There are two binders containing the validation. Binder 1 includes Mx for 2-person mixtures, tiered peak height ratios, and MixMaster functionality, cycle, and randomization testing. Binder 2 includes known mixture studies and authentic case samples evaluated through MixMaster as well as Mx Calculator validation.

Validation performed by:

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BFS Identifier Mixture Validation Summaries

Mx, Tiered PHRs, and MixMaster

Because an individual's contribution to a mixed biological sample is generally proportional to their yield in PCR product, the interpretation of DNA results from mixed samples may be further refined through qualitative and quantitative assessments. Examples of such assessments include identification of foreign alleles, minimum peak height ratio expectations, and mixture proportion evaluations. The deconvolution of a mixed DNA sample is the separation of contributors based on such assessments and underlying assumptions.

This validation assessed the reliability of using mixture proportion and minimum peak height ratio expectations for the deconvolution of Identifier mixed DNA profiles. The validation also included the use of the Excel program MixMaster (final version Beta 2.12) to aid in mixture interpretation and a sister tool, Mx CALculator (version Beta 1.0). Summaries of the various studies are described herein.

This validation addresses guidelines specified in the SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (Jan. 2010). These studies also address the developmental validation requirements in Standard 8.2.1h and supplement the previous BFS internal validation of Identifier data interpretation per Standard 8.3.1b 4 of “The FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories” document (Sept. 2011).

Use of Mx for 2-person Mixtures

SWGDAM 3.5. Interpretation of DNA Typing Results for Mixed Samples

An individual's contribution to a mixed biological sample is generally proportional to their quantitative representation within the DNA typing results. Accordingly, depending on the relative contribution of the various contributors to a mixture, the DNA typing results may potentially be further refined.

- **SWGDAM 3.5.3.** A laboratory may define other quantitative characteristics of mixtures (e.g., mixture ratios) to aid in further refining the contributors.

- **SWGDAM 3.5.3.1.** Differential degradation of the contributors to a mixture may impact the mixture ratio across the entire profile.

Mixture proportion (Mx) is the RFU contribution of one individual within the total RFU for all contributors. This is in contrast to donor ratio (DR) which is the ratio of one individual's RFU contribution to another individual's RFU. The purpose of these studies was: 1.) to assess the variation of Mx among the loci of a single amplification, and 2.) to examine how often an Mx range includes the Mx of the true genotype pairing when the average Mx is estimated based upon an incomplete knowledge of the profiles.

Known mixture studies

To address these questions, 46 mixture studies from analysts across BFS were evaluated on 310 (28), 3100 (2), and 3130 (16) Genetic Analyzers. There were 351 ratios that had been amplified at ~ 1 ng and 32 at ~ 0.5 ng total input DNA. A typical set of nine ratios (~ 1 ng) included 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, and 1:19, whereas a typical set of five ratios (~ 0.5 ng) included 9:1, 4:1, 1:1, 1:4, and 1:9. Variation in the observed "true" Mx for each 2-person mixture was assessed using a residuals approach (Gill et al., 1998, FSI).

Three different Mx scenarios were considered:

- 4-allele loci
- 3-allele loci
• 3-allele and 4-allele loci

When there is no assumed contributor, the 4-allele loci are solely relied upon for the Mx calculation. When there is an assumed contributor, the Mx is refined to include not only the true 4-allele pairings but also the 3-allele loci in which the assumed contributor is homozygous; this is called a “4-allele locus” throughout the validation. Both of these situations involve the calculation of an average Mx based solely upon an assessment of loci with no shared alleles.

The 3-allele loci are those in which there may be a homozygous-heterozygous pairing or a heterozygous-heterozygous pairing with a shared allele. Both possibilities are considered when there is no assumed contributor. The use of 3-allele loci is invoked when there is a lack of 4-allele loci. An example of when this might occur is in a mixture of a parent and child, where it is expected that the two will share an allele identical by descent at each locus and they may share an additional allele identical by state. Another situation where this may be useful is with a mixture that has only one locus with four alleles, especially if this locus has the minor alleles in stutter positions of the major alleles.

