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Performance of EuroForMix deconvolution on PowerPlex® Fusion 6C profiles

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ABSTRACT

Deconvolving mixed autosomal short tandem repeat profiles can be useful for database searching, profile comparisons and weight of evidence evaluations. This study describes results for the Top Marginal deconvolution within EuroForMix. Ratio To Next values were defined above which the major contributor's genotype in PowerPlex® Fusion 6C profiles was predicted correctly and can be used as thresholds for storage in a national DNA database.

1. Introduction

High-template DNA profiles are readily deconvolved in case of an unmistakable major and a single minor component. Complete deconvolution of complex mixed profiles is more challenging and is addressed through probabilistic genotyping software that generate a probability per genotype combination. One such software is EuroForMix (EFM) that displays the deconvolved genotypes in two formats: 1) as 'Top Marginal' in which for each locus a probability for the most probable (top) genotype for each contributor is given accompanied by the ratio to the next (RTN) most probable genotype (see Table 1 for an example), and 2) as 'All Joint' in which per locus a probability is presented for the considered contributors together. This study focuses on the prediction of the major contributor's genotype using the Top Marginal results.

2. Materials and methods

Two- and three-person (2p&3p) PowerPlex® Fusion 6C (PPF6C) profiles of donors with known genotypes were used that varied for donor combination, mixture proportion (2:1(:1), 10:1(:1), 5:1(:2), 20:1(:2)), amount of DNA (600-30pg per donor), level of drop-out and allele sharing. Amounting to a total of 69 2p profiles and 66 3p profiles including 3 replicates [1]. Laboratory procedures, analysis settings and EFM parameters were applied as described in [1]. Deconvolution was performed under Hd, using the true number of contributors, using the individual replicates as well as using the three replicates simultaneously. For each deconvolved mixture the Top Marginal table was compared to the genotypes of the known contributors.

3. Results and discussion

In this study, we aimed to predict the major's genotype with high confidence from 2p&3p PPF6C profiles using the Top Marginal table from EFM. Table 1 presents exemplar probabilities and RTN values for a 2p mixture. The height of the probability for a genotype may be indicative of the true genotype being deconvolved. However, occasionally we observed high probabilities (> 90%) for incorrectly inferred genotypes (3x observed) and low probabilities (< 10%) for correctly inferred genotypes (6x observed). Interestingly with such results, the RTN values accompanying the high probability were always relatively low (the highest probability given for an incorrect genotype was 99% with an associated RTN of 182). This implies that the RTN value may present a more practical selection method to derive the major's genotype with high confidence. Such an RTN threshold was used in a previous study [2], where it was set at two. An RTN of two may be too low to achieve inference of the major contributor's genotype sufficiently reliable for storage in a DNA database. Thus, we tested higher RTN values in this study: when regarding the individual replicates RTN values of > 100 and > 1000 were used for the 2p and 3p mixtures; when three replicates were analyzed jointly RTN values > 10 were applied for both the 2p and 3p mixtures. Loci with an RTN above these values were included in the inference and loci with an RTN below these values were excluded. These thresholds were functional as the genotype inference was correct at all included loci. When regarding the genotype inference for the excluded loci, both correct and incorrect inferences were obtained (Fig. 1, light green and light red bars respectively). This implies that the RTN threshold is functional but comes at cost of missing some of the correctly predicted genotypes (Fig. 1). Mixtures in which the

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Table 1
Example of the Top Marginal results table for a two-person mixture presenting the most probable genotype per contributor (C) per marker.

Locus	Genotype_C1	Probability_C1	RTN_C1	Genotype_C2	Probability_C2	RTN_C2
D1S1656	13/15.3	99.99%	1.59E+04	16/18.3	99.99%	1.59E+04
TPOX	8/8	100%	8.45E+04	8/11	99.95%	1.88E+03
D2S441	11/11	100%	1.28E+37	11/11	100%	1.46E+11
D2S1338	18/20	99.94%	2.66E+03	17/18	86.81%	7.71E+00
D3S1358	15/17	97.52%	5.04E+01	14/15	82.13%	7.83E+00
FGA	22/24	99.99%	1.51E+04	21/23	99.99%	1.51E+04
D5S818	…	…	…	…	…	…
CSF1PO	…	…	…	…	…	…

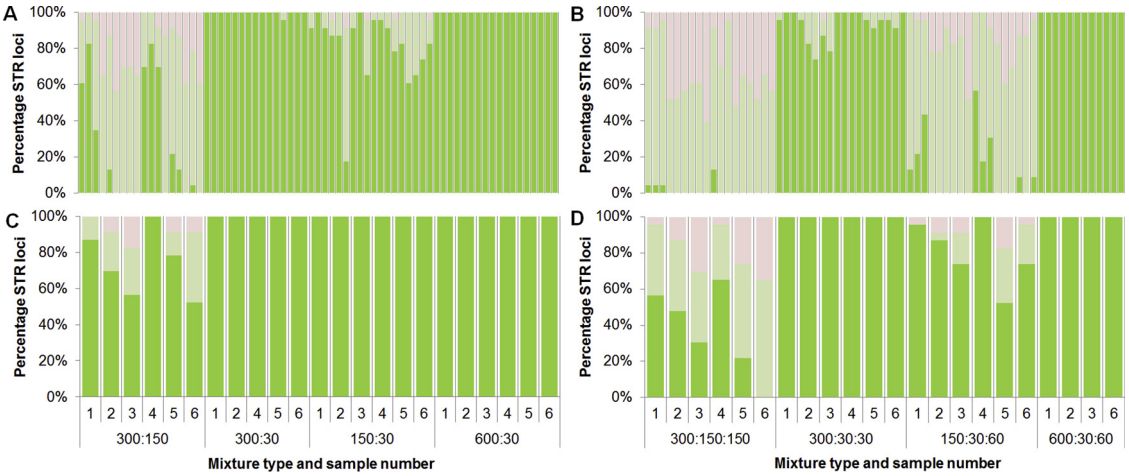

Fig. 1. Effect of RTN thresholds on 2p (A&C) and 3p (B&D) profiles with an individual replicate (A&B) or three replicates jointly (C&D). Loci with an RTN above the threshold and a correct inference are marked green, loci with an RTN below the threshold are marked light green in case of a correct inference and light red in case of an incorrect inference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

contributions are more close (2:1 instead of 10:1) performed less optimal than more unbalanced mixtures. With replicates, the percentages of correctly predicted genotypes above the RTN thresholds increased from 75% to 93% for 2p and 47% to 77% for 3p mixtures.

4. Concluding remarks

This study demonstrates that EFM can reliably infer the major contributor's genotype from 2p&3p PPF6C profiles when using the Top Marginal results and RTN thresholds. The RTN thresholds enable inference of the major contributor's genotype with high confidence and we consider these inferences suitable for uploading and storage in a national DNA database.

Declaration of Competing Interest

None.

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