STANDARDIZATION OF DNA PROFILING TECHNIQUES

IN THE EUROPEAN UNION (STADNAP) MEETING

DUBLIN, IRELAND, 6 MAY 2000

Host: Geraldine O'Donnell

Chairman: Niels Morling

A list of participants is attached at Annex 1

1. Welcome

Geraldine O'Donnell welcomed members to the meeting in Dublin.

2. Update on WP exercises

2.1 mtDNA from head hair samples I — Gillian Tully

Results have now been received from 10 laboratories and data is now available on 55 head hairs. Further sequencing is required for some of the hairs.

Criteria for additional sequencing:

Data only received from one laboratory
One section of the hair or sections from one laboratory only showing differences to other sections/reference sample
Apparent pattern of difference along length of hair.

Experimental/manuscript status:

21 hairs required further work – 17 are complete but 4 require a volunteer lab to provide a sequence as soon as possible. Walther Parson volunteered.
If not already supplied, laboratories should send a complete summary of their method from extraction onwards.

Analysis of results:

10 hairs match the reference exactly
14 hairs have at least one section which matches exactly
14 hairs have heteroplasmic difference at 16234
1 hair has heteroplasmic difference at 16093
5 hairs have heteroplasmic differences at 16234 and 16093
Heteroplasmic differences at 16234 and poly (C)

1 hair has heteroplasmic differences at 16234, 16093 and poly (C)
2 hairs show homoplasmic difference 16093
1 hair has homoplasmic difference at poly (C) and heteroplasmic difference at 16234
1 hair has homoplasmic difference at 16129 and heteroplasmic difference at 16234
Of hairs that are exactly the same in at least one section, up to 2 confirmed heteroplasmic differences were observed in another section.

Discussion
The discussion section of the manuscript will have to address the findings in the recent paper: Grzybowski, T., (2000) Extremely high levels of human mtDNA heteroplasmy in single hair roots. *Electrophoresis* **21**, 548-553.

In this communication, there were no duplicate analyses and contamination was not given as a possible explanation for some results. The author also suggests that only hair is a suitable reference sample for comparison with evidential hair. STADNAP results do not support this unless very many reference hairs are typed.

The importance of ISFG guidelines and EDNAP considerations on working practices will also be emphasised and the conclusion section will contain an interpretation based on these considerations.

A first draft of the manuscript will be prepared as soon as possible.

Angel Carracedo provided a pre-publication copy of the ISFG Guidelines which is attached at Annex 2.

2.2 mtDNA from head hair samples II — Gillian Tully

Experimental status

Hairs from the first donor proved unsuitable
Hairs from a new donor will be sent to laboratories during April
The target date for the results will be end of September.

2.3 Y chromosome pentaplex — Angel Carracedo

A draft manuscript was presented to the meeting (Annex 3) in which the common problems with Y chromosome analysis have been discussed. The data show that there is relative homogeneity within European populations with the exception of the Finnish population.

It was suggested that the manuscript is submitted to Forensic Science International for publication as soon as possible. Members were asked to provide Angel with comments by the 20 May at the latest. It was further suggested that there is one author who writes “on behalf of the EDNAP group”. The participant members will be named in an appendix.

2.4 Analysis of mixtures — Peter Gill

All details are shown in Annex 4

To date not all participants have submitted returns. The results show that there is considerable variation between laboratories and that the use of singleplex systems is no better than using multiplexes.

The assessment of stutters, particularly in relation to the Penta STR loci, was discussed. It was decided to perform a comparison of stutters of the Penta STRs and SGM Plus. Peter Gill will send out a spreadsheet that should be completed by the members.

2.5 Degraded DNA — Peter Schneider

Peter Schneider has now designed a protocol for producing suitable amounts of degraded DNA. All details are given in Annex 5.

Laboratories who have not submitted results should do so as soon as possible. It was suggested that future experiments should involve samples with a greater level of degradation.

2.6 EMD-mtDNA — Angel Carracedo

While it was acknowledged that the methodology has potential for laboratories with a high throughput of samples in casework, the general lack of interest in the subject has arisen because there has been no profitable dialogue with APB.

Angel Carracedo will contact APB and offer relevant mtDNA samples in order to facilitate the fine-tuning of the kit for mtDNA investigations. We expect ABP to do this before further action is taken by the members.

2.7 Short STRs on telogen hairs — Hermann Schmitter

Because telogen hairs are those generally submitted in casework, this study has particular practical relevance. So far the study is incomplete but it has shown that it is possible to obtain a 9 locus multiplex result from a single telogen hair. One result from Peter Schneider is complete and correct. Hermann Schmitter and colleagues now have a total of 8 or 9 short STRs that are well suited for this kind of analysis.

