Further Advances in DNA Amplification for PGT

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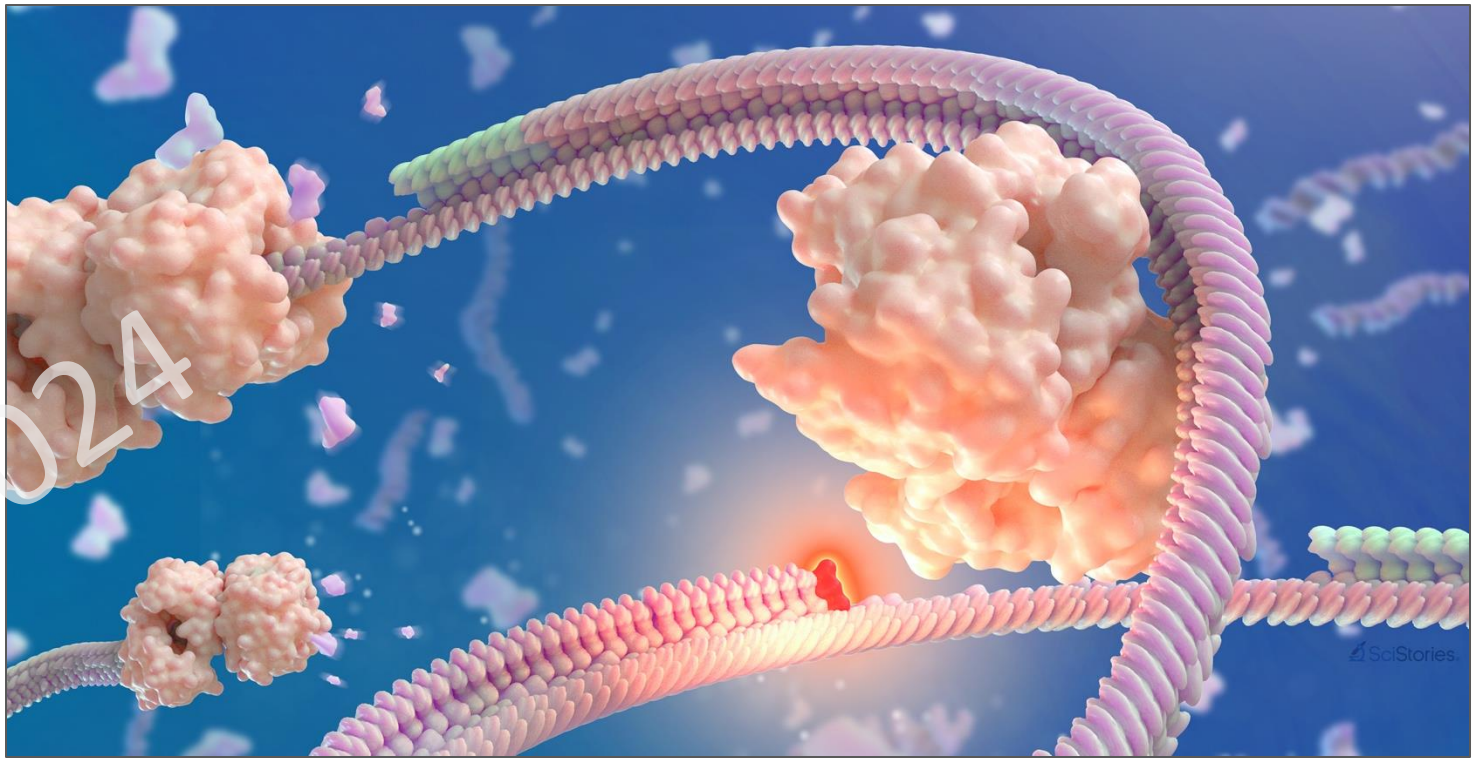
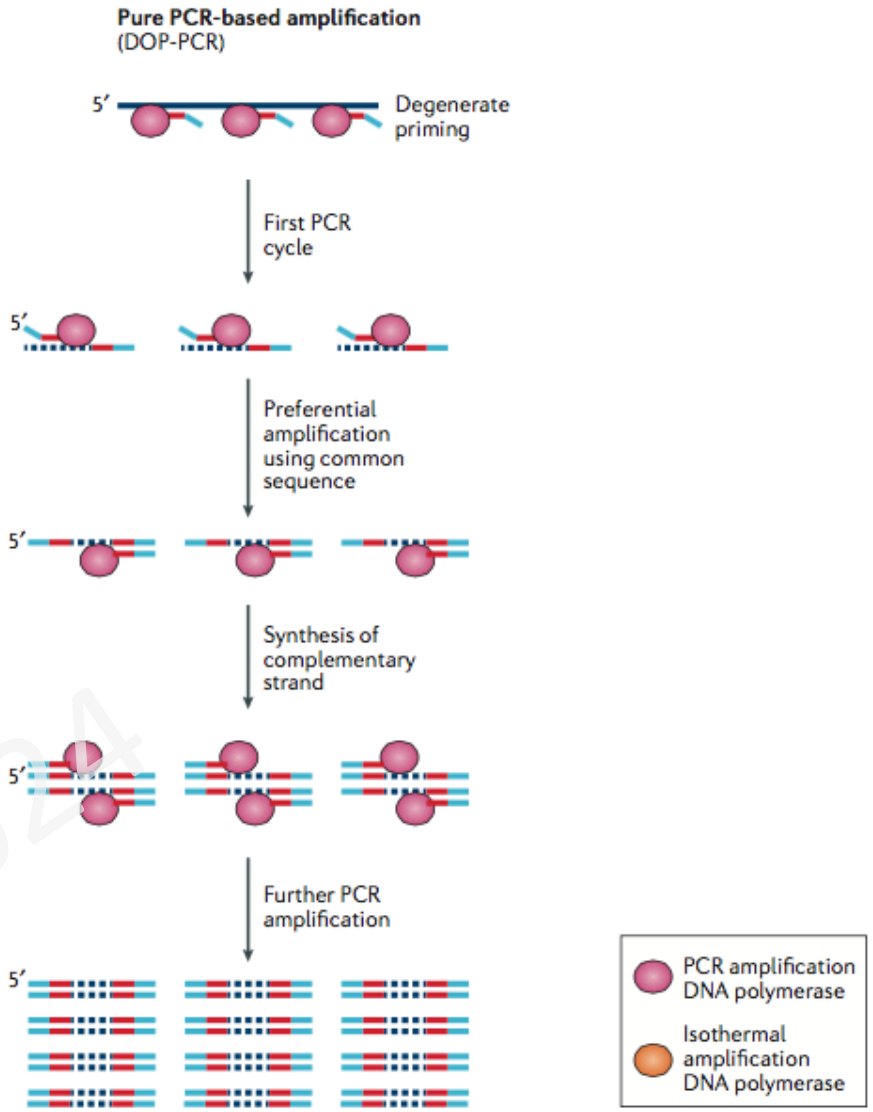


