Standardization of DNA Profiling Techniques in the European Union

– STADNAP –

FINAL REPORT

A network project of the European Commission - DG XII - programme

"Standards, Measurement and Testing"

Contract 97-7506

01.09.1997 – 30.08.2000

Internet Homepage: http://www.STADNAP.uni-mainz.de

Table of contents

1. Summary

The project has successfully achieved most of the initial objectives defined in four work packages related to a survey of the state-of-the-art of DNA profiling, a series of intercomparison exercises, a technology transfer programme, as well as to the establishment of a European population database for the commonly used DNA markers.

The working groups have developed their respective programmes based on these work packages with specific roles assigned to each partner. In addition, all the laboratories actively participated in the inter-comparison exercises agreed on by the consortium.

A total of seven meetings were held in this period plus an expert hearing on new methodologies.

The appreciation of the importance of DNA profiling in forensic casework in Europe was acknowledged by the group, STR profiling with PCR multiplexes being widespread. However the importance of new systems such as mtDNA and Y chromosome polymorphisms is continuously increasing and the group has made a significant effort towards the standardization of this new group of markers during these last three years.

After an expert hearing on new methodologies held in Mechelen, the conclusion was that the DNA microsatellites in current use will continue being used in the short to medium time-scale, but new markers such as SNPs, through the implementation of new methodologies like DNA microarrays are promising markers for the analysis of variation in mitochondrial DNA and the Y chromosome.

The collaboration with the associated industrial partners was excellent and it was clearly shown that feedback from the companies is necessary to explore the applicability of the new methodologies and to promote common European standards.

In addition to the intercomparison exercises carried out on mtDNA and Y chromosome polymorphisms, other exercises concerning new technologies (new methods of mutation analysis, SNP detection) have been designed. Two exercises were particularly important: one concerning the analysis and interpretation of mixtures, one of the most important challenges in DNA analysis; and the other concerning degraded DNA. This latter is crucial for the evaluation of DNA markers and methodologies, due to the importance placed on comparing their performance against control standard degraded DNA. For this reason the standard degraded DNA produced by the group was used in an inter-collaborative exercise open to the whole European forensic community with a high degree of participation.

The exchanges of personnel between European laboratories have also proven to be very successful and of great importance to the harmonization of methodologies and the promotion of common standards. A total of 14 exchanges of researchers among European laboratories were supported by the programme.

A compilation of gene frequencies of commonly used short tandem repeat systems (STRs) for European populations is being undertaken with very important contributions by a large number of European laboratories. Overall, more than 38,000 genotypes for 38 STR loci were provided. In addition, a high quality mtDNA sequence database is being set up.

A STADNAP World Wide Web Homepage has been created where detailed information can be obtained (http://www.STADNAP.uni-mainz.de).

Although the programme has been quite successful, all the partners are keenly aware of the requirement to continue working on the standardization of DNA profiling in Europe, and recommend that the EU takes the action necessary to support this need.

2. Definition of acronyms, terms and abbreviations

allele – the form of a gene, alternative alleles which segregate in the population are the basis of a genetic polymorphism

amplification – the process in which the number of target molecules is increased exponentially during PCR

bp – basepair, smallest unit to describe the length of a DNA molecule or fragment, comprises two complementary bases

chromosome –a physical structure in the cell nucleus, made of DNA, RNA and proteins. The genes are arranged in linear order along the chromosome.

DNA – desoxyribonucleic acid, double-stranded molecule built from the four bases Adenin (A), Thymin (T), Cytidin (C) and Guanin (G); A and T as well as G and C are complementary to each other

D-loop – displacement loop, part of the mtDNA control regions exhibiting polymorphic sequence variations

EDNAP – European DNA Profiling Group

ENFSI – European Network of Forensic Science Institutes

gene – genetic information required to define a protein (= gene product), includes control sequences (= promotor) to regulate its expression

genome – the genetic information required to define all traits of an individual; the human genome has a total length of 3 x 10^9 bp and codes for approx. 50,000 genes

heteroplasmy –mixtures of more than a single mtDNA type within an individual

HV – hypervariable regions in the D-loop of mitochondrial DNA

ISFG – International Society for Forensic Genetics

kb – kilo basepairs = 1000 bp

locus – a defined segment on a chromosome coding for a gene, or describing a polymorphic segment, e.g. a STR sequence

marker – an easily detected gene or chromosome region used for identification

mitochondrial DNA (mtDNA) – Maternally inherited double stranded DNA in the mitochondrias. There are thousands of copies of mtDNA in each cell compared to two copies of nucDNA, making mtDNA analysis a more sensitive assay, and thus, more successful on highly degraded specimens

nucleic acid –DNA or RNA

PCR – polymerase chain reaction, an in-vitro method allowing the targeted amplification of short segments of DNA by the use of two primers flanking the sequence of interest as well as the enzyme DNA polymerase

polymorphism – the presence of more than one allele at a locus in a population

primer – a short single-stranded DNA molecule, also termed oligonucleotide, and used as "starter" molecule for the PCR

SNP – single nucleotide polymorphism; a DNA sequence polymorphism defined by two alternative bases at a given position

STADNAP – Standardization of DNA Profiling in Europe

STR – short tandem repeat, microsatellites typically charactized by arrays of tandemly arranged repeats of 2-5 bp length

system – a genetic locus within the human genome, which is genetically polymorphic, and which can be studied using a particular set of molecular-biological methods (e.g. PCR analysis using distinct oligonucleotide primers, amplification conditions, and allelic ladders for the identification of alleles).

VNTR – variable number of tandem repeats

Y chromosome – one of the two sex chromosomes (X, Y) exhibiting male inheritance

3. Project description

3.1. Background

Forensic DNA profiling has become a standard technique in criminal investigations, since results can be obtained from any source of biological material provided it contains nucleated cells with genomic DNA. In contrast to conventional blood group analysis, the results of DNA profiling do not depend on the nature of the material or cell type analyzed, since the entire genetic information is contained in every single somatic cell of a given individual.

Due to the rapid progress in the field during the last fifteen years, parallel developments of methods as well as typing systems have been made in the laboratories involved in forensic DNA profiling. Further complication has arisen from the fact that there are significant differences regarding the degree of acceptance by the criminal justice systems in the European countries as well as regarding the acceptance of DNA typing in general by the public opinion (e.g., on issues concerned with privacy rights and data protection). This has resulted in heterogeneity of typing procedures as well as genetic systems used for forensic casework within the European Union (and even more in comparison to countries outside Europe, e.g. the USA).

However, intercomparison of DNA typing results becomes not only desirable, but absolutely necessary within Europe. Individual mobility has been facilitated significantly and is encouraged by the policy of the European Union. As restriction of movement becomes less, this inevitably means that cross border crime will increase – mobile serial offenders will not be detected by DNA profiling unless methods are standardized.

The introduction of typing methods based on the polymerase chain reaction (PCR) in combination with the analysis of highly variable microsatellite or short tandem repeat (STR) loci has led to a breakthrough in the forensic use of DNA methodology. A typical STR locus is composed from a variable number of 4-basepair repeats (ususally between 5 and 20 repeat units) of which one (in case of homozygosity) or two alleles (in case of heterozygosity) are present at this locus in a single genome. The total length of STR alleles is in the range between 150 - 400 bp which makes it possible to analyze degraded DNA from old stain material or decomposed tissues.

Furthermore, automated DNA fragment analysis using fluorescence-labelled PCR primers has become the standard method in most forensic labs facilitating a standardized interpretation of typing results.

The rapid progress in the human genome project has provided a continuously growing number of STR systems potentially suitable also for forensic DNA profiling. More than 1000 STR loci have been described which are distributed evenly throughout all chromosomes. If no general agreement is reached on a common set of STR systems suitable for forensic DNA profiling which will be used as standard systems in all major forensic laboratories, exchange of typing results will be virtually impossible due to fact that different STR systems are being selected by each laboratory for routine use, so that no common language exists for intercomparison.

In 1992, the Council of Europe has published a recommendation "on the use of analysis of deoxyribonucleic acid (DNA) within the framework of the criminal justice system" [No. R(92)1] and states (page 24, paragraph 45): "It is therefore important when the technique (i.e. DNA analysis) is used in the criminal justice system that only such laboratories are used which meet the highest standard of scientific integrity and technical skills and experience. One way of ensuring this would be to institute some form of accreditation and control system." Paragraph 57 (page 28) states that where transborder comparisons of DNA profiling are required, courts must be assured that the standards laid down in the recommendation are met.

The political leadership of the member states of the European Union has repeatedly expressed the importance of a close collaboration and coordination of police and law enforcement agencies and institutions. In the U.K., a National DNA Database has been introduced in 1995 to store STR profiles from suspects in 1995, convicted offenders as well as from stain materials collected from scenes of unsolved crimes. Since then, several other European countries have followed this example (see Schneider and Martin, DNA databases: The European experience. Forens. Sci. Int. In press). Exchange of database records between EU member states also requires an agreement on a common set of systems, comparable to speaking a common genetic language. The present STADNAP network application is specifically designated to establish the scientific basis for these European efforts.

3.2. Objectives

Based on these considerations, as well as the continuing rapid development in this area of research, the network participants have agreed to carry out a programme entitled "Standardization of DNA Profiling Techniques in the European Union" with the following objectives:

1. to form a network of European scientists working in the field of forensic stain analysis related to criminology and using DNA-based typing methods (termed "DNA profiling");
2. to evaluate and consolidate research objectives, to identify research priorities for a better management of national and EU resources, and to accelerate the development process as well as the implementation of basic research findings;
3. to identify criteria for the standardization of DNA-based typing systems, and to develop and propose recommendations which could form the basis of a European laboratory standard;
4. to carry out a series of collaborative intercomparison exercises intended to achieve harmonization of DNA-based typing systems;
5. to promote technology and experience transfer by offering short-term secondments for exchange of scientific personnel between the participants;
6. to compile a collection of frequency databases of DNA-based typing systems for the European populations, and to provide general access for the scientific community to this compilation by using the resources of electronic data communication.

3.3. Partners

The participants represent scientists from leading forensic DNA laboratories both from university institutes and departments as well as from police institutions from all EU member states. Thus it has been ensured that both academic researchers and routine casework practitioners are closely collaborating in this network.

a) Main Contractor

1 Prof. Angel Carracedo (SPAIN)
Institute of Legal Medicine,
San Francisco, s/n, E-15705 Santiago de Compostela
(phone) +34 81 582327, (fax) +34 81 580336
apimlang@usc.es

b) Associated Contractors

2 HD Dr. Peter M. Schneider (GERMANY)
Institut für Rechtsmedizin, Universität Mainz
Am Pulverturm 3, D-55131 Mainz
(phone) +49 6131 3932687, (fax) +49 6131 3933183
pschneid@mail.uni-mainz.de

3 Dr. Ioulia Skitsa (GREECE)
Hellenic Police, Forensic Science Div. ,DNA Laboratory
14 Sevastopoulos Str., GR-11526 Athens
(phone) +30 1 748 6791, (fax) +30 1 645 2575

4 Dr. David J. Werrett (UNITED KINGDOM)
Forensic Science Service, Headquarters
Gooch Street North, Birmingham B5 6QQ, UK
(phone) +44 121 607 6871, (fax) +44 121 622 2051

5 Dr. Bernadette Hoste (BELGIUM)
Institut National de Criminalistique
Chaussée de Vilvorde 98-100, B-1120 Bruxelles
(phone) +32 2 240 0473, (fax) +32 2 241 6105

6 Dr. Maria Conceicao Vide (PORTUGAL)
Instituto de Medicin Legal
Largo da sé Nova, P-3000 Coimbra
(phone) +351 39 20000, (fax) +351 39 20549

7 Prof. Niels Morling (DENMARK)
Retsgenetisk Institut
11 Frederik Den Femtes Vej, DK-2000 Copenhagen
(phone) +45 35 32 61 10, (fax) +45 35 32 61 20
nmorling@forensic.ku.dk

8 Dr. Geraldine O'Donnell (IRELAND)
Forensic Science Laboratory, Garda Siochana
Phoenix Park, Dublin 8
(phone) +353 1677 1156, (fax) +353 1679 4667
fsl@indigo.ie

9 Prof. Richard Scheithauer (AUSTRIA)
Institut für Gerichtliche Medizin
Müllerstr. 44, A-6020 Innsbruck
(phone) +43 512 507 3301, (fax) +43 512 567319
richard.scheithauer@uibk.ac.at

10 Dr. Ann Jangblad (SWEDEN)
National Laboratory of Forensic Science, SKL
S-58194 Linköping
(phone) +46 13 24 1430, (fax) +46 131 45715

11 Dr. Denise Syndercombe-Court (UNITED KINGDOM)
St. Bartholomew's and the Royal London School of Medicine and Dentistry
Turner Street, London E1 2AD, UK
(phone) +44 171 377 7076, (fax) +44 171 377 7677
y.d.syndercombe-court@mds.qmw.ac.uk

12 Prof. Bernd Brinkmann (GERMANY)
Institut für Rechtsmedizin
Von Esmarch-Str.86, D-48149 Münster
(phone) +49 251 835 5161, (fax) +49 251 83 55158
brinkma@uni-muenster.de

13 Prof. Björnar Olaisen (NORWAY)
Rettsmedisinsk Institutt
Rikshospitalet, N-0027 Oslo
(phone) +47 2286 8676, (fax) +47 2220 9583
bjornar.olaisen@rh.uio.no

14 Dr. Patrick Fanardjian (FRANCE)
Direction de la Police Judiciare, Laboratoire de Police Scientifique
3 Rue de l'Horloge, F-75001 Paris
(phone) +33 1 53 73 5579, (fax) +33 1 46 34 7740

(This laboratory has not signed the final contract, although
the initial grant application was supported.)

