Project Final Report

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EUROFORGEN NoE

Logo: 

Project title: EUROPEAN FORENSIC GENETICS – Network of Excellence

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1.1 Executive summary

Forensic genetics is a highly innovative field of applied science with important relevance for the security of citizens. The European Forensic Genetics Network of Excellence represents academic and public institutions, as well as SME's, and includes 16 partners; among them some of the leading groups in European forensic genetic research. It has aimed to create a closer integration of existing collaborations, as well as establishing new interactions in the field of security, as all key players are addressed: scientists, stakeholders, end-users, educational centres and scientific societies. These efforts have been combined with identifying and selecting the most innovative ideas to meet the challenges of analysing biological samples recovered from crime scenes and suspects to help with the identification of perpetrators and victims.

The final results of the EUROFORGEN network are expected to have a direct impact the following areas: scientific research & development, integration of the community of scientists and experts on forensic genetics in the criminal justice system, education and training in the field of forensic genetics, and interdisciplinary collaboration for research on the societal dimension of applying forensic genetics in criminal investigations. Our scientific results have already led to new research proposals in the context of a recent Horizon 2020 call. New technologies such as massively parallel DNA and RNA sequencing have been evaluated for their forensic efficiency regarding the prediction of biogeographic ancestry in casework. These will lead to an extension of currently available methods in forensic DNA phenotyping. New epigenetic and RNA-based body fluid identification methods will help to address and resolve the relevance of crime scene traces in criminal investigations, and open source software tools are applying advanced interpretation methods for challenging low level and mixed traces. The software EuroForMix allows a quantitative deconvolution of DNA mixtures and can be readily implemented following extensive validation studies carried out among consortium partners, and it is freely available both to casework laboratories and defence experts. The “Train the trainers” workshops have made a long-lasting impact to spread knowledge about these advanced interpretation methods to all participating countries. A series of local satellite workshops has been initiated addressing the training needs of DNA experts, and more workshops are expected to take place. Furthermore, these activities will be continued by organising annual summer schools beyond the end of EC funding. For academic education, a post-graduate curriculum for forensic genetic studies has been developed and has already been implemented with plans to continue in the future.

Our research on the ethical and legal aspects and the societal dimension of forensic genetics has led to a series of open publications on “Ethical, Social and Policy Aspects”, “Public perspectives on established and emerging forensic genetics technologies”, an “Audit of legislative frameworks within the EU for the collection, retention and use of forensic DNA profiles”, and “A Guide to Legal and Ethical Principles and Practices”. There is a large diversity among European countries regarding public acceptance or critical awareness of these technologies. Our results will raise the overall awareness of opportunities and challenges related to DNA profiling and to stimulate the public debate on these issues. It is envisaged that the development of ethical guidelines for research and deployment of forensic genetic technologies used in criminal investigations and prosecutions will have a major impact both on the scientific community as well as the general public.

The European Virtual Centre for Forensic Genetic Research will become a corner stone for the long-term integration of research efforts at the European level. It will ensure a continuing and sustainable impact on the quality of science and forensic laboratory services to the benefit of the security sector in Europe. Through this centre, and its directory, it will become easy to identify and recruit experts with specialist knowledge and the technologies to address specific scenarios with relevance to DNA-based genetic identification such as terrorism, disaster victim identification, organised crime and other threats to the public security. A close relationship has been established with scientific working groups and societies such as the European DNA Profiling Group (EDNAP), the working groups of the International Society of Forensic Genetics (ISFG), as well as the DNA working group of the European Network of Forensic Science Institutes (ENFSI),. The EUROFORGEN network will continue to exist following its integration as a working group of the ISFG.

1.2 Summary description of project context and objectives

Background and Aims

Forensic Genetics is a field in continuous evolution. From the discovery of DNA, different types of markers and technologies were introduced looking for a better sensitivity, discrimination power and robustness. Highly informative DNA typing systems have been developed which are extremely efficient for the individualization of biological material of human origin and a large effort has been dedicated to standardization. In the last few years, due to the wide ranging and accelerating advances in genomics, new DNA markers and new applications have arisen including both human and non-human DNA typing. A new type of marker, Single Nucleotide Polymorphisms (SNPs), has opened novel applications such as the analysis of externally visible traits, or the assignment of the biogeographic ancestry of biological samples. Scientists are working to identify genetic markers that influence physical characteristics such as hair, skin or eye colour, in the hope of one day using DNA samples from crime scenes to reconstruct a perpetrator's visible appearance. However, at the beginning of this project numerous parallel scientific efforts were not harmonized making it difficult to know the exact state of the art in the field, and to identify experts at the national level when the expertise is required in an investigation.

While some standardization groups on human DNA typing exist in Europe, i.e. the European DNA Profiling Group (EDNAP), and the DNA Working Group of the European Network of Forensic Science Institutes (ENFSI), the absence of coordinated research at the European level is a factor limiting progress in the area. A network coordinating high level collaborative forensic genetic research by combining local resources from academic institutions with work carried out by police and security forces was therefore urgently needed.

The same is true of education and the dissemination of knowledge for experts, police officers, judges, and all other end-users in general. There is a complete lack of common educational and training programs in state-of-the-art forensic genetics in Europe. The harmonization of education and the implementation of expert qualification schemes were as well urgently needed.

Progress in this rapidly developing field of applied science also impacts strongly on the societal dimension of security. Thus it was essential to identify the social, legal and ethical risks associated with the application of forensic genetics in support of criminal investigations and national security. Public concerns about criminal DNA databases and the protection of individual privacy rights needed to be understood and addressed in the context of maintaining the safety of citizens and the security of European societies. Research, industry, stakeholders and end-users needed a common framework of reference to facilitate the exchange of ideas and opinions, and to improve and moderate both the practical applications and the social acceptance of these new technologies.

The European Forensic Genetics Network of Excellence (EUROFORGEN-NoE) aimed to connect these efforts and lay the foundations of a European virtual centre of research in forensic genetics. The network represents a significant number of the **leading groups in Europe on forensic genetic research** and proposed an integration of existing collaborations, as well as establishing new ones, in this security field by incorporating the relevant parties (research centres, decision makers, stakeholders, end-users). This process started around specific technical projects and aimed to achieve a long lasting collaboration based on a joint programme of work leading to the emergence of a **virtual research centre** in the security domain. An **advisory board, with highly recognized experts** from the fields of ethical, legal, and forensic sciences, was set up to ensure that the challenges defined in the network programme would be met.

The main challenges and specific purposes of EUROFORGEN NoE are summarized in Table 1, the most important being:

- Identification of challenges and needs at European level to facilitate the exchange of information between research institutions, stakeholders and end-users, and to ensure long-term sustainability.
- Integrative and multidisciplinary projects within an open research framework contributing to the solution of practical casework projects.
- Develop standards for the ethics of phenotype prediction and protection of privacy in security context.
- Develop efficient dissemination actions and training forums and promote education in the field.

Table 1: Summary of the main challenges and specific objectives of EUROFORGEN-NoE

Challenge	Specific Objectives	Approach
Long term stable collaborative network structures	Identification of challenges and needs at European level to facilitate the exchange of information between research institutions, stakeholders and end-users, and to ensure long-term sustainability	- Integration of efforts towards the creation of Virtual Centre of Research - Approach stakeholders and decision makers for support, offer feedback and expert advice, identify independent funding sources
Integrating projects: Projects from different groups in Europe joining efforts	Integrative and multidisciplinary projects within an open research framework contributing to the solution of practical casework projects	- Exemplar projects among the members of the consortium - Competitive call for projects from other groups in Europe
Evaluation of the ethical, legal, societal issues related to the use of new forensic genomics technologies	Develop standards for the ethics of phenotype prediction and protection of privacy in security context	- Describe diversity in ethical and legal, debate of genomic privacy and security, - Develop discussion programs to stimulate societal debate,
Lack of educational resources, common specialization programs and accredited training	Develop efficient dissemination actions and training forums	- Organisation of training courses, professional dissemination programs, and workshops - Pilot postdoctoral curriculum

Work strategy and general description

The work strategy, summarized in Figure 1, essentially comprised five, parallel linear work package activities:

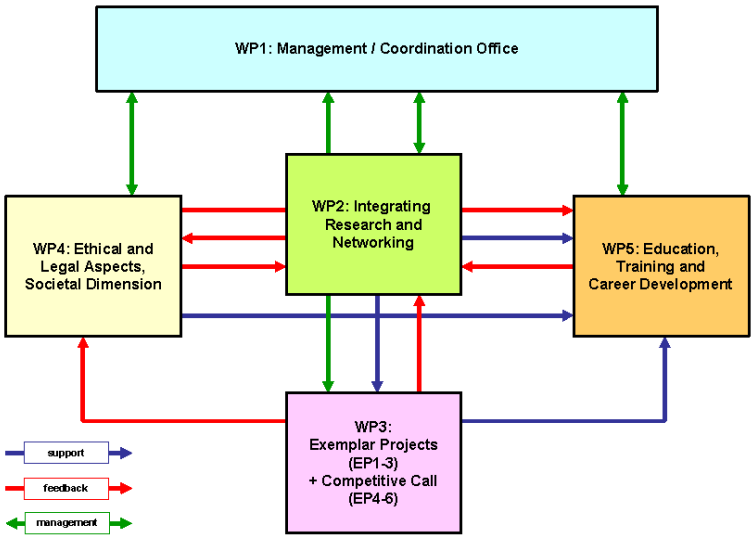

Figure 1: Graphical representation of the working packages showing their interdependencies

WP 1 - Coordination and communication office
WP 2 - Integrating research and networking: towards the creation of a European Virtual Center of Forensic Genetic Research
WP 3 - Three exemplar projects and competitive calls inviting new proposals
WP 4 - Ethical and legal aspects, and the societal dimension of forensic genetics
WP 5 - Education, training and career development

The main elements of the work of the project were:

1. The creation of a network in this field started with the identification of the key members of the scientific community actively carrying out research in forensic genetics across Europe and the description of the relevant processes in DNA evidence handling and processing with regard to the particular needs of stakeholders and end-users. For these objectives the implementation of the **website EUROFORGEN.EU** was key. The website provides a framework for the exchange of expertise and data, not only between consortium members but with any other European centre working in forensic genetics. It has brought together the knowledge and resources centred on forensic genetics tools and education at a European level, and allowed researchers, forensic practitioners, stakeholders or industry to interact with/or integrate into the network. This allowed the forensic genetics research community as a whole to contribute significantly to the improvement of forensic genetics research and expertise. The website has evolved into the central website of the European Virtual Centre of Forensic Genetic Research.
2. The **exemplar projects**: Different research projects were proposed to serve as a model of integrating forensic research in Europe. The projects included aspects from sample collection at the crime-scene to genotyping, data analysis and the preparation of custom marker multiplexes to address a specific investigative need. These main activities provided the focus for other important parts of the overall project, such as dissemination activities. The overall strategy is summarised as follows: use existing data and expertise from the project partners to develop the 'next generation' of integrated DNA profiling methodologies to maximize the chances of identifying perpetrators of crime in major investigations. Furthermore the work in these projects was constantly adjusted to adopt new technical and methodological developments, such as the wider availability of high-throughput massively parallel DNA sequencing leading to an adjustment of the objectives in WP3.
   In a second step of research activities, additional projects were identified following the opening of a **call for competitive research proposals** in forensic genetics to the scientific community. The best three projects were selected on the basis of criteria such as being multidisciplinary or their contribution to the solution of practical problems in forensic genetic casework. This allowed the identification of new partners, research and to extend the network towards the creation of the European Virtual Centre.
3. The **roadmap** to the creation of such a virtual centre rquired the implementation of a series of specific actions:
   - To establish a directory of forensic genetics research institutions across Europe.
   - To identify the processes involved in handling and analysing forensic genetic evidence from crime scene to courtroom, and to describe the relevance, strengths and potential flaws of forensic genetic evidence with regard to the specific needs of stakeholders and end-users.
   - To facilitate the exchange of information between research institutions, stakeholders and end users.
   - To integrate research needs and capacities into a virtual network for the coordination of research in forensic genetics with a solid basis of sustainability – the *European Virtual Centre of Research in Forensic Genetics.*
4. The comprehensive **consideration of ethical and legal issues** and the **societal needs** within WP4 were an essential part of the above processes and their integration into forensic practice in Europe. Ethical issues in forensic genetics were addressed individually and ranged from ethical considerations regarding criminal databases to the use of coding DNA variants for the identification of physical traits. There are other issues constituting socially sensitive areas and these required specific actions including clear and thorough information for the public, ethical analysis and social debate. The Virtual Centre played a vital role in the integration of our work with the wider public voice.
   An adequate response to public concerns regarding a potentially too intrusive use of new forensic DNA applications is seminal for a wider application of these methods in the near future. It needs to be prepared in the context of a responsible, ethically and legally acceptable research framework. Only then the consequences and future perspectives can be addressed adequately. The publication of an ethical guideline on forensic genetics was a major element in this process.

5. The final component was driven by the **needs of scientists and reporting officers** handling the results of forensic genetic analyses, to accommodate the impact of new technologies, and DNA typing approaches of low level biological trace evidence. The activities within WP5 were designated to reach into all European regions and institutions and to facilitate exchange of information and common educational standards. Short term fellowships to enable exchange visits have been a major component to facilitate this exchange. Again, EUROFORGEN-NoE provided a platform for the integration of educational activities, including online learning modules. This was complemented by the design of a common **postgraduate curriculum** for the education of DNA experts and the implementation of a pilot project within an academic institution. All partners and work packages have provided support, as training and education needs to integrate research & development, as well as ethical and legal aspects.

Management structure and procedures

The Project Coordinator ensured the smooth operation of the project and guaranteed that all efforts were focused towards the objectives. He submitted all required progress reports, deliverables, financial statements to the European Commission, and, with the assistance of GABO:mi (until June 2016) and ARTTIC (from July 2016 onwards), he was responsible for the proper use of funds and their transfers to the project participants. The EUROFORGEN Project Office was established by and based at the coordinator in Cologne and at GABO:mi/ARTTIC in Munich. The Project Office at the Coordinator was concerned with the scientific management and the co-ordination of all research activities. The Project Office at GABO:mi/ARTTIC was responsible for the administrative, financial and contractual management and the organisational co-ordination of the project activities.

As specified in Annex I, the General Assembly was responsible for the proper implementation of the Partners' respective rights and obligations in accordance with the contractual framework of the project and the CA. It was in charge of the political and strategic orientation of the project and met once a year.

The Steering Committee consisted of all WP Leaders and was in charge of monitoring all activities towards meeting the objectives of the project in order to deliver as promised, in due time and according to the budget. The Steering Committee met half-yearly. Furthermore, a Scientific Advisory Board was implemented consisting of experts representing the key aspects of EUROFORGEN's activities, to ensure a high standard of research and monitor the progress of the project by taking part in the annual General Assembly Meetings.

In addition, an Ethics Group was established in order to ensure that EUROFORGEN's activities were conducted in accordance with the laws and regulations applicable to the countries and in order to implement the ethical follow-up of the project.

