NicheVision Sole Source Justification
STRmix Mixture Interpretation Software
Justification for Non-Competitive Procurement

Award Number:	2013-DN-BX-0074
Is the award open?	Yes
Type of Procurement	Non-competitive bid (Sole Source)
Does the budget include purchases with a single vendor that totals over $ 100,000?	Yes
Are there vendors in the budget which may cumulatively reach $ 100K?	Yes

Brief description	The objective of the Bureau of Forensic Services Laboratories (CA DOJ) funded by award number 2013-DN-BX-0074 is to handle, screen, and analyze backlogged forensic DNA casework and databank samples and to improve DNA laboratory infrastructure and analysis capacity so that forensic DNA samples can be processed efficiently and cost effectively, and so that future backlogs can be prevented. One specific goal is improving mixture interpretation capabilities within the casework section. CA DOJ has developed rapid-processing programs for sexual assault evidence that require assault-to-CODIS search turnaround times of less than a month, and sometimes as short as two weeks. Manual mixture interpretation can be a laborious, time consuming process that is not amenable to such rapid processing. To that end, CA DOJ previously implemented an in-house mixture interpretation tool that applies our current binary, threshold based protocol to two-person mixtures. The field of forensic DNA testing in the United States, however, is starting to move beyond binary approaches, and complex mixtures with more than two contributors are an ever-present challenge. This sole source relates to the purchase of mixture interpretation software that will integrate well into the current suite of kits, instrumentation, and data processing software already in use at CA DOJ and familiar to our criminalists. The CA DOJ Bureau of Forensic Services has evaluated two mixture interpretation software packages, TrueAllele and STRmix. Since the beginning of our evaluation of TrueAllele, the STRmix software has become commercially available. STRmix, developed by ESR and

	distributed in the United States by NicheVision, uses the same fundamental statistical concepts as TrueAllele. After extensive testing over the course of a year, STRmix was found to be a better fit to our needs and goals. Under this project, the laboratory will make expenditures with NicheVision in the equipment, training, and maintenance categories. The contracts/purchases are anticipated to total over $100,000. NicheVision is the only vendor that can provide the required items and services in order for the laboratory to meet project goals and continue to be in compliance with the guidelines defined in the grant solicitation.

Explanation of dollar amounts of the sole source request:

2013 Budget Line No	Instrument or Equipment Item	Cost per Unit	# Units	Define Unit	Vendor	Cost
430	*STRmix user licenses*	*$ 15,000.00*	*20*	*each*	*Niche Vision*	$300,000.00

2013 Budget Line No	Instrument or Equipment Item	Cost per Unit	# Units	Define Unit	Vendor	Cost
432	*STRmix Training*	*$ 45,000.00*	*1*	*3 days of training for up to 20 people*	*Niche Vision*	$45,000.00

2013 Budget Line No	Instrument or Equipment Item	Cost per Unit	# Units	Define Unit	Vendor	Cost
431	*STRmix Support package*	*$ 16,000.00*	*1*	*80 hours of software and scientific support*	*Niche Vision*	$16,000.00

Explanation of why it is necessary to purchase noncompetitively.	As with any crime laboratory, the Bureau of Forensic Services finds that many of the samples we test consist of complex mixtures (e.g., degraded and/or with more than two contributors.) The previous decision to pursue TrueAllele Casework, and not other available software, as an interpretation assistant was made with the hope that such challenging samples could be interpreted with precision and sensitivity. However, after comparing TrueAllele to STRmix, we determined that STRmix was better suited to our goals. Three key factors in this decision are 1) increased sensitivity, especially with degraded samples; 2) increased precision when maintaining a set number of runs; and 3) decreased

