

EUROPEAN DNA PROFILING GROUP (EDNAP) MEETING

STRASBOURG - 10 MAY 2003

Host: Bertrand Ludes/Christine Keyser-Tracqui

Chairman: Niels Morling

A list of participants is attached as Annex 1.

Welcome

Bertrand Ludes welcomed members to Strasbourg.

2. Update on EDNAP exercises

2.1 mtDNA from head hair samples - 1

Niels Morling

A manuscript concerning mtDNA typing of hairs from an individual with known mtDNA heteroplasmy was submitted at the end of 2002 to the Forensic Science International. The comments of the reviewers were returned to Gillian Tully who is on maternity leave.

2.2 mtDNA from head hair samples - 2

Niels Morling/Walther Parson

Results have been obtained from 8 of 11 participating labs. Coimbra is still working on the project, hairs were lost in the AFDIL lab, and Rome has referred their hairs to Innsbruck who has offered to type the last hairs of the project. Münster will investigate if they need more hairs. It will be checked if some of the hair fragments should be typed in another lab than Innsbruck in order to avoid that all results of a hair are obtained in Innsbruck.

The number of hairs (50-60) to be sequenced in Innsbruck will make it necessary to use high throughput technologies including extraction and sequencing in a 96-well format.

Walther Parson shortly presented the GenoM system which is a 48/96 well extraction instrument using magnetic beads. Innsbruck has worked with mtDNA sequencing of DNA extracted by GenoM from telogen hairs in a 96 well format with good results. The price of the GenoM robot is approximately 70,000 €. The lab at Charite in Berlin has used GenoM for extraction of DNA from more than 10,000 stains.

2.3 Degraded DNA

Peter Schneider

The final version of the manuscript on STR typing results of degraded DNA by Peter Schneider et al was circulated. Based on suggestions by the participants, data on the mtDNA results as well as thresholds for allele calling were included. The manuscript was submitted three days earlier together with the accompanying manuscript on the production of degraded DNA (by Bender et al.) to For. Sci. Int.

2.4 EMPOP

Walther Parson

Walther Parson presented an update from the EMPOP project collaborative exercise. Until 1 May 2003, 21 participating laboratories have submitted results and raw data. For 11 of the laboratories, PHRED (GeneCodes) quality values were determined from the raw sequence data in order to evaluate whether PHRED values would be useful for monitoring the quality

of the sequencing analyses. The results of the exercises have been analysed and a manuscript will be sent to the participants for comments.

Phylogenetic analyses can identify certain kinds of errors. Peter Foster offers a freeware computer programme (see the web) that investigates for the likelihood of data based on a phylogenetic analysis. Innsbruck uses this programme among others to scrutinize data.

The EMPOP database now consists of more than 1,600 haplotypes.

Groups from the universities of Zagreb, Oslo and Bydgoszcz have visited the Innsbruck lab and contributed to the project.

2.5 FSS-SNPs

Angel Carracedo had indicated that Peter Gill had informed him that the FSS and SWGDAM had been in contact in order to select common SNPs and that 25 common, autosomal SNPs had been agreed upon.

2.6 Y chromosome SNPs – Santiago II

Angel Carracedo

Santiago has circulated information on 11 Y chromosome SNPs together with two SNP typed samples (control samples) and two untyped samples (test samples). Results from 7 labs and population data from 5 labs have been received. The results were concordant. Unexpected extra reactions were observed in P25 and 92R7 reflecting duplications on the Y chromosome. Copenhagen, Innsbruck and Santiago are working on a publication on the duplication phenomenon. Maria Brion is working on a draft concerning the main results of the exercise.

Participants are asked to check if results have been submitted and submit results by 1 June 2003.

2.7 SNP status in EDNAP labs

Brussels: Works on Y SNPs with Snapshot.

Copenhagen: Works on Y, autosomal, pharmacogenetic SNPs and soon also on SNPs correlated with physical traits. Focus on multiplexing. Snapshot is used as the reference method. Maldi-Tof is promising. The present version of Nanochip technology seems to be difficult to implement for forensic applications due to low sensitivity with long amplicons and difficulties in using multiplex amplicons. Contamination problems were observed with amplicons below 60 bp.

Innsbruck: Works on mtDNA with Snapshot, MS electrospray, and realtime-PCR

Mainz: Works on Y, autosomal and pharmacogenetic SNPs with Snapshot, Nanogen and glass spotted array technology.

Münster: Works on the EDNAP Y SNP exercise with Snapshot. Looking into real-time PCR.

Rome: Works on Y SNPs with PCR-RFLP on AB373 and AB310.

Strasbourg: Works on Y SNPs with MS and sequencing.

Santiago: Works on Y and mtDNA SNPs, mainly with PCR–RFLP for Y SNPS and Snapshot markers that are not digestible. Other technologies including Nanogen, Maldi-Tof and glass slide micro–array are being explored.

London: Works on Y SNPs with Snapshot and real-time PCR.

Wiesbaden: Works on Y SNPs with Snapshot and real-time PCR.

Other laboratories: SNP work is being done in Wreijswijk and Birmingham but no representatives were present to report. SNP work is not being done in Athens, Dublin, Linköping and Zürich. No information was available from Coimbra and Oslo.

