Analysis of four PCR/SNaPshot multiplex assays analyzing 52 SNPforID markers

Abstract

The SNPforID consortium identified a panel of 52 SNPs forensic analysis that has been used by several laboratories worldwide. The original analysis of the 52 SNPs was based on a single multiplex reaction followed by two single-base-extension (SBE) reactions each of which was analyzed using capillary electrophoresis. The SBE assays were designed for high throughput genetic analyzers and were difficult to use on the single capillary ABI PRISM 310 Genetic Analyzer and the latest generation 3500 Genetic Analyzer, as sensitivity on the 310 was low and separation of products on the 3500 with POP-7™ was poor. We have modified the original assay and split it into four multiplex reactions, each followed by an SBE assay. These multiplex assays were analyzed using polymer POP-4™ on ABI 310 PRISM® and polymers POP-4™, POP-6™ and POP-7™ on the 3500 Genetic Analyzer. The assays were sensitive and reproducible with input DNA as low as 60 pg using both the ABI 310 and 3500. In addition, we found that POP-6™ was most effective with the 3500, based on the parameters that we assessed, achieving better separation of the small SBE products; this conflicted with the recommended use of POP-7™ by the instrument manufacturer. To support the use of the SNP panel in casework in Malaysia we have created an allele frequency database from 325 individuals, representing the major population groups within Malaysia. Population and forensic parameters were estimated for all populations and its efficacy evaluated using 51 forensic samples from challenging casework.

1 Introduction

While short tandem repeat (STR) analysis remains the primary tool in forensic genetics additional markers to analyze degraded DNA are desirable. A number of SNP panels have been evaluated for individual identification (IISNPs), including the SNP*for*ID panel, which was developed by a European consortium [1]. In the original SNP*for*ID study 52 SNPs were amplified in a single PCR followed by two single-base extension (SBE) reactions, using capillary electrophoresis (CE). The two SBE reactions contained 23 and 29 primers; the PCR products size ranged from 59 bp to 115 bp, whilst the SBE products size ranged from 16 to 92 nt [1].

The original system was developed using SNaPshot® Multiplex System to detect the SNPs on three genetic analyzers: ABI 310 PRISM®, ABI 3100 PRISM® and ABI 3100 PRISM® Avant, all with 36 cm capillary arrays and performance optimized polymer POP-4™ (Applied Biosystems, USA). The reported sensitivity of the ABI 310 PRISM® was only 20% of the sensitivity of the ABI 3100 PRISM®, and therefore most of the development studies were carried out using ABI 3100 or ABI 3100-Avant PRISM® Genetic Analyzers. The system has subsequently been validated for use in ISO 17025 accredited laboratories [2], but is not without challenges, in particular peak imbalances between alleles [1, 3]. These challenges have been addressed by developing alternative techniques, such as oligo-ligation [3] and more recently systems developed for next generation sequencing have gained attention in the forensic community as a technology that allows the typing of large SNP arrays [4-6]. Modifications of the multiplexes have also been reported, splitting the PCR into two or more separate amplification reactions, which helped to increase sensitivity, allowing the ABI 310 platform to be used, and facilitating the interpretation of SNaPshot® data [7, 8].

The SNP markers from the SNP*for*ID 52plex were selected for evaluation as a supplementary genetic marker for casework in Malaysia using capillary electrophoresis platforms. During the development the platforms available were the ABI 310 Prism® and the 3500 Genetic Analyzer; the primary aim of the work was to redesign the multiplexes to facilitate interpretation using these platforms. In this study we describe the development of four 13-plex PCR/SNaPshot assays (designated as 13_{st}, 13_{nd}, 13_{rd} and 13_{th}) and the analysis using polymer POP-4™ with ABI 310 and POP-4™, POP-6™ and POP-7™ with a 3500 Genetic Analyzer. The reproducibility and sensitivity

of the assays were assessed during this study and population data for the three major ethnic groups of Malaysia, Malay, Malaysian Chinese (M-Chinese) and Malaysian Indian (M-Indian), were generated as a reference source for casework. The efficacy of SNPs in challenging casework using the redesigned assays was also assessed.

2 Materials and Methods

2.1 Sample, DNA extraction, purification and quantification

For Malay, M-Chinese and M-Indian populations 325 samples were collected after gaining informed consent on FTA® Classic Card and purified using either phenol/chloroform/isoamyl alcohol or QIAamp® DNA Investigator Kit (Qiagen, UK) according to the manufacturer's protocol. DNA was quantified using Quantifler™ Human DNA Quantification kit (Applied Biosystems, UK) with a 7500 Real-Time PCR system (Applied Biosystems). The procedure was carried out according to the manufacturer's protocol, but with half volumes.

9947A and TaqMan® Control Genomic DNA (Applied Biosystems) were used as DNA controls throughout study.

2.2 Multiplex SNPs assay set-up

Several combinations of the PCR and SBE primers were evaluated before selecting the most robust combinations. Finally, four 13-plex assays were developed, named 13_{st}, 13_{nd}, 13_{rd} and 13_{th}. All sequences and sizes of PCR/SBE primers were the same as previously described [1], except for 14 SBE primers that were modified to balance the designated assays. Each modification involved the addition or reduction of poly thymidine (poly T) or poly cytosine (poly C) to the 5′ end of the SBE primer. The new SBE length (bp) and the final combinations and concentrations of all primer pairs in each multiplexed (both PCR and SBE reactions) are presented in Table 1.

2.3 Amplification and PCR Purification

The total reaction volume of 15 µl comprised 1X AmpliTaq Buffer II, 3.0 mM $MgCl_2$ solution, 0.4 mM dNTPs, 2U AmpliTaq®DNA Polymerase and 1 µl DNA sample (all the PCR components from

Applied Biosystems). All other steps of PCR and purification reactions were performed as described previously [1].

2.4 SBE reaction and products clean-up using SAP (USB, Affymetrix, USA)

The SBE reactions were carried out using ABI Prism SNaPshot® Multiplex Kit (Applied Biosystems). The optimized SBE multiplex reactions contained 2.0 µl of SNaPshot mix, 1.5 µl of purified PCR product and 1 µl of mixed SBE primers. The nuclease-free water was added to make up the total reaction volume to 5 µl. Thermal cycling conditions and SAP purification steps were as described previously [1].

2.5 Capillary Electrophoresis with ABI 310 PRISM® Genetic Analyzer

1 µl of SAP- treated SBE products was diluted in 11 µl of Hi-Di™ formamide and 0.4 µl GeneScan™ 120-LIZ Internal Size Standard (Applied Biosystems). Samples were initially analyzed using an ABI 310 PRISM® Genetic Analyzer with POP-4™ polymer, 36 cm capillary array, injection time: 5 s, injection voltage: 1.2 kV and run time: 1800 s.

2.6 Concordance Study using 3500 Genetic Analyzer

20 control and population samples were re-analyzed using a 3500 Genetic Analyzer with different parameters: (i) POP-4™ polymer, with 36 cm capillary array, injection time: 10 s, injection voltage: 1.2 kV, run time: 2800 s, dye set: E5, application type: HID, run module: HID36_POP4, (ii) POP-6™ polymer, with 50 cm capillary array, injection time: 10 s, injection voltage: 1.6 kV, run time: 2800 s, dye set: E5, application type: fragment, run module: fragmentAnalysis50_POP6, and (iii) POP-7™ polymer, with 50 cm capillary array, injection time: 10 s, injection voltage: 1.6 kV, run time: 2800 s, dye set: E5, application type: fragment, run module: fragmentAnalysis50_POP7.

2.7 SNP typing with polymer POP-6™ and ABI 3500 PRISM® Genetic Analyzer

The volume for each SBE multiplex assay was reduced to 1.7 µl of SNaPshot mix, 1 µl of purified PCR product and 1 µl of mixed SBE primers, made to a final volume of 5 µl with nuclease-free water. Following final optimization, all control DNA samples, with template amounts ranging from 0.01 ng to 2.5 ng (in triplicates) and population samples were analyzed with polymer POP-

6™ on a 3500 Genetic Analyzer. An example of the profiles generated from a sensitivity study are shown in Supplementary Figure 1.

2.8 Data collection and evaluation

SNaPshot data from the ABI 310 PRISM® Genetic Analyzer were analyzed using GeneMapper™ ID Software version 3.2; data collected from the 3500 PRISM® Genetic Analyzer were analyzed using GeneMapper® ID-X version 1.2. For all analysis peak thresholds were set to a minimum of 120 RFU (blue), 60 RFU (green) and 30 RFU (yellow, red and orange). The acceptable peak height ratio for heterozygote allele calls was 3:1 (maximum) and a minimum of 5:1 peak height ratio (with a minimum peak height of 400 RFUs (blue), 200 RFUs (green) or 100 RFUs (yellow and red)) for homozygote alleles. These were based on the criteria previously described [1] and verified using commercial DNA 9947A and thirty full profiles generated on both the 310 and 3500.

2.9 Analysis of population data

F_{ST} values and the exact test for Hardy Weinberg equilibrium (HWE) and linkage equilibrium (LE) were carried out using Arlequin version 3.5 [9]. Forensic parameters were calculated using PowerStats version 1.2 [10]. Population attribution was estimated using Arlequin version 3.5 and Snipper version 2.5 [11].

