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Detection of a minor contributor in a DNA sample mixture from human milk

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Abstract. We describe a method to detect very small amounts of DNA in mixed samples using commercially available multiplexes. DNA from whole breast milk samples was successfully extracted using the QIAamp® 96 DNA Blood Kit. We created volume/volume mixtures of milk samples to determine the minimum amount of a minor component that could be detected. Using modified amplification conditions and interpretation guidelines, we can detect the presence of a mixture containing 2% or less volume from the minor contributor. Thus, so long as the two donors provide equivalent DNA mass per milliliter of milk the minor component can be scored with as little as one part in 50 contribution. However, the DNA yield varies significantly among milk samples so the volume/volume ratio does not always reflect the DNA mass/DNA mass ratio in the mixture. In practice, we can generally detect the minor component of a mixture when this sample is mixed with 6 other samples and even when the minor component has a lower DNA yield per milliliter of milk.

Keywords: Breast milk; Mixtures; STR

1. Introduction

We received a number of breast milk samples from human donors and were asked by our supplier to determine whether pooled milk samples originate from one donor or from multiple donors. We evaluated methods of extracting DNA from milk samples and developed a method to directly compare a putative mixture sample to a reference sample in order to identify contaminating minor donor alleles.

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2. Materials and methods

Milk samples (200 μl) were extracted using the QIAamp® 96 DNA Blood Kit (QIAGEN, Inc., Valencia, CA) as recommended, except that during lysate preparation the amount of ethanol added was increased from 200 μl to 500 μl. DNA yield was measured with BodeQuant LCN, a real-time human DNA quantification method developed at The Bode Technology Group. DNA samples were amplified with AmpFLSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) for 30 cycles. Amplification products were separated and detected using the ABI PRISM® 3100 Genetic Analyzer and analyzed with GeneScan® and Genotyper® software (Applied Biosystems).

3. Results and discussion

We investigated different DNA extraction methods for purification of DNA from whole or fractionated human breast milk samples, including DNA IQ™ (Promega, Madison, WI USA), two QIAamp® DNA Blood Kits (QIAGEN), and FTA® Reagent extraction of samples spotted on FTA cards (Whatman, Florham Park, NJ USA). Whole milk samples and dilutions thereof were also added directly to amplification reactions to determine whether profiles could be generated from the original samples. The QIAamp DNA Blood Mini Kit and the QIAamp 96 DNA Blood Kit were equivalently successful in extracting DNA from whole milk samples, while the other methods provided poor yield or poor amplification results. Fractionation of the milk samples proved unnecessary for successful results with the QIAGEN kits.

The yield from 25 whole milk samples isolated with the QIAamp 96 DNA Blood Kit was measured and ranged from 12.7 ng to over 10 μg human DNA, with a median yield of 70.8 ng. The majority of cells in human breast milk are epithelial cells, and the significant variability seen is likely caused by the number of cells shed into the milk by different source individuals [1]. This may be correlated with the number of weeks since lactation began [2]. In each case, the DNA yield was more than sufficient to obtain a DNA profile with the Identifiler kit.

Mixtures of two donor milk samples were created to evaluate the minimum amount of a minor component that could be detected. Four donor milk samples (A–D) were tested separately and mixed in ratios of 98:2, 96:4, 92:8, and 88:12. Each was amplified and analyzed as described in Materials and methods. In order to maximize the detection of small allele peaks, DNA samples were not diluted prior to amplification and were amplified for 30 cycles. During analysis of the amplification reactions in Genotyper, the Kazam macro was used with no additional filter. These conditions led to a very high background in the genetic profile. To distinguish background peaks from true minor

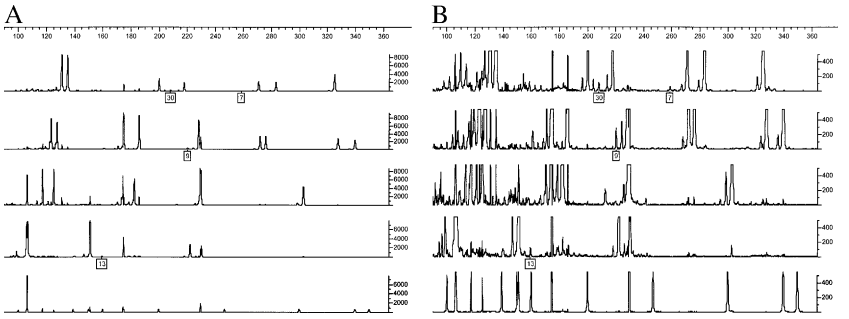

Fig. 1. Genetic profiles showing all peak labels removed except those from the contaminating minor donor DNA. (A) Default *y*-axis (RFU) values. (B) *y*-axis maximum set at 500 RFU to detect the true minor alleles more easily.

Table 1
Correlation between original sample volume in mixture, percent minor donor DNA actually present in mixture, and number of minor alleles detected

Percent volume milk sample per donor		Minor donor DNA in amplification reaction (ng)	Number of minor donor alleles detected
98% A	2% D	0.016	0
98% A	2% B	0.064	4
98% B	2% A	0.047	7
98% C	2% A	0.100	13

donor alleles, the mixture profile was directly compared with the major donor profile (reference sample) analyzed in the same way. The *y*-axis of each profile was set at 500 RFU to maximize visualization of small peaks. The comparison was done either on paper or within the Genotyper program. Major donor alleles and background peaks (stutter, pull up, and random noise) that appeared in both the reference and mixture profiles were eliminated from consideration, leaving only the true minor donor alleles. Fig. 1 shows an example of a mixture sample in which four minor donor alleles were detected.

The four donor samples used to create mixtures in this study ranged in concentration from 1 to 4 ng/μl. Thus, even when the volume of the minor donor sample was the same among mixtures, the mass amount of minor DNA was variable. This variability affected the ability to detect minor donor alleles, since in some cases the amount of minor DNA present was extremely low. Table 1 shows the calculated mass of minor component DNA present in each sample mixed together at the 98:2 volume ratio. The percentage of minor donor DNA ranged from 0.7% to 4.8% of the total DNA mass in the sample. In each amplification reaction, the total amount of DNA amplified was greater than 2 ng with 0.1 ng or less contributed by the minor donor. Despite this low amount of minor donor DNA, we were able to detect minor donor alleles in three of the four mixtures at the 98:2 ratio.

While as little as 2% by volume of a contaminating minor component may be detected using this comparative method, the variability in sample DNA concentration is a limiting factor. However, we find that even with a low concentration minor donor sample, we can generally detect a contaminating sample in a mixture of 1 part minor contributor to 6 parts major contributor. This method is most effective when the reference and mixture samples are derived from the same type of source tissue.

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