VeriSeq™ PGS Solution on the MiSeq® System

A next-generation sequencing solution for PGS, providing accurate results in aneuploidy screening and extending future opportunities.

Highlights

- **Industry-Leading Data Quality**
  90% of sequencing data in the world was generated using Illumina technology
- **Fast, Streamlined Workflow**
  Sample to result is approximately 12 hours
- **High-Throughput Scalable Solution**
  Screen up to 24 samples per run

Figure 1: The MiSeq System. The VeriSeq PGS Solution leverages Illumina SBS chemistry on the MiSeq System for reliable, accurate PGS.

Introduction

Chromosome aneuploidy (an abnormal number of chromosomes) is a major cause of *in vitro* fertilization (IVF) failure as most embryos with aneuploidy will not implant or will miscarry during the first trimester of pregnancy.[1,2] Preimplantation genetic screening (PGS) for euploid embryos, those embryos with a normal number of chromosomes, increases the chance that a viable embryo will be selected for transfer and the likelihood of successful implantation and pregnancy.[3]

The VeriSeq PGS Solution takes advantage of next-generation sequencing (NGS) technology to provide comprehensive, accurate screening of all 24 chromosomes for selecting euploid embryos. PGS results generated using the VeriSeq PGS Solution are comparable to those achieved using the widely used array-based 24sure® technology.

Highest NGS Accuracy

VeriSeq PGS relies on industry-leading Illumina sequencing by synthesis (SBS) chemistry, the most widely adopted NGS technology. In fact, 90% of the world's sequencing data are generated using Illumina technology. The proprietary reversible terminator–based method enables massively parallel sequencing of millions of DNA fragments, detecting single bases as they are incorporated into growing DNA strands. This method minimizes sequencing-related errors.

Illumina sequencing delivers the most accurate human genome at any coverage, the highest yield of error-free reads, and the highest percentage of base calls above Q30* in the industry. Such high data quality results in low false positive and false negative rates, reducing the need for extensive downstream validation while providing full confidence in the data.

Flexible MiSeq System

The MiSeq System (Figure 1) takes advantage of proven Illumina SBS chemistry to offer unparalleled sequencing reliability and accuracy. With the ability to generate up to 15 Gb of data with 25M sequencing reads, this desktop sequencer enables any laboratory to perform various sequencing applications, including targeted gene sequencing, metagenomics, small genome sequencing, targeted gene expression, amplicon sequencing, and HLA typing.

Fast, Efficient Workflow

The VeriSeq PGS solution offers a fast, end-to-end PGS method that is completed in about 12 hours (Figure 2). It begins with DNA extraction and whole-genome amplification (WGA) from a single embryonic cell using the SurePlex™ DNA Amplification Kit. Amplified samples

* Q30 = 1 error in 1000 base calls or an accuracy of 99.9%.

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
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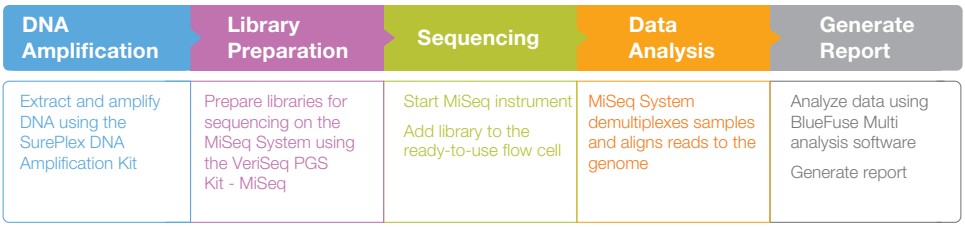

Figure 2: Streamlined VeriSeq PGS Workflow. The VeriSeq PGS process provides a fast, efficient method for screening up to 24 samples.

undergo streamlined library preparation using the VeriSeq DNA Library Kit. Prepared libraries are loaded onto a flow cell for sequencing on the MiSeq System. An on-instrument computer performs data analysis. Generated files are then imported into BlueFuse Multi Software for analysis, data management, and results reporting.

Streamlined Transition From 24sure Arrays to VeriSeq PGS

The well-established, reliable 24sure assay[4] was leveraged for development of the VeriSeq PGS solution. A common amplification process using the SurePlex Amplification Kit facilitates parallel processing of samples on both array and NGS platforms for validation purposes, and the ability to maximize machine availability and volume benefits. Data from both the 24sure array and VeriSeq PGS is analyzed in a common view within BlueFuse Multi Software, ensuring a single interpretation workflow across both platforms. This common approach supports an incremental transition from 24sure arrays to the VeriSeq PGS solution.