3 Results

3.1 Development and optimization of the assays

Four sets of 13-plex PCR/SBE reactions were developed in this study; the organization of the markers and concentration of primers are shown in Table 1. Studies using ABI 310 demonstrated that multiplex assays were amplified effectively under the optimal conditions and parameters as detailed in Sections 2.3 to 2.5. The size and labeling of the SBE products was as expected and the profiling of a QC sample (9947A) was compared to an external reference and showed full concordance. Using DNA template amounts of (0.01, 0.02, 0.03, 0.06, 0.13, 0.25, 0.5 and 1.0) ng complete profiles were generated from all reactions that used 0.06 ng or more template. An example of the 13_{th} assay is shown as Fig. 1A.

Profiles were generated from 325 population samples with input DNA as low as 0.1 ng up to 500 ng. No locus drop-out or unusual peaks were observed. However, peak imbalances and baseline noise was observed in samples with that less than 0.06 ng and higher than 30 ng DNA.

3.2 Analysis of 13-plex assays with different parameters using a 3500 Genetic Analyzer

Initially, the SNPs genotyping of the same SAP-treated products from the previous internal validation study using ABI 310 was carried out using POP-7™ with a 50 cm capillary array on a 3500, following the recommended manufacturer's protocol [12]. However, the resolution of the alleles was poor, especially for the SBE products below 60 nt; we carried out further analysis using POP-4™ and POP-6™. Results obtained are shown in Fig. 1, where the same samples of the 13_{th}-plex assay were run using different types of polymer. Peak heights were similar using all polymers (5000 to 7000 RFU) for all profiles when using the 3500, but each showed different allelic migration patterns, especially for SNP alleles below 60 nt. Samples analyzed using POP-6™ on the 3500 showed the best separation (POP-4™was not tested with a 50 cm capillary). As the sensitivity of the 3500 was higher than the 310 the amount of reaction mixture could be reduced and comparable results, in terms of peak height, obtained (see Section 2.7).

Fig. 2 shows an example of full SNP profile with designated 13-plex assays (*13_{st}, 13_{nd}, 13_{rd} and 13_{th}*), obtained from 1 ng of DNA sample using POP-6™ with a 3500 Genetic Analyzer. All four 13-plex assays were arranged as 4 panels per individual (52 autosomal SNP markers) for better visualization and easier interpretation.

3.3 Population data

Malaysia is multi-racial country with more than 70 identified ethnic groups. These are broadly classified into four main groups: Bumiputera, including Malay and indigenous groups; M-Chinese; M-Indians and others. In Malaysia it is standard practice to generate reference databases for Malay, M-Chinese and M-Indian populations before using new genetic markers in casework. In total, 325 samples were analyzed, 109 Malay and M-Indian and 107 M-Chinese, with the optimized assay using a 3500 Genetic Analyzer. Minimum allele frequencies were 0.079 in M-Chinese and 0.096 in Malay (both SNP marker 29) and 0.142 in M-Indian (at SNP marker 16); no value was below the minimal allele frequency typically used in casework, i.e. 5/2N (0.023). The allele frequencies are shown in Supplementary Table 1 along with allele frequencies from other populations for comparison [13]. The combined match probabilities for the 52 SNPs were: Malay $3.9654e^{-19}$, M-Chinese $5.3964e^{-18}$, and M-Indian $1.7459e^{-19}$ (Supplementary Table 2).

The exact test for departures from Hardy Weinberg equilibrium detected significant differences, after Bonferroni correction, at one locus in Malays (SNP code 26), two loci in M-Chinese (SNP codes 46 and 54) and 5 loci in M-Indians (SNP codes 12, 21, 36, 38, and 54) (Supplementary Table 3). The exact test for linkage equilibrium identified linkage (p = <0.05) at 73 pairs of loci in Malays, 55 pairs in M-Chinese and 80 pairs in M-Indians; however, after Bonferroni correction (p = <0.0000377) only one pair of loci was significant, this was between SNP 3 (chromosome 3) and 53 (chromosome 22) in the Malay population.

Between Malay and M-Chinese the F_{ST} was estimated at 0.00711, Malay and M-Indian 0.03460 and M-Chinese and M-Indian 0.04133. While the IISNP marker panels have not developed for differentiating between populations there were clearly differences between the allele frequencies in the East Asian populations compared to the M-Indian population. The data were examined with both Arlequin and Snipper to assess the power of the 52 SNPs to classify individual

samples into the correct population (Table 2 and Supplementary Figure 2). Following cross validation a high proportion of M-Indian samples, 92%, were correctly classified; the majority of Malay and M-Chinese samples are also correctly classified, but over 25% of Malays were classified as M-Chinese and *vica versa*.

3.4 Application to casework

A small number of samples, 51 samples in total from 10 different cases, which had been processed with varying degrees of success were re-extracted and amplified using the PowerPlex® 16 System (PP16) and the SNP panels. Of the 51 samples, following re-extraction, 19 gave a full profile with PP16, all of these profiles gave a full 52 SNP profile. Nine of the samples gave partial STR profiles ranging from 13 loci to 7 loci, of these full 52 SNP profiles were possible for 7 of the samples and partial profiles (26 and 33 SNPs) for the remaining 2. Of the 23 samples that gave no STR profile 10 could not be profiled at any SNP, 10 could be profiled at all 52 SNPs and 3 gave partial profiles with between 39 and 50 SNPs (Supplementary Table 4)[14]. The match probabilities of the full profiles, when applying an F_{ST} value of 0.01, ranged from $1e^{-18}$ to $2e^{-23}$; partial profiles ranged from $2.8e^{-10}$ to $5.1e^{-19}$ (Supplementary Table 5). Some caution has to be exercised when interpreting these results as reference samples were not available for comparison (i.e. from the donor), so the accuracy of the profiles could not be verified. However, apart from that partial nature of some profiles, they were otherwise judged to be of good quality, meeting the criteria detailed in Section 2.8, and identical profiles, apart from some locus dropout, were obtained from at least two different pieces of evidence from each case.

The SNP assays were also used to process 11 paternity cases that had been examined previously using STR analysis with PP16. These comprised four duos, with child and alleged father, six trios, and one case with mother, three children and alleged father: one case had a single mutation between the child and father and a case had two mutations between the child and the alleged father (the cases with mutations had been examined to exclude avuncular relationships). For the nine children tested with both a mother and father the combined paternity index (CPI) for all of the cases were all in excess of $2e^{6}$ using the 52 SNPs, which translated to a probability of paternity of 99.99995% when using a prior probability of 0.5 (Supplementary Table 6). The CPI for the four

children tested with only the father for reference was lower, with three of the cases below 1000, and one below 100; the 15 STR markers were more informative in the duo cases.

4 Discussion

The SNP*for*ID panel is one of the IISNP panels adopted by the forensic community. Other IISNP panels have also been developed, such as a 92 IISNP panel, 86 of which did not show strong linkage and is commonly referred to as the KiddLab panel [15], a 21-plex [16] and an 18-plex specifically designed to be more robust when amplifying degraded DNA [17]. The KiddLab panel and SNP*for*ID 52-plex have formed the basis for many of the commercially developed platforms, such as the Genoplex typing system from Applied Biosystems, that utilized 49 of the 52 SNPs from the SNP*for*ID panel [3], the iPLEX® Sample ID Plus Panel from Sequenom that utilized 47 of the 52 SNPs from the SNP*for*ID panel [18], the AmpliSeq™ Identity panel (Applied Biosystems) and ForenSeq™ Signature Prep Kit (Illumina, USA) that incorporate SNPs from both the SNP*for*ID panel and KiddLab panel [5, 6, 19]. When selecting SNPs for forensic analysis the SNP*for*ID and KiddLab panels have been developed to ensure that the associated forensic parameters, such as power of discrimination and lack of genetic linkage in multiple tested populations, make them robust genetic markers.

The approach taken in this study to increasing the sensitivity of the assay was to split the initial PCR and subsequent SBE reactions into four separate reactions. This has the advantage of making the assays easier to balance and also separate out the loci, reducing the number of loci amplified also increased the sensitivity, with only 60 pg of DNA needed to generate balanced profiles; this compares to > 200 pg needed when all amplicons were amplified on a single reaction [1]. The increased sensitivity is commonly seen when amplifying fewer amplicons, for example the IrisPlex, which co-amplified just 6 loci needed only 31 pg of DNA [20]. The disadvantage of splitting the assay is that four separate reactions are required to generate a full profile; however, in the application to casework, the SNPs would be typically used as a supplementary tool and all four panels may not be needed in order to reach an acceptable likelihood threshold (or exclusion). An alternative approach to increasing the sensitivity of the analysis used a PCR mix

optimized for STR analysis and increased SBE cycles, which enabled a 49-plex reaction to be stably amplified from only 50 pg DNA [21]. Massive parallel sequencing approaches have been also been reported to increase the sensitivity of assays to as little as 31 pg [6], but range to 0.5 pg to 1 pg of input DNA [4, 5, 22].

Best practice in forensic genetics requires the generation of appropriate population reference databases so that the use of approximate/regional populations is not necessary. It was therefore imperative to generate reference databases for the three main population groups in Malaysia, i.e. Malay, M-Chinese and M-Indian. Five loci were shown not to be in Hardy Weinberg equilibrium in the M-Indian population, one loci in Malays, and two in M-Chinese; this could be due to sampling effects or reflect a degree of selection or inbreeding within the tested populations. It could also be due to technical issues, in particular primer binding site mutations, that could result in an excess of homozygotes. Sequencing of primer sites in the target populations would identify potential problems and allow alternative/additional primers to be developed, but was beyond the scope of this project.

