains smaller peaks and can often fall

victim to PCR quality issues. When minor contributors have low mixture weights, the major contributor can often mask them, making individual alleles hard to distinguish.

Minor contributor profile

In the Profile window (Figure 31), we will focus on the minor contributor's genotype. To view only the minor contributor's profile, we want to hide the major contributor (victim) genotype. We do this by selecting the orange "2" button from the menu bar, and then turning off the blue "1" button. Now we can view the inferred genotypes for only the minor contributor. Many of the loci have genotype probabilities around 100%, and so we would expect the profile to be highly informative.

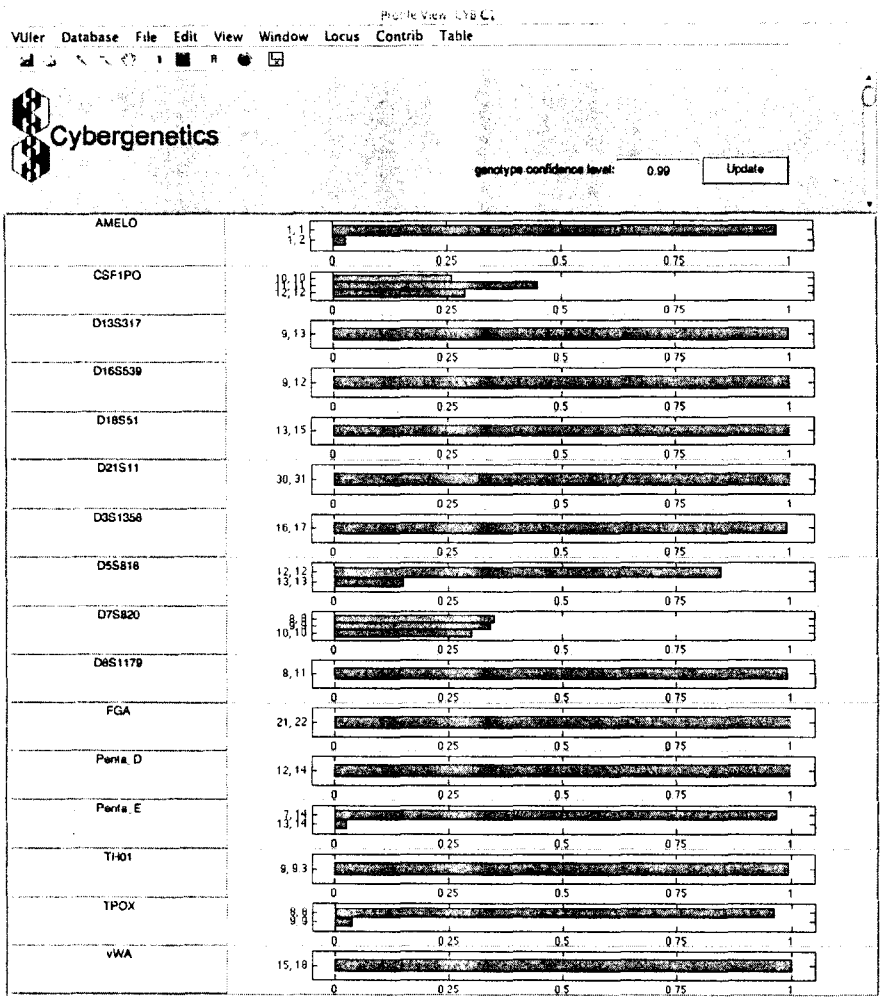


Figure 31. *The minor contributor in Profile view.*

There are several loci where only one genotype was found to be possible, with its probability at 1 (for example, TH01). There are other loci where there are several possible genotypes, each with its own probability. As with the major contributor, although a single, unique profile was not obtained, the inferred profile is highly informative for the minor contributor.

Minor contributor match

We can see the match results for the minor contributor by selecting **Window ▸ Match ▸ 2 ▸ 20 C1 G1,** which tells us that evidence item C1 matches reference G1

with an LR of 20. The match information can be viewed in the match window (Figure 32).

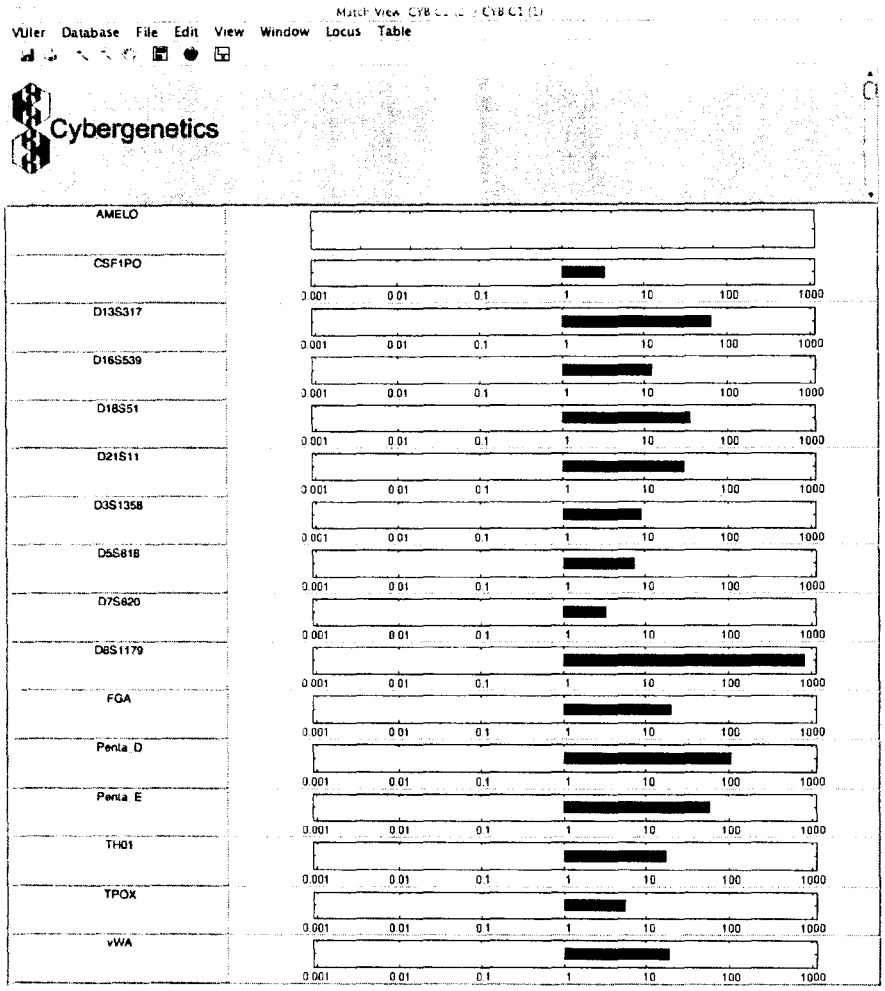


Figure 32. The *Match window* shows the likelihood ratios for the minor contributor.

The Match window shows a match between the minor contributor and the suspect profile G1 with a value of $10^{20.01}$, or 102 quintillion. We expect a strong match here because the inferred minor contributor profile is informative, and more information leads to higher match strength. Even the loci with multiple genotype possibilities add information to the match strength, and the cumulative result is seen in the table.

10.3 Reducing Uncertainty

Now that we have proven the victim is included in the evidence, we can use this additional information to increase the match strength by reducing uncertainty. We did this in the Request Module by including the victim reference with the evidence in a single, one unknown request. By providing this additional information in our question, we reduce the uncertainty in the inferred genotype, and "sharpen" the resulting unknown contributor profile.

One unknown request

We can view the answers to this new question by retrieving the request from the database. We use **Database ▸ Select** to open the Select window. When the Select window opens, we choose A1C1, the one unknown contributor request that includes the victim reference information.

Unknown contributor profile

The Profile window opens, showing the assumed victim genotype in gray and the inferred unknown contributor genotype in blue. We will focus on the inferred unknown contributor (Figure 33). We can hide the victim genotype by pressing the gray 1 in the toolbar.

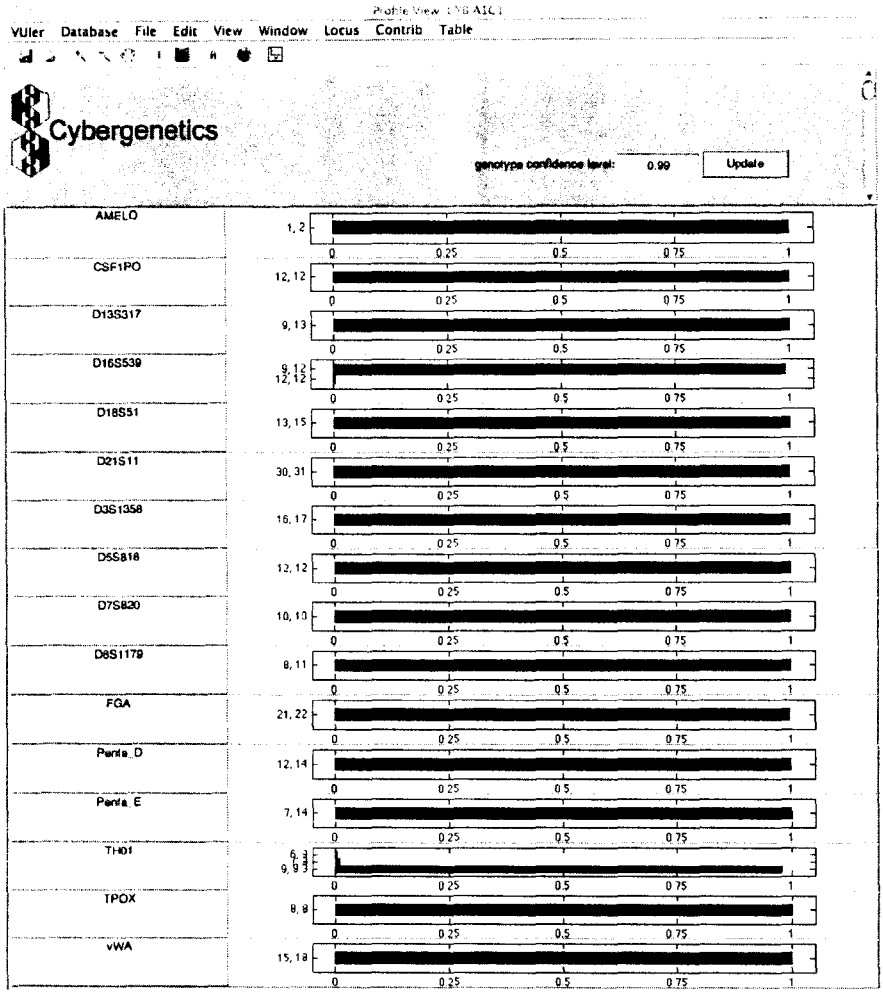


Figure 33. *The sharpened minor contributor genotype.*

For the unknown contributor, all but one locus has a single unique genotype, with a probability of 100%. Qualitatively, we can see that the genotype has sharpened compared to the previous two unknown request.

Unknown contributor match

We can view the match information for this contributor by selecting **Window ▸ Match ▸ 2 ▸ 21 AC1 G1**. The Match window opens, showing the likelihood ratios for

each locus (Figure 34). Note how the likelihood ratios (black bars) are greater than the minor contributor values we saw earlier for the two unknown request.

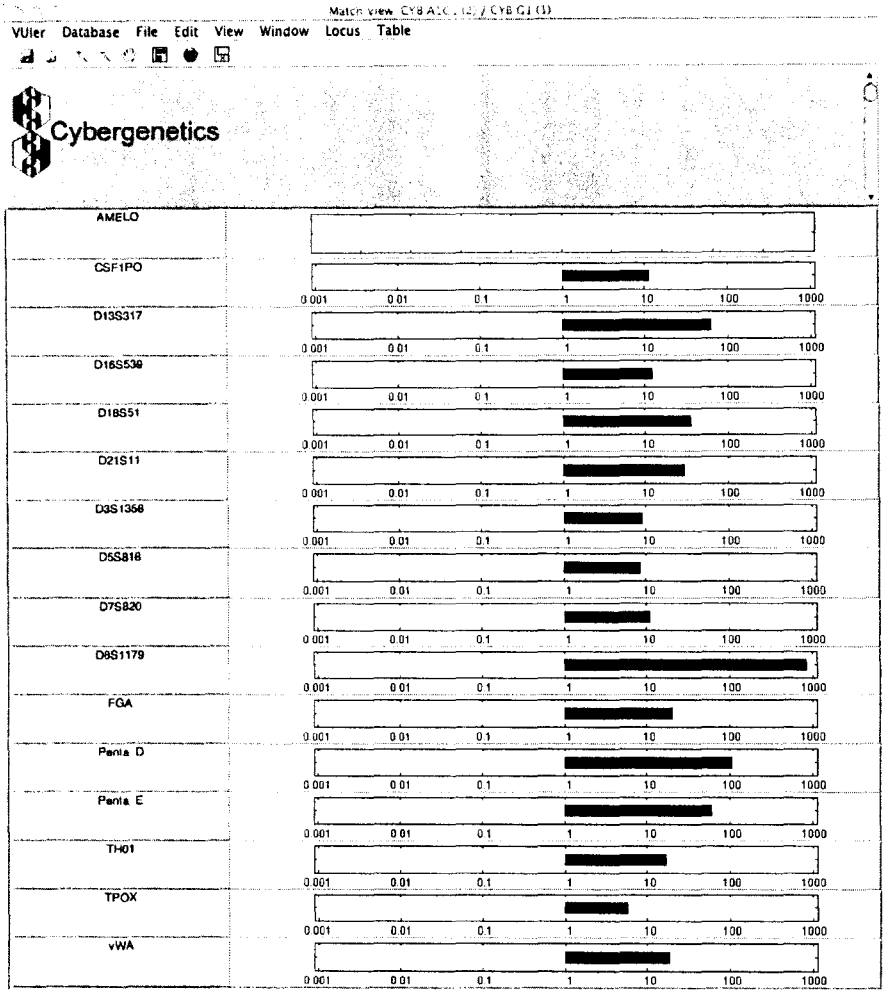


Figure 1. *The match information for the sharpened minor contributor.*

If we view the match table, we see that the inferred contributor profile matches the suspect with a strength of $10^{21.17}$ or 1.48 sextillion. By adding the victim reference information to the same DNA evidence, we sharpen the match to the suspect from $10^{20.01}$ to $10^{21.17}$.

10.4 Conclusion

We asked the system the question, "Who contributed to the DNA evidence?" Assuming 2 unknown contributors, the system inferred genotypes and matched them to references. We used Review to see that the victim and suspect contributed to the DNA evidence. We also saw the strength to which each contributor matched the references. By adding more information to our request (the victim reference), we sharpened the results by reducing uncertainty.

11 Appendix

11.1 Advanced Searching

This section provides a description of all the available options to limit search results. Some search fields may not be applicable to all labs. Search criteria are based on the user's personal preference and the specific task. It is not necessary to fill in each search field.

Each field can be used in isolation or in combination with other search fields to narrow a search. If no requests fulfill the search criteria, a blank list is returned. In this case, broadening search criteria or using wildcards (*) may be helpful. Wildcards match any characters before or after a search entry. For example, entering *2010* in a search field will return all requests with 2010 somewhere in their labeling.

Client

Some labs may have multiple clients set up on a database as a form of data organization. This field provides the option of selecting a Client from the drop down menu or typing in a specific client.

Request

This field searches for all requests with the entered request name. A user can type in an exact request name or use wildcards (*) to search the database of requests.

Case

Searching by *Case* will return all requests included in the case folder created in the Request Module. We can search by selecting a case from the drop down or by entering a case name. This field is the most commonly used filter.

Part

The *Part* is often associated with the role of the STR sample and is established when the request is created. Therefore, it is customizable and can differ between labs and users. For example, if only the suspect reference requests are wanted, the user can enter *suspect* in the Part search field if the requests were labeled as such in the Request module. Other common parts are *victim* and *elimination*.

Process

The *Process* determines how many genotypes the computer should infer. By default, the field is set to include all varieties of computer processing. A drop down menu allows us to limit a search to references, one unknowns, two unknowns, three unknowns or four unknowns.

Status

Searching by *Status* narrows results down by the current state of a request. By default, the field is set to include all possible states. A drop down menu allows us to limit a search to requests that are Done, Ready, Solving, Pending, or have No Data.

Recency

This field allows us to sort the search results by the age of the request. By default, the newest requests are listed first.

