21ST

PGDIS CONFERENCE

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

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Kuala Lumpur

Malaysia

PGT and BEYOND...

Multiomic integration of transcriptome and genome analysis to enable novel biomarker discovery

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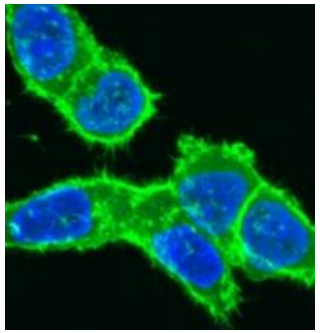
Takara Bio USA, 2560 Orchard Pkwy, San Jose, California, 95131

Takara Bio: core capabilities



that's GOOD science!®

NGS

- SMARTer® and SMART-Seq® RNA-seq library preparation kits
- PicoPLEX® and ThruPLEX® DNA-seq library preparation kits

PCR, qPCR, RT-PCR

- TaKaRa Ex Premier™, LA Taq™, PrimeSTAR® GXL, SeqAmp™,Titanium® polymerases, & PrimeScript™ RT
- EcoDry™ lyophilized enzymes and kits

Cloning

- In-Fusion® Snap Assembly Cloning

Nucleic acid purification

Gene delivery

- Lenti-X™, Adeno-X™, Retro-X™, and AAVpro® systems | Xfect™ transfection reagent
- RetroNectin® reagent

Functional genomics

- Tet systems and iDimerize™ systems
- Guide-it™ CRISPR/Cas9 genome editing products
- Living Colors® fluorescent proteins

Protein expression & purification

- TALON® and His60 Ni protein purification

OEM

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New biomarkers beyond copy number status to improve embryo selection

Looking beyond PGT-A to obtain a fuller picture

- PGT-A may fail to produce interpretable results in up to 5.7% cases*
- DNA copy number provides only partial insights

Comprehensive understanding of the molecular landscape requires multiomic approaches

- DNA-seq: genetic makeup; including copy number, single nucleotide, and structural variants
- RNA-seq: gene expression patterns, transcriptional profiles, single nucleotide variants
- Other omics

PicoPLEX technology

- Highly reproducible whole genome amplification
- Quasi-random priming + suppression PCR
- Widely used in PGT-A workflows around the world