15 Dr. Ate D. Kloosterman (NETHERLANDS)
Gerechtelijk Laboratorium
Volmerlaan 17, NL-2288 GD Rijswijk
(phone) +31 70 340 8131, (fax) +31 70 398 9259

16 Dr. Ernesto d'Aloja (ITALY)
Istituto Medicina Legale, Universita Cattolica
Largo Francesco Vito, 1, I-00168 Roma
(phone) +39 6 3350 7031, (fax) +39 6 355 07033

17 Prof. Bertrand Ludes (FRANCE)
Institut de Médecine Légale
11 rue Humann, F-67085 Strasbourg Cedex
(phone) +33 88 35 2859, (fax) +33 88 24 0085

18 Dr. Vivian Johnsson (FINLAND)
National Bureau of Investigation, Crime Laboratory
Jokiniemenkuja 4, SF-01370 Vantaa
(phone) +358 0 8388 6374, (fax) +358 0 8388 6303
vivian.johnsson@krp.poliisi.mailnet.fi

19 Dr. Hermann Schmitter (GERMANY)
Bundeskriminalamt
Thaerstr. 11, D-65193 Wiesbaden
(phone) +49 611 55 2661, (fax) +49 611 5 53875

20 Prof. Walter Bär (SWITZERLAND)
Institut für Gerichtsmedizin
Winterthurerstr. 190, CH-8057 Zürich
(phone) +41 1 257 5611, (fax) +41 1 364 0804

c) Industrial Consultants

The following two Industrial Consultants have agreed by a letter of committment to support the project programme by ensuring a mutual exchange of information without signing the contract:

1 Perkin-Elmer Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404, USA
(phone) +1 415 638-5343, (fax) +1 415 638-6333

2 Amersham Pharmacia Biotech
Postfach 5480, D-79021 Freiburg
(phone) +49 761-4519 0, (fax) +49 761 4519 159

3.4. Work Packages and Managers

Based on the objectives listed under 3.2, the following four work packages were agreed among the participants:

WP1: State-of-the-Art Survey

Manager: Ate Kloosterman (Rijswijk, Netherlands)

Members: Innsbruck; Austria

Linköping, Sweden

Coimbra, Portugal

The basis of identifying future research activities includes an accurate description of the situation of DNA profiling approaches in Europe. This includes the current typing systems used for DNA profiling, a definition of criteria relevant for the selection of efficient DNA typing systems in stain analysis, and the requirements for accuracy and reproducibility of typing methods.

WP2: Intercomparison Exercises

Manager: Peter Gill (Birmingham, UK)

Members: Oslo, Norway

Vantaa, Finland

Wiesbaden, Gemany

Zürich, Switzerland

Collaborative exercises are one of the foundations of achieving standardization of laboratory methods. Therefore, a number of such exercises are an essential part of the network activities. This includes the establishment of generally agreed typing protocols for transnational comparison, approaches to achieve reproducible typing results independently from the laboratory equipment, an agreement on technical criteria for DNA profiling systems improving the sectorial harmonization, as well as the implementation of basic research findings from the field of DNA profiling into routine casework. Collaborative exercises are also an excellent strategy to explore the potentials of new technologies prior to their potential implementation into routine applications.

WP3: Technology Transfer Programme

Manager: Bernd Brinkmannn (Münster, Germany)

Members: Athens, Greece

Copenhagen, Denmark

Dublin, Ireland

Santiago de Compostela, Spain

The transfer of technology and methods between laboratories and across national borders enhances the perspectives for achieving harmonization. The exchange of methods and experimental strategies promotes collaborations in forensic applications of DNA profiling. Short-term secondments for laboratory visits are provided to promote more intensive collaboration, preferably such that one laboratory from each Member and Associated State may be entitled to only one secondment.

WP4: Population Database Compilation

Manager: Denise Syndercombe-Court (London, UK)

Members: Bruxelles, Belgium

Mainz, Germany

Rome, Italy

Strasbourg, France

The "forensic value" of DNA profiling relies on a reliable estimate of a genotype frequency in the relevant population. This is the basis of successfully introducing the results of DNA typing in a court trial. Due to the numerous DNA systems currently applied in this field, biostatistical data of allele frequencies are not available for many of the present STR systems. Therefore, the network should establish a European database collection for all relevant DNA systems and populations. A database in this context is a collection of anonymous genotype or allele frequencies from representative population samples, which can only be used to determine genotype frequencies for biostatistical calculations in forensic stain cases and paternity analyses. This database should be disseminated to the relevant scientific community using the internet.

4. WP 1: State-of-the-art survey

4.1 Objective 1: The appreciation of the importance of DNA profiling in forensic casework in Europe

In the short time that molecular biology, in the form of DNA profiling, has been available to criminal investigative work, the various law enforcement authorities have come to rely more and more on the results which are presented to them by the scientists. The technology has provided the police and lawyers with the confidence to eliminate a suspect from the investigation or to press ahead with a prosecution based on the DNA results supplied from the laboratory. There is, therefore a considerable onus on the forensic scientists to ensure that there are adequate facilities to meet the needs of the law enforcement authorities and that the information imparted is accurate and intelligible. Now that the EU has an open border policy there is the potential for more cross-border crimes and therefore there is the added requirement for the possible exchange of DNA profiles between the laboratories throughout Europe. With the increasing choice in technologies and systems it is imperative that forensic science organisations settle on a common approach which allows for the effective interchange of data. This will include standard systems of analysis and an accepted nomenclature which is common to all participants.

From the onset of the use of DNA technology it was realised by the scientists involved that the day would come when the interchange of genetic information for crime investigation became commonplace but, at present, the progress is hampered by the disparate criminal justice systems which exist within the EU. Forensic scientists within each member country are bound by different laws which affect not only the way in which the technology is progressed but also the method of data and sample retention and the consequent problems with database compilation. The use of databases are now becoming widespread with most countries either having a national DNA database or legislation is being enacted to provide for their formation. At present the UK is in the vanguard with a national DNA database which is designed to hold profiles from suspects and those found guilty of crimes together with DNA profiles obtained from crime scenes. This database will soon hold close to a million

personal DNA profiles and provides the police with about 800 matches per week. While such an enterprise requires a massive input in finance and staff it is efficient in the amount of police time which is saved during the investigations. The success of the venture is to a large extent due to the comprehensive legislation which was enacted in 1995 and permitted the storage of a wide range of information and also allowed the scientist to retain the DNA samples for future analysis should it be required. Other member states have taken a different view, based largely on the needs to address human rights issues, and allow for only a limited amount of personal information to be held and often prevent the retention of the DNA sample. With the success of the UK system lawyers in other EU countries may need to rethink their strategies.

With the formation of such databases quality issues become paramount as it is important to ensure that samples are correctly typed and that transpositional errors do not occur with entry onto the database. Also, maintenance of the database and confidentiality must be constantly addressed. Therefor it is necessary to have quality assurance programmes for all those laboratories which input to the database and proficiency programmes for all operatives.

At present the technology used focuses on the use of STR multiplexes which have been agreed through the EDNAP inter-laboratory exercises but as the science progresses, new and more efficient systems will emerge which may not be compatible with those in current use (see objective 5, page 30). Should it be agreed that new methodology will be adopted at some time in future, due perhaps to the financial imperative, it will be necessary to re-type samples for those currently held on the database; a situation which could prove difficult for some laboratories.

While the bulk of the forensic DNA analysis has involved the use of multiplex autosomal STR based technology, there are a number of samples encountered in casework situations where this technology cannot be used. In these circumstances the forensic scientist must adopt the ‘tool-box ‘ approach in which the more esoteric methodology is used to deal with the particular situation. The requirement might be for the use of singleplex reactions in which special conditions apply or for the analysis of mitochondrial DNA when there is insufficient genomic DNA available.

Also in a number of cases, particularly those of sexual assault, the typing of Y chromosome STR loci can be of particular benefit. These subjects are discussed in Objective 5 (page 34).

References:

Martin PD, Schmitter H and Schneider PM (2001) A brief history of the formation of DNA databases in forensic science in Europe. *Forens. Sci. Int.* 119:225-231

Schneider PM and Martin PD (2001) Criminal DNA databases: The European situation. *Forens. Sci. Int.* 119:232-238

4.2 Objective 2: Typing systems used for DNA profiling in Europe

Leading up to 1997 there was a considerable range of STR loci in use in the various forensic science laboratories around Europe which included:

Polymarker Kit, Perkin Elmer Amp*Fl*STR kit, APO A11, CSF1PO, GABA, GATA4FO3, GGAA309, LPL, D1S518, D1S1656, D3S1358, D3S1514, D6S502, D6S962, D6S972, D8S1043, D10S516, D11S554, D12S808, D12S826, D14S306, D16S688, D19S283, D20S85, D20S438

When the Forensic Science Service (FSS) first introduced a multiplex which could be used for the formation of DNA databases in the UK, many European laboratories showed a considerable interest in its use for routine crime analysis. Unfortunately the multiplex only contained 4 loci, THO1, VWA, F13A and FES and while the latter 2 loci did not find popularity with a number of laboratories it was also considered to be too small to provide sufficient discrimination within populations.

A second attempt, however, showed a vast improvement and became acceptable to all the major forensic science laboratories in Europe. The system contained the following STR loci: THO1, VWA, FGA, D8S1179,D18S51 and D21S11. This, and all subsequent multiplexes, contained the amelogenin locus for sex determination.

An initiative by Interpol required the production of a set of loci which could be used to compile a register of sex offenders. Following initial recommendations by EDNAP and subsequent work by the European Network of Forensic Science Institutes (ENFSI) seven loci were identified :
THO1, VWA, FGA, D21S11, D3S1358, D8S1179 and D18S51. This multiplex became known as the ‘core European loci’.

Due to the success of the FSS multiplex commercial companies expressed an interest in the production of kits which would be available as off-the-shelf products. This is an attractive concept to forensic scientists as it alleviates the problems of production and maintenance of reagents and removes the complexities of licensing agreements. A kit has been produced which has been thoroughly tested by the EDNAP and ENFSI groups, through inter-laboratory exercises using a variety of methods, and comprises the following set of loci:
THO1, VWA, FGA, D2S1338, D3S1358, D8S1179. D16S539, D18S51, D19S433 and D21S11. While this multiplex has gained wide acceptance around Europe, with most laboratories becoming proficient in its use, the commercial companies involved are now planning to market new kits which contain 16 loci. There is, however, considerable overlap between these multiplexes such that, whichever kit is used, there will be the potential for an effective exchange of data.

4.3 Objective 3: Criteria relevant to the selection of efficient DNA typing systems in stain analysis

There is a general desire on the part of the law enforcement agencies for the scientists to be able to obtain results from extremely small amounts of template material and while this development brings its own set of peripheral problems many laboratories are responding positively. Thought must be given to changes in the way in which detection systems will be used in the future and different laboratories will have different requirements. The laboratories with a large throughput of samples will benefit from robotics in sample preparation and analysis while the smaller laboratories might not be able to afford the capital expense. With capillary

electrophoresis a reality and chip technology just around the corner there are some obvious changes in view.

In general the following criteria are suggested for the selection of DNA profiling systems to lead to a co-ordinated approach throughout Europe and allow for the production of pan-European DNA databases:

1. Accuracy of analysis
2. Robustness of systems
3. Cost of reagents and equipment
4. Speed of analysis
5. Compatibility between laboratories
6. Links to existing systems
7. Ability to transfer to new systems in the future

4.3.1. Accuracy of analysis

It is now accepted that, for the foreseeable future the use of STR based multiplexes will form the basis of the ideal system. In the past the driving factor appears to have been concerned with providing a system which will give a good discrimination power using a minimal number of loci. This theme has continued with the production of the commercial kit systems in which there is compatibility of allele amplification by re-engineering primers. This provides a much more uniform primer mix and alleviates the necessity for empirical juggling with the component primer concentrations.

These kits have been tested through inter-laboratory exercises using a variety of methods to ensure that compatible results will be obtained from all samples.

4.3. 2. Robustness of systems

The methodology should provide the same results from analysis in any of the laboratories. If the technology or component systems are too difficult to handle or the

results too complicated for accurate interpretation then the robustness test has failed.

At the present time there are systems in place determining whether peaks produced are true or spurious and for the interpretation of the results from mixtures of body fluids especially when one is present as a minor component. It is important that there is complete co-operation between participating laboratories to ensure that each would interpret the results in the same way.

4.3.3 Cost of reagents and equipment

Priorities will vary according to the needs of the various laboratories. Laboratories with a large throughput of samples will benefit from the introduction of automated systems to deal with sampling, extraction and analysis. The cost of such equipment for smaller laboratories could be prohibitive and might not provide any real benefit.

Reagents, in the form of commercially available kits, are especially attractive to small laboratories who do not have sufficient resources for the manufacture and quality control of the necessary reagents.

4.3.4 Speed of analysis

Analysis time might not be so important in the processing of database samples but there are occasions when the analysis of crime scene samples is a limiting factor in the overall investigation. In order to meet the latter requirement it is important to identify where `bottle-necks' occur in the analysis procedure. At present it can take a considerable time to carry out the interpretation of the results and there is now an initiative on the part of commercial companies to produce more advanced software for this procedure.

4.3.5 Compatibility between laboratories

During the course of the STADNAP project it has been necessary to determine whether it is preferable for all laboratories to be using an identical set of loci or

whether a core package of STRs is sufficient. Almost all laboratories in Europe are now using kits in which there is sufficient overlap of STR loci to ensure compatibility of results.

