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VeriSeq™ NIPT Analysis Software (48 Samples)

A fast, accessible CE-IVD software solution enabling clinical labs to analyze sequencing data for noninvasive prenatal testing (NIPT) in their own lab.

Highlights

- **Accessible Informatics Solution**
  Reduce informatics burden with an easy-to-use CE-IVD marked NIPT analysis software
- **Innovative Analysis**
  Access proven test performance[1,2] combined with paired-end sequencing[3,4]
- **Scalable Throughput**
  Analyze 48 samples per run for more efficient use of lab resources

Introduction

NIPT using cell-free DNA (cfDNA) performed with next-generation sequencing (NGS) offers a reliable, quick screen that generates highly sensitive and specific results for common chromosomal aneuploidies.[5,6] Unfortunately, the bioinformatics aspect of analyzing this complex data can be challenging to many clinical labs. The CE-IVD marked VeriSeq NIPT Analysis Software (48 Samples) removes this barrier, providing an easy-to-use informatics solution for labs interested in bringing NIPT in-house.

VeriSeq NIPT Analysis Software (48 Samples) is designed to remove the burden of bioinformatics development (Table 1). The software analyzes NGS whole-genome sequencing (WGS) data to aid in the detection and differentiation of fetal aneuploidy status for chromosomes 21, 18, 13, X, and Y. Analysis occurs on a secure, onsite server and requires only 5 hours for each 48-sample run. Concise output files provide clear results. Combining accessibility, reliability, and scalability, VeriSeq NIPT Analysis Software (48 Samples) provides a ready-to-use data analysis solution that enables clinical labs to expand their current offerings to include NIPT.

Table 1: VeriSeq NIPT Analysis Software (48 Samples) Features

Feature	Description
Method	Whole-genome sequencing (WGS)
Chemistry	Paired-end sequencing
No. of Samples	48 samples analyzed per batch
Time to Result	~ 5 hours
Analysis Offered	Aids in the detection and differentiation of fetal aneuploidy status for chromosomes 21, 18, 13, X, and Y

Accessible Informatics Solution

Clinical labs pursuing NGS-based assays traditionally required highly trained bioinformaticians and a dedicated infrastructure to develop, validate, and perform data analysis. The CE-IVD marked VeriSeq NIPT Analysis Software (48 Samples) automates the analysis aspect of NIPT using NGS, minimizing the informatics burden and making this powerful assay available to more clinical labs. The software integrates with automated sample preparation methods, enabling sample batching of up to 48 samples for higher throughput.

Fast Turnaround Time

Using VeriSeq NIPT Analysis Software (48 Samples), it takes just 5 hours to go from sequencing run data to results that aid in the detection and differentiation of fetal aneuploidy status. This can enable a 1-day turnaround time for the entire NIPT screen, from blood sample to analyzed results, when used with an 8-hour, automated sample and library preparation protocol and overnight sequencing run on an NGS system that meets the criteria outlined in Table 2.

Table 2: NGS System Performance Requirements

Parameter	Specification
Read Length	2 × 36 bp
Sequencing File Type	BCL file
Sequencing Output	400M reads
Sequencing Run Time	~ 14 hours
Multiplexing	48 samples per run

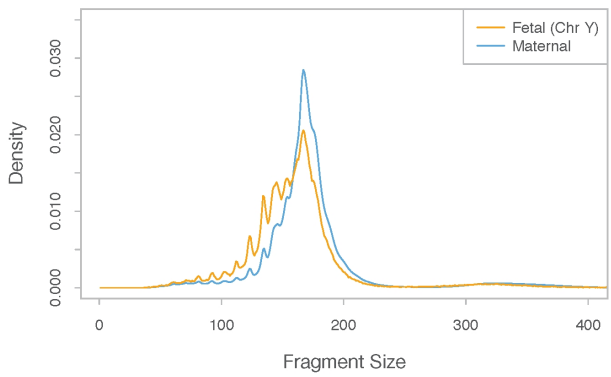

Figure 1: Size Comparison of Maternal and Fetal cfDNA Fragments—Paired-end sequencing is able to discriminate cfDNA fragments based on size.[10]

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Innovative Analysis

VeriSeq NIPT Analysis Software (48 Samples) improves NIPT by combining well-established NIPT methods with advanced analysis techniques.

Proven Technology

The software analyzes data generated by WGS-based NIPT, a method proven to minimize test failures.[1,7,8] VeriSeq NIPT Analysis Software (48 Samples) relies on Illumina NGS technology, the technology used to generate more than 99.7% of NIPT samples in published studies.[9] Combining this proven technology with CE-IVD validated software generates a trusted solution for NIPT analysis.

Improved with Paired-End Sequencing

VeriSeq NIPT Analysis Software (48 Samples) leverages paired-end sequencing for WGS analysis of fetal aneuploidy status of key chromosomes. During paired-end sequencing, both ends of each DNA fragment are analyzed.