Limit

The number in this field establishes the maximum number of requests that will be listed in the *Select* window. If we are conducting a broad search, then we will want to expand the number so that our results are not cut off and the entire query is returned. This number is set to 100 by default.

11.2 Tables

We may want to save any of the tables in the Review Module as an electronic or hard copy. We can do this by selecting the cells from any of the tables and copying and pasting them into a spreadsheet program. The row headings cannot be copied; these must be manually typed into the spreadsheet.

LIMS & CODIS Export

Exporting a list of alleles as a text file can be useful for a Laboratory Information Management System (LIMS). We can select which contributor to export from the Allele table by checking the boxes to the left of the "contrib" column. To save the table as a text file, we select **File ▸ Export txt**. Once the file is saved, the list of alleles for each locus selected can be viewed in any spreadsheet program. If the obligate feature is enabled, these alleles will be designated with a "+" sign.

CODIS uses a Common Message Format (CMF) to facilitate the exchange of data between systems. The allele information for a contributor's inferred profile can be exported in this format by checking the corresponding export boxes. To save the table as a CODIS CMF, we select **File ▸ Export cmf**. This file can then be directly uploaded to CODIS. If the obligate feature is enabled, these alleles will be marked within the file.

11.3 Troubleshooting

Convergence

Cross matching

When we see a 50/50 mixture, it is often happens that the computer cannot completely separate the templates. In these instances, the inferred genotype profiles for each contributor will be almost identical, causing cross matching to occur. When cross matching occurs, both contributors will match to the same reference sample. This does not necessarily mean that the Markov chains have not converged; if the sample is truly a 50/50 mixture, this may be the correct answer. We could also try running the request longer to see if greater sampling time allows for full separation to occur.

Identifying convergence

It may sometimes be challenging to tell when a Markov chain is converged. Here, we will see several contrasting examples of properly and poorly converged Markov chains.

Proper convergence

When a sample has converged on the appropriate answer, we can visually see that the Markov chains have explored options and settled in on an answer (Figure 35).

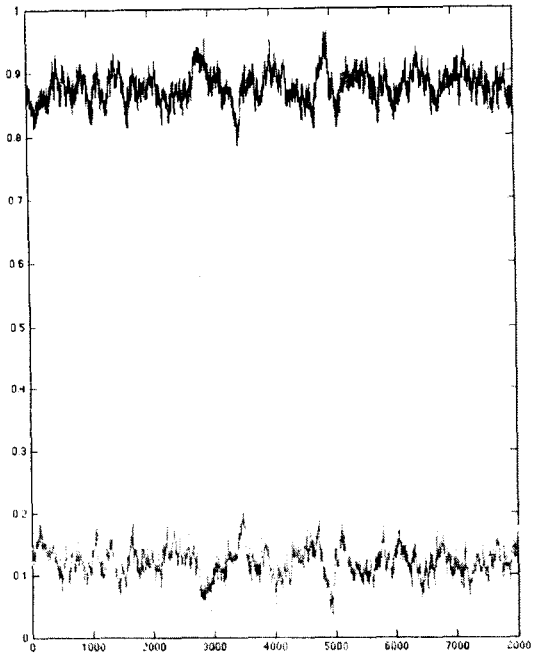


Figure 35. *Example of a converged Markov chain*

Poor convergence

Poor convergence can indicate the need for further action. Often, we can visually identify when a Markov chain needs more time for sampling. When this occurs, the Markov chains look as if they have wandered and never settled on an answer (Figure 36).

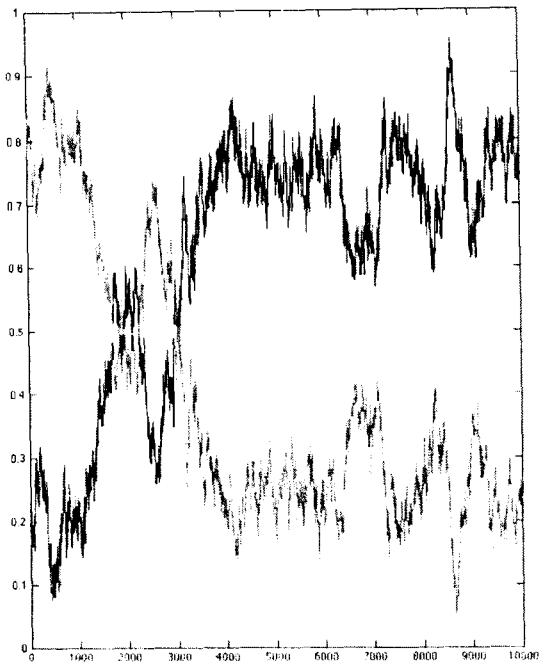


Figure 36. *Poor Convergence*. This chain needs more sampling time.

Here, the chain started to sample the right region, but needed a longer sampling time in order to converge on the correct answer.

It can also happen that the Markov chain gets stuck and never explores other options. We sometimes see this in a 50/50 or a 90/10 mixture. When this occurs, the Markov chains look like ropes; very thin and stationary (Figure 37).

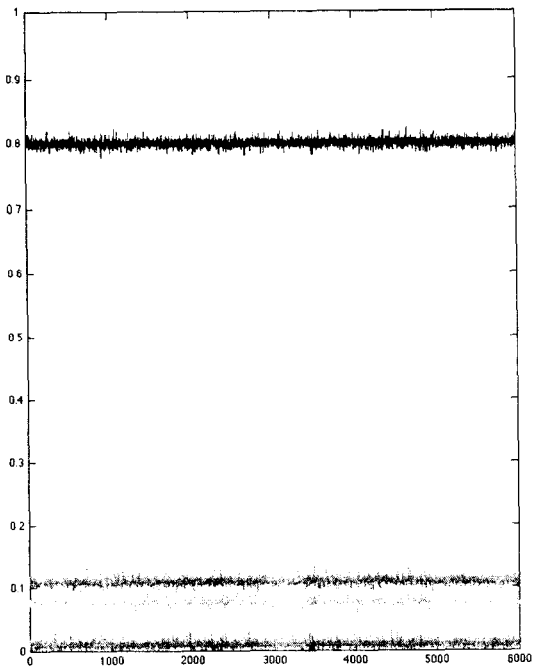


Figure 37. *Poor Convergence.* The chains got stuck and did not explore all options.

In a four-person mixture, we would expect much more sampling, but in Figure 37 the Markov chains appear to be stuck. When we see this rope-like chain, it is a clear indicator that the request needs to be reset.

Resetting a request

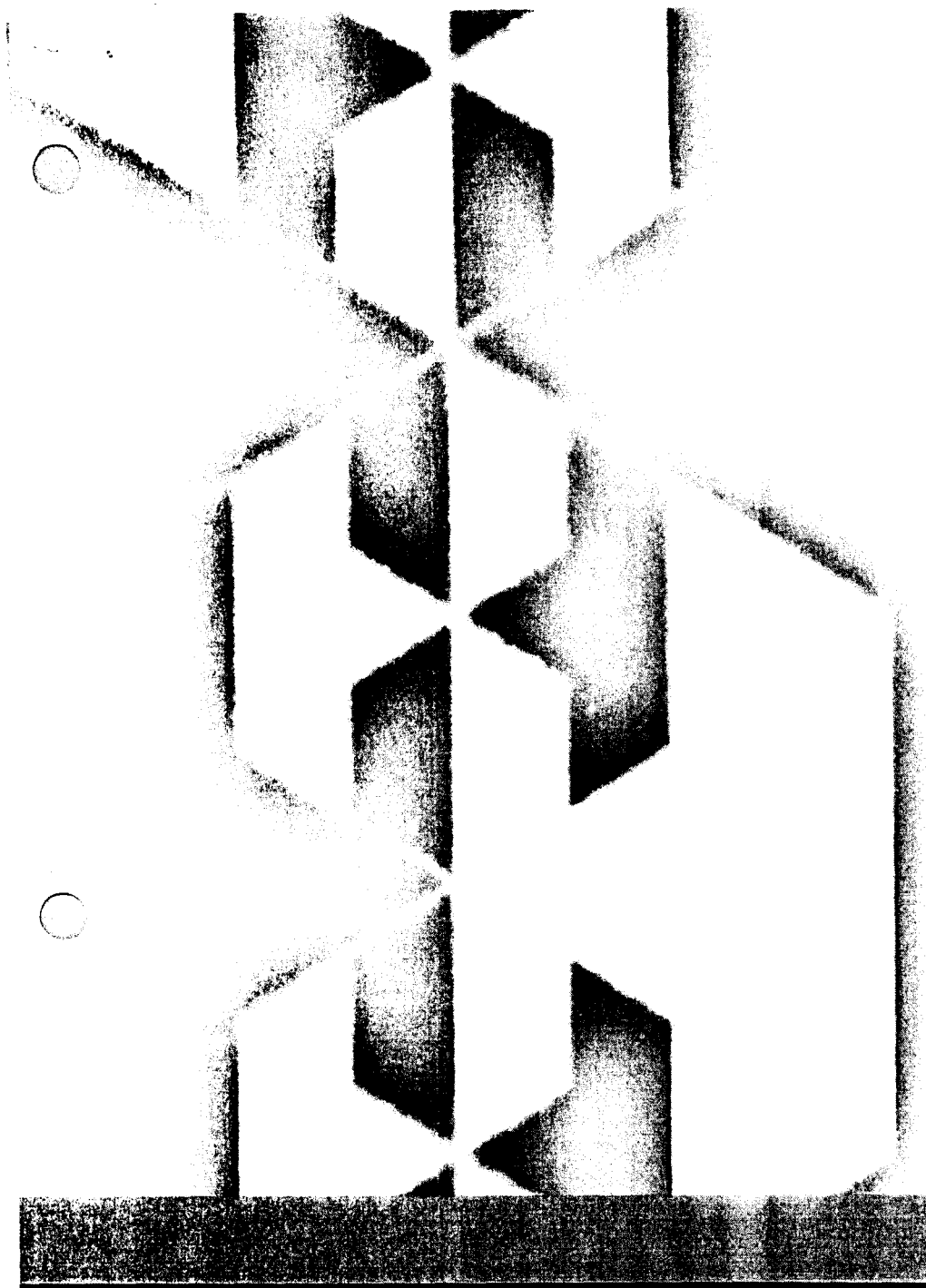
For various reasons, we may want to reset a request to solve again under the same run conditions. To do this we can select **Edit ▸ Reset** from the menu bar in any of the windows. This feature is useful when we think the computer may have gotten stuck on a point in the Markov chain and did not explore all options.

Alternatively, we may want a request to run under different parameters. These situations could include noticing a pattern of DNA degradation, seeing off ladder peaks, or identifying the need for more sampling time. By selecting **Edit ▸ Reset Request...** the Reset Request window opens. Although the Request and Case tabs

cannot be changed, the Setting and Option tabs can be altered. Note that when we reset a request, the original results will be overwritten.

Data

If we find any further artifacts that affect the genotype interpretation after reviewing our results, we can turn these peaks off. Just as we did in the Request Module, we can right click on a labeled peak and choose the "Peak off" option. This action should only be performed if we see a spike or other artifact that negatively affects the genotype interpretation. After turning a peak off, we must then reset the request to solve again.



TrueAllele® VUIer™ Report Module

Reporting Results

Cybergenetics

Table of Contents

1 Overview

Once we have finished reviewing the case information, we can use the Report Module to document the match results and prepare for court presentation. The Report Module supplies us with the tools to understand and present our match information.

The Report Module is the final step in the case investigation process. This module allows us to view visual and textual displays of case evidence, reference and population (Q, S and R) genotype probability distributions. After a final review of the information, we can produce the necessary documentation for our case.

2 Genotype Retrieval

After connecting to a TrueAllele world, we load the case into Report. There are two ways to load our case:

1) find each part (evidence, reference or population) of the case, or
2) import a report specification file.

We will use the first option and search for the individual parts of the case using the buttons in the Report window. More information about creating and importing a report specification file can be found in the **Appendix ▸ Exporting**.

Find Evidence

First, we retrieve the evidence requests from the TrueAllele World where they are stored. We can search for the evidence by clicking the 'Find Evidence' button to open the search dialog window. This search dialog is the same window used to retrieve requests in the Review Module. There are tips on advanced searching in the Review Manual under **Appendix ▸ Advanced Searching**.

Searching produces a list of requests we can select to load in the report interface (Figure 1). Each contributor inferred in a request is listed separately. We can bring in as many or as few genotypes as needed using the checkboxes on the left, but a large batch of contributors will take a few moments to load.

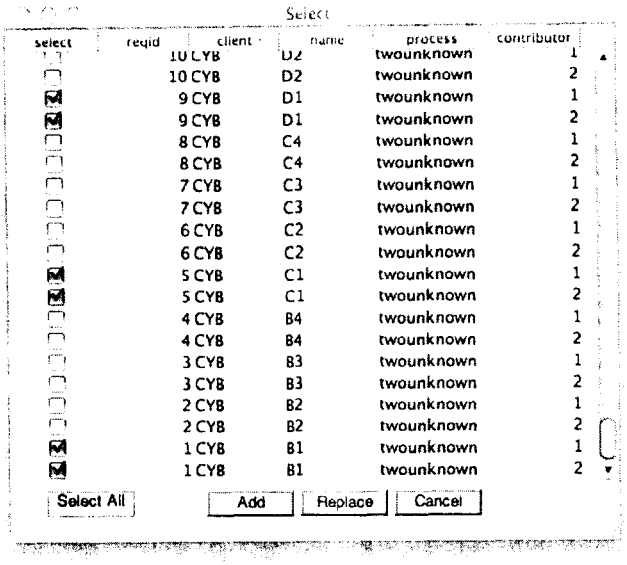


Figure 1. *Select window.* After searching, the Select window displays each contributor inferred in a request that satisfies the search criteria. The options to "Add" or "Replace" inferred profiles to our evidence list are only available after loading an initial list of profiles.

After we select our inferred genotypes of interest, we can click OK. Selected contributors will appear under the "Find Evidence" category (Figure 2). Each inferred profile has a Q (questioned) designation. For example, if we have three evidence items Q1, Q2 and Q3 will be listed under Find Evidence.

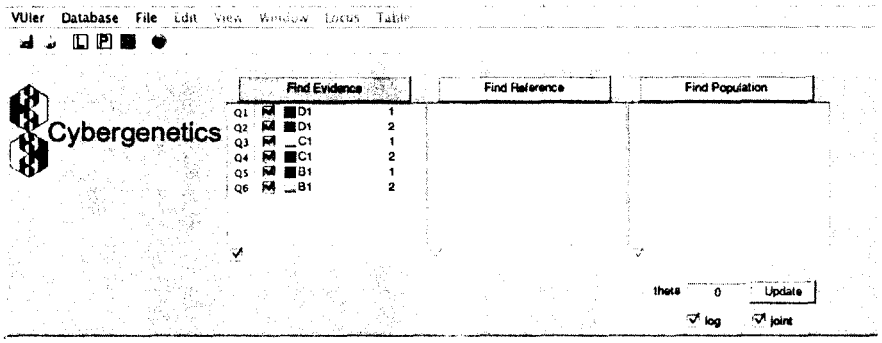


Figure 2. *Report window.*

Find Reference

Similarly, we can find reference requests by clicking the 'Find Reference' button in the Report window. Typically, we will set the *Part* in the search dialog to "suspect," which limits the search results to references where the part was specified as suspect. To include all references in the search results, we can change the setting to "All." If we leave the Part or Contributor fields blank, nothing will be assumed and all options will be shown. Any selected references are displayed with an S (subject) and numeric designation.

Find Population

The last piece of information necessary to compute a likelihood ratio is the population database. The 'Find Population' button generates a list of all the population databases that have been uploaded to a specific TrueAllele World. We can select multiple populations, and a likelihood ratio will be computed using each of the selected databases. Each selected population database is given an R (random) and numeric designation.

Information about uploading population databases to a TrueAllele World is described in the Data Manual.

3 Likelihood Ratio

The main function of the Report Module is to compare inferred profiles and generate match statistics to document the identification information from a case. Depending on the case, it may not be necessary to go through each mode and option in the Report Module.

We now have the three probability distributions necessary to calculate a likelihood ratio and can view the match results.

3.1 Rarity Mode

Applicable Icon

R Rarity Mode

The *Rarity* mode displays the joint likelihood ratio (i.e. match rarity) of a Q, S and R (Figure 3). By default, the Report viewing mode is set to the Rarity as our first view.

