EUROPEAN DNA PROFILING GROUP (EDNAP) MEETING

ROME, ITALY

12 – 13 SEPTEMBER 2007

Host: Alberto Intini and Renato Biondo.
Chairman: Niels Morling.

A list of participants is attached.

Welcome
Alberto Intini and Renato Biondo welcomed members to Rome.

Update on publications Niels Morling
The exercise on typing of mtDNA SNPs is in press and a proof has been produced.

A manuscript of the exercise on typing of autosomal SNPs with a part of the SNPforID 52 SNP multiplex PCR set was circulated in August 2007. The final version will be written 20 September 2007 and comments must be submitted before.

A letter to the editor of FSI: Genetics to communicate the support from EDNAP, ENFSI, the German Stain Commission and The technical DNA Working Group in the UK concerning the ISFG recommendations on the interpretation of DNA mixtures has been published: Morling N, Bastisch I, Gill P, Schneider PM. Interpretation of DNA Mixtures - European Consensus on Principles. FSI: Genetics 2007; 1: 291-2. Furthermore, an official English version of the German recommendations will be published in the Int. J. Legal. Med.

Update on exercises and other activities
EMPOP Walther Parson
Walther Parson presented an update on mtDNA alignment and notation of mtDNA sequences. While the alignment is straight forward for the majority of sequences, ambiguities arise in the alignment of homopolymeric sequence stretches, where multiple solutions are possible. Earlier recommendations invoking a single most parsimonious alignment to a fixed sequence (rCRS) have shown to produce artificial haplotypes (jumping alignments) that put the sequence at further distance in a database search as the actual mutations would require and therefore bear the risk of underestimating the frequency of a mtDNA sequence. In addition, these recommendations have never been strictly followed. This is why a number of databases contain inconclusive sequence notations.
EMPOP supports a different alignment strategy that is based on the phylogenetic history of a sequence and that is performed by a weighted nearest neighbour search. The sequence is reported according to the rCRS. The first results with the sequences stored in the first release of EMPOP (5.173 haplotypes) suggest that this approach is better suited for the forensic

analyses and interpretation of mtDNA. Currently, EMPOP is developing software tools that will ease the use of phylogenetic alignments.

Collaborative exercise on AB MiniFiler
The results were presented at the ISFG congress in Copenhagen in August 2007. Some members expressed concerns about the data presentation, the relevance of the DNA investigated and the conclusions. At the joint EDNAP-ENFSI meeting, Lindsey Dixon presented the results of the exercise again, and the group discussed the results. It was the general opinion that the exercise did not answer the crucial question on the performance of Minifiler on partially degraded DNA (DNA fragments in the size range 80-225 bp) and on samples with a significant degree of inhibition of the PCR. There is no doubt that the DNA investigated was degraded in a way so that there was not much partially degraded DNA in the samples, but mainly 'high'-molecular (> 400 bp) or highly degraded DNA (< 80 bp). Thus, the results of the exercise mainly reflected the sensitivity of the kits in case of very little or no PCR inhibition.

No laboratory was able to offer artificially, partially degraded DNA with the relevant size distribution.

It was agreed that a possible way forward was to expand the exercise with investigations in 3-6 laboratories of 3-6 case work samples that are known to have a substantial proportion of partially degraded DNA, no high molecular weight DNA and/or significant inhibition of the PCR. Copenhagen, Innsbruck, Cologne and Tbilisi showed interest in such a study.

It was suggested to try to collect and compare data from real case work that has been investigated with both MiniFiler and another kit, e.g. SGM Plus. Lindsey Dixon offered to collate and compare data. The following laboratories expect to be able to deliver results: Coimbra, Copenhagen, Cracow, Innsbruck, LGC-UK, Madrid (Antonio Alonso) and Tbilisi.

The results and the conclusions were discussed with representatives from AB who will take the suggestion concerning a supplement exercise forward within AB.

AB presented the status of the development of a Pan European Kit based on SGM Plus and supplemented with 5 short, 'new' systems: D1S1656, D2S441, D10S1248, D12S391, and D22S1045. Four labs (FSS, London, Santiago and Wiesbaden) are expected to test or have already tested the present version of the kit. Further testing of an improved version of the kit is planned in probably 7 laboratories. Coimbra, Copenhagen, Innsbruck and Strathclyde volunteered as additional testers.

Updates from other groups
ENFSI Ingo Bastisch
Ingo Bastisch reported on the activities concerning DNA mixture interpretation, including the presentation and discussion of the recommendations during the last ENFSI meeting that lead to a unanimous decision to support the recommendations. The document concerning an update of the European Standard Set of DNA markers is being processed by the ENFSI steering group. The work of the various subgroups was briefly summarized.

AFDIL N Morling, W Parson

A PowerPoint presentation from Mike Coble was shown and commented (attached as pdf-file).

ICMP Niels Morling

Since the last meeting, the idea that the ICMP might be developed e.g. into a common resource for DVI situations has also been discussed between the ICMP and Copenhagen. Representatives from Wiesbaden, The Hague, Copenhagen and the ICMP will write a manuscript with a presentation of the challenges and a discussion of the possible ways forward.