Table 1 summarizes the observed Mx variation when considering each of the different Mx scenarios, with and without assumed contributors. In each scenario, the majority of individual locus Mx values varied by < ± 0.2 from the average corresponding Mx.

Table 1 – Known Mixture Study Data

<table>
<thead>
<tr>
<th>Average Mx</th>
<th>Assumed contributor?</th>
<th>Mx Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-allele*</td>
<td>Both</td>
<td>All data points &lt; ± 0.23</td>
</tr>
<tr>
<td>4-allele</td>
<td>No</td>
<td>All data points &lt; ± 0.23</td>
</tr>
<tr>
<td>4-allele</td>
<td>Female</td>
<td>All data points &lt; ± 0.23</td>
</tr>
<tr>
<td>4-allele</td>
<td>Male</td>
<td>All data points &lt; ± 0.23</td>
</tr>
<tr>
<td>3-allele</td>
<td>No</td>
<td>All data points &lt; ± 0.3</td>
</tr>
<tr>
<td>3-allele</td>
<td>Female</td>
<td>All data points &lt; ± 0.3 except one -0.34 @ Amelogenin</td>
</tr>
<tr>
<td>3-allele</td>
<td>Male</td>
<td>All data points &lt; ± 0.3</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>No</td>
<td>All data points &lt; ± 0.23</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Female</td>
<td>All data points &lt; ± 0.23 except the one -0.34 @ Amelogenin</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Male</td>
<td>All data points &lt; ± 0.23</td>
</tr>
</tbody>
</table>

* When both contributors were assumed to be present, all loci in which the contributors did not have identical genotypes were used.

The following figures represent a subset of the scenarios outlined above in Table 1.
Mx variation when the genotypes of both contributors are known (a best-case scenario) is shown in Figure 1.

**Figure 1**

*Mixture Proportion Variation (Mixture Studies)*

Mx variation is shown in Figure 2 when the genotypes of neither contributor are known and using only the 4-allele loci:

**Figure 2**

*Mixture Proportion Variation (Mixture Studies)*
Shown in Figure 3 is the Mx variation when the genotypes of neither contributor are known and the average Mx is based solely upon 3-allele loci:

**Figure 3**

**Mixture Proportion Variation (Mixture Studies)**

![Mixture Proportion Variation (Mixture Studies)](image)

Shown in Figure 4 is the Mx variation when the genotypes of the female contributor are assumed and the average Mx is based solely upon 3-allele loci. Note the one data point outside the ±0.3 range is from Amelogenin.

**Figure 4**

**Mixture Proportion Variation (Mixture Studies)**

![Mixture Proportion Variation (Mixture Studies)](image)
Shown in Figure 5 is the Mx variation when the genotypes of the male contributor are assumed and the average Mx is based solely upon 3-allele loci:

**Figure 5**

Mixture Proportion Variation (Mixture Studies)

![Mixture Proportion Variation (Mixture Studies)](image)

Shown in Figure 6 is the Mx variation when the genotypes of neither contributor are known and the average Mx is based upon both 4-allele and 3-allele loci:

**Figure 6**

Mixture Proportion Variation (Mixture Studies)

![Mixture Proportion Variation (Mixture Studies)](image)
Overall, a majority of data points were within a ±0.2 range for each scenario examined and all but one (Amelogenin when including 3-allele loci and the female as an assumed contributor) were within a ±0.3 range.

An Mx average based solely upon 4-allele loci when there is no assumed contributor may be underestimated. Because the Mx is calculated for the minor contributor, the two peaks with the lowest RFU are used in the numerator and may not truly pair. This may lead to an underestimation of the true Mx. The effect of this is most pronounced when approaching an Mx of 0.5 as the minor contributor may truly be the major contributor at another locus. Nevertheless, the average Mx was shown to closely model the respective individual locus Mx values, with the majority of data points falling within a ±0.2 range.

