European DNA Profiling (EDNAP) Group
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Hosts: Prof Bjørnar Olaisen, Benta Mevåg

Chairman: Peter Martin

Proficiency Testing and linking EDNAP with other continents

Peter Gill CRSE UK

The aims and objectives of EDNAP were restated. The group has advanced to a position where it is possible to:-

1. demonstrate that European laboratories conform to standards
2. achieve uniformity of results
3. co-ordinate action by providing collaborative inter-laboratory exercises.

The advantage of uniform systems have become evident. They would provide a common European protocol which already includes:-

1. common enzyme - Hinf 1
2. common probes - MS43A and YNH24
3. common marker ladder - BRL
4. common genomic control

This can be extended to include the same electrophoresis and general working conditions.

EDNAP has become a Working Group of the ISFH. It is now important that each country represented within EDNAP has its own Working Group which reports to the central group responsible for setting the proficiency trials. Already there are a number of working groups in existence.A further aim is to collaborate with other continents. Any inter-continental exercises can be co-ordinated via the DNA Commission.

The last proficiency trial was presented and the results demonstrate that the size of the match window has been reduced.

Three laboratories showed divergent results from the rest and these were removed before re-analysis of the data. The band weights then recorded showed very good agreement.

Peter Gill considered that we are unlikely to improve on this result.
CRSE, Aldermaston will be willing to produce further proficiency trials with two per year at a cost of £150.

During the following debate it emerged that there is already some collaboration with the USA via the FBI laboratory. Some Short Tandem Repeat (STR) systems are being exchanged and this could aid in the formation of databases as well as providing linking systems. Paul Debenham, Cellmark, has been collecting European frequency databases for collation and comparison by Bruce Budowle at the FBI laboratory. It is planned that, once the exercise is complete, the results will be distributed by Cellmark to the European laboratories.

The following short presentations were then made to the meeting:-

1. **DQA 1 using Amplitype** **M.Stenersen,Oslo**

The use of Chelex, for the extraction of DNA was described.
A study was made of 20 crime cases in which DQA 1 was compared with RFLP results.

67 cases have been examined in which DQA 1 was used and this equates to 191 samples. 126 of these samples were amplifiable and 65 were not. 242 reference samples were included.

APO B

In the study using the APO B system problems were experienced due to changes in migration distance (band weights) when different separation media were used.

This finding indicates that it would be difficult to make inter-laboratory comparisons.

2. **COL 2A1** **E.Berg, Oslo**

In the initial stages problems were experienced with the amplification. This was remedied by redesigning the primers.

An inheritance study was performed on 50 small family groups from a village in Norway. Some of the alleles obtained did not correspond with components of the allelic control ladder. When a new ladder was used there were no ambiguous alleles; all could be assigned.
All alleles found have been sequenced and this has shown that the basis of the polymorphism is complex. It is not a simple increase in the number of core repeats; the core sequence can vary between 31 - 34 bp.
The most common allele was 40% with the system showing 73% heterozygosity. Deletions could only be demonstrated on sequencing gels and no mutations were found in the family studies.

19 allelic variants were found and no extra information was obtained from sequencing alleles of the same length.

3. Cryptic Markers — H.Schmitter, BKA

At the BKA cryptic markers (T7 phage - Promega) are added to the slp analyses. Six bands are produced which are spread over the complete range of the separation (2.6 - 13Kb). These T7 markers have many Hinf 1 restriction sites and therefore the enzyme in the buffer must be denatured before addition of these markers.

Measurement is according to the Promega instructions and, using a linear progression, correction factors for the band weights of the slp, can be calculated.

The results have indicated band shift in every gel and these shifts were in both directions. No obvious pattern was obtained which argues against the correlation of band shifts.

The question was raised as to whether these findings would affect the likelihood ratios calculated for intra- and inter-plate analyses.

