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Development of a multiplex PCR assay detecting 52 autosomal SNPs

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SNPforID Consortium

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Abstract. An efficient method that can be used to simultaneously amplify a set of genetic loci across the genome with high reliability can provide a valuable tool for single nucleotide polymorphism (SNP) forensic genotyping. A crucial element is the number of individual biochemical reactions that must be performed. The SNPforID consortium (www.snpforid.org) was established in 2003 with the principal goal of developing a SNP-based system of DNA analysis that would have comparable discrimination power and ease of use to those of existing short tandem repeat (STR) based techniques. Here, we describe a strategy for amplifying 52 genomic DNA fragments, each containing one SNP, in a single tube, and accurately genotyping the PCR product mixture using two single base extension reactions. This multiplex approach reduces the cost of SNP genotyping and requires as little as 0.5 ng of genomic DNA to detect 52 SNPs. We used a multiple injection approach for DNA sequencers that can effectively detect all the SNPs amplified in a single electrophoretic run. We present SNP data for 700 unrelated individuals from 9 populations.

Keywords: Single nucleotide polymorphism; Multiplex PCR; Human identification; Paternity testing

1. Introduction

SNPs are useful markers for human identification. Approximately 50 SNP markers are required to reach the discriminatory power used in most forensic investigations [1]. Here, we describe the

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development of a 52 SNP-plex assay with amplification of 52 fragments (59–115 bp in length) in one PCR reaction and detection of 52 SNPs in two single base extension (SBE) reactions analysed by capillary electrophoresis.

2. Materials and methods

A total of 700 samples from Denmark (156), Greenland (149), Somalia (104), Turkey (96), China (63), Germany (49), Taiwan (43), Thailand (33) and Japan (7) were typed in duplicate. We used blood on FTA cards (Whatman) and DNA purified by phenol/chloroform extraction or the QIAamp DNA blood mini kit (Qiagen).

PCR primer designs and PCR conditions were chosen to ensure equal PCR amplification efficiency for all amplicons as previously described [2]. The primers were HPLC purified and checked for homogeneity by MALDI-TOF mass spectrometry (DNA Technology A/S). Excess primers and dNTPs were removed by adding 0.75 μl 1 U/μl shrimp alkaline phosphatase and 0.023 μl 10 U/μl Exonuclease I (Amersham Pharmacia Biotech) to 2.5 μl PCR product and incubating the mixture at 37 °C for 1 h and 75 °C for 15 min.

SBE reactions were performed in 8 μl with 1 μl purified PCR product, 4 μl SNaPshot reaction mix (Applied Biosystems), 1 μl SBE primer mix (0.01–0.27 μM) and 2 μl Milli-Q water. Excess nucleotides were removed after the SBE reaction by addition of 1 μl 1 U/μl SAP to the SBE mix and incubation at 37 °C for 30 min and 75 °C for 15 min. Detection of the SBE products was performed using ABI Prism 310 or ABI Prism 3100 genetic analysers (Applied Biosystems). A GeneScan method file was created to allow sequential injection of the two SBE multiplexes without a capillary polymer fill stage between the two injections, by modifying the run module that fill the syringe and the capillary array along with the pre-run.

The matching probability and power of discrimination were calculated as described [3]. The mean exclusion probability and the typical paternity index were calculated as described [4].

3. Results

Fifty-two autosomal SNPs that were highly polymorphic in Caucasian, Asian and African populations were selected mainly from the distal parts of the p- and the q-arms of each chromosome. The selected loci were positioned at least 100 kb from known genes and minimally 1.3 Mb from STRs used in standard forensic case work.

The multiplex PCR amplification strategy allowed the simultaneous amplification of all 52 amplicons from as little as 0.5 ng DNA. The optimal amount of DNA in the PCR was 1–10 ng, but amplification was also successful with 100 ng DNA; thus, quantification of DNA was not mandatory prior to PCR amplification. SNP detection was based on capillary electrophoresis of two sets of multiplexed SBE reactions with 23 and 29 SBE primers, respectively.

Pair-wise comparisons of 700 samples from nine different populations were performed. An overview of the population differentiation is shown in Fig. 1. Except for one SNP, no deviation from Hardy-Weinberg expectations was observed ($P > 0.05$) and no linkage disequilibrium between any of the loci was seen. The highest average heterozygosity was found in Europeans (0.44). The combined mean match probability was between 5.0×10^{-19} (Asians) and 5.0×10^{-21} (Europeans). The average number of identical SNP loci found in two unrelated individuals (244,650 comparisons) was 21 with a minimum of 7 and a maximum of 41 matching loci. The typical paternity indices calculated from the obtained allele frequencies ranged from over 300,000 in Asians to about 550,000 in Europeans.

4. Discussion

This study introduces a set of 52 SNP markers carefully tailored for use in human identification and readily genotyped with an already well-established technology in many forensic laboratories.

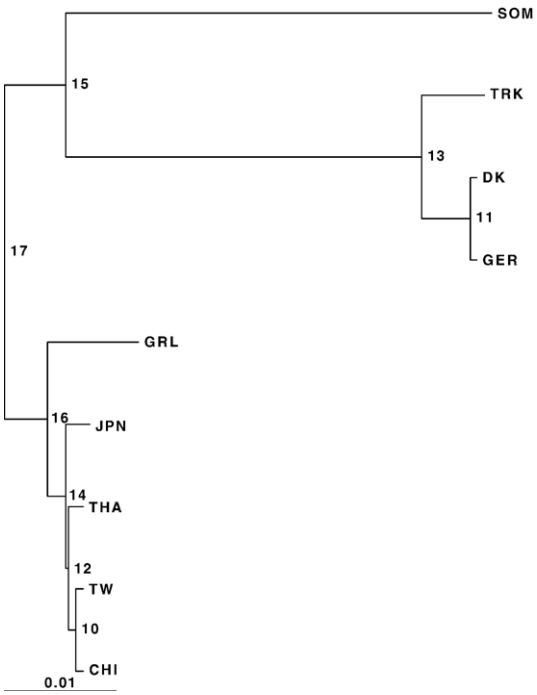

Fig. 1. Hierarchic tree obtained by pair-wise analyses of the genetic distances between the populations studied: Somalis (SOM), Turks (TRK), Danes (DK), Germans (GER), Greenlanders (GRL), Japanese (JPN), Thais (THA), Taiwanese (TW) and Chinese (CHI). The genetic distances were computed using the F_{ST} values between two populations and clustered using the UPGMA methods. The numbers on the branches represent the bootstrap values.

The mean match probability and the typical paternity indices calculated on basis of the 52 SNP-plex would be satisfactory in both crime and paternity cases. All 52 SNPs were successfully typed from DNA isolated from human cadavers where only partial STR profiles had previously been obtained using the AmpF*l*STR SGM Plus STR amplification kit (data not shown), suggesting that the short amplicons in the 52 SNP-plex (59–115 bp) are more efficiently amplified from degraded DNA than the longer amplicons in the STR kit. Furthermore, the low mutation rate of SNPs offers a significant advantage in paternity and other relationship investigations that are needed in immigration casework, inferring that SNPs may be an important supplementary set of markers for future forensic casework.

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