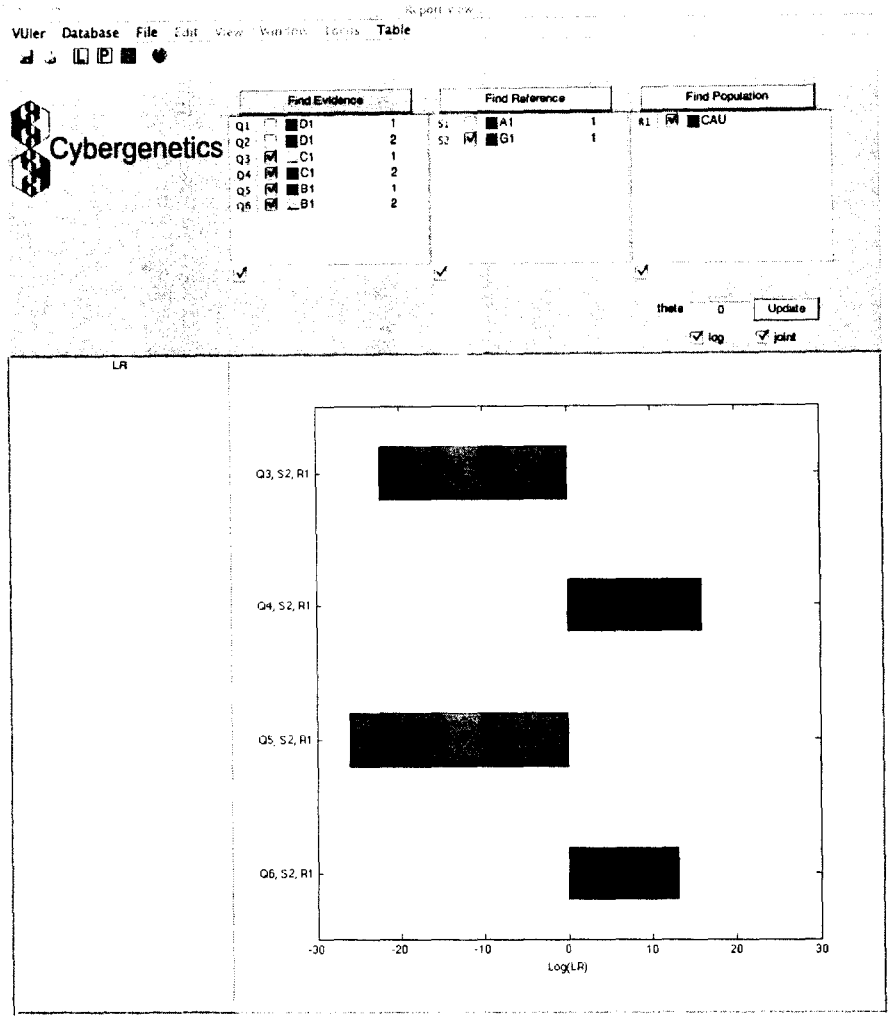


Figure 3. *Report window in Rarity mode.* Here we see the Reference (S2) has a positive likelihood ratio for items Q4 and Q6, shown in blue. The other inferred contributor profiles produce negative likelihood ratios for the reference, shown in red.

A blue bar extending to the right indicates a more likely match (LR>1). A red bar extending to the left indicates a likelihood ratio less than one. The specific contributor in an evidence item, reference and population used in each LR calculation are labeled on the left (ex. Q3,S2,R1).

3.2 Options

Item Selection

We can select which evidence items, references and populations are displayed by clicking the checkboxes to the left of each item. Limiting our view to a few items or to only matching contributors helps us focus on a few relevant matches.

New Searches

We can add or replace the listed probability distributions by conducting additional searches. These searches are done in the same manner as the Find Evidence, Find Reference, and Find Population as noted in the previous section. When the new search results are returned, we see that the button options are now 'Add' and 'Replace'. Clicking on 'Add' will include the newly selected items in addition to those previously viewed. Selecting 'Replace' will open the newly selected items in place of those that we just reviewed.

View

By default, the log(LR) is displayed in the Report window. We have the option to view the LR on a non-logarithmic scale by deselecting the "log" checkbox in the upper right corner.

The LR ratio calculation for each locus is viewed by deselecting the "joint" checkbox in the upper right corner (Figure 4). If we scroll up on the upper scroll bar we can display more loci on the screen. To view fewer loci at once, we scroll down. The lower scroll bar moves through the various loci or, as in other modules, we can select a particular locus from the **Locus** menu.

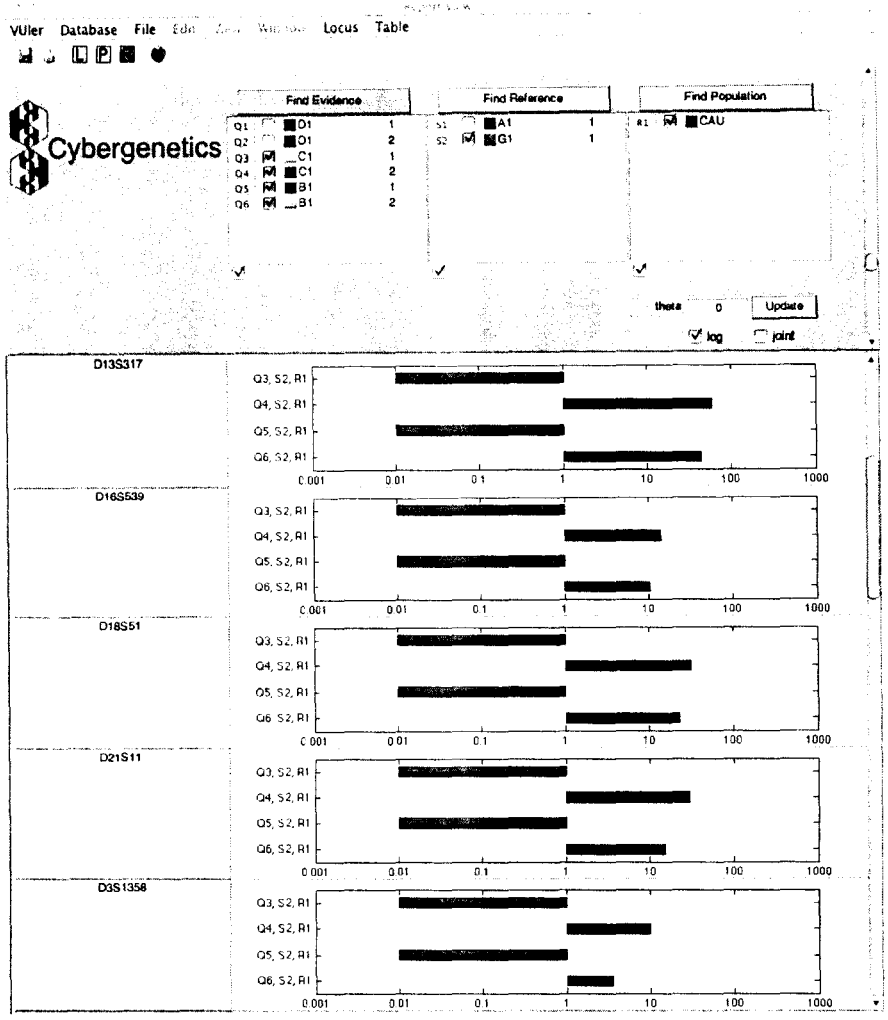


Figure 4. *Rarity mode locus by locus.* Here we see each likelihood ratio calculated locus by locus. Adding the log(LR) from each locus forms the joint likelihood ratio.

Coancestry

If we want to consider population substructure in the LR calculation, we can update the theta value in the Report window. By default, we assume no population substructure by leaving theta equal to 0. For example, to apply a theta value of 3%, we enter .03 in the "theta" field and click 'Update'. Applying a coancestry coefficient updates the likelihood ratio and the theta assumption is documented in any reports produced.

4 Genotype Probability

The genotype probability displays in Report are similar to the Profile view in the Review Module, as they show probability distributions.

4.1 Probability Mode

Applicable Icons

P Probability Mode

The Probability mode displays the genotype probability distribution for the evidence contributors, references and population database (Figure 5). For Q and S, the bars indicate the probability of each genotype as interpreted by the TrueAllele server. For R, the bars indicate the probability of each genotype as defined by the allele frequencies of the population.

Each inferred genotype, reference and population database selected are displayed according to its color code. Evidence genotypes are shades of blue. Reference genotype probabilities are shades of green. The population genotype frequencies are shades of brown.

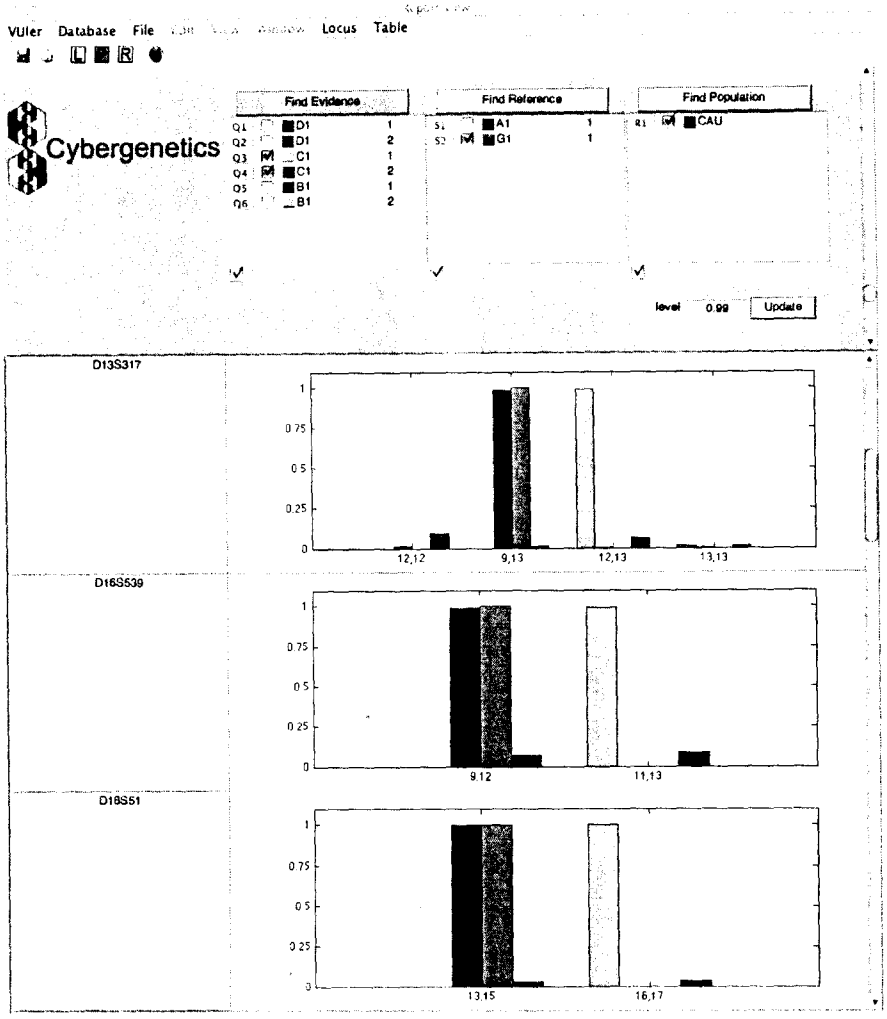


Figure 5. *Probability mode.*

As in the Review Module, we can set the confidence level of the probabilities displayed. Updating the "level" changes the limit of the genotype probabilities displayed. By default, a 99% confidence level is set.

4.2 Likelihoods

Applicable Icons

L Likelihood Mode

The Likelihood mode focuses on the fit of the model and not a genotype probability. Here, the population frequencies have not been considered and we can see the

equivalent likelihoods for each genotype. We can then see how the population frequencies affect each genotype possibility in the Probability mode and compare the result to TrueAllele's genotype probability distribution.

5 Genotype Review

In Report, we have the option to view the original electropherogram data and mixture weight window. To view the data for either an evidence or reference sample, we select the item of interest and a window containing request information will be displayed (Figure 6).

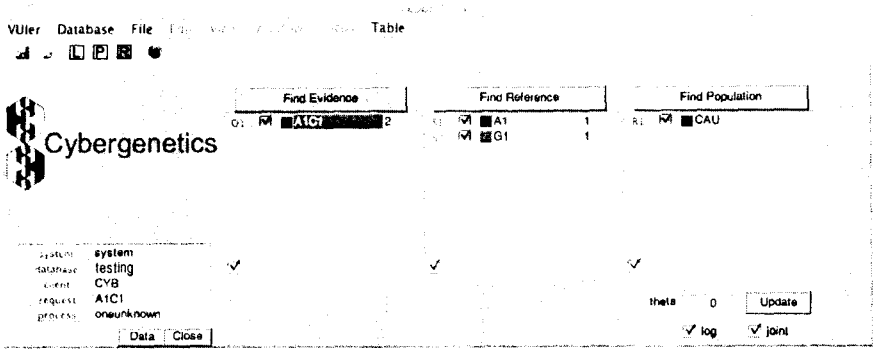


Figure 6. *Data Access.* After highlighting the A1C1 sample, a window containing the system, database, client, request and process information appears. The "Data" button provides a link to the original electropherogram associated with this request.

From this window, we can select the 'Data' button to retrieve the electropherogram in the *Data View window*. Any notes that were made to the lanes of data in Request or Review are also viewable here and can be annotated. For mixture samples, we can access the *Mixture View window* by selecting **Window ▸ Mixture** from the toolbar. These are the same windows we see in the Review Module and the Review manual describes them in further detail.

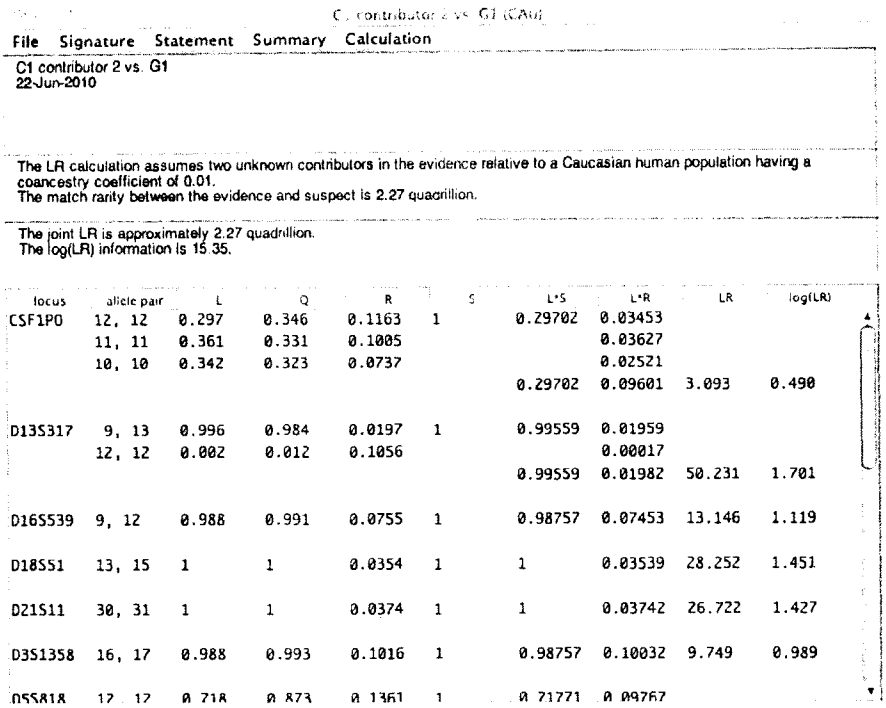
6 Report Calculation

The Report Module automatically generates a match report to document the match information results for court. A likelihood ratio statement that summarizes the match information is calculated from a questioned (Q), a suspect (S) and a population (R) genotype distribution.

From the **Table** menu, we select the Q, S and R combination for the item of interest. Each combination of the evidence items, references and populations selected in the Report View window is displayed in the **Table** menu. The **Table ▸ All** option will produce each of the reports listed in this menu.

The report documents the case identification information, reference population, theta value (if applicable), and likelihood ratio calculation (Figure 7) for a specific Q, R, and S set.

Instructions on how to save a report are available in the **Appendix ▸ Exporting.**



C1 contributor 2 vs. G1 (Cau)

File Signature Statement Summary Calculation

C1 contributor 2 vs. G1
22-Jun-2010

The LR calculation assumes two unknown contributors in the evidence relative to a Caucasian human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 2.27 quadrillion.