For any instances where it may be desirable to estimate the Mx using the maximum number of loci, an average Mx incorporating all 3-allele and 4-allele loci can be calculated by combining the different scenarios (i.e., the 3-allele and 4-allele loci above). While the addition of the 3-allele loci seemed to reduce the accuracy of the average, incorporating 3-allele loci into the average Mx will increase the number of mixtures that can be deconvolved. Only subtle differences were noted between the 3-allele and 4-allele combined approach versus the use of only 3-allele loci.

When including the Mx values for 3-allele loci and accounting for the female as an assumed contributor, a data point was calculated beyond the ±0.3 range at approximately 0.34. This was at the Amelogenin locus, which MixMaster does not include in deconvolution due to increased PCR and genetic variability at this locus.

Authentic case samples
Mx variation was further assessed by evaluating 2-person mixtures from 79 authentic cases using data from 310 (17), 3100 (16), and 3130 (46) Genetic Analyzers. These mixtures were considered “solved,” a majority of which comprised a simple sexual assault situation in which an epithelial cell fraction (containing DNA from the victim and sperm DNA carryover) was used as the mixture in question. A victim reference profile was the assumed female contributor and a single-source sperm fraction result was the assumed male profile.

Table 2 summarizes the variation results when considering each of the different Mx scenarios, with and without assumed contributors, for the tested case sample mixtures. In each scenario, the majority of individual locus Mx values varied by < ±0.2 from the average Mx.

Table 2 – Case Sample Data

<table>
<thead>
<tr>
<th>Average Mx</th>
<th>Assumed contributor?</th>
<th>Considering Mx variation, all data points &lt; ±0.3 except:</th>
<th>Number of Amelogenin data points &gt; ±0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-allele</td>
<td>Both</td>
<td>One - Amelogenin</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>4-allele</td>
<td>No</td>
<td>Three - 2 Amelogenin, 1 D19S433</td>
<td>2/3 (66%)</td>
</tr>
<tr>
<td>4-allele</td>
<td>Female</td>
<td>Two - both Amelogenin</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>4-allele</td>
<td>Male</td>
<td>Two - both Amelogenin</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>3-allele</td>
<td>No</td>
<td>Six - 2 Amelogenin, 1 D19S433, 1 D16S539, 1 TPOX, 1 D2S1338</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>3-allele</td>
<td>Female</td>
<td>Three - 2 Amelogenin, 1 D2S1338</td>
<td>2/3 (66%)</td>
</tr>
<tr>
<td>3-allele</td>
<td>Male</td>
<td>Five - 3 Amelogenin, 1 D19S433, 1 D2S1338</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>No</td>
<td>Five - 2 Amelogenin, 1 TPOX, 1 D2S1338, 1 D8S1179</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Female</td>
<td>One - Amelogenin</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Male</td>
<td>Four - 3 Amelogenin, 1 D19S433</td>
<td>3/4 (75%)</td>
</tr>
</tbody>
</table>

The following figures represent a subset of the scenarios outlined above in Table 2.
Mx variation when the genotypes of both contributors are known (the best-case scenario) is shown in Figure 7.

Figure 7

Mixture Proportion Variation (Cases)

shown in Figure 8 is the Mx variation when the genotypes of neither contributor are known and the average Mx is based solely upon 3-allele loci:

Figure 8

Mixture Proportion Variation (Cases)
Shown in Figure 9 is the Mx variation when the genotypes of neither contributor are known and the average Mx is based upon 3- and 4-allele loci:

**Figure 9**

![Mixture Proportion Variation (Cases)](image)

In terms of outliers, most were observed when no contributor was assumed to be present, whereas fewer outliers were observed when accounting for at least one known contributor. Of the outliers that were $\geq \pm 0.3$, all were $\geq +0.3$, which means the calculated Mx average was lower than expected. All case sample outliers $> 0.3$ were accounted for by four cases. In two of the four cases, the only locus outside the $\pm 0.3$ range was Amelogenin; the known contributors for these two cases were correctly included when examined in MixMaster.

