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Laboratory Experience Using Illumina Next-Generation Sequencing for Noninvasive Prenatal Testing

Noninvasive prenatal testing results generated on the NextSeq® 500 System are equivalent to those achieved with the HiSeq® System.

Introduction

Prenatal screening provides pregnant women with the option of identifying particular fetal chromosomal abnormalities, including Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13), and sex chromosome–related syndromes such as Klinefelter and Turner syndromes. As shown in prior studies, noninvasive prenatal testing (NIPT) performed with next-generation sequencing (NGS) offers a reliable, quick screen for chromosomal abnormalities.[1,2] NGS generates high-sensitivity, high-specificity NIPT results with low false-positive and false-negative rates and maintains a low test failure rate, minimizing the need for invasive testing procedures.[3]

The verifi Prenatal Test* is an NGS-based noninvasive test that uses the Illumina HiSeq System for sample sequencing. To demonstrate that the Illumina NextSeq 500 System can generate data equivalent to that of the HiSeq System, 2 experiments sequencing a total of 663 maternal samples for NIPT on both systems were performed.

Illumina NGS Systems

The Illumina HiSeq and NextSeq 500 NGS Sequencing Systems provide the throughput, read length, and depth required for NIPT. The HiSeq System is a massive-throughput sequencing instrument offering power and efficiency for large-scale genomic studies. The NextSeq 500 System is a desktop instrument that provides a fast, integrated, sample-to-results workflow with push-button sequencing, simple data analysis, and minimal hands-on time. The NextSeq 500 System delivers the benefits of high-throughput sequencing at a lower capital expense than the HiSeq System.

* The verifi Prenatal Test is a laboratory developed test and its performance characteristics were determined by Verinata Health, Inc., a wholly owned subsidiary of Illumina, Inc. The U.S. Food and Drug Administration has not approved or cleared this test.

Experimental Studies

The following 2 experiments demonstrate the potential use of the NextSeq 500 System for NIPT.

Experiment 1—Design

In the first experiment, 551 maternal blood samples were prepared and sequenced on both the HiSeq and NextSeq 500 Systems following the workflow outlined in Figure 1. Libraries for sequencing were prepared using TruSeq® Nano DNA Library Prep Kits. Sequencing on the NextSeq 500 and HiSeq Systems used the run parameters outlined in Table 1. Each instrument generated initial base calls. Additional data processing was done using analysis methods based on the verifi Test analysis software.

Table 1: NGS Parameters for NIPT

	HiSeq 2500 System	NextSeq 500 System
Samples per Run	15	15
Reads Passing Filter	330 M	458 M
Read Length	36 bp	36 bp
Tags per Run	268 M	331 M

Experiment 1—Analysis and Results

Data generated for all 551 samples were compared across 2 important NIPT metrics: ability to identify copy number changes on chromosome X for male fetus samples and variation of signals for unaffected (diploid) samples.

Copy number changes on chromosome X can be measured by relative representation of chromosome X in the background of sequences from other ("reference") chromosomes, or chromosomal ratio X. Ratio X in

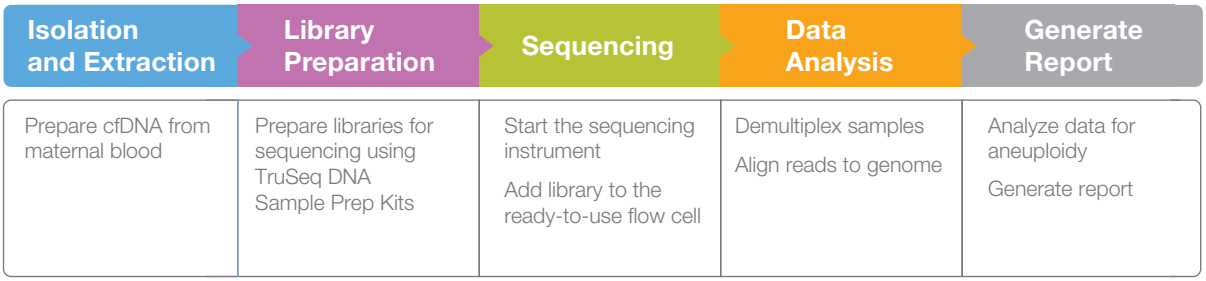

Figure 1: NGS Workflow for NIPT—To demonstrate the performance of the verifi Prenatal Test on the NextSeq 500 System, experiments using both the NextSeq 500 and HiSeq Systems followed the same workflow.

male samples characterizes the response to fetal fraction and relates directly to the ability of the system to detect aneuploidy. As shown in Figure 2, the value of ratio X was similar when measured on HiSeq and Next 500 Systems. This result indicates similar amplitude of copy number variation in response to fetal fraction.