4.3.6 Links to existing systems

Decisions which were made in chapter 5 (page 30) had to allow sufficient scope for those laboratories who already had databases in operation to be able to upgrade their systems without the costly and time consuming exercise of retyping samples which were already entered.

4.3.7 Ability to transfer to new systems in the future

Upgrading systems will always be important and all scientists would wish to take advantage of new developments but these must be well thought out against a well developed strategy. While the mid-term strategy is to continue with the use of STR based multiplexes, the introduction of non-compatible systems e.g. SNP technology, will require considerable thought with regard to database formation.

4.4 Objective 4: Future research orientations and method developments

4.4.1 Future methods

During the course of the STADNAP initiative it became apparent that the first requirement was to refine the systems in current use and to arrive at a consensus for the use of a multiplex system which would be used by all laboratories for the formation of a pan-European DNA database. In the main this has been achieved (see objective 2, page 20)

The expanding use of the mitochondrial DNA (mtDNA) analysis in cases where there is insufficient genomic DNA available has spawned a number of inter-laboratory

exercises to develop the methodology and to ensure the accurate analysis of the results. For similar reasons the elucidation of Y chromosome STR markers has also involved the group in collaborative exercises to find the most informative loci and also to develop ways of expressing the results in terms of individuality.

Head hairs are commonly received by forensic scientists as evidential samples and, in the past, it has been the practice to look only for mtDNA polymorphisms. A project is now underway to detect genomic DNA from telogen hairs using a modified amplification programme via singleplex reactions. This project will continue.

As it is normal for laboratories to receive samples in which the DNA is highly degraded an exercise is now being progressed to prepare a batch of degraded material for future experiments. It is suggested that commercial companies could use this as reference material. There may be advantage in producing samples from two cell-lines which contains different alleles and, in future, the preparation could be passed to an organisation who routinely produces standard reference material. A strategy will be decided at a future EDNAP meeting.

In order to keep abreast of current developments commercial organisations have been invited to STADNAP meetings to make presentations on new technologies, equipment and reagents. These presentations have helped to focus the debate on the likely future of DNA profiling within forensic science.

4.4.2 Future technology for DNA profiling - presentations by commercial companies STADNAP meeting Mechelen 1999

4.4.2.1 Automated DNA analysis by MALDI-TOF

Dirk van den Boom (Sequenom GmbH, Hamburg)

As with most of the current examples of DNA analysis this relies on a microchip platform but Sequenom have incorporated the use of mass spectrometry for the detection and sizing of the DNA fragments. Basically, very small volumes of

amplified DNA are co-crystallised with an organic matrix and robotically immobilised on a two-dimensional array spectrochip. Measurement of the DNA fragments is done via matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). The short pulse of laser irradiation volatilises and ionises the DNA/matrix mix and the resulting accelerated gas-phase ions are measured by the time taken to pass through a field-free area. The time taken is proportional to the molecular weight of the DNA fragment. The organic matrix absorbs the laser energy leaving the DNA fragments intact.

So far the majority of the research has focussed on SNP analysis where it has been demonstrated that the system is consistently accurate to one base pair. For each particular system which has been investigated (e.g. Hb) the differences can be determined from `look-up' tables. Some work has been carried out on microsatellites and a portfolio is being prepared for further study. It has been found necessary to engineer primers which anneal adjacent to the repeating sequences. The addition of di-deoxy nucleotides prevents elongation into the primer sites so that the number of repeat units becomes a function of molecular weight. Stutter peaks etc. will still occur as they are part of the PCR process.

'Look-up' tables have been prepared for some of the human microsatellites used in forensic science but the software for fully computerised result analysis is still being developed. At present there is a limitation on size and the upper limit for a microsatellite is 100bp.

4.4.2.1 Novel tools for creating and reading microarrays

Myles L Mace Jr. (Genetic Microsystems, Woburn, Maryland)

This company does not have a complete technology but has developed equipment for the preparation and detection of DNA samples analysed in the two dimensional array chips. In particular they have manufactured a loop and solid cylinder device for accurate dispensing of the microdrops. The equipment is machined to very small tolerances and it is claimed that there is extreme reproducibility. A scanner has also been produced which is said to be rapid, rugged and reliable.

The use of silanisation of spots for immobilisation to glass is being studied as it is considered that this method does not provide for homogeneity of reaction with probes etc.

4.4.2.3 STR analysis by electronic microarrays

Lana Feng (Nanogen, San Diego California)

A microchip has been developed which consists of one series of electrodes in the centre and another set on the periphery. Biotinylated probes are immobilised directly above the electrodes in the centre of the chip. Test samples which are added to the peripheral sites will migrate to the centre when a current is passed. Those samples which do not hybridise with the probes can be removed by changing the polarity; a method which provides for very high stringency. (This methodology can also be used for antigen-antibody reactions). Although the technique has been designed for SNPs, a STR based system has also been developed which relies on a stacking system of repeat units in which a stable structure is formed only when there is an exact match.

Nanogen have also developed a novel amplification method; strand displacement assay (SDA). In the first round of amplification dCTPαS is incorporated into the new strand. Following polymerisation the new strand is `nicked' with an endonuclease (the thiol groups in the template prevent complete restriction) which releases the tail and displaces the strand. The whole process is run at constant temperature and amplification takes 15 to 30 minutes.

4.4.2.4 Microdevice for automated, ultra-high speed and portable DNA forensics

Daniel Erlich (Whitehead Institute, MIT, Cambridge, Massachusetts)

The technology described is based on a chip form of capillary electrophoresis in which very small volumes of sample can be injected and run in a few minutes. The capillary has side arms which incorporate electrodes and, by changing polarity, only small volumes are allowed to enter the main capillary for separation. Although the separation system is fast it currently requires manual loading. The support polymer also needs to be exchanged after a few hours of operation.

While the system is small and eminently portable, for scene of crime work it would still be necessary to have amplification equipment on site.

4.4.3 Future methods: Presentations by commercial companies STADNAP meeting, Santiago de Compostela 2000

4.4.3.1 Advances in capillary electrophopresis for genotyping
Nicola Oldroyd (Applied Biosystems)

The new 3100 16 capillary model uses exactly the same technology as the 310 with automated polymer filling, sample injection and analysis. At present there are 36 and 50 cm capillaries with 22 and 80 cm being developed. The capillary array loading header is now more robust. Run time is approximately 46 mins and the throughput is 736 samples/24 hrs. (This compares with 48 samples/24 hrs for the 310 and 384 for the 377). Instrument validation is underway and it is hoped for a completion date sometime in December 2000.

A 5-dye genotyping technology is being developed which will contain the reagents to amplify and analyse the alleles from a 15 locus multipex. The genemapper software will be made available for PCs with Windows NT and not for the Mac operating system.

AB Genemapper v1.0 software incorporates a combination of Genescan/Genotyper and GenBase functionalities. It includes a built-in database and is primarily for allele identification. It will not be available for the Mac.

New initiatives include new extraction procedures, a real-time PCR quantitation method and sensitivity evaluations. AB are organising a series of user meetings focused at information to the forensic science community.

4.4.3.2 A novel detection system for PCR products
Tom Mozer (Promega Corp.)

Promega have developed the READIT technology with SNP analysis in mind. This methodolgy can determine base matches via a polymerase reaction with detection

using a luciferase mediated light emission. Activity is only achieved with a perfect match and therefore sequence specific results are obtained.

The method can also be used for quantitation of DNA with a low signal to noise ratio. This is possible even with high levels of bacterial contamination. The technology has the capability of quantitating mtDNA and, following singleplex reactions, it can also be used for determining SNPs. Microtitre plates with a suitable light detector can be used for reading results.

The product should be ready within the next six months and any interested members should contact Promega for information.

4.4.3.3 A new capillary-based analyser

Jackie Evans (Amersham Pharmacia Biotech)

Amersham have developed the MegaBACE 1000 sequencing system which is capable of both sequencing and genotyping. The equipment consists of a 96 glass capillary system (in sets of 16 capillaries) in which the gel medium is loaded at one end and the samples at the other. It is possible to load 96 samples, wait for 2 minutes for electrophoresis and then add a further 96 samples. This can be repeated for approximately 10 times. Primers and dyes of choice can be used. The cost is expected to be approximately £140,000.

The Genetic Profiler analysis system requires minimal editing time and can cope with multiple run analyses. Validation has been carried out on sizing precision, reproducibility, resolution and sample/capillary success.

There is a method for SNP analysis (SNuPe) and Amersham are looking for collaboration on both SNP and STR development.

Website at www.megabace.com

4.5. Objective 5: Recommendations for future work

4.5.1 DNA profiling technology continues to move forward at an unrelenting pace. We can confidently expect that there will be many new methods available to forensic scientists in the very near future. However, most of the new methodology is aimed at medical diagnostics and will require adaptation for use in individualisation assays.

4.5.2 Single nucleotide polymorphisms (SNP)

The EDNAP group is aware that the currently used set of STR markers will remain state-of-the-art for the coming years. Refinement of their use will allow for the analysis of the smallest stains and provide results which approach individuality. Nevertheless there are cutting-edge developments within other fields of DNA research one of which, SNP (single nucleotide polymorphisms) technology, appears as the most promising for forensic science. SNPs are the most prevalent form of genetic polymorphism and are now of great interest as markers for mapping genes for inherited traits and pharmacogenetic indicators in the development of new drugs. SNPs are expected, in the future, to replace STRs in the same way that microsatellite technology replaced that of restriction fragment length polymorphism analysis. The main reasons for the popularity of SNPs in genetic research are their frequent occurrance in the genome and their stable Mendelian inheritance pattern with a mutation rate of less than 1 in 10^8. All members have agreed that the potential forensic use of SNPs should be further explored. When designing a project of this nature it is important to consider the preferences of all the member laboratories to ensure that there are no conflicts in terms of incompatible technologies. An example is the use of minisequencing which will enable all members to be involved in an inter-laboratory exercise using their current technology and instrumentation. SNPs will be amplified in a singleplex format and analysed using the automatic DNA sequencer technology for the identification of point mutations. The laboratories of Birmingham, Santiago De Compostela, Münster and Rijswijk will play key roles in developing the groundwork of this exercise and ensure that there is a continuing programme which can be operated by all subsequent participants independent of their technical platform.

4.5.3 Collaborative exercise on the use of Y chromosome STRs

This is, in part, already being progressed to find the best multiplexes of the most informative loci. It will be expanded to incorporate an SNP study.

4.5.4. Collaborative exercise on the efficiency of different typing systems and kits for the identification of markers in mixtures of body fluids

The elucidation of individual DNA profiles from stains formed from mixtures of body fluids contributed by different individuals has always presented a problem. This project aims to address the difficulties.

4.5.5. The formulation of a standard degraded DNA sample

This project is already started (see 5.6.3) but will require considerable extra resources to complete a collaborative exercise.

4.5.6 The use of re-engineered primers for detecting STRs in degraded DNA and hair samples

This exercise has just been identified and, in essence, requires that primers are used which anneal closely to the sequences to be analysed. More amplification cycles (than those used in routine methods) will probably be necessary due to the low copy number present in the degraded samples. A successful outcome to this project could be of considerable value for the analysis of samples commonly recovered from crime scenes.

4.5.7 Legal and ethical considerations

In the last 10 years co-operation and understanding between forensic scientists involved with DNA analysis in Europe has continued to grow. The scientific basis for the formation of DNA databases is in place and there is the ability for member laboratories to exchange casework information. Unfortunately the progress of the effective production and use of these databases is hampered by legal constraints

most of which are based on civil rights issues. In 1996 the Mainz laboratory organised a meeting to appraise lawyers and legislators of the requirements for the effective use of the interchange of scientific information [Schneider PM, Rittner C & Martin PD (eds) (1997) Proceedings of the European Symposium “Ethical and Legal Issues of DNA Typing in Forensic Medicine”. Forensic Sci Int 88: 1-110]. As there are such wide differences in the approaches taken by the different criminal justice systems within Europe it is suggested that there could be considerable benefit from revising this subject.

5. WP 2: An account of the STADNAP collaborative projects

5.1 Preamble

During the period of the STADNAP grant, the group has been actively engaged with at least 4 collaborative projects that cover the whole field of forensic DNA profiling. So far, the group has published 4 papers derived from these activities; these have strongly influenced the entire direction of DNA profiling strategies not only in Europe but throughout the world. The focus of the STADNAP papers has been to demonstrate inter-laboratory reproducibility and robustness of techniques that are in their infancy. The group has worked on the development of STRs, mitochondrial DNA, Y chromosome DNA, mixture analysis, and the development of control standards.

STRs

Building on previous work that demonstrated that both simple STRs (HUMVWA, HUMTH01) and the complex STRs showed reproducibility, they have all been universally adopted as standards by the forensic community. During the course of the STADNAP period, three different experiments were undertaken:

a) Comparison of the performances of different multiplexes in relation to stutter and mixture interpretation.
b) Development of a degraded DNA control standard to assess the efficacy of different multiplexes using standard protocols and protocols designed to boost the sensitivity of the assay
c) Evaluation of short STRs for the analysis of telogen hairs.

Mitochondrial DNA

The utilisation of mitochondrial (mt) DNA in forensic analysis has followed a similar evolutionary path to that described for STRs. Analysis can be carried out by following different protocols – yet previously there had been no demonstration that the use of

different protocols were comparable. An experiment was organised to test this. A number of samples were sent to different laboratories and complete reproducibility was demonstrated. A second experiment was organised to compare heteroplasmy in hair samples taken from a known a heteroplasmic individual with individuals with no known heteroplasmy. This experiment is on-going.

Y-chromosomal DNA

Y chromosome analysis is probably the newest technique that is being widely used by forensic laboratories. Bloodstains (from 6 males) were tested using the STR Y-linked system DYS385. Reproducibility between laboratories was demonstrated. A second experiment was organised to test the reproducibility of a multiplexed system. Again reproducibility was demonstrated.

