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Investigation of 74 microhaplotypes for kinship testing in US populations

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ABSTRACT

Microhaplotypes are markers that consist of haplotype blocks of SNPs, which can be analyzed by massively parallel sequencing technologies. These allow determining the haplotype phase at every locus by clonal sequencing each DNA strand. MHs are polymorphic loci with same size alleles, no stutter, and lower mutation rate than STRs. They can provide the same power of discrimination of STR-kits, thus useful for mixture deconvolution, but more accurate ancestry prediction than STRs. In this study we investigated the potential of a recently developed 74plex-MH panel for kinship testing using the Familias software.

Samples from families of four major US population groups were collected and genotyped using the 74plex-MH panel. MH allele frequency data from 347 individuals were imported into Familias software along with STR allele frequency data of 29 loci (NIST dataset) from 1036 individuals. Different family scenarios were tested and these included unrelated vs parent-child, unrelated vs full siblings, unrelated vs half siblings, unrelated vs cousin pairs. The prediction of the kinship relation for the four populations of interest was reported as Log10 of the likelihood ratio (LR).

Overall, the panel of 74MHs and 29STRs showed similar performance in predicting the correct kinship scenarios tested. Correct prediction was reported for parent-child, full siblings, and half sibling scenarios, but not for the cousin pairs scenario. The panel of 74 MHs showed larger Log10LR values than the 29 STR-assay, thus demonstrating the effectiveness of this biomarker as a tool for kinship testing in addition to mixture deconvolution and ancestry prediction.

1. Introduction

There is growing interest in novel biomarkers for forensic applications. Microhaplotypes (i.e., MHs), an example of such new markers, are haplotype blocks of SNPs that can supplement STR analysis [1]. These have recently gained significant ground within the forensic community. Massively parallel sequencing (MPS) allows determining the haplotype phase at each locus (the *cis/trans* relationship of heterozygous SNPs) by clonal sequencing each individual allele. MHs can be highly polymorphic, have same size alleles, no stutter, and lower mutation rate than STRs. They can reach the same power of discrimination as a panel of STRs and provide information on human identification, mixture deconvolution, ancestry prediction and kinship identification [2,3]. In this study, a recently developed 74 MH-assay [4] was tested for kinship analysis in four major US population groups using Familias software, and data were further compared to STR data.

2. Material and methods

In this study, samples from families of four population groups including African Americans, Caucasians, Hispanics and Asian Americans were genotyped using a previously developed 74plex MH assay [5]. To assess the potential of MHs in kinship prediction, simulation tests were performed in parallel using the panel of 74 MH and 29 STR (NIST dataset) markers through the open source Familias software [6,7], which was adapted for MH data intake.

A total of 1000 simulation tests for each test population/kinship scenario were performed in order to establish reliable likelihood ratio thresholds using population specific allele frequency databases. Allele frequency database of 74 MHs [4] and 29 STRs [8] tested on a total of 347 and 1036 individuals, respectively were imported into Familias software. The family scenarios investigated included unrelated vs parent-child, unrelated vs full siblings, unrelated vs half siblings, unrelated vs cousins.

