Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 1 (2007) 180–185
FSI GENETICS
www.elsevier.com/locate/fsig

Evaluation of the Genplex SNP typing system and a 49plex forensic marker panel

C. Phillips [a,*], R. Fang [b], D. Ballard [c], M. Fondevila [a], C. Harrison [c], F. Hyland [b], E. Musgrave-Brown [c], C. Proff [d], E. Ramos-Luis [a], B. Sobrino [a], A. Carracedo [a], M.R. Furtado [b], D. Syndercombe Court [c], P.M. Schneider [d]

The SNP*for*ID Consortium

[a] *Forensic Genetics Department, Genomic Medicine Group, University of Santiago de Compostela, Galicia, Spain*
[b] *Applied Markets Group, Applied Biosystems, Foster City, CA, USA*
[c] *Department of Haematology, ICMS, Queen Mary's School of Medicine & Dentistry, London E1 2AT, UK*
[d] *Institute of Legal Medicine, University of Cologne, Germany*

Received 29 January 2007; accepted 3 February 2007

Abstract

Using a 52 SNP marker set previously developed for forensic analysis, a novel 49plex assay has been developed based on the Genplex typing system, a modification of SNPlex™ chemistry (both Applied Biosystems) using oligo-ligation of pre-amplified DNA and dye-labeled, mobility modified detection probes. This gives highly predictable electrophoretic mobility of the allelic products generated from the assay to allow detection with standard capillary electrophoresis analyzers. The loci chosen comprise the 48 most informative autosomal SNPs from the SNP*for*ID core discrimination set supplemented with the amelogenin gender marker. These SNPs are evenly distributed across all 22 autosomes, exhibit balanced polymorphisms in three major population groups and have been previously shown to be effective markers for forensic analysis. We tested the accuracy and reproducibility of the Genplex system in three SNP*for*ID laboratories, each using a different Applied Biosystems Genetic Analyzer. Genotyping concordance was measured using replicates of 44 standardized DNA controls and by comparing genotypes for the same samples generated by the TaqMan®, SNaPshot® and Sequenom iPLEX® SNP typing systems. The degree of informativeness of the 48 SNPs for forensic analysis was measured using previously estimated allele frequencies to derive the cumulative match probability and in paternity analysis using 24 trios previously typed with 18 STRs together with three CEPH families with extensive sibships typed with the 15 STRs in the Identifiler® kit.

Keywords: SNP; Genotyping; Oligo-ligation assay; OLA; Genplex

1. Introduction

The SNP*for*ID consortium (www.snpforid.org) has been funded by the EU Growth programme to develop single nucleotide polymorphisms (SNPs) for forensic use. A major outcome of the development work of SNP*for*ID was the creation of a core single-tube 52plex PCR that can be used as the preparatory step in a variety of assays, each based on different SNP genotyping chemistries [1]. Although the consortium chose the SNaPshot® (AB: Applied Biosystems, Foster City, CA, USA) single base extension system as the ''benchmark'' technique for analysis of forensic samples and for the allele frequency validation process [2], peak imbalances and high background signal with this assay have hampered its application in the analysis of challenging DNA. Furthermore despite considering the AB SNPlex™ oligo-ligation system as a potentially useful technique since it can genotype up to 48 SNPs, it was evident that this approach lacks sufficient sensitivity for forensic analysis, requiring 37 ng of DNA to ensure successful ligation. Using oligo-ligation as a forensic technique only became a viable prospect once the system was adapted to begin with a pre-amplification of SNPs prior to the ligation stage. This is easier to achieve with fixed SNP sets

* Corresponding author. Tel.: +34 981582327; fax: +34 981580336.
E-mail address: c.phillips@mac.com (C. Phillips).
URL: www.snpforid.org

1872-4973/$ – see front matter
doi:10.1016/j.fsigen.2007.02.007

where the pre-amplification step can be carefully adjusted to give a balanced yield of target DNAs for ligation. Therefore the appropriate step was to incorporate the previously optimized 52plex PCR as the first stage in an adapted SNPlex™ system (termed Genplex) using a universal dye-labeled probe set to detect the alleles of each SNP with capillary electrophoresis analyzers. This report details the Genplex assay developed by AB to genotype 48 of the 52 SNP*for*ID loci (plus the amelogenin gender marker) and outlines the concordance studies performed to measure the accuracy of the technique. In addition, estimates are given of the informativeness of the 48-SNP set in comparison to widely used STR marker sets from the standard parameters of match probabilities in European and African populations together with exclusion probabilities and paternity indices estimated from both normal trios and extended families that allowed first-degree relatives of the true father to be compared.

