High-throughput fetal-fraction amplification increases analytical performance of noninvasive prenatal screening

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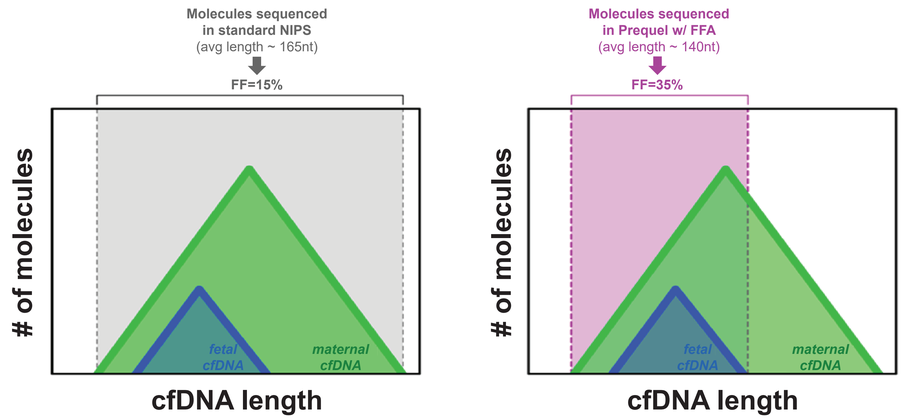
INTRODUCTION

- Noninvasive prenatal screening (NIPS) based on cell-free DNA (cfDNA) has provided millions of pregnant women with information about their risk for fetal chromosomal abnormalities.
- A primary driver of NIPS sensitivity and specificity for aneuploidy in a given maternal plasma sample is the fetal fraction (FF), which describes the proportion of cfDNA fragments that originate from the placenta.
- For the majority of samples, FF values are between 4% and 30%, and many laboratories fail samples with FF< 4% to diminish the risk of issuing false-negative reports.
- On certain NIPS platforms, however, >20% of women with high body-mass index (and >5% overall) receive a test failure due to low FF (< 4%).

METHODS

- A scalable fetal-fraction amplification (FFA) technology that is routinely applied to all samples undergoing whole-genome sequencing (WGS)-based NIPS in our laboratory was analytically validated on 1,264 samples tested with and without FFA (Figure 1).

Figure 1. FFA increases the relative concentration of fetal-derived cfDNA fragments via size selection.



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RESULTS

- Zero samples had FF< 4% when screened with FFA, whereas 1 in 25 of these same patients had FF< 4% without FFA (Figure 2).
- The average increase in FF was 3.9-fold for samples with low FF (2.3-fold overall) and 99.8% had higher FF with FFA (Figure 3).
- For all abnormalities screened on NIPS, z-scores increased 2.2-fold on average in positive samples and remained unchanged in negative samples, powering an increase in NIPS sensitivity and specificity (Figure 4).
- With FFA, the combined sensitivity for five common microdeletions is 97.2% with a specificity of 99.8%. For DiGeorge Syndrome in particular, FFA has an expected analytical sensitivity of 95.6% with an analytical specificity of 99.95% (Table 1).

Figure 2. Fetal fraction amplification (FFA) technology increases fetal fraction (FF) across all BMI levels.

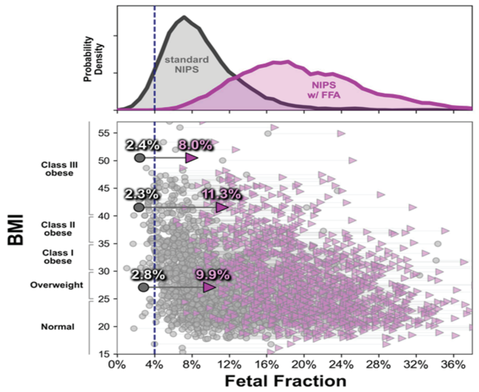


Table 1. Estimated analytical performance.

	Analytical Sensitivity	Analytical Sensitivity
Common aneuploidies (aggregate)	99.988% ± 0.004%	99.968% ± 0.005%
T21	99.990% ± 0.005%	99.996% ± 0.001%
T18	99.990% ± 0.002%	99.996% ± 0.001%
T13	99.978% ± 0.005%	99.976% ± 0.005%
RAAs (aggregate)	99.695% ± 0.305%	99.981% ± 0.010%
Microdeletions (aggregate)	97.172% ± 0.054%	99.767% ± 0.012%
DiGeorge Syndrome (22q11.2)	95.633% ± 0.071%	99.949% ± 0.005%

Figure 3. FFA increases FF for 99.8% of samples tested and most appreciably for low-FF samples.

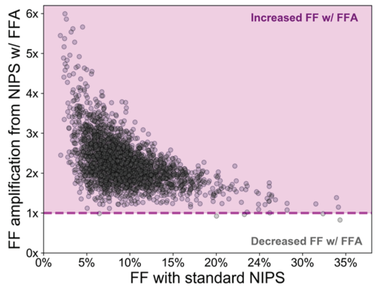
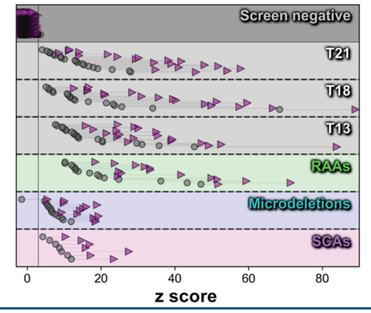


Figure 4. FFA increases the analytical accuracy of fetal sex calling.



CONCLUSION

- FFA transforms low-FF samples into high-FF samples. By combining FFA with WGS-based NIPS, a single round of NIPS can provide nearly all women with confident results about the broad range of potential fetal chromosomal abnormalities across the genome.
- Please reference our entire study for more details: Welker, N. C., Lee, A., Kjolby, R. A. S., Wan, H. Y., Theilmann, M. R., Jeon, D., . . . Chu, C. (2020). High-throughput fetal-fraction amplification increases analytical performance of noninvasive prenatal screening. *Genetics in Medicine*. e-pub ahead of print.