# DNA TECHNIQUES AVAILABLE FOR USE IN FORENSIC CASE WORK

After 60 years of DNA discovery, DNA profiling continues to revolutionise forensic science and the investigation of crime worldwide

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# STANDARD DNA AND SPECIALIST TECHNIQUES AVAILABLE FOR USE IN FORENSIC CASEWORK

It seems hard to believe that two decades ago, using DNA to fight crime was unheard of. The development of DNA profiling revolutionised forensic science and the investigation of crime worldwide.

# In New Zealand, DNA profiling is now used routinely to:

- investigate a wide range of crimes from burglaries to homicides
- identify suspects and exclude the innocent, reducing police investigation time
- solve historic cases
- assist in the reconstruction of crimes and crime scenes
- assist in identifying human remains, including those from disaster events
- assist in forensic paternity investigations.

ESR is at the forefront of research and development internationally with a thriving research and development programme.

Our expertise is recognised throughout the world.

Here we outline the range of techniques currently offered by ESR to the criminal justice community based on DNA and its sister compound RNA.

The techniques you will find described are:

- STANDARD DNA PROFILING tests used to deliver highly discriminating results in most cases
- LOW COPY NUMBER (LCN) DNA profiling tests for highly sensitive testing of very small amounts of DNA
- MINIFILER™ DNA profiling used for analysing very degraded DNA
- Y STR DNA profiling used for selective analysis of the Y chromosome found only in males

Buccal cells

- mRNA analysis for the detection of body fluids
- LASER MICRODISSECTION for the isolation of specific cells for further testing.

All of these techniques use a method known as the Polymerase Chain Reaction (PCR) to obtain profiles. This is a standard technique used to amplify or selectively copy specific regions of DNA or RNA many times. In this way, minimal amounts of DNA or RNA isolated from small or degraded samples can be increased to a level where they are able to be detected, profiled and compared with other samples.

In addition, we are now using  $STRmix^{TM} - a$  powerful tool developed to help interpret complex mixtures of DNA.



# STANDARD (AUTOSOMAL) DNA PROFILING

Human DNA is packaged in 46 chromosomes: 22 autosomal pairs, and either two X chromosomes in females or one X and one Y chromosome in males. The majority of DNA profiling carried out at ESR uses systems that target autosomal DNA sites. These DNA sites are found in both males and females and are highly variable amongst individuals, thereby delivering highly discriminating results. Currently, the standard DNA profiling test is used on routine sample types such as blood, semen and saliva stains as well as body tissue, hair and bone. It includes a gender test.

### These DNA profiles can be:

- compared with reference DNA profiles from suspects, complainants and those providing samples for elimination purposes
- used in missing person enquiries and DVI (Disaster Victim Identification).

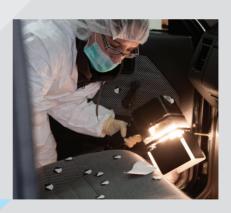
**DETECTS:** Currently fifteen autosomal DNA regions (sites) and a gender test.

**SENSITIVITY:** Good sensitivity for routine samples.

DISCRIMINATING POWER: Very discriminating. It is highly unlikely that two unrelated people will have matching DNA profiles using the current system.

#### **CASE EXAMPLE:**

DNA profiles from post mortem samples were used to identify the deceased after the Christchurch earthquake.



# LOW COPY NUMBER (LCN) DNA TESTING USING SGM PLUS®

Low Copy Number (LCN) DNA profiling is used mostly on contact samples where there are very small amounts of DNA. It is the only DNA profiling test currently available that can be used on these types of samples. The sensitivity of the test requires strict procedures for the entire testing process.

**DETECTS:** 10 variable DNA regions and a gender test. It is fully compatible with standard DNA profiling.

**SENSITIVITY:** LCN DNA profiling is the most sensitive DNA test currently available. It provides results with only trace amounts of DNA present.

**DISCRIMINATING POWER:** High. It is very unlikely that two unrelated people will have matching DNA profiles.

**LIMITATIONS:** Due to the sensitivity of the test, mixtures of DNA are frequently detected which may make it uninterpretable. Careful targeted sampling may assist in reducing potential mixed cell samples.



## MINIFILER™ DNA ANALYSIS

Minifiler™ DNA profiling is useful for samples containing DNA that has been degraded either due to the age of the sample or because of environmental conditions. This is because this test targets smaller lengths of DNA and the smaller the target length, the higher the chance that there will be no breaks in the DNA sequence.

Minifiler™ may also be useful when samples contain inhibiting substances, such as soil and fabric dyes that could interfere with the standard DNA profiling test.

The Minifiler<sup>™</sup> DNA profiling test analyses eight of the DNA sites used in the standard DNA test. Because fewer DNA sites are analysed, results are not as discriminating as standard DNA results. The Minifiler™ DNA profiling test is however more sensitive than the standard DNA profiling test and may be useful when the amount of DNA present in a sample is low. Also it may be useful when partial DNA profiling results have been obtained from standard and/or LCN DNA profiling tests. Partial DNA profiles are those where results have not been obtained from all the DNA sites tested. Minifiler™ results may be able to be combined with results. from these other tests to provide a more complete profile.

