Accurate Fetal Fraction from NIPS using Whole Genome Sequencing

Counsyl

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Introduction

The ability of noninvasive prenatal screening (NIPS) to identify fetal aneuploidy in a maternal blood sample is proportional to the fetal fraction (FF), which is the percentage of cfDNA derived from the pregnancy. For NIPS offerings that utilize whole-genome sequencing (WGS), FF ascertainment in male-fetus pregnancies is straightforward using reads from chrX and chrY; for female-fetus pregnancies, statistical inference is required to estimate FF. Since the accurary, precision, and relative prevalence of different FFs is important for proper interpretation of NIPS results, we endeavored to further improve FF inference from WGS data.

Conclusions

The ability of noninvasive prenatal screening (NIPS) to identify fetal aneuploidy in a maternal blood sample is proportional to the fetal fraction (FF), which is the percentage of cfDNA derived from the pregnancy. For NIPS offerings that utilize whole-genome sequencing (WGS), FF ascertainment in male-fetus pregnancies is straightforward using reads from chrX and chrY; for female-fetus pregnancies, statistical inference is required to estimate FF. Since the accuracy, Although fetal fraction is not strictly required for WGS-based NIPS to detect fetal aneuploidies with high clinical sensitivity and specificity, it is recommended by clinical guidelines and provides a valuable quality-control metric that can further improve overall test performance. Here we have demonstrated that a large training dataset combined with careful statistical analysis yields accurate and precise fetal fraction estimates. Importantly, consideration of fetal fraction as a percentile allows for better comparison of FF methodologies, both inter-laboratory and intra-laboratory to aid in the clinical interpretation of results.

References

[1]Kim SK, Deciu C, Ehrich M, Geis J, Hannum G, Hogg G, et al. "Determination of fetal DNA fraction from the plasma of pregnant women using sequence read counts." Prenat Diagn. 2015;35:810-15.

[2]Nygren, A. O. H., Dean, J., Jensen, T. J., Kruse, S., Kwong, W., van den Boom, D., & Ehrich, M. "Quantification of Fetal DNA by Use of Methylation-Based DNA Discrimination." Clinical Chemistry, 2010;56(10), 1627–1635.

[3]Xu, X.-P., Gan, H.-Y., Li, F.-X., Tian, Q., Zhang, J., Liang, R.-L., et al. "A Method to Quantify Cell-Free Fetal DNA Fraction in Maternal Plasma Using Next Generation Sequencing: Its Application in Non-Invasive Prenatal Chromosomal Aneuploidy Detection." PLoS ONE, 2016; 11(1), e0146997–13.

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Comparison between different sources of FF

$$FF_{XY} = \frac{FF_{chrX} + FF_{chrY}}{2}$$

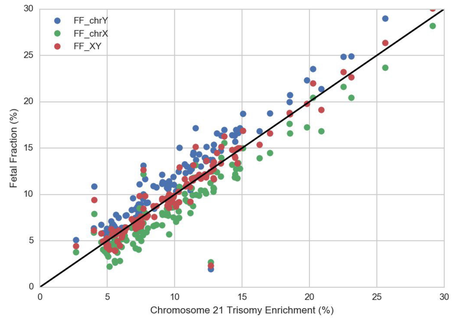
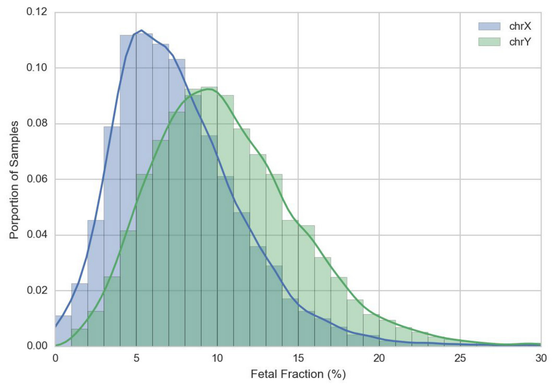


Figure 1: Allosomal estimates of FF (chromosome Y enrichment ("FF_chrY"), chromosome X depletion ("FF_chrX"), and their average ("FF_XY")) versus the median fold-change enrichment among bins on chromosome 21 for 129 male T21 samples.

Range of clinically observed fetal fractions

The difference between the median FF measured in males from Y-chromosome enrichment was ~3% higher than the FF estimated from depletion on chromosome X. Taking the average of these two sources provides the best agreement to chromosome 21 trisomy samples. Further, in showing that specific FF values depend on the reference chromosome used, we have illustrated the importance of considering the relative FF value (e.g., as a percentile) rather than the absolute FF value.



Percentile	1st	5th	25th	50th	75th	95th	99th
Fetal Fraction (FF_XY)	1.6%	3.3%	6.1%	8.1%	11.6%	17%	22.4%

Figure 2: Distribution of fetal fraction from 16,434 XY male samples, for the two different possible measures of fetal fraction: chromosome Y enrichment "FF_chrY", and chromosome X depletion "FF_chrX". Additionally the fetal fraction percentiles from the average "FF_XY" are shown in the above table.

