Forensic SNP Genotyping with SNaPshot: Technical Considerations for the Development and Optimization of Multiplexed SNP Assays

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ABSTRACT: This review explores the key factors that influence the optimization, routine use, and profile interpretation of the SNaPshot single-base extension (SBE) system applied to forensic single-nucleotide polymorphism (SNP) genotyping. Despite being a mainly complimentary DNA genotyping technique to routine STR profiling, use of SNaPshot is an important part of the development of SNP sets for a wide range of forensic applications with these markers, from genotyping highly degraded DNA with very short amplicons to the introduction of SNPs to ascertain the ancestry and physical characteristics of an unidentified contact trace donor. However, this technology, as resourceful as it is, displays several features that depart from the usual STR genotyping far enough to demand a certain degree of expertise from the forensic analyst before tackling the complex casework on which SNaPshot application provides an advantage. In order to provide the basis for developing such expertise, we cover in this paper the most challenging aspects of the SNaPshot technology, focusing on the steps taken to design primer sets, optimize the PCR and single-base extension chemistries, and the important features of the peak patterns observed in typical forensic SNP profiles using SNaPshot. With that purpose in mind, we provide guidelines and troubleshooting for multiplex-SNaPshot–oriented primer design and the resulting capillary electrophoresis (CE) profile interpretation (covering the most commonly observed artifacts and expected departures from the ideal conditions).

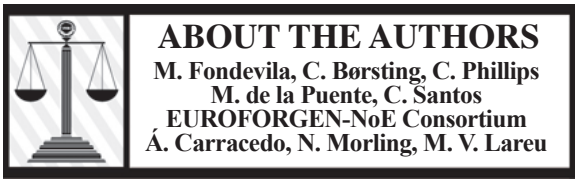
KEYWORDS: Artifacts, capillary electrophoresis, CE, multiplex development, profile interpretation, single-base extension, SBE, single-nucleotide polymorphism, SNP.

INTRODUCTION

Forensic SNP Analysis Is a Specialized and Complementary Technique to Mainstream DNA Profiling

Single-nucleotide polymorphism (SNP) genotyping has a relatively long history in the forensic genetics field [1–5,7,11,12,15,16,19-23,27,28,32,33,35,36,39,40,43–53,55,57,59–61,72,75]. SNPs underlie most of the protein polymorphisms originally analyzed in forensic identification systems prior to the use of DNA polymorphisms. However, with the advent of short tandem repeat (STR)–based tests as the system of choice for forensic DNA analysis, SNPs were only relevant when a nucleotide variant altered the target sequence of a PCR primer and a linked allele in the target STR dropped out, causing a null allele. More recently, standalone SNP assays have been suggested as supplementary tests to STR analysis [2,23,34,43,59,61] that enable amplification of very short PCR fragments from highly degraded DNA samples [4,15,16,19,20,27,55,61,75], or provide tests for the prediction of biogeographical ancestry [11,39,49,51,52] and externally visible characteristics [10,24,36,37,41,54,56,69]. SNPs also provide complimentary data to STRs for the analysis of complex pedigrees, particularly when such tests seek to analyze distant family relationships or when the pedigree is deficient [2,23,33,43,59]. One particular benefit of SNPs in kinship analyses is their mutational stability, with mutation rates three orders of magnitude lower than those of STRs, giving a much lower probability of a genotype incompatibility due to mutation that appears as an exclusion.

Despite the simplicity of the binary variation of SNPs and their evident benefits to forensic analysis, SNP genotyping has yet to be widely adopted as a routine forensic identification tool. So far, few laboratories have incorporated SNP genotyping into routine analyses, although a considerable number are actively developing SNP sets for specific forensic purposes. One reason for the slow uptake has been a lack of suitable SNP genotyping systems and dedicated software for SNP data analysis. Length polymorphisms such as STRs or Indels can be readily genotyped by multiplex PCR using fluorescent dyes linked to the PCR primers, followed by capillary electrophoresis (CE) on validated automatic sequencers, originally developed for sequencing. The CE signal data is analyzed using well-established software that has undergone refinement and optimization for more than 20 years. In a well-balanced multiplexed PCR, both the intra- and interlocus electrophoretic signal balance is reproducible and this simplifies data analysis as well as aiding the interpretation of mixtures of multiple DNA



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Manuel Fondevila obtained his B.S. degree in biology at the University of Santiago de Compostela (Santiago de Compostela, Spain) in 2001. In the same year he started his thesis research work in the Forensic Genetics Unit, Institute of Forensic Sciences at the same university, obtaining his Ph.D. in 2009. In 2010 he achieved a Barrie de la Maza fund grant and from then on worked as a post-doc researcher at the US National Institute of Standards and Technology (NIST), on the forensic research team of John Butler until 2012. In that year he returned to the Institute of Forensic Sciences, University of Santiago de Compostela, where he has been conducting research up to the present time.