2. Materials and methods

2.1. Selection of SNP markers for Genplex

Genplex routinely analyzes 48 SNPs so 4 of the original 52 SNP markers were not incorporated: rs2016276, rs826472, rs2830795 and rs1028528. SNP rs2016276 had several proximal SNPs, previously avoided in the SNaPshot® extension primer design, that were likely to interfere with the binding of ligation primers on both sides of the substitution site. The other three SNPs had been given revised positions in dbSNP build 118 (NCBI genome build 34) that made them less well separated from other more informative SNPs retained in the Genplex set. Amelogenin was added as a gender marker with the assay able to successfully differentiate the 6 base pair (bp) deletion of the X chromosome from the full Y sequence with the same oligo-ligation chemistry used to analyze the SNP substitutions.

2.2. DNA samples

Genotyping concordance was measured using a standardized Applied Biosystems control plate available as a common reference panel for the SNPlex™ system (PN 4366135) and containing 44 duplicated DNA samples sourced from the European Collection of Cell Cultures (www.ecacc.org.uk). The control plate was typed with Genplex in all three laboratories to gauge between-replicates concordance. In addition the plate was parallel genotyped with TaqMan® assays as part of the assay optimization process at AB, and in one of the SNP*for*ID laboratories with SNaPshot® and Sequenom iPLEX® MALDI-TOF SNP genotyping systems for cross-platform concordance analysis. The informativeness of the 48 SNPs in paternity analysis was studied using 24 German paternity trios previously analyzed with 18 STRs together with 3 extended CEPH Utah families: 1333; 1340 and 1345 each comprising three generations of European origin and sibships of 9, 4 and 7 offspring, respectively (http://ccr.coriell.org/nigms/nigms_cgi/fam.cgi?1333).

2.3. The Genplex assay

The main principle of Genplex and SNPlex™ assay systems is to identify the products of a 48plex oligo-ligation assay (OLA) using probes specific to each SNP/allele combination [3]. Specificity is controlled using a standard set of 96 non-human sequences: one at the end of each allele-specific oligo. These sequences in turn hybridize complementary oligos termed ZipChute® probes that carry FAM™ or dR6G dye labels and proprietary mobility modifiers. The Genplex assay comprises the following reaction steps (outlined in Fig. 1, with reference to the numbered steps below) performed in 96-well micro-titre plate (MTP) format:

(i) *PCR*: pre-amplification of 48 SNPs used the same primers developed for the core discrimination set PCR designed to give amplicon lengths 59–115 bp as previously described [1] plus a primer pair for amelogenin developed de novo by AB (giving a 170 bp product).
(ii) *Post PCR cleanup*: removal of unincorporated bases and primers with ExoSAP-IT® (USAB Corp., Cleveland, OH, USA).
(iii) *OLA*: ligation of biotinylated locus-specific oligos (LSOs) binding to sequence immediately downstream of the SNP site (~20 bases) with allele-specific oligos (ASOs) binding directly to the alleles and upstream sequence. Each ASO identified by a pre-assigned reporting sequence complimentary to a ZipChute® detection probe.
(iv) *Binding of OLA products to capture plate*: a streptavidin-coated MTP binds the biotinylated OLA products to provide a solid phase for ZipChute® hybridization.

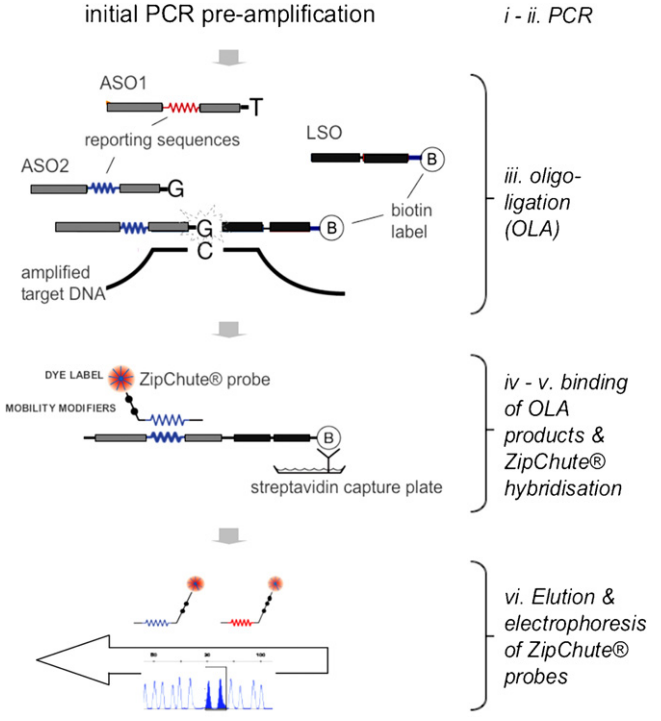

Fig. 1. Graphical summary of Genplex assay steps. Numerals refer to the description in the text of each step.