Opportunities for PGT Innovation

- Sample Collection
- Sample Identification
- Nucleic Acid Amplification
- Sequencing
- Analyses
- Results Interpretation

Overview of WGA Strategies

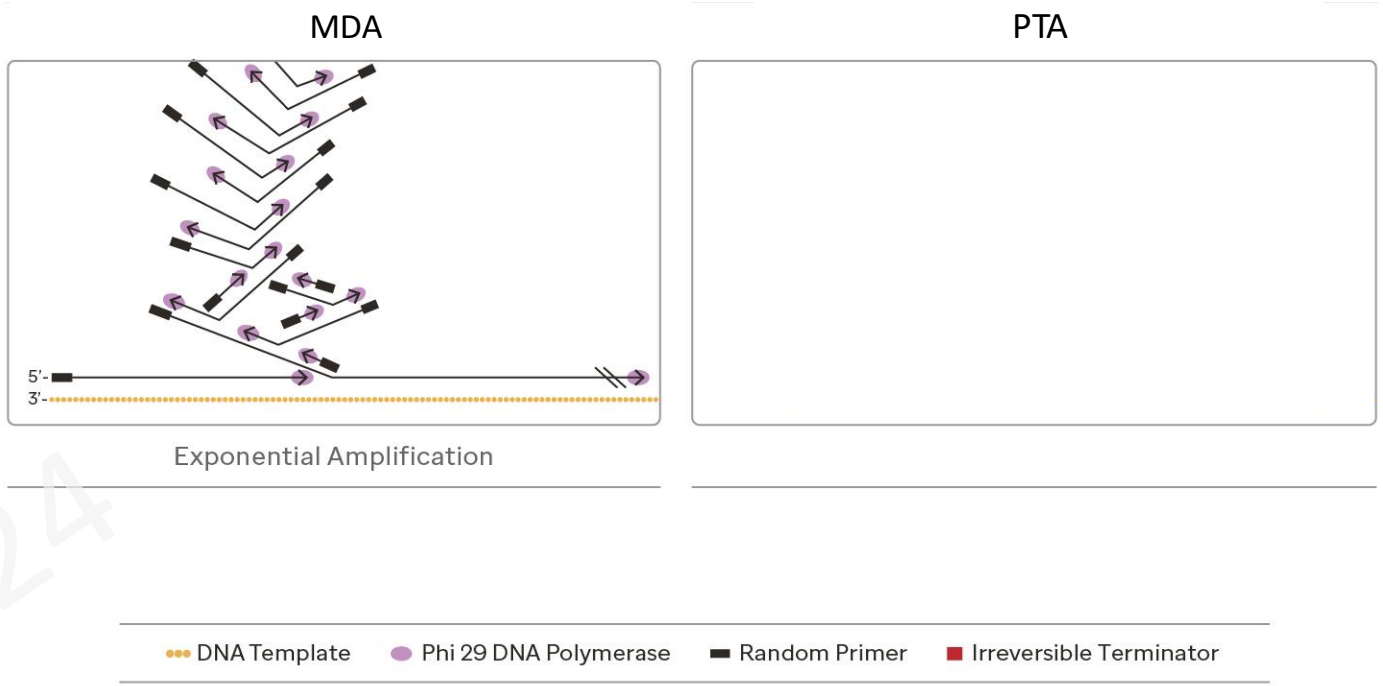


(Gawad et al Nature Reviews Genetics 2016)

Limitations of Previous Single-Cell WGA Methods

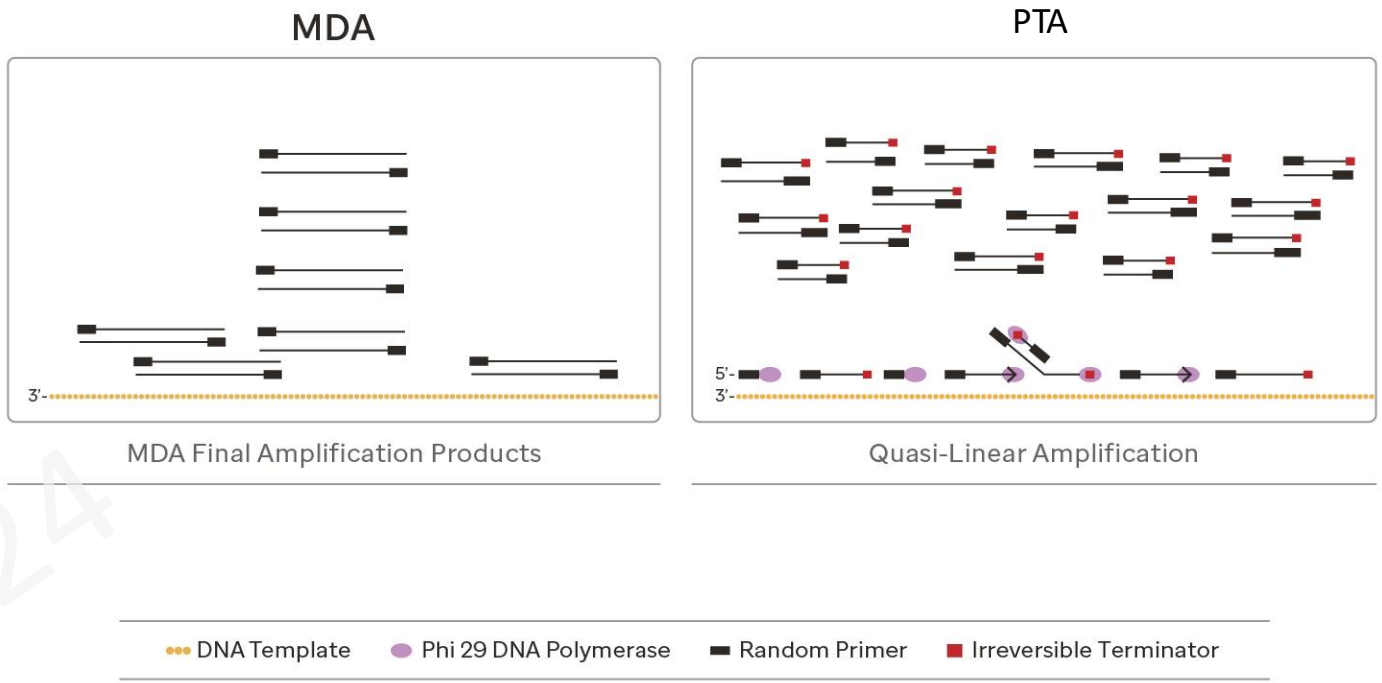
- Poor whole genome amplification uniformity and/or coverage
- Low single nucleotide variant detection sensitivity
- Introduction of false positive variant calls leading to poor precision
- Lack reproducibility between cells/samples

