Generation and Validation of Reference Standards for Non-Invasive Prenatal Testing (NIPT)

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Non-invasive prenatal testing (NIPT) has been widely adopted in clinical practice as a screening tool for fetal chromosomal abnormalities. However, there is little regulatory oversight on test performance underlining the need for appropriate reference standards that accurately mimic the complexity of NIPT samples to establish assay sensitivity, specificity and reproducibility. Here we describe our unique approach on the generation and validation of NIPT reference material from clinical fetal samples with or without chromosomal aneuploidies, and from corresponding maternal and paternal blood samples. We are the first to generate commutable, cell line-derived NIPT reference standards containing defined fractions of fetal DNA in a matched maternal DNA background that can be used by platform developers and laboratories to validate their assays and routinely monitor assay performance.

1. Project outline

Women carrying a fetus with high risk for a chromosomal aneuploidy
Based on NIPT, ultrasound, combined or quadruple test

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Invasive testing
Amniocentesis or chorionic villus sampling

Maternal & paternal blood

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QF-PCR for trisomy 13, 18 & 21 on fetal sample
(and Turner syndrome if scan is indicative)

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Culture of fetal cells for karyotyping or array
(if QF-PCR results positive)

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Results and clinical testing completed
Surplus fetal cell cultures used for this project

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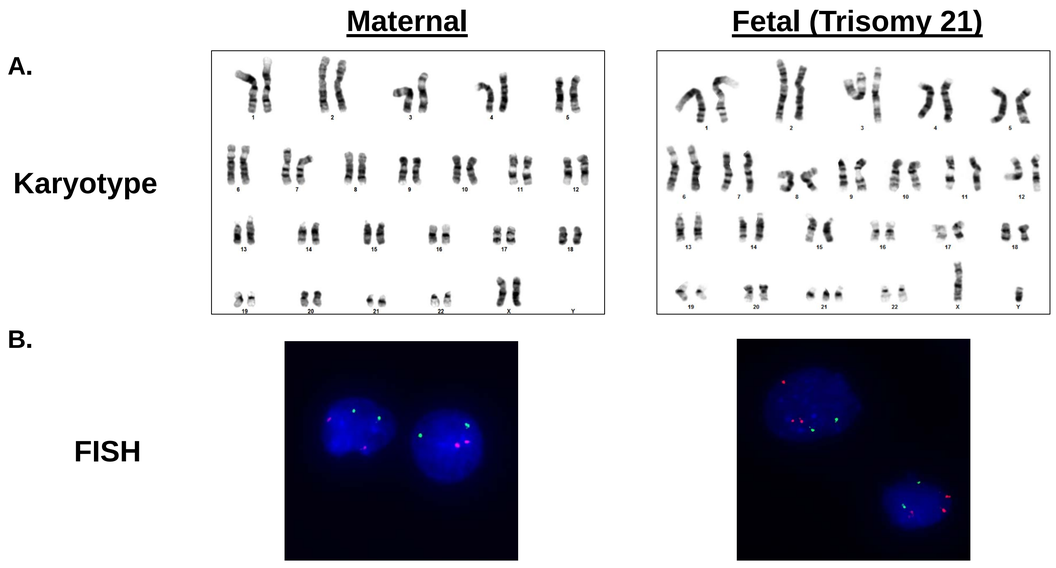
Immortalization, DNA extraction, fragmentation, blending, & characterization

- Euploid (Normal Karyotype)
- Trisomy 13,18, 21
- Klinefelter syndrome
- Turner Syndrome

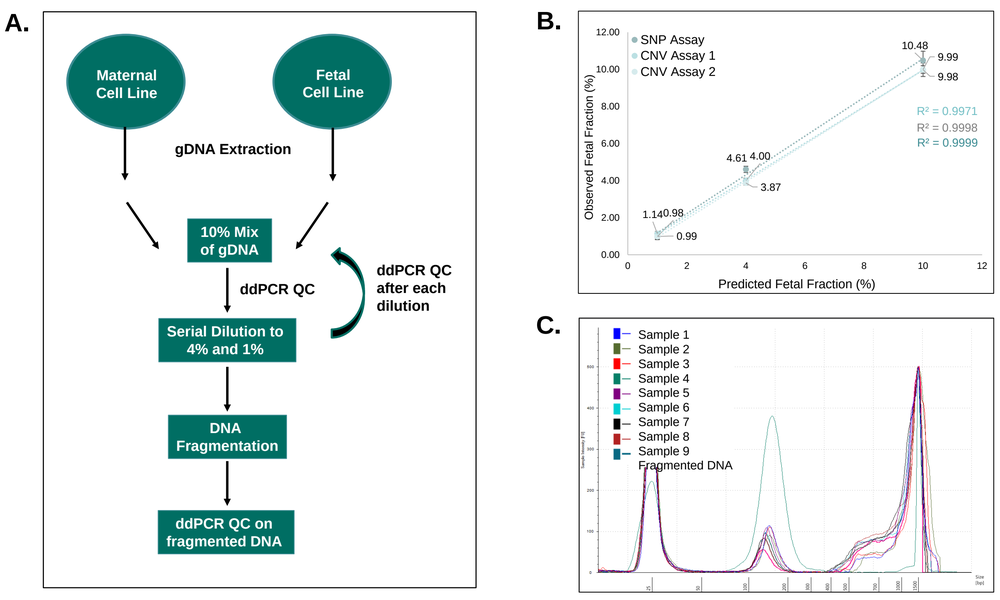
Different fetal fractions ranging from 1 to 20%

2. Cell Line Characterization



A. Representative karyotype analysis of immortalized cell lines showing a normal maternal karyotype (46, XX) and an abnormal fetal karyotype (47, XY, +21) in all cells analyzed. **B.** FISH analysis using Cytocell Prenatal Enumeration Probe Kit showing three copies of chromosome 21 (red) and two copies of chromosome 18 (green) in the fetal cell line and two copies both chromosome 21 and 18 in the maternal cell line.

3. Fragmentation / Quality Control



A. Flowchart describing how the fetal fraction test material was generated using matched maternal and fetal cell lines. **B.** Precise fetal fraction is determined using either copy number variation or SNP ddPCR assays. **C.** Agilent TapeStation size distribution analysis of fragmented DNA compared with 9 clinical cfDNA samples.

4. Conclusions and Outlook

- We have generated immortalized cell lines from matched clinical samples that remain stable over several passages, and can therefore be used as an unlimited source of cell line-derived reference DNA for NIPT
- We have shown that fragmentation of maternal and fetal DNA to 160 base pairs closely mimics the size distribution profile of clinical cfDNA
- Future studies are under way to characterize the performance of these preparations on different NIPT platforms

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