**Detection of a 1% to 2% Minor Contributor in a DNA Sample Mixture from Human Milk**

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We describe a method to detect very small amounts of DNA in a mixed sample using commercially available multiplexes. We have received a number of breast milk samples from human donors and have been asked by our supplier to determine whether pooled milk samples originate from one donor or from multiple donors. We determined that it is possible to extract DNA from whole milk samples using the QIAamp® DNA Blood Mini Kit. Total DNA yields from 200 microliters of milk were measured using the BodeQuant LCN method described in the accompanying work and these ranged from 6.5 ng to 205 ng.

The significant observed variability could be due to many causes such as sample age, care in handling by the original donor, or method of shipment to us. The primary cause of variability is likely differences in the numbers of cells shed into the milk by different source individuals. However, in each case, enough DNA was obtained to generate a DNA profile with the AmpFlSTR® Identifiler® kit. We then created volume/volume mixtures of milk samples in ratios of 98:2, 96:4, 92:8, and 88:12 to determine the minimum amount of the 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 of the total DNA content 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, we learned in our initial evaluations that the DNA yield per milliliter of milk varies significantly from sample to sample so that the volume:volume ratio does not always reflect the DNA mass:DNA mass ratio in the sample.

In practice, we can generally still detect the minor component of a mixture even when this sample is mixed with 6 other samples and even when the minor component has a lower DNA yield per milliliter of milk. We will discuss the methods that allow detection of mixtures at these low levels and how these results relate to evaluation of blood mixtures of similar imbalance.