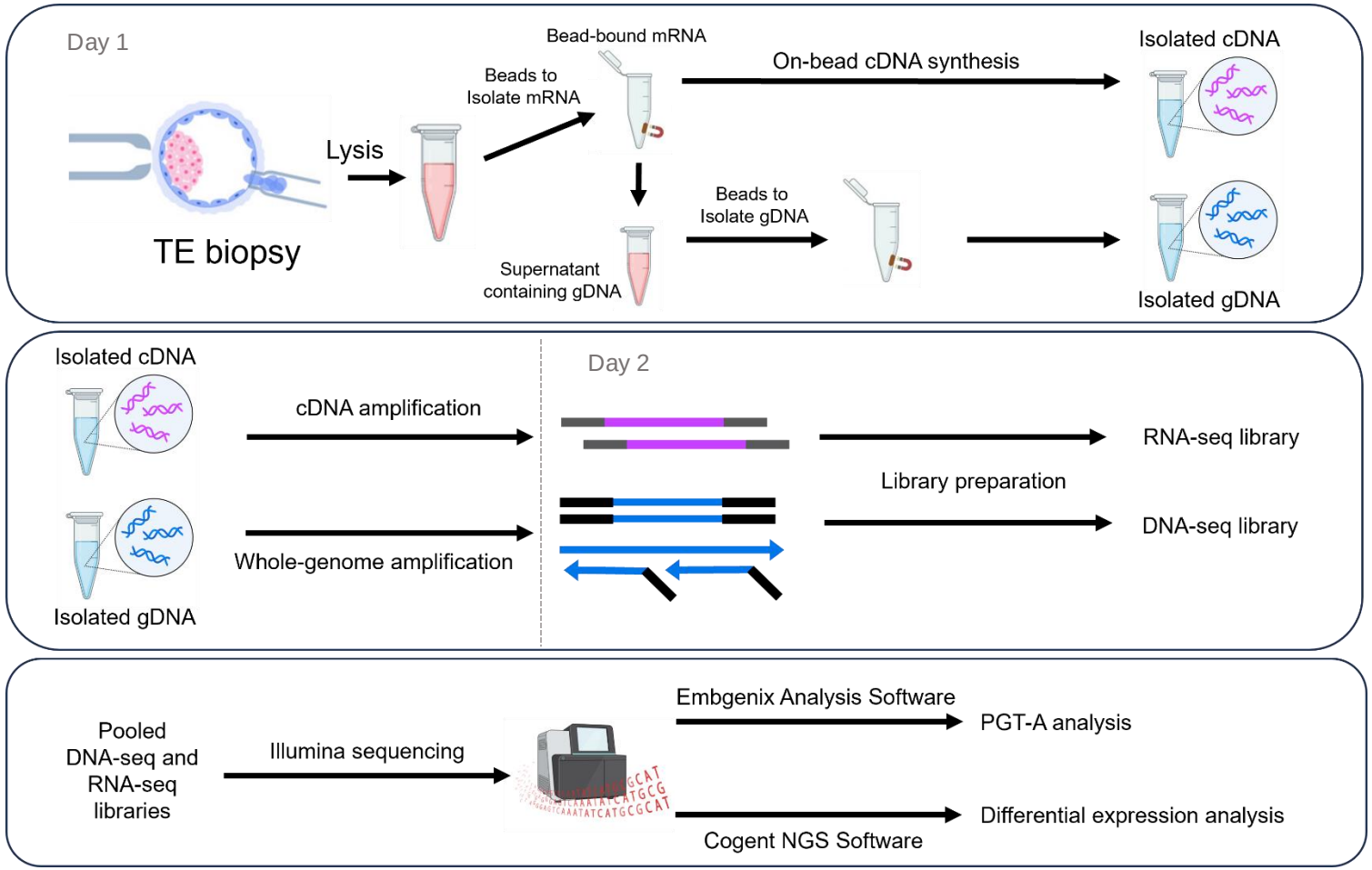
SMART-Seq technology

- Full-length transcript information
- Highest sensitivity and uniform gene-body coverage
- Industry gold standard for low-input and single-cell RNA-seq

*Benjamin S. Harris, et al. Preimplantation genetic testing: a review of current modalities. *F&S Reviews* **2**, 43-56 (2021).

Multiomic workflow for genome and transcriptome analysis

- A tailored method for combined PGT-A and differential expression analysis
- >95% success rate (passing combined QC criteria for PGT-A and RNA-seq), improved from 75.6% in the original publication*
- Accompanying bioinformatic analysis solutions
  - PGT-A: Embgenix™ Analysis Software
  - RNA-seq: Cogent™ NGS analysis pipeline

