VeriSeq PGS Kit – MiSeq System

Rapid aneuploidy screening using the MiSeq System.

Chromosome aneuploidy (abnormal number of chromosomes) in embryos is a major cause of in vitro fertilization (IVF) failure, pregnancy loss, and, in rare cases, abnormal pregnancy resulting in live birth.[1,2] Preimplantation genetic testing for aneuploidy (PGT-A) offers a way to detect the chromosome number of an embryo and make sure that only euploid embryos—those with a normal number of chromosomes—are implanted during IVF procedures. Next-generation sequencing (NGS) is proving to be a reliable tool for PGT-A.[3,4]

The VeriSeq PGS Kit - MiSeq uses NGS on the Illumina MiSeq System to screen all 24 chromosomes for aneuploidy in a single assay. The assay can be used on a single cell or a few cells from an embryo biopsy. The protocol can be completed in approximately 12 hours. The VeriSeq PGS Kit is designed for multiplexing up to 24 samples per run on the MiSeq System. Each VeriSeq PGS Kit - MiSeq provides sufficient reagents for 4 runs on the MiSeq System (up to 96 samples).

Supply

The VeriSeq PGS Kit - MiSeq (Table 1) includes all reagents required to run VeriSeq PGS on the MiSeq System, including:

- SurePlex™ DNA Amplification System for amplifying DNA from an embryo biopsy. The SurePlex System provides enzymes and buffers required to perform the amplification protocol in clear screw-cap tubes with colour-coded lids as referenced in the SurePlex laboratory protocol. It is shipped on dry ice.
- VeriSeq Library Preparation Kit-PGS for preparing sequencing-ready libraries from samples amplified using the SurePlex System. It is supplied in 2 boxes. Box 1 is shipped on dry ice. Box 2 is shipped at room temperature and must be stored at 2°C to 8°C until first use.
- VeriSeq Index Kit-PGS provides 24 indexes for use during library preparation, enabling multiplexing of up to 24 samples in a single sequencing run. The kit is shipped on dry ice and must be frozen until use. A complementary pack of index adapter replacement caps is included and shipped at room temperature.
- MiSeq Reagent Kit v3-PGS provides all reagents required for sequencing on the MiSeq System. It is supplied in two boxes; one MiSeq PGS Cartridge shipped on dry ice and one PGS flow cell shipped at 2°C to 8°C and must be refridgerated until use.

Complete kit contents are in the 'Contents' section of this document. BlueFuse Multi Analysis Software provides analysis and reporting capabilities for results generated using the VeriSeq PGS Kit - MiSeq. It is available from your technical support representative.

Table 1: VeriSeq PGS Kit - MiSeq Components

Kit	Description
2 × SurePlex DNA Amplification System	Each kit contains reagents for 50 amplification reactions
1 × VeriSeq Library Preparation Kit-PGS	Kit contains reagents for preparing 96 samples
1 × VeriSeq Index Kit-PGS	Kit contains 24 indexes for multiplexing up to 24 samples with sufficient reagent for processing up to 96 samples
4 × MiSeq Reagent Kit v3-PGS	Each kit contains 1 cartridge and 1 flow cell with sufficient reagents for sequencing 24 libraries

Technical specifications

The SurePlex DNA Amplification System amplifies the genomic DNA from an embryo biopsy—a single blastomere biopsy from day 3 embryos, or a few cells from a day 5 blastocyst biopsy. The VeriSeq Library Preparation Kit-PGS uses an engineered transposome to simultaneously fragment and tag ("tagment") SurePlex input DNA, adding unique adapter sequences in the process. A limited-cycle PCR uses these adapter sequences to amplify the SurePlex insert DNA. PCR also adds index sequences to both ends of the DNA, enabling dual-indexed sequencing of pooled libraries on the MiSeq System. Up to 24 samples can be multiplexed in a single MiSeq run. Choose between performing a fast run (12-plex single-index library) or a standard run (24-plex dual-index library).

Sample sheets, used by both the MiSeq System and BlueFuse Software, are generated using BlueFuse Workflow Manager. Prepared VeriSeq PGS libraries are pooled and run on the MiSeq System. The on-instrument MiSeq Control Software (MCS) v2.5 or above performs secondary analysis of the sequencing data, demultiplexing and aligning the reads to the reference genome. BAM files from the MiSeq System are imported directly into the BlueFuse Multi Analysis Software using the prepared sample sheet.