	interpretation time due to reduced run time and fewer re-runs. 1) Increased sensitivity, especially with degraded samples Degraded samples are a common observation in forensic DNA testing. Especially challenging instances involve what is sometimes referred to as differential degradation where the different contributors' DNA are each degraded to a different extent. This can happen when the biological material consists of different tissue types and/or the various contributors' biological material was deposited at different times. In some circumstances, the result can be a DNA profile in which an individual might be a majority contributor to the results in one molecular weight range, but a minority contributor in another molecular weight range. This phenomenon can be difficult or impossible for an examiner to discern without the assumption of one or more of the contributors' genotypes. Both STRmix and TrueAllele can incorporate an assessment of degradation and differential degradation into their process. For STRmix, it is automatic. For TrueAllele, it is an optional setting. Testing a series of two-person mixtures with differential degradation as described above, STRmix was clearly better at detecting and adjusting for this. STRmix had 0 Type I errors, here defined as likelihood ratios (LR) below 1.0, while TrueAllele had Type I errors in 18% of the contributor-to-mixture comparisons. Additionally, a number of TrueAllele interpretations did not include the true contributors' genotypes. This was masked in the reporting statistic by TrueAllele's minimum locus LR threshold of 0.01. Overriding the LR threshold, Type I errors increased to 60% with 56% of the comparisons being complete exclusions (LR = 0.) Similar results were observed for a 3-person, 6:3:1 mixture with the middle contributor degraded. Type I errors occurred in 3.7% of the STRmix comparisons, the one error being a complete exclusion for the degraded donor. TrueAllele had no Type I errors under the standard settings, but 56% of the comparisons were Type I errors (all complete exclusions) when overriding the LR threshold. Our current process has been shown to be relatively robust and effective for interpreting 2-person mixtures, albeit without the benefits that come with a probabilistic assessment of peak height ratios, stutter, and drop-out. However, we remain severely limited in how we can address 3-person mixtures. For the validation at hand, multiple sets of 3-person mixtures were created using four different ratios (1:1:1, 8:1:1, 6:3:1, and 4.5:4.5:1) at three different total template quantities (1.5 ng, 0.75 ng, and 0.375 ng.) Replicate interpretations were performed on each mixture using STRmix and

	TrueAllele, with the goal of comparing precision and sensitivity. In this study, 3.4% of the overall STRmix comparisons were Type I errors. The two interpretations that lead to complete exclusions were due to a limitation in the Java programming environment, and a clear diagnostic was discovered with which to evaluate and prevent that issue. The remaining Type I errors occurred in the low template samples (0.375 ng amplifications), primarily with minor donors that contributed approximately 6 diploid cells worth of DNA to the mixture. 5.1% of TrueAllele comparisons were Type I errors under standard settings, which is similar to the levels observed for STRmix. However, overriding the LR threshold increased TrueAllele Type I errors to 9.2%, with 5.6% of comparisons being complete exclusions. 2) Increased precision Randomness is one of the hallmarks of the Markov Chain Monte Carlo (MCMC) process at the heart of both STRmix and TrueAllele. Because of that, it is expected that successive MCMC runs using the same starting profile will give somewhat different results. The magnitude of these differences, however, is ideally kept as low as possible. Comparing log(LR) values between replicates, precision was measured as the proportion of replicate pairs that differed by less than 1 log unit (i.e., a factor of 10 difference in the LRs.) The data from the differential degradation and 3-person mixture studies were used for this purpose. With the differential degradation samples, 1.6% of STRmix pairs differed by more than one log unit, while 46% of TrueAllele pairs differed by more than one log unit. Overriding TrueAllele's LR threshold decreased this to 38%, but that was due at least in part to replicates that were both now complete exclusions (i.e., both replicates were LR = 0.) 3) Decreased interpretation time due to reduced run time and fewer re-runs. Both STRmix and TrueAllele were tested using settings designated as appropriate for challenging samples, under the theory that an evidence sample may appear to be simpler than it truly is. With these settings, TrueAllele required significantly longer analysis times when compared to STRmix. The mixture interpretation phase for a 2-person mixture using TrueAllele typically required an overnight analysis (8+ hours), while STRmix would be complete within minutes. For 3-person mixtures, TrueAllele's analysis time increased to 3-4 days, while STRmix run times ranged from minutes to less than 1 day. TrueAllele has the capability to