Typical forensic parameters were, as expected, very similar to previously typed populations [1, 23], in addition there was very little evidence for linkage at the population level. F_{ST} between Malay and M-Chinese was low, which was expected given their well-characterized genetic relationship [24]; between the M-Indian and M-Chinese/Malay the F_{ST} values were above 0.003. While the identification of bio-geographical origin was not the aim of this research the apparent differences in allele frequencies between the Indian population and Malay/M-Chinese in particular and could be exploited for this purpose. Assessment using both Arlequin and Snipper correctly predicted the origin of most M-Indian samples, whereas Malay and M-Chinese were more challenging to separate. The predictive power of analysis could well be improved by utilizing assays that have been specifically designed for this purpose, such as the SNP*for*ID 34-plex, with SNPs selected in part on the different allele frequencies between populations [25] and the KiddLab ancestry panel [26, 27]. However, the implementation of this type of analysis is not currently undertaken in Malaysia and no decision has been made on whether it will be used; its implementation would be especially sensitive as it could be seen to discriminate against one minority population.

A selection of problematic casework samples was processed as part of this study. The results were promising, with approximately 50% of samples that gave no results using STR analysis with PP16 giving full or highly informative SNP profiles. The increased success rate was in part down to the increase sensitivity of the SNP assay in comparison to the PP16; this was apparent when small amplicons in the PP16 profile were not detected whereas larger amplicons in the SNP profile were successfully amplified. In some cases DNA degradation made it challenging to obtain full profiles, especially using STRs and the short amplicon sizes in the SNP assay facilitated profiling of the highly degraded samples. The sensitivity of the SNP assay could potentially be further enhanced by applying the amplification and SBE protocols described by Borsting et al (2013)[21]. Similarly, the latest generation of STR kits, such as ESI (Promega), NGM (Applied Biosystems and DNA Investigator (Qiagen) [28-30] also have greater sensitivity than the PP16 and so may have yielded results in a greater proportion of the cases sampled in this study, although the STRs still have larger amplicons than the SNPs. The processing of the kinship cases provided resolution of all the cases where two parents were available, supporting the use of the SNP panel as a supplementary method in kinship analysis for the current STR-based systems [7, 31-35]. However, in deficient cases additional STR markers may be more productive as the paternity indexes generated from the duo cases was lower than with PP16. In conclusion, the SNP*for*ID panel has been shown to be of potential value for forensic casework in Malaysia, providing increased sensitivity and capability to profile highly degraded samples; population reference databases are available should it be implemented in this format or using a massive parallel sequencing platform.

References

[1] Sanchez, J. J., Phillips, C., Børsting, C., Balogh, K. *et al*. *Electrophoresis* 2006*, 27*, 1713-1724.

[2] Musgrave-Brown, E., Ballard, D., Balogh, K., Bender, K. *et al*. *Forensic Sci. Int. Genet.* 2007*, 1*, 186-190.

[3] Phillips, C., Fang, R., Ballard, D., Fondevila, M. *et al*. *Forensic Sci. Int. Genet.* 2007*, 1*, 180-185.

[4] Daniel, R., Santos, C., Phillips, C., Fondevila, M. *et al*. *Forensic Sci. Int. Genet.* 2015*, 14*, 50-60.

[5] Churchill, J. D., Schmedes, S. E., King, J. L., Budowle, B. *Forensic Sci. Int. Genet.* 2016*, 20*, 20-29.

[6] Elena, S., Alessandro, A., Ignazio, C., Sharon, W. *et al*. *Forensic Sci. Int. Genet.* 2016*, 22*, 25-36.

[7] Schwark, T., Meyer, P., Harder, M., Modrow, J. -., Von Wurmb-Schwark, N. *Transfus. Med. Hemotherapy* 2012*, 39*, 187-193.

[8] Bulbul, O., Phillips, C., Argac, D., Shahzad, M. S. *et al*. *Forensic Sci. Int. Genet. Suppl. Ser.* 2009*, 2*, 129-130.

[9] Excoffier, L., Laval, G., Schneider, S. *Evolutionary Bioinformatics Online* 2005*, 1*, 47-50.

[10] Tereba, A. *Profiles in DNA* 1999*, 3*, 14-16.

[11] Santos, C., Phillips, C., Gomez-Tato, A., Alvarez-Dios, J. *et al*., in: Goodwin, W. (Ed.), *Forensic DNA Typing Protocols*, New York, Springer 2016, pp. In Press.

[12] Applied Biosystems. *Applied biosystems 3500/3500xL Genetic Analyzer User Guide*, Foster City, CA, Applied Biosystems 2009.

[13] Amigo, J., Phillips, C., Lareu, M., Carracedo, Á. *Int. J. Leg. Med.* 2008*, 122*, 435-440.

[14] Alimat, S., Hadi, S., Goodwin, W. *Forensic Sci. Int. Genet. Suppl. Ser.* 2013*, 4*, e178-e179.

[15] Pakstis, A. J., Speed, W. C., Fang, R., Hyland, F. C. L. *et al*. *Hum. Genet.* 2010*, 127*, 315-324.

[16] Dixon, L. A., Murray, C. M., Archer, E. J., Dobbins, A. E. *et al*. *Forensic Sci. Int.* 2005*, 154*, 62-77.

[17] Freire-Aradas, A., Fondevila, M., Kriegel, A. -., Phillips, C. *et al*. *Forensic Sci. Int. Genet.* 2012*, 6*, 341-349.

[18] Johansen, P., Andersen, J. D., Børsting, C., Morling, N. *Forensic Sci. Int. Genet.* 2013*, 7*, 482-487.

[19] Eduardoff, M., Santos, C., De La Puente, M., Gross, T. E. *et al*. *Forensic Sci. Int. Genet.* 2015*, 17*, 110-121.

[20] Walsh, S., Liu, F., Ballantyne, K. N., Van Oven, M. *et al*. *Forensic Sci. Int. Genet.* 2011*, 5*, 170-180.

[21] Børsting, C., Mogensen, H. S., Morling, N. *Forensic Sci. Int. Genet.* 2013*, 7*, 345-352.

[22] Børsting, C., Fordyce, S. L., Olofsson, J., Mogensen, H. S., Morling, N. *Forensic Sci. Int. Genet.* 2014*, 12*, 144-154.

[23] Santos, C., Phillips, C., Fondevila, M., Porras-Hurtado, L. *et al*. *Forensic Sci. Int. Genet.* 2011*, 5*, e25-e26.

[24] Abdulla, M. A., Ahmed, I., Assawamakin, A., Bhak, J. *et al*. *Science* 2009*, 326*, 1541-1545.

[25] Fondevila, M., Phillips, C., Santos, C., Freire Aradas, A. *et al*. *Forensic Sci. Int. Genet.* 2013*, 7*, 63-74.

[26] Kidd, J. R., Friedlaender, F. R., Speed, W. C., Pakstis, A. J. *et al*. *Invest. Genet.* 2011*, 2*.

[27] Kidd, K. K., Speed, W. C., Pakstis, A. J., Furtado, M. R. *et al*. *Forensic Sci. Int. Genet.* 2014*, 10*, 23-32.

[28] Poetsch, M., Bayer, K., Ergin, Z., Milbrath, M. *et al*. *Int. J. Leg. Med.* 2011*, 125*, 733-739.

[29] Pajnic, I. Z., Pogorelc, B. G., Balažic, J., Zupanc, T., Štefanic, B. *Croat. Med. J.* 2012*, 53*, 17-23.

[30] Tucker, V. C., Hopwood, A. J., Sprecher, C. J., McLaren, R. S. *et al*. *Forensic Sci. Int. Genet.* 2011*, 5*, 436-448.

[31] Børsting, C., Morling, N. *Forensic Sci. Int. Genet.* 2011*, 5*, 236-241.

[32] Børsting, C., Mikkelsen, M., Morling, N. *Transfus. Med. Hemotherapy* 2012*, 39*, 195-201.

[33] Børsting, C., Morling, N. *Transfusion* 2012*, 52*, 425-430.

[34] Pontes, M. L., Fondevila, M., Laréu, M. V., Medeiros, R. *Transfus. Med. Hemotherapy* 2015*, 42*, 385-388.

[35] Phillips, C., Fondevila, M., García-Magariños, M., Rodriguez, A. *et al*. *Forensic Sci. Int. Genet.* 2008*, 2*, 198-204.