The joint LR is approximately 2.27 quadrillion.
The log(LR) information is 15.35.

locus	allele pair	L	Q	R	S	L*S	L*R	LR	log(LR)
CSF1PO	12, 12	0.297	0.346	0.1163	1	0.29702	0.03453		
	11, 11	0.361	0.331	0.1005			0.03627		
	10, 10	0.342	0.323	0.0737			0.02521		
						0.29702	0.09601	3.093	0.490
D13S317	9, 13	0.996	0.984	0.0197	1	0.99559	0.01959		
	12, 12	0.002	0.012	0.1056			0.00017		
						0.99559	0.01982	50.231	1.701
D16S539	9, 12	0.988	0.991	0.0755	1	0.98757	0.07453	13.146	1.119
D18S51	13, 15	1	1	0.0354	1	1	0.03539	28.252	1.451
D21S11	30, 31	1	1	0.0374	1	1	0.03742	26.722	1.427
D3S1358	16, 17	0.988	0.993	0.1016	1	0.98757	0.10032	9.749	0.989
D5S818	12, 12	0.718	0.873	0.1361	1	0.71771	0.09767		

Figure 7. *Example Report.* This report compares contributor 2 of the C1 questioned item to reference G1. A coancestry coefficient of .01 is used, as well as a Caucasian population database.

6.1 Calculation

For each locus, the inferred genotype possibilities are listed. Each allele pair in the list has a likelihood (L), which is determined by the fit of the data before conditioning on population frequencies. The inferred genotype probability distribution (Q) comes from the probability model.

The allele frequencies from the population database are used to calculate column R. For example, when theta is equal to zero, the population's genotype probability is equal to 2pq for heterozygotes and p^2 for homozygotes.

The suspect's genotype probability distribution is listed in column S. Reference samples are typically unique profiles, reflected in the 100% genotype probabilities.

The likelihood ratio (LR), or match rarity for each locus, is calculated by dividing the sums of the probability products in columns L*S and L*R. The joint LR is calculated by multiplying the individual LRs from each locus. The logarithmic form of the match statistic provides the measure of information.

6.2 Customizable Options

In the menu bar of the calculated report, we have several options to customize our reports. These options may be set as a lab policy to generate standard reports.

To anonymize the report, we can select **Signature ▸ Off** from the menu bar. This action takes the case identifier and date out of the report. Typically, it is only useful to anonymize our reports for scientific presentations.

Similarly, the match statement can be turned on and off using the **Statement ▸ Off** option in the menu bar. We also have the option of selecting the language used in our match statements. The *Rarity* option states the match statistic as follows: "The match rarity between the evidence and suspect is 2.27 quadrillion." Alternatively, the *Likelihood* option states the match statistic as: "The likelihood ratio for the identification hypothesis is 2.27 quadrillion."

The *Summary* statement reports the joint likelihood ratio and the logarithmic form of the likelihood ratio. We can turn this statement on or off from the **Summary** menu.

Depending on our audience, a simplified version of the report calculation may be helpful as a presentation tool. Selecting **Calculation ▸ Simple** reduces the report and displays only the matching allele pair possibility.

7 Match Table

The *Match Table* provides a summary of the match statistics for a group of requests. Creating a match table is useful when we have a larger number of evidence and reference samples to compare. We can generate a match table by selecting **Table ▸ Match** (Figure 8). This table displays match statistics for the items and references that are selected in the Report View window.

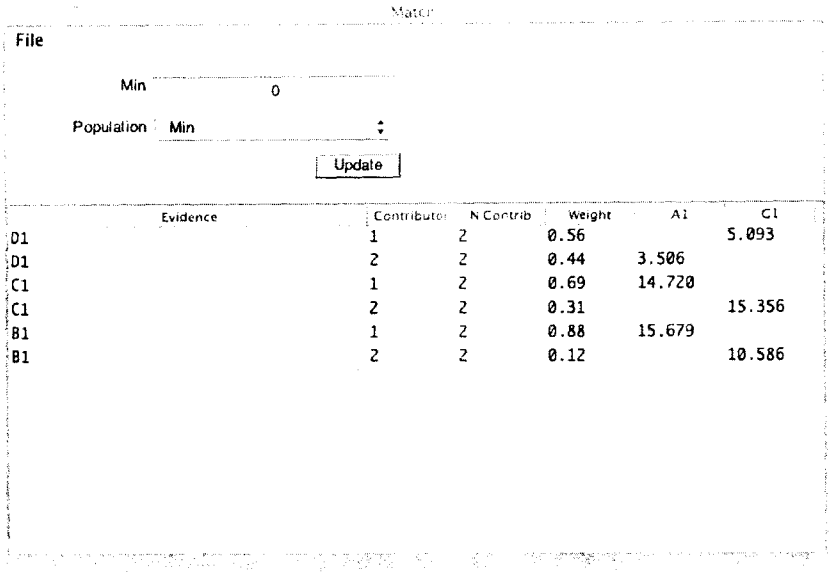


Figure 8. *Match Table*. This table displays the match rarity for a set of evidence items and their mixture weights. In this table, we have filtered the results to matches with a log(LR) greater than zero relative to the minimum population.

In the *Match* window, we can filter the results by a minimum log(LR). To do so, we can enter a value in the *Min* field and 'Update' the calculation. Similarly, we can choose the population database used to calculate the match statistics. The *Population* drop down menu contains all the populations uploaded to a TrueAllele World. Selecting the minimum population (shown as "min"), displays the lowest LR calculated using each population database.

Instructions on how to save this table are available in the **Appendix ▸ Exporting.**

7.1 Using the match table for complex cases

When we have cases with numerous pieces of evidence and multiple references, the match table is often our best resource. In smaller cases, it is easy to save the one or two Report files that we need. For larger cases, we may want to determine what Report files would be the most informative.

To determine the most informative matches, we can view all of the evidence and reference items in the match table. After identifying the most probative matches, we can produce reports for those specific items. For cases with multiple rounds of processing, the match table can provide a visual view of the information gain.

8 Case Example

In this section, we will continue with the case example that we begin in the Request and Review manuals.

8.1 Did the Suspect Contribute to the DNA Evidence?

To document the match information for a case, we can acquire the evidence, suspect and population genotype distributions necessary to produce a match statistic. We can review the answer to our question by first adding the unknown contributor of the evidence A1C1 request, the suspect (G1) and population databases to the Report window.

8.2 The joint likelihood ratio

To determine if there is a match between the unknown contributor in the evidence and the suspect, we look for a positive joint likelihood ratio (blue bar) (Figure 9).

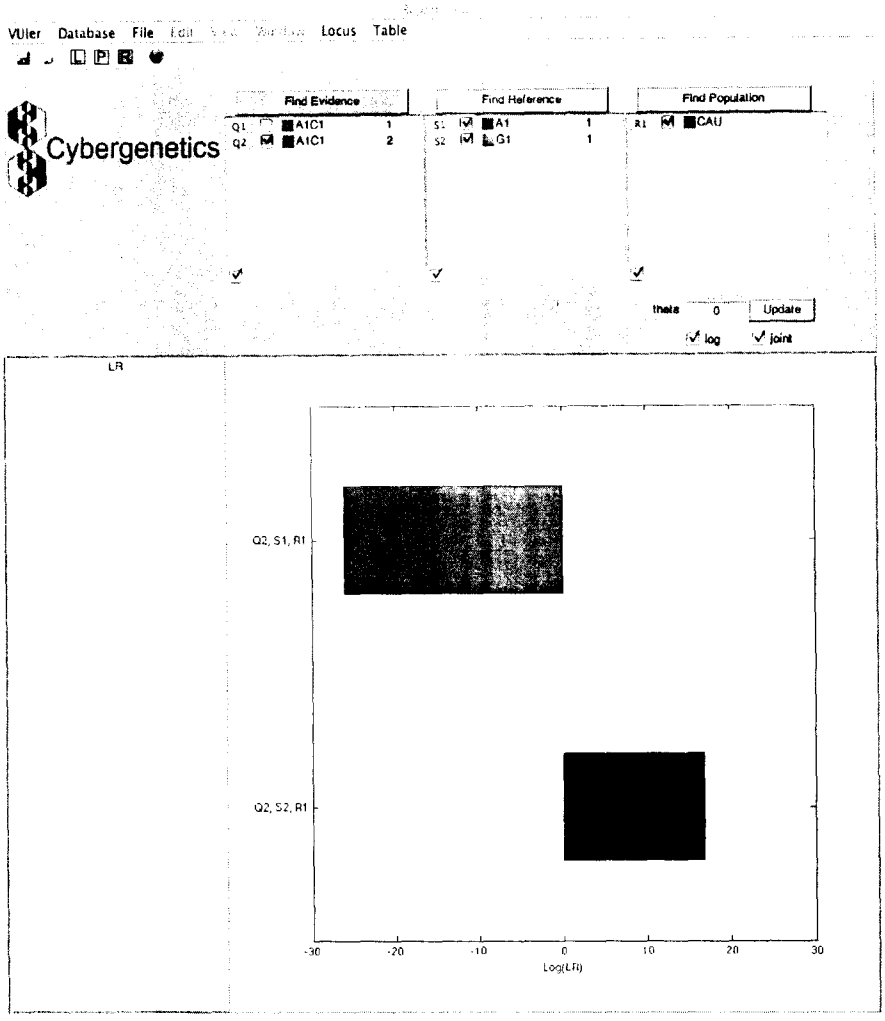


Figure 9. *Joint Likelihood Ratio.* The red bar indicates that the second contributor's inferred profile does not match the victim reference (A1). The blue bar indicates a positive log(LR) between the second contributor and the suspect (G1).

Here, the bottom blue bar shows a log(LR) between 10 and 20 for the unknown contributor of A1C1 to the suspect G1. This positive log(LR) tells us that we should generate a match report to determine the exact value.

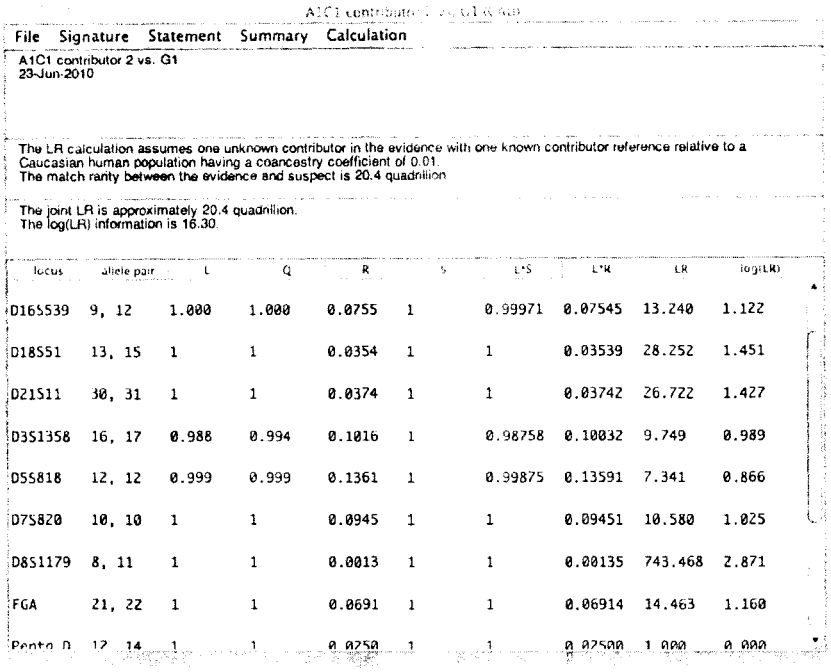
8.3 Creating the report

We can now report on the match results found between the unknown contributor in the A1C1 evidence request and the suspect. In this example, we will account for population substructure by adjusting the theta value to .01 to see how the likelihood ratio is affected.

To create the report, we select the match of interest from the **Table** menu (Q2&S2&R1). This selection indicates that the report uses evidence Q2, reference S1 and population R1.

Report

A detailed text report opens and we can see that, after accounting for population substructure, our LR drops to 16.30 (Figure 10).



File Signature Statement Summary Calculation

A1C1 contributor 2 vs. G1
23-Jun-2010

The LR calculation assumes one unknown contributor in the evidence with one known contributor reference relative to a Caucasian human population having a coancestry coefficient of 0.01.
The match rarity between the evidence and suspect is 20.4 quadrillion.

The joint LR is approximately 20.4 quadrillion.
The log(LR) information is 16.30.

locus	allele pair	L	Q	R	S	L*S	L*R	LR	log(LR)
D16S539	9, 12	1.000	1.000	0.0755	1	0.99971	0.07545	13.240	1.122
D18S51	13, 15	1	1	0.0354	1	1	0.03539	28.252	1.451
D21S11	30, 31	1	1	0.0374	1	1	0.03742	26.722	1.427
D3S1358	16, 17	0.988	0.994	0.1016	1	0.98758	0.10032	9.749	0.989
D5S818	12, 12	0.999	0.999	0.1361	1	0.99875	0.13591	7.341	0.866
D7S820	10, 10	1	1	0.0945	1	1	0.09451	10.580	1.025
D8S1179	8, 11	1	1	0.0013	1	1	0.00135	743.468	2.871
FGA	21, 22	1	1	0.0691	1	1	0.06914	14.463	1.160
Penta D	12, 14	1	1	0.0250	1	1	0.02500	1.000	0.000

Figure 10. *A1C1 contributor 2 vs. G1 Report.*

Our case identification information is listed in the first section. Next, we have our assumptions about the case that we used in building our requests. A statement of match is also included that can be useful when presenting to a jury. In the third section, we see the joint likelihood ratio and the log(LR).

The table illustrates where the likelihood ratio is coming from. In the table, we see each locus, the inferred genotype's allele pair possibilities, their likelihoods, its

probability of occurrence in the evidence, the reference population, the suspect genotype and the likelihood ratio.

Example Locus

We can walk through the locus D3S1358 to see how the likelihood ratio is reached. Here, the matching allele pair is [16, 17]. We begin by taking the probability of this allele pair for the evidence genotype (.988) and multiplying it by the probability of this allele pair for the suspect (1). This creates the numerator of the likelihood ratio (L*S). Next, we form the denominator (L*R) by multiplying the probability of this allele pair for the evidence genotype (.988) and the CAU population (.1016). This creates the likelihood ratio .98758/.10032 or 9.749, as we see in the LR column. We can take the log of this number to find the log(LR), which comes to .989.

8.4 Using Report to Explain Results

The images in the Report Module are useful to communicate match results. We are able to see the gain in identification information by comparing the probability of a genotype in a population database to the inferred probability based on the data. To begin sharing a match result, we view the data alongside its probability distribution (P mode) for each locus (Figure 11).

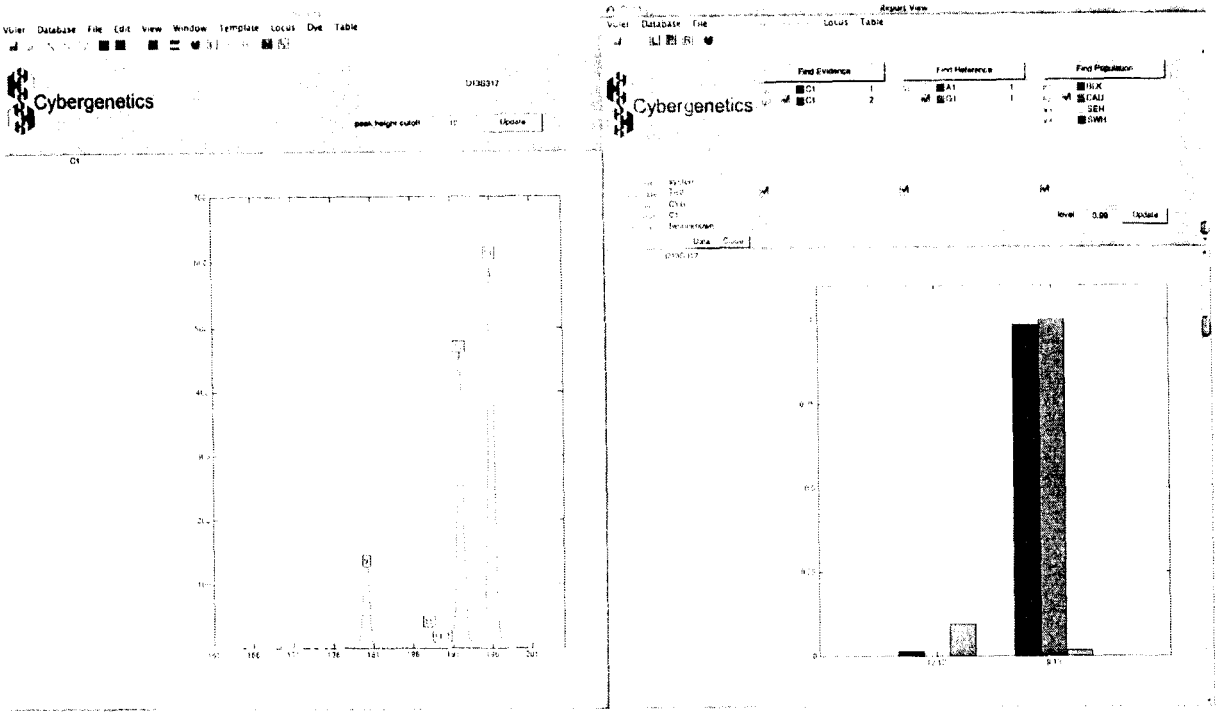


Figure 11. *Individual Locus.* The left image shows the STR data for the D13S317. The right image shows the locus genotype probability distributions for the inferred questioned genotypes (Q, blue), a Caucasian population (R, brown), and the suspect reference (S, green).