**DETECTS:** Eight variable DNA regions and a gender test and is fully compatible with standard DNA profiling.

**SENSITIVITY:** The sensitivity falls between the standard DNA profiling test and the LCN DNA profiling test. Minifiler™ is not as sensitive as LCN DNA testing.

DISCRIMINATING POWER: On their own, Minifiler™ results are not as discriminating as standard testing. However, Minifiler™ results can be combined with profiling results obtained from other techniques for greater discrimination. A full Minifiler™ profile will still provide a statistic in ESR's most discriminating 'extremely strong support' category.

#### **CASE EXAMPLE:**

A bone washed up on a beach gave no results using the standard DNA test but sufficient results were obtained from Minifiler™ to allow comparison to reference samples.

## Y STR - Y CHROMOSOME DNA ANALYSIS

The Y STR profiling test targets only male DNA that may be present in a particular sample by analysing DNA sites on the Y chromosome only. Therefore, DNA from females is not detected using this technique.

The main advantage of the Y STR DNA profiling system is that it selectively targets male DNA even in the presence of large amounts of female DNA. This means that results can be obtained from very small amounts of male DNA, which were not previously possible.

Y chromosomes are passed from father to son; therefore paternally related male individuals cannot be distinguished using this current Y STR technique.

Lower genetic diversity has been found in populations of Polynesian origin due to the nature of recent population movements within these groups. In some cases this is reflected in a decrease in statistical significance of any correspondence found.

### Y STR DNA profiles can be:

- compared to reference samples from male individuals
- used to support family relationships and are often used in missing persons or disaster victim identification work.

**DETECTS:** Twelve STR loci on the Y chromosome.

**SENSITIVITY:** Results can be obtained from very small amounts of male DNA.

**DISCRIMINATING POWER:** Cannot currently discriminate between father and sons or other paternally related males.



## **mrna analysis for body fluid identification**

The identification of body fluids can be important in determining the body fluid source of a DNA profile or in corroborating different versions of events.

mRNA is an intermediary compound between DNA in the cell nucleus and the cell proteins. The mRNA profile of a cell is unique for each cell type. By exploiting these differences ESR has developed an mRNA test that can be used to detect blood, menstrual blood, vaginal fluid, saliva and semen (with or without sperm).

## mRNA testing can be used when:

- regular chemical tests have not been successful in identifying the body fluid
- there is no chemical test available (vaginal material, menstrual blood).

As mRNA is a more fragile molecule than DNA, and more susceptible to environmental influences, it is possible that results may not be obtained from all samples for which DNA profiles are available. Conversely, the amount of mRNA per cell can be greater than the amount of DNA therefore the opposite is also possible.

**DETECTS:** the body fluid source of DNA – blood, menstrual blood, vaginal fluid, saliva and semen (with or without sperm).



# LASER MICRODISSECTION FOR THE ISOLATION OF SPECIFIC CELLS FOR FURTHER TESTING

LMD is a microscopic technique for the isolation of a particular cell type from a mixture of cells. It provides improved analysis of challenging forensic samples.

It is particularly useful in the analysis of semen stains when sperm numbers are low as it is possible to isolate the sperm and provide a useful DNA profile from these cells alone. It can be used on samples where there is a small amount of sperm mixed in with a large amount of female cells such as a vaginal swab, or to recover other cell types such as vaginal cells, or buccal cells when mixed with sperm or skin cells.

After cell recovery standard, LCN, Minifiler  $^{\text{TM}}$  and/or Y STR DNA profiling techniques can be applied. The choice of profiling technique will depend on the number of the cells recovered, quality of the DNA recovered from the cells and the results available from other samples for comparison.

LMD can be used on samples where there is a small number of sperm mixed with a large amount of female cells, such as a semen stained vaginal or oral swab. It can also be applied to samples that contain small numbers of epithelial cells (vaginal or buccal cells) when mixed with sperm, or skin cells on samples such as penile swabs or male underclothing.

# X/Y FISH – X/Y Fluorescent in situ hybridisation

X/Y FISH analysis is a gender-specific labelling method for separating male and female epithelial cells in mixed cell samples prior to LMD. Identification is achieved via fluorescent labelling of the sex chromosomes of the cells recovered from a case sample, for example, a vaginal swab containing semen from a vasectomised male (semen with no sperm). Male cells display one green fluorescent signal and one red fluorescent signal (see image), and can be distinguished from female cells that display two red fluorescent signals. Targeted cells are then laser microdissected from the sample, and DNA analysis is undertaken specifically on the recovered cells.

## INTERPRETING DNA

STRmix™ is expert forensic software, developed by ESR and Forensic Science South Australia (FSSA), that can resolve previously unresolvable mixed DNA profiles.

As well as improving interpretation of DNA profiles from a single source, it can also determine the contributors to complex DNA mixtures of up to four individuals.  $STRmix^{TM}$  has been in routine use at ESR since August 2012.

#### STRmix™ enables ESR to:

- ▼ interpret DNA results faster
- compare profiles against a person of interest and calculate a likelihood ratio
- ▼ resolve previously unresolvable, complex DNA mixtures
- use more of the information in a DNA profile





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