

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 cancer¹, and for screening for fetal trisomies during pregnancy². 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 DNA³. 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

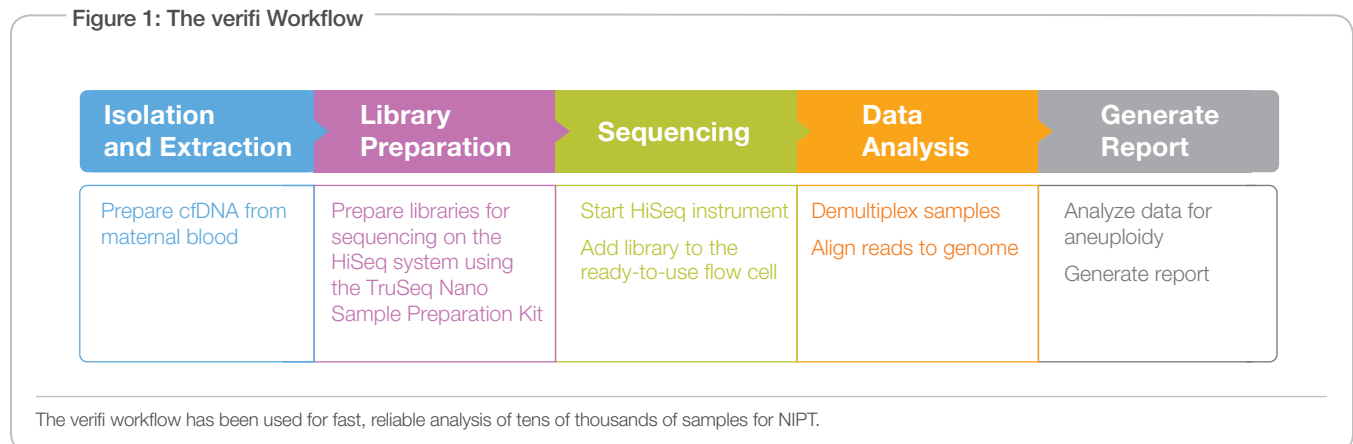
Illumina sequencing technology. Employing the in-house developed **verifi** test in their CLIA-certified, CAP-accredited laboratory, Verinata laboratory scientists have achieved a low failure rate of 0.07%⁴ with sensitivity > 99.99% and specificity > 99.8% in trisomy 21 detection⁵. Screening is usually 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



* 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.

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