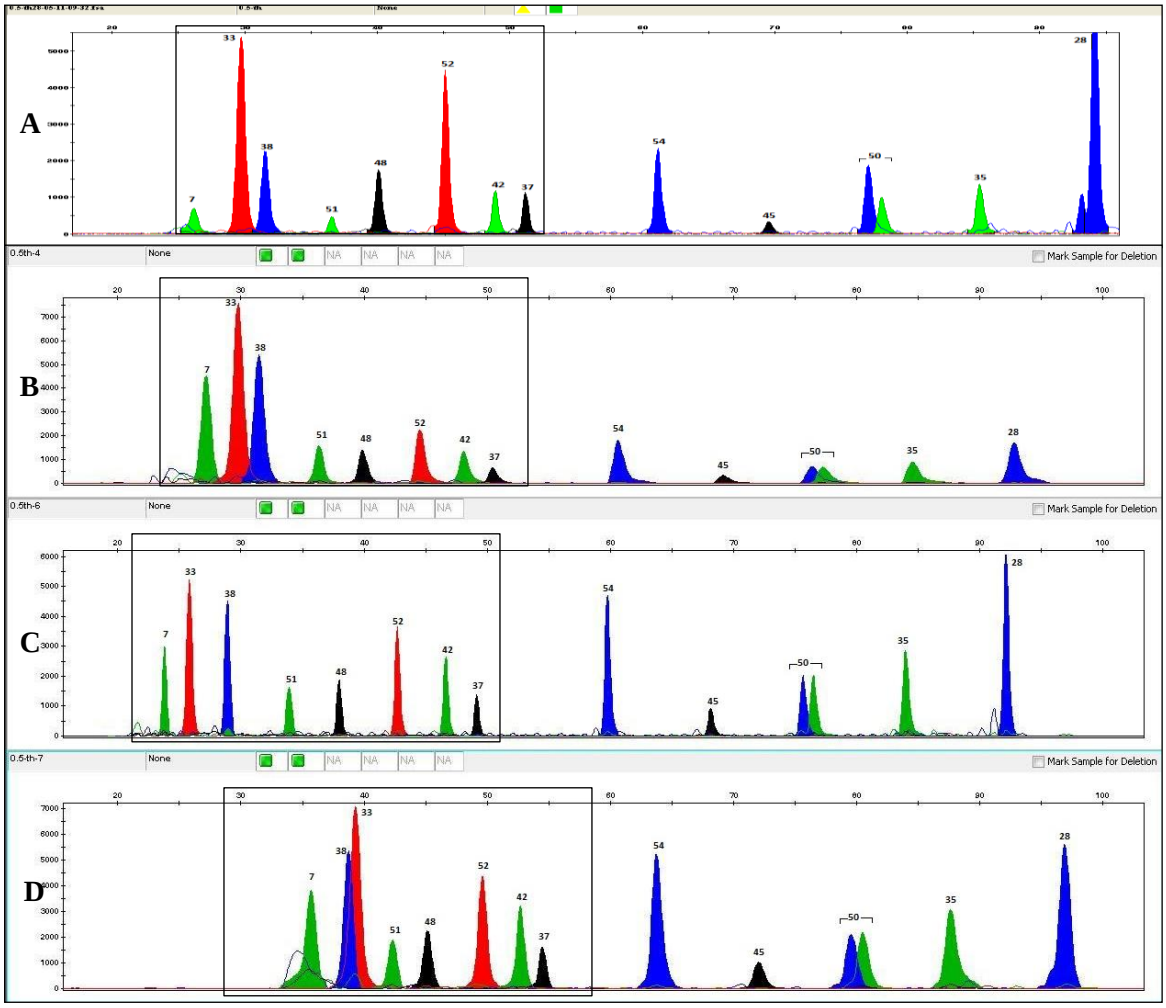


Figure 1 Comparison of SNaPshot profiles of *13$_{th}$* multiplex assay using polymers; A) POP-4™ on an ABI 310 PRISM® Genetic Analyzer, B) POP-4™, C) POP-6™ and D) POP-7™ on a 3500 Genetic Analyzer. Boxes show the most mobility-affected SNPs. 1 ng of DNA template was used.

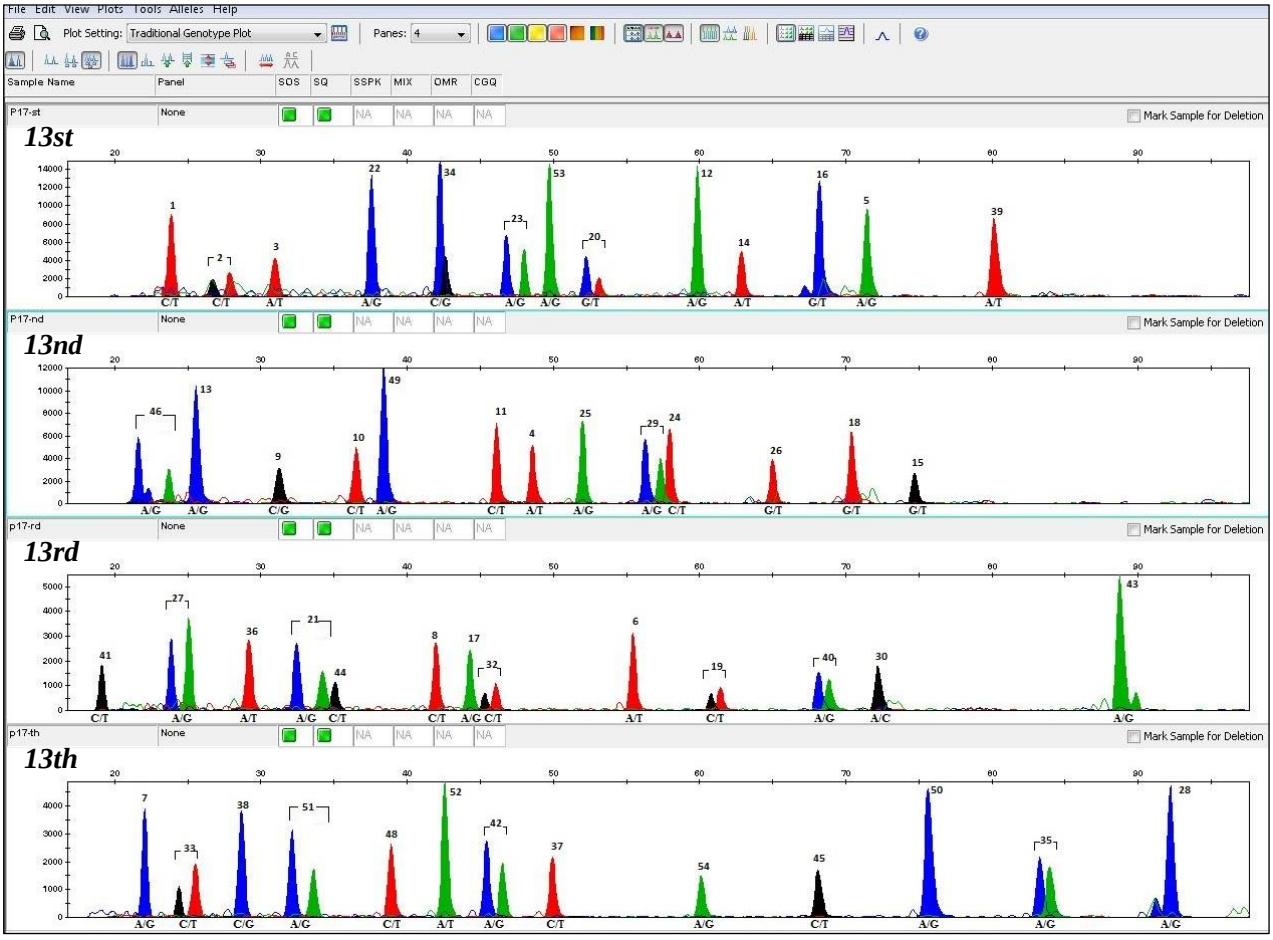


Figure 2 Full SNaPshot® profile of designated *13st, 13nd, 13rd and 13th* assays with 1 ng template DNA using POP-6™ with a 3500 Genetic Analyzer.

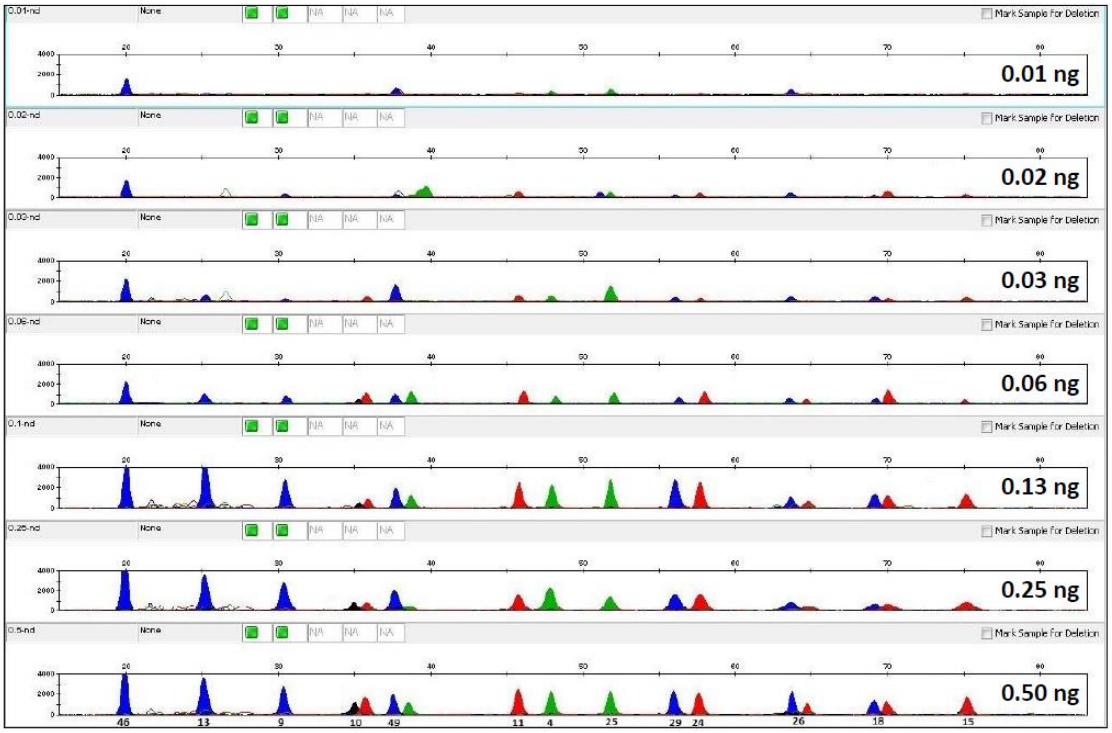
Table 1: Four sets of 13-plex assay reactions developed in this study.