Paired-end sequencing enables discrimination of cfDNA fragment sizes within each sample (Figure 1).[10] Previous studies have shown that a maternal blood sample contains different lengths of cfDNA; longer lengths tend to be of maternal origin, while shorter lengths are generally of fetal origin.[10] Using the cfDNA fragment size information from paired-end reads, the algorithm in the VeriSeq NIPT Analysis Software (48 Samples) enriches for fetal signal, increasing the signal-to-noise ratio of the assay.[3] This maintains a high level of analytical performance using one-third less data compared to other sequencing assays.[2,3]*

Software Output and Built-In Quality Assessment

Generated WGS data are streamed to the VeriSeq NIPT Analysis Server where the software filters and aligns WGS reads to a reference genome. The software uses a sophisticated counting-based algorithm to generate Log Likelihood Ratio (LLR) scores for each sample for each of the 5 test chromosomes: 13, 18, 21, X, and Y. Clinical labs perform their own clinical validation studies to establish guidelines for calling aneuploidy based on the LLR scores and quality assessment (QA), and then use the data to generate a clinical report.

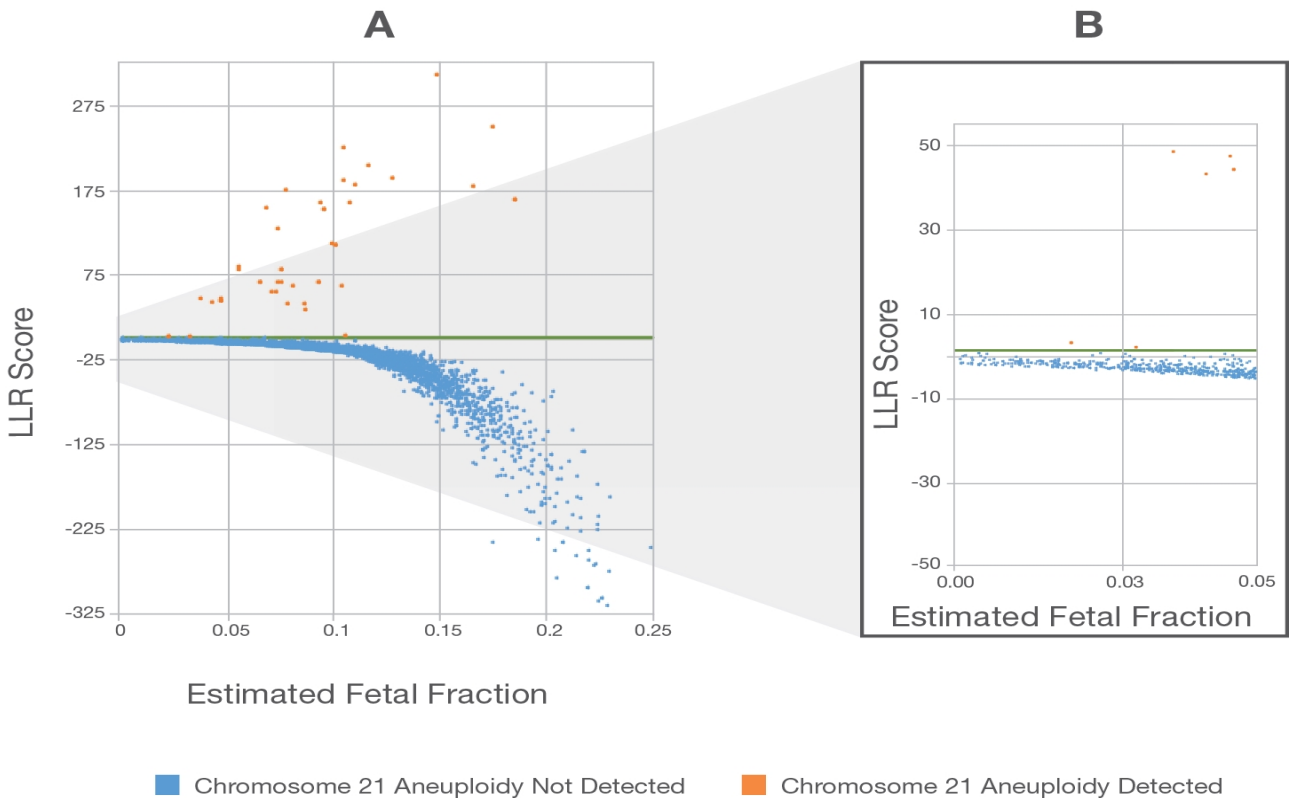

Figure 2: Distinct Identification of Aneuploidy Samples Using LLR Scores — A. Chromosome 21 LLR scores and estimated fetal fraction for a collection of 6094 samples generated by the VeriSeq NIPT Analysis Software (48 Samples) based on WGS data from a compatible sequencing system. An LLR threshold of 1.5 was used in this study (Data on file. Illumina, Inc. 2016.); individual labs should perform their own clinical validation studies to establish LLR thresholds. Sample classifications shown are based on verifi® Test results. Results of this study show 100% concordance between the verifi Test and the VeriSeq NIPT Analysis Software (48 Samples) when used with a 1.5 LLR threshold. B. The same data set showing separation for samples at fetal fraction < 5%.

