Poster ID # PRE347

Validation of Fetal and Maternal Recessive Disease Genotyping with FirstGene: a Combined, Non-Invasive Prenatal cfDNA Assay for Fetal Aneuploidy, Recessive Diseases, and Serological Screening

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Background

- The success of identifying couples at risk of having fetuses affected by recessive conditions depends on both partners receiving carrier screening. However, fewer than 50% of male partners complete the screening even when the maternal result is positive.[1]
- The FirstGene assay combines multiple prenatal risk assessments into a single blood draw, predicting fetal and maternal recessive disease status simultaneously, without the need for paternal screening **(Figure 1)**.

Objective: Here we describe the analytical validation of FirstGene for detecting fetal and maternal single nucleotide variants (SNVs) and small insertions or deletions (INDELs) in genes associated with serious recessive conditions.

Methods

- FirstGene is a hybridization capture-based deep sequencing assay. Powered by a novel dynamic *in silico* insert size analysis **(Figure 2)**, FirstGene allows observation of how allele balance (AB) signals change with fetal fraction (FF), termed the "AB trajectory". AB and AB trajectory are used to disambiguate fetal and maternal genotypes **(Figure 3)**.
- Fetal SNV and INDEL calling performance was evaluated using 79 plasma samples from 59 pregnant patients and 93 cell-line mixtures from 19 unique mother-child pairs with rare genotypes of interest.
- Maternal SNV and INDEL calling performance was evaluated using 264 plasma samples from 244 pregnant patients.
- FirstGene maternal and fetal variant calling in the recessive genes was evaluated by running corresponding genomic DNA (gDNA) on the previously validated Foresight Carrier Screen.[2] For patients with plasma samples, the gDNA was isolated from maternal whole blood or cultured fetal cells (gathered via chorionic villus sampling or amniocentesis). For cell-line mixtures, the single source gDNA samples were purchased from Coriell.

Conclusions

- FirstGene achieves high accuracy in detecting SNVs and INDELs in both fetuses and pregnant patients.
- The FirstGene screening approach streamlines the identification of at-risk pregnancies, especially in cases where carrier status is unknown for the reproductive partner.

Results

Figure 1. FirstGene provides a streamlined workflow for prenatal genetics risk assessment

1 person

1 test

FirstGene prenatal cfDNA screening

Prenatal cfDNA aneuploidy screening:
- T21, T18, T13, 22q, XX, XY, X, XXX, XXY, XYY

Maternofetal blood compatability:
- RHD

Maternal carrier screening:
- Core guidance genes: *CFTR, SMN1*
- Hemoglobinopathies: *HBB, HBA1/2*
- Expanded content: *HEXA, PMM2, ACADM, ASPA, DHCR7, PAH*, etc.
- Maternal-only: *FMR1* (via capillary electrophoresis)

Fetal recessive disease status (affected, carrier, or normal):
- Same genes as maternal carrier screening (except *FMR1*)

Fetal variant calling performance (Figure 4)

- FirstGene accurately detected 5090 SNVs and 122 INDELs in fetuses across 79 plasma samples. Overall sensitivity was 99.20%, and specificity was 99.79%.
- FirstGene correctly identified 5649 SNVs and 166 INDELs in fetuses among the 93 cell-line mixtures, resulting in 98.21% sensitivity and 99.00% specificity.
  - 13/15 (86.7%) cystic fibrosis fetuses with 2 *CTFR* variants were identified correctly (4 were caused by a homozygous CFTR ΔF508 variant and 9 were caused by a heterozygous state for 2 pathogenic variants).
  - The 2 mis-predicted as carriers had a 3% mixing ratio, with their FFs close to 3%. At low FF, clinical samples would be routinely failed.

Maternal variant calling performance (Figure 5)

- FirstGene correctly called the 17148 SNVs and 598 INDELs in the pregnant patients across 264 plasma samples, resulting in 99.90% sensitivity and >99.99% specificity.
- The majority of the maternal miscalls were in benign repetitive regions.

Figure 2. AB trajectory analysis to differentiate fetal and maternal genotypes

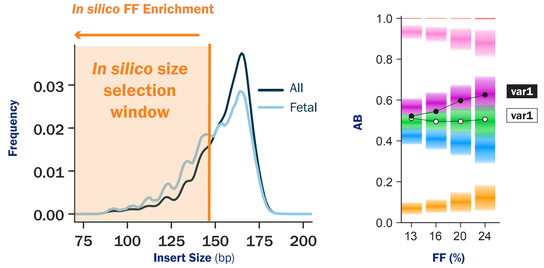
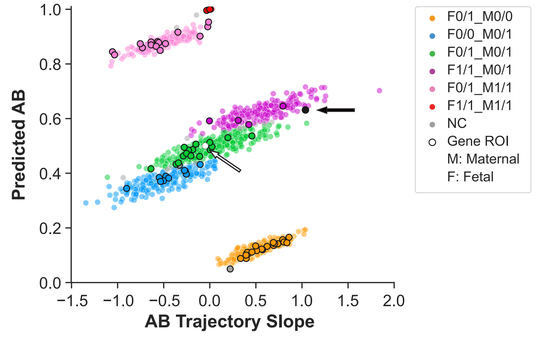


Figure 2: (left) Dynamic *in silico* FF enrichment by selecting reads across different insert sizes. **(right)** AB distributions shift with increasing FF from *in silico* size selection, allowing easy separation of variants (var1, var2) with different genotypes.

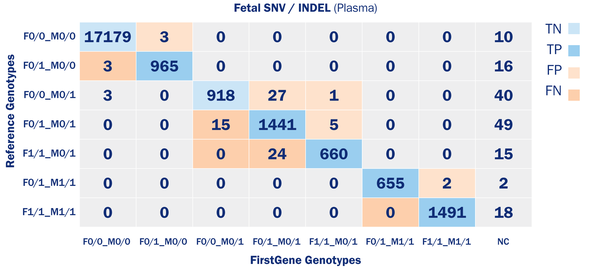
Figure 3. Fetal and maternal genotype prediction using an AB trajectory-based Gaussian Mixture Model (GMM)



Arrows highlight the locations of the same variants as in the right panel of Figure 2.

Figure 3: Each dot represents a single variant position. Arrows highlight the locations of var1 and var2 in the GMM space

Figure 4. Fetal variant calling performance



Sample Type		Variants	Sensitivity (%), 95% CI	Specificity (%), 95% CI
Fetal Results	Plasma (n=79)	All Variants	**99.20** (98.92 - 99.41)	**99.79** (99.71 - 99.85)
		SNV	**99.36** (99.10 - 99.54)	**99.81** (99.74 - 99.87)
		INDEL	**93.13** (87.46 - 96.34)	**99.31** (98.50 - 99.68)
	Cell-line Mixture (n=93)	All Variants	**98.21** (97.84 - 98.52)	**99.00** (98.83 - 99.14)
		SNV	**98.36** (98.00 - 98.66)	**99.07** (98.91 - 99.21)
		INDEL	**93.26** (88.59 - 96.10)	**97.56** (96.26 - 98.41)

Figure 5. Maternal variant calling performance

Sample Type		Variants	Sensitivity (%), 95% CI	Specificity (%), 95% CI
Maternal Results	Plasma (n=264)	All Variants	**99.90** (99.84 - 99.94)	**>99.99** (99.89 - 100)
		SNV	**99.98** (99.10 - 99.54)	**>99.99** (99.99 - 100)
		INDEL	**97.71** (96.20 - 98.63)	**99.96** (99.84 - 99.99)