SNPforID Peter Schneider

The Genplex forensic validation study based on a presentation prepared by Esther Musgrave-Brown, London, for the 22nd ISFG Congress 2007 was presented (enclosed). In contrast to SNP typing using SNaPshot technology, Genplex is based on a multiplex PCR followed by an allele-specific oligo ligation assay with reagents also used in the SNPlex assay (Applied Biosystems). Genplex is a microtitre plate-based assay for medium to high throughput typing. Major advantages are more balanced signals within the two alleles of a given locus, as these are both labelled in the same colour, thus leading to a more reliable automated allele calling using cluster analysis (a feature provided by the GeneMapper 4.0 software – but not part of GeneMapper ID). Although the entire typing procedure involves multiple amplification, hybridization and washing steps, which can be facilitated using robotics, the results are reliably and clear-cut even by first-time users.

The forensic validation study involved a comparison of a 48-SNP Genplex assay based on the original SNPforID 52plex plus Amelogenin with the standard STR multiplex kits PowerPlex 16 and SGM Plus kits. The investigations included a sensitivity study, analysis of artificially and naturally degraded DNA samples, and mixtures in ratios of up to 1 in 10. It was found that Genplex had a reduced sensitivity compared to STRs, but a superior performance on heavily degraded DNA samples. Thus, Genplex typing could be a useful addition to currently available methods for accurately typing poor quality DNA. Initial results indicate that mixtures up to 1 in 10 could be typed. This is facilitated by using the features built into cluster analysis. Applied Bioystems considers making the SNPforID primers and reagents commercially available "for research only" together with the protocol for performing the Genplex assay. Furthermore, Chris Phillips (Santiago de Compostela) is developing another 48-SNP Genplex assay based on ancestry-informative markers (AIMs) for the prediction of geographic origin in collaboration with AB. This assay is expected to distinguish among three major populations (European, African, and East Asian) with an error rate of only 1%.

The Helios Airways Crash - Victims DNA Identification Maria Vouropoulou

Maria Vouropoulou presented the work of the laboratory in Athens in relation to the Helios Airways Crash.

Future activities

Future of EDNAP Herman Schmitter

Herman Schmitter gave a brief overview of the achievements of the EDNAP Group and suggested the following issues to be studied:

- identification of cells/tissues by mRNA measurements
- identification of ancestry by SNP typing of ancestry informative markers
- identification of physical traits by SNP typing.

After a discussion, the following possible projects were outlined:

Identification of cells/tissues by mRNA measurements
The representative from Zürich will take the idea forward with the Zürich group that has experience with identification of peripheral blood, menstruation blood, salvia and sperm cells with mRNA. More than 10 laboratories expressed interest.

SNP typing of ancestry informative markers
The representative from Santiago will take the idea forward with the Santiago group that has experience in typing of AIM SNPs – including a 34 SNP multiplex that can be typed with the SNaPshot kit, CE and multicolour detection. More than 10 laboratories expressed interest.

Investigation of the AB Genplex SNP typing platform
Collaborative EDNAP exercises have shown that the SNaPshot technology works rather well in EDNAP laboratories. However, the present version is not optimal and there are a number of drawbacks. It is desirable to agree upon another platform. One possibility is the AB Genplex system. Cologne, Copenhagen, London and Santiago have experience with the AB Genplex system. The advantages include robustness, clear discrimination between positive and negative reactions and the possibility for high throughput analyses. The disadvantages include the need for robots and equipment that may not be found in a routine forensic laboratory, the need for reagents that presently are provided only from AB, and lack of feasibility for typing of small numbers of samples. The following laboratories expressed interest: Coimbra, Rome Catholica, LGC and Tbilisi.

EDNAP web site update (www.isfg.org/ednap/ednap.htm) Peter Schneider
Peter Schneider introduces the new EDNAP website, which has been completely redesigned and is integrated into the new ISFG homepage that will be launched within a few weeks. The new ISFG web site has been programmed by Sascha Willuweit, Berlin. The new EDNAP web site features all information currently present including the summaries and the final report from the STADNAP network project, extended pages on activities, publications, the history of EDNAP, and a new list of all EDNAP meetings since 1988 with major topics from each event (prepared with the help of Hermann Schmitter). EDNAP members coming across old or new photographs from any of the previous meetings are kindly asked to send the photos to peter.schneider@uk-koeln.de as we will celebrate the 20th anniversary of EDNAP in 2008. The advanced features of the new ISFG website including registration and login procedures for individual access for ISFG members were demonstrated.

Any other business
There was no other business.

Next meeting
The next EDNAP meeting will be held in conjunction with the next ENFSI DNA Working Grouping Meeting in Prague – most likely 22-23 April 2008.

Closing of the meeting
The meeting closed with sincere thanks to Renato Biondo and Alessandra La Rosa for hosting the meeting.

Attachments

- List of participants (Word)
- AFDIL update from Mike Cobble (pdf)
- Genplex slides from Esther Musgrave-Brown (pdf).