1.3 Description of the main S&T results/foregrounds of EUROFORGEN-NoE

Following the work program of EUROFORGEN-NoE, significant progress regarding the research of forensic genetics has been achieved. This is highlighted by the detailed progress reports of the work packages (2-5), which are summarized below.

WP2: Integrating Research and Networking

This Work Package is devoted to "Integrating research and networking" and the main objectives that were accomplished during the entire project period were:

1. Identification of key members of the scientific community actively carrying out research in forensic genetics across the Europe
2. Description of the relevant processes in DNA evidence handling and processing with regard to the particular needs of stakeholders and end-users.
3. Stimulation of increased communication and information exchange between forensic genetics service providers across national borders, and among different stakeholders.
4. The establishment of a sustainable framework for a European Virtual Centre of Research in Forensic Genetics.

Significant actions have been undertaken during this five-year period, most of them related to more than one objective. Aiming to create a close network within the Forensic Genetics field, one of the first actions was to identify experts of the 25 Western and Central European countries in order to act as **National Contact Points** for the EUROFORGEN-NoE network. A meeting (deliverable D2.1) was held in Munich in September 2012, supported by the attendance of all the NCPs proposed. The Coordinator and the WP2 leader presented EUROFORGEN-NoE network and the expected role of the NCPs. Each National Contact Point gave a short presentation on his/her institutional and regional situation, focusing on casework and research, education and training, acceptance of DNA evidence in the courtroom and public opinion on forensic DNA applications. The NCP meeting served as a most useful source of information for planned activities in WP 2, WP 4, and WP 5. Following a suggestion made by the Scientific Advisory Board, some additional persons were invited to support the NCPs from less active countries in order to strengthen the impact of the network. Once the NCPs were involved in our project, we carried out an exhaustive search in order to **identify every single institution performing forensic genetics in Europe**. A general questionnaire was prepared in collaboration with the other consortium partners in order to collect information from the participating institutions. Each centre was asked to reply to a number of specific questions combined into the following sections: (A) Institution & General Information, (B) Practical aspects (including case work, presumptive tests, DNA quantification and statistical evaluation), (C) Research activities, (D) Education and (E) Standardization, research and implementation activities. The questionnaire for forensic genetic laboratories (http://www.euroforgen.eu/eu/) was distributed by WP2 partners, NCPs and dissemination activities (conferences, meetings, training workshops). National Contact Points provided a valuable support both during the identification of the participating laboratories and the contact process.

A map together with a list of addresses (**European landscape in forensic genetics**) is available on the EUROFORGEN-NoE website displaying the forensic genetic institutions performing research and/or casework in the field that kindly have replied to the general EUROFORGEN-NoE questionnaire. This online directory represents a search mechanism to find partners for specific project collaborations or to identify of experts at a European level. New questionnaire replies from forensic genetic researchers and providers expressing their interesting to join the network have been received up until the end of the project and they are still being received. By the end of the project (date 31/12/16) 251 replies to our questionnaire were received. We have also received a number of replies from non-European countries.

A "**Directory of forensic genetic research institutions across Europe**" was compiled and statistical analysis of the questionnaire results obtained was carried out including data on the type and numbers of genetic markers used, number of cases, statistical interpretation, presumptive tests and DNA quantification. The directory is available for downloading from the EUROFORGEN-NoE website, and a continuously updated directory of European forensic genetic institutions is openly accessible online: https://www.euroforgen.eu/networking-activities/european-landscape-in-forensic-genetics/. Among the conclusions extracted it is worth mentioning the widespread concern regarding the need for high-level education and training in forensic genetics. On the other hand, a positive conclusion that can be extracted from this data is the extent of the European leadership in this field, both in terms of casework and research activities. Initiatives like EUROFORGEN-NoE have helped to maintain and strengthen this leadership.

A document describing the whole process of handling biological evidence from crime scene to court room, identifying

the main challenges of this process from different perspectives, and determining crucial targets to be considered in order to improve the current situation of forensic genetics in Europe (“**State-of-the-art-description of handling biological evidence from crime scene to court room**”) was prepared and made available on the EUROFORGEN website. A statistical analysis of the questionnaire results received until end of June 2014 (194 questionnaires from 34 countries) was also included. Again, among the challenges identified, it should be mentioned that there is widespread concern for the need for education and training in forensic genetics, for both scientists and members of the legal community, and to improve the quality of communication between scientific experts and the judiciary. Other weaknesses encountered along the process are difficulties in the identification of experts, the increasing case load, the poor funding for research topics, and a lack of information on social, legal and ethical risks. Taking these findings into consideration, the proposed targets for improving the current situation in our field can be achieved by:

- Improving education and training programs
- Promoting standardization
- Improving communication between the players in the process
- Increasing funds devoted to research in this field
- Promoting the establishment of collaborative research projects

In order to stimulate the communication and information exchange between forensic genetics service providers, a call for applications for **short-term fellowships for visits** among scientists and experts was prepared and opened (http://www.euroforgen.eu/dissemination/short-term-fellowships/). This initiative was targeted not only between research laboratories, but also involving members of the security organizations and the justice system, to permit an increase in the understanding for the specific opportunities and needs of forensic genetic research. The evaluation and selection criteria considered were: relevance of the visit to the objectives described and the order of the application. Since the beginning of the project, three calls for short-term fellowships for visits among scientists and experts were published, offering 20 fellowships in each of them. The first call was open between 1 November 2012 and 31 December 2013, and 14 fellowships were awarded. Subsequently, a second phase call was opened in March 2014 and a final call was held in the period between 1st January 2016 and 31st July 2016. Overall, 45 fellowships have been awarded. After completion of the visits, the applicants were asked to provide a written report and reply to a questionnaire summarizing the strengths and pitfalls of their experience. An analysis of the reports received shown that the main purpose of the visits was by far the possibility of being trained on DNA mixture interpretation, which clearly represents the main educational challenge in the field. However, a variety of other purposes led participants to apply for a EUROFORGEN fellowship grant: kinship analysis, learning different lab techniques, analysis of social, ethical and legal issues, “new” research fields in forensic genetics as External Visible Characteristics (EVCs) or Ancestry Informative Analysis (AIMs). The number of applicants from labs outside of the consortium (42%) is a good example for the success of our dissemination efforts regarding information about the activities of our network and the opportunities offered to collaborators. The overall satisfaction of the visits was very high (4.8/5 on average).

Colleagues of the forensic genetic field have been informed about the EUROFORGEN project progress by means of **regular newsletters** that have been prepared and distributed during the project. These newsletters provide updates on the Consortium work, as well as announcements such as important publications and invitations for fellowship applications. These newsletters have been sent to all the registered laboratories who had responded to our initial questionnaire, to all the EUROFORGEN members that have asked for individual access, to the EUROFORGEN National Contact Points (NCP) and to the Consortium members. Additionally, the newsletters are also available for downloading from the website, and promoted with announcements through EUROFORGEN’s social media activities (Facebook, Twitter).

One of the milestones achieved in our project was the development of the **EUROFORGEN-NoE website** and the **European Virtual Centre for Research in Forensic Genetics website**. Initially it was planned to substitute gradually the EUROFORGEN website by a newly designed website of the Virtual Centre under the acronym "VICFORGE". However, during one of our annual General Assemblies, it was decided that this change could lead to a less effective integration because the network, and its acronym EUROFORGEN-NoE, were already quite well known in our field. Therefore, the final website was structured preserving the current EUROFORGEN-NoE website, but adding a restricted area devoted to EUROFORGEN members.

Thus, the EUROFORGEN-NoE website is open and accessible to the public. It includes general information regarding the project (group, project, activities, calls, meetings and workshops, publications), training topics (list of forensic genetic courses available and sorted by countries), the “European landscape in forensic genetics”, a virtual resource bank on "Ethical, Legal and Social Aspects of Forensic Genetics: a selection of the most significant commentaries on forensic genetic policies and practices", and short instructive videos entitled "Forensic genetics explained". These videos have been planned in order to answer the possible questions that members of the public can have regarding

the forensic genetic field. All of them have been made in English language, and in addition, versions in some major European languages (Spanish, German, Polish and Portuguese) have been produced.
The member-restricted area, within the EUROFORGEN-NoE website, is available to colleagues belonging to institutions on the "European landscape" registry who have requested individual access. This membership is available not only for forensic genetic scientists, but for all experts involved in this field (scientists, members of the legal system, policemen, forensic experts, stakeholders, and end-users). The content included within the members' area with restricted access is divided into the following sections:

- EUROFORGEN Course Material: Up-to-date lectures and presentations on major topics of forensic genetics derived from the "Train the Trainers" workshop series.
- EUROFORGEN publications: Original publications from EUROFORGEN Consortium members available for downloading.
- Recommended Open Software: a list with open software tools is displayed together with a brief description on their applications.
- Online Training Academy - Webinars: presentations and recordings of the EUROFORGEN online webinar series. Very successful series of educational webinars on forensic genetics trending topics were launched with a wide international participation
- Online Training Academy - Lectures: recorded lectures prepared by EUROFORGEN consortium partners on a variety of state-of-the-art topics in the field of forensic genetics. A system of 21 online tutorials and recorded lecturers are available on the member's area of EUROFORGEN-NoE website

The European Virtual Centre of Research in Forensic Genetics is a research platform that facilitates the information exchange about ongoing and new research, of the establishment of direct networking among scientists of the field, the barrier-free availability of high quality information about forensic genetics for the general public and experts related with our field on the major aspects covered by the research work of our consortium, and also includes helpful documents regarding ethical, legal and societal aspects about forensic genetics.
Long term support is needed for ensuring the sustainability of the European Virtual Centre of Research in Forensic Genetics – public funding, sponsorships and fee-based summer courses have been considered. The consortium has planned to organize a **European Forensic Genetics Summer School** in Santiago de Compostela, Spain (July 2017). This will serve two purposes, as it represents an attractive continuation of the network's very successful activities in training and education, and, if successfully established, can become a reliable source of income from tuition to support the Virtual Centre for Research in Forensic Genetics beyond the end of the funding period. Due to the already very close collaboration with the EDNAP Group, which is embedded as a working party into the non-profit scientific society ISFG, we are planning to integrate our Virtual Centre and the associated summer school activities into the EDNAP working group. An agreement with the Executive Board of the ISFG was reached in November 2015 to confirm the integration into the EDNAP Group and the support by the ISFG.

The EUROFORGEN-NoE partners have been extremely active in organizing **dissemination activities** that have greatly increased the network visibility. A public relations conference, under the headline "Millions of genetic traces and no suspects – what can be done?" was held in Brussels on September 30th 2014, and the main objective was to increase the awareness of the society and stakeholders on the importance of forensic science in the context of security and the need of research in the field. The main topic of the first public relations event is addressing the problematic research funding situation at the national and European level. To address these important issues, Members of the European Parliament, scientists, the legal community, police and the press were invited to attend the conference. As a direct consequence, the consortium members P. Schneider (UHC) and W. Parson (IMU) had a meeting in Brussels with DG "Migration and Home Affairs in March 2015. The funding situation and future calls for research topics in the Security Programme of H2020 were discussed.
On 23rd June 2016, the EUROFORGEN Network of Excellence held the **International Dissemination Conference "Forensic DNA analysis in the light of the new security needs"** in Venice, Italy, in connection with the Intersocietal Symposium of the International Academy of Legal Medicine (IALM). This International conference aimed to present the network's activities, to discuss current developments and new technologies in the field and to disseminate the Consortium results addressing the relevant stakeholders, end users, and the public. The conference was an opportunity to exchange information and ideas among scientists, experts and stakeholders from the security sector, and the judiciary. The conference was an opportunity to exchange information and ideas among scientists, experts and stakeholders from the security sector, and the judiciary. In addition, a separate and well attended session on "Forensic Genetics and Genomics" was held at the main IALM Symposium on June 24th featuring four prominent consortium

members, A. Carracedo, M. Kayser, W. Parson and P.M. Schneider, to give overview presentations about forensic genetics and genomics.

As a final output, the UK Charity "Sense about Science" has been subcontracted to produce the **public guide "Making Sense of Forensic Genetics"**. Leading EUROFORGEN researchers worked together with support from "Sense about Science" to develop this public engagement project in order to address misconceptions about the application of DNA analysis in criminal casework. This guide shares what DNA analysis can currently do in the criminal justice system, what its limitations are, and what might be possible in the future. It includes graphics and real-life cases where DNA evidence has been a game changer in investigations as well as where its misuse has led to miscarriages of justice. The 38-page booklet is available both online as a free PDF version as well as in printed form. It includes an assessment of the strengths and potential flaws of forensic genetic evidence with regard to the specific needs of stakeholders and end-users, and to address these in a suitable language not requiring prior knowledge of the scientific context. The public launch of the guide was arranged for mid-January 2017 and has resulted in a strong media echo not only in the UK and Europe, but also in the US and Australia.

WP3 - Exemplar Project 1: Crime scene investigation and human DNA discovery

The objective of this exemplar project was to further forensic investigations by focussing on three aspects:

1) Improve sampling of evidentiary items to direct laboratory efforts to those specimens that provide the greatest potential to result in informative DNA profiles. The importance of this derives from the fact that in most European countries not all samples present or collected at crime scenes are forwarded to forensic laboratories for DNA analysis due to financial and capacity restrictions.
2) Study common causes of miscarriages of justice relating to erroneous, negligent or biased interpretation of DNA evidence to help both scientists and legal professionals with limited scientific backgrounds to understand the intricacies of DNA use in the justice system and to promote a constructive debate on trace sample best practise;
3) Develop tools that assess what type of cell material resides in an evidentiary stain, which may be informative on the activity that led to deposition of cell material.

In pursuing this objective the following approaches were adopted and results were obtained:

1) Since six partners are laboratories performing forensic casework, data on DNA yields were collected for over 24 thousand crime scene samples that were grouped in 44 functional categories. These data sets were used to predict DNA profiling success rates for various types of forensic traces and published in *Forensic Magazine*, which has a large worldwide readership of forensic practitioners. Crime scene workers may use this information to select the most promising specimens –whilst keeping in mind the criminalistics context- for DNA processing.
2) DNA has become the gold standard by which a person can be placed at the scene of a crime, and the past decade has seen great advances in this powerful crime solving tool. To help both scientists and legal professionals understand the intricacies of DNA use in the justice system, the book '*Misleading DNA Evidence: A Guide for Scientists, Judges, and Lawyers*' was written by Peter Gill (NIPH). The book examines crucial topics such as characterization of errors,determination of error rates, reporting DNA profiles and the source and sub-source levels, and the essentials of statement writing. High-profile and somewhat contentious cases are included to illustrate these points. There are 12 recommendations that make up preventative actions in the book's corrective action plan.
3) Molecular approaches for cell type inference rely on special features in specific cell types and three approaches, namely expression of specific messenger RNAs (mRNAs) or microRNAs (miRNAs) and the occurrence of specific DNA methylation marks were studied regarding their marker potential. For miRNAs and DNA methylation marks, interesting markers were found but data interpretation appears not as straightforward as required for an implementation in forensic casework, especially when multiple cell types are involved. mRNA markers, on the other hand, were incorporated in large multiplex assays that allow simultaneous assessment for the presence of body fluids (blood, saliva, semen, vaginal mucosa, menstrual blood, nasal mucosa, supplemented with skin and gender) or organs (brain, heart, kidney, liver, skeletal muscle, lung also supplemented with skin and gender). Not only were these assays subjected to extensive validation at the EUROFORGEN laboratory that developed these assays, they were also shared between partners in an inter-laboratory exercise of which the results were

published. Some EUROFORGEN partners have proceeded to apply RNA typing to casework and ~200 cases have included RNA analysis. Results have already been challenged in 7 court appearances.

