Design and validation of an improved non-invasive prenatal screen for fetal aneuploidy

Counsyl

Chuba Oyolu PhD, Makan Azarshahy MS, Chris Bremner, Eric Chou, Davian Contreras, Nathan Copeland, Kevin D'Auria PhD, Dustin Hite PhD, Greg Hogan PhD, Andrew Horn, Claire Jun, Henry Lai, Annie Lee, Kaying Lui, Dale Muzzey PhD, Sandra O'Donoghue, Thi Tran, Helen Wan, Carrie Haverty MS, Imran S. Haque PhD, Eric Evans PhD

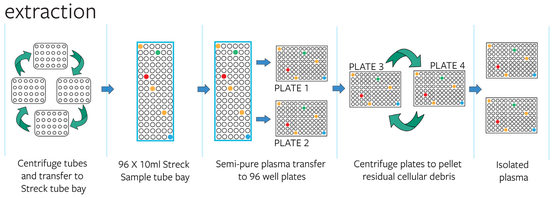
We designed and validated a non-invasive prenatal screen using custom cfDNA extraction chemistry and whole genome next-generation sequencing (NGS) techniques.

Abstract

Non-invasive prenatal screening (NIPS) can identify pregnancies at significantly increased risk for genetic aneuploidies using cell-free DNA (cfDNA) purified from maternal blood samples. We describe the development and validation of the Counsyl *Informed Pregnancy Screen* (IPS), an NIPS test for chromosome aneuploidies including Down syndrome (T21), Edwards syndrome (T18), and Patau syndrome (T13). To verify the results from Counsyl Informed Pregnancy Screen, Counsyl and Verinata Health, Inc.®, a wholly owned subsidiary of Illumina, conducted concurrent prenatal screens, testing a total of 940 samples, including 65 aneuploidy positives detected by Verinata's verifi® test. This concordance testing showed greater than 99% concordance between the Counsyl and Verinata laboratories for T21, T18, and T13.

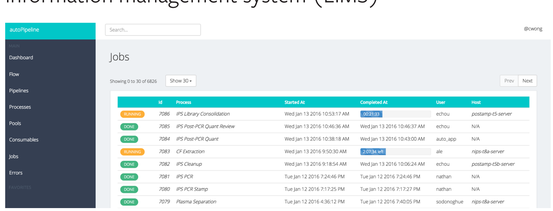
Methods

1. Automated plasma separation and bead based cfDNA extraction



2. Whole genome shotgun sequencing

3. Custom sample tracking software and Laboratory information management system (LIMS)



Assay improvements

Sample ID fidelity ↑

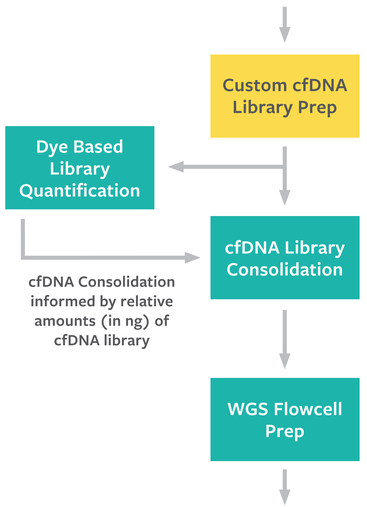
We implemented plasma separation and cell-free DNA extraction on the same automated platform to increase the fidelity of information carried throughout sample processing. Multiple automated barcode scans are performed and recorded for every single sample to ensure we maintain correct mapping between sample and end result.

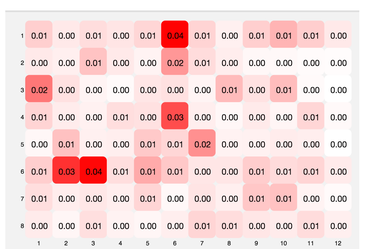
Sample TAT ↓

We integrated a custom designed consolidation algorithm which automatically normalizes the amount of each sample that is added to the total sample pool prior to sequencing. This ensures that we seldom need to re-sequence samples as >99% of samples receive at or above the requisite number of reads required to accurately determine aneuploidy.

Quality control ↑

We implemented a unique contamination metric that follows each sample right from plasma separation through to data analysis. This contamination metric is designed to detect any well to well contamination that may occur in each sample processing batch.





Validation study results

Concordance: Autosomal aneuploidy

A. Confusion matrices showing correlation between verifi® and Counsyl Validation results on samples tested.

VERIFI \ COUNSYL	T21	T18	T13	Normal	No call
T21	40	0	0	0	0
T18	0	17	0	0	0
T13	0	0	8	0	0
Normal	0	1	0	2709	3

B. Sensitivity, Specificity, and Accuracy values recorded for the Counsyl Validation sample set

	Sensitivity	Specificity	Accuracy
T21	100.0% (87.7–100.0)	100.0% (99.5–100.0)	100% (99.6–100.0)
T18	100.0% (73.5–100.0)	100.0% (99.6–100.0)	100.0% (99.6–100.0)
T13	100.0% (39.8–100.0)	100.0% (99.6–100.0)	100.0% (99.6–100.0)

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