4. Short Tandem Repeats (STRs) — M.Greenhalgh, MPFSL

PCR is still in the research stage at the MPFSL but, having identified several problems associated with PCR-based VNTRs, a programme of collaborative work with CRSE has been agreed to progress work on STRs.
There are several reasons for choosing STRs in preference to other PCR-based VNTRs, among them being:-

resistance to degradation
ease of multiplexing
general robustness
short repeats - less chance of deletions

The following examples were used to illustrate the choice available:-

name	core repeat	No of alleles
HumTHO 1	(TCAT)n	7
SE33	(AAAG)n AA(AAAG)n	21

MPFSL and CRSE have agreed to concentrate on SE33 and HumTHO 1 in the initial stages.

There was some considerable discussion on the ethical problems on the use of polymorphisms from non-coding regions which have some linkage association with disease problems related to coding parts of the genome. It was decided that we should keep a watching brief on this situation and return to the subject at our next meeting.

5. Minisatellite Variant Repeat (MVR) P.Debenham, Cellmark

The presentation included the progress made by Cellmark with regard to the use of the MVR system in forensic science.
The kit which has been developed by Cellmark and Alec Jeffreys has been issued to 14 laboratories in Europe and 7 in The USA. Each lab will evaluate the system and Cellmark will co-ordinate the responses.

Cellmark have assessed the following parameters:-

comparison of flanking primers
optimisation of Tag A and Tag B primers
optimisation of gel length
'hot start' and annealing temperature
effect of degraded DNA
sensitivity
extraction methods
optimisation of pH and buffer
use of synthetic gel support

Basically, the method used included:-

200ng of DNA
19 cycles
3 hour exposure with non-isotopic probe

an annealing temp of 66C is preferred to 70C
pH 8.8 is better for extension of larger alleles than pH 8.0

A comparison of slp and MVR analysis was made of mixtures of body fluids following preferential extraction. As long as the ratio of DNA from each fluid is 10:1 or higher the system does not detect the contaminant.

(Walter Bar pointed out that there were faint bands, from the contaminant, showing on the autoradioograph and he asked how he would distinguish this from faint bands caused by mispriming.
Paul Debenham thought that this would cease to be a problem in the future.)

Future developments

Minisatellite MS31 has a 20bp repeat unit with a heterozygosity higher than MS32. The intention is to multiplex MS31 and MS32 and use differential NICE hybridisation.
The MPFSL scanner has been modified by Cellmark to scan the MVR autoradiographs and read the code.

The advantages of the MVR system:-

DNA length measurement is obviated
codes are ideal for databases
codes include information redundancy
standard DNA confirms code authenticity
band match criteria are not subjective
codes are unaffected by shift
large communal databases are possible

with regard to mixtures, work is progressing on the use of hypervariable regions in the flanking DNA. More information will be forthcoming.

6. Further collaborative exercises

Peter Gill proposed that the next collaborative exercise should be based on STRs and that the group should have STRs as the linking systems for future casework.
In the first instance HumTHO 1 and SE33 should be used and form the basis of the collaborative exercise. This is without prejudice as other STRs may be identified at a later date which have better characteristics for casework.

This was accepted unanimously.

CRSE will prepare the trial and Peter Schneider offered to make the primers. Details will be sent to members of the group.

The DNA Commission is attempting to find a way of standardising the nomenclature.

There was some discussion as what decisions could be made by the EDNAP group without recourse to the ISFH. It was more-or-less decided that those present form the committee as they are all members of the ISFH and the contribution should be Quality Assurance, standardisation, exchange of data etc.
It was reiterated that each member country should form their own working groups with a single representative attending the EDNAP meetings.

7. Licensing agreements with Roche.

Peter Gill informed the group that it has now been decided that government departments will be exempt from royalty payments as long as they use Roche Taq, thermocyclers, etc.
There was some discussion on the status of university laboratories and it was suggested that

they should contact Roche for clarification.

8. National Academy of Sciences Report

There was some disquiet concerning the NAS report especially with regard to the way of calculating frequencies and the composition of the national committee on forensic DNA profiling.
Peter Martin offered to write something for the ISFH newsletter after consultation with members.

9. DNA databases

Peter Martin asked members if they could write to him with details of the law in each country in respect of forming databases from named individuals.

The date and location of the next meeting was not discussed. Peter Martin will write to members with suggestions following the next collaborative exercise.