In addition to characterizing response to fetal fraction in male samples, signal variation for unaffected samples on target chromosomes (13/18/21) was characterized by percent coefficient of variation (%CV) of chromosomal ratios. Even though %CV is measured on unaffected samples, it determines analytical sensitivity for detecting aneuploidies. The %CV for chromosomes 13,18, and 21 was consistent between platforms (Table 2), suggesting nearly identical assay performance regarding detection of fetal aneuploidy for these chromosomes across the HiSeq and NextSeq 500 Systems.

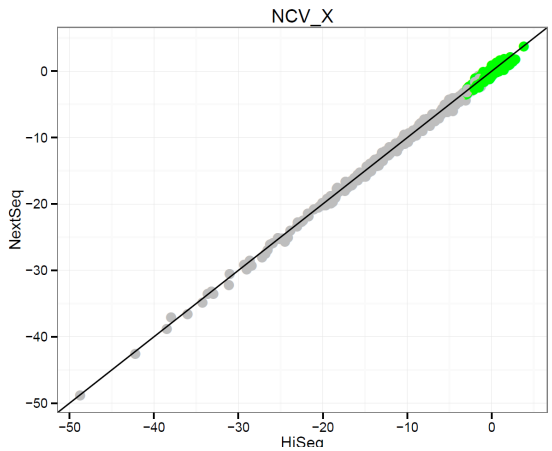

Figure 2: High Concordance of Ratio X Values—The high level of concordance achieved when plotting the ratio X of each sample generated on the NextSeq 500 and HiSeq Systems demonstrates that both produce the chromosomal ratios required for detection of fetal aneuploidy. Grey: male fetus. Green: female fetus.

Table 2: Low, Consistent %CV Values Achieved With the HiSeq and NextSeq 500 Systems

	% CV HiSeq 2500 System	% CV NextSeq 500 System
Chr. 13	0.197%	0.202%
Chr. 18	0.217%	0.200%
Chr. 21	0.294%	0.274%

Experiment 2—Design

A second sample set of 112 samples was sequenced on both the HiSeq and NextSeq 500 Systems. The second sample set contained 9 samples that tested positive for fetal trisomy 21 by the verifi Prenatal Test. Samples were prepared and sequenced according to the protocols outlined in the "Experiment 1—Design" section.

Experiment 2—Analysis and Results

Data obtained for the second set of 112 samples was used to generate normalized chromosome 21 values (NCV 21, or the z-scores used for NIPT classification). As shown in Figure 3 and predicted by previous %CV analysis, the sequencing instrument used did not affect z-scores or the ability to detect aneuploidy in paired samples.

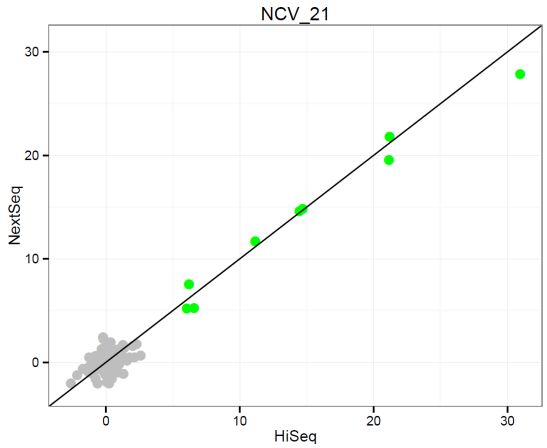

Figure 3: Nearly Identical NCV 21 Values—Plotting NCV 21 values of 112 samples sequenced using both the HiSeq and NextSeq 500 Systems shows nearly identical values, indicating the ability of both systems to determine the presence of fetal trisomy 21 in NIPT. Grey: unaffected. Green: affected T21.

Summary

This study assessed data relating to key NIPT metrics for 663 matched samples processed on both the HiSeq and NextSeq 500 Systems. Results indicate that the NextSeq 500 System generates ratio X, %CV, and NCV 21 data that is comparable to the HiSeq System.

Learn More

To learn more about Illumina solutions for NIPT, visit www.illumina.com/nextseqnote.

Reference

1. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol.* 2012;119(5):890-901. doi:10.1097/AOG.0b013e31824fb482.

2. Futch T, Spinosa J, Bhatt S, de Feo E, Rava RP, Sehnert AJ. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. *Prenat Diagn*. 2013;33(6):569-574. doi:10.1002/pd.4123

3. Bianchi DW, Rava RP, Sehnert AJ. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med.* 2014; 7;371(6):578. doi: 10.1056/NEJMc1405486.

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