In one of the other two cases, differential degradation was observed in the electropherogram results. When assessed in detail through MixMaster, inclusion results varied from the expected results when relying on 3-allele loci only; see Table 3 below.

**Table 3 – Case 3 Data**

<table>
<thead>
<tr>
<th>Average Mx</th>
<th>Assumed contributor?</th>
<th>Known(s) included?</th>
<th>All data point variation less than $\pm 0.3$?</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-allele</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele</td>
<td>No</td>
<td>No – one or both excluded</td>
<td>No (D2S1338)</td>
</tr>
<tr>
<td>4-allele</td>
<td>Male</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Male</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele</td>
<td>Male</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>4-allele</td>
<td>Female</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Female</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele</td>
<td>Female</td>
<td>No – male excluded*</td>
<td>No (D2S1338)</td>
</tr>
</tbody>
</table>

*Major/minor flipped; inclusion contingent upon analyst observation.*

When plotting the average molecular weight for the alleles at each locus (x-axis) versus the true Mx variation (using both known contributors), the overall pattern is generally consistent with differential degradation; see
Figure 10 below. It is recommended that great caution be exercised in using an Mx mixture deconvolution approach with differentially degraded samples.

For comparison, Figure 11 shows a known mixture designed to have differential degradation.
Finally, the fourth case was also closely examined. Differential degradation may be contributing to the issue but a clear pattern was not apparent. For example, the victim’s Mx is 0.715 at D19S433 (lowest MW locus in yellow) and decreases to 0.271 at D18S51 (highest MW locus in yellow), while the Mx at D5S818 (lowest MW locus in red) is 0.287. When assessed in detail through MixMaster, the following summarized results were obtained:

Table 4 – Case 4 Data

<table>
<thead>
<tr>
<th>Average Mx</th>
<th>Assumed contributor?</th>
<th>Known(s) included?</th>
<th>All data point variation less than ±0.3?</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-allele</td>
<td>No</td>
<td>No – both excluded at D19S433</td>
<td>No (D19S433, Amelogenin)</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>No</td>
<td>No – both excluded at D19S433</td>
<td>No (D16S539, D19S433, Amelogenin)</td>
</tr>
<tr>
<td>3-allele</td>
<td>No</td>
<td>No – both excluded at D19S433</td>
<td>No (D16S539, D19S433, Amelogenin)</td>
</tr>
<tr>
<td>4-allele</td>
<td>Male</td>
<td>Yes</td>
<td>No (Amelogenin)</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Male</td>
<td>Yes – with maximum stutter subtraction at D19S433</td>
<td>No (D19S433, Amelogenin)</td>
</tr>
<tr>
<td>3-allele</td>
<td>Male</td>
<td>No – female excluded*</td>
<td>No (D19S433, Amelogenin)</td>
</tr>
<tr>
<td>4-allele</td>
<td>Female</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele &amp; 4-allele</td>
<td>Female</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3-allele</td>
<td>Female</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Major/minor flipped; inclusion contingent upon analyst observation.

In Figure 12, the average molecular weight for the alleles at each locus (x-axis) was plotted for this fourth case versus the true Mx variation (using both known contributors). Differential degradation is possibly seen for loci with no overlapping alleles, but an overall pattern is not otherwise evident. The overall range observed was 0.715 (D19S433) – 0.271 (D18S51) = 0.444, which is within a ±0.3 range. However, the average Mx was not in the middle of the actual variation and was driven downward when there was no assumed contributor, especially when including 3-allele loci.

Figure 12
**Overall Mx Conclusions**

Locus Mx variation generally ranged between ±0.3 from the profile average Mx, with almost all of the data points falling between ±0.2 from the profile average. For mixtures involving relatively intact DNA (i.e., not degraded), it was acceptable to estimate the average Mx using the 4-allele, 3-allele, and 3-allele/4-allele scenarios, with or without an assumed contributor. These scenarios captured all STR results in the known mixture studies, and almost all of the STR results in the authentic case mixtures.

