Fairfax Diagnostics

Preimplantation Genetic Testing (PGT)
Practitioner Information

Next Generation Sequencing (NGS) using Illumina's MiSeq sequencer with the VeriSeq library prep protocol and BlueFuse Multi software is used for PGT-A aneuploidy screening. PGT-A using NGS is designed for full and balanced coverage of all chromosomal regions and has a much higher genomic coverage and resolution for detecting chromosomal defects compared to standard microarray testing. This system is ideal for cases of repeated pregnancy loss, advanced reproductive age, and multiple IVF failures despite the presence of morphologically good embryos. NGS PGT-A is performed following whole genome amplification using the 24Sure™ SurePlex kit (BlueGnome Ltd., Cambridge, UK). The VeriSeq library prep protocol (Illumina, Inc., San Diego, CA) generates over 1 million individual genomic reads per embryo spanning all 24 chromosomes with an average resolution of 1Mb. NGS using VeriSeq is designed to identify whole chromosome aneuploidy in samples obtained from embryo biopsy. Results are interpreted using BlueFuse Multi data analysis software which empirically determines chromosomal copy number values and is optimized for preimplantation genetic testing. Testing is available for Day 5/6 trophectoderm biopsy and frozen transfer. Results are available 2 days following sample receipt.

PGT for gender selection. For gender determination, PGT-A using NGS is available to determine gender of embryos (XX or XY) as well as provide chromosome copy number of autosomes to eliminate embryos displaying aneuploidy.

PGT using NGS for patients with chromosomal Structural Rearrangements (PGT-SR). Recent research studies indicate enhanced pregnancies rates with NGS when compared to FISH or microarray testing. No pre-test workup is needed for translocation carriers but advanced notification of the rearrangement along with submission of a laboratory report documenting the breakpoints or fusions is required. PGT-SR using NGS has expanded genomic coverage and resolution for detecting small unbalanced chromosome segments. NGS analysis is performed using whole genome amplification (24Sure+™ SurePlex kit, BlueGnome Ltd., Cambridge, UK) followed by VeriSeq library prep protocol (Illumina, Inc., San Diego, CA) providing an overall resolution of 1Mb with an approximately 0.5Mb and 0.25Mb resolution in the pericentromeric and subtelomeric regions, respectively. PGT-SR using NGS is designed to identify unbalanced rearrangements and whole chromosome aneuploidy in the same sample obtained from embryo biopsy. Results are interpreted using BlueFuse Multi data analysis software, which empirically

determines chromosomal copy number values and is optimized for preimplantation genetic testing. Testing is available for Day 5/6 trophectoderm biopsy and frozen transfer. Results are available 2 days following sample receipt.

PGT for Monogenic disorder detection (PGT-M). Fairfax Diagnostics has expanded our team of molecular geneticists devoted to monogenic disorder testing-PGT-M. Recent advances in medical genetics have now identified the molecular basis for over 6,000 Mendelian diseases and for most of these disorders a patient-specific genetic test can be designed. Our test design takes into account the affected gene and specific mutation as well as flanking polymorphic markers for added diagnostic accuracy. Single gene disorder testing is also be coupled with PGT-A for chromosomal aneuploidy detection. Due to the complexity of genetic testing for these disorders, patients should have a full consultation with a medical geneticist to determine if this option would fit their family's needs. Prior to the PGT-M cycle, the laboratory must be notified in order to develop a patient-specific test for each disorder. In addition to testing for the inherited disorder, aneuploidy screening is also included on each sample and included on the PGT-M report.

Accuracy and Test Limitations. Next Generation Sequinning (NGS) is a method used to sequence whole genome amplified DNA (see sections above) biopsied from blastocyst embryos. Sequence alignment data is converted by dedicated software to display chromosomal copy number. This method does not identify the presence or absence of genetic diseases or detect all types of polyploidy, balanced chromosome abnormalities such as translocations or inversions, low-level mosaicism, microdeletions, microduplications, point mutations, uniparental disomy, or genomic imbalances. It is estimated that about 4% of the time the biopsied cells fails to get into the buffer solution or the DNA is degraded to the point where it cannot be analyzed. As performed at Fairfax Diagnostic, the technical accuracy of NGS testing from successfully amplified samples is about 98% for whole chromosome aneuploidy and unbalanced rearrangements. The technical accuracy for monogenic disorders and aneuploidy testing is also about 98%. Genetic counseling is recommended to discuss test results with patients. The use of intracytoplasmic sperm injection (ICSI) is recommended to reduce the risk of misdiagnosis due to contamination by sperm DNA. Preimplantation genetic testing (PGT) is not intended to replace prenatal testing. These tests are validated and their performance is determined by Fairfax Diagnostics Laboratory.