Here we see the gain in identification information. Our prior belief, without looking at the STR data, is represented by the population database (R, brown). After interpreting the STR data, we see how the genotype probability distribution is updated based on the data in blue (Q). The height difference in these probabilities represents the gain in identification information. Based on modeling the data, we see that the genotype [9, 13] has become much more probable than our belief prior to looking at the data. When we compare this inferred genotype probability distribution to the suspect's genotype (S, green), we expect a large likelihood ratio due to this increase in probability (Figure 12).

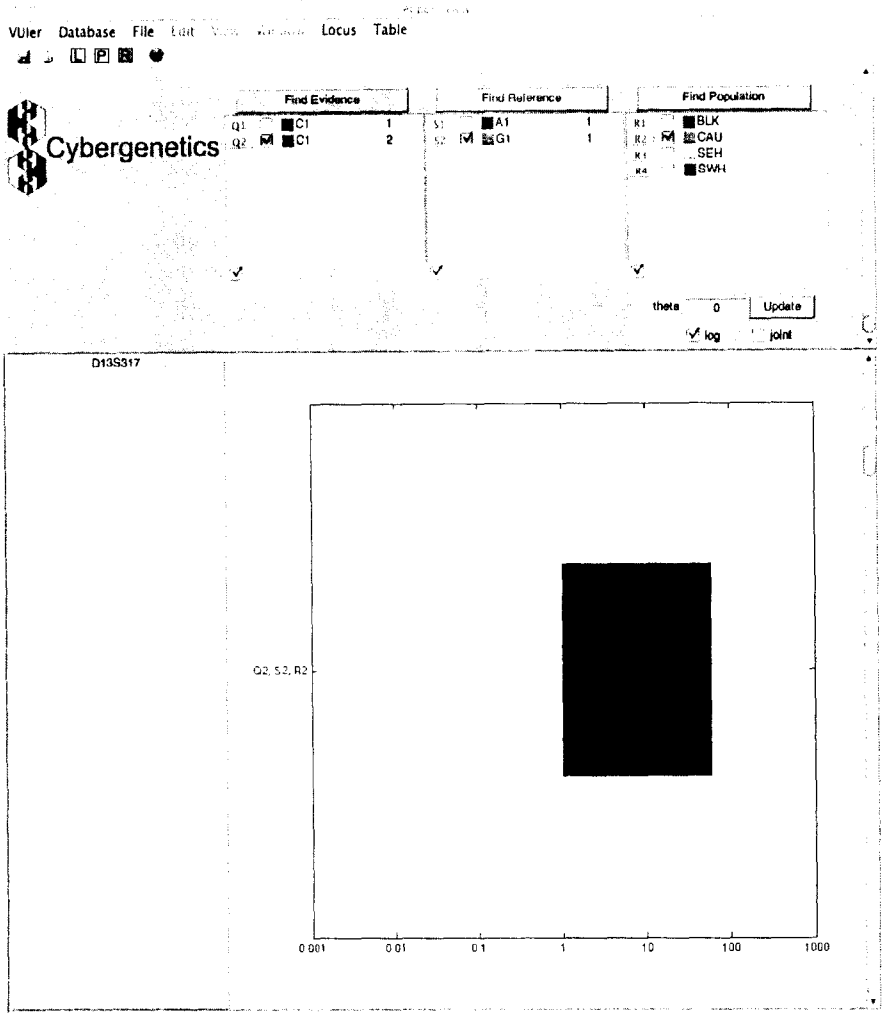


Figure 12. *Individual LR.* The report window shows the likelihood ratio for the D13S317.

When we change the report window to rarity mode (R), the LR for D13S317 is displayed. The LR quantifies the increase in identification information we observed. With the large increase in the matching genotype probability, we expect a large contribution to the match strength at this locus. In this example the log(LR) is 1.772, supporting these expectations. We can review this information for each locus by continuing in a similar manner.

The report module also enables us to demonstrate reproducibility. If we have two independent interpretation requests of the same STR data, we can display the LR

results simultaneously (Figure 13). Here, we can visually show the reproducibility of the likelihood ratio at each locus.

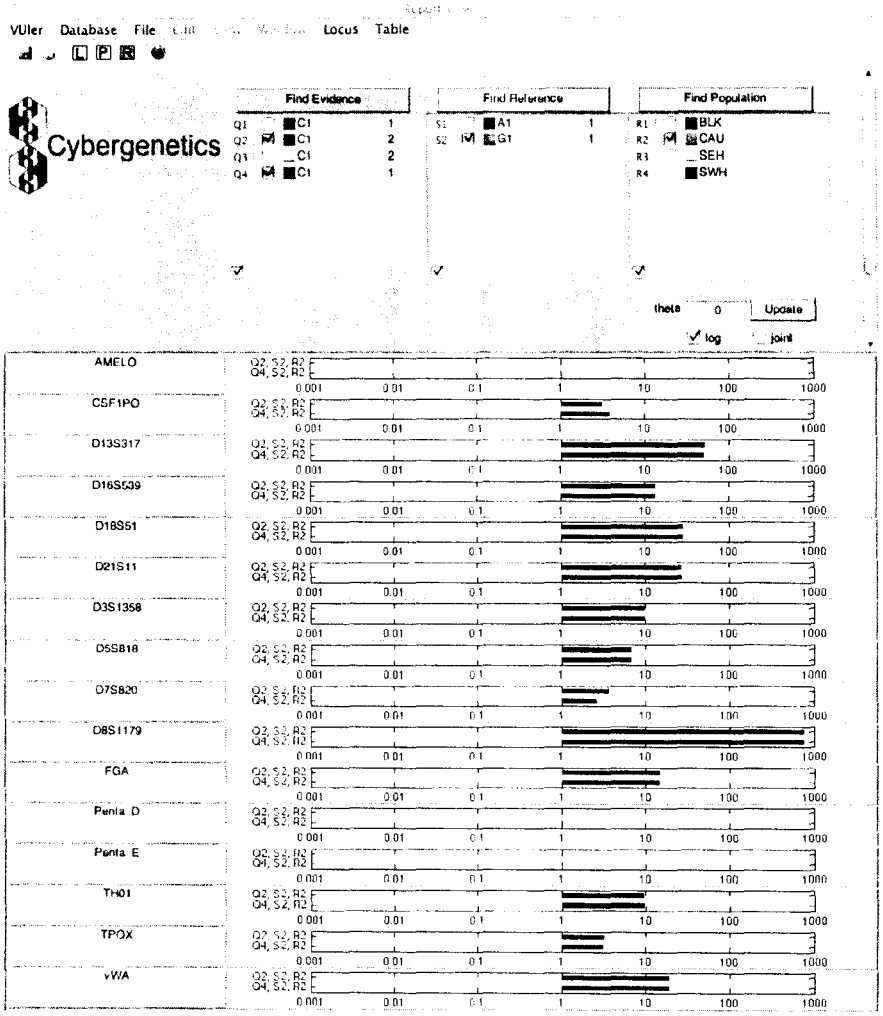


Figure 13. *Duplicate Runs.* The report window shows LRs at each locus for two independent TrueAllele computer runs.

To show the effect population substructure has on likelihood ratios, we can set various theta values. Using the images, we can visually show how much the values decrease the likelihood ratio.

9 Appendix

9.1 Exporting

Specification Files

A report specification file, also called a "spec file," documents a set of evidence items, references, and populations that have been loaded into the Report Module. It is useful to save the state of the Report Module to prevent the reloading of each genotype distribution set multiple times. To export a spec file, we select **File ▸ Export** from the toolbar and choose where to save the file. The spec file saves the system, database, case name, case part, client, request name, request id and contributor number.

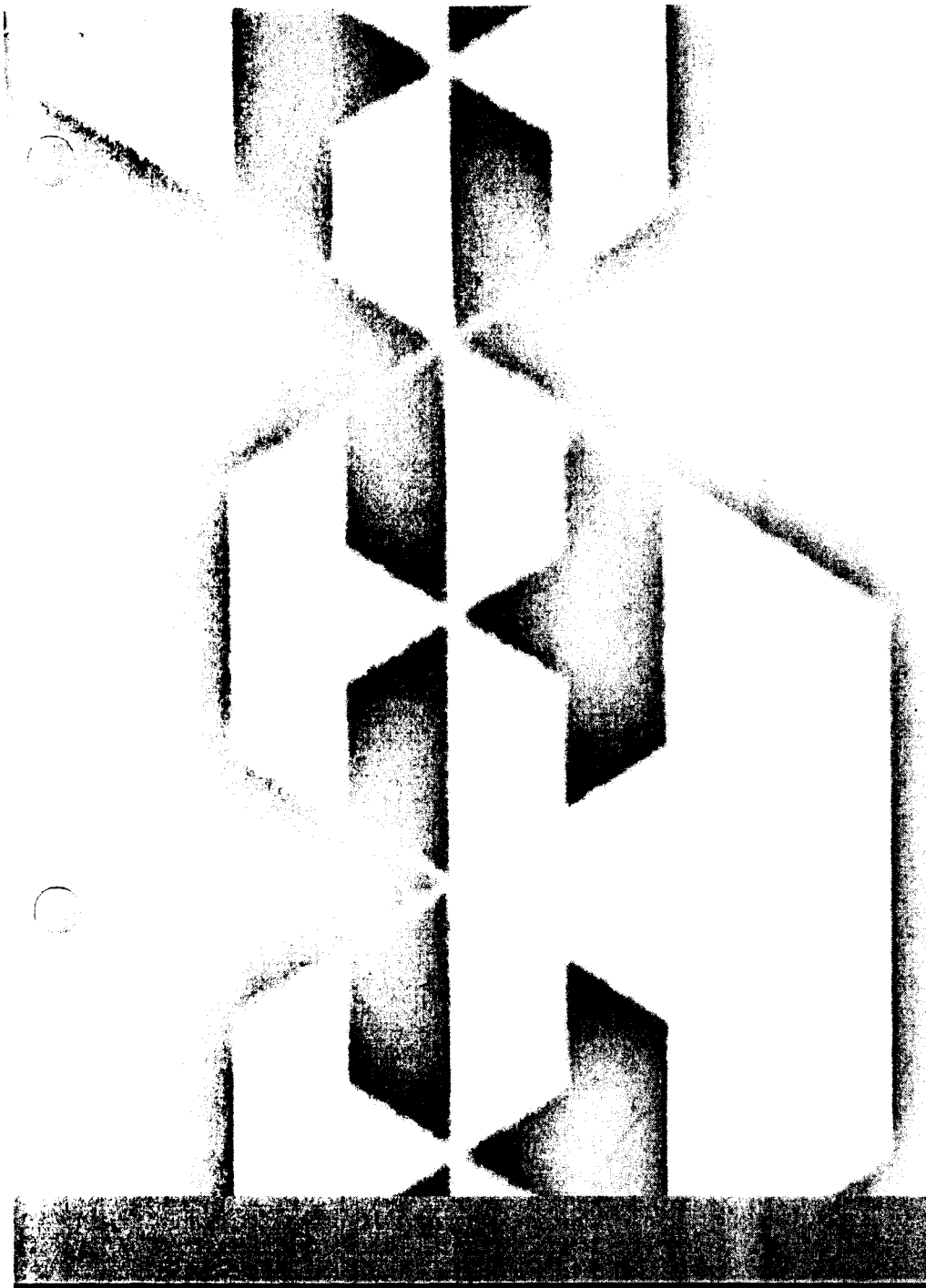
The files can be shared between users for review by importing the saved spec file. To import a file we select **File ▸ Import** and navigate to the location of the saved file. This action brings in the evidence items, references, and population databases from the saved state.

Reports

A report file generated in the Report module can be saved as a text (.txt) file. To save a report, select **File ▸ Save** from the report's toolbar. The text file can be opened in a spreadsheet program and printed for case documentation.

Match Table

A match table generated in the Report module can be saved as a text (.txt) file. To save a report, select **File ▸ Save** from the table's toolbar. The text file can be open in a spreadsheet program and printed for case documentation.



TrueAllele® VUIer™ Tools Module

Administrative Guide

Cybergenetics

Table of Contents

1 Overview

The Tools Module is the administrative area of the TrueAllele system. It allows us to make changes across an entire database, alter preferences and modify specific requests. In this module, we can also create new storage areas for our data, known as TrueAllele Worlds. The menu bar of the Tools Module has four different categories: VUIer, Database, File and Tools.

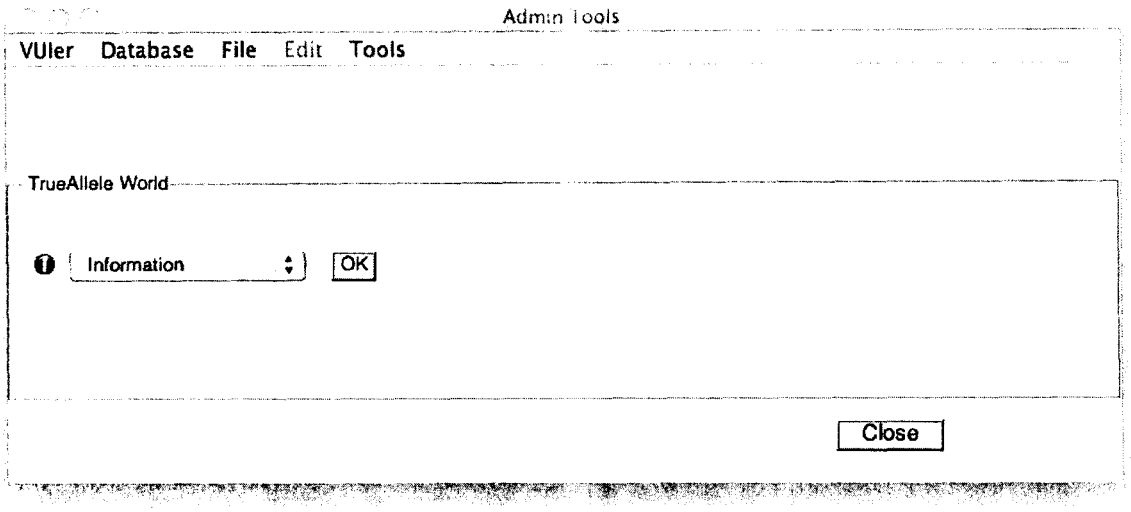


Figure 1. *The Tools Module.*

A person can log into the Tools Module in several ways. The most common is as a user. Unless otherwise noted, the options described below are available to users. Other options are available when logged in as an owner. The owner is the person who created the world and is given special rights and privileges. The third option is COM, which is described in the COM Database section.

2 Menus

2.1 VUler

Selecting **VUler ▸ About** displays the current VUler version and the date of download. This feature helps us ensure that the software is up to date.

Selecting **VUler ▸ Quit** closes the program entirely, along with any connections that are currently open.

2.2 Database

The Database menu option allows us to connect to or disconnect from a TrueAllele world.

In Tools, we connect to a world in the same way as we do in the other four modules. After selecting **Database ▸ Connect**, a Connect window opens nearly identical to those in other modules. The single difference is the "Owner" checkbox under the login information (Figure 2). We check this box if we are the creator or designated owner of the world. Checking this box when logging in allows us to make administrative changes.

