Moalin2

European DNA Profiling (EDNAP) Meeting

Coimbra, Portugal. 22 October 1994

Welcome address by host, Maria Vide.

Chairman: Peter D Martin

List of members attending is attached.

REPORT OF THE MEETING

1. EDNAP STR Exercise. Julia Andersen, MPFSL (London)

Each member received a copy of a report of the results from the exercise (a copy is attached to this report).

As all EDNAP members had successfully profiled each of the bloodstains and identified those which were mixtures, there was little discussion on this aspect of the exercise. It was agreed that a manuscript should be prepared and submitted to Forensic Science International for publication.

There was considerable debate on the various other loci which were used in addition to the two designated loci; in particular SE33. There was general agreement that the results of any additional loci examined should be included in the publication but these results must be reported in context ie the exercise was intended to test the ability of members to identify the alleles from HUMTHO1 and VWA loci.

2. Frequency calculations

In answer to the question of why the UK public sector laboratories (FSS and MPFSL) used a 5.0% default value, **Peter Gill** replied that until we have bigger databases we feel it necessary to use a conservative value. Additionally, if confidence intervals are used, they should be calculated on the total number of loci used and not individually.

Peter Gill presented some population information generated from the quadruplex of loci THO1, VWA, F13 and FES. Among fifteen populations obtained from various laboratories, he reported that the biggest variation in allelic frequency between different ethnic populations was in THO1. He reported a similar variation in allelic frequency between White Caucasian populations. Although the FSS and MPFSL routinely use a default frequency value of 5.0%, they also include an Fst kinship factor according to the formula produced by Nicholls and Balding:

$$P = C + (1-C)p \quad \text{where C is the inbreeding coefficient}$$

As many of the possible sub-populations have never been sampled, **Peter Gill** recommended that for the present an Fst should be included which is considered equivalent to C. He said that the Fst is very small when comparing white populations and the same is found for a comparison of Asian populations (Hindu, Sikh, Bangaladesh and Birmingham Asians). 0.001 would appear to be an appropriate Fst value but, for the present, a higher value will continue to be used by the FSS and MPFSL.

Locus-locus interactions were observed by the FSS and MPFSL when using the Weir exact tests but they were not consistent within samples from the same population or between ethnic groups when studies were made of the databases from various laboratories. Explanations offered were sampling bias or that this was an effect of the statistics.

Christian Rittner reported that studies with the Polymarker, on a small database, had shown no deviation from Hardy Weinberg with the exception of MN. The deviation seen was not compatible with the original results from the serological typing. He could offer no explanation.

In a study of 300 Norwegians using THO1 and VWA **Bjōrnar Olaisen** obtained a skewed distribution when assuming that null alleles were absent.not present. If, however, he assumed that the population included 3-4% null alleles he found a normal distribution.
Two approaches were made to investigate the occurrence of null alleles:

to consider informative meioses

to use external primers

He concluded from his study that null alleles could not account for the original skewed distribution. Possible alternative explanations advanced were segregation distortion, population substructure or chance occurrence. Further work will include the use of phenotype frequencies, rather than counting alleles, or resampling the populations.

Bernd Brinkmann reported that in an investigation of approximately 1,700 meioses he had observed only 4 or 5 mutations. These were all insertions or deletions of 4 nucleotide repeat units and were not associated with homozygotes. He suggested that it could be worthwhile to compare multiplexing with single locus amplifications to determine whether there is preferential amplifications of alleles which could indicate the presence of null alleles. **Colin Kimpton** reported that the FSS have already shown that this is not the case and the results are in press awaiting publication.

Bernd Brinkmann presented a study on microheterogeneity by sequencing alleles in a number of the loci which have been discussed within EDNAP. The results showed that there is a considerable range of variation between loci. His results were as follows:

In general THO1, F13A and FES show little microheterogeneity.

FES and SE33 show deviations in the flanking regions.

SE33 studies showed one site which was prone to insertions or deletions. The

insertion of a hexamer was also observed at variable sites.

Microvariation was observed within the repeat sequences of D21S11.

F13B showed a deletion in the 3' flanking region.

Apes had also been studied using the THO1 and VWA loci and it was found that as the apes evolved (apes - humanoids - humans), the longer the repeat sequences became.

Colin Kimpton outlined the developments which the FSS is proposing for the next multiplex system. He referred back to the previous meeting in Copenhagen when the Hex + Sex was first discussed. Since then problems have been encountered with the amplification of D19S253 which appear to be due to a mutation in the primer binding site. This locus has now been replaced in the multiplex by two additional loci, D6S502 and D20S085 to give a total of seven loci plus AMG. For white caucasians the DP = 1 in 5 X 10^8. The largest product of this proposed multiplex is 340 nucleotides. The system will only be suitable for fluorescence detection.

In general the peak heights are uniform. Occasional artefact peaks can be easily distinguished from those of true allelic products.

Of the commercial non-fluorescent STR multiplexes the Promega system (2 triplexes combined) has a DP = 1 in 10^5

Peter Martin asked the meeting whether, in light of these developments, members should be considering any other loci which can be used for inter-laboratory harmonization. No decision was reached but **Colin Kimpton** offered to provide primers for any of the members who wished to experiment with the loci being developed by the FSS.