Human biological traces have the potential to present strong evidence for placing a suspect at a crime scene for which DNA profiling has become gold standard. The motivation for the research addressed in exemplar project 1 is schematically represented in Figure 2 below. The first two aspects aim to direct efforts to the evidentiary stains that have the highest DNA typing potential while ensuring stain quality and preventing contamination. The third aspect aims to provide forensic scientists with tools to infer cell type identity, which can assist interpretation at the activity level. Within the forensic sphere, propositions can be placed in three generic classes that centre on source, activity or offence level. Typical questions at these three hierarchical levels are: "Could the semen present in the evidentiary stain have originated from the defendant?" (source level), "What actions resulted in the observed bloodstaining pattern?" (activity level), "Is the defendant guilty of the crime?" (offence level, which is clearly duty of the court and not the forensic expert). The activity that led to the deposition of the cellular material is more and more assessed. Growing societal forensic awareness, increasing appeals to "the right to remain silent" and contradictory testimonies have stimulated this. Also the research described in exemplar projects 4 and 5 relates to cell type identification. Other aspects of human biological stain analysis are studied in other exemplar projects such as: improved statistics for complex evidentiary traces in exemplar project 3 and surveying ethnic and phenotypic traits of perpetrators in exemplar projects 2 and 6 respectively.

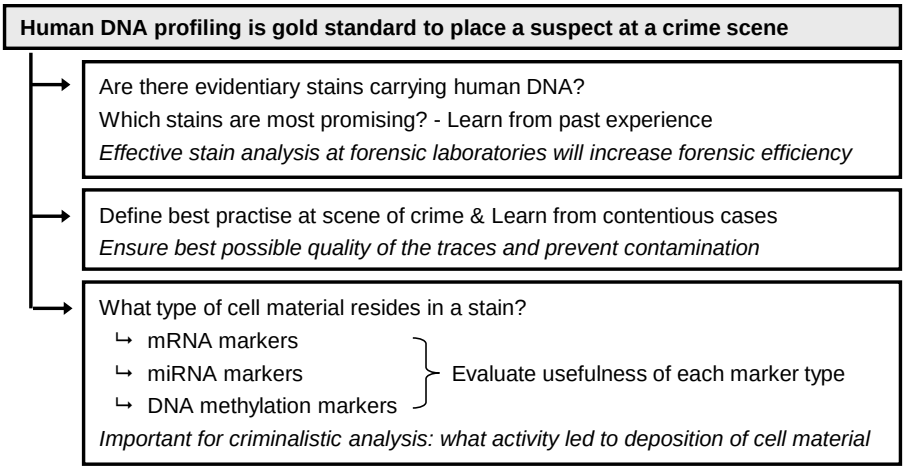

Figure 2. Context and objectives of exemplar project 1.

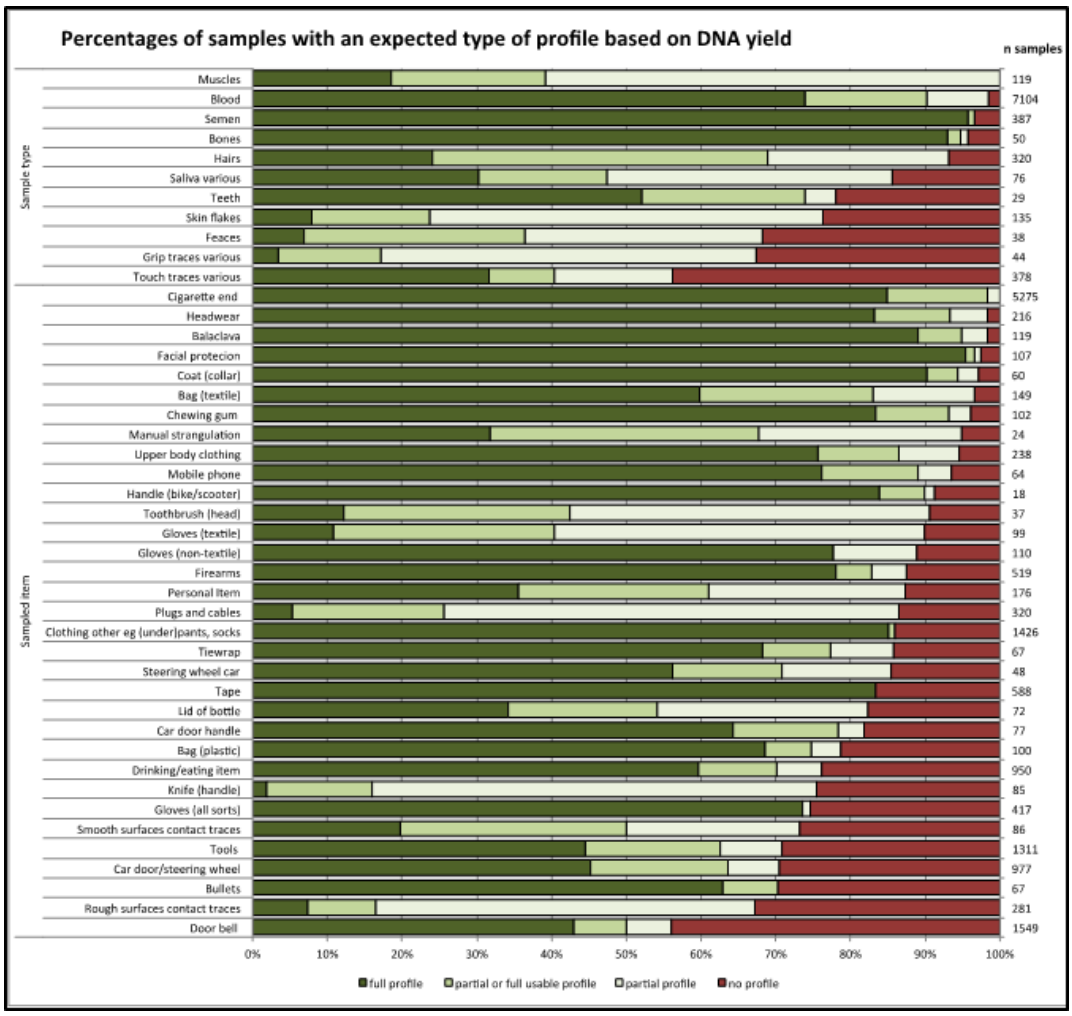

Figure 3. Outcomes sample types and expected success rates

1) In most European countries not all samples present or collected at crime scenes are forwarded to forensic laboratories for DNA analysis due to financial and capacity restrictions. From past experience it can be learned which evidentiary specimens hold most potential to result in informative DNA profiles. Over 24,466 crime-related samples collected in six forensic EUROFORGEN laboratories were categorized based on biological source or sampled item type and the DNA yield was used to predict the DNA profiling result. Four categories were chosen based on in-house experience: 1) full profile, 2) usable partial or full profile, 3) partial profile possibly useful, and 4) no informative profile. The results were shared with the forensic community through publication and are shown in Figure 3. Dark green bars indicate full profile; medium green bars usable profile; light green bars partial profile and dark red bars no informative profile. The items are indicated on the left, the number of samples per item type on the right.

2) A study on common causes of miscarriages of justice relating to erroneous, negligent or biased interpretation of DNA evidence resulted in the book '*Misleading DNA Evidence: A Guide for Scientists, Judges, and Lawyers*' by EUROFORGEN member Prof. Peter Gill. Twelve recommendations are presented that are listed below:
    1. Inform on all possible modes of transfer of DNA evidence
    2. Regard the implications of peaks in negative controls
    3. Investigator-mediated contamination outside the laboratory environment cannot be excluded
    4. The association of body fluid and donor is risky
    5. Sets of alternative hypotheses need to mirror each other
    6. With two or more individuals assessment of the probative value requires formal probabilistic analysis
    7. Replicates and/or multiple stain analysis reduce the impact of contamination
    8. Assess 'trace-DNA' profiles only at sub-source level; different statistic needed for source-level

9. An opinion must be qualified by experimental evidence.
10. The assessment of non-DNA evidence should be carried out independently of the DNA evidence to mitigate conformation bias.
11. Error rate discovery is needed more; consider proxy when no error rate
12. Train investigators on various aspects

Type		Forensic relevance
Body fluids	Blood	Violence, human-specific assay
	Semen, fertile	Sexual assault, confirmation sampled area
	Semen, sterile	Sexual assault, confirmation sampled area
	Saliva	Sexual assault e.g. licking, kissing or inoffensive stain
	Vaginal mucosa	Sexual assault, confirmation sampled area
	Menstrual secretion	Sexual assault or inoffensive alternative scenario
Touch	Skin	Confirmation sampled area
Other secretions	Expirated blood	Violence, confirmation bloodstain pattern analysis
	Nasal blood	Thump on the nose or inoffensive alternative scenario
	Nasal secretion	Inoffensive alternative scenario
	Sweat	Confirmation witness report, cross-reactivity?
	Urine	Confirmation sampled area, cross-reactivity?
	Tears	Possible inoffensive scenario if cross-reactive
	Breast milk	Possible inoffensive scenario if cross-reactive
	Vomit	Contains saliva and stomach content, inoffensive scenario
	Faeces	Anal sexual assault
Organs	Brain	Head injury
	Heart, lung	Chest injury
	Kidney, liver	Abdominal injury
	Skeletal muscle	Injury

Figure 4. Forensic relevance cell type inference.

3) Three approaches were tested to infer what cell types reside in a forensic stain, namely messenger RNA expression, micro RNA occurrence and DNA methylation signatures. The RNA-based approaches have the advantage that no valuable DNA extract is consumed; but RNA needs to be specifically extracted and the method cannot be applied when only DNA extract remains in an old case. For all approaches, multiple candidates were found for various cell types, although the specificity of markers is higher for some cell types (eg highly specific semen markers are readily found) than other cell types (eg mucous membranes such as vaginal mucosa, nasal mucosa and saliva tend to be more difficult to discern). The forensic relevance of inferring these cell types is indicated in Figure 3. The marker type for which forensic data interpretation appears most expedient is mRNA markers. Two assays were developed, validated and applied to casework: one identifying body fluids (blood, saliva, semen, vaginal mucosa, menstrual blood, nasal mucosa, supplemented with skin and gender) and a second identifying organs (brain, heart, kidney, liver, skeletal muscle, lung also supplemented with skin and gender).

WP3 - Exemplar Projects 2: Guiding investigations by genetic analysis of physical traits and tailored multiplex development

The context of tailored multiplex development for ancestry and physical trait prediction

During the lifetime of the EUROFORGEN project, the transition in forensic DNA testing from capillary electrophoresis (CE) to sequence-based analysis with compact massively parallel sequencing (MPS) systems, started in earnest. This major technological change has consequences for the level of genetic detail that can be obtained from contact traces and the number of markers that can be analysed in a single amplification test in the coming years. EP2 maintained a clear focus, throughout its three-year funding period, on the need for tailored multiplex development to provide investigators with the most detailed possible description of a DNA donor who had not been matched to a DNA database profile or described by an eyewitness. That focus was centred on the adoption of MPS in forensic DNA analysis regimes, whether specialised or mainstream pipelines; and on the establishment of 'triaging' approaches where simple CE tests could answer an investigative question more efficiently and in a quicker timeframe than MPS is currently able to do. The two key arms of the tailored multiplex test development in EP2 comprised: extension of physical trait predictive tests to encompass new externally visible characteristics; and enhancement of forensic ancestry inference

tests to reach better detail about population-of-origin. Both arms of this field of forensic DNA testing, that is supplementary to mainstream DNA profiling, were brought much further forward compared to the depth of data and scope of such tests available to the forensic analyst at the start of the EUROFORGEN project.

The major achievements of EP2 in the field of forensic ancestry tests were: development of a large-scale MPS 128-SNP ancestry multiplex; development of a smaller subset of these ancestry markers in a 31-plex SNP test for CE analysis and roll-out of an Indel-based ancestry test, with provision of an optimised forensic test system to laboratories outside the project (both allow triaging of DNA samples between CE and MPS pipelines); development of a complimentary ancestry multiplex able to differentiate European and Middle East populations. The continued refinement of reference population data resources and online statistical analysis tools has also accompanied multiplex development for all ancestry test multiplex development in EUROFORGEN.

The major achievements of EP2 in the field of physical trait predictive tests were: genetic analysis of early-onset male pattern baldness and development of an initial forensic test for this trait; initial exploration of hair morphology genetics as a preamble to the fully-fledged EP6 project to develop a hair morphology predictive test; and exploration of the effect of sex on forensic eye colour predictive tests. In all cases, newly introduced statistical approaches to making a prediction from genetic data were explored. These included simple Bayes likelihood calculations as the basis for all classifications, extended to logistic regression, neural networks and classification and regression trees.

EP2 also coordinated important inter-laboratory evaluations of the first commercial MPS SNP test plus the 128-SNP ancestry test developed by EUROFORGEN. These have both progressed the validation of MPS technology applied to forensic DNA analysis (USC-IMU-UCH-KCL partners). There was special emphasis on assessment of sequence read balance; establishing criteria for the optimum loading of the combined sequence library onto the chip; and mixed DNA analysis – a challenging aspect of MPS arising from its raised sensitivity and the use of binary polymorphisms where sequence read imbalance may not always be apparent in the data.