5.2. List of the STADNAP publications

1 Carracedo A, d'Aloja E, Dupuy B, Jangblad A, Karjalainen M, Lambert C, Parson W, Pfeiffer H, Pfitzinger H, Sabatier M, Syndercombe-Court D, Vide C. (1998) **Reproducibility of mtDNA analysis between laboratories: a report of the European DNA Profiling group (EDNAP)**. *Forensic Sci. Int. 97:155-164*

2 Gill P, d'Aloja E, Dupuy B, Eriksen B, Jangblad A, Johnsson V, Kloosterman AD, Lareu MV, Mevag B, Morling N, Phillips C, Pfitzinger H, Rand S, Sabatier M, Scheithauer R, Schmitter H, Schneider PM, Skita I, Vide MC. (1998) **Report of the European DNA Profiling group (EDNAP) - an investigation of the hypervariable loci ACTBP2, APOA11 and D11S554 and the compound loci D12S391 and D1S1656.** *Forensic Sci. Int. 98: 193-200*

3 Schneider PM, d'Aloja E, Dupuy BM, Eriksen B, Jangblad A, Kloosterman AD, Kratzer A, Lareu MV, Pfitzinger H, Rand S, Scheithauer R, Schmitter H, Skitsa I, Syndercombe-Court D, Vide MC (1999) **Results of a collaborative study regarding the standardization of the Y-linked STR system DYS385 by the**

European DNA Profiling (EDNAP) group. Forensic Sci. Int. 102: 159-165

4 Carracedo A, Beckmann A, Bengs A, Brinkmann B, Caglia A, Capelli C, Gill P, Gusmão L, Hagelberg C, Hohoff C, Hoste B, Kihlgren A, Kloosterman A, Myhre Dupuy B, Morling N, O´Donnell G, Parson W, Phillips C, Pouwels M, Scheithauer R, Schmitter H, Schneider PM**,** Schumm J, Skitsa I, Stradmann-Bellinghausen B, Stuart M, Syndercombe Court D , Vide MC (2001) **Results of a collaborative study of the EDNAP group regarding the reproducibility and robustness of the Y chromosome STRs DYS19, DYS389 I and II, DYS390 and DYS393 in a PCR pentaplex format**. *Forensic Sci. Int.* 119:28-41

5.3 Results of a collaborative study regarding the standardization of the Y-linked STR system DYS385 by the European DNA Profiling (EDNAP) group

Schneider PM, d'Aloja E, Dupuy BM, Eriksen B, Jangblad A, Kloosterman AD, Kratzer A, Lareu MV, Pfitzinger H, Rand S, Scheithauer R, Schmitter H, Skitsa I, Syndercombe-Court D, Vide MC (1999) Forensic Sci. Int. 102, 159-165

1. Introduction

In contrast to autosomal short tandem repeat loci, the Y-chromosomal STR systems are characterized by a male inheritance pattern. As the male-specific portion of the Y chromosome does not recombine during meiosis, Y-STR´s are inherited as a closely linked haplotype, which appears to remain stable in a given paternal lineage over many generations. Thus these systems are quite informative for anthropological studies as well as the study of large family pedigrees and in paternity deficiency cases when the putative father is not available for testing (Jobling et al., 1997). In forensic cases, Y-linked STR´s are particularly useful for the identification of human remains as well as in rape cases with mixed male/female stain samples, as there is no need to separate the male cells from the mixture prior to PCR amplification. This may circumvent the risk of allelic drop-out, when the male cells are the minor component in the mixture. A large number of Y-linked STR loci have already been characterized in a multi-center population study (de Knijff et al., 1997; Kayser et al., 1997). Whereas the majority of Y STR loci are only moderately polymorphic with

allele numbers ranging between four and seven, there are a few loci which exhibit a more extensive polymorphism, as they are derived from tandemly duplicated segments of the Y chromosome thus giving rise to two fragments of variable length which do not behave like alleles but genotypes. One of these loci, DYS385, has recently been characterized in more detail regarding the internal repeat structure as well as its variability among different populations (Schneider et al., 1998; Kayser et al., 1997). Due to this heterogeneity, mean exclusion chances betweeen 0.87 and 0.96 have been observed for different populations making DYS385 one of the most informative single Y-linked STR described so far.

Based on previous experiences with difficulties encountered in the standardization of complex STR loci (Gill et al., 1994), and to evaluate the usefulness of this Y-linked system for reliable inter-laboratory exchange of typing results, the European DNA Profiling group has carried out a collaborative exercise among 14 participating laboratories using DYS385 for typing of unknown bloodstains as well as to collect population data from different European countries.

2. Results and discussion

Typing results were reported by 14 laboratories as follows: sample 1: 13-14; sample 2: 14-18; sample 3: 11-12; sample 4: 13-16; sample 5 (reference): 11-14; sample 6: 11-13. In addition, 7 laboratories provided population data with sample sizes between 91 and 150 male individuals. The typing results of the 6 bloodstains were in agreement with the data obtained by the organizing laboratory except for two instances. In the first case, the results of samples 2 and 3 had been exchanged. As cause, an inadvertent mislabelling of the filter papers carrying the stains 2 and 3 (all stains were provided in separate envelopes) by the organizing laboratory could be identified. In the second case, a 4 bp allele shift (approximately minus one repeat) was observed for stains 2, 4 and 6 by laboratory 12 (using PAA gel electrophoresis and silver staining), and the results were reported to be inconclusive. As a control, the same PCR products were analysed side-by-side with the corresponding local samples by the organizing laboratory, and no migration differences could be observed. Thus it was concluded that the PCR had been performed correctly, but that the resolution of the gel system used had not been adequate.

For electrophoretic separation and detection, two laboratories used native horizontal

or vertical PAA gels followed by silver staining, and the other twelve laboratories used automated DNA sequencing equipment with fluorescent detection. Of these, nine used Applied Biosystems ABD 373/377 sequencers, and three used the Amersham Pharmacia Biotech ALF/ALF Express sequencers. One laboratory used the ABD 310 capillary electrophoretic system in parallel to the 373 gel system, and reported about more focused fragment peaks but identical results using the capillary system. Recently it was demonstrated that the sizes of the amplified DYS385 fragments can be reduced in length by 112 bp by replacing primer DYS385-2 with primer DYS-3852B, which is located closer to the tandem repeat sequence (Schneider et al., 1998). This allows for a better electrophoretic resolution which should help to overcome the problem encountered in the present study. Furthermore, the reduced total size of the target sequences will also enhance the robustness of amplification from partially degraded forensic specimen.

The population data comprised results from Portugal (n=103), Spain (n=91), Italy (n=150), Greece (n=121), Netherlands (n=110), Norway (n=150), England (n=94), and Germany (n=103)

In summary, these results confirm previous observations that DYS385 is one of the most informative Y-linked STR systems. It could also be demonstrated that reproducible results can be obtained independently from the electrophoretic separation and detection methods used. Thus DYS385 should be included in a panel of Y-linked systems for routine casework. Alternatively, it could be used in exclusion cases as a single system by laboratories who do not plan to establish a set of 4-5 single fragment Y-STR's for their routine casework.

3. References

P. Gill, C. Kimpton, E. D'Aloja, J.F. Andersen, W. Bär, B. Brinkmann, S. Holgerssen, V. Johnsson, A.D. Kloosterman, M.V. Lareu, L. Nellemann, H. Pfitzinger, C.P. Phillips, H. Schmitter, P.M. Schneider, M. Stenersen, Report of the European DNA profiling group (EDNAP) - towards standardization of short tandem repeat (STR) loci. Forensic Sci. Int. (1994) 65:51-59

M.A. Jobling, A. Pandya, C. Tyler-Smith, The Y chromosome in forensic analysis and paternity testing. Int. J. Legal Med. 110 (1997) 118-124

M. Kayser, A. Caglia, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A.

Piccinini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G. Weichhold, P. de Knijff, L. Roewer, Evaluation of Y-chromosomal STRs: a multicenter study. Int. J. Legal Med. 110 (1997) 125-133

C. Kimpton, P. Gill, E. D'Aloja, J.F. Andersen, W. Bär, S. Holgerssen, S. Jacobsen, V. Johnsson, A.D. Kloosterman, M.V. Lareu, L. Nellemann, H. Pfitzinger, C.P. Phillips, S. Rand, H. Schmitter, P.M. Schneider, M. Stenersen, M.C. Vide, Report on the second EDNAP collaborative STR exercise. Forensic Sci. Int. 71 (1995) 137-152

P. de Knijff, M. Kayser, A. Caglia, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccinini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G. Weichhold, L. Roewer, Chromosome Y microsatellites: population genetic and evolutionary aspects. Int. J. Legal Med. 110 (1997) 134-149

P.M. Schneider, S. Meuser, W. Waiyawuth, Y. Seo, C. Rittner, Tandem repeat structure of the duplicated Y-chromosomal STR locus DYS385 and frequency studies in the German and three Asian populations. Forensic Sci. Int. 97 (1998) 61-70

5.4 Results of a collaborative study of the EDNAP group regarding the reproducibility and robustness of the Y chromosome STRs DYS19, DYS389 I and II, DYS390 and DYS393 in a PCR pentaplex format

Carracedo A, Beckmann A, Bengs A, Brinkmann B, Caglia A, Capelli C, Gill P, Gusmão L, Hagelberg C, Hohoff C, Hoste B, Kihlgren A, Kloosterman A, Myhre Dupuy B, Morling N, O´Donnell G, Parson W, Phillips C, Pouwels M, Scheithauer R, Schmitter H, Schneider PM**,** Schumm J, Skitsa I, Stradmann-Bellinghausen B, Stuart M, Syndercombe Court D , Vide MC (2001) Forensic Sci. Int. 119:28-41

1. Introduction

Y-chromosome polymorphisms are increasingly being used in forensic casework. The Y chromosomes show low levels of polymorphisms when compared with autosomal chromosomes (Malaspina et al, 1990), however these markers have been shown to be very useful in some cases, namely in criminal casework in sexual crimes and complicated paternity testing cases (Jobling et al, 1997; Prinz et al, 1997; Honda et al, 1999; Gusmao et al, 1999. Y-chromosome analysis can be particularly helpful to detect male DNA fractions in stains involving male/female mixtures, the most common biological material available in sexual crimes.

Although different kinds of polymorphisms were described on the haploid male-specific portion of the human Y chromosome, STRs proved, until now, to be the most suitable markers in forensic genetics due to their diversity levels, relatively low

mutation rates and technical simplicity (Roewer et al, 1992; Santos et al, 1993, Kayser et al, 1997a,b; Knijff et al, 1997; Perez-Lezaun, 1997). Some of the Y STRs have proved to be prone to artifacts (Gusmao et al, 2000; Dupuy et al, 2000). However since the number of Y-STRs is limited, the presence of artifacts in the amplification does not necessarily imply discarding the use of a particular STR in forensics, but to be aware of possible problems in interpretation. In this context the reproducibility of results is crucial especially when multiplex formats are used.

For this reason the European DNA Profiling Group (EDNAP) in the frame work of the STADNAP program (Standardization of DNA Profiling in Europe), has carried out a collaborative exercise among 18 participating laboratories to determine whether uniformity of results could be achieved among different European laboratories using a Y chromosome STR pentaplex (Gusmao, 1999), which includes the loci DYS19, DYS389 I and II, DYS390 and DYS393 for typing unknown bloodstains and a male-female mixed stain.

This exercise represents the continuation of the Y STR inter-collaborative exercises of the group initiated with the DYS385 system (Schneider et al 1999).

In addition a population genetic survey was carried in 8 different European population. This population data is of special interest to evaluate the extent of population stratification when common reference European databases are used.

2. Materials and methods

For analysis, six bloodstains of 200 µl each from voluntary donors were sent out to the 18 participant laboratories. These samples included: (a) two control samples with known Y-STR haplotypes, (b) three male samples and (c) a 1 to 20 male/female blood mixture.

In addition, the laboratories received aliquots of sequenced allelic ladders, individualized by systems, as well as a balanced allelic ladder for the PCR pentaplex.

The participating laboratories were asked to type all the samples for the five Y STRs using the pentaplex amplification protocol that was distributed with the stains.

2.1 Primers and PCR conditions

Primers with sequences obtained from the GBD and standard amplification protocols were provided

2.2 Allelic ladder and nomenclature

The pentaplex allelic ladder and individual sequenced allelic ladders were prepared according to Gusmão et al. (1999) and Pestoni et al. (1998).

Allele nomenclature was as proposed by Kayser et al. (1997) with the exception of the DYS389 locus. The nomenclature of this locus was according to Roewer et al (1996) considering that the sum of p and q stretches corresponds to DYS389I. However DYS389II alleles were named by subtracting the DYS398I stretch to the total number of repeats [(n + m + p + q) – (p + q)] (Rolf et al, 1998).

2.3 Population samples

Population data was also requested from the participants. A total of 930 male samples from 10 different populations from Europe were analysed for all the loci included in the pentaplex. Eight of these ten populations also included haplotype data.

Allele and haplotype frequencies were estimated by gene counting. Gene and haplotype diversities were estimated according to Nei (1987).

Analysis of Molecular Variance (AMOVA) was performed by a Markov test using the Arlequin software 1.1 (Weir et al, 1984). AMOVA yields Φ_{st} values which, similar to Wright´s F_{st}, reflect the proportion of total molecular variance among two populations attributable to population differences.

Genetic distance matrices between populations were obtained by using the pairwise difference genetic distance. UPGMA (unweighted pair group method with arithmetic means) tree was built from the distance matrix using the options NEIGHBOUR and DRAWTREE in the PHYLIP package (Felstein, 1989).

