It’s not just the multiplex kit!

The new kits offer a greater level of sensitivity (30 cycles v 28 for SGMplus), have better chemistry which ensures more input DNA, allow faster amplification and have more Mini-STRs to make them more effective when working with degraded samples, so.....

“Why do different companies have different success rates despite using the same multiplex kits? “

Because it’s not just about the kit!

There are a number of other factors and processes that can influence the end result e.g. DNA extraction, electrophoresis and interpretation.

- Cellmark’s DNA scientists are renowned for their ability to get results from the most challenging samples.
- Our scientists have developed innovative new techniques to aid recovery such as Sperm Elution.
- Cellmark has developed robotic protocols to reduce the time in the lab.
- Our in-house software specialists have developed and implemented sophisticated programmes to analyse complex mixtures
- And our staff are highly skilled at interpreting results - an area which will be vital given the additional complexity of the results produced by DNA17.

Cellmark was the world’s first private DNA fingerprinting laboratory, and has been assisting the Police to solve crime since 1987.

IDENTIFICATION

INTERPRETATION

INNOVATION

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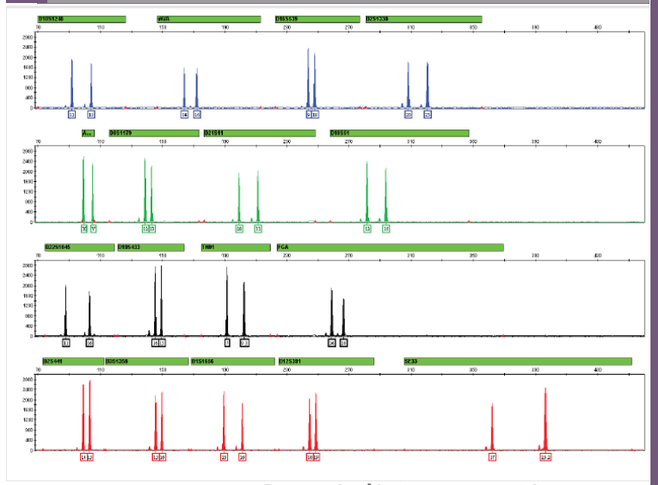
DNA17-02/16

Cellmark
Forensic Services

DNA17 MULTIPLEXES
NGM SElect™ & NGM SElect™ Express



Specialist expertise
in recovery, preservation,
enhancement & analysis

IDENTIFICATION

INTERPRETATION

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Introduction

“The biggest change to DNA testing since 2000”. DNA17 multiplexes are replacing the standard SGMPlus test previously used n the UK.

3 manufacturers, Life Technologies, Promega and Qiagen, supply kits and they all provide significant advantages over the old SGMplus kits.

After careful consideration Cellmark has chosen to use the Life Technologies NGM SElect™ (for Crime Scene Stain DNA) and NGM SElectPlus™ (for PACE) multiplex kits developed by Life Technologies™.

However the STR multiplex is just one component in a multi-stage laboratory process that determines the successful outcome of a DNA profile. All the other components in the laboratory process, particularly DNA extraction, play key roles in determining the final result.

DNA17 - NGM SElect & NGM SElectExpress

New Improved Chemistry

The SGMplus chemistry was designed over 15 years ago and STR technology has advanced significantly since then. The new kits provide vastly improved reaction chemistry, which makes for more robust amplification, less affected by any inhibitory chemicals (although with good DNA extraction it is rare to encounter any inhibition even with SGMplus) and with 30 cycle amplification we expect to see stronger profiles, more distinct from a cleaner background baseline.

