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September 19, 2016

Department of Forensic Biology is Implementing New Technologies – a New STR Kit, a New STR Analysis Software and a New Probabilistic Genotyping Software

Starting this month, the OCME is training its staff and starting to implement a trio of new technologies. The suite of products is the Promega PowerPlex Fusion STR amplification kit, SoftGenetics GeneMarker HID analysis software and STRmix fully continuous probabilistic genotyping software.

New Technology	Retiring Technology
Promega PowerPlex Fusion	ABI AmpFlSTR Identifiler
SoftGenetics GeneMarker HID	ABI GeneMapper ID
STRmix	RMP, Forensic Statistical Tool (FST)

New STR Kit
Promega PowerPlex Fusion is replacing ABI AmpFlSTR Identifiler.

Since 2015, the OCME has been performing internal validation studies on a new STR Kit. The kit we have chosen is the Promega PowerPlex Fusion kit. This kit is designed to meet the new CODIS and European standards to achieve the most inter-database compatibility and highest discrimination of any autosomal STR kit. The kit contains 24 loci – 22 autosomal loci, 1 Y-STR locus and Amelogenin sex determination locus. As comparison, the ABI AmpFlSTR Identifiler kit contains 16 loci – 15 autosomal loci and Amelogenin sex determination locus. Therefore, we will generate more genetic information for each sample we analyze.

New STR Analysis Software
GeneMarker HID is replacing GeneMapper ID.

GeneMarker HID is a DNA genotyping analysis software program developed and sold by SoftGenetics, LLC. It takes the sample files from the capillary electrophoresis collection software and depicts the DNA sample in a visual, graphical format. GeneMarker HID is used to identify the fragments based on their size and illustrates them into a DNA profile. At this point, scientists can view the DNA profiles, interpret them, and identify if samples are from male or female donors, or if the samples are from a mixture of male(s) and/or female(s).

New Probabilistic Genotyping Software
STRmix is replacing the use of random match probabilities, source attribution statements and the OCME FST software.

After a sample has been genotyped with the GeneMarker HID software it will be evaluated by the reporting analyst and, if appropriate, analyzed using STRmix. STRmix, a fully-continuous probabilistic genotyping software, will be used primarily for the following:

- To assist in the deconvolution of mixtures (determine possible genotypes present in the mixture)
- To calculate the LR ratio for a match to a single source sample
- To calculate the LR ratio for a comparison to a mixed sample

The second and third functions are performed after a sample is compared to DNA profile(s) of known or reference sample(s). If a match or inclusion occurs then the statistical meaning of that match or inclusion must be determined. This software uses the fundamentals of probabilistic theory and incorporates biological parameters such as peak height ratios, drop-in/drop-out, and forward/reverse stutter in order to interpret single source samples as well as DNA mixtures. Ultimately, STRmix will provide a statistical weight to each possible genotype.

Path Forward
The OCME plans to entirely cease using Identifiler, GeneMapper and FST on new casework on or around January 1, 2017. These technologies will be replaced by PowerPlex Fusion, GeneMarker HID and STRmix.

FST will continue to be used to calculate statistics on comparisons to samples tested using Identifiler.

Low Copy Testing (LCN), also called high sensitivity testing and ID-31, will be discontinued because Fusion shows reliable results in most of the high sensitivity range.

In our current procedures, the OCME laboratory validated the amplification of evidentiary samples in ID-28 and ID-31. For Fusion, our validation has shown reliable results at a level nearly as low as ID-31, without the extra cycles and other processes associated with LCN testing. See the table below. We estimate that due to this new lower threshold for routine testing with Fusion, we will be amplifying ~20% more samples than are currently being tested with Identifiler.

	ID-28	**ID-31**	**Fusion**
Minimum total DNA input in pg	100 pg	20 pg	37.5 pg
Minimum concentration in pg/uL	20 pg/uL	4 pg/uL	5 pg/uL

The testing thresholds selected for Fusion are supported by extensive validation data.

In the last 18 months only 27 cases (42 total samples) had samples that were below 37.5 pg/ul. In that same time period, we extracted 60,099 samples (42 samples represents only 0.07% of the total volume). Of these 27 cases, 10 cases were current cases, 10 cases were cold homicides and 7 were for outside jurisdictions. Two-thirds of the samples tested below 37.5 pg/ul failed to give conclusive results. The remaining one-third resulted in partial DNA profiles of marginal utility (<13 loci; many inconclusive loci, many inconclusive alleles (Z's)).

The OCME is fully committed to staying on the cutting edge of new technology to best serve the City of New York.