3. Results and discussion

3.1 Intercomparison exercise: Performance of the pentaplex

All the participating laboratories submitted correct results for samples 1 to 3. For the mixed stain (sample 4) all the labs also submitted correct results except the labs 2, 12, 13 and 15 which reported to obtain inconclusive or no results for this sample. A new bloodstain was sent again to these laboratories and 3 of these 4 laboratories reported correct results.

In conclusion no incorrect results were reported by any of the participating laboratories, all the labs reported correct results for normal bloodstains and only one lab reported an inability to obtain results for the mixed stain.

3.2 Population genetic data

Observed allele frequencies for each STR in the different populations were analyzed, together with the calculation of gene diversity value which is equivalent to the power of discrimination and chance of exclusion.

A first look at the gene frequencies shows the lower gene diversity in Western European populations compared with Germany, Italy and Greece. This pattern is compatible with a demographic expansion from Eastern to Western European countries. The picture is very similar to that shown by mitochondrial DNA variation (Salas et al, 1998).

The bimodal pattern shown by the population of Finland is very interesting. The bimodal pattern observed in the Finnish population is compatible with the existence of two populations with different genetic backgrounds in that population sample.

A more robust population comparison can be performed using haplotype frequencies instead of single locus frequencies. A total of 222 haplotypes were observed.The most common haplotype (h67) in all the European populations was absent in the Finnish population. The most frequent haplotypes in this population (h38 and h103) are rare in the other European populations (the haplotype h103 was only found in the Galician population sample in one individual). The high frequency of h63 in the Dutch population was also noticeable.

Large collections of Y STR haplotypes are necessary for interpretation purposes and several databases have been compiled. Some examples are the database collection of the STADNAP group (http://www.STADNAP.uni-mainz.de), the collection of the GEP-ISFG Working group (http://www.gep-isfh.usc.es) and especially the haplotype data collated in an SQL database at the Institute of Legal Medicine, Humboldt University, Berlin, Germany (http://ystr.charite.de) which is the most important collection of Y STR data.

These large database collections are also necessary to obtain adequate Φ_{st} estimates to correct for population stratification if necessary. Y-chromosomal

haplotypes are confined to patrilines, so they are more prone to genetic drift than autosomal loci. Φ_{st} estimates of large databases collections for Central European populations were estimated to be lower than 0.1. Our findings are in agreement with these values and the Φ_{st} estimates in our population samples, with the exception of the population from Finland, are lower than 0.05.

4. References

B. Dupuy, T. Gedde-Dahl Jr., B. Olaisen. DXYS267: DYS393 and its X chromosome counterpart. Forensic Sci. Int. (in press).

J. Felsenstein, PHYLIP: Phylogeny Inference Package (Version 3.2). Cladistics 5 (1989) 164-166.

J. Gómez, A. Carracedo, The 1998-1999 collaborative exercises and proficiency testing program on DNA typing of the Spanish and Portuguese Working Group of the International Society for Forensic Genetics (GEP-ISFG). Forensic Sci. Int. (in press).

L. Gusmão, M. Brión, A. González-Neira, MV. Lareu, A.Carracedo, Y Chromosome specific polymorphisms in forensic analysis, Legal Med. 1 (1999) 55-60.

L.Gusmão, A. González-Neira, C. Pestoni, M..Brión, MV. Lareu, A. Carracedo, Robustness of the Y STRs DYS19, DYS389I and II, DYS390 and DYS393: optimization of a PCR pentaplex, Forensic Sci. Int. 106 (1999) 163-172.

L. Gusmao, A. González-Neira, P. Sánchez-Diz, M.V.Lareu, A. Carracedo, Alternative primers for DYS391 typing:advantages of their application to forensic genetics, Forensic Sci. Int. (in press).

K. Honda, L. Roewer, P. de Knijff, Male DNA typing from 25-year-old vaginal swabs using Y chromosomal STR polymorphisms in a retrial request case, J. Forensic Sci. 44 (1999) 868-872.

M.Jobling, A. Pandya, C. Tyler-Smith, The Y chromosome in forensic analysis and paternity testing, Int. J. Legal Med. 110 (1997) 118-124.

M. Kayser, A. Cagliá, D. Corach, N..Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S . Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawzczak, K. Leim, S. Meuser, E. Meyer,. W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccini, M. Prinz, C. Schmitt, PM. Schneider, R Szibor, J. Teifel-Greding G., Weichhold, P. de Knijff, L. Roewer, Evaluation of Y-chromosomal STRs: a multicenter study, Int. J. Legal Med. 110 (1997) 125-133

M .Kayser, P. De Knijff, P. Dieltjes, M. Krawczak, M. Nagy, T. Zerjal, A. Pandya, C. Tyler-Smith, L. Roewer, Applications of microsatellite-based Y chromosome haplotyping, Electrophoresis. 18 (1997) 1602-1607.

P. de Knijff, M. Kayser, A. Caglià, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G. Weichhold, L. Roewer, Chromosome Y microsatellites: population genetics and evolutionary aspects, Int. J. Legal Med. 110 (1997) 134-149

P. Malaspina, F. Persichetti, A. Novelletto, C. Iodice, L. Terrenato, J. Wolfe, M. Ferraro, G. Prantera, The human Y chromosome shows a low level of DNA polymorphism, Ann. Hum. Genet. 54 (1990) 297-305.

M .Nei. Molecular Evolutionary Genetics, Columbia University Press. New York, NY, USA, (1987).

A..Peréz-Lezaun, F. Calafell, M. Seielstad, E .Mateu, D. Comas, E. Bosch, J. Bertranpetit, Population genetics of Y-Chromosome Short Tandem Repeats in humans, J. Mol. Evol. 45 (1997) 265-270.

C. Pestoni, M.L. Cal, M.V. Lareu, M.S. Rodríguez-Calvo, A. Carracedo A, Y chromosome STR haplotypes: genetic and sequencing data of the Galician population (NW Spain), Int. J. Legal Med. 112 (1998) 15-21.

M. Prinz, K. Boll, H. Baum, B. Shaler, Multiplexing of Y chromosome specific STRs and performance for mixed samples. Forensic Sci. Int. 85 (1997) 209-218.

L. Roewer, J. Arnemann, N.K. Spurr, K.H. Grzeschik, J.T. Epplen, Simple repeat sequences on the human Y chromosome are equally polymorphic as their autosomal counterparts, Hum. Genet. 89 (1992) 389-394.

L.Roewer, M. Kayser, P.Dieltjes, M.Nagy, E.Bakker, M.Krawczak, P. de Knijff,. Analysis of molecular variance AMOVA of Y-chromosome specific microsatellites in two closely related human populations, Hum. Mol. Genet. 7 (1996) 1029-1031.

L. Roewer, M. Kayser, P. De Knijff, K. Anslinger, A. Caglià, D. Corach, S. Füredi, G. Geserick, L. Henke, M. Hidding. H.J. Kärgel, R. Lessig, M. Nagy, V.L. Pascali, W. Parson, B. Rolf, C. Schmitt, R. Szibor, T. Teifel-Greding, Y-chromosomal short tandem repeat (STR) haplotyping of Caucasian males: population genetic and forensic aspects. Forensic Sci. Int. (in press).

A. Salas, M.V. Lareu, D. Comas, J. Bertranpetit, A. Carracedo. mt DNA analysis of the Galician population: A genetic edge of European variation. Eur. J. Hum. Gen. 6 (1998) 365-375.

F.R. Santos, S.D. Pena, J.T. Epplen, Genetic and populational study of a Y-linked tetranucleotide repeat DNA polymorphism with a simple non-isotopic technique. Hum. Genet. 90 (1993) 655-656.

P.M. Schneider, E. D´Aloja, B.M. Dupuy, B. Eriksen, A. Janblad, A.D. Kloosterman, A. Kratzer, M.V. Lareu, H. Pfitzinger, S. Rand, R. Scheithauer, H. Schmitter, I. Skitsa, D. Syndercombe-Court, M.C. Vide, Results of a collaborative study regarding the standardization of the Y-linked STR system DYS385 by the European DNA Profiling (EDNAP) Group, Forensic Sci. Int. 102 (1999) 159-165.

S. Schneider, JM. Kueffer, D. Roessli, L. Excoffier, Arlequin ver. 1.1. University of Geneva, (1997).

5.5 Reproducibility between laboratories of mtDNA analysis: a report of the European DNA Profiling Group (EDNAP)

Carracedo A, d'Aloja E, Dupuy B, Jangblad A, Karjalainen M, Lambert C, Parson W, Pfeiffer H, Pfitzinger H, Sabatier M, Syndercombe-Court D, Vide C. (1998) Forensic Sci. Int. 97:155-164

1. Introduction

Mitochondrial DNA (mtDNA) in humans displays considerable sequence variation between individuals (Cann et al. 1987; Piercy et al.1993). Much of the variation is within the non-coding region, which contains the origin of replication for one strand, both origins of transcription, and the D-loop region (Anderson et al. 1981). This sequence variation is specifically concentrated in two hypervariable regions usually designated as HV1 and HV2 (Greenberg et al. 1983). Due to the high copy number per cell (1000-10000) mtDNA analysis is especially appropriate when studying degraded samples. In forensic casework mtDNA analysis is particularly important for the individualisation of certain types of evidence, notably hair shafts, which contain little or no genomic DNA.

MtDNA variation can be analysed by a variety of strategies (Cann et al., 1987, Stoneking et al. 1991, Thomas et al. 1994, Alonso et al. 1996, Sullivan et al. 1996, Barros et al. 1997) but the combination of PCR amplification with direct DNA sequencing is usually the ultimate choice for identification.

A potential drawback to mtDNA sequencing is the labour intensive nature of the technique. For routine forensic analysis the process must be highly automated to maximise sequence throughput and minimise errors in data handling as well as laboratory errors. The variety of strategies for PCR and sequencing can complicate matters further.

The aim of this collaborative exercise was to determine whether uniformity of mtDNA sequencing results could be achieved among different laboratories using the whole range of different methodologies employed.

2. Material and Methods

2.1 Samples

EDNAP laboratories were asked to sequence the mtDNA HV1 region (16024-16365) from a total of three bloodstains previously submitted to the laboratories in 1995 for a previous EDNAP exercise (samples 1, 3 and 5). Analysis of samples proceeded in accordance with the protocol and strategies currently used in each individual laboratory. Laboratories were asked to fill out a short questionnaire about their sequencing strategy, in addition to submitting results, and also to state whether this technique was used routinely and whether screening methods were used prior to sequencing. A total of 12 laboratories submitted results.

2.2 Results and discussion

Despite the diversity of methodologies used, all the laboratories reported the same results. PCR amplification of overlapping fragments (4 laboratories) and semi-nested PCR (4 laboratories) were the most commonly used strategies. There was no uniformity in the primers used as different primers were selected by the different laboratories and only 3 labs coincided in their choices (Lab. 1, 4 and 6).

In general, cycle sequencing was preferred (11/12 laboratories) with only one laboratory using solid phase sequencing (laboratory 2). Automated sequencers and sequence analysis software were used in all cases.

Most of the laboratories reported using mtDNA analysis in casework (laboratories 1, 2, 5, 7, 8, 10, 11 and 12). Two other laboratories (laboratories 3 and 4) reported not using mtDNA routinely in casework, and no information was provided by a further two labs.

Most of the laboratories using mtDNA analysis in casework did not include screening procedures for analysing mtDNA. Three laboratories used screening procedures; laboratory 1 used single stranded conformational polymorphism analysis (SSCP) or SSCP-RE (SSCP of restriction enzymes fragments; Barros et al, 1997). Laboratory 2 used a minisequencing approach (Sullivan et al, 1996). Laboratory 4 reported using Amplification Created Restriction Site (Lien et al, 1993).

MtDNA typing reliability and efficiency are primarily dependent on the quality of the DNA extract and quality control measures are used to limit external contamination.

Methods to detect heteroplasmy, some aspects of the nomenclature and statistics may need further discussion and refinement. The successful result of this exercise, however, confirmed that PCR based mtDNA typing by automated sequencing is a valid, robust and reliable means of forensic identification despite the different strategies and methodologies used by the different laboratories.

The publication that resulted from this work played an important role in a recent North American case to demonstrate laboratory reproducibility.

3. References

Alonso A, Martin P, Albarran C, García O, Sancho M (1996). Rapid detection of sequence polymorphisms in the human mitochondrial DNA control region by polymerase chain reaction and single-strand conformation analysis in mutation detection enhancement gels. Electrophoresis 17:1299-1301.

Anderson S, Bankier AT, Barrell BG, De Bruijn MH, Coulson AR, Sanger F, Schreier PH, Smith AJH, Staden R, Young G (1981) Sequence and Organization of the human mitochondrial genome. Nature 290:457-465.

Barros F, Lareu MV, Salas A, Carracedo A (1997) Rapid and enhanced detection of mitochondrial DNA variation using single-strand conformation analysis of superposed restriction enzyme fragments from polymerase chain reaction-amplified products. Electrophoresis 18:52-54.

Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31-36.

Greenberg BD, Newbold JE, Sugino A. Intraspecific nucleotide sequence variability surrounding the origin of replication in human mitochondrial DNA (1983) Gene 21:33-49.

Lien S, Kaminski S, Alestrom P, Rogne S (1993). A simple and powerful method for linkage analysis by amplification of DNA from single sperm cells. Genomics 16(1):41-44.

Pyercy R, Sullivan KM, Benson N, Gill P (1993). The application of mitochondrial DNA typing to the study of white Caucasian genetic identification. Int J Leg Med 106:85-90.