Tailored multiplex development for forensic ancestry inference – highlights

- **Development, optimisation and forensic evaluation of the 'Global' 128-SNP ancestry analysis panel designed for Ion Torrent-based MPS.** This panel compiled precisely balanced SNP loci with the highest possible population differentiation for comparison of the five major global population groups. This focussed the panel's power on the ability to detect and measure population admixture, typical in the modern urban demographic profiles of most developed countries. Subsequent analysis of 1000 Genomes admixed populations (Fig. 4) indicated this to be an efficient forensic tool for high quality ancestry assignments both in un-admixed and admixed population samples. *C. Phillips, W. Parson, et al., 2014, Building a forensic ancestry panel from the ground up: The EUROFORGEN Global AIM-SNP set, Forensic Science International: Genetics, 11: 13–25. (**21 citations**, Scopus, 1-2-17)*

- **Assessment of the MPS forensic assay conversion success rate for a custom SNP panel provided by Thermo Fisher.** Of 128 ancestry-informative SNP candidates, 125 were successfully incorporated and three could be substituted. At the end of detailed evaluation of sequencing performance for forensic applications this set provided reliable SNP genotyping data for 127 of 128 markers – representing an assay conversion rate of more than 99%. *M. Eduardoff, T.E. Gross, et al., 2016, Inter-laboratory evaluation of the EUROFORGEN Global ancestry-informative SNP panel by massively parallel sequencing using the Ion PGM™, Forensic Science International: Genetics 23: 178–189. (**1 citation**, Scopus, 1-2-17)*

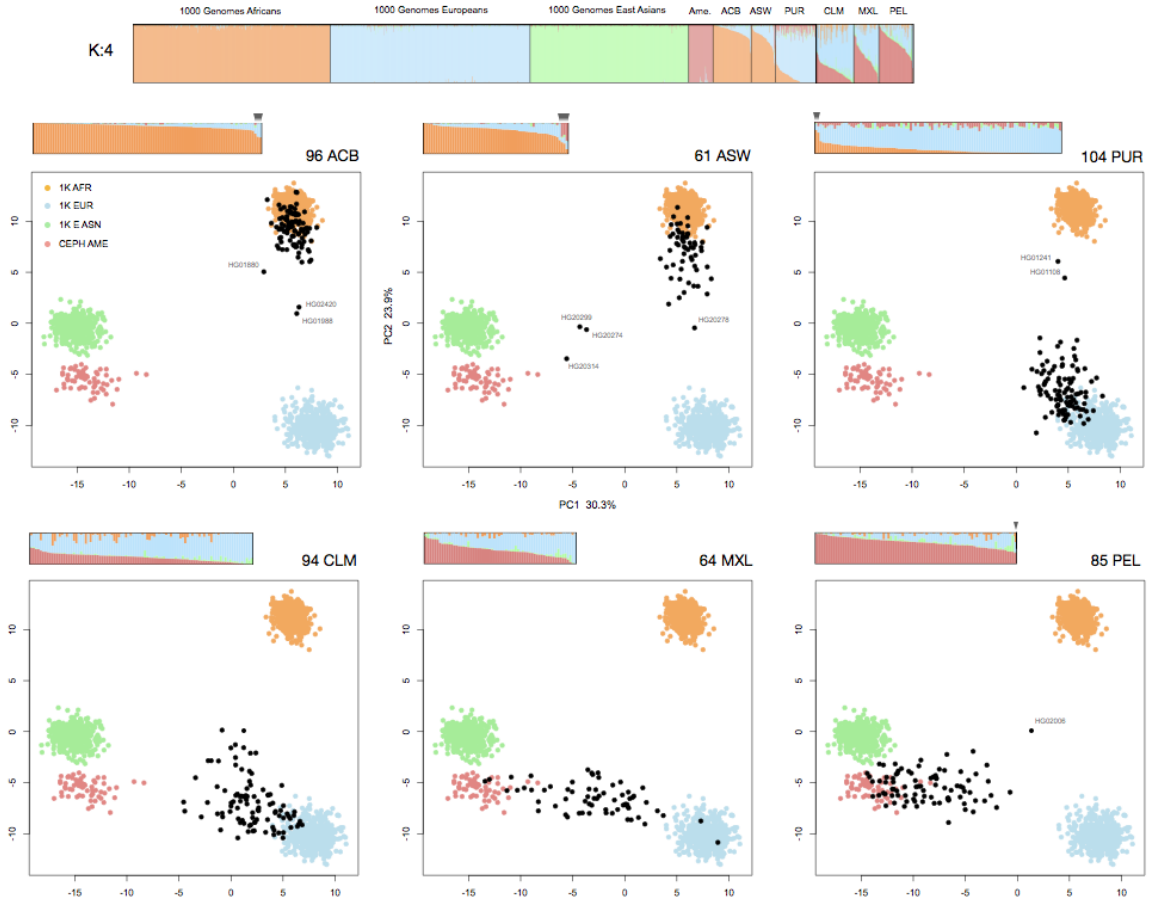

Figure 4. Analysis of admixed American populations from the 1000 Genomes project (black points) using the EUROFORGEN Global AIM-SNP panel. ACB: Barbadians; ASW: African Americans; PUR: Puerto Ricans; CLM: Colombians; MXL: Mexicans; PEL Peruvians.

- **Generation of AIM-Indel population data by EUROFORGEN EP2 partner laboratories and provision of an optimised AIM-Indel 46-plex 'kit' to EDNAP collaborators and other forensic institutions interested in establishing a simple, fast ancestry test.** This enabled the building of a population reference database (forInDel); gave the opportunity for laboratories to use this assay for the first time and successfully detect a mixture with a binary marker set; brought a simple ancestry analysis system into mainstream use in criminal investigations and missing persons ID. Sharing of the Indel ancestry test primer mix with non-specialists has been treated as a networking initiative in EUROFORGEN and has led to its adoption in criminal casework in Lyon, France and use as a missing persons identification tool 'in the field' in Ethiopia, following a refugee-migrant ship sinking incident in 2015. AIM-Indels were used to successfully resolve the cold case investigation of the murder of Eva Blanco Puig in October, 2015. In 2017, USC will establish the AIM-Indels as the main 'triage by CE' ancestry panel ahead of MPS analyses in selected casework. *C. Santos, M. Fondevila, et al., 2015, Forensic ancestry analysis with two capillary electrophoresis ancestry informative marker (AIM) panels: Results of a collaborative EDNAP exercise, Forensic Science International: Genetics, 19: 56–67. (**4 citations**, Scopus, 1-2-17)*
- **Development of a supplementary 111-plex ancestry-informative SNP panel for differentiating North African/Middle East populations from Europeans and South Asians, for use alongside the Global AIMs in MPS tests.** *V. Alves, A. Freire, et al., 2017, Development and validation of the EUROFORGEN NAME (North African and Middle Eastern) ancestry panel; publication in preparation for submission to Forensic Science International: Genetics.*

Tailored multiplex development for physical trait prediction – highlights

- **Development of an early-onset male pattern baldness (MPB) predictive test using 5 SNPs, taken from a candidate pool of 50 markers associated with the trait.** Addition of a further 15 SNPs to this core set gave a predictive accuracy for MPB in men less than 50Y of age of 42.2%, raised to 67.7% accuracy for men older than 50Y. Indicating the basis for a first forensic MPB test has been made by EUROFORGEN but more SNP predictors

need to be added in future. *M. Marcin□ska, E. Pośpiech, Evaluation of DNA Variants Associated with Androgenetic Alopecia and Their Potential to Predict Male Pattern Baldness, PLoS One, 10: e0127852 http://dx.doi.org/10.1371/journal.pone.0127852. (**3 citations**, Scopus, 1-2-17)*

- **First exploration of hair morphology-associated SNPs to begin development of a forensic test for hair type.** Of six loci strongly associated with straight, wavy and curly hair, SNPs rs11803731, rs7349332, rs1268789 have the highest association to straight hair, with AT, GG, GG genotypes giving almost 80% probability to have this hair type. This initial work with 6 SNPs has led into the more detailed genetic explorations of hair morphology in EP6. *E. Pośpiech, J. Karłowska-Pik, et al., 2015, Evaluation of the predictive capacity of DNA variants associated with straight hair in Europeans, Forensic Science International: Genetics 19: 280–288. (**3 citations**, Scopus, 1-2-17)*
- **Evaluation of the effect of sex on eye colour**. Sex was found to be significantly associated with eye colour with males having ~1.5 higher odds for blue eye colour comparing to females (p=0.002) and was ranked as the third most important factor in blue/non-blue eye colour determination, after variation in SNPs rs12913832 and rs1800407. *E. Pośpiech, J. Karłowska-Pik, et al., 2016, Further evidence for population specific differences in the effect of DNA markers and gender on eye colour prediction in forensics, International Journal of Legal Medicine, 130: 923-934.*

Coordinated evaluation and optimisation of forensic MPS SNP assays - highlights

- **Both a commercial forensic ID-SNP set and the custom Global AIM-SNP set, designed for MPS analysis with the Ion PGM™, have been extensively evaluated within a coordinated inter-laboratory validation framework** that assessed: sequence read balance; genotyping concordance; chip loading considerations (for casework and reference sample DNAs); non-specific allele calls; ability to detect and analyse mixtures; and sensitivity when testing low level DNA. From these studies, criteria have been established for further optimisation of forensic SNP assays using MPS and factors identified for the critical improvement of SNP allele calling software applied to forensic analyses. *M. Eduardoff, C. Santos, et al., 2015, Inter-laboratory evaluation of SNP-based forensic identification by massively parallel sequencing using the Ion PGM™, Forensic Science International: Genetics, 17: 110–121. (**16 citations**, Scopus, 1-2-17)*

WP3 - Exemplar Project 3: Bioinformatics in silico modelling and statistics

Objectives

1. To carry out in silico simulations to determine the best strategies to identify perpetrators at major crime scenes.
2. Optimize a strategy to provide intelligence to investigators when STR analysis fails to find suspect(s).
3. To measure effectiveness of customized multiplex systems.
4. To provide modules for complex mixture determinations.

Forensic bioinformatics of autosomal markers

STR-validator is a free and open source R-package developed mainly for internal validation of forensic STR DNA typing kit. However, it is equally suited for validation of other methods and instruments, or for process control. Its graphical user interface makes it very easy to analyse data exported from e.g. GeneMapper® software, without any knowledge about R commands. It provides convenient functions to import, view, edit, and export data. After completed analysis the results, generated plots, heat-maps, and data can be saved in a project for easy access. Currently, analysis modules for stutter, balance, drop-out, concordance, mixtures, precision, pull-up, result types, and analytical threshold are available. STR-validator can greatly increase the speed of validation by reducing the time and effort needed for analysis of the validation data. It allows easy exploration of the characteristics of DNA typing kits according to ENFSI and SWGDAM recommendations. Another area of use is monitoring of the contamination level which is essential to estimate the probability of drop-in. In this way STR-validator facilitates the implementation of probabilistic interpretation of DNA results.
Link to STR validator: http://cran.r-project.org/web/packages/strvalidator/index.html , where there are further links to the:

a) Reference manual; b) Package source; c) Windows binary; and d) Archived sources, e) Current Version 1.5.2, and previous versions downloads available from the main website https://sites.google.com/site/forensicapps/strvalidator. f) Manuals with Installation instructions for STR-validator (written for STR-validator version 1.5.0): [strvalidator_installation.pdf]. g) The STR-validator manual (written for STR-validator version 1.3.0): [strvalidator_manual.pdf] h) Italian translation of the STR-validator manual by Massimiliano Stabile and Stella Eugenia Cirillo (written for STRvalidator version 1.3.0): [strvalidator_manual_it.pdf]. i) Short instruction for how to estimate analytical thresholds (written for STR-validator version 1.5.0): [estimate_analytical_thresholds.pdf]

Forensic bioinformatics of haploid markers

The work for this task has been completed as a freely available and documented. **R-package eurohaplo** has been developed and can be downloaded from http://arken.umb.no/~theg/eu/ . A brief summary of the documentation follows. The work on Y-chromosome haplotypes extends the work of Egeland and Salas [PloS one 3.12 (2008): e3988]. For instance Brenner's kappa corrected formula for match probability of a haplotype previously not observed has been included, documented and exemplified. The coverage of a database is also estimated. For instance, a coverage of 0.7 indicates that 70% of all haplotypes in the population are represented in the database. This figure is useful as it indicates whether or not a specific database is sufficiently large. For more advanced work on simulation and alternative estimates of haplotype frequencies and match probabilities as well as freely available R software, we refer to work done by Meyer Andersen (Aalborg University, Denmark) http://people.math.aau.dk/~mikl/ . For mtDNA, the package is based on Egeland and Salas [PloS one 6.10 (2011): e26723]. The most relevant part of eurohaplo addresses the following generic case: an mtDNA profile, a haplotype, is recovered from a stain. There is reason to believe that this is a mixture as no similar haplotype has been observed previously. There is functionality to deconvolve the mixture, i.e., find combinations of haplotypes in a database provided that matches the evidence. If there are several combinations, these can be ranked according to the computed likelihood.

In silico experiments to determine robustness and efficiency of proposed solutions

An example to test robustness and efficiency of the 'Euroformix program' (described below) is as follows. In this example, a real casework sample reported in the UK court of appeal: Regina v. Dlugosz was reanalyzed. The main conclusions were as follows [1]: The fact of a 20-allele match during a database search may not be a rare event, and does not necessarily coincide with the 'true-donor'. It may be a false positive. In addition the LR of a false positive may be many orders of magnitude greater than that of the true donor. False positive results may have more alleles matching than the true donor. The risks are increased with three or more contributors to a crime-stain. The LR method was shown to be more efficient than the MAC = x-alleles method. Although more matching alleles globally correspond to bigger LRs, there is no direct link between the probability of exclusion of the mixture and the LR for a defendant. A consequence of applying a LR to each sample on a database is that match vs non-match criterion no longer applies to a database search. With complex LRs, especially those that are three or more contributors, it is likely that LRs observed from a false positive may be greater than that of the true-donor.

Relationship tests and SNPs

This work has been carried out primarily by Thore Egeland and Guro Dørum (UMB). References [2, 3] present a regression model for mixtures. The basic idea is that a parametric model is used. The statement "A person contributes to a mixture" is equivalent to stating that the fraction the person contributes is greater than 0. In this way classical hypothesis testing can be performed, i.e., standard statistical theory can be used. The examples use SNP-s (simulated and real data) and all peak height information is used. The complexity described in reference [4] arises as the contributors can be related. Previous papers only considered certain specific close relationships; the present paper imposes no principal restrictions on how contributors can be related. Reference [4] also presents an approach to power calculation for kinship problems. Before DNA profiles have been collected, it is of interest to consider if there will be sufficient data to reach a reliable conclusion. This approach is relevant also for complex mixture problems potentially involving related contributors. Finally, reference [5] is relevant for the discussion on how to present evidence. While the likelihood ratio approach is accepted as the preferred approach in many labs, the Random Man Not Excluded (RMNE) continues to be reported. The paper demonstrates the mathematical relation between LR and RMNE. Kinship problems are used to exemplify, but the mentioned relationship also apply to complex mixtures.

Publications

[1] Bleka, Ø., et al. Database extraction strategies for low-template evidence. Forensic Sci Int Genet 9 (2014): 134-141.
[2] Egeland T, Dørum G, Vigeland MD, Sheehan N. Mixtures with relatives: a pedigree perspective. Forensic Sci Int Gen, Volume 10, May 2014, 49–54.
[3] Kaur N, Fonneløp AE, Egeland T. Regression Models for DNA-mixtures. Forensic Sci Int Gen, Volume 11, July 2014, 105–110.
[4] Egeland, T, Pinto, N, Vigeland MD. A general approach to power calculation for relationship testing. Forensic Sci Int Gen Vol. 9, March 2014, 186–190.
[5] Slooten K, Egeland T. Exclusion probabilities and likelihood ratios with applications to kinship problems. Int J Legal Medicine, May 2014, Volume 128, 415-425.