	interpret multiple samples at the same time, but the purchase of multiple STRmix licenses yields the same result. During the studies of sensitivity and precision, the diagnostic tools available in TrueAllele suggested, albeit on a somewhat subjective level, that the MCMC process had not always proceeded in an ideal manner, and reruns could be in order. These questionable results at times contributed to poor precision between replicates. Other times, it led to replicates that were very precise but with poor sensitivity. Examples were also observed of identical results occurring in sample types where that would not be expected, despite the randomness associated with MCMC. As a counterpoint, STRmix results rarely warranted re-runs. Where they were called for in this study, the diagnostic was objective, and the time required to complete the reanalysis was lower than with TrueAllele. Overall, we found that STRmix will give more reproducible and sensitive results with fewer reanalyses.
Timeliness	The project period for award 2013-DN-BX-0074 is set to end on March 31, 2015. The purchases will be made very soon after the Sole Source GAN is approved. Implementation of STRmix should be more rapid than with TrueAllele. TrueAllele requires the use of its own data analysis software. Raw files from genetic analyzers are imported into TrueAllele for fragment sizing and comparisons to allelic ladders. In contrast, STRmix would simply replace our existing mixture interpretation software, stepping in at the same point of our well established process, while maintaining all other components. As with our current system, data created by our genetic analyzers would be processed through GeneMapper ID or ID-X, and peak tables would be exported for import into the mixture interpretation software. By maintaining much of our current work flow, we anticipate both a reduction in required training and an increase in initial comfort level with the process. Without the ability to purchase the STRmix within the project period, the goals of the project would not be met.
Uniqueness	There are still only two mixture interpretation systems commercially available from US distributors that allow for more than three contributors, automatically consider stutter artifacts as possible alleles, use fully probabilistic interpretation approaches, and automatically account for degraded DNA. Regarding the number of contributors allowed, TrueAllele Casework will allow for up to 6 unknowns, while STRmix will test

	up to a 4-person mixture. We feel it is clearly a benefit to be able to test mixtures with more than three contributors, but the general consensus among criminalists within our bureau is that there is less of a desire to interpret mixtures of 5+ individuals. Beyond the results of our comparison studies, one additional factor distinguishes STRmix: STRmix is a standalone piece of software that can be loaded on to any of our agencies current laptops or desktops. Should the computer cease to function, all that is required to continue operations is to load the STRmix onto another computer and acquire a new license file from NicheVision. With multiple copies of STRmix in each of our DNA testing laboratories, this process should not interfere with casework processing. TrueAllele is server based, and the server and software are purchased as a unit from Cybergenetics. Should a server crash, interpretation of new mixtures would cease to happen until the server could be repaired or replaced.
Other	Concerns were raised about the sizing and allele calling approach in TrueAllele. The data analysis component of that system is such that capillary data is converted into a virtual gel, and the sizing algorithm takes adjacent lanes into account. Despite the data being from a capillary, the size of fragments can vary depending upon the other samples imported into this virtual gel. Similarly, heights and allele designations were seen to occasionally change from the values listed prior to upload to the server and those listed after processing in the server. This was mostly limited to low RFU peaks, but in a system that examines peaks down to a 10 RFU threshold, this remains a concern. In contrast, the source of size and allele designations for STRmix is GeneMapper, and our experience has shown this software package to be completely uniform when analyses are performed under identical settings. As with other crime laboratories throughout the country, our agency is anticipating a transition to a new autosomal STR multiplex. However, it is not yet decided which one we will use. New multiplexes can be added to STRmix in-house, without the assistance of NicheVision or ESR. TrueAllele, on the contrary, is the source of all software updates for new typing systems, both multiplexes and capillary electrophoresis systems. We favor the ability to add systems at our discretion. We acknowledge that there are free software systems for the interpretation of complex mixtures, but to date these are limited to systems that apply very rough approximations to complex issues. The shift from our current approach to one involving probabilistic genotyping will require a significant commitment from our caseworkers. It is in our best interest to make such a change to a

	package that is leading the movement.
Declaration that this action is in the best interest of the agency	This expenditure is in the best interest of the Bureau of Forensic Services CA DOJ. Our current approaches are both time consuming and highly limiting as to the amount of information that can be obtained from complex DNA mixtures. The STRmix software will greatly increase our capacity and capabilities.