Model for why bin-count regression predicts FF

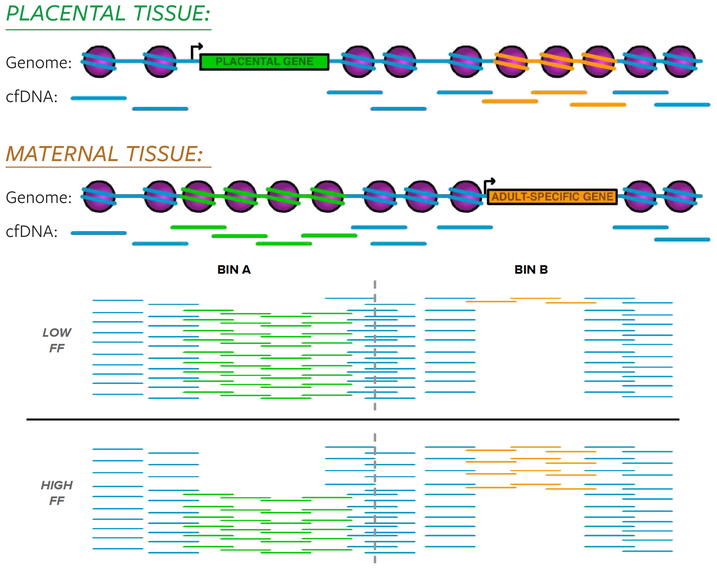


Figure 3: Schematic of how nucleosomes are depleted in regions of actively expressed genes and therefore less cell free DNA is observed in these regions. The differences in gene expression between placenta and mother result in varying prevalence of cell free DNA within 20,000 base pair bins from whole genome sequencing. Additionally, as the fetal fraction increases this difference is enhanced and thus can be used as a signal of fetal fraction.

Methodology for determining fetal fraction

WGS NIPS data from 16,434 male-fetus pregnancies and 15,064 female-fetus pregnancies was retrospectively analyzed to develop and characterize a FF inference algorithm. To train the model, we used male samples with FF directly measured on chromosomes X and Y. WGS reads in bins tiled across the genome can detect relative enrichment of fetal-derived cfDNA in specific parts of the genome between mother and fetus. This relative enrichment in chromosome 1-22 was mapped to known FF values using ridge regression. This was followed by both polynomial smoothing and an error reduction scaling process. Model fitting performance was evaluated from 10-fold fully cross-validated data.

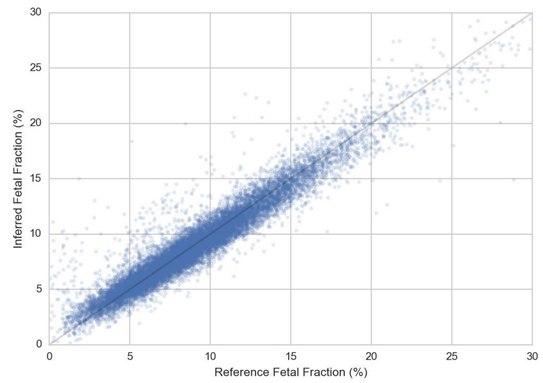


Figure 4: Comparison to the chrX and chrY derived fetal fraction "FF_XY" for 16,434 XY male samples from Counsyl production NIPS assay which serves as the reference fetal fraction for algorithm training, and the inferred fetal fraction from our regression algorithm 10-fold cross validation.

Validation Results

The correlation between the inferred FF using our algorithm and the FF observed in male-fetus pregnancies (FF_XY) is 0.957, in excess of previously published reports[1-3]. Further, the median absolute error in inferred FF was 0.68%, far smaller than the range of FF. The observed percentiles of FF are shown in Figure 2 from the chrX and chrY derived data. Analysis of trisomy 21 samples shows a correlation of 0.954 for male samples and 0.905 for female samples between the inferred FF and the FF calculated from chr21 enrichment (Figure 5).

	Fetal Fraction (FF_XY) vs. Inferred Fetal Fraction
R^2 score	0.969
Median absolute error	0.67%
Correlation	0.957

Table 1: Accuracy of fetal fraction prediction method in comparison to the chrX and chrY derived fetal fraction "FF" for 16,434 XY male samples from Counsyl production NIPS assay. Results used for comparison are from singleton pregnancies and satisfied production sequencing quality control criteria for clinical reporting.

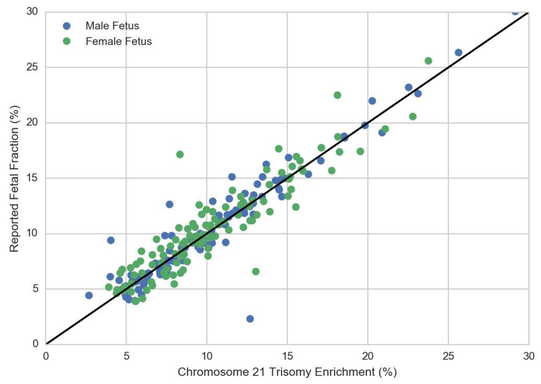


Figure 5: Comparison between the reported fetal fraction and the enrichment of chromosome 21 from 219 male and 214 female T21 aneuploid samples. The reported fetal fraction is the average "FF_XY" for male samples and "FF inferred" from our algorithm.