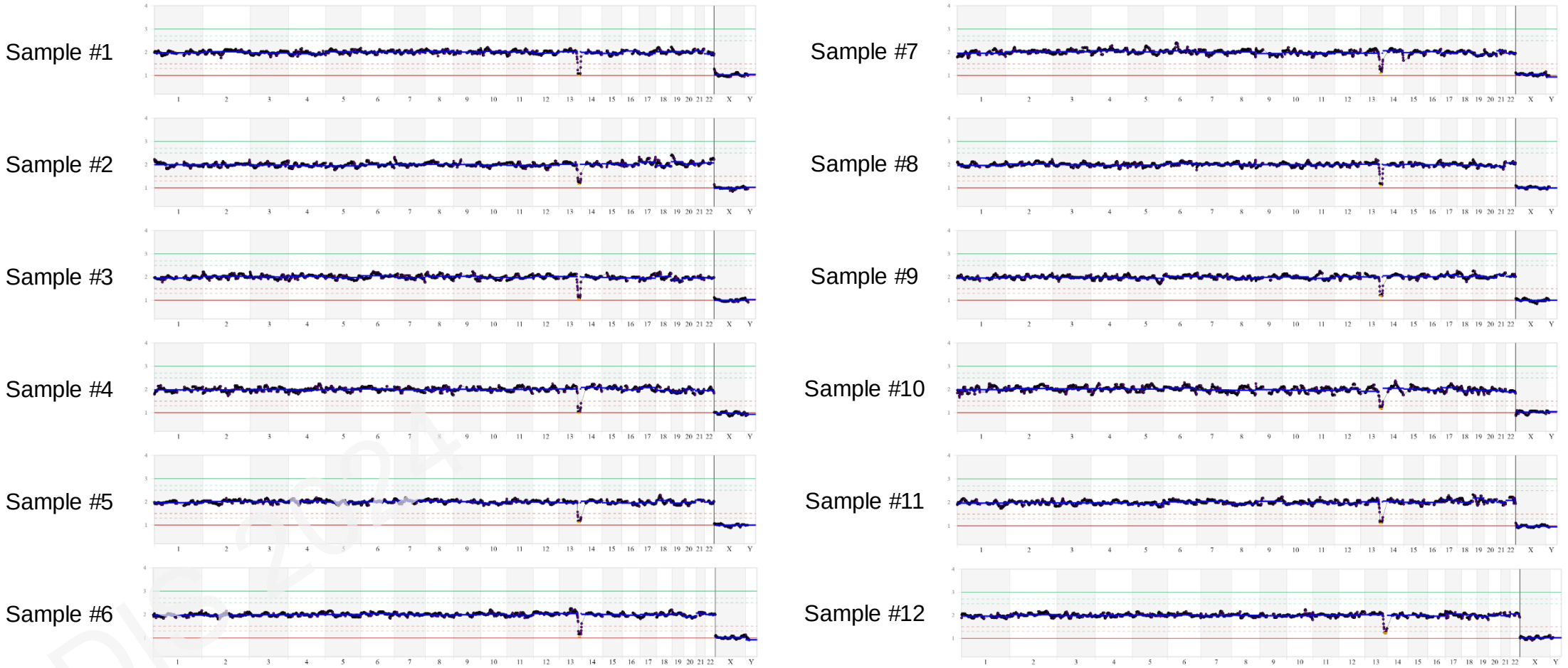


*Macaulay, I. et al. G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. *Nat Methods* **12**, 519-522 (2015).

Analysis methods for lymphoblastoid cells

- Starting material:
  - GM08331 cell line (Coriell Institute)
    - Male, 46, XY, del(13)(q32q33)
    - 12.1 Mb deletion
- Experimental workflow:
  - Five cells were sorted into independent wells of a 96-well PCR plate
  - 12 replicate samples were processed using the multiomic workflow
  - Libraries were sequenced on an Illumina® NextSeq® using 2 x 75 bp paired-end (PE) reads
  - Data were down-sampled to 1.5 x 10^6 reads (750,000 PE clusters) for DNA-seq results and 4 x 10^6 reads (2 x 10^6 PE clusters) for RNA-seq results