Sanchez *et al*., 2006 (Original SBE Multiplexes)			Designated multiplex assays	Marker Code	SNP (rs#)	PCR		Single base extension (SBE)	
SBE Multiplex	Marker Code	SNP (rs#)				Final primer concentration (μM)	Amplicon size (bp)	Final primer concentration (μM)	Amplicon size (bp)
23 SBE Set	1	rs1490413	13_{st}	1	rs1490413	0.15	68	0.07	18
	2	rs876724		2	rs876724	0.1	83	0.1	24
	3	rs1357617		3	rs1357617	0.15	90	0.08	29
	4	rs2046361		22	rs733164*	0.1	68	0.1	36 (34)
	5	rs717302		34	rs1979255	0.15	86	0.15	40
	6	rs1029047		23	rs826472*	0.2	85	0.2	45 (46)
	7	rs917118		53	rs1028528	0.1	113	0.1	48
	8	rs763869		20	rs1031825	0.15	98	0.2	50
	9	rs1015250		12	rs2107612	0.15	93	0.3	58
	10	rs735155		14	rs1454361	0.1	73	0.15	62
	11	rs901398		16	rs729172*	0.1	60	0.2	68 (70)
	12	rs2107612		5	rs717302*	0.15	86	0.3	70 (74)
	13	rs1886510		39	rs354439	0.1	93	0.2	80
	14	rs1454361	13_{nd}	46	rs1360288	0.1	103	0.07	16
	15	rs2016276		13	rs1886510*	0.1	86	0.06	26 (25)
	16	rs729172		9	rs1015250*	0.1	95	0.15	30 (29)
	17	rs740910		10	rs735155	0.15	100	0.15	34
	18	rs1493232		49	rs1005533	0.1	107	0.1	36
	19	rs719366		11	rs901398*	0.15	70	0.1	44 (46)
	20	rs1031825		4	rs2046361*	0.3	79	0.15	47 (78)
	21	rs722098		25	rs873196	0.1	63	0.15	52
	22	rs733164		29	rs1024116	0.1	76	0.1	56
	23	rs826472		24	rs2831700	0.1	62	0.15	56
29 SBE Set	24	rs2831700		26	rs1382387	0.13	69	0.1	64
	25	rs873196		18	rs1493232*	0.1	59	0.2	68 (66)
	26	rs1382387		15	rs2016276	0.15	90	0.3	80
	27	rs2111980	13_{rd}	41	rs737681	0.1	96	0.07	16
	28	rs2056277		27	rs2111980	0.05	72	0.05	23
	29	rs1024116		36	rs2076848	0.1	89	0.08	27
	30	rs727811		21	rs722098*	0.15	81	0.15	32 (38)
	32	rs1413212		44	rs914165	0.1	100	0.15	32
	33	rs938283		8	rs763869*	0.15	100	0.2	40 (42)
	34	rs1979255		17	rs740910	0.1	87	0.15	42
	35	rs1463729		32	rs1413212	0.3	84	0.3	44
	36	rs2076848		6	rs1029047	0.25	100	0.4	54
	37	rs1355366		19	rs719366*	0.2	105	0.2	60 (58)
	38	rs907100		40	rs2040411	0.1	94	0.1	68
	39	rs354439		30	rs727811	0.15	78	0.15	72
	40	rs2040411		43	rs251934	0.15	98	0.2	88
	41	rs737681	13_{th}	7	rs917118*	0.1	87	0.15	20 (18)
	42	rs2830795		33	rs938283	0.08	85	0.1	22
	43	rs251934		38	rs907100	0.15	91	0.15	27
	44	rs914165		51	rs891700*	0.1	109	0.1	31 (32)
	45	rs10495407		48	rs964681	0.15	106	0.15	36
	46	rs1360288		52	rs1335873	0.15	110	0.1	40
	48	rs964681		42	rs2830795	0.1	97	0.15	44
	49	rs1005533		37	rs1355366	0.15	90	0.15	48
	50	rs8037429		54	rs1528460	0.15	115	0.15	60
	51	rs891700		45	rs10495407	0.4	102	0.2	68
	52	rs1335873		50	rs8037429	0.2	108	0.15	76
	53	rs1028528		35	rs1463729	0.1	87	0.15	84
	54	rs1528460		28	rs2056277	0.07	73	0.1	92

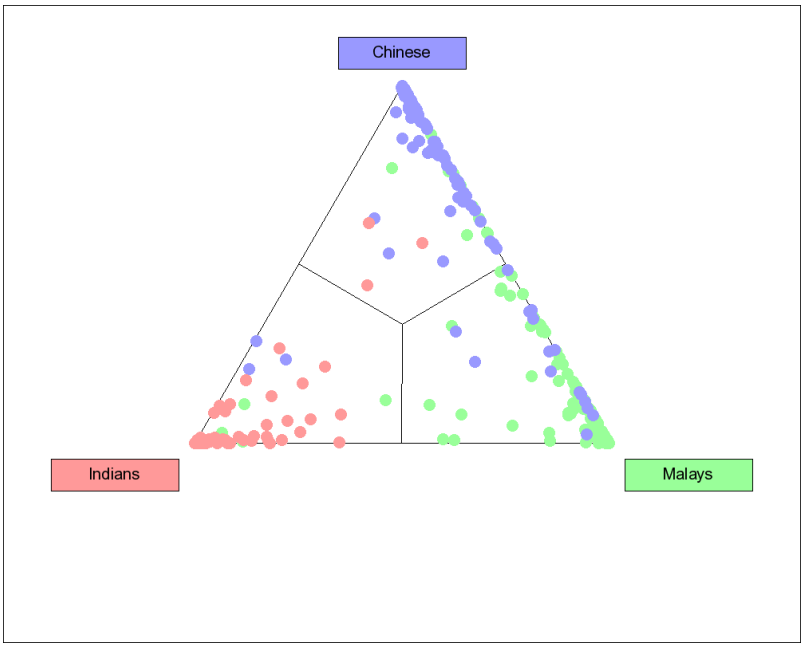
Note: * indicates modified SBE primers length. Number in brackets show the original SBE amplicon size [3].

Table 2 Ancestry predictions with A) Snipper, B) Arlequin and C) Snipper with cross validation.

	A	B	C	A	B	C	A	B	C
Population of Malay origin	82.57%	70.64%	64.22%	11.93%	17.76%	26.61%	5.50%	8.26%	9.17%
Population of Chinese origin	13.08%	21.10%	28.04%	84.11%	81.31%	68.22%	2.8%	2.75%	3.74%
Population of Indian origin	0.00%	8.26%	2.75%	2.75%	0.93%	4.59%	97.25%	88.99%	92.66%



Supplementary Figure 1 13ndplex profiles with input template DNA from 0.01 ng to 0.5 ng.



Supplementary Figure S2 Graphical representation of Snipper analysis. Each coloured dot represents an individual and the area that they are placed in represents the most likely population that they belong to based on their profile frequencies using each of the three reference allele frequency databases.

Supplementary Table S1 Allele frequency data for Malaysia and neighboring regions

Individual Identification SNPs (IISNPs)			Allele frequencies						
Marker Code	SNP	Allele	Malaysia			East Asia (N = 378)	Central - South Asia (N = 234)	Oceania (N = 28)	Europe (N = 1140)
			Malay (N=109)	M-Chinese (N=107)	M-Indian (N=109)				
1	rs1490413	C	0.427	0.444	0.486	0.423	0.482	0.333	0.564
		T	0.573	0.556	0.514	0.577	0.518	0.667	0.436
2	rs876724	C	0.647	0.682	0.674	0.564	0.724	0.981	0.685
		T	0.353	0.318	0.326	0.436	0.276	0.019	0.315
3	rs1357617	A	0.161	0.178	0.275	0.188	0.293	0.054	0.293
		T	0.839	0.822	0.725	0.812	0.707	0.946	0.707
4	rs2046361	A	0.454	0.449	0.385	0.417	0.432	0.593	0.350
		T	0.346	0.551	0.615	0.583	0.568	0.407	0.650
5	rs717302	A	0.734	0.855	0.601	0.848	0.625	0.696	0.503
		G	0.266	0.145	0.399	0.152	0.375	0.304	0.497
6	rs1029047	A	0.339	0.290	0.495	0.357	0.370	0.240	0.396
		T	0.661	0.710	0.505	0.643	0.630	0.760	0.604
7	rs917118	A	0.225	0.285	0.413	0.267	0.448	0.232	0.283
		G	0.775	0.715	0.587	0.733	0.552	0.768	0.717
8	rs763869	C	0.431	0.421	0.216	0.346	0.287	0.429	0.486
		T	0.569	0.579	0.784	0.654	0.713	0.571	0.514
9	rs1015250	C	0.573	0.491	0.596	0.535	0.716	0.179	0.786
		G	0.427	0.509	0.404	0.465	0.284	0.821	0.214
10	rs735155	C	0.179	0.126	0.339	0.190	0.393	0.643	0.536
		T	0.821	0.874	0.661	0.810	0.607	0.357	0.464
11	rs901398	C	0.257	0.294	0.335	0.265	0.274	0.214	0.326
		T	0.743	0.706	0.665	0.735	0.726	0.786	0.674
12	rs2107612	A	0.706	0.738	0.500	0.871	0.728	0.411	0.673
		G	0.294	0.262	0.500	0.129	0.272	0.589	0.327
13	rs1886510	A	0.142	0.159	0.271	0.117	0.209	0.161	0.436
		G	0.858	0.841	0.729	0.869	0.700	0.804	0.505
14	rs1454361	A	0.564	0.570	0.615	0.492	0.476	0.143	0.531
		T	0.436	0.430	0.385	0.508	0.524	0.857	0.469
15	rs2016276	C	0.349	0.350	0.261	0.377	0.214	0.054	0.231

