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Non-Invasive Prenatal Testing (NIPT)

Using next-generation sequencing (NGS) for NIPT.

Introduction

Analyzing cell-free DNA (cfDNA) shows promise as a non-invasive method for investigating genetic biomarkers that may indicate disease progression, as in cancer[1], and for screening for fetal trisomies during pregnancy[2]. Over the past few years, researchers have refined the process of capturing cfDNA circulating within plasma. The remaining challenge was finding a technology with the sensitivity required to access the genetic information contained within cfDNA. With its high levels of sensitivity and accuracy, next-generation sequencing (NGS) produces the data quality needed for reliable analysis of the trace amounts of cfDNA found circulating within blood plasma. The result is access to these previously inaccessible samples for genomic investigation.

This application note discusses the use of Illumina NGS technology in the verifi® test*, a prenatal screen that detects fetal aneuploidies by analyzing fetal cfDNA obtained from a single maternal blood draw. Based on this information, laboratories can better understand use of NGS for NIPT.

An Overview of the verifi Test

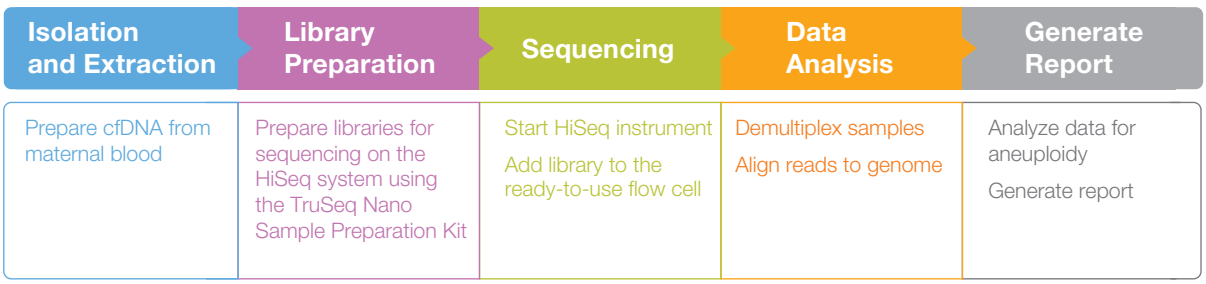
During the early stages of pregnancy, fetal cfDNA represents approximately 3% of the genomic content found within maternal plasma DNA[3]. Through the power of NGS, this fetal DNA can be analyzed to identify potential chromosomal aberrations. Verinata, an Illumina company, has experience screening tens of thousands of fetal cfDNA samples for chromosomal abnormalities using the proven Illumina sequencing technology. Employing the in-house developed verifi test in their CLIA-certified, CAP-accredited laboratory, Verinata laboratory scientists have achieved a low failure rate of 0.07%[4] with sensitivity > 99.99% and specificity > 99.8% in trisomy 21 detection[5]. Screening is usually completed in < 4 days of receipt of the blood sample in the lab.

The verifi Workflow

For the verifi test, Verinata uses a five-step workflow that starts from fetal cfDNA and ends with analysis (Figure 1). DNA is isolated and extracted following the standard procedure in the verifi test. Verinata fully automates this step for processing 64–96 samples simultaneously. This step can be performed manually for batches of 16 samples. For both methods, libraries are prepared using an Illumina library preparation kit, such as the TruSeq® Nano DNA Library Preparation kit, which is optimized to generate sequencing libraries from low sample inputs. The verifi test currently uses the Illumina HiSeq® 2000 sequencing system to generate sequencing data. The sequencing run generates short reads from all fragments of DNA in the sample (1 × 25 bp) and is optimized to decrease run time and provide a rapid turnaround from sample to report (Table 1). For an even faster turnaround time, sequencing can be performed on the Illumina HiSeq 2500 system in rapid-run mode using the TruSeq Rapid SR Cluster Kit-HS and the TruSeq Rapid SBS Kit-HS (50 cycles)†.

Verinata performs primary data analysis using the on-board HiSeq instrument computer for primary analysis and internal servers for downstream analysis and report generation. A highly

Figure 1: The verifi Workflow



The verifi workflow has been used for fast, reliable analysis of tens of thousands of samples for NIPT.

* Not available in the United States. Contact an Illumina representative for regional availability.

† Performance parameters stated within this application note are specific for the verifi test on the HiSeq 2000 system.

Table 1: The verifi Workflow for NIPT on the HiSeq System

Parameter	verifi Workflow	Alternative Workflow‡
Run mode	High output	Rapid run
No. tags per flow cell	1.5 B	300 M
No. samples per run	64	16
No. tags per sample	28 M	18 M
Time per run	19 h*	9 h
Samples per year per instrument	> 18,700†	> 3,000

* Based on 1 flow cell per run on a HiSeq 2000 system.

† Running 360 samples per week.

‡ Based on published throughput specifications for the HiSeq 2500 system in rapid run mode.

optimized algorithm, based on the counting method described by Sehnert AJ, et al.[6], calculates a Normalized Chromosome Value (NCV) for each chromosome to provide clear, informative results. Results with quality metrics are returned from the algorithm to the laboratory to use when generating a report. All analysis is done in-house.

Bring NGS In-House

Laboratories interested in following this NGS method can choose to implement either a manual processing workflow or a fully automated workflow combining Illumina sequencing with automation setup. Illumina scientists test all workflow steps to ensure the easiest and smoothest setup in your lab. For customers needing assistance setting up computing and storage infrastructure, Illumina offers the IlluminaCompute System.

Additionally, certified Illumina Field Application Scientists are available to perform complete in-house laboratory training and technical validation. This service includes full installation and validation of the lab setup and all equipment used throughout the entire workflow.

Learn More

To learn more about the Illumina technology used by Verinata, visit www.illumina.com.

To learn more about how Illumina can assist with implementing sequencing workflows in-house, contact your local account manager.

References

1. Gormally E, Caboux E, Vineis P, Hainaut P (2007) Circulating free DNA in plasma or serum as biomarker of carcinogenesis: practical aspects and biological significance. Mutat Res. 635: 105–117.
2. Bianchi DW, Wilkins-Haug L (2014) Integration of noninvasive DNA testing for aneuploidy into prenatal care: what has happened since the rubber met the road? Clin Chem. 60: 78–87.
3. Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, et al. (1998) Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet. 62: 768–775.
4. Data on file.
5. Verinata Health, Inc. (2012) Analytical Validation of the verifi® Prenatal Test: Enhanced Test Performance For Detecting Trisomies 21, 18 and 13 and the Option for Classification of Sex Chromosome Status. Redwood City, CA.
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Ordering Information

System	Catalog No.
HiSeq 2500 Sequencing System	SY-401-1501
Sequencing Reagent Kit	
TruSeq Rapid SR Cluster Kit-HS	GD-402-4001
TruSeq Rapid SBS Kit-HS (50 cycles)	FC-402-4002
Library Preparation Kits	
TruSeq Nano DNA LT Library Preparation Kit Set A (24 samples)	FC-121-4001
TruSeq Nano DNA LT Library Preparation Kit Set B (24 samples)	FC-121-4002
TruSeq Nano DNA HT Library Preparation Kit (96 samples)	FC-121-4003

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