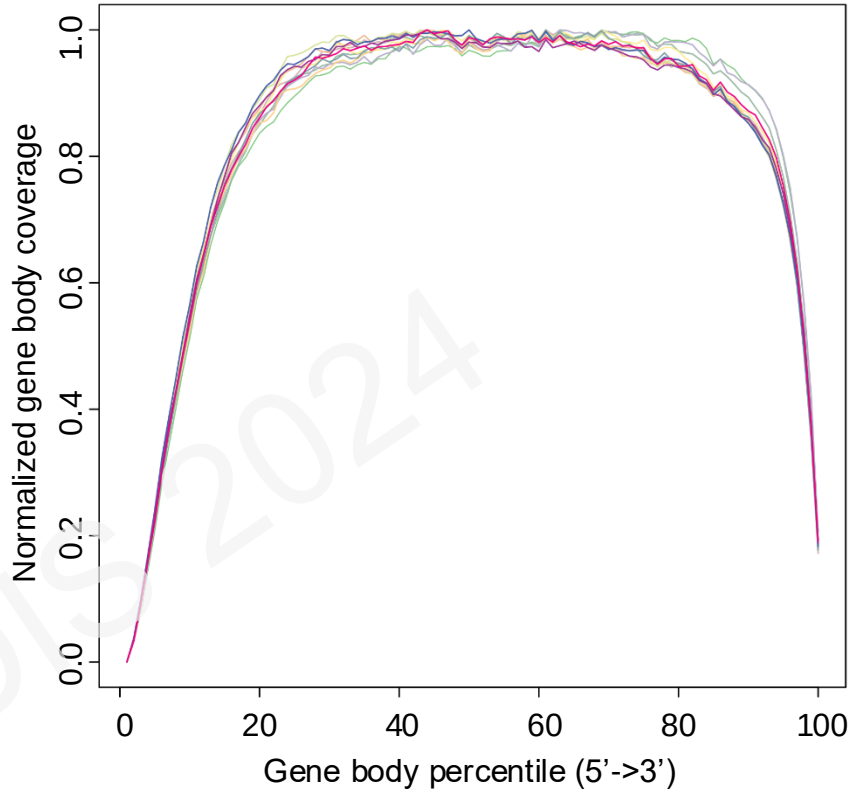
Reproducible results for CNV analysis

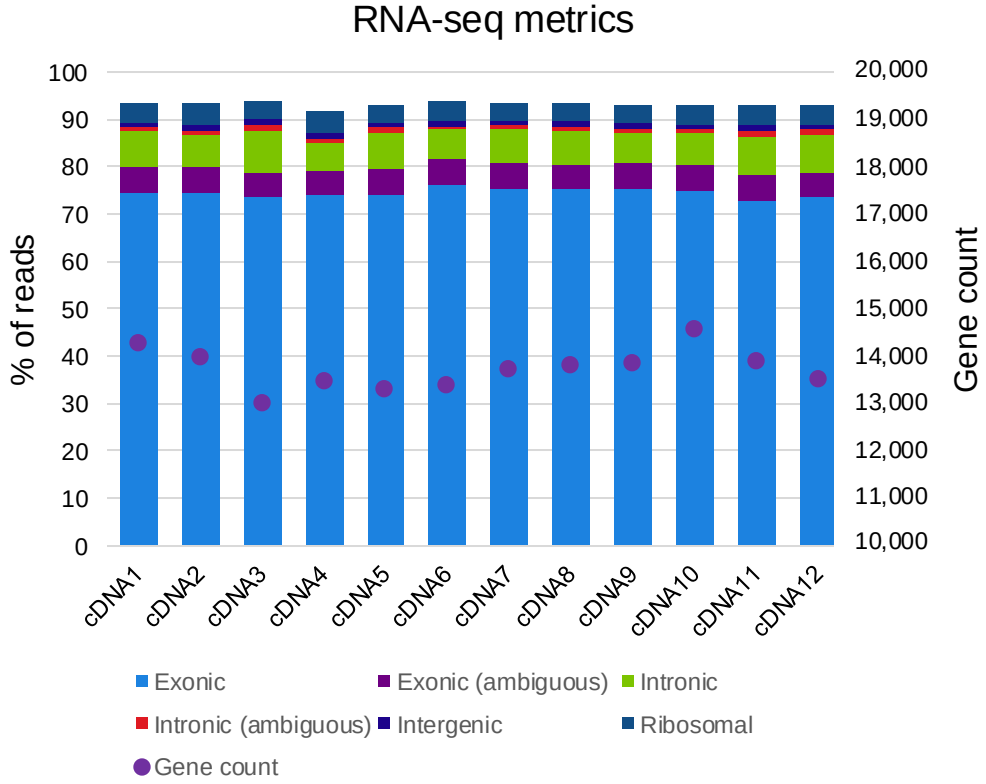


Del(13)(q32q33) was reported for all replicates by Embgenix Analysis Software, indicating that the sensitivity and specificity for detection of CNVs ≥10 Mb at 1.5 x 10^6 reads was comparable to the standalone PGT-A assay.

Uncompromised sensitivity and data quality for mRNA analysis

- Data analysis with Cogent NGS analysis pipeline at 4M reads per sample
- Unbiased gene body coverage with >10,000 genes detected and >70% of reads within exons

