eurofins | Forensic Services

KING'S College LONDON

Specialist Forensic DNA testing using Next Generation Sequencing technologies

Next Generation Sequencing (NGS), also known as Massively Parallel Sequencing (MPS), is a term applied to a number of different methods for ultra-high throughput DNA sequencing. NGS technologies have revolutionised genomic sequencing in other sectors and applications for forensic casework are now coming to fruition

EFS are able to offer a range of NGS applications utilising the expertise within our Eurofins Genomics Division and our strategic academic partner King's Forensics at King's College London. King's College have been at the forefront of NGS research and method development for a number of years and we are excited to now be able to make use of these services in forensic casework. Eurofins Genomics Division routinely use NGS technology for high throughout genomic sequencing for other sectors, such as agrobiotechnology, and we are delighted to be able to share knowledge and expertise across our divisions to be able to apply some of these methods for forensic casework.

This brochure will describe some of the applications of NGS which are now available from EFS.


1. Ancestry Prediction

Prediction of the geographical origin of an individual's ancestry can provide useful intelligence information to help direct an investigation. Whether this is a bloodstain left by a fleeing perpetrator, or a body part from an unidentified victim, knowledge of that person's ancestry could be a valuable lead.

DNA testing to predict ancestry is often referred to as Biogeographical ancestry (BGA) testing. Most commonly, this is achieved by testing a number of specific DNA regions called Ancestry Informative Markers (AIMs). AIMs are selected because they exhibit substantially different frequencies between different populations from different geographical regions of the world. Panels of such AIMs can be tested using Next Generation Sequencing (NGS) to predict the geographical origins of the ancestors of a person of interest typically in terms of the continent of origin (e.g. Africa, Asia, or Europe), but in some cases by smaller geographical regions (e.g. East African or South Asia (India/Pakistan/Bangladesh))

In addition to AIM panels, genetic information from mitochondrial sequencing (see section 4) and from Y-STR analysis can be used to refine the ancestry prediction. These tests can provide information on the ancestry of the maternal and paternal lines specifically. In most cases, the inclusion of such tests in addition to AIM will provide the most reliable prediction of ancestry.

Prediction likelihoods are available for each of six different populations.

- European
- Sub-Saharan African
- North East African (e.g. Somalia, Ethiopia, Eritrea)
- South Asian (e.g. Pakistan, India, Bangladesh)
- East Asian (e.g. China, Korea, Japan)
- South East Asia (e.g. Indonesia, Malaysia, Thailand)

2. Phenotype Prediction

Using NGS technology it is possible to predict an individual's externally visible characteristics (EVCs). This is often termed phenotype prediction.

Whilst there are a wide range of different appearance traits for which tests could be developed, in practice only a few characteristics have been shown to have relatively simple and well-defined genetic determinants such that DNA tests can be developed with a reasonable predictive power.

At present, the main tests are for hair colour, eye colour and skin colour, which can be determined with a high (but not absolute) degree of accuracy. In future this may extend to other markers such as propensity to male pattern baldness, and hair type (straight, curly).

Whilst prediction of hair, eye and skin colour may provide useful intelligence information in some instances, it should be noted that, even if the prediction is correct, the characteristic may not be observed in the individual themselves. This is most problematic for hair colour, where baldness, greying and artificial dying of hair can all alter or eliminate hair colour as a useful indicator. Furthermore, hair colour can change over time, most commonly seen as the progressive darkening of blond hair, resulting in a difference between predicted and actual hair colour in some cases.

3. Age Prediction

The ability to predict the age of the person who left blood at a crime scene can provide useful intelligence information to an investigator.

Although the DNA sequence of a human genome is essentially identical in every cell of an individual over their lifetime, there are some specific modifications to the DNA that do change over time. In particular, a chemical modification called methylation occurs cumulatively at specific sites in the DNA, and measurement of the degree to which a particular DNA sample is methylated can provide an indication of the chronological age (in years) of that individual.

At present, tests based on this characteristic are limited to certain body fluids as it is known that methylation patterns vary between different tissues. To date, most studies and publications have focussed on blood, but some development work is also being carried out on saliva and other tissues.

Tests developed for this application generally interrogate a small number of methylation sites which then provide an estimate of chronological age. Estimates are characterised by the average error in the prediction. At present, most estimates are currently within +/-7 years so cannot be used for accurate prediction, but as a general guide of age. Ongoing research in this area will be able to make the estimate more specific.

At present, because the age prediction test is more complex than other NGS tests, it does require more DNA than other tests. A good quality bloodstain will therefore be required to give the best chance of a successful result with this method.



4. Mitochondrial DNA Analysis

Mitochondrial sequencing has been part of the forensic toolbox for many years (previously using older "conventional" sequencing technologies). The high copy number and small size of mtDNA makes it a robust source of DNA in very old or damaged samples (e.g. burnt bones) and in samples which lack nuclear DNA (such as hair shafts). The maternal inheritance pattern can make it a useful tool in kinship investigations.