		T	0.651	0.650	0.739	0.623	0.786	0.946	0.769
16	**rs729172**	G	0.844	0.860	0.858	0.836	0.726	0.946	0.598
		T	0.156	0.140	***0.142***	0.164	0.274	0.054	0.402
17	**rs740910**	A	0.867	0.916	0.839	0.922	0.738	0.815	0.702
		G	0.133	0.084	0.161	0.078	0.262	0.185	0.298
18	**rs1493232**	G	0.601	0.631	0.390	0.511	0.242	0.714	0.336
		T	0.399	0.369	0.610	0.489	0.758	0.286	0.664
19	**rs719366**	C	0.335	0.215	0.312	0.231	0.377	0.778	0.373
		T	0.665	0.785	0.688	0.769	0.623	0.222	0.627
20	**rs1031825**	G	0.463	0.486	0.546	0.568	0.577	0.714	0.731
		T	0.537	0.514	0.454	0.432	0.423	0.286	0.269
21	**rs722098**	A	0.583	0.486	0.555	0.446	0.680	0.500	0.782
		G	0.417	0.514	0.445	0.554	0.320	0.500	0.218
22	**rs733164**	A	0.280	0.164	0.183	0.145	0.252	0.463	0.296
		G	0.720	0.836	0.817	0.855	0.748	0.537	0.704
23	**rs826472**	A	0.202	0.121	0.390	0.174	0.312	0.648	0.376
		G	0.798	0.879	0.610	0.826	0.688	0.352	0.624
24	**rs2831700**	C	0.427	0.542	0.225	0.493	0.335	0.519	0.408
		T	0.573	0.458	0.775	0.507	0.665	0.481	0.592
25	**rs873196**	A	0.839	0.911	0.812	0.864	0.703	0.679	0.599
		G	0.161	0.089	0.188	0.136	0.297	0.321	0.401
26	**rs1382387**	G	0.413	0.393	0.408	0.304	0.293	0.185	0.319
		T	0.587	0.607	0.592	0.696	0.707	0.815	0.681
27	**rs2111980**	A	0.610	0.603	0.509	0.619	0.491	0.648	0.456
		G	0.390	0.397	0.491	0.381	0.509	0.352	0.544
28	**rs2056277**	A	0.170	0.121	0.206	0.133	0.167	0.056	0.259
		G	0.830	0.879	0.794	0.867	0.833	0.944	0.741
29	**rs1024116**	A	***0.096***	***0.079***	0.284	0.121	0.412	0.500	0.573
		G	0.904	0.921	0.716	0.879	0.588	0.500	0.427
30	**rs727811**	A	0.541	0.640	0.674	0.688	0.513	0.661	0.552
		C	0.459	0.360	0.326	0.312	0.487	0.339	0.448
32	**rs1413212**	C	0.422	0.341	0.390	0.545	0.656	0.574	0.697

		T	0.578	0.659	0.610	0.455	0.344	0.426	0.303
33	**rs938283**	C	0.284	0.150	0.161	0.150	0.103	0.019	0.169
		T	0.716	0.850	0.839	0.850	0.897	0.981	0.831
34	**rs1979255**	C	0.459	0.444	0.372	0.436	0.435	0.268	0.339
		G	0.541	0.556	0.628	0.564	0.565	0.732	0.661
35	**rs1463729**	A	0.495	0.533	0.454	0.449	0.507	0.058	0.556
		G	0.505	0.467	0.546	0.551	0.493	0.942	0.444
36	**rs2076848**	A	0.252	0.322	0.436	0.340	0.446	0.286	0.441
		T	0.748	0.678	0.564	0.660	0.554	0.714	0.559
37	**rs1355366**	C	0.330	0.164	0.518	0.157	0.473	0.096	0.421
		T	0.670	0.836	0.482	0.843	0.527	0.904	0.579
38	**rs907100**	C	0.468	0.533	0.463	0.550	0.550	0.786	0.606
		G	0.532	0.467	0.537	0.450	0.450	0.214	0.394
39	**rs354439**	A	0.408	0.421	0.569	0.431	0.638	0.875	0.418
		T	0.592	0.579	0.431	0.569	0.362	0.125	0.582
40	**rs2040411**	A	0.326	0.332	0.472	0.271	0.461	0.750	0.629
		G	0.674	0.668	0.528	0.729	0.539	0.250	0.371
41	**rs737681**	C	0.835	0.864	0.606	0.827	0.651	0.982	0.594
		T	0.165	0.136	0.394	0.173	0.349	0.018	0.406
42	**rs2830795**	A	0.399	0.537	0.706	0.516	0.770	0.821	0.709
		G	0.601	0.463	0.294	0.484	0.230	0.179	0.291
43	**rs251934**	A	0.867	0.888	0.688	0.875	0.758	0.696	0.614
		G	0.133	0.112	0.312	0.125	0.242	0.304	0.386
44	**rs914165**	C	0.706	0.640	0.578	0.689	0.571	0.286	0.604
		T	0.294	0.360	0.422	0.311	0.429	0.714	0.396
45	**rs10495407**	C	0.720	0.696	0.844	0.671	0.740	0.978	0.643
		T	0.280	0.304	0.156	0.329	0.260	0.022	0.357
46	**rs1360288**	A	0.220	0.350	0.486	0.347	0.373	0.074	0.335

		G	0.780	0.650	0.514	0.653	0.627	0.926	0.665
48	**rs964681**	C	0.335	0.360	0.436	0.298	0.423	0.375	0.417
		T	0.665	0.640	0.564	0.702	0.577	0.625	0.583
49	**rs1005533**	A	0.197	0.290	0.335	0.332	0.399	0.519	0.536
		G	0.803	0.710	0.665	0.668	0.601	0.481	0.464
50	**rs8037429**	A	0.422	0.374	0.390	0.456	0.543	0.583	0.516
		G	0.578	0.626	0.610	0.544	0.457	0.417	0.484
51	**rs891700**	A	0.472	0.458	0.422	0.504	0.417	0.357	0.475
		G	0.528	0.542	0.578	0.496	0.583	0.643	0.525
52	**rs1335873**	A	0.716	0.617	0.702	0.658	0.728	0.923	0.691
		T	0.284	0.383	0.298	0.342	0.272	0.077	0.309
53	**rs1028528**	A	0.555	0.589	0.550	0.688	0.709	0.232	0.742
		G	0.445	0.411	0.450	0.312	0.291	0.768	0.258
54	**rs1528460**	A	0.528	0.477	0.523	0.616	0.603	0.250	0.690
		G	0.472	0.523	0.477	0.384	0.397	0.750	0.310

*Bold and italic number(s) represent minor allele frequency in three ethnics: the Malays, Chinese and Indians.

Supplementary Table S2 Forensic parameters calculated for 52 SNPs for each Malaysian population group. MP (match probability); PD (power of discrimination); and PIC (polymorphisms information content).