Interpretation of complex mixtures

EuroForMix (EFM) is open-source software to analyse STR and SNP-DNA profiles in a user-friendly graphical user interface within R. The software implements a model to explain the allelic peak height on a continuous scale in order to carry out weight-of-evidence calculations for profiles which could be a mixture of contributors. Through a properly parameterized model it is possible to carry out inference on mixture proportion, the peak height properties, stutter proportion and degradation, for any number of unknown and known contributors. In addition, EuroForMix accommodates allele drop-out, allele drop-in and sub-population structure. EuroForMix supports two inference approaches for likelihood ratio calculations. The first approach uses maximum likelihood estimation of the unknown parameters. The second approach is Bayesian which requires prior distributions to be specified for the parameters involved. EuroForMix is **the first fully freely open source, continuous model** (accommodating peak height, stutter, drop-in, drop-out, population substructure and degradation), to be reported in the literature. It therefore serves an important purpose to act as an unrestricted platform to compare different solutions that are available.
DNAmatch is a fast contamination search algorithm which has been implemented in R to carry out searches between trace samples (mixtures) and reference samples within a range of time. The match statistic uses likelihood ratio (LR) calculation based on allele frequencies to create a decreasing sorted table of the most likely matches. The user can select the threshold for LR to assign matches and can change the model to take into account drop-in/out. For instance, the algorithm is capable of doing 23 million comparisons within 8 minutes requiring only 1.3 Gb.
Homepage: http://euroformix.com/
Current version: http://euroformix.com/sites/default/files/euroformix_0.3.3.zip
Manual: http://euroformix.com/sites/default/files/euroformixManual.doc
Tutorial: http://euroformix.com/sites/default/files/euroformixTutorialv1_2_0.doc
Presentation: http://euroformix.com/sites/default/files/EuroForMix.pdf

Extension of LRmix to interpret complex SNP mixtures: A series of two- and three-person mixtures of varying ratios were prepared and analysed with Life Technologies' HID-Ion AmpliSeq™ Identity Panel v2.2 using the Ion PGM™ massively parallel sequencing system. This panel includes 136 autosomal SNPs and 33 Y-chromosome SNPs. Using the reference samples of the mixture donors, we evaluated the strength of evidence with likelihood ratio (LR) calculations using the open-source LRmix program. This program was designed for multi-allelic STRs, but can be extended to bi-allelic SNPs without modification.

WP3: Exemplar research projects, Exemplar Project 4: Association of a Body Fluid with a DNA Profile by Targeted RNA and DNA Deep Sequencing

Objectives

- To set up a targeted mRNA/miRNA NGS approach for body fluid/tissue identification and to establish a probabilistic approach to call/predict the presence of a body fluid.
- To select a set of SNPs for each body fluid/tissue and ethnicity, that discriminates individuals the most. Specific assays will be developed for DNA and RNA biomarkers.
- To combine the RNA analysis with gDNA STR sequencing, allowing simultaneous forensic tissue identification and human individual identification.

mRNA NGS approach for body fluid/tissue identification

We introduced a final messenger RNA next generation sequencing (NGS) assay for body fluid/tissue identification. We presented a protocol designated for the two NGS instruments “Illumina MiSeq” and “IonTorrent PGM” to detect 33 and 29 body fluid/tissue specific mRNA markers for the identification of blood, saliva, semen, vaginal secretion, menstrual blood and skin. A manuscript on this assay is currently in progress.

A probabilistic approach to calling/predicting the presence of a body fluid was developed based on our results with the MiSeq assay results on 183 samples. We evaluated the performance of the model by its ability to correctly predict a sample’s body fluid. The model’s estimated prediction accuracy is 95 %, i.e. 95 % of the samples were correctly predicted. A manuscript on this probabilistic approach is currently in progress.

miRNA NGS approach for body fluid/tissue identification

For analyzing miRNAs we used a whole miRNome approach, optimized for the Illumina HiSeq platform. Our results from two experiments look promising, in that we will be able to use miRNAs to differentiate between body fluids. The probabilistic approach described for the mRNA data was also applied for the miRNA data. Since we are not using a targeted assay with a few body fluid specific markers, we are interested in expression patterns over a large number of markers that characterise a particular body fluid. The prediction accuracy of the model suggests good predictive ability based on the miRNA markers, but these are only preliminary results based on a very limited sample size. More data is needed to build a reliable model. We will prepare a separate assay and manuscript, as soon as we have enough data. Because the experimental procedure for miRNA extraction and whole miRNome library preparation is complicated, and the results are not as significant as the mRNA results, we decided not to include the miRNAs in the final NGS body fluid assay.

Body fluid/tissue specific SNPs

We performed an evaluation of ‘off the shelf’ RNA cSNP assays using the Illumina platform. 35 cSNPs were selected for a proof-of-concept study, including the following markers: blood: 11 cSNPs (from 5 genes); semen: 8 cSNPs (from 4 genes); saliva: 3 cSNPs (from 2 genes); vaginal: 3 cSNPs (from 2 genes); menstrual blood: 3 cSNPs (from 2 genes); skin: 7 cSNPs (from 3 genes). The specificity of the RNA cSNP assay was high for blood, semen, menstrual blood and skin. Additional work will be needed for saliva and vaginal secretions. For the first time we were able to assign the body fluids in an admixture to the respective donors on the basis of RNA coding SNPs in body fluid specific transcripts.

Since many (27 of 35) of the off-the-shelf cSNP amplicons are in the same exons, residual DNA contamination in the RNA extract could interfere with RNA profiling. Although it is unlikely that significant DNA input levels would survive the DNase treatment used in the isolation of RNA from samples. Nevertheless this is an area that requires more work.

A similar Illumina assay was designed on gDNA level including the same 35 cSNPs. We analyzed a population sample of 188 European individuals. To assess the usefulness of these 35 markers we estimated how powerful these loci are to discriminate between individuals. We calculated the probability of a match between two random profiles. Since the markers are intended to associate donors with body fluids in mixture stains, we considered match probabilities for sets of markers from two different body fluids. The lowest match probability is seen for mixtures of blood and semen, where

19 markers are involved. The most challenging mixture is saliva and vaginal secretion, where only 6 markers are available.

RNA/DNA co-analysis

In a last stage we wanted to combine the RNA analysis with gDNA STR sequencing, allowing simultaneous forensic tissue identification and human individual identification. We successfully tested a prototype AmpliSeq HID STR 25-plex and the Precision ID GlobalFiler NGS STR Panel (33 markers) from Applied Biosystems on the IonTorrent PGM. Both assays showed some issues, like constant low chip loading, .1/.3 artefacts, poor D21 performance with low template DNA, high stutters and being not well suited for mixtures. For the moment we are reluctant to include one of these assays into our pipeline, because they are technically not mature yet. Other companies came up with their own NGS STR-kits, which we will test for suitability and compatibility with our mRNA and cSNP assays.

Collaborative exercise

We organized a collaborative exercise within the EUROFORGEN and EDNAP laboratories, to test our targeted mRNA sequencing assay for the identification of human body fluids using 2 NGS platforms (Illumina MiSeq and IonTorrent PGM). 16 laboratories participated in the exercise, including EUROFORGEN partners UHC, USC, NIPH, UCPH, NFI, IMU, JU, KCL, UZH, WWU and Erasmus MC. They had to analyse 8 samples on PGM or 24 samples on MiSeq, respectively. Overall the results were comparable, in that highly expressed markers showed high read counts, and less expressed markers lower counts. There was some inter-laboratory and platform-related variability in read counts. The IonTorrent workflow seemed to be more sensitive to low input samples, in that only manually extracted samples resulting in higher RNA yields showed a meaningful result. We performed a partial least squares analysis (PLS) on the data, where the blood, menstrual blood, saliva and semen markers and samples clustered nicely, better for Illumina than for IonTorrent.

Conclusion

Our final goal was to have a ready-to-use NGS solution for DNA and RNA analyses of casework samples. We introduced mRNA and miRNA NGS assays for body fluid/tissue identification, including probabilistic approaches for reliable result interpretation. Our proof-of-concept cSNP marker set that we established for RNA and DNA analyses allowed the assignment of body fluids to the respective donors in 2-fluid mixtures. For a final cSNP marker set custom solutions will be designed with the help of the companies. A careful selection of cSNPs that work on DNA and RNA level will be included in a second collaborative exercise within the same laboratories, as a continuation of our project, outside of EUROFORGEN. We wanted to combine the RNA analysis with gDNA STR sequencing, allowing simultaneous forensic tissue identification and human individual identification. At the moment we are reluctant to include an NGS STR assay into our pipeline, because the company solutions are technically not mature yet.

WP3 - Exemplar Project 5: Development of innovative electrochemical biosensor technologies for the detection of tissue specific DNA methylation

Exemplar project 5 entailed two objectives. The first was the identification of 5-10 candidate tissue specific differentially methylated regions (tDMRs) for saliva, semen, blood, menstrual fluid. The second involved the development of an ultrasensitive ECL biosensor technology for methylation detection with optimized signal-to-noise ratio and shifting of luminescent emission beyond the absorption range of whole blood for tDMR recognition without the need for extraction or pre-concentration. Work in this work package led to two deliverables:

Identification of tDMRs for tissue identification

In total 45 tDMRs were identified by an extensive literature study, from which the 13 most promising tDMRs (USP49, DACT1, PFN3, PRMT2, SE1, SE2, SE3, BL1, BL2, SA1, VF1, VF2, and EFS) were investigated in detail. Evaluation criteria for the assessment of the initial markers were defined as high tissue specificity of markers and robust assay performance. Initial singleplex assay studies led to the conclusion that a set of only four tDMRs is needed for reliable identification of semen, saliva, blood and menstrual blood. These markers were subsequently combined into a multiplex assay. Marker VF2 (menstrual blood-specific) expressed partial to strong methylation in menstrual blood from all three days of the menses and nearly complete lack of methylation in all other body fluids ($p < 0.001$). Marker SA1 (saliva-specific) exhibited significantly saliva-specific hypermethylation ($p < 0.001$) with an even higher specificity compared to literature data. Marker SE2 (semen-specific) expressed significant semen-specific hypermethylation ($p<0.001$) with complete lack of methylation in the remaining body fluids. Marker BL1 (blood-specific) showed a significantly higher methylation state in venous blood compared to semen ($p < 0.001$), saliva and menstrual blood ($p < 0.001$). All markers showed to be highly stable with results from the initial multiplex analyses being reliably reproduced in the final multiplex assay. Not only was the difference in relative methylation amongst the different body fluids highly statistically significant but also allowed for visual distinction by evaluation of the according electropherograms. A validation study of the multiplex assay designed to analyse the array of four tDMRs comprised body fluid mixtures and mock crime scene stains. This exercise showed the huge potential of DNA methylation in assessing crime scene traces, even if mixtures are present. The main advantage of using DNA methylation assays over conventional presumptive tests or RNA based methods is that it can be applied even after DNA testing. In all other test methods, a decision on whether or not body fluid identification is necessary needs to be made by the analyst before a DNA typing result was obtained. Methylation analysis is hitherto the only available method for body fluid identification at a later time point in the investigative process. This can be very helpful in cases in which the presence of a mixture of body fluids becomes obvious only after DNA typing.

Ultrasensitive ECL detection of DNA methylation

The aim of this deliverable was to develop a novel, innovative method for ultrasensitive detection of DNA methylation for forensic body fluid identification. This work examined the exploitation of near-infrared (NIR) quantum dots as effective electrochemiluminescent (ECL) labels and their application to the detection of artificial sequences in bodily fluids, specifically blood samples. The use of NIR quantum dots as ECL labels was to address the challenge of gaining a detectable response in blood. Due to the absorption characteristics of blood, it is a particularly challenging matrix for both fluorescent and ECL approaches without incorporating a pre-treatment and/or extraction step to the analysis procedure. The application of novel NIR-ECL labels which shift the standard peak emission wavelength into the transmission window of blood will allow the detection of light without these extraction or pre-treatment steps. Within this work it was clearly shown, that the novel NIR-ECL labels could produce a peak emission, at ~ 800 nm, outside the transmission window of blood and that this could be achieved when surface confined, confirming that they maintained the same properties as shown when freely dissolved in solution. This work also successfully illustrated that a detectable response could be achieved in peripheral and menstrual blood samples which had been spiked with the artificial sequences. This showcases the proof of principle that DNA methylation could be detected by ECL within a complex matrix typical of a forensic sample.

Future work to expand to other bodily fluids, using the same electrochemical-based sensor, is ongoing and will also include examination of its potential use at crime scenes. Once this multiplexed analysis is undertaken, validation using mock crime scene samples will also be undertaken. *This work highlights the vast potential of electrochemical sensors for forensic analysis of methylated DNA sequences without the need of amplification or bisulfite conversion.*

WP3 - Exemplar Project 6: Forensic DNA phenotyping of hair structure for investigative purposes

Forensic DNA phenotyping (FDP) describes the inference of human externally visible characteristics (EVCs) from DNA traces such as those found at crime scenes with the goal to guide police investigations and reduce the pool of potential suspects. This is important in cases where the trace donor with his/her standard DNA profile is completely unknown, and therefore cannot be identified via standard DNA profile matching. Over recent years, progress in understanding the genetic basis of human pigmentation traits, particularly eye and hair colour, already allowed to develop FDP tools.

Such DNA test systems for predicting pigmentation traits from trace DNA comprise multiplex genotyping systems of the most predictive DNA markers, and statistical prediction algorithm or prediction model to translate the observed genotypes into individual probabilities of pigmentation categories by using reference datasets. Some of these tools have already been forensically validated and are applied in forensic casework in various countries where FDP has been legalized or is allowed otherwise. Prior to this project, besides pigmentation, the genetic knowledge of no other EVC was complete enough to allow application to FDP. The main challenging factor is that the genetic architecture of all other EVCs seems to be more complex than that of pigmentation traits, although their heritability is similarly high. The small effects of individual genes, as is typical for such complex traits, provides challenges to find them. This can be overcome by using large-enough datasets in the gene search.

With this project, we investigated whether hair structure variation i.e., the form of the head hair being straight, wavy, or curly, may be added to the list of EVCs to be used for FDP purposes. Hair structure is highly heritable, serving as prerequisite for successful genetic studies. Previous studies had identified DNA variants mainly at the trichohyalin gene (TCHH) with significant association to hair structure variation in Europeans, while findings for some other genes were promising, but not conclusive. Although, TCHH only explains about 6% of phenotypic hair structure variation, the previous genetic knowledge on hair structure was more promising than for any other non-pigmentation EVCs (perhaps with the exception of male pattern baldness already investigated by EUROFORGEN-NoE when we joined the Consortium). Thisprovided the scientific motivation for this study. Regarding the forensic motivation, although already useful in forensic investigation, the restriction on pigmentation traits puts serious limitation on practical FDP. Adding any additional EVCs would therefore be highly welcomed from the investigative perspective.

