PGT-A COMBINING COPY NUMBER VARIATION AND ALLELIC BALANCE CONFIRMS WHOLE CHROMOSOME AND SEGMENTAL MOSAICISM IN PREIMPLANTATION EMBRYOS

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OBJECTIVE:

The primary objective of this study was to establish the utility of a PGT-A method leveraging both copy number variation and allelic balance for detecting whole chromosome and segmental mosaic aneuploidy. A second objective was to survey a large cohort of preimplantation embryos for the distribution of mosaic length, degree and complexity.

BACKGROUND:

Mosaic embryos present a challenging clinical decision-point.

- Results indicate an occurrence rate of 5-25%.
- Differences in analysis methods, sample variability, and data interpretation may create discrepancies.
- Classification uncertainty blurs true clinical outcomes and makes reliable assessment of transfer options difficult.

Most PGT-A platforms are limited to detecting mosaicism by either allelic balance from single nucleotide polymorphisms (SNPs) or normalized copy number variation (CNV) across the genome.

- Underpowered CNV or SNP data may mask the true ploidy level and lead to misclassification.
- A method combining CNV and SNP data may generate more confident transfer data.

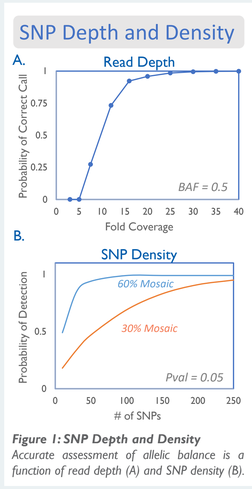
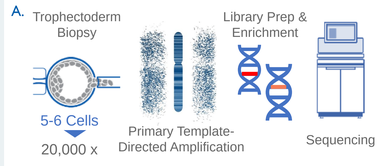


Figure 1: SNP Depth and Density
Accurate assessment of allelic balance is a function of read depth (A) and SNP density (B).

ASSAY DESIGN:

Trophectoderm biopsies are amplified using Primary Template-directed Amplification (PTA), library-prepped, target-enriched, and sequenced with NGS. Normalized CNV and genome-wide SNP data were used to identify and confirm regions of aneuploidy, including whole chromosome and segmental mosaic regions to >5 mb.



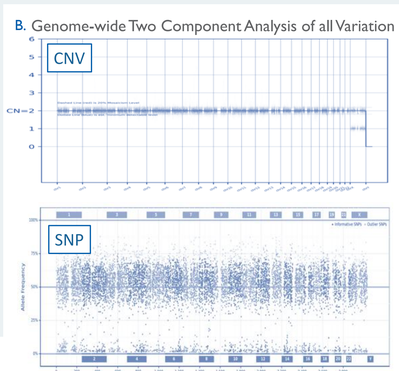


Figure 2: PGT-A Assay with Combined CNV/SNP Analysis.
(A) Component laboratory methods in the PGT-A pipeline. (B) Pipeline data from a euploid embryo showing sensitive CNV based on normalized read depth across the genome, and genome-wide SNP data translated in to a B-Allele Frequency (BAF) chart. The steady line present at copy number (CN=2) and uniform BAF centered at 0.5 indicate a diploid genome with no identifiable regions of aneuploidy.

METHODS DEVELOPMENT:

The method generated deep, distributed SNP data, averaging 108K quality-filtered SNPs per genome (~33 SNPs/mb) at an average depth of 51 reads/SNP.

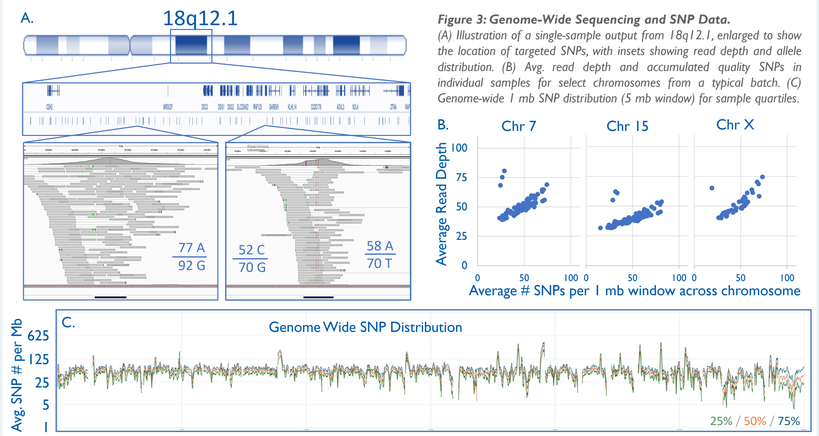


Figure 3: Genome-Wide Sequencing and SNP Data.
(A) Illustration of a single-sample output from 18q12.1, enlarged to show the location of targeted SNPs, with insets showing read depth and allele distribution. (B) Avg. read depth and accumulated quality SNPs in individual samples for select chromosomes from a typical batch. (C) Genome-wide 1 mb SNP distribution (5 mb window) for sample quartiles.