*Estimated 66% less sequencing as compared to previously published, single-end WGS methods (eg, Sehnert AJ, et al.[2]). Data on file. Illumina, Inc. 2016.

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VeriSeq NIPT Analysis Software includes built-in QA of each sample to ensure the accuracy of generated LLR scores. Data input for each sample is assessed for DNA library yield and sequencing data quality and quantity. Batch-level quality and consistency are also monitored.

Clear, Reliable Outputs

LLR scores from VeriSeq NIPT Analysis Software (48 Samples) can aid in the detection and differentiation of aneuploid samples (Figure 2). These scores reflect normalized coverage of the test chromosome. The VeriSeq NIPT Analysis Software (48 Samples) algorithm combines additional method development with an optimized version of the counting method described by Sehnert AJ, et al.[2,3]

Lower Test Failure Rates

VeriSeq NIPT Analysis Software (48 Samples) includes the individualized fetal aneuploidy confidence test (iFACT) sample quality scoring metric. iFACT indicates whether the system has generated sufficient sequencing coverage and data quality, given the fetal fraction estimate for each sample, to determine LLR scores for samples with low fetal fraction.[3,4,8]

Competitor NIPT technologies, including those based on arrays and targeted sequencing, employ a minimum fetal fraction cutoff; if the fetal fraction of a sample is below this threshold, no result is reported.[11,12] This cutoff requirement leads to higher test failure rates, even upon redraw (up to 65% NIPT redraw failure rate).[12] iFACT allows VeriSeq NIPT Analysis Software (48 Samples) to report on many of these low fetal fraction samples, resulting in lower test failures compared to other NIPT technologies.[8,11,12]

How iFACT Works

Samples with sufficient coverage and fetal fraction pass iFACT. Samples with insufficient coverage are flagged for a repeat test using plasma from the same maternal draw. For samples with low fetal fraction, a dynamic cutoff based on the quantity of sequencing reads for that sample is used to help maintain test accuracy.[3†] If these samples do not have sufficient data coverage, they fail iFACT. Samples do not fail on fetal fraction alone.

Scalable Throughput

VeriSeq NIPT Analysis Software (48 Samples) enables analysis of up to 6 batches of 48 samples per VeriSeq Server per day. Analysis and reporting are automated, requiring no hands-on time or labor. Additionally, WGS using paired-end sequencing requires one-third less sequencing data per sample than other sequencing approaches to generate comparable analytical performance.[2,3*] These features minimize costs, improve overall workflow efficiency, and support growing NIPT demand. Labs can increase capacity by adding multiple NGS systems.

Dedicated Analysis Server

The CE-IVD marked VeriSeq NIPT Analysis Software (48 Samples) runs on a dedicated VeriSeq NIPT Analysis Server. The onsite server offers secure data analysis that does not require an internet connection. Data remains on site and accessible only over a private network.

Figure 3: VeriSeq NIPT Analysis Server—Data analysis using VeriSeq NIPT Analysis Software (48 Samples) occurs on an onsite server, eliminating the need to send out samples for analysis and protecting sample identity.

Supported Implementation

For seamless laboratory integration, the VeriSeq NIPT Analysis Software (48 Samples) and VeriSeq NIPT Analysis Server include installation by a skilled Illumina Field Service Engineer and 30+ hours of hands-on instruction. Knowledgeable Illumina scientists train laboratory personnel on data analysis and results interpretation. Consultation on NGS workflows is also available. When laboratories are up and running, continued support is provided by the Illumina Technical Support team.

Summary

Bringing NIPT analysis capabilities in-house can require a sizable investment in bioinformatics expertise. The VeriSeq NIPT Analysis Software (48 Samples) provides a ready-to-use, accessible, onsite solution that overcomes this barrier.

Learn More

To learn more about the VeriSeq NIPT Analysis Software (48 Samples), visit www.illumina.com/NIPTsoftware.

†Data on file. Illumina, Inc. 2016.

Ordering Information

Product	Catalog No.
VeriSeq NIPT Analysis Server provided preloaded with the VeriSeq NIPT Analysis Software (48 Samples)	20016240

Intended Use Statement

The VeriSeq NIPT Analysis Software (48 Samples) generates quantitative scores to aid in the detection and differentiation of fetal aneuploidy status for chromosomes 21, 18, 13, X, and Y by analyzing sequencing data generated from cell free DNA (cfDNA) fragments isolated from maternal peripheral whole blood specimens in pregnant women of at least 10 weeks gestation. The quantitative scores are Log Likelihood Ratio scores associated with under- or over representation of a target chromosome relative to an expectation for a diploid genome.

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