Stoneking M Hedgecock D, Higuchi RG, Vigilant R, Erlich HA (1991). Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-specific oligonucleotide probes. Am J Hum Genet 48:370-382.

Sullivan KM, Tully G, Alliston-Greiner R, Hopwood A, Bark JE, Gill P (1996). A Two Stage Strategy for the Automated Analysis of Mitochondrial DNA. In: Carracedo A, Brinkmann B, Bär W(eds) Advances in Forensic Haemogenetics 6. Springer, Berlin Heidelberg, pp 11-13.

Thomas AW, Morgan R, Sweeney M, Rees A and Alcolado J. (1994) The detection of mitochondrial DNA mutations using single stranded conformation polymorphism (SSCP) analysis and heteroduplex analysis. Hum. Genet 94:621-623.

Thompson JD, Higgins DG, Gibson TJ: ClustalW (1994) Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673-4680.

5.6 Continuing collaborative exercises

5.6.1 Mitochondrial DNA

It is well established that the mitochondrial DNA (mtDNA) mutation rate is substantially higher than that encountered with genomic DNA. Consequently, it is not uncommon for differences to be observed in the DNA sequence when comparing close maternal relatives (such as mother and child) (Parsons *et al*., 1997). Substitution has also been observed in somatic tissues, presumably due to segregation of an existing mixture of mtDNA types within the individual, a condition known as heteroplasmy. This means that differences may be observed between different hairs/tissues within an individual (Wilson *et al*., 1997; Sullivan *et al*., 1997).

Heteroplasmy probably exists in all individuals, although it is often at such a low level that it cannot be detected by the routine sequencing techniques presently used. In order for a mutation to be detected by sequencing, it must be present at a level approaching 20% to be distinguished from background. In addition, the chance of detection of heteroplasmy is dependent upon the sequencing chemistry used. Furthermore, detection may be more efficient at certain nucleotide positions than at others, and differences in detection may be also observed between the two DNA strands.

In order to study this phenomenon more closely, two collaborative exercises were undertaken.

Hair Study 1

The aims of this study were twofold:

1. to characterise the amount of heteroplasmy and mutation detected within and between multiple hairs from a single individual;
2. to test the reproducibility of mtDNA sequencing of hairs between laboratories.

The study was designed such that each individual hair would be tested by at least two laboratories and the position of the segments analysed, relative to the hair root, was known. A donor who displayed heteroplasmy in reference buccal samples was chosen and multiple hairs collected. Ten laboratories have returned results from a total of 55 hairs, showing existence and segregation of heteroplasmy at multiple base positions. Final experimentation to ensure results from two laboratories are available from every hair is now in progress, as is preparation of a paper for publication in the peer-reviewed press.

Hair Study 2

In light of the multiple heteroplasmy observed in Hair Study 1, a second study was initiated, using a donor in whom no heteroplasmy had previously been observed. The experimental design was the same as for the first study. Eleven participating laboratories have received segments from a total of 55 hairs, and results have to date been returned from one latoratory. The target date for return of results is 31/10/00, after which the data will be collated and published.

References

1. Parsons, T.J., D.S. Muniec, K. Sullivan, N. Woodyatt, R. Alliston-Greiner, M.R. Wilson, D.L. Berry, K.A. Holland, V.W. Weedn, P. Gill, M.M. Holland (1997). A high observed substitution rate in the human mitochondrial DNA control region. *Nature Genet.* 15: 363-368.
2. Wilson, M.R., D. Polanskey, J. Repogle, J.A. DiZinno, B. Budowle (1997). A family exhibiting heteroplasmy in the human mitochondrial DNA control region reveals both somatic mosaicism and pronounced segregation of mitotypes. *Hum. Genet.*, 100: 167-171.
3. Sullivan, K.M., R. Alliston-Greiner, F.I.A. Archampong, R. Peircy, G. Tully, P. Gill, C. Llyod-Davies (1997). A single difference in mtDNA control region sequence observed between hair shaft and reference samples from a single donor. In: Proceedings from the Seventh International Symposium on Human Identification; 1996 Sept 19-21; Scottsdale (AZ):1996; Promega Corporation, pp 126-129.

5.6.2. An experiment to evaluate the efficacy of different multiplexes to analyse mixtures

Introduction

An experimental protocol was devised in order to test the efficacy of mixture analysis when different multiplexes were used. To do this a series of male: female mixtures were supplied to laboratories in the following ratios 1:2, 1:4, 1:8, 1:16, 1:50, 1:100, 1:300. Laboratories were requested to analyse and interpret samples using the multiplex systems in current use in their laboratories, as well as an experimental multiplex that comprised a series of pentameric STRs, that was provided by Promega. The purpose of the exercise was to compare different multiplex systems to determine the point at which the male component could no longer be visualised. In addition, the effect of stutter on interpretation was assessed and compared between the different multiplexes.

The multiplexes compared were as follows:

Promega Powerplex 16; Applied Biosystems Profiler, Profiler Plus, SGM plus and the FSS SGM systems. In addition, Promega supplied a trial multiplex of 4 different pentameric STRs. A series of Y- chromosome STRs supplied by A Carracedo was also distributed.

Results and Discussion

The results of the exercise demonstrated that the male component of the mixture could be detected at levels down to 1:10 – 1:30 for the STR multiplexes analysed. For the Y-chromosome markers, the limits of detection varied between 1:10 – 1:100. There was considerable variation between laboratories. However, the exercise seems to confirm the utility of Y-chromosome markers to detect low levels of male DNA in the presence of large amounts of female DNA.

Participants of the exercise are currently compiling data related to stuttering effects in relation to their effect on mixture interpretation.

5.6.3. STR Systems for the Genetic Analysis of Degraded DNA – Preliminary Results of a STADNAP Collaborative Exercise

Degradation of human DNA extracted from forensic stains is the result of a natural process due to the exposure of the stain samples to the environment. Light, humidity, elevated temperatures as well as bacterial and fungal contaminations followed by the growth of these microorganisms lead to physical, chemical and biochemical degradation of the genomic DNA. Once the average DNA fragment length is reduced to sizes smaller than 300 bp, a loss of genetic information occurs due to the lack of suitable template DNA and the subsequent failure of STR systems to generate any useful or reproducible result.

Experience with degraded DNA from casework samples shows that every sample may exhibit different properties in this respect, and that it is difficult to systematically assess the performance of routinely used typing systems to analyze degraded DNA samples. To learn more about the efficiency of STR systems, a standardized reference sample of degraded DNA in sufficient amounts would be quite helpful. Therefore, a collaborative exercise on degraded DNA was planned by the STADNAP network, and participation was offered to 50 forensic laboratories across Europe. Briefly, the exercise was carried out as follows:

1. A large batch of high molecular weight genomic DNA was prepared at the Institute of Legal Medicine in Mainz from the two human cell lines HepG2 and P118 with male and female genotypes, respectively. The DNA samples were degraded under standardized conditions to an average fragement size of less than 200 bp, using a combination of physical and biochemical methods, i.e. sonication and treatment with DNAse I. The degradation process was closely monitored to control the resulting fragment sizes, as well as the suitability to obtain at least partial results by multiplex STR typing. It was observed that the final fragment size distribution heavily depends on the DNA quality and concentration, the reaction conditions and volumes, as well as the properties and the concentration of the DNAse I digestion. Batch to batch reproducibility could only be achieved by testing the degraded DNA by STR analysis (K. Bender, M.J. Farfan, P.M. Schneider: manuscript in preparation).

2. Two aliquots of 20 µl each were shipped at the end of July 2000 to the 50 participating laboratories with the following instructions:

   - first, apply your standard combination of forensic STR systems, e.g. the SGM Plus or any other multiplex using regular PCR conditions,
   - then, if your first amplification fails, try to modify these conditions as you would do in casework, or use singleplex systems,
   - record the results for each locus with successful amplification by allele designation and peak height in a tabulated form, and enclose prints showing the DNA profiles using the GeneScan or GenoTyper format,
   - if you routinely perform mtDNA typing, please try to sequence HV-I and HV-II.

The results from 20 laboratories were received by the end of August, and are the basis of the preliminary results described below. These results were presented at the XVIIth Congress of the International Academy of Legal Medicine (IALM) in Santiago de Compostela, Spain, September 6-9, 2000, in a session of the STADNAP network initiative. The final results of the exercise will be obtained until the end of October, and will be subsequently analyzed and published.

Only results obtained for the SGM Plus Kit (Applied Biosystems) were included in the preliminary analysis, as this kit is the most widely used multiplex system currently used. The submitted data were recorded for each STR locus based on the peak height (in relative fluorescence units – *rfu*) for each allele observed. To allow a comparison between laboratories, the peak heights were pooled into three categories: strong signal: >150 rfu, low signal: 150 – 30 rfu, very low or no signal: 30 – 0 rfu. Furthermore, two categories of data were defined based on the exercise instructions (see above): "standard" PCR conditions using 0.5 – 2 ng degraded DNA and 28 PCR cycles, and "enhanced" PCR conditions using 1 – 5 ng DNA and 28 – 35 PCR cycles.

The results are summarized in Figs. 5.1 (page 53) and 5.2 (page 54) across all loci analyzed for both categories. Using standard conditions, a significant loss of information occured around 180 bp (the size of the VWA system), whereas successful amplification was achieved for the majority of labs up to approx. 220 bp (D21S11 system). However, not all alleles could be identified correctly for all loci.

A number of common problems were identified based on these results. A strong peak imbalance was observed in heterozygous genotypes in particular for the larger STR systems and for "enhanced" conditions applying more than 28 cycles. Artifact

signals ("pull-ups") occurred due to overamplification mimicking alleles not present in the sample. Allelic drop-out (i.e. the complete loss of one allele in a heterozygous genotype) occurred frequently, and sometimes the smaller of two alleles at a given locus was affected. The use of the Genotyper software for automatic data analysis sometimes prevented the detection of an allele due to the threshold settings of peak height detection, but also prevented the inclusion of artifact bands in some cases.

From these results, some preliminary conclusions can be drawn:

- To overcome the effects of template DNA degradation, a PCR protocol optimized for all parameters such as template DNA and Taq polymerase concentration as well as the number of PCR cycles has to be established.
- Strict guidelines for the interpretation of the observed DNA profiles have to be applied.
- A careful visual control of all results in addition to a computer-based analysis (e.g. using the GenoTyper software) is required.
- The use of standardized degraded control DNA is helpful to understand and optimize the parameters affecting the success of STR typing.
- The availability of such a degraded control DNA in sufficient amounts would enable the forensic laboratories to enhance the efficiency of their typing methods.

It can be expected that the complete exercise results from all laboratories, and for all STR typing systems investigated, will add further informations to understand the performance of STR systems to type degraded DNA.

It is the intention to produce larger batches of degraded material from DNA extracted from one or more cell lines for future experiments. Ideally the group would like an independent organisation to take over the preparation and distribution of the material at a later stage of development so that it could benefit the entire forensic community.

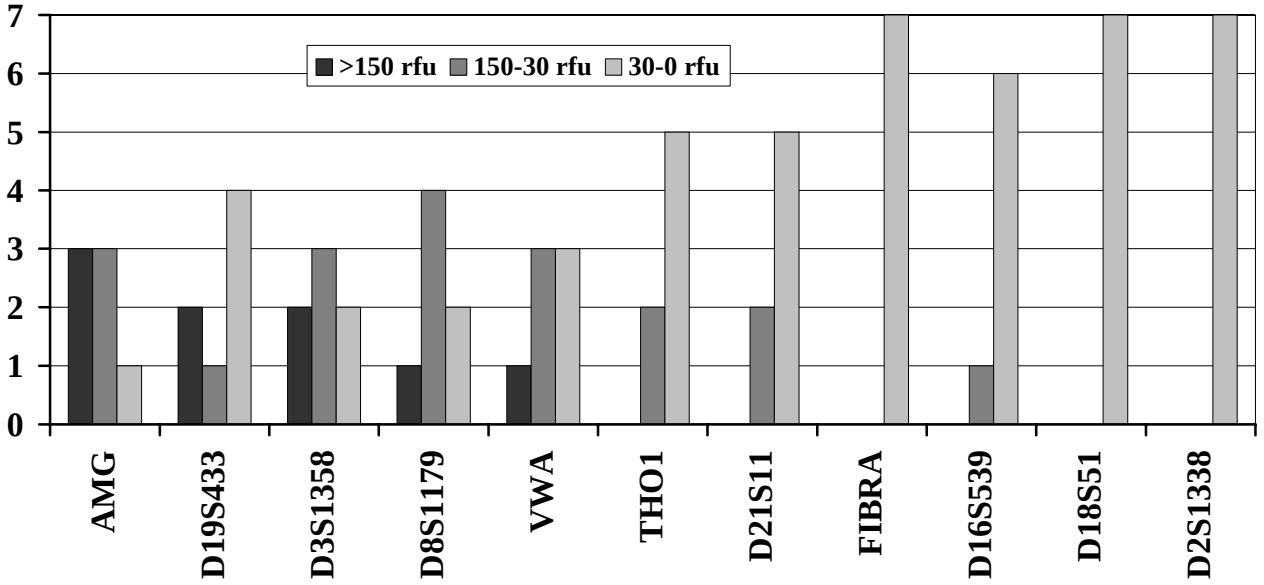

Fig. 5.1: Results of SGM Plus multiplex PCR analysis using standard PCR conditions (0.5 – 2 ng DNA and 28 cycles). The smallest fragment (from the amelogenin system – AMG, 106 bp) is on the left end, and the largest fragment (D2S1338, 312 bp) is on the right end. The total number of fragments scored in each category (based on peak height in *rfu*, se box) is shown.