In this project, we started-out by searching for DNA predictors of human hair structure variation via performing genome-wide association studies (GWAS) in different European cohorts and conducting a GWAS meta-analysis in more than 12.000 individuals. Moreover, we used another 12,000 individuals to replicate our findings. This allowed us to identified 12 genetic loci expressing genome-wide association with hair structure variation. 10 of these were novel and two were previously known (including TCHH). In particular, we found 700 single nucleotide polymorphisms (SNPs) with genome-wide significant hair structure association. Because analyzing such large number of SNPs in trace DNA is impossible with currently available technologies suitable for forensic DNA analysis, we performed statistical analyses to select the most hair structure informative SNPs out of the 700, resulting in identifying the 50 most predictive SNPs. A model based on these 50 SNPs achieved prevalence-adjusted average prediction accuracies expressed as area under the receivers operating characteristics curve (AUC) of 0.66-0.63 in different population samples. AUC considers both aspects of prediction accuracy i.e., sensitivity (proportion of positives correctly identified as such) and specificity (proportion of negatives correctly identified as such) and ranges from 0.5 (representing random prediction) to 1.0 (representing completely accurate prediction). To further investigate the predictive power of the identified 50 SNPs, we tested them together with 40 SNPs selected from other studies in independent <2000 European individuals in a collaboration of 9 EUROFORGEN-NoE partners and one external partner. For this, we developed and validated multiplex genotyping assays for these 90 SNPs using three different DNA technologies suitable for forensic DNA analysis including two massively-parallel sequencing platforms. These three multiplex genotyping protocols were developed by three EUROFORGEN-NoE partners and shared with others for validation and final application to genotype the samples. Using the data of the 90 SNPs in >2000 Europeans for predicting straight vs. non-straight hair revealed an AUC value of 0.67.

Although this project did not deliver DNA markers and prediction models that allow appearance trait prediction to the accuracy of eye or hair colour, where for instance AUC of 0.95 were obtained for blue and brown eye colour, respectively, the project results brought us an important step further towards prediction with more externally visible characteristics from DNA, than pigmentation traits alone. Given that AUC represents average prediction accuracy, even with an AUC of 0.67, there are individuals that do predict with high probability for either straight or non-straight hair structure, which will be useful in future forensic investigation. As always in Forensic DNA Phenotyping, it will be the degree of the obtained prediction probability of the individual whose DNA was tested, being high or low, which will determine how useful this information is in the investigation of a case. Moreover, this project demonstrated the use of targeted massively parallel sequencing for simultaneous genotyping of a larger number of SNPs that is too large for currently used genotyping technology for forensic SNP analysis. Notably, with more and more SNP predictors for more and more EVCs to become available through future research, the number of SNPs available for FDP will drastically increase in the future. This will only be analyzable simultaneously by means of targeted massively parallel sequencing from forensic DNA, which this project has exemplified.

WP4: Ethical and legal aspects, and the societal dimension of forensic genetics

Background

The history of the use of genetics in support of criminal justice objectives is recent and short. It began in England in 1984 with discoveries made in one university laboratory and has spread rapidly across the globe since that time. Thirty years after those discoveries, forensic genetics is now regularly represented as the epistemic leader among all forensic science disciplines because of the strength of its underlying scientific foundations, the reliability of the laboratory and IT technologies used in its application, and its development of a robust statistical approach to the interpretation of the analytical results produced in the course of criminal casework. The EUROFORGEN consortium has been dedicated to advancing and disseminating reliable knowledge of the expanding capacity of this technoscience within key forensic science networks in Europe and beyond. In addition, the consortium has also recognized the need to consider the social, ethical and legal aspects of the many scientific, technological and operational innovations that make up the contemporary forensic genetics landscape. Work Package 4 has provided a focus for this latter consideration both within the consortium and more widely. Within the Consortium, members of the Work Package have engaged with their colleagues in a variety of ways. They have made personal visits to the majority of the partners to discuss with them, as critical friends, those legal and ethical aspects of existing and developing forensic genetic technologies that have engendered recent public and academic interest. They have circulated drafts of four reports on ethical, legal and social issues in forensic genetics for commentary by Consortium members before completing these reports. They have presented ongoing and completed work at annual meetings of the Consortium. They have promoted the work of the Consortium to wider audiences by disseminating work at meetings and conferences involving academic audiences and criminal justice practitioner groups. They have published work in academic journals and on the EUROFORGEN website. All of these efforts have informed forensic scientists within and beyond the Consortium of extra-scientific understandings (and some misunderstandings) of their work. The interaction between social scientists and natural scientists facilitated through WP 4 activities have also meant that the social scientists in the Consortium were better informed of what (socially, ethically and technically) mattered most to leading researchers in this field of inquiry.

The four substantial reports together provide an authoritative account of recent and current work on ethical, legal and social aspects of forensic genetics and are a vital corollary to the scientific, technical, and organizational preoccupations of the other Work Packages undertaken within the Consortium. The aims and contents of these four reports are described in the following paragraphs.

Report One: 'Ethical, Social and Policy Aspects of Forensic Genetics: A Systematic Review'.

This 34,000 word review provides a novel sociologically informed history of forensic genetics, noting the key technical and organisational advances that make up successive 'waves of innovation' in this field of applied science. It introduces to readers the main ethical and social considerations that have both informed the drive for these innovations and also emerged as responses to the trajectory of their introduction into the criminal justice systems of Europe and beyond. It maps the key civil society commentaries on the nature and uses of forensic genetics that have published in the United States of America and in Europe.

The review does not take sides in the commonly encountered contrast between benefit and risk, or between promise and threat that is typical of social and ethical analysis of forensic genetics. Instead, it presents the typical arguments and concerns that have been expressed within liberal democracies about innovations in forensic genetics and their operational applications over the past 30 years. The review concludes by noting the need for criminal justice actors, policy makers and publics to be better informed about the developing uses of forensic DNA, as well as the need for those involved in the development of science and technology in this domain to engage more fully with civil society organisations, policymakers and publics across the European Union.

Report Two: 'Public perspectives on established and emerging forensic genetics technologies in Europe'

This 46 page publication is the first effort of its kind to collect, analyse and disseminate what is known about European public perspectives on forensic genetics. The report argues that knowledge of the concerns and (mis-) conceptions voiced by techno-scientific publics is necessary to inform both lay and expert participation in debates about current and emergent social aspects and impacts of forensic genetics and its developing uses. The report catalogues instances of such debates in a variety of European States and describes the variety of 'public agents' that have promoted them.

It describes some of the key themes that recur – for example the concern to manage unrealistic expectations arising from the 'CSI effect'. However, the report, which draws on requests made to National Ethical Councils in 26 EU States, notes the limited involvement of these and other public agents in efforts to improve public understanding or facilitate public engagement in forensic genetics policy and practice. It argues that this lack of public attention to existing and forthcoming issues in forensic genetic technologies is not to the advantage of any of the main stakeholders in forensic science and criminal justice. Finally it warns that failure to engage with publics who are increasing subject to the investigative uses of new kinds of forensic genetic knowledge and practice can produce unwanted and expensive consequences since informed trust in forensic science is essential to its effective use in criminal justice.

Report Three: 'A Comparative Audit of Legislative Frameworks within the European Union for the Collection, Retention and Use of forensic DNA profiles.

This document provides a comprehensive survey of how national jurisdictions across the European Union seek to regulate existing and developing uses of DNA profiling and databasing in support of policing intelligence and criminal prosecutions. In 103 pages, it provides a detailed, up-to-date and authoritative account of the variety of ways in which European Nation States have sought to introduce and shape the permissible uses of these technologies into routine investigative and prosecutorial practice. It documents the ways in which such states have attempted to reach a balance between the needs of security and public safety on the one hand, and the rights of citizens to remain free from state intrusion into private and family life on the other hand.

Report Four: 'A Guide to Ethical and Legal Principles and Practice in Forensic Genetics'.

This multi-authored substantial 30,000 word document has two main aims. The first is to enhance the capacity, and encourage the willingness, of forensic geneticists, researchers and practitioners to participate in legal, social and ethical deliberations of their work and its deployment in the criminal justice system, especially with respect to recent and emerging technologies. A secondary aim is to inform other criminal justice stakeholders and publics about current legal frameworks for, and ethical aspects of, these technologies.

The guide begins by describing in detail, the nature of emerging innovations in forensic genetics, focusing in particular, on those innovations that have emerged during the time of the existence of the Network of Excellence. Having laid this scientific and technological groundwork, the guide goes on to review the European legal landscape into which these innovations have emerged, and which, in turn they may come to affect. Finally, the guide turns to introduce to its potential users, the three dominant approaches to 21st century ethical deliberation – teleology, deontology, and virtue ethics – before showing how the key ethical principles of dignity, bodily integrity, justice, equality, and solidarity are variously engaged by current and emerging innovations in forensic genetics.

The guide argues that forensic geneticists need to be willing to participate in conversations and deliberations about legal, social and ethical aspects of innovations in forensic genetics since they will know more than lay people or legal actors about what particular innovations do and do not make possible in support of criminal investigations – what can be done. At the same time, the guide reminds readers that this scientific expertise cannot be used to decide what should be done. Ethical consensus may not always be achievable, but an understanding of what matters ethically can only improve the quality of policy making and practice in this domain. The guide does not offer a set of prescriptive ethical rules that must be followed by forensic genetic practitioners, but instead urges the adoption of Ladd's view of ethics as 'an open-ended, reflective and critical intellectual activity' consisting of 'issues to be examined, explored, discussed, deliberated, and argued.' It provides an introduction to the conceptual resources necessary to participate in such explorations, discussions and deliberations.

Resource Bank on Ethical, Legal and Social Aspects of Forensic Genetics

The production of each of the reports on ethical, legal and social issues in forensic genetics, described above, necessitated the identification, collection, and assimilation of a large amount of existing information from a wide variety of sources, including academic journal papers, State and civil society publications, legislative instruments, and judicial commentaries. The Consortium believes that it is important that such source material is readily available to current and future practitioners and scholars of forensic genetics. Accordingly, Work Package 4 has established a virtual resource bank of information about current and emerging uses of forensic DNA profiling. The material – comprising a collection

of more than 200 separate items has been divided into ten folders which reflect the topics that have most interested EUROFORGEN collaborators, national contact points, the EUROFORGEN Ethics Group, as well as a variety of other scholars and members of civil society groups who have been consulted during the life of the Network of Excellence. Access to this unique assemblage of material is unrestricted. Its availability will support the continuation of informed policy deliberation on the nature and consequences of forensic genetic innovations.

The Consortium Ethics Group

Work Package 4 was the home of the Consortium Ethics Group. This group was established in the first six months of the life of the Network. Its members comprise representatives of Work Package 3 and 4 along with two ethics experts from the Network Scientific Advisory Board. One of these is Professor of Legal Medicine at Zurich University, and the other is the Chair of the UK National DNA Database Ethics Group. The Group has met at each annual gathering at the Consortium, has overseen the ethical approval process that all exemplary research projects have undergone at the appropriate local and national levels, and has facilitated communication on ethical issues between research project leads and the Research Executive Agency. It has published minutes of its deliberations. The Chair of the Group was involved in the selection of the second round of Exemplary Research Projects, and the Group has promoted its availability to any EUROFORGEN researchers who wanted to raise ethical issues relating to their own research. Members of the group contributed strongly to the EUROFORGEN Guide to Ethical and Legal Principles and Practice described above. Finally, the Chair of the Group has provided two detailed accounts of the ethical review process undertaken by members of the Consortium who have conducted empirical research using human samples. These accounts include details of the approvals granted by local and national research ethics committees along with a commentary on the ways in which the Research Executive Agency has interpreted its role in the ethical governance of empirical research.

EUROFORGEN-NoE was subject to an **ethical review**. Following a meeting in Brussels on 09/12/2015 and the review of EUROFORGEN documents, the Ethics Reviewer submitted a Report which raised a number of issues concerning the Ethics approval and oversight of processes to which EUROFORGEN research project had been subject. This report was accompanied by the rejection of Deliverable D4.09 which had contained details of these processes. The Consortium replied to this ethics review in a letter and by revising the deliverable.

A second Ethics Follow-Up Report, responding to the consortium's work was submitted on 03/04/2017. In this Follow-Up Report, and amongst other things, the Ethics Reviewer noted that the revised (249 page) D4.09 deliverable showed 'an increased and highly appreciated awareness of the Consortium about more practical Ethics issues. It includes not only a summary of the Ethical Approval Process in EUROFORGEN-NoE, as included in the first version, but also an analysis of the procedures and activities practically carried out to ensure the respect of the Ethics national and European rules during the research activities carried out by each one of the Exemplar Projects.'

All projects had submitted informed consent forms and information sheets to the relevant local and national research ethics committees. However, the Reviewer noted that not all of these forms were included in the documents available to her. Equally, although sample and data storage and destruction regimes were the subject of local and national ethical committee deliberation, the details of these were not always described in detail in the reports, but they had been taken into account fully at the time of the research.

However, and despite these criticisms, the Reviewer rated the Consortium as being in **Acceptable Compliance with the FP7 ethical guidelines (with some deviations/omissions).** It should be noted that the Reviewer also set **no further requirements** for the Consortium and **did not recommend** a further audit.

WP5: Education, Training and Career Development

Objectives

- Introduction of a postgraduate curriculum for a specialization in forensic genetics.
- Organization of workshops and seminars for scientists, stakeholders, and end-users.
- Creation of a European Virtual Training Academy for Forensic Genetics.

Forensic DNA analysis has been one of the most rapidly evolving fields of applied research in the last 25 years, the rapid pace of change has affected both typing technologies and genetic systems and this has resulted in a situation where most scientists currently responsible for analysing the results have not been formally educated in this field, but have rather been "trained on the job". Consequently, this has led to an ever-increasing demand for continuing education to keep up-to-date with these developments. The DNA Commission of the ISFG, the ENFSI DNA Working Group and other groups have voiced a clear demand for more education in this field. For the time being, no institution has the capacity to provide special seminars or workshops to meet this demand, due to the lack of funding and, equally important, the lack of trained staff ready to take up this challenge. The strategies developed in this Work Package was intended to overcome this "bottleneck", and to establish a solid basis for a European-wide system of training, education and career development for forensic analysts, as well as to invite members of the legal profession to establish interdisciplinary educational events.

Although the main areas of interest for education and training in forensic genetics are basically known, to obtain a realistic picture it was important to get a reliable feedback about educational needs from the forensic community, and to identify individuals from all European countries who have the background and interest to become "multiplicators" by acting as forensic genetic teachers at the national level. For this purpose, a European-wide survey was organized by sending out requests to respond to a questionnaire in the form of online interviews. This survey was extended to police investigators and members of the legal profession who often do not have a clear knowledge about the potential and the limits, as well as the appropriate interpretation of results from forensic DNA analysis. This data became the basis for organizing a working group meeting during the first year of the EUROFORGEN-NoE network, where those individuals with a strong interest and background to advance high-level education will get an opportunity to develop ideas and coordinate their activities.

The responses and conclusions were collated into a "White Book on Education and Training in Forensic Genetics in Europe", March 2013. This White Book summarized the current situation of the educational background of scientists and technical staff in forensic casework laboratories across Europe, as well as provided a road map for the training requirements following the rapid progress of forensic genetics typing technologies during the last five years, such as enhanced detection methods, and the need for a comprehensive interpretation of complex DNA profiles, including the biostatistical evaluation of allelic drop-out events due to enhanced sensitivity. The subjects for the most urgently needed courses were:

- Interpretation of results and weight of evidence in crime cases
- Interpretation of result in complex relationship cases
- Biostatistics in general
- Disaster victim identification
- Ethical and legal aspects.