BlueFuse Multi Analysis Software processes and displays the data to provide genomic profiles of each sample in a run. Whole chromosome aneuploidy is called automatically. The effective resolution of the assay is 20 Mb. Reports are generated automatically for each sample and for the cycle.

Contents

The VeriSeq PGS Kit - MiSeq, Catalog No. RH-101-1001, provides sufficient reagents for processing 96 samples. It includes the SurePlex DNA Amplification System, VeriSeq Library Preparation Kit-PGS, VeriSeq Index Kit-PGS, and MiSeq Reagent Kit v3-PGS (Tables 2–5). Certain kit components are stored at different temperature than at which they are shipped. Upon kit receipt, review the storage instructions indicated in the

Table 2: SurePlex DNA Amplification System

Design Element Catalog no.	**Parameter** PR-40-415101-00
Part No.	15043067
No. Kits Supplied	2
Storage	–25°C to –15°C
Components	
Cell Extraction Buffer Extraction Enzyme Dilution Buffer Cell Extraction Enzyme SurePlex Pre-Amp Buffer SurePlex Pre-Amp Enzyme SurePlex Amplification Buffer SurePlex Amplification Enzyme Nuclease-Free Water	

Table 3: VeriSeq Library Preparation Kit-PGS

Design Element	**Parameter**
Box 1 Part No.	15050111
Box 2 Part No.	15050128
No. Reactions per Kit	96
No. Kits Supplied	1
Box 1 Storage	Store frozen until first use
Box 2 Storage	Store refrigerated until first use
Box 1 Components	
Nextera® Amplicon Tagment Mix Hybridization Buffer Tagment DNA Buffer Nextera PCR Master Mix Resuspension Buffer Library Normalization Additives Library Normalization Wash	
Box 2 Components	
Library Normalization Storage Buffer 1 Neutralize Tagment Buffer Library Normalization Beads 1	

Table 4: VeriSeq Index Kit-PGS	
Design Element	**Parameter**
Box 1 Part No.	15050106
Box 2 Part No.	15026762
No. Indexes	9624
No. Samples per Kit	96
No. Kits Supplied	1
Box 1 Storage	–25°C to –15°C
Box 2 Storage	Room temperature
Box 1 Components	
Index Adapters ID Nos. N701, N702, N703, N704, N705, N706, N707, N708, N709, N710, N711, N712	
Box 2 Components	
Index Adapter Replacement Caps	

Table 5: MiSeq Reagent Kit-PGS	
Design Element	**Parameter**
Box 1 Part No.	15050048
Box 2 Part No.	15050051
No. Reactions per Kit	24
No. Samples per Kit	4
No. Kits Supplied	1
Box 1 Storage	–25°C to –15°C
Box 2 Storage	2°C to 8°C
Box 1 Components	
Hyb Buffer	
Reagent Cartridge	
Box 2 Components	
Incorporation Buffer	
Flow Cell MiSeq v3.0 PGS	

Software specifications

Illumina recommends using the BlueFuse Workflow Manager for preparation of the sample plate and sample sheet prior to library preparation. To analyze VeriSeq PGS results, Illumina recommends using BlueFuse Multi Analysis Software v4.1, or later (Table 6).
Sample sheets, used by both the MiSeq System and BlueFuse Software, are generated using BlueFuse Workflow Manager. Prepared VeriSeq PGS libraries are pooled and run on the MiSeq System. The on-instrument MiSeq Control Software (MCS) v2.5 or above performs secondary analysis of the sequencing data, demultiplexing and aligning the reads to the reference genome. BAM files from the MiSeq System are imported directly into the BlueFuse Multi Analysis Software using the prepared sample sheet.

Table 6: Minimum Hardware Requirements for Running BlueFuse Workflow Manager and BlueFuse Multi Analysis Software	
Memory (RAM)	4 GB
Processor	2 GHz
Screen Resolution	1024 × 768 or higher
Network Speed	1 GB
Disk Space	1 BAM file per sample is 70 MB
Operation System	Windows 7 or Vista

References

1. Scott RT Jr, Ferry K, Su J, et al. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril. 2012; 97(4):870-875.

2. Tobias E, Connor JM, Ferguson-Smith M. Essential medical genetics. 6th edition: 243–247. Chichester, West Sussex, UK. Wiley-Blackwell.

3. Fiorentino F, Biricik A, Bono S, et al. Development and validation of a next-generation sequencing–based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril. 2014; 101(5):1375-1382.

4. Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod. 2014; 29(12):2802-2813.