Chris Konialis described an X-linked locus (DXS101) which he has investigated in his laboratory. This is an informative locus of trinucleotide repeats with at least 17 alleles. No 'stutter' bands were observed and the system was reported to amplify well with the FSS quadruplex and with AMG. A database of approximately 155 individuals (male and female) have been examined and there is good agreement with HW equilibrium for the Greek population. However, he reported considerable differences between Caucasian, Asian and Black populations (the numbers in the Asian and Black samples were much smaller than those in the White Caucasian sample). He plans further studies using CEPH family samples.

Peter Martin raised the question of the relative merits of multiplexing and single locus amplifications. **Peter Gill** led the discussion on the theme of maximising the use of resources to obtain results which are applicable to national databasing and not prohibitive in terms of cost per sample. He said that multiplexing is performing reliably in the hands of the FSS scientists and the US Army laboratory. The MPFSL has experienced some preferential amplification of small molecular weight products at the expense the locus specific repeat sequences.

Hermann Schmitter reported that he had experienced variable peak heights and that he prefers to use single amplifications which are combined prior to electrophoresis. He stated

that he does not measure the quantity of DNA before amplification. **Chris Konialis** agreed with other workers that quantification is an important step, saying that there is a narrow window of 1-5ng which must be observed to prevent problems with amplification. **Bernd Brinkmann** considered that there might be a political problem if cost efficiency conflicts with evidential value. He commented that if amplification is carried out in separate tubes, contamination might occur in only one tube rather than affecting all loci. Also, because of the variability of peak height, he questioned whether allelic 'drop-out' might arise. He also commented on a need for careful balancing of the primer concentrations in a multiplex system to obtain maximum sensitivity and avoid the production of 'stutter' bands and the possibility of allelic 'drop-out'.

Julia Andersen confirmed that the MPFSL is experiencing a problem with amplification which she suspects is due to primer-primer interaction with the preferential amplification of small products. Although this phenomenon is not uncommon it does not always prevent amplification of the quadruplex loci. **Colin Kimpton** considered that there will always be some primer-dimer formed but that this should not normally prevent amplification of the various loci present.

Niels Morling said that his laboratory had experienced problems with multiplexing using their own prepared primers. However since they changed to the FSS 'multimix' and protocol they have been achieving good quality results. **Bernd Brinkmann** asked if we should settle on some quality systems eg the relative peak heights of the loci being multiplexed should not vary by more than a specified amount. **Peter Gill** reiterated that the systems had been very successful in the FSS and other laboratories and had been subjected to large and rigorous validation procedures. He suggested that all laboratories should have adequate quality control and quality assurance procedures. **Angel Carracedo** said that he was in favour of working towards validation of multiplex systems with standardisation being on a minimum basis. However, he saw value in the use of single amplifications so that prosecution and defence laboratories could standardise on systems without having to invest in expensive equipment such as ABI sequencers.

3. Capillary electrophoresis

Ate Kloosterman reported that Beckmann have made considerable advances with the development of capillary electrophoresis for DNA analysis. It is now possible to obtain STR separations with a single base pair resolution and a throughput of 2 to 3 samples per hour. In order to increase the throughput work is continuing on automated sampling with multi-channel analysis. Each column is capable of 1000-2000 sample separations but each new column must be separately calibrated. He considered that it will be 1 to 2 years before the technology is sufficiently advanced for use in forensic science. **Peter Gill** informed members that ABI are also developing the technology.

4. SE33

A number of the members wished to continue using SE33 in casework so it was decided that there should be a separate working group to determine the best way to progress this study. **Bernd Brinkmann** offered the Münster allelic ladder for use in this study and it was agreed that the aim of the exercise would be to produce a standard form of nomenclature for the use

of SE33. It was also agreed that the same samples used for the THO1/VWA exercise should be used in this study. The MPFSL will provide the necessary samples. **Ate Kloosterman** agreed to be the co-ordinator of the SE33 exercise. The following laboratories expressed an interest in participation: Coimbra, Linköping, Münster, Oslo, Rijswijk, Santiago de Compostela, Wiesbaden and Zurich.

5. Databases

The MPFSL and the FSS would co-operate on the preparation and publication of any population database information. Any laboratories who are willing to share their information should contact either **Julia Andersen** or **Colin Kimpton**.

6. Next meeting

Bernd Brinkmann suggested that the next meeting should contain a workshop on some statistical issues associated with new DNA technology. It was agreed that the aims of such a workshop need to be clarified. The only positive proposal was a need for recommendations on ways in which to treat the data which is currently being obtained by member laboratories. **Peter Martin** agreed to invite Ian Evett and David Balding to address the meeting and to canvass EDNAP members for their opinions on the content of the workshop.

Birmingham, London and Athens were offered as venues for the next meeting. There was an overwhelming vote for Athens. Requests were made to avoid the weekends of 5.5.95 and 17.5.95.

The meeting closed with thanks to **Maria Vide** for providing such an excellent venue, for first class organisation and for such a lavish social programme. Delegates also wished to thank members of her family for all their hard work in associated with the meeting.
There followed an address by the Professor of Legal Medicine of the University of Coimbra.