The need to establish a system of short-term training opportunities was addressed by building a **framework for continuing education workshops** in parallel at several levels:

1. The direct action was implemented by organizing three central "Train the Trainers" (TTT) workshops. These ‘train the trainers’ workshops were held in 2013, 2014, and 2015 at the University of Copenhagen (UCPH). The leading topic of all three workshops was ‘Statistical methods in forensic genetics’ - identified as the major area of interest in the educational survey. The presenting scientists were experts in the field of forensic biostatistics, and most of them are members of the EUROFORGEN consortium. The participants of the TTT series workshops were appointed by the national contact points from European countries. The participants have the background and interest to become "multiplicators" by acting as teachers of statistical methods in forensic genetic at the national level. The number of participants was 49.
2. The second level involved satellite workshops at the national and local level by trainees, who have participated in the TTT workshops organized. The national participants of the EUROFORGEN TTT workshops were expected to take on the organisation of workshops in their home country with support from Consortium partners, and 22 workshops were held in 11 countries.
3. The third was established internationally in collaboration mainly with the International Society for Forensic Genetics (ISFG) to organize pre-congress workshops at the biannual conferences attended regularly by 500- 600 scientists during the conferences in 2013 and 2015, with another workshop in preparation for 2017.

The design of a **curriculum for postgraduate education** within an academic institution represented one of the most ambitious objectives of the consortium since the academic education for forensic DNA analysts is quite diverse in

Europe. Currently, scientists, working either in academic or in police laboratories, have a standard education in classical biology, molecular biology, genetics or other biomedical sciences, and used to have at most a master of science degree, more exceptionally a PhD, but they hardly have been formally educated to cover the special demands of forensic genetics.

The EUROFORGEN-NoE Consortium designed a specific educational program in forensic genetics, and identified a University in Europe with a master degree program in Forensic Genetics, the *University of Rome Tor Vergata*. This university already offered a specific master's degree in our area, entitled *Master Universitario di II livello in Genetica Forense*. At the same time this is a highly reputed academic institution, particularly in genetics and forensic science. After the approval of the Rector of this University and fruitful negotiations with the coordinator of the Master, we reached an agreement and the curriculum developed by EUROFORGEN-NoE was adopted by the *Master Universitario di II livello in Genetica Forense*. An agreement between our Consortium and the University of Rome was approved and recently signed. The new course will be offered for the first time in the academic year 2018-2018..

Within the EUROFORGEN website we developed a specific **training section** (http://www.euroforgen.eu/training/) The training page includes the following five options: "Upcoming courses", "Regular courses", "Exchange options", "Online resources" and "Online Training Academy" and offers information on courses with links to the generic information about forensic genetic courses in Europe as well as other information that may be useful for students and colleagues searching for further education.

One of the objectives included developing and offering online training material in order to facilitate the education and training of experts related to the forensic genetics field. In order to approach this, the Consortium planned and organized two complementary initiatives: the preparation of educational material to be uploaded on the EUROFORGEN-NoE website and the organisation of online real time webinars, these webinars have also been made available as recorded video presentations for individual viewing. These contents are accessible for registered members of the Virtual Centre of Research in Forensic Genetics.

- The recorded learning lectures prepared by Consortium´s members cover some of the main topics in the forensic genetics field, and these videos have been uploaded on the EUROFORGEN-NoE website (https://www.euroforgen.eu/members-area/online-training-academy-lectures/). A system of 20 online tutorials and recorded lecturers are available at the Online Training Academy of the EUROFORGEN-NoE member's area.
- As a second initiative, a series of live webinars have been introduced. These activities consist of an online educational presentation or seminar allowing participants in different locations to see and hear the presenter, and during which participating viewers can submit questions and comments. This was achieved by using a technical interface (WebEx) with online video and sound connections between the viewers and the presenter. Registration was required to provide the online link connection. Participants were invited to answer a questionnaire after the event was held, in order to have the option to receive a certificate of participation. Five webinars have been held with 495 participants registered from 40 countries distributed worldwide. Individuals belonging to Institutes of Legal Medicine and Forensic Sciences, universities, police laboratories, and private companies were among the participants, but also experts coming from the judicial system have attended our EUROFORGEN webinars.

1.4 The potential impact

Socio-economic impact and the wider societal implications of the project

The consortium's approach to impact has been directed by three main objectives:

1. To build capacity in forensic genetics
2. To build robust knowledge of forensic genetics amongst a wide variety of forensic genetics stakeholders
3. To build the contribution of the consortium and its partners to forensic genetic policy domains and publics.

Both the scientific and the societal impact of research and research networks takes time to emerge and develop. In the UK, the Research Councils 'Impact Agenda' recognises that successful impact is 'developed over many years of research activity, aided by supportive institutional environments and plenty of practice and reflection with colleagues and users (http://www.esrc.ac.uk/research/impact-toolkit/what-is-impact/). EUROFORGEN's work in support of the objectives listed above has established the foundations of impact. However, its further development will depend not only on work already done by Consortium members – much of which is described below – but also on the willingness of others to grasp the opportunities for capacity building, partnership, influence, and reflection, that the Consortium has now put into place. The following paragraphs provide examples of what we have done during the lifespan of the Consortium, how we have disseminated our work, and looks forward to extend the impact trajectories that we have launched in the last five years. Full details of all of our dissemination activities can be found in an appendix.

1. Capacity building in forensic genetics.

Earlier paragraphs in this section have already described the achievements of the Consortium in making scientific and technological advances in forensic genetics analysis and interpretation through the series of exemplary projects that were at the heart of our work for the last five years. We have also provided details of the dissemination of scientific findings from those exemplary projects. However, the Consortium recognises that these advances remain irrelevant to the successful introduction of such innovations into real-world criminal justice contexts unless they are available to forensic science laboratories for use by suitably qualified practitioners. This kind of intended impact then is simultaneously social – more specifically organisational - and scientific. The Consortium has undertaken a number of activities to disseminate its work on such social capacity building.

They include:

- The publication of two important documents on the EUROFORGEN website: The Directory of Forensic Genetic Research Laboratories in Europe; and the White Book on Education and Training in Forensic Genetics.
- An extended series of Practitioner Workshops – specifically 'Training the Trainers' to support and extend the capacity of state and private laboratory scientists to reliably interpret and report forensic genetics findings to investigators and to courts.

A further aspect of capacity building is unique to this Consortium: the building of ethical capacity amongst forensic science practitioners. Whilst there is no shortage of codes of ethics (often described as 'professional ethics') that assert their capacity to govern the conduct of forensic scientists, there is an ongoing dispute about the extent to which such codes effectively develop ethically informed conduct. In addition, even if adherence to such codes does produce the kind of conduct that was sought, some critics have argued that there is an essential contradiction in their very existence. For these critics, and for the EUROFORGEN consortium, ethical conduct is not determined by rules imposed by external authorities but an open-ended, reflective and critical intellectual activity consisting of issues to be examined, explored, discussed, deliberated, and argued.' In this sense, ethical decision-making should be contrasted with rule-making or law-following since there is no indisputable authority which rules the former; ethical disputes cannot be done by any formal edict.

This understanding of ethics has informed the work of the consortium. It has directed our effort to enhance the capacity, and encourage the willingness, of forensic genetics researchers and practitioners to participate in social and ethical deliberations of their work and its deployment in the criminal justice system, wherever and whenever these deliberations happen. In the latter stages of the life of the Network we have especially focussed on ethical and the

recent and emerging technologies that have been the focus of our exemplary projects. This effort has been disseminated through the following means:

- In three EUROFORGEN publications (totalling about 100,000 words) which exemplify this view of ethics and provide resources for ethical capacity building in the forensic genetics community. The publications are 'Ethical, Social and Policy Aspects of Forensic Genetics: A Systematic Review'; Public Perspectives on Established and Emerging Forensic Genetics Technologies in Europe'; and 'A Guide to Ethical and Legal Principles and Practice in Forensic Genetics'.
- In three academic publications targeted to forensic genetics audiences (Wienroth, Morling & Williams, 'Technological Innovations in Forensic Genetics: Social, Legal and Ethical Aspects' *Advances in DNA and Gene Sequences,* 2014; Williams, 'When global science meets local legality: Deliberating and regulating forensic genetics '*FSI Genetics Supplement Series 5* 2015; and Williams & Wienroth 'Social and Ethical Issues in Forensic Genetics' *Forensic Science Review*, forthcoming).
- A 103-page 'Comparative Audit of Legislative Frameworks within the European Union for the Collection, Retention and Use of forensic DNA profiles' has provided a comprehensive survey of how national jurisdictions across the European Union seek to regulate existing and developing uses of DNA profiling and databasing in support of policing intelligence and criminal prosecutions. It documents the ways in which such states have attempted to reach a balance between the needs of security and public safety on the one hand, and the rights of citizens to remain free from state intrusion into private and family life on the other hand.
- In two half-day workshops and a plenary lecture given at the 26th Biennial International Congress of the International Society for Forensic Genetics held in Krakow in 2015. One of the Workshops ('Ethical, Legal & Social Aspects of Forensic Genetics') – the first of its kind given at a ISFG meeting, was organised directly by EUROFORGEN, and the other Workshop (Next Generation Sequencing for Forensic Genetics) provided time for a EUROFORGEN presentation by Williams entitled 'Biolegal innovations and the public good' which dealt with ethical, legal and social aspects of this particular innovation. The plenary lecture, given by Robin Williams was called 'When global science meets local legality: Deliberating and regulating forensic genetics'. It has already been agreed that a workshop on Ethical, Legal and Social Aspects of Forensic Genetics will also be held at the 27th Congress to be held in Seoul in 2017.
- By the establishment of a virtual resource bank of information about current and emerging uses of forensic DNA profiling. The material – comprising a collection of more than 200 separate items has been divided into ten folders which reflect the topics that have most interested EUROFORGEN collaborators, national contact points, the EUROFORGEN Ethics Group, as well as a variety of other scholars and members of civil society groups who have been consulted during the life of the Network of Excellence. All individuals and agencies that have registered as members of the Network have been given access to this unique assemblage of material on ethical, legal and social aspects of forensic genetics.

2. Knowledge building amongst forensic genetic stakeholders.

The successful impact of innovations in forensic genetics depends on the willingness of non-scientific actors, especially those involved in the criminal justice process – as law-makers, police or legal actors - to support the introduction of new forms of laboratory analysis and interpretation into criminal investigations and prosecutions. Such support depends on the transmission of sound knowledge of the technical and evidential strengths and limitations of particular innovations as well as the social and ethical benefits and harms that might accompany their various uses. The Consortium's efforts at disseminating this knowledge building has targeted a variety of agents and agencies that have a stake in the development and use of forensic genetics in modern liberal societies. Dissemination actions include:

- A significant and widely commended book by Peter Gill 'Misleading DNA evidence: Reasons for miscarriages of justice'. The book provides a guide for practitioners, judges and lawyers, detailing common causes of errors of DNA interpretation and the effect of cognitive bias on interpretation of complex evidence.
- Two linked 2014 journal publications by Denise Syndercombe Court and Kristiina Reed (Radical Changes in DNA Analysis I and II) in *Criminal Law and Justice Weekly* have specifically targeted legal actors in order to raise their understanding of innovations in forensic genetics.

- Partnership building activities in which the EUROFORGEN collective have engaged with a wide variety of such forensic genetic stakeholders, including national legislators, international research agencies, public sector and commercial professionals, and non-governmental organisations. Two major examples of such efforts involving the EURORFORGEN collective include:
    - The Public Relations Conference 'Millions of Genetic Traces and No Suspects: What Can be Done' held in Brussels on September 30th 2014 addressed the significance of EUROFORGEN's work for policing and security priorities, the shaping of our work by the 'Responsible Research and Innovation' agenda, and a guide to topics for advanced and innovative research in forensic genetics.
    - the International Dissemination Conference 'Forensic DNA analysis in the light of the new security needs' in held on 23rd June 2016 in Venice, Italy, in connection with the Intersocietal Symposium of the International Academy of Legal Medicine (IALM). This International conference presented the network's activities through a discussion of current developments and new technologies in the field. Brussels Meeting and Venice Meeting.
- Individual meeting and conference presentations by EUROFORGEN personnel whose work has focused on ethical, legal and social aspects of forensic genetics. A full list of these presentations is already available in this final report, but recent examples during 2016 include: Matthias Wienroth 'Governing anticipatory technologies. Forensic DNA phenotyping in Europe' (4S/EASST Barcelona); Robin Williams 'Themes and Variations in The Social Life of Forensic Genetics: Credibility, Legitimacy and Utility.' (ESRC Seminar Newcastle); and Robin Williams 'Beyond Rigid Designation: Operational and Policy Issues in Third Wave Forensic Genetics' (International Conference Coimbra).

3. Contributing to forensic genetics policymaking domains and publics.

The first two impact objectives described above are instrumental in character: they seek to influence professional practice, service provision, and legal acceptance. The third intended impact is conceptual in nature: it seeks to improve the understanding of scientific and policy issues, and to resource and to reframe debates concerning developments in forensic genetics. Members of the Consortium have facilitated, supported and encouraged this perspective on policy and practice deliberation through participation in a number of local, national and international fora. Whilst the Consortium may not have a single voice when evaluating the legitimacy and effectiveness of forensic genetic policy and practice, its members have sought to provide sound advice to several existing and emerging scientific and social deliberations on innovations in forensic genetics. Dissemination actions have included:

- Presentations by Denise Syndercombe Court to the UK National DNA Database Ethics Board on issues in forensic DNA phenotyping.
- The use of two EUROFORGEN publications ('Ethical, Social and Policy Aspects of Forensic Genetics: A Systematic Review' and 'Guide to Ethical and Legal Principles and Practice in Forensic Genetics') by the UK National DNA Database Ethics Board during their most recent deliberations on the ethical implications of recent forensic genetic innovations.
- The establishment of a prestigious UK Economic and Social Research Council funded Seminar Series 'The New Genetics, Security and Justice. Crossing, contesting and comparing boundaries'. This seminar series, which is based at Northumbria University and led by two EUROFORGEN collaborators, provides a forum for the discussion of opportunities, challenges and risks that relate to the adoption of genetic technologies for criminal justice and security purposes in socially acceptable ways, and with a focus on engendering good practice. The seminar series has opened up and connected existing research areas, thus generating cross-disciplinary perspectives, from Sociology, Science and Technology Studies, ethics and socio-legal studies, in conversation with geneticists, police, forensic laboratories and policy makers as well as representatives from NGOs. By bringing representatives from these communities together, it has made it possible for their differing perspectives to both inform and be informed by others, helping to learn from experiences and opening up new fields of engaging with genetic innovations, exploring the boundaries between a variety of applications, and considering how such boundaries are contested, negotiated, and penetrated. The series began in December 2015 and ends in July 2017.
- Finally, the Consortium has subcontracted a leading external science communication charity (Sense About Science) to provide support for producing and disseminating a unified perspective on the nature of contemporary forensic genetics, with the particular aim of educating the wider public about what this science can and cannot do

when applied to criminal investigations and prosecutions in modern liberal democracies. This effort has resulted in the publication of the brochure 'Making Sense of Forensic Genetics' in January 2017, a guide to the field which has already received widespread press attention and has been commended by a number of key forensic science actors and agencies.

