Validation of the VeriSeq PGS Solution

Large, multisite validation of preimplantation genetic testing for aneuploidy (PGT-A) by the VeriSeq PGS Solution using both cell line and embryo biopsy samples

Preimplantation genetic testing for aneuploidy (PGT-A) is a test for chromosome copy number that can be used during in vitro fertilization (IVF) to help determine the chromosomal status of an embryo from a biopsy of one or more cells. The results of PGT-A aid in the selection of an embryo likely to have a normal number of chromosomes (euploid) for transfer, and help avoid those with abnormal copy number (aneuploid) that may result in IVF failure or miscarriage.

Several approaches have been developed to enable comprehensive chromosome screening of preimplantation embryos, including qPCR, SNP arrays, array-CGH, and next-generation sequencing (NGS).

NGS has been proposed as a potentially more advantageous PGT-A approach and recent studies, most with small samples sizes, have suggested that NGS-based PGT-A is highly accurate.[1-6]

What is the VeriSeq PGS solution?

VeriSeq PGS is an NGS solution for PGT-A. VeriSeq PGS provides comprehensive testing for copy number on all 24 chromosomes from the embryo biopsy.
In the PGT field there are relatively few published studies describing the extensive product validation steps that are needed for preclinical validation of new technology and quality assurance.

Study design and methods

A multisite, retrospective study analyzing the chromosomal status of both cell lines and embryo biopsy samples by array-CGH and NGS was performed. A total of 170 cell line samples and 680 embryo biopsy samples were included in the study. Single cell and three-cell samples, mimicking blastomere and blastocyte biopsies, respectively, were obtained from six commercially available cell lines. Embryo biopsy samples (cleavage-stage or trophectoderm-stage) were obtained from four international reference laboratories or IVF centres with patients undergoing clinical IVF cycles with PGT-A. For all cell line and embryo biopsy samples, array-CGH was carried out using 24sure™ microarrays and NGS was carried out using the VeriSeq PGS Kit. Analysis of results for both PGT-A approaches was carried out using BlueFuse Multi software. Copy-number calls automatically generated by BlueFuse Multi were assessed manually by the respective sites by two independent observers in a blinded fashion; any unresolved manual call underwent further blinded analysis by a third observer.
Concordance assessment of the ploidy results by NGS (index test) with respect to the array results (reference test) was performed at the individual chromosome-arm level. The copy numbers for all 24 chromosomes in each sample tested were compared between the two methods, and for the overall sample diagnosis of aneuploidy or euploidy.
To assess the reliability of NGS for aneuploidy detection, the sensitivity and specificity of the test were calculated. The sensitivity is the proportion of embryos with an aneuploid array-CGH result that have an aneuploid NGS result, TP/(TP+FN)*. The specificity is the proportion of embryos with a euploid array-CGH result that have a euploid NGS result, TN/(TN+FP)†.

Results

Characterized cell line samples

- Sample-level positive and negative agreements were 100% for single-cell and three-cell samples
- Chromosome-level positive agreements were 96.0% (48/50) and 98.9% (88/89) and negative agreements were 99.8% (1,626/1,630) and 99.9% (2,045/2,047) for single cells and three-cell samples, respectively

Embryo biopsies:

- 86 samples were excluded: 71 with failed quality control (QC) metrics for array-CGH and/or NGS, 13 without array-CGH and/or NGS plots available for independent analysis, and 2 suspected triploids
- 594 embryos with array-CGH and NGS results were used for concordance analysis
- At an embryo level, the overall concordance between array-CGH and NGS was 99.2% (589/594), with a sample-level sensitivity and specificity of 99.0% (401/405) and 99.5% (188/189), respectively
- At a chromosome level, the sensitivity and specificity of NGS-based PGS were 97.7% (1,041/1,065) and 99.9% (23,866/23,883), respectively).

Conclusions

VeriSeq PGS was validated for detection of aneuploidy in preimplantation embryos, with both cell lines and embryo biopsy samples, in a large, preclinical analysis across multiple sites.
The dataset contained a good representation of chromosome losses and gains across all chromosomes, which ensured a thorough evaluation of assay performance. Overall sample-level positive and negative agreements were 99.0% and 99.5%, respectively
VeriSeq PGS reliably detected chromosome aneuploidies in low DNA input samples, across multiple sites, whether from biopsy of a single cell or a few cells

References

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Study sites: Colorado Center for Reproductive Medicine, Lone Tree, CO, U.S.A.; Genoma Laboratory, Molecular Genetics Laboratory, Rome, Italy; Melbourne IVF, Suite 10/320 Victoria Parade, East Melbourne, VIC, Australia 3002); Reprogenetics UK, Institute of Reproductive Sciences, Oxford, United Kingdom.

Consent and ethical approvals

The study was approved and written informed consent was obtained from each enrolled couple, as approved by the Institutional Review Boards: Melbourne IVF (Melbourne IVF Human Research Ethics Committee), Reprogenetics UK Institute of Reproductive Sciences (WIRB 20060680), and GENOMA laboratory (Institutional Review Board of Genoma). Samples from the Colorado Center for Reproductive Medicine were obtained from patients that had consented to PGS by array-CGH and the use of discarded materials for research.