Claus Børsting received his M.Sc. (1994) and Ph.D. (1999) degrees in molecular biology from Odense University (Odense, Denmark). Dr. Børsting is currently the manager of the research group at the Forensic Genetics Section, University of Copenhagen, Denmark). Upon completion of his Ph.D. degree, Dr. Børsting continued with the same line of research as a postdoctoral fellow at Albany Medical College (Albany, NY). In 2001, he was employed as a forensic geneticist at the Forensic Genetics Section, University of Copenhagen. He was trained as a reporting officer in paternity and immigration casework. His research activities earned him positions as an assistant professor in 2004 and a senior advisor in 2010. In 2007, he validated and implemented the SNP*for*ID HID assay for relationship casework according to the ISO 17025 standard. He became the manager of the SNP laboratory and continued to have this function until 2012 where he became the daily manager of the research group. The main focus of Dr. Børsting's current research is to explore the use of next-generation sequencing methods in forensic genetics.

Christopher Phillips studied genetics at Birmingham University (Birmingham, UK) between 1974 and 1977 and in 1978 obtained his M.Sc. degree in applied genetics at the same institute. Mr. Phillips is currently a researcher in the Forensic Genetics Unit of the University of Santiago de Compostela. Mr. Phillips started his forensic genetics career in 1979 at the Biochemistry Division of the Metropolitan Police Forensic Science Laboratory (London, UK). He then moved to the Forensic Haematology Department, Barts Health NHS Trust (London, UK) and the London School of Medicine and Dentistry (London, UK) and worked there until 2001. Since 2001 he has been a full-time researcher in the Forensic Genetics Unit of the University of Santiago de Compostela. Mr. Phillips's Research interests include SNP analysis applied to medical, population, and forensic genetics, the development of novel forensic polymorphisms, and building open-access online genomics search tools for the genetics and forensic communities.

Maria de la Puente studied biology, concentrating on biotechnology and molecular biology, at the University of Santiago de Compostela between 2006 and 2011. She completed her M.Sc. degree in biomedical investigation at the same university on 2012. She is now completing her Ph.D. research on the forensic applications of new genomic technologies at the Forensic Genetics Unit of the University of Santiago de Compostela, supported by funding awarded by the Xunta de Galicia as part of the Plan Galego de Investigación, Innovación e Crecemento 2011–2015.

Carla Santos studied biology at the University of Aveiro (Aveiro, Portugal). Since 2008 she has been continuously conducting research at the Forensic Genetics Unit of the University of Santiago de Compostela focusing on binary markers, particularly the development of SNP assays dedicated to forensic ancestry analysis. She recently finished her Ph.D. in forensic sciences and pathology at the University of Santiago de Compostela.

The EUROFORGEN-NoE Consortium (European Forensic Genetics Network of Excellence) consists of 16 participating institutions based in nine different European Union member states. The activities of this network of excellence are centered on the mobilization of the synergies of the major relevant European research groups to investigate new technologies and methods for the application of forensic genetics in the context of security-relevant issues and the justice system. The EUROFORGEN-NoE consortium is formed by renowned researchers from all over Europe, each one with a strong scientific background in the field of forensic genetics research and its application in casework.

Ángel Carracedo received his degree in medicine from the University of Santiago de Compostela. Dr. Carracedo is professor of legal medicine at the University of Santiago de Compostela, director of the Galician Foundation of Genomic Medicine, and director of the Spanish National Genotyping Centre. Dr. Carracedo was formerly director of the Institute of Legal Medicine at Santiago and president of the International Society of Forensic Genetics (ISFG). Dr. Carracedo's research lines include the genetics of complex traits with different applications ranging from forensic to clinical genetics.

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Maria Victoria Lareu received her M.D. degree from the University of Santiago de Compostela in 1984. Dr. Lareu is professor of legal medicine at the University of Santiago de Compostela and the director of the Institute of Legal Medicine at Santiago, directing the forensic science services provided by the Forensic Genetics Unit. Dr. Lareu is the principal investigator of many research projects undertaken in the department and directed the Ph.D. studies of the following coauthors of this article: Manuel Fondevila, Maria de la Puente, Carla Santos, and Christopher Phillips.