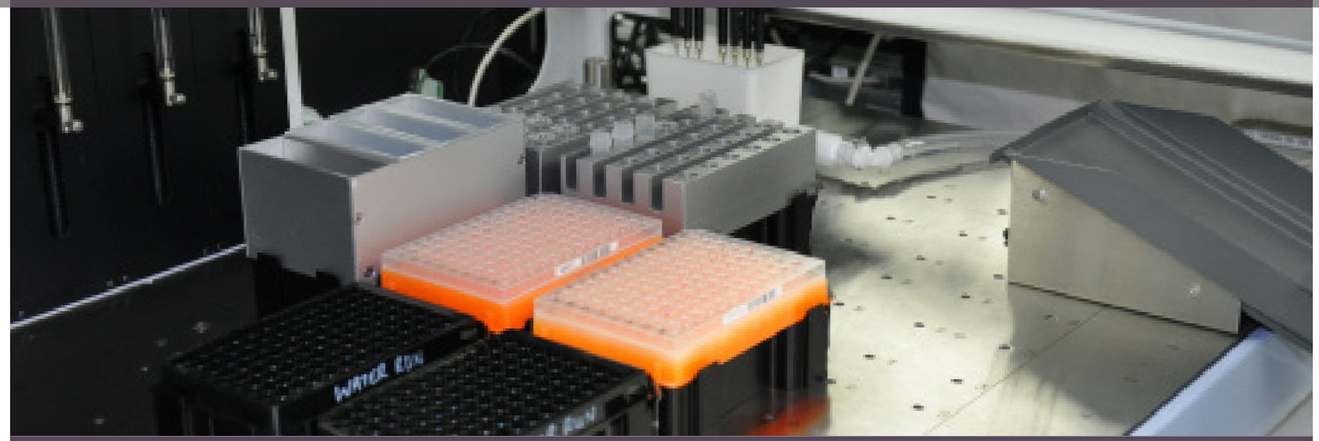
Mini-STRs

Shorter (and therefore lower molecular weight) markers are more likely to be able to produce profile information from degraded samples. For some years we have therefore used the STR kit MiniFiler to maximise the chance of obtaining a profiling result from degraded samples because the primer sets had been re-engineered to shorten the PCR product of each STR marker in the multiplex – so called 'mini-STRs'.

Both the Life Technologies (NGMSElect) and the Promega (ESI) kits benefit from having an increased number of mini-STRs and so both kits should assist with analysing degraded samples.

	<150bp	150 - 220bp	220 - 250bp	Total Mini-STRs
NGM SElect	5	4	1	10
ESI 17	4	3	0	7

However, although NGMSelect has more mini-STRs overall, ESI has one more mini-STR in the SGMplus equivalent loci than NGMSElect.



Non-Concordance

If different primers are used in kits targeting the same STR markers, there is a possibility that a "primer binding site mutation" for one of the markers could affect one kit differently to another.

The un-affected multiplex would give a heterozygote result for a specific marker, but the multiplex that is affected would give a 'null' result on one allele, therefore producing what appears to be a homozygote result. This is called a discordant result - the results will not match despite coming from the same person.

NGMSelect uses the same primers for the SGMplus loci as those found in the actual SGMplus multiplex, so there will be no discordant results when comparing with the SGMplus results already on the NDNAD.

The Home Office carried out a study before the kits were approved and it determined that the expected frequency of non-concordance where one profile is SGMplus and the other is ESI (which uses different primers), is 1 in 300. (The discordancy rate between an ESI and an NGMSElect DNA17 profile is expected to be 1 in 150.)

More Mixtures

Everyone who has used the new generation of multiplexes has reported an increase in the number of mixed profiles from forensic crime scene samples.

Approximately 12 months ago the Irish forensic laboratory (Forensic Science Ireland) introduced DNA17 (using NGMSElect) and more recently the FSNI (Forensic Science Northern Ireland) has moved to DNA17 having validated both NGMSElect and ESI.

They both report that with stronger profiles, and more mini-STRs comes the interpretation challenges of identifying more mixtures. Whichever kit is used by the FSPs in the England and Wales it is likely that the interpretation of profiles will take longer (potentially as much as 50%) and there will be additional challenges of identifying individual contributors in mixed samples.

To assist with this we expect to see the increased use of sophisticated probabilistic profile interpretation software.

Load Criteria

Currently, to load a profile to the NDNAD there is a minimum load criteria of 4 complete loci (of the original 6 SGM loci). Although the NDU was originally considering increasing the minimum load criteria to 8 SGMplus loci when DNA17 is launched, the NDU has now decided to keep the load criteria exactly as it is now, i.e. a profile will need to have 4 complete SGMplus loci to be loaded to the NDNAD.

There is therefore no reason to believe that there will be any difference in the load success rates attributable to use of different kits.

Conclusion

Cellmark believes that there is likely to be little difference between the Life Technologies and Promega DNA17 kits, and it is perhaps too early to be sure. They are both going to provide significant advantages by way of sensitivity and their use on degraded samples but there will also be some interpretation challenges resulting from the increase in mixtures.

Further to that all suppliers are required to validate the use of two kits to allow them to respond to non-concordance issues so although Cellmark's primary kit will be NGMSElect we will also have the ESI kit validated which will ensure that we gain experience of using both multiplexes, and it will allow us to review their comparative performance on live casework samples.

Cellmark expects to continue to work with the Home Office and the NDNAD to ensure that we continue to deliver industry leading success rates with the new DNA17 kits.