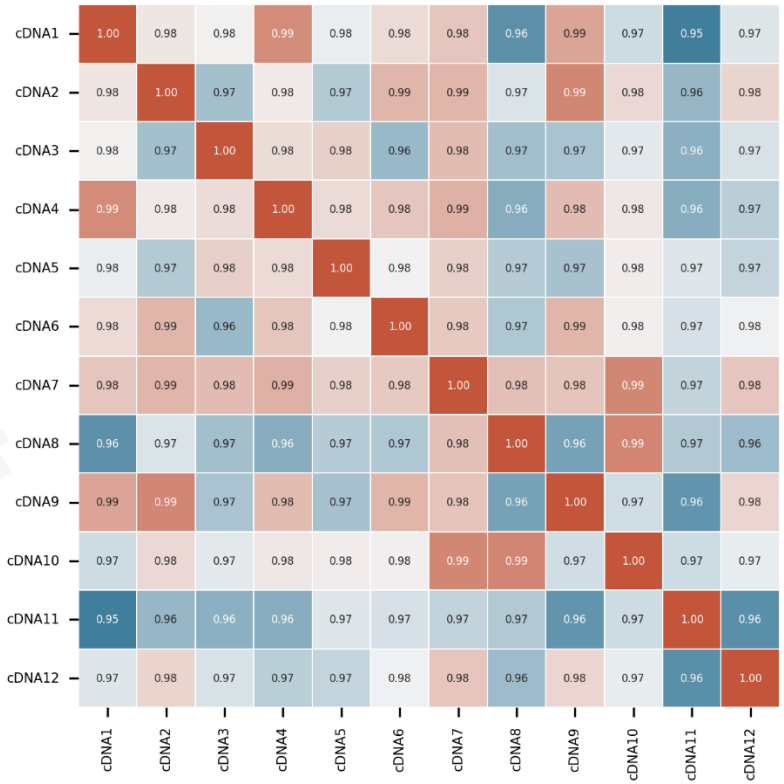




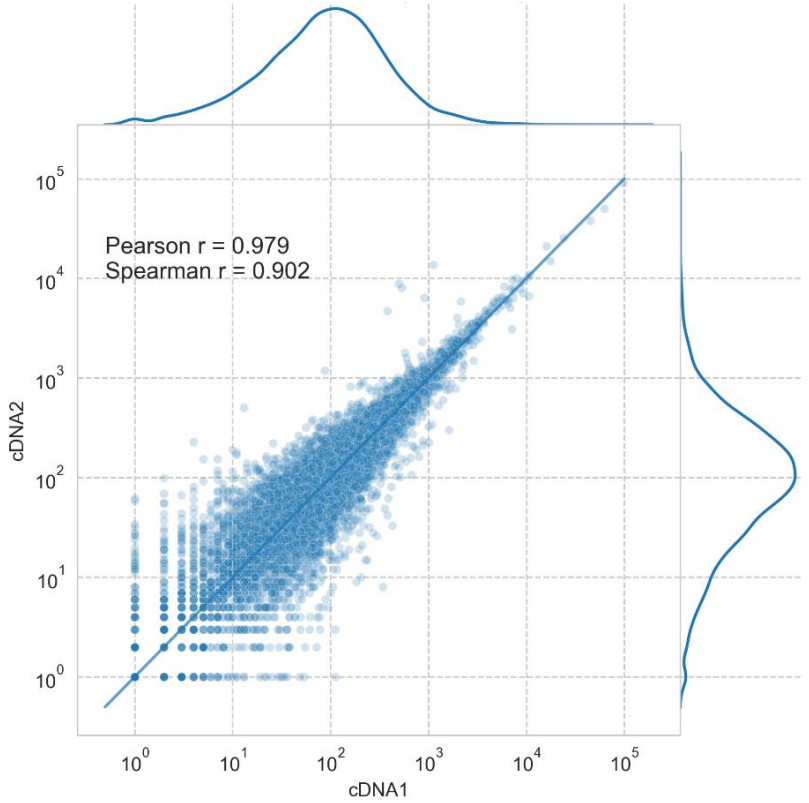
Reproducible transcriptomic profiles

- High correlation in gene counts across experimental replicates
  - RNA-seq results analyzed with Cogent NGS analysis pipeline
  - Pearson correlation >0.95 for all comparisons

