Cellmark Guidance Notes for SFRs

A. *Human Mitochondrial DNA (mtDNA) sequencing:*

Mitochondrial DNA (mtDNA) sequencing analyses the base pair sequence of the DNA found in intra cellular objects called mitochondria.

Mitochondria are small organelles present inside most human cell types. They are the site of energy production within cells and each cell can contain several thousand mitochondria. Each mitochondrion contains numerous copies of its own genetic material consisting of a small circular strand of DNA 16,569 base pairs long. Due to a relatively large degree of DNA sequence variability compared to the rest of the mitochondrial genome, two regions known as hypervariable region 1 (HV1) and hypervariable region 2 (HV2) are useful for the identification of individuals.

DNA sequence data is generated from HV1 (mtDNA bases 16024 to 16365) and HV2 (mtDNA bases 73 to 340). The sequence obtained is compared to a standard reference sequence known as the 'revised Cambridge Reference Sequence' (rCRS). Any base positions in which the sample sequence differs from the rCRS are tabulated to produce the "mitochondrial haplotype" of the sample tested.

The high number of mitochondria present in cells and the small size of the mitochondrial genome mean that that it is often possible to detect DNA from very small, old or degraded samples. It is also possible to obtain sequence data from samples such as hair shafts and old or burnt bone when there may not be sufficient nuclear DNA present to obtain a conventional STR DNA profile.

The mitochondrial genome is inherited unchanged via the maternal line and consequently all the offspring of a mother will have the same mtDNA sequence unless a mutation event has occurred. It is possible to search databases of mitochondrial sequences to obtain an estimate of the relative frequency of a particular sequence in the particular population(s) of interest.

On some occasions an individual may have two slightly different mitochondrial DNA sequences present in their mitochondria. This is known as heteroplasmy. As a result it is not unusual to detect two slightly different sequences of mitochondrial DNA in samples such as hair taken from the same individual.

The results of mitochondrial DNA sequencing are not suitable for direct comparison with standard STR DNA profiling results. There is no equivalent to the National DNA Database for mitochondrial DNA sequences. Instead, mitochondrial DNA sequencing results are almost exclusively used for direct comparison between samples and individuals.

B. *Mitochondrial DNA Analysis to determine species of origin:*

Mitochondria are small organelles present inside many types of cells in all multi-cellular organisms. They are the site of energy production and each cell can contain several thousand mitochondria. Each mitochondrion contains numerous copies of its own genetic material consisting of a small circular strand of DNA.

The high number of mitochondria present in cells and the small size of the mitochondrial genome mean that it is often possible to detect mitochondrial DNA from very small, old or degraded samples. It is also possible to obtain sequence data from samples such as hair shafts and feathers as well as burnt bone when there may not be sufficient nuclear DNA present to obtain a conventional STR DNA profile.

The gene for 12S ribosomal RNA is located in the mitochondrial genome. The DNA sequence of this gene is highly conserved within a species but shows variability between most species. Approximately 100 bases of this region are sequenced for species determination. Due to the region of the 12S ribosomal RNA gene used for sequencing, this assay can be used to identify vertebrate species (although there are exceptions) but is less useful for invertebrates.

12S sequences obtained from samples of interest are compared against a database of 12S DNA sequences held by the National Center for Biotechnology Information (BLAST database) in the USA, in an attempt to identify the species of origin. This database contains a wide range of DNA sequences. If a match to the 12S sequence of particular species is obtained then this provides evidence that the tested sample has also originated from the same species. For species/groups of species with low representation in the database, the level of sample identification is determined on a case by case basis.

C. DNA Enhancement

High sensitivity STR profiling involves specialised procedures that can significantly increase the sensitivity of the STR profiling method, such that very low levels of DNA may yield a DNA profile suitable for interpretation. 'DNA Enhancement' is a validated and accredited technique to increase the level of detection of amplified DNA components from forensic samples.

In the initial stage of DNA enhancement, the amplified DNA is subjected to a slight modification of the run conditions used to detect the DNA. For subsequent stages of DNA enhancement, chemicals that can limit the detection of the DNA components are removed from the sample to be analysed. This clean up procedure (sometimes referred to as 'post-PCR clean-up') can significantly increase the level of detection compared with results from the same DNA following standard analysis conditions.

DNA enhancement is generally performed on at least two separate portions of the DNA extracted from a sample.

D. Items pre-screened in police force laboratories

The exhibit(s) in this case has been examined by the submitting Police Force, prior to any submission to Cellmark Forensic Services for analysis/further examination. As such questions relating to any examination should be directed to the submitting Police Force.