Main dissemination activities and exploitation of results

Crime scene investigation and human DNA discovery

The forensic community has long sought a human-specific DNA screening test applicable to the crime-scene. This can assist in the prioritisation of contact trace recovery before samples are sent to the lab. EUROFORGEN dedicated considerable time and resources to initiate the development of a sensitive human-specific DNA test, but this is very much dependent on the interest of a commercial partner. As this was not forthcoming the project did not progress further, although a promising technology with gold particles was evaluated. The scientific impact of this work takes the form of a clear message that DNA-based contact trace characterisation that can be run at the crime-scene is not feasible technology at the current time. However, this focuses the attention of forensic genetics towards lab-based tests of tissue-of-origin, which has been the major area of achievement for EP1 and has had the greatest impact on the development of forensic genetics tests that can state which tissue source a DNA sample has originated from rather than who the donor was.

Identification of the tissue source of DNA samples obtained from the crime-scene is a major area of development in forensic genetics. At the start of EUROFORGEN's scientific work, three forms of analysis were being considered at very early stages of knowledge about the efficiency of each approach: microRNA typing (UHC leading), RNA typing (NFI) and epigenetic-based analyses (EPTS-KCL). The impact of the work of EP1 has been to place firm emphasis on the need for continued optimisation of RNA typing to enhance tests that characterise the tissue sources. Much of this work has been achieved by EP1 led by NFI with key contributions from UHC, KCL and EPTS, but also the addition of Partner 14, Zurich University, with a focus, amongst other fields, on MPS-based RNA typing. The bulk of Y2-Y3 EP1 work was taken up with a comparison of the efficiency, specificity and sensitivity of tissue source tests. MicroRNA analysis had considerable interest as a way to identify tissues, because the fragments tested are shorter than normal RNA molecules and likely to be more resistant to degradation, but EP1 findings consistently indicated these tests are inferior, in all three forensic criteria, to those typing normal RNA. To a large extent, the forensic community has benefitted from these comparative assessments and it is clear the scientific impact of EUROFORGEN's work has been to emphasise the key role of mainstream RNA analysis to establish the tissue source of DNA samples. Much work needs to be done, but EP1 research has progressed the field considerably in terms of identifying the most specific markers and applying rules of interpretation of relative signal strength in cases of non-specific peak patterns that have affected the accuracy of tests for vaginal secretions and saliva; venous and menstrual blood. The organisation of inter-laboratory trials by EP1 has made RNA tests more robust and consistent. NFI have also used EUROFORGEN to introduce organ-specific RNA tests; a sex test based on RNA; species-specific tests and a new test for nasal mucosa able to distinguish, e.g., nose-bleed from venous bloodstains.

Detailed analysis of methylation-based tests for tissue source were run in EP1 and the scientific impact of this work matches that of MicroRNA, in that this system for testing tissue of origin lacks the sensitivity and general utility of RNA tests and has served to instruct the forensic community that RNA typing is the best approach. It is significant that the leading experts in forensic methylation analysis are all part of EUROFORGEN and this emphasises that this technique is not suited to tissue source analysis. The importance of this impact is that DNA-only analyses (e.g. cold cases) will require the development of new tests to establish tissue source, and despite big strides in forensic methylation analysis, no epigenetic test of tissue source can be applied yet to DNA-only cases.

The second most impactful outcome from EP1 has been the work to define best practice at the crime-scene and measure the DNA profiling success rates assessed by type of evidential material. Until EUROFORGEN instigated this work, no detailed examination had been made of crime-scene practice with regard to optimal DNA recovery and profiling efficiency. More than 24,000 crime samples were collected by six EUROFORGEN partners and assessed for DNA typing success - far more than would be possible by any other laboratory single handed. The results and guidance documents from this work have helped inform crime-scene practice and have had a major influence on how evidence is collected or which exhibits are prioritised at the scene. This is all the more important in its impact when considering that not all exhibits collected are sent for DNA analysis due to budget constraints, thus a form of prioritisation is critical to the successful completion of DNA tests in any one case.

Finally, detailed knowledge of the source and significance of a DNA sample (as well as likelihood of profiling success from any one exhibit); which has been brought much further forward by EP1, has greatly contributed to the debate between scientists and the legal profession about the complexities of interpreting forensic genetic evidence. These days the importance of finding a DNA profile in a particular location takes precedence over its origin from a suspect. The wealth of experience amongst EUROFORGEN partners in this field has meant almost 200 cases have benefitted from tissue source characterisation, with seven court appearances by NFI to date.

Tailored multiplex development

EP2 released a series of major multiplex SNP tests that aimed to enhance forensic ancestry analysis and extend the scope of predictive tests of externally-visible characteristics, with significant scientific impact on forensic analysis. These developments are timely as compact MPS systems are being increasingly adopted to supplement mainstream DNA profiling tests and offer higher multiplexing capacity and increased sensitivity. In parallel to developing PCR chemistries, the community was served with extensions of online analysis systems hosted in Snipper and specialised forensic variant population databases hosted in SPSmart. Lastly, attention was focussed on CE-based triage systems that can provide a way to resolve the inference of ancestry in many cases without resorting to expensive and time-consuming MPS tests.

The most impactful achievements of EP2 in the field of forensic ancestry tests were: development of a large-scale MPS 128-SNP ancestry multiplex; development of a smaller subset of these ancestry markers in a 31-plex SNP test for CE analysis and roll-out of an Indel-based ancestry test, with provision of an optimised forensic test system to laboratories outside the project (both allow triaging of DNA samples between CE and MPS pipelines); development of a complimentary ancestry multiplex test able to differentiate European and Middle East populations. The continued refinement of reference population data resources and online statistical analysis tools has also accompanied multiplex development for all ancestry test multiplex development in EUROFORGEN and these are widely used to aid population analyses by forensic labs.

The most impactful achievements of EP2 in the field of physical trait predictive tests were: genetic analysis of early-onset male pattern baldness and development of an initial forensic test for this trait using 20 SNPs with a predictive accuracy for MPB in men less than 50Y of age of 42.2%, raised to 67.7% accuracy for men older than 50Y. Another major step was the initial exploration of hair morphology genetics as a preamble to the fully-fledged EP6 project to develop a hair morphology predictive test; and exploration of the effect of sex on forensic eye colour predictive tests.

Newly introduced statistical approaches were refined to making a prediction from genetic data. These included simple Bayes likelihood calculations as the basis for all classifications, extended to logistic regression, neural networks and classification and regression trees. This has impacted ongoing research into forensic predictive tests handling complex genetic data and starting to assess the effects of more complex genetic interactions such as epistasis.

The third impactful forensic research field coordinated within EP2 was two important inter-laboratory evaluations of the first commercial MPS SNP test and the 128-SNP ancestry test developed by EUROFORGEN. These initiatives have progressed the validation of MPS technology applied to forensic DNA analysis by identifying the key sequence analysis parameters and promoting the idea that the SNP analysis software of the two forensic MPS platforms require much more optimisation and tailoring to forensic needs. There was special emphasis on assessment of sequence read balance; establishing criteria for the optimum loading of the combined sequence library onto the chip; and mixed DNA analysis – a challenging aspect of MPS arising from its raised sensitivity and the use of binary polymorphisms where sequence read imbalance may not always be apparent in the data.

Bioinformatics for in-silico modelling and statistics

EP3, of all EUROFORGEN exemplar projects, has arguably had the most significant impact on forensic genetics practice, because it is centred on improving the interpretation of complex DNA profiles derived from tests in place for almost 20 years. The ethos of EP3 reflects EUROFORGEN as a whole in promoting the idea of open-source analysis regimes that make use of open software solutions - i.e. they can be adapted for particular forensic genetics scenarios with transparency. The whole community can assess the statistical validity of the adaptations incorporated after each iteration made. This has led to disputes about how easily equivalent commercial solutions for complex profile analysis,

as “black boxes”, can be evaluated independently - whether to settle a disputed interpretation in court or to evaluate their relative efficiency to find the most applicable likelihood ratio for a mixture of profile contributors. This is a very hotly debated issue in forensic DNA analysis at this moment. Therefore the release of a series of open-source software solutions by EP3, many with the link to EUROFORGEN, such as EuroForMix, can be said to be extremely impactful, as well as demonstrating best practice with regard to analytical transparency. Some exploration has been made in EP3 of the relationship between reporting likelihood ratios and a value termed “random man not excluded” which is still widely used in reporting the statistical value associated to the presence of a set of peaks in a mixed profile. This work is going to continue to impact the community and contribute significantly to the debate about use of appropriate statistical values in courtroom discussion concerning complex profile interpretation.

The software package dedicated to forensic bioinformatics of autosomal markers is STR-validator. This analyses key STR kit parameters influencing DNA profile quality: stutter, peak balance, mixtures, electrophoretic precision, peak pull-up, result type and analytical threshold. STR-validator speeds up the time taken to validate a new forensic STR kit run on any given instrument; therefore it plays a significant role in enabling all labs to set up their analytical regimes correctly, which will have a major impact on maintaining forensic DNA profiling quality and minimising the false negative rate.

The scope of forensic bioinformatics of haploid markers is covered by release of eurohaplo. The eurohaplo software package addresses the issue of estimating the frequency of rare or unobserved haplotypes in community databases of Y or mtDNA variation. The impact on forensic genetics practice is the feature of eurohaplo that measures whether a database is large enough to provide an accurate haplotype frequency estimate. Of particular relevance for use of haplotypic markers is the ability of eurohaplo to allow mixture combinations to be explored (e.g. the combination of two mtDNA haplotypes creates a pattern that does not match entries in a database).

In addition, in-silico experiments to test the robustness of the developed software packages were carried out. The most impactful work relates to calculating the likelihoods that occur when searching NDNADs for a profile derived from mixed profiles. The occurrence of up to 20-allele matches is not necessarily a rare event and can present a significant false-positive rate as the likelihood is sometimes several orders of magnitude greater than the true donor. The statistical analysis of mixed DNA has also been extended to binary SNP loci and the future impact of this work will be considerable if SNPs are increasingly relied on for generating forensic data from degraded DNA, when STRs fail. The binary polymorphisms of SNP loci means that the analysis of mixtures is challenging, but by adapting a parametric model which uses the premise that a person’s contribution to a mixture is greater than zero progress was made towards developing a system to detect contributors in simple mixtures. Further complexities can arise when related individuals are compared in the same mixture. LRmix was extended to accommodate SNP data to anticipate increasing use of MPS analysis in forensics.

Our work on the interpretation of complex mixtures has had the most significant scientific impact leading to the release of the EuroForMix software suite. EuroForMix is the first fully open-source continuous-model system to take account of peak height, stutter, drop-in, drop-out, population sub-structure and degradation. The second most impactful software release for this task was DNAmatch that could search for trace contaminant DNA signals. Accompanying manuals, recorded tutorials and community presentations ensured widespread adoption of these software solutions.

Association of body fluid identification with a DNA profile

EP4 developed the first comprehensive MPS-based RNA tests (i.e. analysing cDNA sequences) for tissue of origin, with 29/33 RNA markers analysed and capable of identifying blood, saliva, semen, vaginal secretion, menstrual blood and skin. Predictive accuracy was estimated to be 95% with both the chemistry and predictive software development due for publication. The demonstration of a viable and accurate tissue of origin MPS test analysing RNA has significant scientific impact in the current period of transition towards forensic MPS for full range of tests that analyse nucleic acid in contact traces. The EP4 discovery phase re-iterated the superiority of mRNA over microRNA as reported by EP1. Off-the-shelf MPS RNA tests for gene expression of selected tissue-indicative markers have also emphasised the difficulty of separating epithelial cell signatures for vaginal and saliva sources of forensic material. The challenge of body-fluid mixtures was also an impactful area of research as this has led to development and optimisation of probabilistic analysis of RNA data. The most difficult mixture being blood and semen due to the level of shared RNA markers. The evaluation of MPS-based RNA analysis was extended to a larger range of operational labs by completing a combined EUROFORGEN-EDNAP collaborative exercise that will impact the increasing adoption of this technology for forensic tissue of origin tests. The combination of normal STR profiling tests and tissue of origin in a single MPS

system requires further optimisation of the technology but when this has been achieved it will represent a significant step forward in forensic MPS tests as the one test will serve the two most common investigative questions.

Development of innovative electrochemical biosensors detecting tissue-specific methylation

EP5 focussed on the use of tissue-differentiated methylation regions (tDMR) to detect the origin of crime-scene stains by development of ultra-sensitive biosensors able to detect tDMR patterns. The potential impact of these developments is that the DNA is not extracted but its source is directly detected at the crime-scene with compact and sensitive devices. In all 45 tDMRs were identified and studies indicated four were sufficient to reliably differentiate semen, saliva, blood and menstrual blood. The impact of this work was outlined in the description of EP1 impact – there is no need to make the decision to co-extract RNA and DNA to establish tissue of origin – the tests can be made post-hoc on the DNA extract used for profiling, should the investigators require it. Possibly the most impactful finding is that the instrumentation to detect methylation patterns can work without bi-sulphite conversion of the DNA structure to infer CpG levels.

Development of the near-infrared electrochemiluminescent (NIR-ECL) instrument required the adaptation of the technology to detect wavelengths beyond those of blood components such as haemoglobin. This was done using quantum dot labels and avoids extraction or pre-treatment steps. In terms of scientific impact, the application of novel NIR-ECL labels which shift the standard peak emission wavelength into the transmission window of blood shows the novel NIR-ECL labels produce a signal at ~ 800 nm, from blood on surfaces which maintains the same properties as that dissolved. Further impact has been achieved from the successful detection of a response from both peripheral and menstrual blood samples spiked with the artificial sequences. This highlights the value of DNA methylation patterns detected by ECL from a complex matrix typical of a forensic sample.

Forensic DNA phenotyping of hair structure

EP6 accomplished a large meta-analysis of genome-wide association studies to identify the most informative markers for hair structure, therefore progressing the work of EP2 to develop a simple 6-SNP predictive test for hair type. The meta-analysis looked at 12,000 European subjects and identified 700 associated SNPs in 12 genes. This finding will have a significant impact on studies of hair morphology for forensic purposes. The associations were successfully replicated and confirmed the widely reported finding that most hair structure associated loci are European-specific.

Applying statistical methods to the 700 hair structure-associated SNPs identified 50 best predictors and to these were added a further 40 SNPs associated with common human physical characteristics (mainly associated with facial features and hair distribution). MPS tests were developed for all 90 loci and this allowed a predictive model for straight, wavy and curly hair structure to be tested with de novo samples from EUROFORGEN partner labs. The final predictive performance of the test (AUC=0.67) did not reach that of established pigmentation trait tests (AUC=0.95); the EP6 development work impacts the field by demonstrating that a large set of SNPs can be readily adopted into a single multiplexed MPS test.

Finally, EP6 findings re-iterate the initial findings of EP2 by showing that a proportion of individuals will show higher than average probability to have straight rather than non-straight hair. The scientific impact of this work on forensic phenotyping tests will result from the imminent publication of the meta-analysis and development of the large-scale MPS tests for hair structure.

Section 2 – Use and dissemination of foreground

Please see ECAS.

Section 3 – Report on societal implications

Please see ECAS.