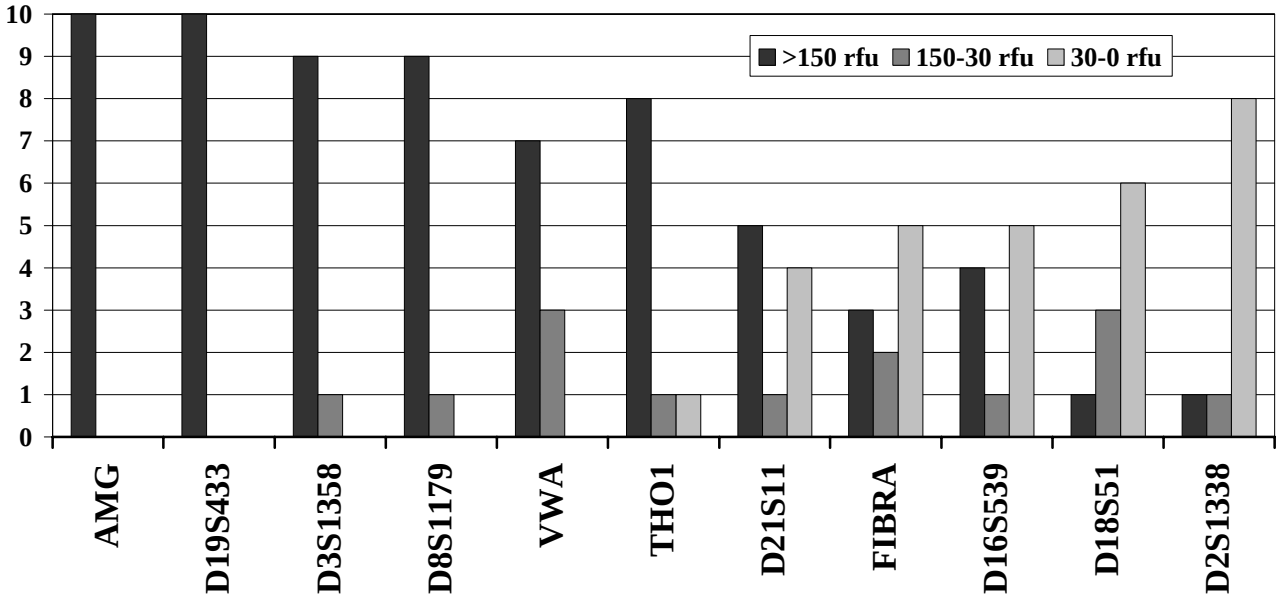

Fig. 5.2: Results of SGM Plus multiplex PCR analysis using enhanced PCR conditions (2 – 5 ng DNA and 28 – 35 cycles). The smallest fragment (from the amelogenin system – AMG, 106 bp) is on the left end, and the largest fragment (D2S1338, 312 bp) is on the right end. The total number of fragments scored in each category (based on peak height in *rfu*, se box) is shown.

5.6.4. Short STRs on telogen hairs

A collaborative experiment to analyse degraded DNA found in telogen hair roots was devised. Primers were designed that flank the STRs of TH01 and TPOX. It was demonstrated that these primers were more effective and sensitive to detect low levels of degraded DNA than the longer published sequences that are used.

The work is currently being expanded to include the analysis of a multiplex system that comprises 9 loci.

Note added in 2004:

The results have been published in the following paper:

Schneider PM, Bender K, Mayr WR, Parson W, Hoste B, Decorte R, Cordonnier J, Vanek D, Morling N, Karjalainen M, Carlotti CMP, Sabatier M, Hohoff C, Schmitter H, Pflug W, Wenzel R, Patzelt D, Lessig R, Dobrowolski P, O'Donnell G, Garafano L, Dobosz M, de Knijff P, Mevag B, Pawlowski R, Gusmão L, Vide MC, Alonso A, García O, Nicolás PS, Kihlgreen A, Bär W, Meier V, Teyssier A, Coquoz R, Brandt C, Germann U, Gill P, Hallett J, Greenhalgh M. (2004) **STR analysis of artificially degraded DNA – results of a collaborative European exercise**. *Forensci Sci. Int.* 139:123-134

6. WP 3: Technology Transfer Programme

Short-term secondments for laboratory visits were provided to promote more intensive collaboration between European Laboratories. In a first phase secondments between STADNAP members were approved for a total of seven. From this seven applications six were completed.

The second phase of the Transfer Programme was initiated after the meeting in Tenerife (January 1999). A call for applications under the STADNAP Fellowship Programme has appeared on the homepage under http://www.STADNAP.uni-mainz.de/fellow.htm. The same announcement was also published in the most important forensic journals.

A total of eight applications were approved and completed in this second phase. The laboratories involved in these exchanges and the purposes of the visits are summarized in Table 6.1.

The scientific objectives of the exchanges included the analysis of the Y chromosome variation, the improvement of mtDNA sequencing, analysis of degraded DNA, new technologies and new population genetic approaches.

The scientists involved in the exchanges reported that the secondments were successful, not only from a scientific point of view, but also to increase awareness and communication. In the light of this and also the prospect of new and innovative techniques which are now being developed, it was concluded that exchanges of personnel between European laboratories should be encouraged to promote harmonisation and technology transfer.

Table 6.1

Person	From	To	Objective
Brigitta Kindstrand	Linköping	Oslo	DNA extraction, mtDNA
Chris Phillips	London	Münster	mtDNA - ABI 310
Catherine Thacker	London	Santiago	Y chromosome SNPs
Luisa Pereira	Porto	Ferrara	Y chromosome SNPs
Marie Luise Sonntag	Wiesbaden	Münster	DNA sequencing
Nathalie Brandt	Brussels	Strasbourg	MtDNA
Michael Rasmussen	Copenhagen	Innsbruck	MtDNA
Michael Rasmussen	Copenhagen	Santiago	MtDNA
Charlotte Hallenberg	Copenhagen	Santiago	Y chromosome
Ioulia Skitsa	Athens	Santiago	MtDNA
Lourdes Solla	Madrid	Santiago	Y chromosome
M.Victoria Lareu	Santiago	Uppsala	Pyrosequencing
Isabelle Clisson	Strasbourg	Santiago	Degraded DNA
Helena Gómez	Madrid	Porto	Y chromosome

7. WP 4: Population Database Compilation

7. 1 Objectives

Laboratories involved in the study of human identification throughout Europe are continuously collecting genetic information about particular populations that they are interested in. Collection and verification of this data is time-consuming and expensive and often the appropriate resources, or particular population material, are not available to the individual laboratories. Provision of a bank of data that everyone in the field can access can be invaluable to the individual researcher and collection of a large amount of genotype information, rather than just population frequencies, will enable much needed empirical research to be undertaken looking at associations across the genome between the various DNA subtypes.

DNA typing of short-tandem repeat (STR) loci is used throughout the world as the principal tool for human identification and it is collection of this data that was sought as part of this project.

The objectives of WP 4 were therefore to:

- Collect STR genotype data from as wide a source as possible
- To produce large, statistically, validated STR databases of frequencies and genotype information for different geographical and ethnic populations thoughout, but not limited, to Europe.
- To make these databases available to researchers via the internet and on CD

7. 2 Work

7.2.1 Laboratories to approach

Discussions amongst the STADNAP members of WP 4, and in consultation with the whole group of STADNAP members led to an initial approach being made to the STADNAP members for information about laboratories in their countries who might

have collections of STR population data that would be useful for the project. Most laboratories that are involved in human identification work will be in associations that are known to the members and by this route we sought to get the widest source of genetic information from the population. Opportunities were also taken at various scientific meetings to publicise the project and invite participation. This information was also made available on the STADNAP web pages.

7.2.2 STRs to collect

A letter was sent to all the members requesting contact information. In this letter we requested the provision of genotyped data for any STR systems where the particular laboratory had typed over one hundred unrelated individuals from any one broad racial grouping. We requested that they provide us with a unique identifier for each individual tested to allow for statistical assessment to be made across the various systems.

It had been agreed that people should be asked to provide information on any STR they utilised, rather than dictate which loci we wanted to collect. Although we were aware of which STRs were used by most of the STADNAP members we suspected that a large number of other STRs would be used by other laboratories, particularly those not involved mainly in criminal work.

7.2.3 Mechanics of the database

A protocol was given to the people when submitting their database to us. Virtually everyone sending us information was able to comply with the request. A few of the laboratories had not preserved the individual genotype information across the different STR systems and so were only able to provide us with allele frequencies.

Laboratories were to be encouraged to send their data in electronic (Excel) format. Data in this form could be imported into various statistical modules for analysis. Consideration was also to be taken of the eventual size of the database so that the appropriate software was used for the purpose. STATISTICA was selected as a

database format because of its virtually unlimited restriction on database size, ability to produce graphical output and speed of statistical analysis.

Data which could not be sent electronically would be entered manually within the collecting laboratory, by two individuals, and conflicts resolved before being assimilated into the main database.

A single dedicated large capacity computer was to be purchased which other people would not have access to, in order to protect the database. The computer would be provided with an integral CD writer to allow rapid regular backup copies of the data to be made and appropriately stored.

7.2.4 Population ethnicity

It was agreed that laboratories should be asked to provide as much detail about the individual's ethnicity as they were able. It was understood that, in some cases, this information would be limited or absent altogether, particularly in those laboratories whose main business was criminal, but we thought that this should not limit the collection of data since the power of any statistical analysis would be increased.

7.2.5 Allelic assignment

Much of the older STR data collected within laboratories was typed without reference to sequenced allelic ladders, which is the common practice now. We did not want to exclude laboratories from providing data that had not been typed according to this, now recommended, procedure, but wished it to be made explicit so that the data could be compared statistically with data that had been appropriately typed. While we may chose eventually not to include data from a particular source if it was shown to be flawed, this would not be made explicit within the published database and the contribution would be acknowledged in the composite data in the same way as other contributors.

7.2.6 Quality of database

In any database collection there will be a question of the reliability of the data. We were, therefore, particularly interested in various measures of quality that could be associated with any particular database and addressed this by asking the laboratories to answer a questionnaire that was specific to each STR system that they provided information on.

Data was sought relating to the base-pair (bp) size of the major alleles detected within a system, plus information on the repeat number range. The base-pair size was to provide information on whether appropriate primers had been used to produce products within a size range where detection would be reliable. The repeat number range allowed us to validate both nomenclature and allele assignment between laboratories.

In addition to asking whether the database samples had been run at the same time as an allelic ladder we requested information on whether the ladder itself had been sequenced and was, therefore, reliable for typing purposes, and what the specific source of the ladder was.

Information on the particular technology used in the production of the database was sought. Much of the older database collection was done using manual methodology where the resolution is not as high as automated methods. Many scientists, however, have considerable experience at these methods and can produce completely reliable results so our aim here was to provide background information and validation to this data, rather than to remove it. The particular methods used, whether denaturing or non-denaturing gels are used, whether agarose or polyacrylamide gels with silver staining are employed, may all affect the efficacy and accuracy of the typing.

Automated equipment is in common use for typing STRs, with some instrumentation providing higher resolution than others, and some manufacturer's equipment being more popular than others. We wished to survey what equipment had been used to

prepare the databased samples because of differences in both resolution and allele (bp) sizes.

We asked laboratories what the source of their samples was so that we could look at biases in sample collections. This would be possible if the collection of data were of sufficient size.

We were also interested to see if the individual laboratory's data had already been evaluated statistically and asked for the specific tests done and results in such cases. Testing for Hardy Weinberg equilibrium was something that we were also going to undertake on behalf of the laboratories, along with tests of independence across loci.

Mutations are not uncommon in STR systems and those laboratories undertaking relationship tests are able to provide information on these. Reliable estimates of mutation rates will be possible if sufficient data is collected and provision of this information would be an invaluable resource to many scientists.

Participation in proficiency testing by the individual laboratory is a surrogate measure of quality of the database produced by the laboratory although, even if a laboratory does not participate in such exercises, it does not necessarily imply that their data is in anyway flawed.

7.2.7 Provision of the database

It was agreed that the composite databases should be made available from the web site but consideration should also be given to making individualised collections of data available only to scientists for research purposes. This is because of concerns of possible abuse of the data even though the data itself has been anonymised. Frequency data could, however, be made freely available on the web site with, perhaps, restricted access to more sensitive information. Alternatively this data could be requested by scientists and provided, individually, on CD ROM.

It was to be made clear to all contributors that the data would remain their property and that they would be free to publish their data elsewhere and that we would make reference to their contribution and also cite reference to any publications that already described the data within their individual database.

7.3 Results

7.3.1 Laboratories

As a result of the appeal to STADNAP partners, a database of 165 laboratories and their contact details was collected. The extent of the contacts provided is shown in table 7.1.

The wide variation in the number of contacts across the different European countries is a reflection of the different ways that the various countries organise and conduct their genetic research. Not all the countries are European. We were provided with names of contacts in non-European countries with which European scientists have had collaboration and sought their help in addition.

7.3.2 STRs

As we suspected, a large variety of STRs are being used amongst the various laboratories; some, like TH01 and VWA, were some of the earliest STRs employed and they continue to remain popular. Others, like FES, and F13A1 used to be very popular but have been replaced, more recently, by more discriminating STRs like D21S11 and FGA. Both of the latter two STRs possess fairly common 2bp subtypes which can be more readily identified with the more modern automated technology. Some of the differences in popularity are due to the provision of multiplexes of particular loci being provided by commercial interests, and also because some loci have been defined as core loci for use within the criminal laboratories within Europe. One loci, SE33, is in common use within Germany, but only one other European country has provided a database. Table 7.2 lists the individual loci (n=38) along with the number of laboratories providing databases.