NGS can readily be used to generate the mtDNA sequence, and indeed with this method it is easy to sequence the entire mtDNA genome rather than just the smaller "hypervariable regions" in the Control Region (CR) of the genome which are commonly targeted when conventional sequencing is used.

The potential value of mitochondrial sequencing needs to be considered on a case-by-case basis. The sequence variants (called "haplotypes") observed offer significant power to discriminate between unrelated individuals but the evidential weight of a match is typically much lower than with STR analysis and the shared sequence among maternal relatives also needs to be considered. mtDNA is often considered a test of last resort if other approaches have failed to provide any usable results because of insufficient or damaged DNA.

Mitochondrial DNA sequencing can also provide information on the haplogroup of the individual indicating the maternal ancestry of an individual (i.e. the bio-geographical origin of the individual's maternal lineage). This can be used to supplement and support other information on bio-geographical ancestry derived from the specific AIM panels described previously.

5. NGS-STR Analysis

Traditional DNA analysis, such as DNA17, uses variation in the length of regions of DNA, known as STRs, to differentiate between individuals. NGS is able to determine the exact sequence of the DNA in these same STR regions and therefore provide more detailed information which may allow differentiation between STRs that are the same length.

Fig.1 *Schematic diagram illustrating the increased discriminating power of STR analysis using Next Generation Sequencing. Using conventional CE-based sequencing which resolves alleles based only on length, three different alleles can be identified at this locus (5, 6 and 7). With NGS, although some alleles are the same length, additional internal sequence variants can be determined (shown as yellow and blue boxes) which increases the number of different alleles to nine*

In addition to the increased information provided by the sequence information, NGS also enables more STRs to be tested simultaneously.

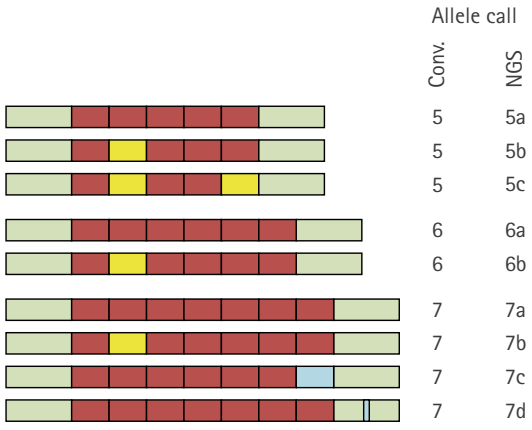


Fig.1

Continued over ▶

Currently available NGS-STR systems typically include three types of STRs;

(1) autosomal STR loci (such as those used in the current DNA17 systems, or in the Globalfiler® 24-locus system);

(2) Y-STR loci (such as those in the PowerPlex® Y23 system); and

(3) X-STR loci (not commonly used in forensic investigations).

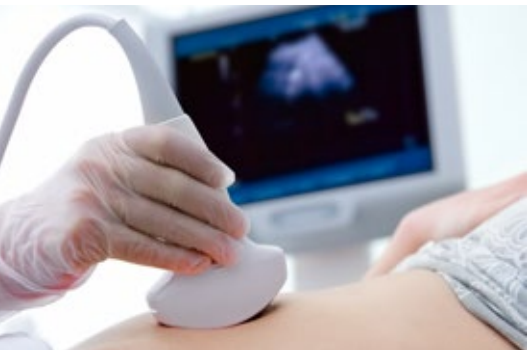
There is also potential that NGS-STR testing may be more effective than traditional STR testing on degraded samples due to the generally smaller PCR amplicon sizes technically possible with NGS-STR testing. However, in general NGS-STR testing is no more sensitive than traditional STR so is very unlikely to provide results on samples with insufficient DNA for current CE STR methods.

6. Differentiation of Identical Twins

Monozygotic (identical) twins are considered to be genetically identical and cannot be differentiated by standard forensic DNA testing. Therefore, a DNA match between a crime stain and an individual who is an identical twin would also be a match to the second twin. However, ultra-deep NGS of the entire DNA genome (the total DNA content of each cell, representing over 3 billion DNA base pairs) can now be utilised to look for rare mutations in the DNA sequence that have occurred during embryonic development and therefore are different between the two twins. If any such mutations are found, it is then possible to test the crime sample specifically for these differences to identify from which twin the crime sample could have originated. This is a lengthy and complex analysis very different from the predictive SNP tests, mtDNA or STR tests described above which target small and specific regions of the DNA for analysis.

This method has been applied in only a handful of cases to date but has successfully distinguished between identical twins in a paternity dispute in Germany, and in a rape investigation in the US. Due to the complexity of the technique, good quality reference DNA is required from both twins to enable the full genome sequence to be obtained.

We continue to collaborate with Eurofins Genomics and King's College London to review the latest NGS research and work with them to develop this into further forensic casework services.



Contact us

General Enquires
Tel: +44 (0)844 2641 999
EFSenquiries@eurofins.co.uk

Media Enquiries
+44 (0)1827 263768

www.eurofins.co.uk/forensic-services