Care should be taken to watch for signs of differential degradation in case samples. When a mixture is affected by this phenomenon, and there is no assumed contributor, miscalls could occur. Similarly, Mx (and PHR) values for highly degraded mixtures with loci exhibiting widely spaced alleles may also be distorted, again leading to miscalls. The presence of an assumed contributor does not guarantee that the average Mx will adequately center the range for all loci. However, in a real case situation, careful examination of the assumed donor’s genotypes would identify a miscall. Given the discriminating power of STR loci, it is worth noting that these miscalls were false exclusions and are likely to be identified should miscalls result with other challenging case samples of this sort.
Tiered PHR Minimums for 2-person Mixtures

**SWGDAM 3.3.1.** The laboratory should establish PHR requirements based on empirical data for interpretation of DNA typing results from single-source samples. Different PHR expectations can be applied to individual loci (e.g., 70% for D3S1358, 65% for vWA, etc.); alternatively, a single PHR expectation can be applied to multiple loci (e.g., 60%).

- **SWGDAM 3.3.1.1.** The laboratory may evaluate PHRs at various DNA template levels (e.g., dilution series of DNA). It is noted that different PHR expectations at different peak height ranges may be established.
- **SWGDAM 3.3.2.** PHR requirements are only applicable to allelic peaks that meet or exceed the stochastic threshold.

Peak height ratios (PHRs) calculated from 22 Identifiler sensitivity studies run on 310 and 3130/3130xl Genetic Analyzers are plotted below in Figure 13.

**Figure 13**

Based on this data, the expected PHR minimums were established as follows:
- When the peak height of the taller allele is 1000 RFU or greater, the minimum PHR is 50%.
- When the peak height of the taller allele is 500 RFU - 999 RFU, the minimum PHR is 40%.
- When the peak height of the taller allele is 200 RFU - 499 RFU, 30% is the minimum PHR.
- When the peak height of the taller allele is below 200 RFU, any PHR is accepted.
These tiers are expected to account for the majority of PHRs routinely observed. However, outliers are expected due to the variable nature of the polymerase chain reaction. This data set included a few such data points. For example, two data points exhibiting dropout (sister peaks at ~30 RFU and 45 RFU) were observed to be greater than 200 RFU. The lowest PHR tier is based on the pre-established stochastic threshold of 200 RFU for the Identifiler PCR Amplification Kit. These outliers reinforce the need for careful examination of low-level results.

**MixMaster**

MixMaster is a two-person mixture deconvolution Excel tool that has been validated for use with Identifiler results. MixMaster is not an expert system. It is not meant to replace the analyst's assessment and judgment. MixMaster is a calculation tool that mimics the manual deconvolution approach.

**MixMaster Functionality**

Functionality testing of MixMaster involved the “function” testing of the program under numerous scenarios for the purpose of ensuring proper operation of the various parameters involved in a run through the program. Parameters tested included: stutter (N-4, N+4), Mx (locus, average, range), Mx override, PHRs, PHR range, analytical threshold, artifact slots, wild card slots, report features, import function, and Mx function with a partial assumed donor. This testing spanned three versions of MixMaster: 2.7, 2.8, and 2.9. Versions 2.8 and 2.9 were created to address four programming issues encountered during the functionality testing of versions 2.7 and 2.8. Each issue was successfully resolved through the revision updates and all parameters were determined to function properly.

**MixMaster Cycle Number Testing**

The purpose of cycle number testing was to determine the minimum number of randomized cycles for which MixMaster will reliably capture the correct genotypes for both DNA contributors to a mixture. Thirteen mixtures from 13 different cases were used for this testing. The PHRs used in MixMaster were adjusted to match those of previous manual analysis. Differences in genotype calls were noted due to the use of donor ratios in the manual analysis, versus Mx in MixMaster. Additionally, there was one case mixture that had a difference attributable to the use of randomized cycles (MixMaster) versus a worst-case scenario calculation (manual approach). Based on 30 runs of this mixture, and the cumulative runs of the 12 other mixtures, a minimum cycle number of 2500 was suggested for use with MixMaster.