Members are asked to submit results before September 2000.

A full report will be given at the next STADNAP meeting.

3. Update from other groups

3.1 ENFSI — Peter Gill

The last meeting was held in March at Weisbaden with 54 participants attending.

The exercise to compare multiplexes from ABD and Promega involved 30 laboratories. The information has now been published.

An exercise to assess the potential of low copy number analysis has been undertaken as an inter-laboratory exercise. Handprints were prepared on acetate sheets and laboratories were

asked to make duplicates for analysis. While large-scale statistical treatments were necessary, the outcome was generally good based on consensus results.

A Quality Assessment Programme has been agreed upon. If possible, the programme will be sent to the EDNAP members.

A population database is being compiled which will incorporate data from all member laboratories. The information will be published.

The next meeting is 12 – 16 September 2000 in Krakow.

3.2 Police Co-operation Working Group (PCWG) Hermann Schmitter

This group wished to increase the European Core Loci from 4 to 7

3.3 Interpol DNA Monitoring Expert Group Richard Scheithauer

At the end of 1998 the group moved from European to international status with the general remit to aid in crime scene investigation. The first DNA user group met in Lyon in 1999 with police representatives and some scientists attending. In February 2000, the Expert Group met in Innsbruck. In order to keep the membership small, the members should represent groups and be prepared to disseminate the information gained from the meetings.

Participating states will send scene of crime results to Interpol for comparison with reference samples in other countries. The PCWG 7 loci package will be used in the knowledge that this will change in the future.

The next meeting will set the terms of reference and other subjects for discussion will include quality issues and available training courses. There is also a need to set a platform for international data exchange. The group will also focus on police training in DNA.

A website does exist but access is not worthwhile at present.

3.4 Prevention of Fraud Workshop Peter Schneider

Peter Schneider attended a meeting in Brussels on 7 December 1999. The workshop encompassed the Standards and Measurement Project and the Research in Identification Systems both of which are funded by the EU.

The aims were to share information in existing projects and to identify future collaboration. In April 2000, there was a call for anti-fraud methodologies for “protecting European economic interests”.

All details of the workshop are included in Annex 6.

4. Update from Companies

4.1 PE Biosystems Nicola Oldroyd

The “PE” has now been removed from the company name.

On new software there will be a disclaimer “For forensic and research only”.

A user bulletin is now available for the 96-lane protocol and validation document is being prepared for the 3700.

All new instruments will use NT workstations but there will be continued support for MacIntosh. The conversion for existing platforms is scheduled for later this year.

Hi-Di formamide has now been repackaged and the purity has been upgraded. This seems to be of special importance in capillary electrophoresis.

The AmpFLSTR kit product line (SGM Plus, Blue kit, Profiler Plus, etc.) has been rationalised. The Universal Databasing multiplex has the 13 CODIS loci plus D2S1338 and D19S433 and this should satisfy all international standards. There is no change to the primer sequences and the new multiplex can be used as an alternative to those in present use.

New dye chemistry has been employed to produce a 5-dye set: 6-FAM, VIC and NED are ready but the 4th and 5th dyes are still in preparation. All instruments are 5-dye compatible and new software is already available.

ABI now have a new instrument: the ABI Prism 3100 Genetic Analyser, which is a 16 capillary machine. This is the result of collaboration with Hitachi. The prize is expected to be at the same level as that of ABI 377.

4.2 Amersham-Pharmacia-Biotech

No representative attended the meeting.

4.3 Promega

No representative attended the meeting. Randy Nagy has now left the company which, in future, will be represented by Tom Moser.

5. Suggestion for an EDNAP mtDNA database — Walther Parson

A draft description of the project is attached at Annex 7

Two mtDNA databases are already in existence; one from the FBI and the other from Germany. The proposal is that there should be an open database on the Internet that is maintained by EDNAP. The acronym EMPOP was suggested for ‘The EDNAP mitochondria DNA Population Database Project’.

The pilot project, which has been set up in Innsbruck, uses software that can check input data to ensure that it is a sensible result. Statistical data on major ethnic types and sub-affiliations is included. The software also records who has accessed the database and what has been searched.

Richard Scheithauer guarantied that the Innsbruck laboratory can maintain a mtDNA database for at least 5 years.

The members agreed that they would be happy to have EMPOP under the EDNAP umbrella.

As it is essential to maintain quality standards it might be necessary to have proficiency testing on those who will input data.

6. Reports from Work Package Managers

6.1 WP1 State of the Art — Ate Kloosterman

The exercise involving the penta STRs did show low stutter. It is a question if the overall results were better than those of the commonly used STRs.

It was considered that an exercise to explore the use of racially linked STRs was not feasible even though many police forces would be in favour of such a project.