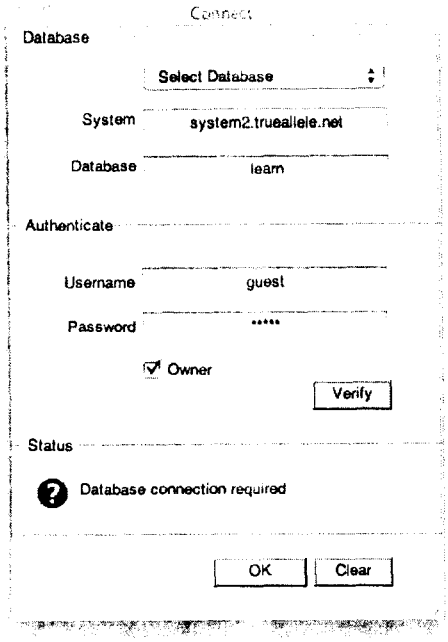


Figure 2. *The Connect window.*

Selecting **Database ▸ Logout** disconnects us from that specific TrueAllele world. We can log out of a world and reconnect to a different world at any time.

2.3 File

To close the Tools module and return to the module chooser, we select **File ▸ Close**. This action closes the Tools window without logging out of the database, so the connection is maintained.

2.4 Tools

The Tools menu option is the core of the module (Figure 3). When a category is chosen from the tools menu, a drop down menu appears in the center of the window (the TrueAllele World drop down is shown here).

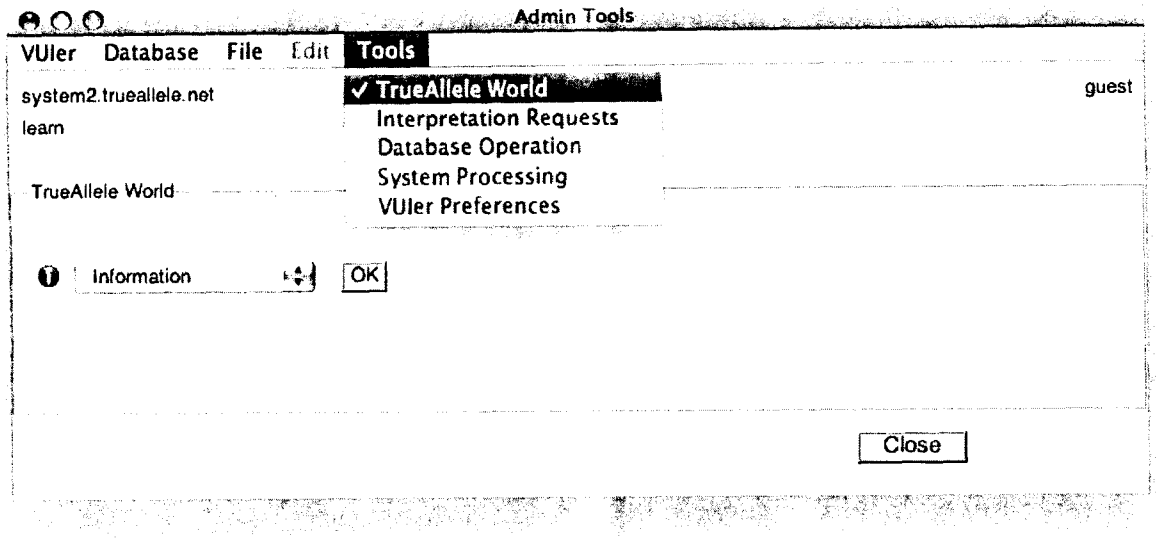


Figure 3. *The Tools menu.*

3 TrueAllele World

The TrueAllele World menu contains information and options that relate to a specific database. This menu consists of the current clients and labs that are included, information about the world, and the ability to copy or create a world. Clicking OK will access the selected features from this drop down menu.

Information

When a world is created, certain annotating information can be recorded. We can view the annotations by selecting *Information* from the TrueAllele Worlds drop down menu.

The World Information window displays seven different fields of information (Figure 4). At the top is the world name. *Label* is a descriptive indicator of the world. *Company* and *Place* reflect the lab and location. The *Reset* option dictates the ability of the user to reset all of the requests in a particular world, while the *Delete* option permits the world to be deleted from the server (both options are set to *Deny* by default). *About* gives a description of the world (up to 1000 characters) and contains any information that the owner feels is pertinent.

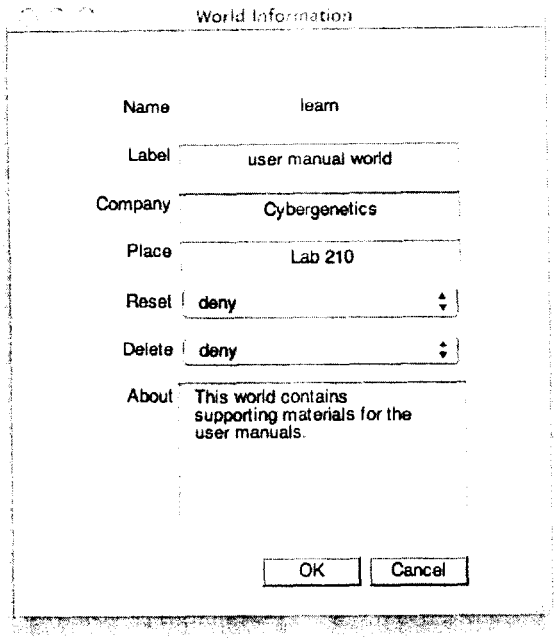


Figure 4. *The World Information window.*

Upon creating the world, an owner can fill in any or all of the fields in the World Information window. Only an owner can make changes in this window.

Create World

In some instances, it is helpful to create separate data storage areas for different case types, research projects, or validation studies. To generate this new storage area, we need to create a new TrueAllele World.

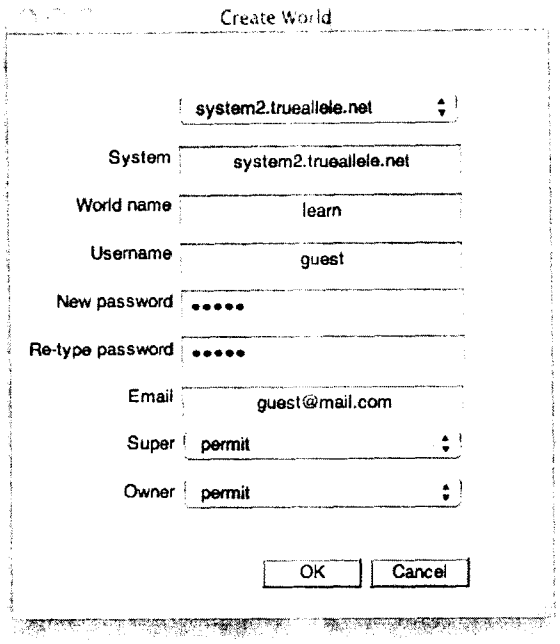


Figure 5. *The Create World window.*

To begin, select "Create World" from the TrueAllele World drop down menu. By choosing this function, a new, empty world is created. The Create World window opens (Figure 5), and allows us to name the world and designate a specific user as owner [Note: it is not necessary to create a different username and password for each world. This information can be the same in each TrueAllele World]. Usually, the person creating the world will designate themselves as owner. An e-mail address can be entered as a contact regarding that world. The owner has the ability to edit the information window, change permissions, and give other users access to the world.

Client

As a form of data organization, a *client* must be designated before uploading a request to the server. The client is typically installed by Cybergenetics, and some

labs may only use a single client name. However, if an additional level of organization is necessary, additional clients are an option.

Selecting "Client" from the TrueAllele Worlds menu will show all clients that are part of the current database (Figure 6). The personal contact, company, and location for each client are also listed.

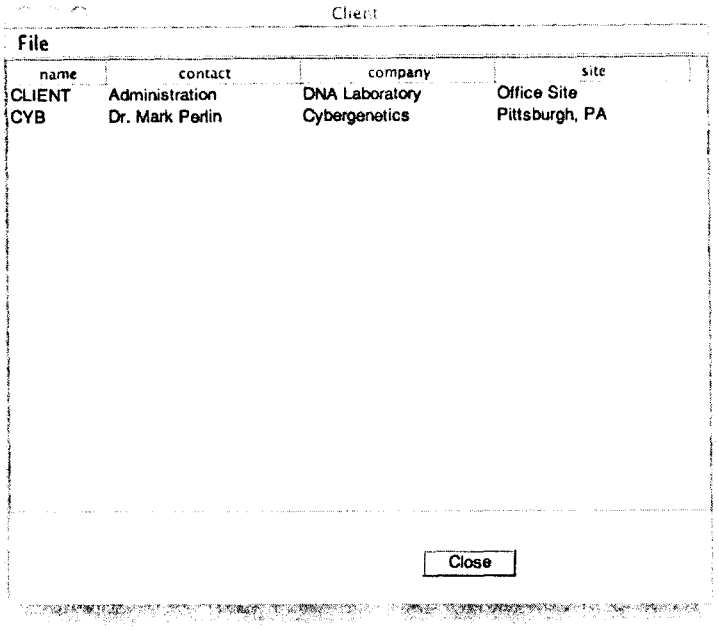


Figure 6. *The Client window.*

If we are logged in as an owner, this information can be edited. To add a new client to a world, selecting **Edit ▸ Add Client** opens a window where the client name, contact person, company and site can be filled in. To edit information for an existing client, select "Edit" under the actions menu.

Lab

Another form of data organization is the *lab* identifier. A lab name must be uploaded with a .gel file to designate where the biological specimen was analyzed. This is

particularly useful in places where various laboratories may be doing the biological analysis, and a separate location performs interpretation.

Selecting "Lab" from the TrueAllele Worlds menu will bring up a window that has a layout similar to the Client window (Figure 7). Here, all of the labs are listed that exist on the current world. The personal contact, company, and location for each lab are also listed.

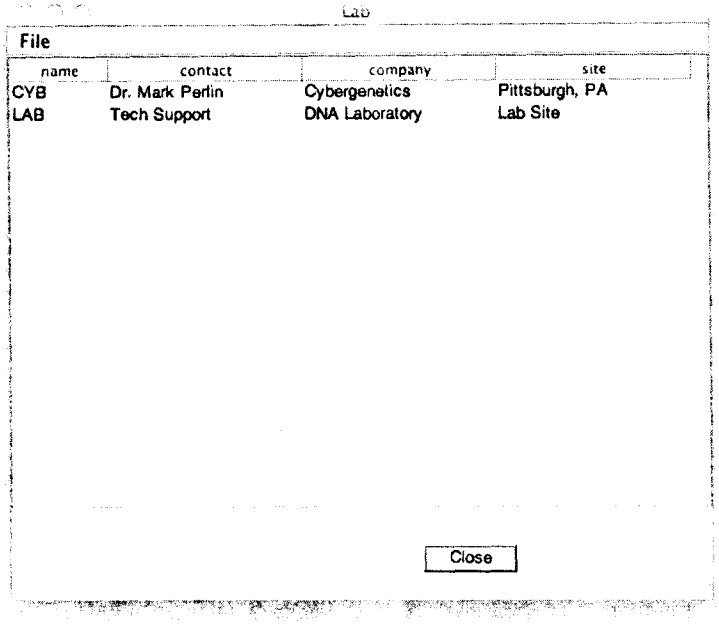


Figure 7. *The Lab window.*

If we are logged in as an owner, this information can be edited and labs can be added. To add a new lab to a world, selecting **Edit ▸ Add Lab** opens a window where the lab name, contact person, company and site can be filled in. To edit information for a current lab, select "Edit" under the actions menu.

When logged in as an owner, some additional options are available from the TrueAllele World dropdown menu. These options are *Users* and *Copy World*.

Users

Typically, multiple analysts are involved in the interpretation of DNA evidence. Having individual user accounts for each analyst provides them with their own workspace on the genetic calculator.

Selecting "Users" from the TrueAllele World menu opens a window listing all of the users in the current world, their email address, and whether they are a superuser or an owner. A superuser is a general user account that has access to everything on that world. When a user account is not listed as a superuser, that account has limited access. User accounts are defined on a per World basis. Therefore, an account that has access to all the data on a specific world does not have access to data on another World on the same system.

When we are logged in as owner, information for these users can be edited and the user can be deleted by selecting "Edit" or "Delete" under the action menu. To add a user to the world, selecting **Edit ▸ Add User** opens a window where a username, password, email address, and role can be set. To give a user limited access, the check box under the superuser column can be deseleted (superuser box is checked by default). An owner can also select another user to be an owner of that TrueAllele world, granting them the same rights and privileges.

Copy World

When doing validations, reproducibility is a frequently measured aspect. Creating an exact copy of a database and asking questions with identical parameters can prove the reproducibility of the TrueAllele process. The owner can make this duplicate world by selecting "Copy World" from the TrueAllele World drop down menu and clicking OK.

This action opens the *Copy World window* (Figure 8). Here, we select the system and world that we are copying, and designate the name of the new, copied world in the "To World" box.

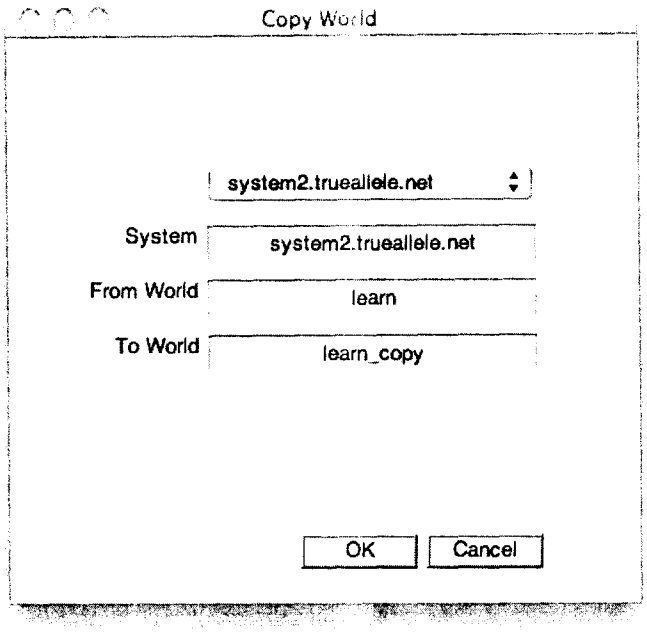


Figure 8. *The Copy World window.*

By copying the world, all the requests are duplicated with the same process and status. It is recommended that a world not be copied while any requests are still solving; these requests would not complete solving in the duplicate world. Note that the requests in the copied world do not rerun automatically and will have to be reset. Resetting a world is described below in the "Global Requests" section.

After copying a TrueAllele World, we are disconnected from the current world. To make any changes to the new world, we must log in to that database.

4 Global Requests

The Global Requests menu is available only when a user is logged in as an owner (Figure 9). This menu enables changes to be made to every request in a world.

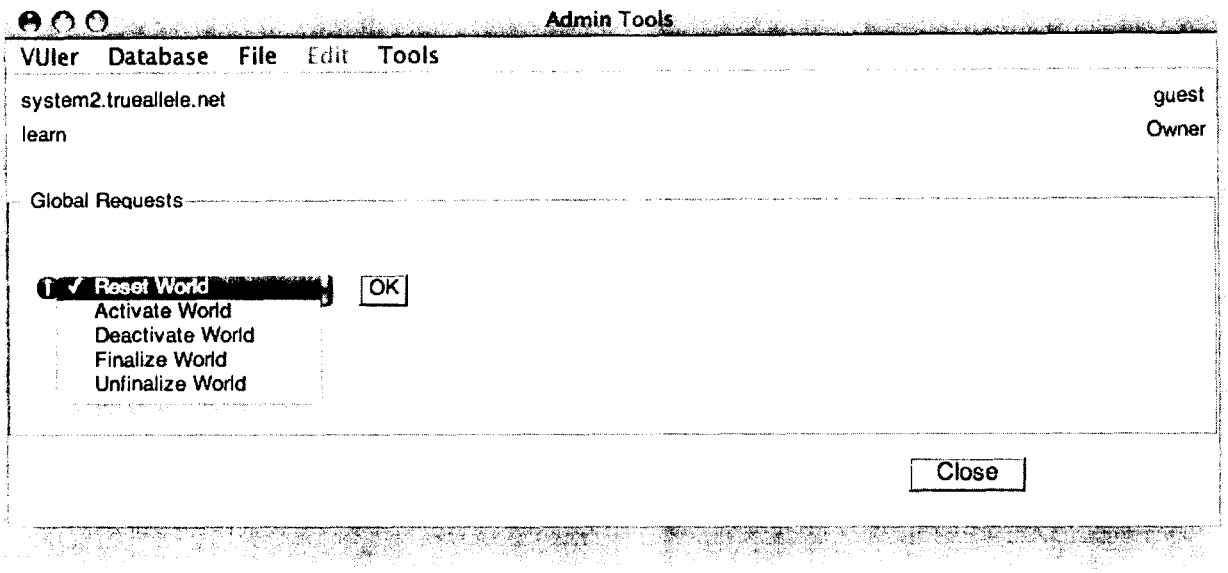


Figure 9. *The Global Requests menu.*

Reset World

After copying a world for reproducibility studies, the requests in the duplicate world need to be reset so that they will solve again. This is done with the Reset World feature.