Gene count: Pearson correlations



Gene count: scatter plot

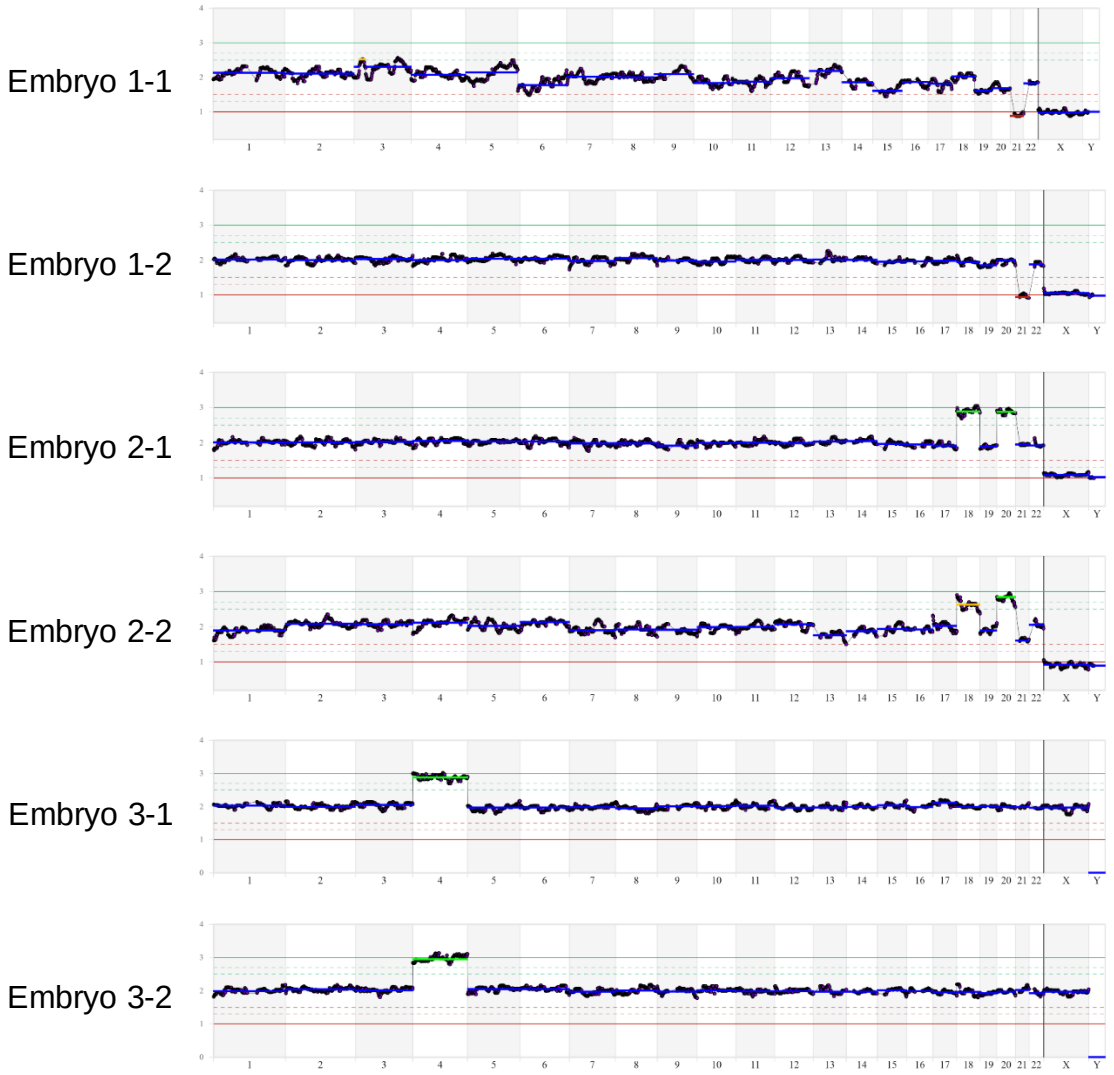


Analysis methods for trophectoderm biopsy samples

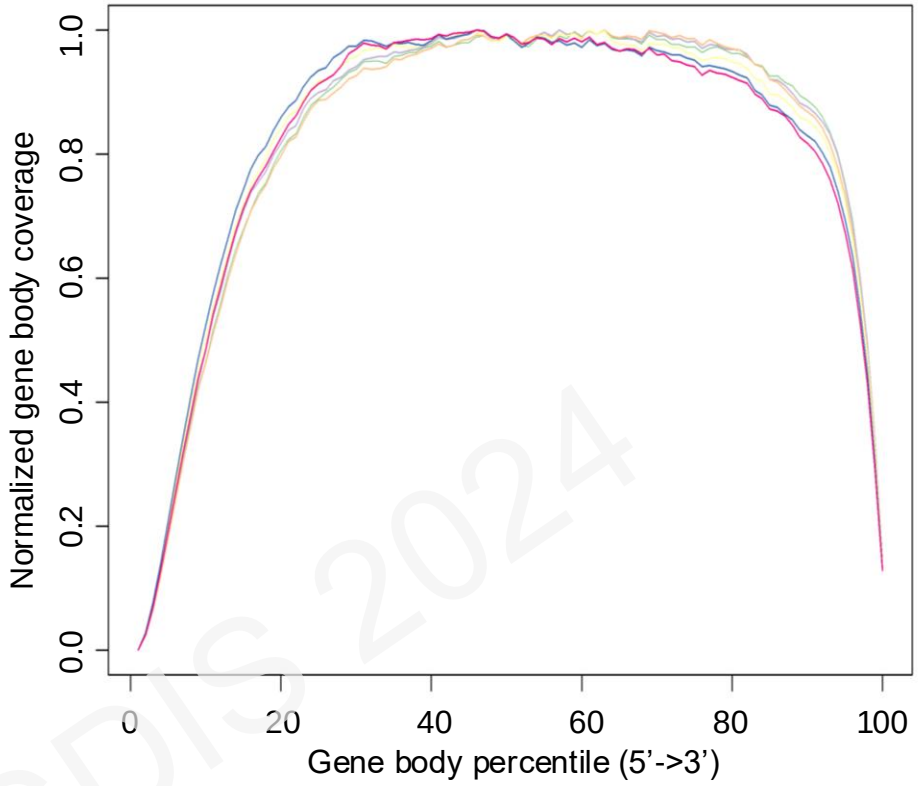
- Starting material:
  - Two trophectoderm (TE) biopsy samples were taken from each of three previously characterized embryos; samples were directly lysed and stored in sample lysis buffer
    - Embryo 1: Monosomy 21, XY
    - Embryo 2: Trisomy 18 & 20, XY
    - Embryo 3: Trisomy 4, XX
- Experimental setup:
  - Six TE biopsy samples were processed with the multiomic workflow
  - DNA-seq and RNA-seq libraries were sequenced on a NextSeq® platform using 2 x 75 bp paired-end (PE) reads
  - Data were down-sampled to 1.5 x 10^6 reads (750,000 PE clusters) for DNA-seq results, and 4 x 10^6 reads (2 x 10^6 PE clusters) for RNA-seq results