2.8 Population database compilation

Denise Syndercombe Court

After the previous set of data had been stolen, the database has now been reconstructed. Preliminary analysis on 12,000 samples typed for TH01 and VWA showed highly significant deviations from H-W equilibrium. Other loci will be scrutinized, and the data will be analysed according to ethnicity and geographical origin. The results and frequencies will be made available through the ISFG-EDNAP web page.

The information on the STADNAP homepage will be moved to the ISFG-EDNAP homepage when possible.

3. Updates from other groups

3.1 ENFSI

Richard Scheithauer

Last meeting in Birmingham 6 Dec 2002. Next meeting in Prague, May 2003, the following meeting in September 2003.

The chairman, Dave Werret, will retire.

An STR database will be made available in September 2003 on www.strbase.org.

ENFSI laboratories participated in the GEDNAP proficiency trials. ENFSI and other labs had similar error rates – normally transcription errors not typical for casework.

Focus was put on competence assurance of personnel.

The FSS has developed the Pendulum software expert system for interpretation of DNA mixtures.

LCN methods for detection of transfer of DNA were presented.

Peter Gill informed about collaboration on SNPs with FBI.

3.2 Interpol

Richard Scheithauer

The DNA activities of Interpol were briefly presented. The information can be found on the website <http://www.interpol.int/forensic/DNA>.

3.3 SNPforID

3.3.1 SNPforID - overview

Peter Schneider

The overview on the project and its current activities is summarized in Annex 2.

3.3.2 SNPforID – SNP selection

Chris Philips

Recommendations for the websites listing all the HGMP and private lists of SNP loci (available both for public and private access) were presented. These include NCBI dbSNP, The SNP Consortium, Celera Discovery System and the ABI Assays on Demand portal. CDS requires an annual fee of €2,000 and offers information on 4.1 million SNPs.

The search criteria used by the SNP selection working group were outlined. The importance of validation status was emphasized with the need to identify both true and unique SNPs as forensic candidates. In addition, allele frequency estimates can vary between those based on small sample sizes (or pooled sequencing) and those on a larger scale and therefore of greater accuracy. CDS frequencies are based on 45 Africans and 45 Caucasians (with Chinese to be incorporated in September) so this provides a valuable cross check on quality of validation of SNPs identified in dbSNP.

Linkage considerations were reviewed both between SNP candidates and genes and between candidate SNPs on the same chromosome. The latest developments in linkage disequilibrium (LD) block analysis were presented with the extent of LD appearing to be more extensive than originally thought and LD values differing between populations. The LDU approach of Maniatis to mapping blocks was discussed – with the aim of directing searches to areas of low LD and high recombination so that ethical issues are avoided and the product rule can be used to construct cumulative frequencies for 50 – 80 SNPs. Inter SNP linkage was considered to be much less important in the light of studies by Charles Brenner on the Orchid SNP set that showed no allelic association between loci as close as 0.05 centiMorgans

Polymorphic information content (PIC) as a selection criterion was discussed. Discrimination Power (DP) values were shown to decrease only slightly between allele values of 0.5 and 0.3. Differences in allele frequency distributions between racial groups are commonly seen, and these may be exploited to provide additional information within a SNP set.

SNP clustering and its implications for multiplex design was discussed. This is a common phenomenon and represents a constraint on SNP candidate selection since primer and probe binding positions become difficult to select. The issue is less important with a reduction in amplicon size as required for forensic amplifications.

4. New exercises

It was discussed to perform an exercise concerning Y chromosome haplogrouping of a smaller number of male samples. Members preferred to await further collaborative exercises until it becomes possible to circulate multiplex SNP ‘kits’ among laboratories. Peter Schneider will send out their present Y-SNP typing protocol using smaller amplification multiplexes and Snapshot detection of multiplexes.

5. New technologies

5.1 SNPplex SNP Typing Technology

Chris Philips

The multiplex strategy devised by ABI for high throughput SNP typing was presented. The technique is based on oligo ligation of a locus specific primer and one or two allele specific primers. This is followed by a PCR amplification using primers incorporating the normal ABI fluorescent dyes and a “drag chute” proprietary mobility modifier - allowing fine tuned fractionation of products with capillary sequencers.

This technology will not be available for 3100/310 sequencers and will require next generation 3730 48/96 capillary array sequencers. The cost will probably be € 300,000 – 400,000. The equipment allows high throughput analyses. Assay kits will be provided for SNPplex typing along the same lines as assay on demand for Taqman.

Santiago intends to purchase a 3730 and use this technology for association studies and large scale genomics. The 3730/SNPplex technology could therefore be assessed for forensic use and SNPplex ligation assays could be tried on the 3100 – as this might be a way of adopting a multiplexing SNP technique for forensic use.

Celera will be offering detailed locus information for SNP selections as part of the SNPplex assay design package and this may be a useful service for mapping physical traits at a later stage in SNP development in forensic analysis.

6. Next meeting

A meeting will be held during the ISFG congress in Arcachon 9 - 14 September 2003. Members who would like to host the following EDNAP meeting in the beginning of 2004 are kindly asked to approach the secretary.

7. Any other business

There was no other business and the meeting closed with sincere thanks to Christine Keyser-Tracqui and Bertrand Ludes.