The Reset World function will set all requests in the current world to rerun by changing their status from "done" to "ready." This action indicates to the server that this request should be reprocessed. The reset function is found in the Global Requests drop down menu.

Activate World

When requests are set up, they can be deferred to "inactive" status, meaning that they aren't immediately sent to the server. If requests in a world are set up as inactive, the requests can be activated globally by using the Activate World option from the Global Requests menu. This sets every request in the current TrueAllele world to "isactive" so that the server can pick up the questions and begin solving.

To activate a set of specific requests and not all requests globally, choose Activate under the "Interpretation Requests" menu.

Deactivate World

We can also indicate to the server that requests should not be picked up for solving. We do this by setting the status of all requests to "inactive."

Inactive status can be set globally by selecting "Deactivate World" from the Global Requests menu. When the server is selecting requests to solve, it will ignore the inactive requests. If we want to inactivate specific requests and not an entire TrueAllele world, choose Deactivate under the "Interpretation Requests" menu.

Finalize World

If a case has finished and a user wishes to lock it from any future processing, a request can be finalized. This option will occur over the entire world, setting all requests to status "Final."

To make a TrueAllele world final, select "Finalize World" from the Global Requests menu. This will change the status of every request in that specific world to final, meaning that the requests cannot be reset or rerun. To finalize only certain requests, choose *Finalize* from the Interpretation Requests menu.

Unfinalize World

In the same way that an owner can set the status of all requests in a world to "Final," they can reverse this action. By selecting "Unfinalize World" from the Global Requests menu, the status of every request in that TrueAllele world will go from "final" to "done." This means the requests can now be reprocessed, if desired.

5 Interpretation Requests

Unlike the Global Requests menu, the Interpretation Requests menu is available to non-owners. This menu allows us to make changes to a subset of requests on a TrueAllele World, as opposed to across all requests.

Reset

At times, we may want to rerun a specific set of requests. This may happen when only specific requests are being used as part of a reproducibility study.

We can reset these specific requests by selecting *Reset* from the Interpretation Requests menu and clicking OK. This action brings up a *Find Request* window identical to the window found in the Review Module. Here, we can enter request criteria to narrow our search. After entering the search criteria, a window opens that is similar to the *Select* window in the Review Module. A new feature is the Reset column. By default, all of the requests that are shown in the window have the reset box checked. Here, we can check only the individual requests that we wish to reset.

We also have the ability to change the parameters of specific requests before we reset them. Once our *Select* window opens, we can click the "Parameters" button, which opens the *Global Parameters* window (Figure 10). In this window, the Setting and Option tabs from the Request module are displayed. Any of these parameters can be changed and are applied to the selected requests. Clicking OK takes us back to the *Select* window; the reset does not occur until OK is clicked in the *Select* window.

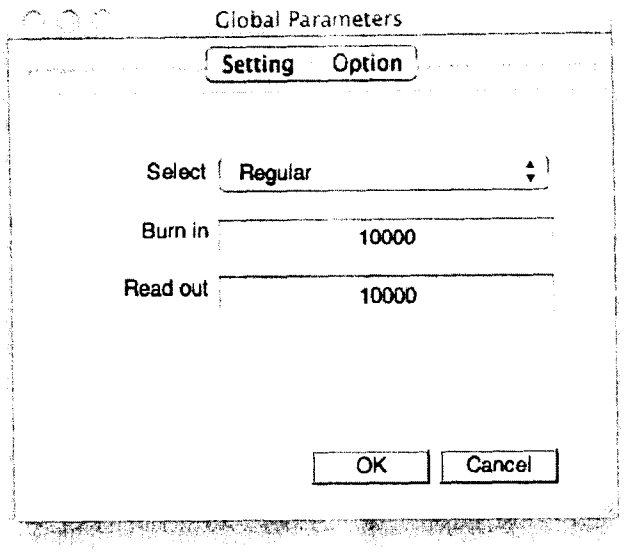


Figure 10. *Global Parameters window*. The parameters for a selected set of requests can be changed and reset.

Activate

At times, only several requests in a TrueAllele World are set up as inactive. Therefore, activating a world globally is not an option. To select specific requests to activate, we can use the *Activate* option from the Interpretation Requests menu.

This action brings up the same *Find Request* window as in the *Reset* option. Just as in the *Reset* option, we can enter our search criteria and check the activate box for those requests that we wish to change from inactive. Note that in the *Select* window, the "isactive" column tells us if the request is already active.

Deactivate

In the same way that we can activate specific requests, we can deactivate requests with the *Deactivate* option from the Interpretation Requests menu. The *Select* window is identical to that of the *Activate* option except that the activate check box is

replaced by the deactivate check box. By default, every request in this window has "deactivate" selected.

Finalize

If multiple cases reside within the same TrueAllele World, we may only want to finalize a subset of requests from the world. We can do this with the *Finalize* option in the Interpretation Requests menu.

After selecting Finalize, the identical *Find Request* window from the previous sections appears. The *Select* window is identical except for the "finalize" column. Again, all of the finalize boxes are checked by default but can be unchecked when not all of the requests in the *Select* window should be finalized.

Unfinalize

To undo a "finalized" request, we can use the *Unfinalize* option under the Interpretation Requests menu. Here, we follow the same steps to unfinalize a request as in the other Interpretation Request menu options.

6 Database Operation

The Database Operation menu tells us information about the server we are working on, as well as the licensing information.

Server Version

Selecting *Server Version* from the Database Operation menu allows us to confirm that our current server version by displaying the version number.

License Information

Selecting *License Information* from the Database Operation menu shows us the total number of users that can be logged on to the server concurrently.

License Usage

Selecting *License Usage* from the Database Operation menu shows us the computers that are currently logged on to the server (Figure 11). Selecting OK opens a window which shows the name of the computers, the process IDs and when the computers logged on to the server. We also have the ability to log any user out of the server by checking the logout box and clicking OK.

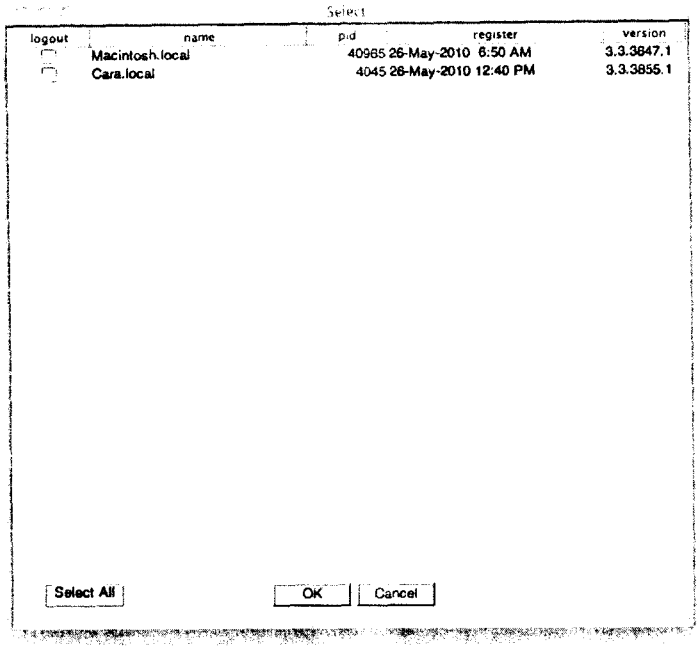


Figure 11. *The License Usage select window.*

7 System Processing

The System Processing menu allows us to see the status of our current requests. We can also check to ensure that the server is working to its fullest capacity.

Server Version

Selecting *Server Version* from the Database System Processing menu allows us to confirm our current server version by displaying the version number.

Request Processing

Selecting *Request Processing* from the System Processing menu allows us to see what requests are solving on the server. A Current Processing window opens, which has a row for each request showing the database name, request ID number, client name and number of total cycles completed.

Interpret Processing

The *Interpret Processing* option under the System Processing menu allows us to check that all of our interpret processes are active. After selecting this option, a message appears telling us the number of interpret processes allocated to the server and the number currently running. When the system is running properly, the processes allocated and processes running on the server should be equal. If the numbers are not equal, please contact Cybergenetics for technical support.

8 VUIer Preferences

This menu is used to set the Connect, Request, and Report preferences for the specific Viewstation™ on which we are working.

Client

When finding a request in the Review Module, having the appropriate Client available in the field's dropdown menu saves time and narrows our search. To change the default client name on a machine, we can select *Client* from the VUIer Preferences dropdown menu. A "client.txt" window appears. Here, we can change the current text from "CLIENT" to the new, desired name and save the file.

Control

When designating parameters at which to run a request, there are four default settings: Reference, Fast, Good and Better. We can change both the cycle numbers and setting names by selecting Control in the VUIer preferences menu. After making any changes, the file can be saved and the new text will be reflected in the Request Module. Please contact Cybergenetics before make any changes to the recommended settings.

Connect

Often there are multiple TrueAllele Worlds on a server, but the worlds may not all be listed in the *Connect* window. To add worlds to the dropdown in the *Connect* window, we can select the *Connect* option from the VUIer preferences menu. The

"connect.txt" file opens, showing four columns: name, url, isdirect and database. The simplest way to add a new world is to copy and paste the information from the first row and replace the "name" and "database" fields. The database field should be the exact name of the TrueAllele World we are adding; the name field can be more descriptive. This file should then be saved, and the new world will appear in the dropdown menu next time the *Connect* window is opened.

Lab

When finding data in the Request Module, having the appropriate Lab name in the dropdown field narrows our search and saves time. To change the default labs available on our machine, we can change the "lab.txt" file by selecting *Lab* from the VUIer Preferences menu. When the text file opens, we can add additional lab names to the file and then save.

Signature

The signature section of the report that is generated in the Report Module is automatically filled from the information found in the signature text file. To create a signature for a specific machine, we can select *Signature* from the VUIer Preferences menu. The "signature.txt" file can be filled in with a name, organization, title and any other information that a user wants to include. The file should then be saved and will be accessed by the system when generating a report.

9 Server Support

9.1 COM Database

The COM database is an administrative database that exists on the TrueAllele Server. This database provides information to the system that allows for automated maintenance and processing. Access to the COM through the Tools Module allows for server upgrades to be conducted from a Viewstation.

Logging into the COM database with a non-owner account will allow access to the Database Operation, System Processing, and VUIer Preference options that are described above. Logging into COM with an owner account adds the Server Upgrade option as well.

9.2 Server Version

Selecting *Server Version* from the Database System Processing menu allows us to confirm our current server version by displaying the version number.

9.3 Server Upgrade

Server Upgrade allows a user to connect to a TrueAllele Server and perform a server upgrade. Cybergenetics will provide support for performing server upgrades when they become available.

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Allegheny County

TrueAllele® Casework Rollout Plan

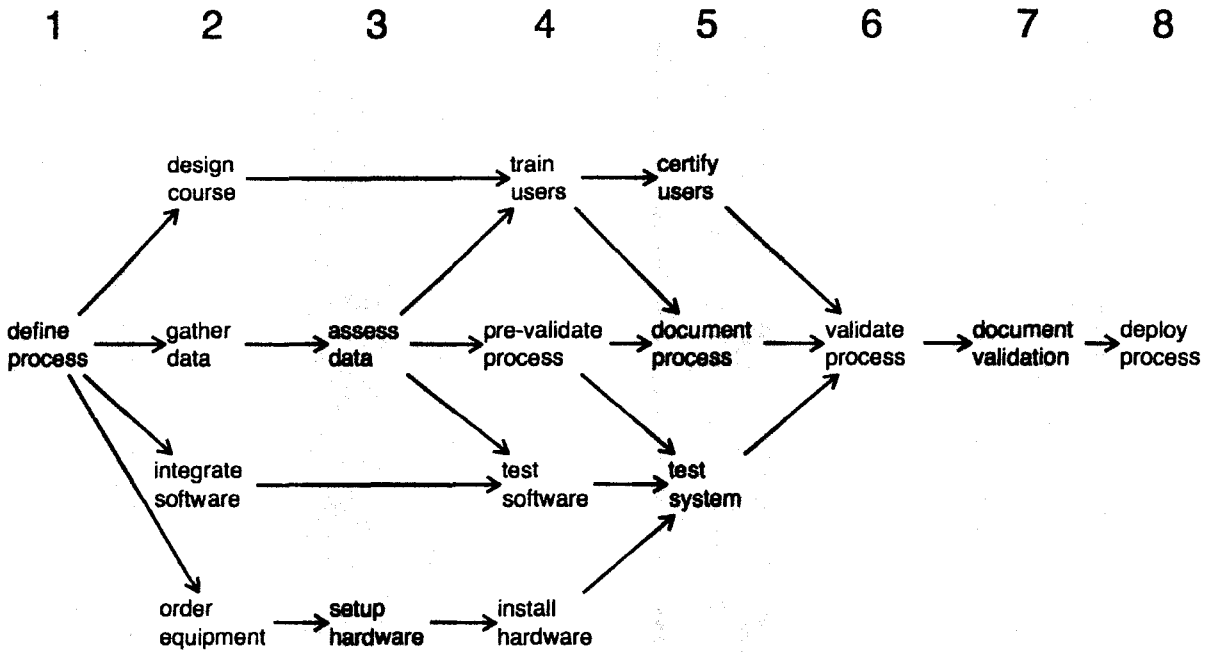
03-Aug-2010

Cybergenetics

Rollout Plan

Task scheduling and dependencies

There are eight rollout stages, each dependent on previous stages.



Roles

There are predefined roles for people having different rollout responsibilities at both the user site and at Cybergenetics.

User

project - A project coordinator responsible for scheduling and assigning action items.

admin - The site's administrative contact person.

legal - A legal contact to aid any legal requirements for the rollout process and data release.

IT - An Information Technology / Information Systems person who facilitates system installation onto the site's network.

LIMS - Typically an external vendor that supports a lab's Laboratory Information Management System.

technical - The technical leader of a laboratory DNA process.

users - The analysts who will be performing the DNA process.

data – The person responsible for supplying data.

Cybergenetics

project - A project coordinator responsible for scheduling and assigning action items.

admin - Cybergenetics' administrative contact person.

science - A scientific leader responsible for the scientific planning and execution of the rollout process.

program - The programmers responsible for software related tasks and integration.

analyst - Cybergenetics analysts who support the TrueAllele process and assess the data.

setup - An IT contact responsible for deploying the system hardware.

trainer - The Cybergenetics training staff who will train and certify users.

support - Support contact responsible for ongoing user support of deployed processes.

data – The person responsible for receiving data.

Tasks

The rollout process is comprised of 17 discrete tasks. Dependent tasks cannot be done until their preceding tasks have been completed. From the initial meeting (stage 1, define process) to the final outcome (stage 8, deploy process), there are eight stages, with tasks grouped by dependencies.

Stage 1

Define process. This planning meeting sets the initial specification that guides the rest of the rollout. The planning meeting minimally includes a Cybergenetics scientist and the user site's technical leader. They complete the rollout plan, and define the TrueAllele DNA interpretation process, taking into account the lab's current practices. They also identify any necessary software integration for the laboratory process. The project data requirements are determined at this point, and so the site's attorneys are contacted to ensure legal clearance for releasing case data to Cybergenetics.

Stage 2

Design course. Cybergenetics designs a weeklong TrueAllele training course focused on a specified DNA interpretation process. The course is adapted to a lab's DNA interpretation needs, based on the planning meeting specification. Customizing TrueAllele training to a lab's process and data facilitates a successful deployment.

Gather data. The site users collect the relevant DNA process data identified during the planning meeting. These case data have multiple rollout purposes, including process customization, system validation, system testing, hands-on software training and proficiency testing. The rollout cannot proceed to the next stage until after the DNA data have been collected and sent to Cybergenetics.

