Method comparison for circulating free fetal DNA (cffDNA) extraction from plasma samples of women during pregnancy in support of noninvasive perinatal sequencing for precision medicine prenatal care.

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Purpose: Conduct a pilot to determine which of two cell free DNA (cfDNA) extraction methods provided the best yields for sequencing work to identify fetal genetic abnormalities in a test set of genes.

Methods: We piloted two methods touted to maximize cfDNA recovery (including cffDNA). Method 1 used the magnetic-bead technology on PerkinElmer's Chemagic Magnetic Bead Separation module (MSM) with kit# CMG1304. Method 2 was a column-based extraction using the Omega Biotek E.Z.N.A cfDNA kit# D3091. For both methods the manufacturers' instructions were followed, using 4 mLs of plasma per extraction. Paired plasma samples from 6 individuals were used, with 4/6 individuals run in duplicate on each system. Anonymized plasma samples for the pilot study were obtained from both pregnant and non-pregnant women according to approved UNC IRB regulations. Quantification and DNA size analysis was performed on an Agilent Tape Station 4200 using the cfDNA screen tape kit.

Results: The median cfDNA yield for the 6 pairs analyzed from the MSM extraction was 11.46 ng (7.6-45.3), while the median yield for the column-based extraction was 3.95 ng (1.7-11.4). Tape Station analysis indicated that the DNA extracted was in the correct size range for cfDNA mono, di, and tri-nucleosome peaks. The median percentage in the cfDNA size range of 50 to 70 bp was 76% for both DNA extraction methods, indicating little genomic DNA contamination. Based on the pilot results, the magnetic bead method was selected to isolate 20 additional plasma samples from pregnant women. Isolates were sent together with their respective infant genomic DNA to the library preparation and sequencing facility. Library preparation on all samples was successful, and sequencing analysis is in progress.

Summary: Based on this pilot assay it was determined that the MSMI magnetic bead system yielded more cfDNA and was chosen for the main study of 20 plasma samples from 2nd-3rd trimester mothers.