Table 7.1

Country	No. of contacts
Argentina	2
Austria	3
Belgium	13
Brasil	1
Canary Islands, Spain	2
Colombia	2
Costa Rica	1
Czech Republic	1
Denmark	1
Estonia	1
Finland	1
France	9
Germany	74
Greece	1
Italy	12
Japan	1
Northern Ireland	1
Norway	1
Poland	2
Portugal	6
Slovakia	1
South Africa	1
Spain	22
Sweden	1
Switzerland	2
The Netherlands	1
UK	3
Uruguay	1
USA	1

Table 7.2

STR database	No. of laboratories	STR database	No. of laboratories
D1S1656	3	D21S11	22
D2S1338	5	CD4	6
D3S1358	18	CSF1PO	12
D3S1744	1	CYAR04	1
D5S818	14	F13A01	7
D6S477	1	F13B	7
D7S820	16	FABP	1
D8S1132	3	FES/FPS	10
D8S1179	21	FGA (FIBRA)	23
D8S306	1	FOLP	2
D12S1090	1	HPRTB	1
D12S391	4	HSACO	1
D13S317	14	LPL	3
D16S539	11	MBPB	1
D18S51	19	PLA2A1	1
D18S535	2	SE33 (ACTBP2)	10
D18S849	1	TH01	25
D19S523	1	TPOX	11
D19S5433	5	VWA	26

7.3.3 Database

Data from around 38,000 individual genotypes has been provided by the laboratories. This data has been accumulated in a STATISTICA database where it has been individually checked for consistency of coding for missing data, homozygotes, country, source and ethnicity. All laboratories provided data electronically, by e-mail, or on Zip or floppy dics. Two laboratories provided separate loci with individualising codes that required sorting in order to accumulate it into the large database. This was rapidly undertaken within the chosen database.

7.3.4 Ethnicity

A large amount of information has been provided by the laboratories about the ethnicity of their database populations. The majority of the database is composed of caucasian individuals.

7.3.5 Allelic assignment

Virtually all databases supplies have been typed with reference to an allelic ladder run at the same time as the samples. This is very encouraging since many databases were previously prepared without such reference and the importance of the reference appears to have prompted most laboratories to include this practice, which may have meant retyping of many of their original samples.

7.3.6 Quality issues

Base-pair size range of detected alleles varies on occasions due to the differing primers used in the amplification process. Generally the base-pair repeat size is consistent between the various laboratories, with one or two reports requiring further clarification from the source. SE33 is an exception providing highly variant ranges but is not unexpected because this is a hypervariable locus and not like any of the other STRs.

Whereas virtually all the data had been typed with reference to an allelic ladder there was more variation in the ladders. Whereas the majority of laboratories used commercially prepared ladders which had been sequenced, in a large minority of loci there were not commercially available ladders (the production of these presumably being linked to the production of primers for loci that these companies support). Generally these allelic ladders had been either home prepared, or prepared by another laboratory. Only a minority were not sequenced.

The technology used was variable. No laboratories reported using agarose for gel preparation but a significant number of laboratories had prepared some of their

databases wholly, or partly, by manual techniques with silver staining and one laboratory reported only using manual techniques.

Automated instrumentation from only two manufacturers was reported in use: Applied Biosystems (ABI instruments) and Amersham Pharmacia Biotech (ALF instruments). The ALF instruments were in the minority, as was the older ABI373 instrument. The ABI 377 was in wide use but the capillary-based ABI 310 was the most popular instrument.

The majority of laboratories provided data that had been obtained from testing of unrelated parents in paternity investigations. Use was also made of samples from staff, unrelated volunteers or hospital patients, bone marrow or blood donors, but these were all relatively small in number. Samples from criminal sources, although not provided by many laboratories, in some cases produced very large amounts of data from single sources. The statistical evaluation of this data has not yet been completed.

Where statistical tests of Hardy-Weinberg equilibrium had already been undertaken the results showed either non-significance or were of marginal ($p < 0.05 > 0.01$) significance. These latter incidences could be chance occurrences and global studies of the data currently being undertaken should clarify this. Often these tests have already been undertaken when the laboratory seeks publication of their data but journal publication of population databases is not as widely welcomed unless accompanied by additional information and we hope that the database will provide a means to acknowledge the work of the laboratory as well as providing them with the statistical validity of their data. The results of the tests will be fed back to the individual laboratories and other selected information will be made available along with the database.

Several laboratories have reported mutations – all have been the apparent addition or subtraction of one repeat, the largest number of mutations being observed in the VWA system. Laboratories that don't, or rarely undertake large numbers of tests of related individuals will be unaware of many of these mutant events.

We asked about participation in proficiency testing. Many laboratories are required to undertake such exercises as part of their local accreditation procedures and it was encouraging to see that the majority of laboratories took part in these exercises, a large number participating in two or more such tests.

7.3.7 Database provision

While much of the data is now in place the statistical assessments need completion. It is planned that, initially, allele frequencies from the different populations, along with quality information, will be made available on the internet and raw data genotypes provided to individual laboratories that request them. We plan in the future to make the database interactive, being able to be continuously updated and searchable, but this will take more time. The data will be made available through the STADNAP website, and efforts are currently under way to install a first version of the population frequency database onto the webserver.

8. Incidences and deviations from the programme

The consortium has met the planned schedule according to the project programme with a few changes. A total of six meeting were carried out during these three years in Rome, Innsbruck, Tenerife, Mechelen, Dublin and Santiago de Compostela. In addition a meeting was held in San Francisco (USA) in August 1999 in connection with the meeting of the International Society for Forensic Genetics.

One partner ("MIFR.DRP.LPS") has had difficulties joining the project. Finally they have decided to attend the meetings of the consortium as observers but not actively participating in the project. So, it was decided by the consortium that this partner be excluded from the STADNAP project and therefore no cost statements are included. No substitutions were possible for the time being. The corresponding amount concerning this partner should be cancelled or reimbursed to the Commission.

The working groups have developed their respective programmes with specific roles assigned to each partner. In addition all the laboratories actively participated in the inter-comparison exercises agreed on by the consortium. The general feeling about the project was that it was very satisfactory and has met the expectations of the group.

9. Conclusions

During the course of the STADNAP initiative it became apparent that the first requirement was to refine the systems in current use and to arrive at a consensus for the use of a multiplex system which would be used by all laboratories for the formation of a pan-European DNA database. In the main this has been achieved.

The growing use of mitochondrial DNA (mtDNA) analysis in cases where there is insufficient genomic DNA available (e.g. single hairs, old stain samples, identification of human skeletal remains), has spawned a number of inter-laboratory exercises to develop the methodology and to ensure accurate analysis of the results. The reproducibility of mtDNA sequencing results among laboratories was proven and an important collaborative research (to analyze and to better understand the heteroplasmic events in hair shafts) has also been completed, thus preparing the basis for a more reliable approach for the interpretation of the results when heteroplasmies are observed in real casework.

For similar reasons, the elucidation of Y chromosome STR markers has also involved the group in collaborative exercises to find the most informative loci and to develop ways of expressing the results in terms of individuality. In this sense the reproducibility of Y STR analysis with PCR multiplex formats was demonstrated for mixed (male-female) stains .

Head hairs are commonly received by forensic scientists as evidential samples and, in the past, it has been the practice to only look for mtDNA polymorphisms. A collaborative exercise has been initiated to detect genomic DNA from telogen hairs using modified PCR primer sequences and amplification conditions via singleplex reactions. This study will continue.

Another important problem in forensics are stains with mixed biological material from different individuals. This was also the objective of an experiment to evaluate the efficacy of different multiplexes to analyse mixtures.

As it is normal for laboratories to receive samples in which the DNA is highly degraded, an exercise is now being undertaken to prepare a batch of degraded material for future experiments. There may be an advantage in producing samples from two cell-lines which contains different alleles and, in the future, the preparation could be passed to an organisation which routinely produces standard reference materials. The batches of degraded DNA produced by the group were the object of an exercise open to all the scientific community.

In order to keep abreast of current developments commercial organisations have been invited to STADNAP meetings to make presentations on new technologies, equipment and reagents. These presentations have helped to focus the debate on the likely future of DNA profiling within forensic science. In this regard, the conclusion was that the DNA microsatellites in current use will continue being used in the short to medium time-scale, but new markers such as SNPs, through the implementation of new methodologies like DNA microarrays are promising markers for the analysis of variation in mitochondrial DNA and the Y chromosome. However the robustness of these new methodologies and their reproducibility among laboratories must be proven before they can be used in practical casework.

Short-term secondments for laboratory visits were provided to promote more intensive collaboration between European Laboratories. These secondments were successful not only from a scientific point of view but also to increase awareness and communication.

DNA typing of short-tandem repeat (STR) loci is used throughout the world as the principal tool for human identification and it was the collection of this data that was sought as part of this project. A compilation of STR genotype data was carried out with the very successful participation of European laboratories. The aim was to produce statistically validated STR databases of frequencies and genotype information for different geographical and ethnic populations throughout, but not limited, to Europe. This database has been made available to researchers via the internet and on CD ROM.

Although the programme was very successful and the general objectives have been achieved, all the partners are keenly aware of the necessity to continue working on the standardization of DNA profiling in Europe, as requested by the Council of Europe recommendation R92/1, and recommend that the EU takes the action necessary to support this need.

Therefore, the STADNAP consortium strongly recommends to support research and development activities as well as coordination and exchange of information particularly in the following areas:

1. Analysis of mtDNA sequence polymorphisms; application and interpretation in casework; introduction of a European mtDNA frequency database for scientific and forensic use;
2. Studies on the usefulness of single nucleotide polymorphisms (SNP's) in forensic casework;
3. Development of certified DNA reference materials such as degraded DNA for the validation of forensic typing methods;
4. Ethical and legal studies related to the European harmonization of the national criminal justice systems as well as to the protection of individual rights on the context of forensic DNA profiling.

10. Publications

1. Carracedo A, d'Aloja E, Dupuy B, Jangblad A, Karjalainen M, Lambert C, Parson W, Pfeiffer H, Pfitzinger H, Sabatier M, Syndercombe-Court D, Vide C. (1998) Reproducibility of mtDNA analysis between laboratories: a report of the European DNA Profiling group (EDNAP).
   Forensic Sci. Int. 97: 155-164
2. Gill P, d'Aloja E, Dupuy B, Eriksen B, Jangblad A, Johnsson V, Kloosterman AD, Lareu MV, Mevag B, Morling N, Phillips C, Pfitzinger H, Rand S, Sabatier M, Scheithauer R, Schmitter H, Schneider PM, Skita I, Vide MC. (1998)
   Report of the European DNA Profiling group (EDNAP) - an investigation of the hypervariable loci ACTBP2, APOA11 and D11S554 and the compound loci D12S391 and D1S1656.
   Forensic Sci. Int. 98: 193-200
3. Schneider PM, d'Aloja E, Dupuy BM, Eriksen B, Jangblad A, Kloosterman AD, Kratzer A, Lareu MV, Pfitzinger H, Rand S, Scheithauer R, Schmitter H, Skitsa I, Syndercombe-Court D, Vide MC (1999)
   Results of a collaborative study regarding the standardization of the Y-linked STR

system DYS385 by the European DNA Profiling (EDNAP) group.
Forensic Sci. Int. 102: 159-165

4. Carracedo A, Beckmann A, Bengs A, Brinkmann B, Caglia A, Capelli C, Gill P, Gusmão L, Hagelberg C, Hohoff C, Hoste B, Kihlgren A, Kloosterman A, Myhre Dupuy B, Morling N, O´Donnell G, Parson W, Phillips C, Pouwels M, Scheithauer R, Schmitter H, Schneider PM, Schumm J, Skitsa I, Stradmann-Bellinghausen B, Stuart M, Syndercombe Court D , Vide MC (2001)
Results of a collaborative study of the EDNAP group regarding the reproducibility and robustness of the Y chromosome STRs DYS19, DYS389 I and II, DYS390 and DYS393 in a PCR pentaplex format.
Forensic Sci. Int. 119:28-41
5. Martin PD, Schmitter H, Schneider PM (2001)
A brief history of the formation of DNA databases in forensic science within Europe.
Forensic Sci. Int. 119:225-231
6. Schneider PM, Martin PD (2001)
Criminal DNA databases: the European situation.
Forensic Sci. Int. 119:232-238
7. Schneider PM, Bender K, Mayr WR, Parson W, Hoste B, Decorte R, Cordonnier J, Vanek D, Morling N, Karjalainen M, Carlotti CMP, Sabatier M, Hohoff C, Schmitter H, Pflug W, Wenzel R, Patzelt D, Lessig R, Dobrowolski P, O'Donnell G, Garafano L, Dobosz M, de Knijff P, Mevag B, Pawlowski R, Gusmão L, Vide MC, Alonso A, García O, Nicolás PS, Kihlgreen A, Bär W, Meier V, Teyssier A, Coquoz R, Brandt C, Germann U, Gill P, Hallett J, Greenhalgh M. (2004)
STR analysis of artificially degraded DNA – results of a collaborative European exercise.
Forensci Sci. Int. 139:123-134

Additional publications about the continuing collaborative exercises (see 5.6) are in preparation.

11. Acknowledgements

The STADNAP consortium sincerely thanks Dr. Peter D. Martin (former deputy director, Metropolitan Police, London) and Prof. Patrick Lincoln (St. Bartholomew's and the Royal London School of Medicine and Dentistry) for the invaluable support and expert advice. The consortium extends its thanks to the colleagues from European forensic laboratories who have provided STR data for the STR population frequency collection. The excellent support by the staff members from the participating laboratories is gratefully acknowledged. Finally, the consortium would like to thank the project officer Christos Profilis for his continuous support on practical and technical details in the execution of the network initiative.