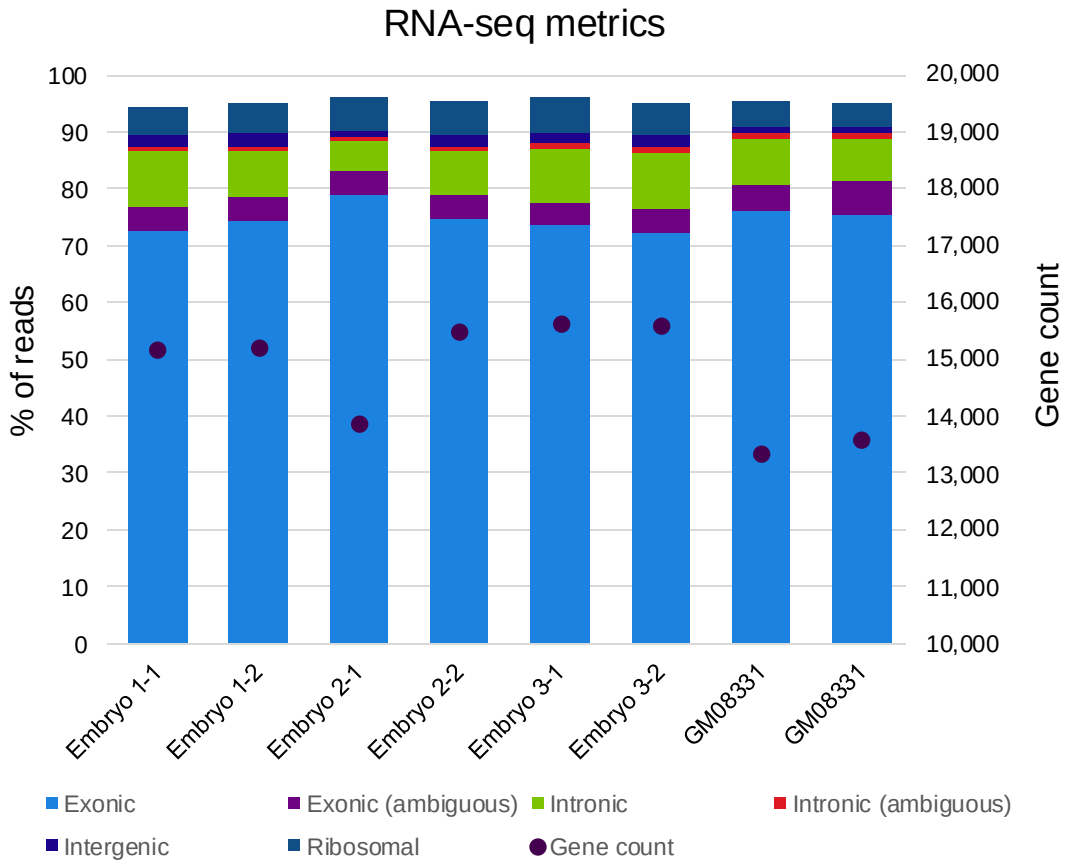
Concordant CNV calls between multiomic analysis and initial PGT-A findings

SAMPLE ID	Embryo 1-1	Embryo 1-2	Embryo 2-1	Embryo 2-2	Embryo 3-1	Embryo 3-2
REFERENCE LAB RESULT	Monosomy 21, XY	Monosomy 21, XY	Trisomy 18 & 20, XY	Trisomy 18 & 20, XY	Trisomy 4, XX	Trisomy 4, XX
QC STATUS	PASS	PASS	PASS	PASS	PASS	PASS
SAMPLE CALL INTERPRE-TATION	Aneuploid	Aneuploid	Aneuploid	Aneuploid	Aneuploid	Aneuploid
NUMBER OF TOTAL READS	1,500,000	1,500,000	1,500,000	1,500,000	1,500,000	1,500,000
SEX	Male	Male	Male	Male	Female	Female
KARYOTYPE	45,XY,seq (21)x1	45,XY,seq (21)x1	48,XY,seq(18)x3,seq(2 0)x3	47,XY,seq(20)x3	47,XX,seq(4)x3	47,XX,seq(4)x3
Concordant with standalone PGT-A	YES	YES	YES	YES	YES	YES



Uncompromised sensitivity and data quality for mRNA analysis from TE biopsy samples