SNPs were thought to constitute the next phase of development and although single SNPs are unlikely to be used, this approach would facilitate an easy introduction to the methodology. The group was confident that an exercise could be achieved as a platform for obtaining future funding.

The exercise would need to be designed for detection on both 310 and 377 instruments. All laboratories should participate in the exercise. Care will be taken in the selection of the test SNPs, taking into consideration the problems in some countries when working with coding areas and flanking regions where other information may reside.

ABI have a "Snapshot" kit and it might be possible to supply these to all participating laboratories. Nicola Oldroyd will explore the possibility of supplying members with Snapshot kits for an exercise.

Ate Kloosterman and Steve Rand will co-ordinate the exercise.

6.2 WP2 Projects — Peter Gill

mtDNA hair exercise I – final data is being generated.
mtDNA hair exercise II - hair samples have been sent out and the results are due to being returned.
mtDNA nomenclature exercise – a draft manuscript has been circulated (Annex 8) and comments are requested by 30 May. In the meantime, a letter to the editor of For Sci Int will be drafted by Gill Tully to address the questions raised by the Grzybowski paper.

EMPOP database – the difficulties associated with obtaining high quality data and the monitoring of quality in general, need to be addressed. It was requested that Walther Parsons should prepare suggestions for finalising the website and for running the database and contact members via e-mail.

Mixtures exercise – more data needs to be collected and the question of "stutters" needs more consideration.

Telogen hairs – Hermann Schmitter will send out more hair samples and the deadline for returning results is 31 August.

Y chromosome exercise – In the near future, it is expected that a better Y chromosome multiplex will be available. Most members would like to take part in the exercise but at a later date. Maviky Lareu will prepare a suggestion and the details will be discussed at the September meeting.

Degraded DNA – results from the current exercise should be returned to Peter Schneider by 30 June. There were suggestions that future exercises could involve new degraded material and, perhaps, mixtures. It was also proposed that the exercise could involve non-EDNAP laboratories.

6.3 WP3 Technology Transfer Programme Steve Rand

Money is still available for new secondments. Following each secondment it is imperative that Steve Rand receives a report (approximately one page, e.g. by email) with the information on when the secondment took place, where it was and what was undertaken. This also counts for old secondments.

6.4 WP4 Population Database Compilation Denise Syndercombe Court

Databases have been received from the following countries:

Argentina (1), Austria (2), Belgium (3), Brazil (1), Denmark (1), France (2), Germany (13), Italy (1), Japan (1), Norway (1), Poland (1), Portugal (3), South Africa (1), Spain (3), The Netherlands (1), UK (3).

The following STR systems have been included:

CD4, CSF1PO, D2, D3, D5, D7, D8, D12, D13, D16, D18, D21, FES, FGA, F13A, F13B, Folp, SE33, THO1, TPOX and VWA.

There was some discussion with regard to placing genotypes on an open network even though they would be anonymous. This will be discussed at the September meeting.

7. mtDNA – Update on considerations Gillian Tully

A draft paper was circulated to members (Annex 8).

The following points were raised for consideration:

As the IFSG have already addressed the nomenclature, it might not be a requirement for this paper.

Working practices show that EDNAP is more detailed than the ISFG.

In the interpretation of results, EDNAP was again more detailed than the ISFG.

The criteria for inclusions and exclusions are less arbitrary than those of the FBI.

Comments on the draft manuscript should be sent to Gill Tully by 31 May.

8. Practical notes on the STADNAP project — Angel Carracedo

So far the Commission has allocated 68% of the total budget.

In the past week, Angel Carracedo has sent money to all laboratories.

All expenditure must be justified. If there is no written justification, there will be no EU contribution.

Angel Carracedo will e-mail all laboratories detailing how much money they have left to spend.

Interim reports are required. Each WP manager should send a two page report **immediately** to Angel Carracedo.

WP managers should start preparing the drafts of their final reports. These reports will be required by the end of August and should be approximately 10 to 15 pages in length and prepared in 12pt Times New Roman with 1.5 spacing.

The final cost statements must be received by Angel Carracedo by 21 September 2000.

WP managers should meet together before the September meeting to discuss the final report.

9. Any other business

No items were identified

10. Enclosures

Annex 1 List of participants
Annex 2 Proof of the ISFG guidelines on mtDNA typing
Annex 3 Draft of manuscript of Y chromosome exercise
Annex 4 Results of mixtures exercise
Annex 5 Status on degraded DNA exercise
Annex 6 Executive summary of the EU workshop on fraud detection
Annex 7 Suggestion for an mtDNA database (EMPOP)
Annex 8 Draft on EDNAP's considerations on mtDNA typing.