Marker Code	SNP (rs#)	Allele (1/2)	Malay			Chinese			Indian		
			MP	**PD**	**PIC**	**MP**	**PD**	**PIC**	**MP**	**PD**	**PIC**
1	rs1490413	T/C	0.379	0.621	0.37	0.351	0.649	0.37	0.383	0.617	0.37
2	rs876724	C/T	0.420	0.580	0.35	0.409	0.591	0.34	0.404	0.596	0.34
3	rs1357617	T/A	0.567	0.433	0.23	0.543	0.457	0.25	0.474	0.526	0.32
4	rs2046361	T/A	0.373	0.627	0.37	0.404	0.596	0.37	0.377	0.623	0.36
5	rs717302	A/G	0.433	0.557	0.31	0.607	0.393	*0.22*	0.419	0.581	0.36
6	rs1029047	A/T	0.397	0.603	0.35	0.431	0.569	0.33	0.357	0.643	0.37
7	rs917118	G/A	0.485	0.515	0.29	0.457	0.543	0.32	0.419	0.581	0.37
8	rs763869	C/T	0.355	0.645	0.37	0.373	0.627	0.37	0.501	0.499	0.28
9	rs1015250	G/C	0.371	0.629	0.37	0.364	0.636	0.37	0.369	0.631	0.37
10	rs735155	T/C	0.546	0.454	0.25	0.628	0.372	*0.20*	0.397	0.603	0.35
11	rs901398	C/T	0.456	0.544	0.31	0.418	0.582	0.33	0.402	0.598	0.35
12	rs2107612	A/G	0.442	0.558	0.33	0.467	0.533	0.31	0.563	0.437	0.38
13	rs1886510	G/A	0.593	0.407	*0.21*	0.566	0.434	*0.23*	0.453	0.547	0.32
14	rs1454361	A/T	0.369	0.631	0.37	0.409	0.591	0.37	0.383	0.617	0.36
15	rs2016276	T/C	0.391	0.609	0.35	0.395	0.605	0.35	0.447	0.553	0.31
16	rs729172	T/G	0.579	0.421	*0.23*	0.610	0.390	*0.21*	0.627	0.373	*0.21*
17	rs740910	A/G	0.609	0.391	0.20	0.720	0.280	*0.14*	0.575	0.425	*0.23*
18	rs1493232	T/G	0.408	0.592	0.36	0.417	0.583	0.36	0.378	0.622	0.36
19	rs719366	C/T	0.423	0.577	0.35	0.496	0.504	0.28	0.416	0.584	0.34
20	rs1031825	T/G	0.390	0.610	0.37	0.399	0.601	0.37	0.414	0.586	0.37
21	rs722098	A/G	0.391	0.609	0.37	0.399	0.601	0.37	0.614	0.386	0.37
22	rs733164	A/G	0.436	0.564	0.32	0.575	0.425	*0.24*	0.534	0.466	0.25
23	rs826472	G/A	0.511	0.489	0.27	0.638	0.362	*0.19*	0.378	0.622	0.36
24	rs2831700	C/T	0.379	0.621	0.37	0.402	0.598	0.37	0.486	0.514	0.29
25	rs873196	A/G	0.580	0.420	*0.23*	0.717	0.283	*0.15*	0.528	0.472	0.26
26	rs1382387	G/T	0.528	0.472	0.37	0.447	0.553	0.36	0.440	0.560	0.37
27	rs2111980	A/G	0.411	0.589	0.36	0.378	0.622	0.36	0.398	0.602	0.37
28	rs2056277	A/G	0.522	0.448	*0.24*	0.652	0.348	*0.19*	0.506	0.494	0.27
29	rs1024116	A/G	0.706	0.294	*0.16*	0.733	0.267	*0.14*	0.426	0.574	0.32
30	rs727811	A/C	0.386	0.614	0.37	0.403	0.597	0.35	0.459	0.541	0.34
32	rs1413212	C/T	0.457	0.543	0.37	0.415	0.585	0.35	0.476	0.524	0.36
33	rs938283	C/T	0.434	0.566	0.32	0.597	0.403	*0.22*	0.575	0.425	*0.23*
34	rs1979255	G/C	0.354	0.646	0.37	0.370	0.630	0.37	0.381	0.619	0.36
35	rs1463729	A/G	0.382	0.618	0.37	0.370	0.630	0.37	0.414	0.586	0.37
36	rs2076848	A/T	0.470	0.530	0.31	0.420	0.580	0.34	0.550	0.450	0.37
37	rs1355366	C/T	0.440	0.560	0.34	0.560	0.440	*0.24*	0.388	0.612	0.37
38	rs907100	G/C	0.336	0.664	0.37	0.349	0.651	0.37	0.343	0.647	0.37
39	rs354439	A/T	0.365	0.635	0.37	0.400	0.600	0.37	0.366	0.634	0.37
40	rs2040411	A/G	0.414	0.586	0.34	0.401	0.599	0.35	0.412	0.588	0.37
41	rs737681	C/T	0.560	0.440	*0.24*	0.609	0.391	*0.21*	0.367	0.633	0.36
42	rs2830795	A/G	0.398	0.602	0.36	0.434	0.566	0.37	0.436	0.564	0.33
43	rs251934	A/G	0.625	0.375	*0.20*	0.666	0.334	*0.18*	0.428	0.572	0.34
44	rs914165	C/T	0.419	0.581	0.33	0.384	0.616	0.35	0.369	0.631	0.37
45	rs10495407	C/T	0.433	0.567	0.32	0.422	0.578	0.33	0.571	0.429	*0.23*
46	rs1360288	A/G	0.490	0.510	0.28	0.524	0.476	0.35	0.417	0.583	0.37
48	rs964681	C/T	0.408	0.592	0.35	0.432	0.568	0.35	0.377	0.623	0.37
49	rs1005533	A/G	0.517	0.483	0.27	0.442	0.558	0.33	0.432	0.568	0.35
50	rs8037429	A/G	0.394	0.606	0.37	0.443	0.557	0.36	0.372	0.628	0.36
51	rs891700	A/G	0.379	0.621	0.37	0.351	0.649	0.37	0.416	0.584	0.37
52	rs1335873	T/A	0.443	0.557	0.32	0.405	0.595	0.36	0.418	0.582	0.33

53	rs1028528	A/G	0.429	0.571	0.37	0.354	0.646	0.37	0.362	0.638	0.37
54	rs1528460	A/G	0.389	0.611	0.37	0.666	0.334	0.37	0.553	0.467	0.37
			MP = 1 in 3.9654e^{-19} PD >99.9999%			**MP = 1 in 5.3964e^{-18} PD >99.9999%**			**MP = 1 in 1.7459e^{-19} PD >99.9999%**		

Supplementary Table S3 Observed ($Obs._H$) and expected ($Exp._H$) heterozygosities for 52 SNPs typed in 325 individuals from three Malaysian ethnic groups.

Marker Code	SNP (rs#)	Malay			Chinese			Indian		
		$Obs._H$	$Exp._H$	P-value	$Obs._H$	$Exp._H$	P-value	$Obs._H$	$Exp._H$	P-value
1	rs1490413	0.50	0.50	1.00	0.41	0.49	0.12	0.51	0.50	0.85
2	rs876724	0.50	0.46	0.40	0.40	0.43	0.51	0.41	0.44	0.52
3	rs1357617	0.28	0.27	0.73	0.29	0.29	1.00	0.50	0.40	***0.0166***
4	rs2046361	0.50	0.50	1.00	0.54	0.50	0.43	0.44	0.48	0.54
5	rs717302	0.33	0.39	0.14	0.21	0.25	0.23	0.55	0.48	0.17
6	rs1029047	0.40	0.44	0.39	0.42	0.42	1.00	0.46	0.50	0.44
7	rs917118	0.32	0.35	0.41	0.48	0.41	0.10	0.55	0.49	0.23
8	rs763869	0.42	0.49	0.17	0.47	0.49	0.70	0.39	0.34	0.15
9	rs1015250	0.47	0.49	0.70	0.48	0.50	0.70	0.44	0.48	0.43
10	rs735155	0.28	0.30	0.35	0.23	0.22	1.00	0.42	0.45	0.53
11	rs901398	0.38	0.38	1.00	0.35	0.42	0.10	0.43	0.45	0.83
12	rs2107612	0.38	0.44	0.13	0.44	0.38	0.20	0.72	0.50	****0.00000***
13	rs1886510	0.28	0.25	0.12	0.32	0.27	0.07	0.43	0.40	0.47
14	rs1454361	0.47	0.49	0.70	0.54	0.49	0.33	0.46	0.48	0.84
15	rs2016276	0.42	0.46	0.53	0.44	0.46	0.68	0.31	0.38	0.08
16	rs729172	0.25	0.26	0.72	0.22	0.24	0.43	***0.17***	0.23	***0.0171***
17	rs740910	0.27	0.23	0.21	0.17	0.16	1.00	0.25	0.27	0.47
18	rs1493232	0.52	0.48	0.43	0.51	0.47	0.40	0.44	0.48	0.43
19	rs719366	0.49	0.45	0.40	0.34	0.34	1.00	0.42	0.43	0.83
20	rs1031825	0.52	0.50	0.70	0.54	0.50	0.44	0.56	0.50	0.25
21	rs722098	0.49	0.49	1.00	0.54	0.50	0.44	0.76	0.50	****0.00000***
22	rs733164	0.39	0.40	0.81	0.23	0.27	0.15	0.31	0.30	1.00
23	rs826472	0.35	0.32	0.56	0.22	0.21	1.00	0.45	0.48	0.55
24	rs2831700	0.49	0.49	1.00	0.54	0.50	0.44	0.35	0.35	1.00
25	rs873196	0.23	0.27	0.15	***0.15***	0.16	0.59	0.32	0.31	0.76
26	rs1382387	0.68	0.49	****0.000***	0.58	0.48	***0.05***	0.58	0.49	***0.05***
27	rs2111980	0.52	0.48	0.42	0.46	0.48	0.69	0.54	0.50	0.45
28	rs2056277	0.32	0.28	0.30	0.19	0.21	0.18	0.34	0.33	1.00
29	rs1024116	***0.16***	0.17	0.25	***0.15***	0.15	1.00	0.33	0.41	***0.05***
30	rs727811	0.51	0.50	0.85	0.48	0.46	0.83	0.54	0.44	***0.0194***
32	rs1413212	0.61	0.49	***0.0194***	0.47	0.45	0.83	0.61	0.48	***0.0025***
33	rs938283	0.40	0.42	0.82	0.22	0.26	0.25	0.25	0.27	0.47
34	rs1979255	0.44	0.50	0.25	0.48	0.50	0.70	0.43	0.47	0.42
35	rs1463729	0.51	0.50	0.85	0.49	0.50	0.85	0.56	0.50	0.25
36	rs2076848	0.43	0.39	0.32	0.46	0.44	0.82	0.71	0.49	****0.00000***
37	rs1355366	0.51	0.44	0.13	0.33	0.27	0.07	0.52	0.50	0.70
38	rs907100	0.35	0.50	***0.0016***	0.43	0.50	0.17	0.27	0.50	****0.00000***
39	rs354439	0.43	0.49	0.32	0.52	0.49	0.55	0.46	0.49	0.56
40	rs2040411	0.45	0.45	1.00	0.42	0.45	0.66	0.56	0.50	0.25
41	rs737681	0.29	0.28	0.73	0.25	0.23	0.69	0.42	0.48	0.23

42	rs2830795	0.50	0.48	0.70	0.59	0.50	0.08	0.44	0.42	0.65
43	rs251934	0.21	0.23	0.40	0.19	0.20	0.62	0.46	0.43	0.66
44	rs914165	0.35	0.42	0.11	0.42	0.46	0.40	0.46	0.49	0.56
45	rs10495407	0.39	0.41	0.64	0.42	0.42	1.00	0.31	0.26	0.07
46	rs1360288	0.33	0.35	0.78	0.65	0.46	***0.00000***	0.57	0.50	0.18
48	rs964681	0.45	0.45	1.00	0.51	0.47	0.40	0.49	0.49	1.00
49	rs1005533	0.32	0.32	1.00	0.45	0.41	0.48	0.50	0.45	0.20
50	rs8037429	0.51	0.49	0.70	0.55	0.47	0.10	0.43	0.48	0.32
51	rs891700	0.50	0.50	1.00	0.43	0.50	0.17	0.54	0.49	0.32
52	rs1335873	0.44	0.41	0.49	0.50	0.47	0.55	0.38	0.42	0.36
53	rs1028528	0.58	0.50	0.12	0.39	0.49	***0.0443***	0.46	0.50	0.44
54	rs1528460	0.52	0.50	0.70	0.80	0.50	***0.00000***	0.70	0.50	***0.00000***
Mean		**0.42**	**0.41**		**0.41**	**0.40**		**0.46**	**0.44**	
s.d.		**0.11**	**0.09**		**0.14**	**0.12**		**0.12**	**0.08**	

Supplementary Table S4 Results obtained from the analysis of 15 STRs and 52 SNPs with 51 casework samples.