Increased Throughput

With the VeriSeq PGS Solution, users can multiplex samples for simultaneous analysis, greatly increasing throughput. Choose to process up to 24 fresh samples, or batch up to 24 frozen samples. This flexibility enables laboratories to begin scaling up today to meet future throughput needs (Table 1).

Table 1: VeriSeq on the MiSeq System.

Samples per Run	24
Protocol Length	~ 12 hours
Required Input	Biopsied cells
Sample Preparation	SurePlex DNA Amplification Kit
Library Preparation	VeriSeq DNA Library Kit-PGS
Analysis Software	BlueFuse Multi Analysis Software

Comprehensive Data Analysis and Information Management

VeriSeq PGS includes a license for BlueFuse Multi Analysis Software, a complete solution for analyzing and reporting VeriSeq results. BlueFuse supports the complete laboratory workflow, from sample receipt to results software (Figure 3).

Sample Database

BlueFuse uses a scalable database architecture to store all sample details, experimental information, and results. Simple filters, powerful queries, and visual representation of each IVF cycle make sure that the right information is available when needed. Within a BlueFuse Multi database, PGS data generated using 24sure microarrays can be analyzed, stored, and viewed alongside VeriSeq PGS data.

Automated Analysis

Demultiplexed sample information is uploaded directly from the MiSeq System, saving time and allowing sample tracking. Single-click shortcuts provide rapid access to run and sample reports for easy QC.

Clear Profiles

Powerful visualization capabilities generate profiles from thousands of pooled measurements from each flow cell, enabling full understanding of the status of each chromosome and results confirmation.

Concise Reports

Sophisticated algorithms calculate and call the status for each chromosome as either “normal” or “abnormal”, and include an estimate of confidence in the call based on assay noise or any underlying ambiguity. In addition to reproducibility and objectivity, this call data enables comparison of laboratory results with results published in the literature. The end product is an automated sample and cycle report.

Accurate Aneuploidy Screening

To demonstrate the accuracy of the VeriSeq PGS solution, a large-scale verification test was performed using cell line material of known karyotype status. The study compared results achieved using the VeriSeq PGS Kit with results from 24sure arrays. In particular, the study focused on how often samples called as aneuploidy or euploid by 24sure arrays were assigned the same status by VeriSeq PGS.

For the experiment, DNA obtained from single-and three-cell samples from different cell lines was amplified using the SurePlex DNA Amplification System. Single-cell samples simulate blastomere biopsies and three-cell samples to simulate trophectoderm biopsies. Automatic calling and data analysis was performed using BlueFuse Multi Software. Results showed a high level of concordance at the chromosome level and complete concordance at the sample level between the VeriSeq PGS Kit and 24sure arrays (Tables 2 and 3).

Table 2: Chromosome Level Agreement Between VeriSeq PGS and 24sure Arrays.

Data Set[a]	Positive % Agreement	Negative % Agreement
Overall	97.8% (n = 139)	99.8% (n = 3677)
Single-cell Samples	96.0% (n = 50)	99.6% (n = 1630)
Three-cell Samples	98.9% (n = 89)	99.9% (n = 2047)

[a] Chaotic and mosaic samples were excluded from analysis.

Table 3: Sample Level Agreement Between VeriSeq PGS and 24sure Arrays.

Data Set[a]	Positive % Agreement	Negative % Agreement
Overall	100% (n = 135)	100% (n = 24)
Single-cell Samples	100% (n = 48)	100% (n = 22)
Three-cell Samples	100% (n = 87)	100% (n = 2)

[a] Chaotic and mosaic samples were excluded from analysis.

Peer-Reviewed Publication Validates VeriSeq PGS

Two recent studies by Fiorentino F, et al.,[5,6] one preclinical and the second clinical report extensive validation of the use of NGS for 24-chromosome aneuploidy screening of embryos and its use in PGS. Dr. Fiorentino, Director, Genoma Molecular Genetics Laboratory, concludes, “given the high level of consistency with an established methodology, such as arrayCGH, NGS has demonstrated a robust high-throughput methodology ready for clinical application in reproductive medicine.”