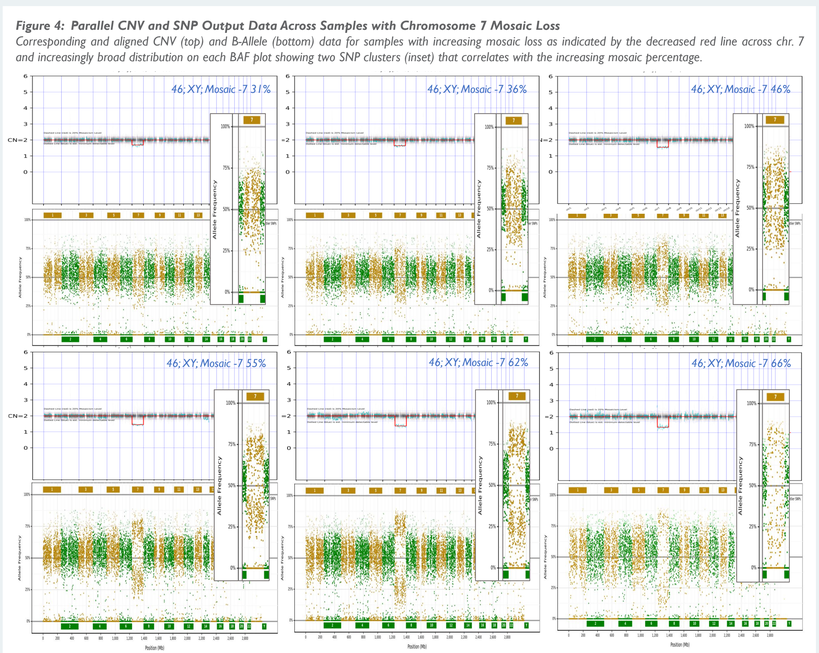


Figure 4: Parallel CNV and SNP Output Data Across Samples with Chromosome 7 Mosaic Loss
Corresponding and aligned CNV (top) and B-Allele (bottom) data for samples with increasing mosaic loss as indicated by the decreased red line across chr. 7 and increasingly broad distribution on each BAF plot showing two SNP clusters (inset) that correlates with the increasing mosaic percentage.

RESULTS:

Initial CNV analysis identified a subset of embryos with a single whole chromosome (1277) or segmental (1930) mosaic region. In both scenarios, mosaicism was confirmed by allelic balance in 65% of regions. Minimal difference was observed in baseline adjusted noise across sample classifications.

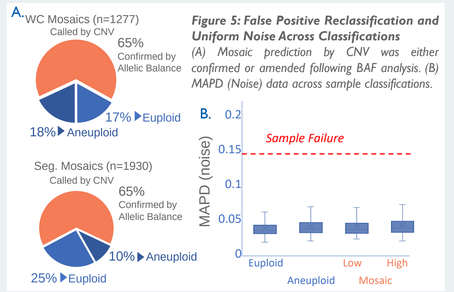


Figure 5: False Positive Reclassification and Uniform Noise Across Classifications
(A) Mosaic prediction by CNV was either confirmed or amended following BAF analysis. (B) MAPD (Noise) data across sample classifications.

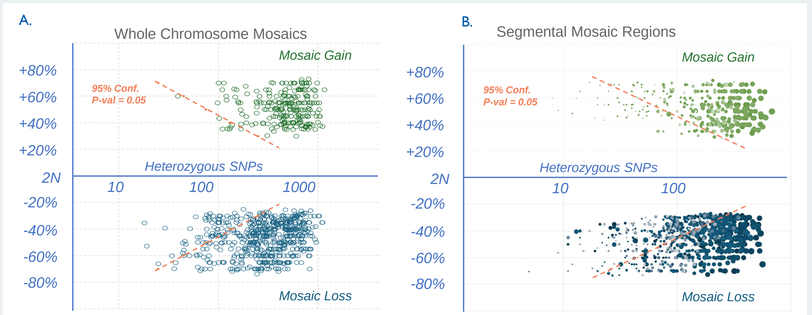


Figure 6: Statistically Significant Mosaic Classification Based on SNP Data
Whole Chromosome (A) and Segmental (B) data indicating the number of heterozygous SNPs within regions of indicated percent mosaic gain or loss. Dashed red lines indicate the approximate position of statistically significant (P-val <0.05) with 95% confidence that the indicated region is "not diploid" and likely contains an intermediate copy number. Segmental shading indicates chromosomal location and size reflects the mosaic segmental length.

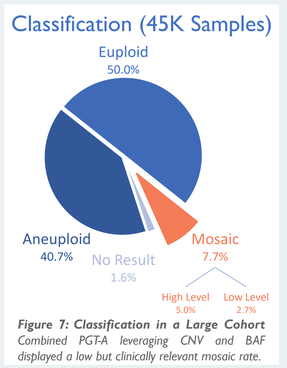


Figure 7: Classification in a Large Cohort
Combined PGT-A leveraging CNV and BAF displayed a low but clinically relevant mosaic rate.

Confident mosaic detection results from the combination of deep individual SNP data and the accumulation of SNPs within the region. Across 45K de-identified clinical embryos analyzed in this study, there was an observed overall mosaic rate of 7.7% with 50.0% Euploid, 40.7% Aneuploid, 1.6% Unclassified. Within the mosaic embryos, 5.0% and 2.7% displayed high and low-level mosaicism, respectively. Complex mosaic aneuploidy (3+ regions) was observed in approximately 0.6% of samples.

CONCLUSIONS:

This study confirmed the utility of a SNP-based and coverage-based PGT-A pipeline to identify and confirm mosaic regions, generating higher confidence and reducing misclassification. The uniform noise level among all classified samples confirms that mosaicism is unlikely a platform artifact or the result of sample variability. This study suggests a relatively low but significant mosaic occurrence rate and emphasizes the need for accurate detection to further clarify clinical outcomes of mosaic transfer.