Case	Samples	In-house Labeled	52 SNPs	Identifiler/Powerplex® Sytem
1	Bloodstains on the pillow case	F1	FP	FP
	Bloodstains on the bedsheet	F2	FP	PP-13
	Bloodstains on the blanket	F3	FP	FP
2	Bloodstains on the mat	F4	FP	FP
	Bloodstains on the T-shirt	F5	FP	FP
	Bloodstains on the pair of jeans	F6	FP	PP-7
	Bloodstains on the panties	F7	FP	FP
3	Bloodstains on the blouse	F8	FP	PP-8
	Bloodstains on the skirt	F9	FP	FP
	Bloodstains on the brassiere	F10	FP	PP-8
4	Bloodstains on the comforter 1	F11	FP	FP
	Bloodstains on the comforter 2	F12	FP	FP
5	Bloodstains on the towel	F13	PP-26	PP-8
	Bloodstains on the pair of jeans	F14	PP-39	NP
	Bloodstains on the blouse	F15	NP	NP
	Bloodstains on the camisole	F16	FP	FP
	Bloodstains on the skirt	F17	FP	FP
	Bloodstains on the sarong	F18	FP	FP
6	Swab of the cable 1	F19	NP	NP
	Swab of the cable 2	F20	NP	NP
7	Bloodstains on the blanket 1	F21	NP	NP
	Bloodstains on the blanket 2	F22	FP	FP
	Bloodstains on the shirt 1	F23	FP	NP
	Bloodstains on the shirt 2	F24	FP	NP
	Bloodstains on the shirt 3	F25	FP	NP
	Bloodstains on the shirt 4	F26	FP	NP
	Bloodstains on the shirt 5	F27	FP	PP-11
	Bloodstains on the shirt 6	F28	FP	PP-13
	Bloodstains on the shirt 7	F29	FP	FP
	Bloodstains on the shirt 8	F30	FP	Weak/Inconclusive
8	Bloodstains on the plastic bag 1	F31	PP-50	NP
	Bloodstains on the shirt	F32	PP-47	NP
	Bloodstains on the pair of jeans	F33	FP	FP
	Bloodstains on the towel	F34	FP	FP
	Bloodstains on the tissue paper	F35	NP	NP
	Bloodstains on the string 1	F36	FP	NP
	Bloodstains on the string 2	F37	FP	NP
	Bloodstains on the plastic bag 2	F38	NP	NP
9	Bloodstains on the pair of short pants	F39	FP	PP-7
	Bloodstains on the T-shirt	F40	NP	NP
	Bloodstains on the underwear	F41	PP-33	PP-7
	Bloodstains on the baton	F42	FP	FP
10	Bloodstains on the shirt 1	F43	FP	FP
	Bloodstains on the shirt 2	F44	FP	FP
	Bloodstains on the pairs of short pants	F45	FP	FP
	Bloodstains on the underwear	F46	FP	NP
	Bloodstains on the singlet	F47	FP	NP
	Bloodstains on the pair of short pants	F48	FP	NP
	Bloodstains on the panties	F49	NP	NP
	Bloodstains on the brassiere	F50	NP	NP
	Bloodstains on the slippers	F51	NP	NP

Notes: FP- Full profile, PP- Partial profile and NP- No profile.

Supplementary Table 5 Statistical evaluation of SNP data using DNA.VIEW.

Case	Samples	In-house Labeled	Source of the samples	52 SNPs	Theta (θ)	
					0.01	0.03
1	Bloodstains on the pillow case	F1	Samples are from the same source.	FP	$1e^{-18}$	$720e^{-15}$
	Bloodstains on the bedsheet	F2		FP		
	Bloodstains on the blanket	F3		FP		
2	Bloodstains on the mat	F4	Samples are from the same source.	FP	$23e^{-18}$	$15e^{-18}$
	Bloodstains on the T-shirt	F5		FP		
	Bloodstains on the pair of jeans	F6		FP		
	Bloodstains on the panties	F7		FP		
3	Bloodstains on the blouse	F8	Samples are from the same source.	FP	$12e^{-18}$	$8.6e^{-18}$
	Bloodstains on the skirt	F9		FP		
	Bloodstains on the brassiere	F10		FP		
4	Bloodstains on the comforter 1	F11	Samples are from the same source.	FP	$2.7e^{-18}$	$1.9e^{-18}$
	Bloodstains on the comforter 2	F12		FP		
5	Bloodstains on the towel	F13	Samples are from the same source.	PP-26	$1.7e^{-12}$	$1.2e^{-12}$
	Bloodstains on the pair of jeans	F14		PP-39	$17e^{-15}$	$11e^{-15}$
	Bloodstains on the blouse	F15		NP		
	Bloodstains on the camisole	F16	Samples are from the same source.	FP	$3.2e^{-21}$	$1.9e^{-21}$
	Bloodstains on the skirt	F17		FP		
	Bloodstains on the sarong	F18		FP		
6	Swab of the cable 1	F19		NP		
	Swab of the cable 2	F20		NP		
7	Bloodstains on the blanket 1	F21		NP		
	Bloodstains on the blanket 2	F22	Samples are from the same source.	FP	$200e^{-21}$	$110e^{-21}$
	Bloodstains on the shirt 1	F23		FP		
	Bloodstains on the shirt 2	F24		FP		
	Bloodstains on the shirt 3	F25		FP		
	Bloodstains on the shirt 4	F26		FP		
	Bloodstains on the shirt 5	F27		FP		
	Bloodstains on the shirt 6	F28		FP		
	Bloodstains on the shirt 7	F29		FP		
	Bloodstains on the shirt 8	F30		FP		
8	Bloodstains on the plastic bag 1	F31	Samples are from the same source.	PP-50	$41e^{-18}$	$28e^{-18}$
	Bloodstains on the shirt	F32		PP-47	$51e^{-18}$	$30e^{-18}$
	Bloodstains on the pair of jeans	F33		FP	$32e^{-18}$	$23e^{-18}$
	Bloodstains on the towel	F34		FP		
	Bloodstains on the tissue paper	F35		NP		
	Bloodstains on the string 1	F36	Samples are from the same source.	FP	$32e^{-18}$	$23e^{-18}$
	Bloodstains on the string 2	F37		FP		
	Bloodstains on the plastic bag 2	F38		NP		
9	Bloodstains on the pair of short pants	F39	Samples are from the same source.	FP	$460e^{-18}$	$280e^{-18}$
	Bloodstains on the T-shirt	F40		NP		
	Bloodstains on the underwear	F41	Samples are from the same source.	PP-33	$28e^{-9}$	$23e^{-9}$
	Bloodstains on the baton	F42		FP	$460e^{-18}$	$280ev^{18}$
10	Bloodstains on the shirt 1	F43		FP	$570e^{-18}$	$360e^{-18}$
	Bloodstains on the shirt 2	F44		FP		
	Bloodstains on the pairs of short pants	F45		FP		
	Bloodstains on the underwear	F46		FP		
	Bloodstains on the singlet	F47		FP		
	Bloodstains on the pair of short pants	F48		FP		
	Bloodstains on the panties	F49		NP		
	Bloodstains on the brassiere	F50		NP		
	Bloodstains on the slippers	F51		NP		

Supplementary Table 6 Paternity cases evaluated using 15 STRs (PowerPlex 16) and 52 SNPs.

Case	Remarks	Combined paternity index (CPI)		Probability of paternity (%)	
		15 STRs	**52 SNPs**	**15 STRs**	**52 SNPs**
1	Trio	$1.09e^{6}$	$2.16e^{6}$	99.99991	99.99995
2	Duo	179401	796.42	99.9994	99.9
3	Trio	$86.55e^{6}$	$7.55e^{6}$	99.999999	99.99999
4	Trio	$43.41e^{6}$	$9.46e^{6}$	99.999998	99.99999
5	*Trio- 1 mutation*	*87851*	*$14.15e^{6}$*	*99.999*	*99.999993*
6	Trio	$11.407e^{9}$	$328.849e^{6}$	99.99999999	99.9999997
7	Duo	108.978	$5.14e^{6}$	99.09	99.99998
8	Control 1-a family of 5 (father and mother) with: Child C Child D *Child E* *(2 mutations)*	 $516.24e^{6}$ $27.72e^{6}$ *54.44*	 $3.96e^{6}$ $325.84e^{6}$ *$3.26e^{6}$*	 99.9999998 99.999996 *98.2*	 99.99997 99.9999997 *99.99997*
9	Duo	275561	77.32	99.9996	98.7
10	Duo	$5.24e^{6}$	409.15	99.99998	99.8
11	Trio	$193.90e^{6}$	$17.43e^{6}$	99.9999995	99.999994