Integrate software. Cybergenetics adapts the software, as necessary, to support the TrueAllele process. Areas of process integration support are identified at the planning meeting. The Cybergenetics lead scientist and programmers meet to determine how to best effect the integration. They then develop a software development and testing plan.

Order equipment. Cybergenetics orders the TrueAllele server and client hardware. To avoid procurement and hardware availability bottlenecks, these orders are placed early on in the rollout in order to avoid delay. To prepare for eventual installation, Cybergenetics discusses the user site server configuration and network integration requirements with IT. The ordered hardware will support the interpretation capacity needed for the defined process.

Stage 3

Assess data. Cybergenetics analysts process all the gathered data in TrueAllele to produce an initial data assessment. This assessment identifies the specific data to be used in the validation studies, user training and for testing software, systems and users. Much of the succeeding rollout effort depends on having these assessed data.

Setup hardware. Cybergenetics installs the TrueAllele Casework software onto the server and client hardware. The server build is a weeklong procedure that installs the operating system, utility functions, database system, MATLAB computational engine and TrueAllele mathematical application software. The hardware is unit tested prior to site installation.

Stage 4

Train users. Cybergenetics trains users on the TrueAllele software with their own data to perform the computer-based DNA interpretation process. The goal of user training is to educate proficient users who can effectively deploy the defined process. The training course provides the necessary background in DNA identification theory, hands-on instruction in TrueAllele software operation, and problem-solving exercises that apply TrueAllele to DNA data situations.

Pre-validate process. Cybergenetics analysts conduct an initial validation on the lab's DNA data. This pre-validation enables Cybergenetics to provide support to the site as they perform their own on-site TrueAllele process validation studies. Cybergenetics validation support includes addressing helping in the study design, adapting the TrueAllele process to the lab's data, preparing a preliminary validation report, and answering user questions.

Test software. Cybergenetics tests the customized TrueAllele software integration on the lab's data. Testing out the defined process helps ensure that the deployment will proceed as planned. The site validation is performed on this fully tested software.

Install hardware. Cybergenetics (or its representatives) install the configured TrueAllele server and client hardware at the site. This installation includes integration of the hardware into the site's computer network, and makes the TrueAllele hardware fully operational for process deployment. Cybergenetics setup team works closely with the user's IT group during this task. The IT group will also provide external secure access to Cybergenetics for remote support. Further, the IT group may need to install Microsoft Office on the ViewStations.

Stage 5

Certify users. Cybergenetics certification ensures that TrueAllele users are fully proficient in their computer interpretation process. Trained users prepare for TrueAllele certification by practicing on case data, and with additional studying. Cybergenetics administers certification tests, comprising problem solving competency tests on case data. Cybergenetics provides follow-on user support for proficient users in a TrueAllele process, particularly during the site's TrueAllele validation study.

Document process. The site's technical leader (and trained users) document their TrueAllele process. They write guidelines and standard operating procedures (SOP) used in process integration, validation and deployment. Cybergenetics provides TrueAllele user manuals that the site can incorporate into their SOPs. Cybergenetics will review the site's completed draft process documentation.

Test system. Cybergenetics conducts a final system test of the installed hardware and integrated software prior validation. The system check uses the case data and TrueAllele databases from the pre-validation study. The user validation is then done on the tested system.

Stage 6

Validate process. The site must validate their TrueAllele interpretation process before it can be deployed. Cybergenetics pre-validation results serve as a useful guide for the lab. The site's trained users conduct their validation on a subset of the gathered lab data set. Cybergenetics provides support for the design and progress of the study.

Stage 7

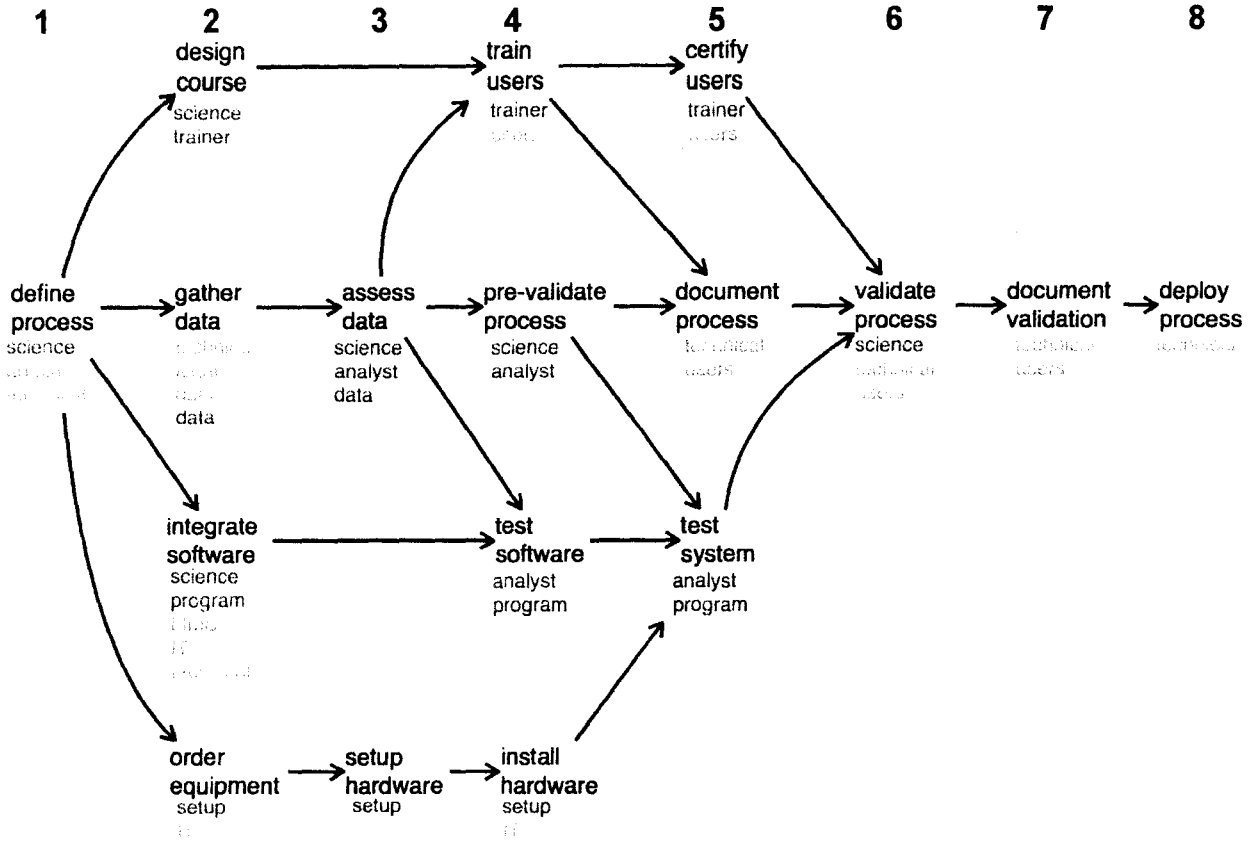
Document validation. A documented validation study is useful when presenting DNA evidence in court. The site's users who performed the validation write up a validation report. Cybergenetics provides technical support. Cybergenetics will review the site's completed draft validation documentation.

Stage 8

Deploy process. The site can deploy their validated TrueAllele process, operated by certified users. Cybergenetics provides user support for certified TrueAllele personnel and processes.

People's roles in the rollout process

This task dependency diagram is annotated with the roles of different people. The Cybergenetics roles are indicated in blue and the user site roles in green.



Schedule

1	2	3	4	5	6	7	8

Specification

To be completed during the planning meeting.

Roles

This section contains the contact information for the staff assigned to each role.

User roles

Project	Jamie
Admin	Jamie
Legal	"Bob" Brigione
IT	Dave Smith
LIMS	"Beast" "STACS"
Technical	Tom
Users	Wally, Nate, Sara, JQ, Tom
Data	Tom

Cybergenetics roles

Admin	Ria David
Science	Mark Perlin
Program	Meredith Clarke
Analyst	Cara Spencer, Jessica Smith
Setup	Matthew Legler
Trainer	
Support	Matthew Legler, Erin Turo
Data	Bill Allan

Discussion

This section outlines each step of the rollout for discussion. Discussion questions and comments are included in some sections.

Define Process

This step is covered by the Process document.

Design Course

The training will cover the entire process including Analysis and decision points in Review.

What hardware is available for training?

Gather Data

A set of data for training and validation will need to be collected by Allegheny County.

Integrate Software

Define a specification for desired output.

Order Equipment

This step has been completed.

Assess Data

Data assessment will be performed on the defined data set.

Setup Hardware

This step has been completed.

Train Users

Schedule the training

Identify trainees.

Pre-Validate Process

Define the exact data set to be used.
Define the measures used for comparison (Information, Time,...).

Test Software

Cybergenetics will test the integrated software with the data Allegheny County has provided.

Install Hardware

This step has been completed.

Certify Users

Schedule the certification.

The certification will be based on problem solving with actual case data.

Document Process

Allegheny County will document the process.

Test System

Cybergenetics will test the system with the data Allegheny County has provided.

Validate Process

Define validation metrics and data set.

Document Validation

The validation team will document the validation process.

Deploy Process

The process can be deployed after a completed validation.

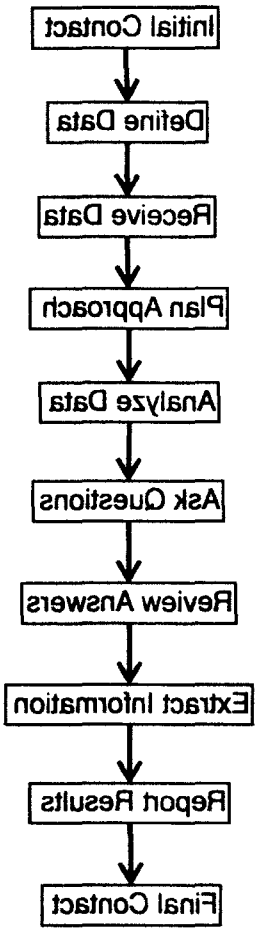
Allegheny County

TrueAllele® Casework Process

02-Aug-2010

Table of Contents

The Ten Step TrueAllele Process Overview



Initial Contact – The initial contact where an analyst first becomes aware of a case for interpretation. Also involve agreeing on the amount of data to process.

Define Data –The front-end processes including determining the panel and controls that will be used during the analysis. Also involves determining what evidence will be interpreted.

Receive Data – An analyst receives the sequencer data in electronic form. At this point an analyst should also receive case context that defines each items role in the case.

Plan Approach – In this step an analyst plans the questions they will ask based on their standard protocols.

Analyze Data – The sequencer data is sized and the controls and allelic ladders are quality checked. The quality checked peak data is then uploaded to the TrueAllele Server.

Ask Questions – The analyst poses questions in the Request module according to the plan.

Review Answers – The analyst reviews the results of interpretation. An analyst is performing a quality assurance check of the interpretation results and may reset a request to allow additional processing time.

Extract Information – Once an analyst has confirmed the results they will select what requests to use for producing a report. Also, in CODIS cases, an analyst may produce CODIS ready output.

Report Results – For cases where a suspect profile is available a Likelihood Ratio (LR) may be produced in the Report module. The analyst can generate report files in this step.

Final Contact – Any final results are gathered and reported to the appropriate contact.

Discussion

This section explores the workflow by walking through each of the ten TrueAllele process steps in the context of the Allegheny County casework process.

Initial Contact

Where do the cases come from?

Define Data

What is the evidence in this process?

Evidence

What is the plate organization?

Verify data information (kit, sequencer).

Could duplicate amplifications be run?

References

What type of references will be available in this process (victim, suspect)?

Receive Data

This step involves moving the electronic sequencer files to the TrueAllele ViewStations.

What will be the source of the data?

How is the sequencer case data organized for the analyst in the lab?

What will be the procedure for gathering the data for TrueAllele processing?

Plan Approach

What references can be provided as knowns?

What are the data parameters (number of contributors, number of items)?

Analyze Data

Who does the Analyze process?

Ask Questions

Who does the Request process?

What references are available?

What data review will an analyst perform?

Review Answers

What information aspects of a request will be reviewed?
- genotype
- mixture weight
- likelihood ratio

When would you examine a request and decide to re-amplify and re-interpret?

Extract Information

After a hit (with LR) is detected then a case can be reported.

What population databases will be used?

Report Results

What goes into the final case report?

Is there an export to CODIS?

Are suspects available for matching?

Final Contact

What goes into the final case folder?

Appendix

Initial Contact

This is the initial contact with a lab and the agreement of what will be analyzed, including the following information:

- Who is the contact?
- Why is the data being brought to us?
- When is the deadline?
- How much data will we be receiving?

Define Data

The "data" is the original DNA sequencer files. The problem-solving context tells why we are interested in this data. The annotating information supports the data and its analysis.

Receive Data

We can obtain the data from the sender in several ways:

- Electronically
- Mailed disk
- Emailed files
- FTP transfer

Data shipping information should be logged upon receipt:

- Who received the data
- When it was received
- Who the data came from

Plan Approach

Standard Operating Procedures will be followed for each case type. These SOPs will be developed and standardized. Electronic documentation forms for planning will be created.

Goals of the Planning phase:

- State the identification hypotheses (who contributed to the evidence?)
- Describe LR formulation of information result
- Will we account for coancestry? If so, at what value?

A specific person will be designated responsible for the case. The following information should be organized for upcoming steps, via the SOP:

- System
- TrueAllele World
- Client
- Lab
- Case name
- Request naming
- Q – questioned evidence
- S – suspect (or reference) profiles
- R – population databases to be used

We should also understand the questioned evidence completely.

- How many items are there?
- Are there multiple cuttings?
- Are there multiple amplifications of the same sample?
- How many contributors are believed to be in this sample?
- Are we looking for a suspect match?

We can discuss or meet about these questions if necessary.

Analyze Data

The purpose of the Analyze Data step is quality control. The EPG signals are transformed into peak events for later processing. Troubleshooting and early problem identification in the data occurs here. This step provides the lab feedback, such as when amplifications fail or sequencer reinjections are necessary. The quality of the control samples is also evaluated.

This step and any findings should be documented. When complete, the quality checked peak data should be uploaded to the designated TrueAllele World.

Ask Questions

The questions that were determined in the planning step should be asked here in the form of Requests. Will requests be uploaded in an automatic batch, or will individual requests be made? Refer to the planning goals when making the requests, and document the requests.

Review Answers

When do we know that we are done? If we were not done, what would we do next? What tools (Review, Report) do we use to understand the answers, and what do they tell us? How does Explain help us understand the inferred genotypes? What does a user actually do here? How does this step support the report writing? How are different problems looked at in different ways?

- Satisfy goals?
- Standardize
- Convergence
- Information
- Meaningful answer
- What did it tell you?
- Review
- Peer Review SOP

Extract Information

What identification information are we trying to extract? How does it relate to our to our goals and plan? When are we done? What do we report when we are done? What do we do if we are not done? What software tools do we use? How do we use these tools (e.g., Report) to explore the inferred identification information? What else should we look at (Data, Explain, Weight) and why?

- Human Identification information relative to a hypothesis
- LR=O(H|d)/O(H)
- Determined by goals

Report Results

Findings are communicated to one person but can be intended for multiple audiences. These findings should answer the goal questions from previous steps. We should confirm the computer results by doing duplicate or longer runs (when applicable). We may not need to report everything; only the essential results should be selected for reporting.

A standardized, tangible document will be created for reporting, which will be dependent on the original task.

- Case - write-up, attachments, CODIS
- Validation - manuscript, tables & figures

A match statistic LR should be reported if there is a reference with which to compare. If a coancestry value was determined, it should be used when reporting results.

A peer review of findings should be completed before results are submitted.

Final Contact

The last step is the completion of the contact loop. The original agreement with the client should be satisfied. The analyst should speak to the contact at the client group.

- How (phone, email, FAX)
- When (time frame, tracking, timeliness)
- What (verbal report, written report, final court-ready)

IN THE PENNSYLVANIA OFFICE OF OPEN RECORDS

IN THE MATTER OF:

CAITRIONA FITZGERALD,
Complainant,

vs. **Docket No.: AP 2015-2380**

ALLEGHENY COUNTY,
Respondent.

PROOF OF SERVICE

I hereby certify that a true and correct copy of the **Statement of Information and Legal Argument** was served upon the persons and in the manner set forth below:

Service By Electronic Mail (E-Mail) and/or First Class Mail Addressed As Follows:

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Date: November 5, 2015

Rachel M. Cipolat, Esquire
Assistant County Solicitor