**MixMaster Randomization Testing**

For peaks that overlap stutter and/or INA and/or “artifact” (non-stutter and not-INA) positions, the amount of the peak considered to be an allele will vary depending upon how many RFU are subtracted for each applicable artifact type. Rather than simply pick one average value to subtract, MixMaster will apply randomized amounts within each variable’s range at each cycle.

An additional use of the MixMaster randomization cycling feature is applied to the division of shared alleles. Rather than treat each person as an equal (or even a proportional) contributor to a shared allele, MixMaster cycles through randomized levels of contribution.

This study examined the VBA (Visual Basic for Applications) code, looking for bias in the VBA formulae using the Excel randomization function. Parameters examined included rounding, cycle Mx, stutter subtraction, F alleles, and artifact alleles; cycle Mx is a random Mx selected from the set range around the average Mx and is used by MixMaster for the division of shared alleles. The randomization functions in the VBA code for MixMaster were found to behave in the appropriate manner.
MixMaster Mixture Validation Studies

Eighteen 2-person mixture studies were evaluated through MixMaster: 15 from Identifiler caseworker training sets from across the Bureau and three from different internal validation studies conducted at the Richmond Laboratory. Each data set included various ratios tested within the range of ~0.5 to 1 ng total input DNA. Most data sets included the ratios 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, and 1:19, while some were subsets of this and others included variations on the same general range. The vast majority of these data sets were tested with and without assumed contributors. The expected genotypes, based on the known contributors, were obtained from each mixture result, with one exception in which an allele at 232 RFU should have been paired with a sister allele that had dropped out (~30 RFU). This exception was an issue related to the stochastic threshold, not MixMaster.

MixMaster Case Validation Studies

One hundred thirty-two case sample evaluations were performed on sixty authentic case samples, the vast majority of which were tested with and without an assumed contributor(s). Of these 132 evaluations, 129 were concordant with previously reported results and the expected genotypes of known contributors. The three discordant results were attributable to one particular sample (no assumed, female assumed, male assumed) that showed differential degradation (see Case 4 described under Use of Mx for 2-Person Mixtures, Authentic case samples).

Mx CALculator Validation

Mx CALculator is an Excel tool that may be used to calculate 3-allele, 4-allele, and 3-allele/4-allele Mx values. The expected common use of this tool will be to calculate an Mx Override for MixMaster. This is typically needed for two-person mixtures that lack loci with four detected alleles (including three alleles with a homozygous assumed contributor). An example of when this might occur is in a mixture of a parent and child, where it is expected that the two will share an allele identical by descent at each locus and they may share an additional allele identical by state. Another situation where this may be useful is with a mixture that has only one locus with four alleles, especially if this locus has the minor alleles in stutter positions of the major alleles.

Mx CALculator considers both a heterozygous genotype combined with a homozygous genotype and two heterozygous genotypes that overlap at one allele. The rules of Mx CALculator were originally tested as part of the Mx validation studies (“3-allele loci”) and eventually incorporated into this Excel tool and the technical procedure.

The correct functioning of Mx CALculator was assessed in situations from both mixture study samples and an authentic case sample in which MixMaster was unable to calculate a locus Mx. Testing included four mixture study samples encompassing 15 3-allele loci and one case sample that had four 3-allele loci. For each of the five samples, 3-allele locus Mx values were calculated by hand (following Section 3.6.6. Appendix I of DNA Technical Procedure Volume I Revision 14) and compared to the values obtained from Mx CALculator. This included 14 heterozygote-homozygote pairings and 30 heterozygote-heterozygote pairings. Concordant results were obtained for each pairing at each locus. The average Mx from Mx CALculator was then entered into MixMaster as an Mx Override for each sample and the correct genotyping results were obtained.