Methods

The preclinical study consisted of 18 single cells and 190 WGA products from single blastomeres, analyzed previously using karyotyping or 24sure arrays. The clinical study consisted of 192 blastocysts obtained from 55 clinical PGS patients. All samples were

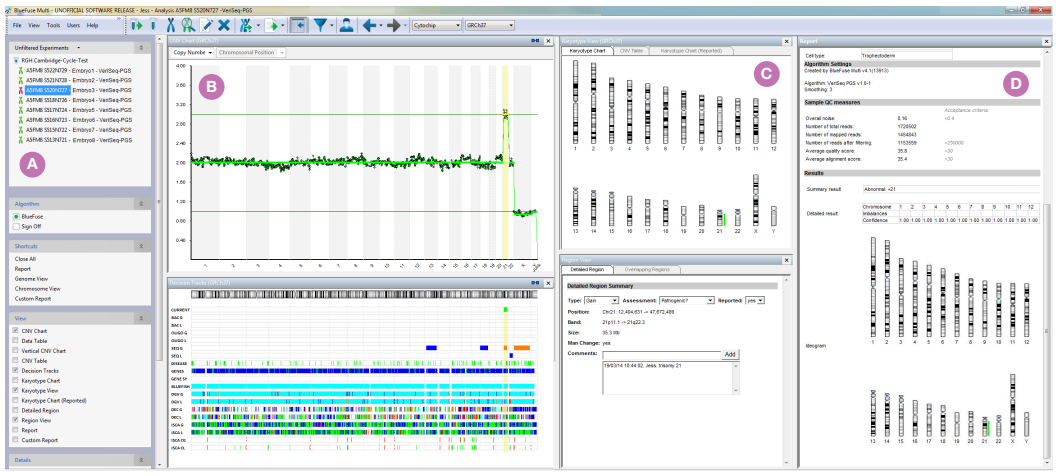

Figure 3: BlueFuse Multi Analysis Software Delivers a Complete Data Analysis and Information Management System. BlueFuse software provides a complete solution for analyzing, storing, and reporting VeriSeq results. A. Sample database shows experimental information. B. Profiles for the sample (top) and DecisionTrack information (bottom). C. Karyotype chart for whole-genome view (top) and region view with the opportunity to annotate (bottom). D. Reports per embryo or per cycle (embryo report shown).

evaluated with NGS for chromosome copy number imbalances. Specificity and sensitivity values obtained in the NGS studies were compared to results from earlier experiments.

Results

NGS specificity for aneuploidy calling (consistency of chromosome copy number assignment compared to current gold standard assay) was 99.98% with a sensitivity of 100%. NGS specificity for aneuploid embryo calling (24-chromosome embryo level diagnosis consistency) was 100% with a sensitivity of 100%. Both positive and negative predictive values of the NGS-based assay were 100% at the embryo level. A secondary outcome of the studies showed a 63.8% clinical pregnancy rate per embryo transfer.

Conclusions

NGS provides reliable, comprehensive screening of 24-chromosome aneuploidy, achieving results with a high degree of concordance to those obtained using established array-based PGS techniques. NGS offers a readily available, high-throughput method for PGS in the clinic with the potential benefits of reduced costs and enhanced precision.

Looking Forward

In a 2014 article in *The Guardian*[7], Dagan Wells, Director, Reprogenetics, UK stated "We are entering a golden age of genetics applied to the understanding of infertility, early human development, and the diagnosis of inherited disease. Several other major technical advances are now on the verge of routine clinical application and the landscape of IVF is likely to be radically altered in the coming months and years." Bringing NGS into the laboratory for PGS is just the beginning. With applications spanning the entire human genome, NGS opens up new possibilities, enabling an incremental increase in portfolio offerings.

Summary

The VeriSeq PGS Kit - MiSeq and MiSeq System bring the power of NGS to IVF, providing the potential to increase pregnancy success rates. The accurate aneuploidy screening results are comparable to the current industry standards. As we learn more, NGS opens up improved workflows for more precise performance and new opportunities.

Learn More

To learn more about the VeriSeq PGS Kit – MiSeq, visit www.illumina.com/VeriSeqPGSSolution.

References

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Ordering Information

Product	Catalog No.
VeriSeq PGS Kit - MiSeq (96 samples)[a]	RH-101-1001
MiSeq System	SY-410-1003

[a] The VeriSeq PGS Kit - MiSeq includes the SurePlex DNA Amplification Kit, VeriSeq DNA Library Prep Kit-PGS, VeriSeq Index Kit-PGS, and MiSeq Reagent Kit v3-PGS.

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