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Using the VeriSeq™ PGS Kit - MiSeq® in Low-Throughput Mode

Guidelines for running the VeriSeq PGS assay with fewer than 24 samples.

Introduction

Chromosome aneuploidy (abnormal number of chromosomes) is a major cause of failure of *in vitro* fertilization (IVF) leading to pregnancy loss and, in rare cases, abnormal pregnancy, and live birth.[1,2] Preimplantation genetic screening (PGS) detects chromosomal copy number variation (CNV) within an embryo and makes sure that only euploid embryos (those with a normal number of chromosomes) are implanted during the IVF procedure. Next-generation sequencing (NGS) is proving to be a reliable means of CNV screening.[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 (blastomere biopsy) or a few cells (blastocyst biopsy) from an embryo. The VeriSeq PGS workflow (Figure 1) goes from sample to result in approximately 12 hours*.

VeriSeq PGS Kit Configuration

The VeriSeq PGS Kit is designed for multiplexing up to 24 samples per run on the MiSeq System. When 24 samples are not available for a run, it is possible to run the assay in a low-throughput, fast-run mode (8-12 samples/run). The VeriSeq assay should not be run with fewer than 8 samples per run. This technical note provides a step-by-step guide using the VeriSeq PGS assay in a low-throughput, fast-run mode.

Running the VeriSeq Assay in Low-Throughput Mode

Using the VeriSeq PGS Kit with fewer than 24 samples follows the original protocol with a few additional steps.

DNA Amplification

Amplify sample DNA using the SurePlex™ DNA Amplification Kit following the standard VeriSeq PGS protocol.

* Learn more about the VeriSeq PGS Kit - MiSeq at www.illumina.com/VeriSeqPGSSolution.

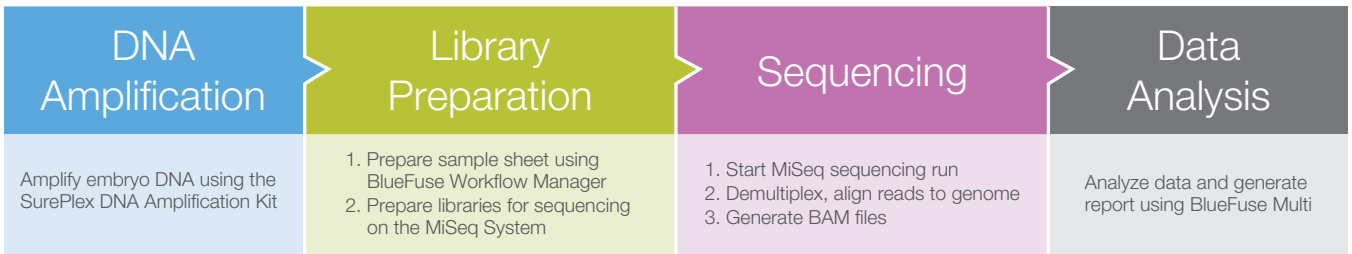

Figure 1: The VeriSeq PGS workflow.

Library Preparation

BlueFuse Workflow Manager Software

Manager Software to create a Worklist before starting the library preparation procedure:

1. Select the 'VeriSeq' category and the 'VeriSeq PGS – MiSeq' workflow.
2. Click 'Create Worklist'.
3. Fill in the Worklist names as usual.
4. Select the 'Up to 12 (Single Index)' option in the Number of samples field† (Figure 2).
5. Fill the remaining sections like usual.

Library Preparation Reagents

The VeriSeq Library Preparation Kit – PGS and the VeriSeq Index Kit – PGS include sufficient reagents for library preparation of 96 samples. The frozen reagents in the VeriSeq Library Preparation Kit – PGS can undergo a maximum of 4 freeze-thaw cycles. To make sure that reagents do not undergo more than the recommended number of freeze-thaw cycles, pipette frozen reagents into smaller volumes for use as single-use aliquots. Use only DNase and RNase-free molecular biology grade tubes for aliquots. Make sure that each tube is labeled and stored correctly to avoid any mix-up of reagents.

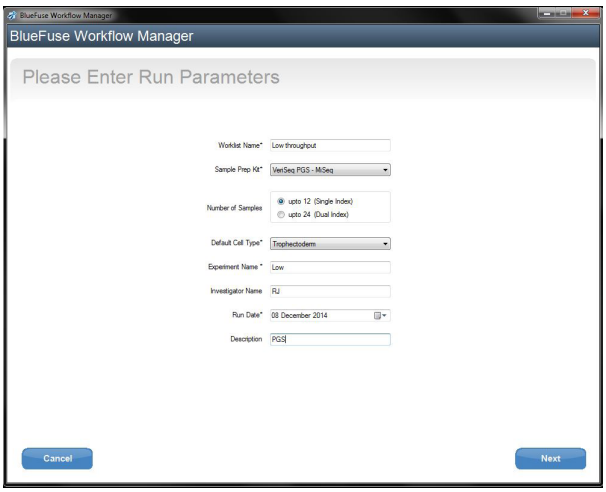

Figure 2: BlueFuse Workflow Manager. When running the VeriSeq PGS Kit - MiSeq in low-throughput, fast-run mode, it is important to select the proper run parameters in BlueFuse Workflow Manager to ensure the correct combination of indexes in your run.

† It is important to select the correct option as this ensures the selection of correct combination of the indexes for your run.

Sequencing

Follow the standard VeriSeq PGS Kit protocol for the sequencing step. The BlueFuse Workflow Manager Software will automatically create a fast-mode run. The sequencing run time for a fast-mode run (single-index read run) is ~70 minutes shorter than for normal mode (dual-index read run).

Data Analysis

Follow the standard VeriSeq PGS protocol for data analysis.

References

1. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, et al. (2012) Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril., 97(4): 870-875.
2. Tobias E, Connor JM, Ferguson-Smith (2011) Essential medical genetics. 6th edition: 243-247. Chichester, West Sussex, UK. Wiley-Blackwell.
3. Fiorentino F, Biricik A, Bono S, Spizzichino L, Cotroneo E, et al. (2014) Development and validation of a next-generation sequencing based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril., 101(5): 1375-1382.
4. Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, et al. (2014) Application of next-generation sequencing for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Human Reprod., Epub ahead of print.

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