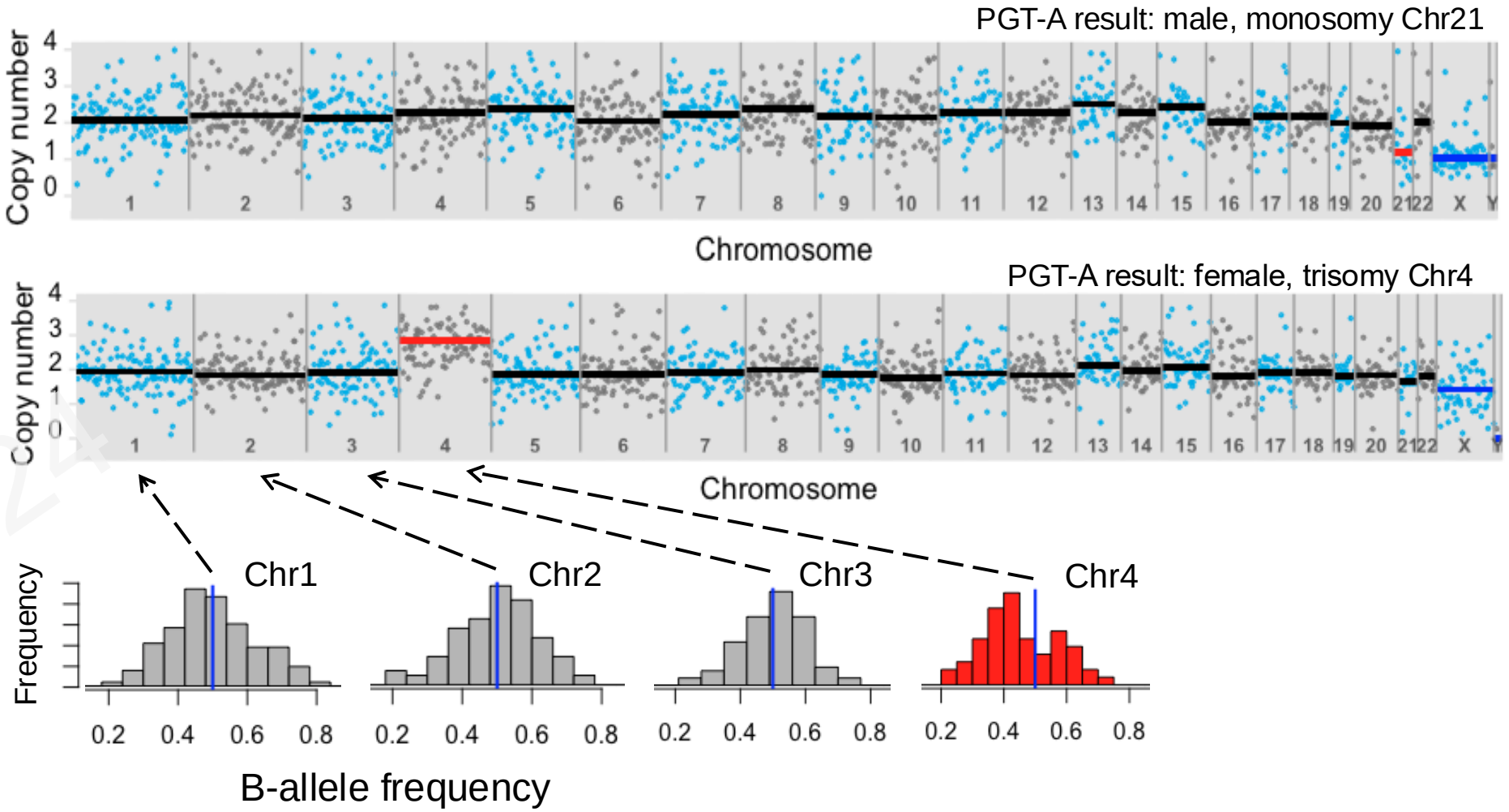




Detecting chromosome-level copy number variation and allelic imbalance using transcriptomic data

CNV detection using transcript counts

- Chromosome copy number
- Aneuploidy detected
- Sex chromosome calling



SNV-based detection of allelic imbalance using RNA-seq. Can also be used to detect polyploidy, UPD, etc.

Conclusions

- We have demonstrated the feasibility of obtaining both genome and transcriptome information from a single biopsy sample by integrating Embgenix and SMART-Seq workflows in a manner that minimizes genomic DNA loss and RNA contamination
- Leveraging transcriptome analysis for discovery of novel biomarkers in this context provides a dynamic and functional view of the embryo, providing a basis for potential improvements to ART

To discuss early access opportunities involving this technology, please contact:

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