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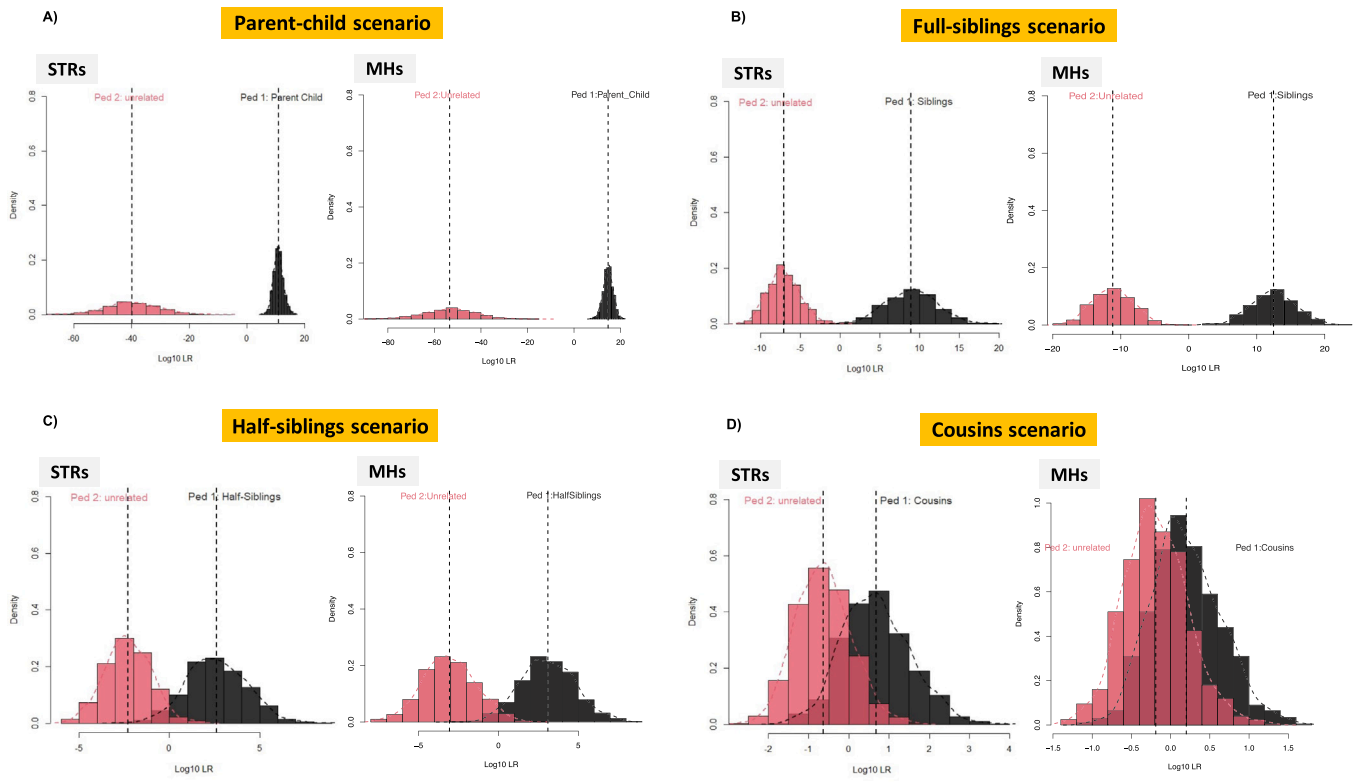

Fig. 1. Log10LR distribution curves of MHs and STRs for (A) parent-child, (B) full siblings, (C) half-siblings and (D) cousin pairs scenarios in African Americans.

3. Results and discussion

The prediction of the kinship relation for the population of interest is shown as Log10 of the likelihood ratio (Log10LR). The Log10LR distribution curves of unrelated pairs and related pairs are displayed in pink and black bars, respectively. In this study, MH and STR data from the African American population group are shown. For parent-child pairs, the Log10LR distribution curves of related pairs (in black) are well separated from the unrelated pairs for both STR and MH marker where MHs show larger LR values (Fig. 1(A)). When testing full-sibling pairs for both STRs and MHs, the Log10LR distribution of related pairs were well separated from unrelated pairs with the exception of few cases where overlap was reported for STR data only (Fig. 1(B)). When testing half-sibling pairs for STRs and MHs, the Log10LR distribution curves of half sibling pairs were separated from unrelated pairs; however, some overlap was reported for both STR and MH data (Fig. 1(C)). Under this scenario MHs showed higher Log10LR distribution values than STRs. Finally, when testing cousin pairs the Log10LR distribution values of related and unrelated pairs overlapped for STR and MH markers (Fig. 1 (D)). Overall, MHs and STR showed effective in predicting the correct kinship scenarios tested where the panel of 74 MHs showed larger Log10LR values than the 29 STR-assay for all scenarios investigated.

4. Conclusions

In conclusion, we investigated the performance of the sequence-based 74plex MH assay in kinship testing. This supports further the promising use of microhaplotype profiling as a versatile biomarker tool for forensically relevant applications.

Conflict of interest

The authors declare no conflict of interest.

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