Primary Template-Directed Amplification (PTA)



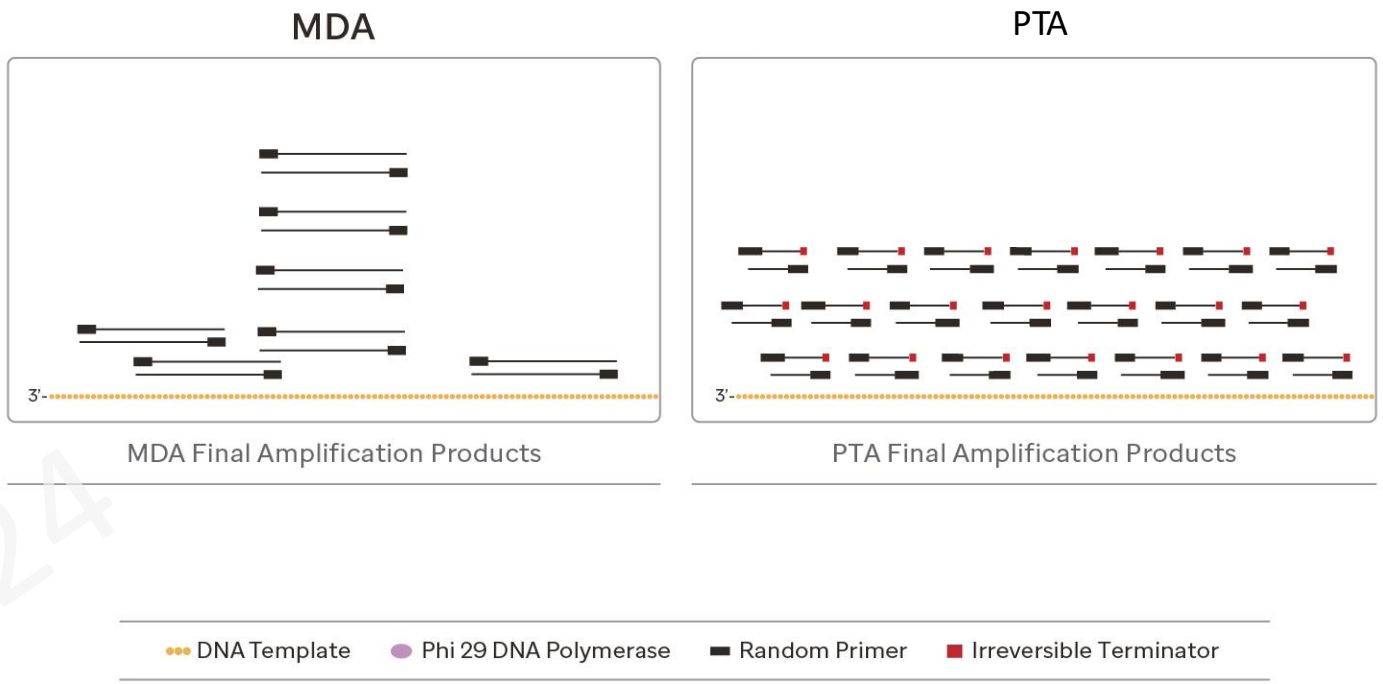
(Gonzalez, V…. Gawad, C *PNAS* 2021)

Primary Template-Directed Amplification (PTA)



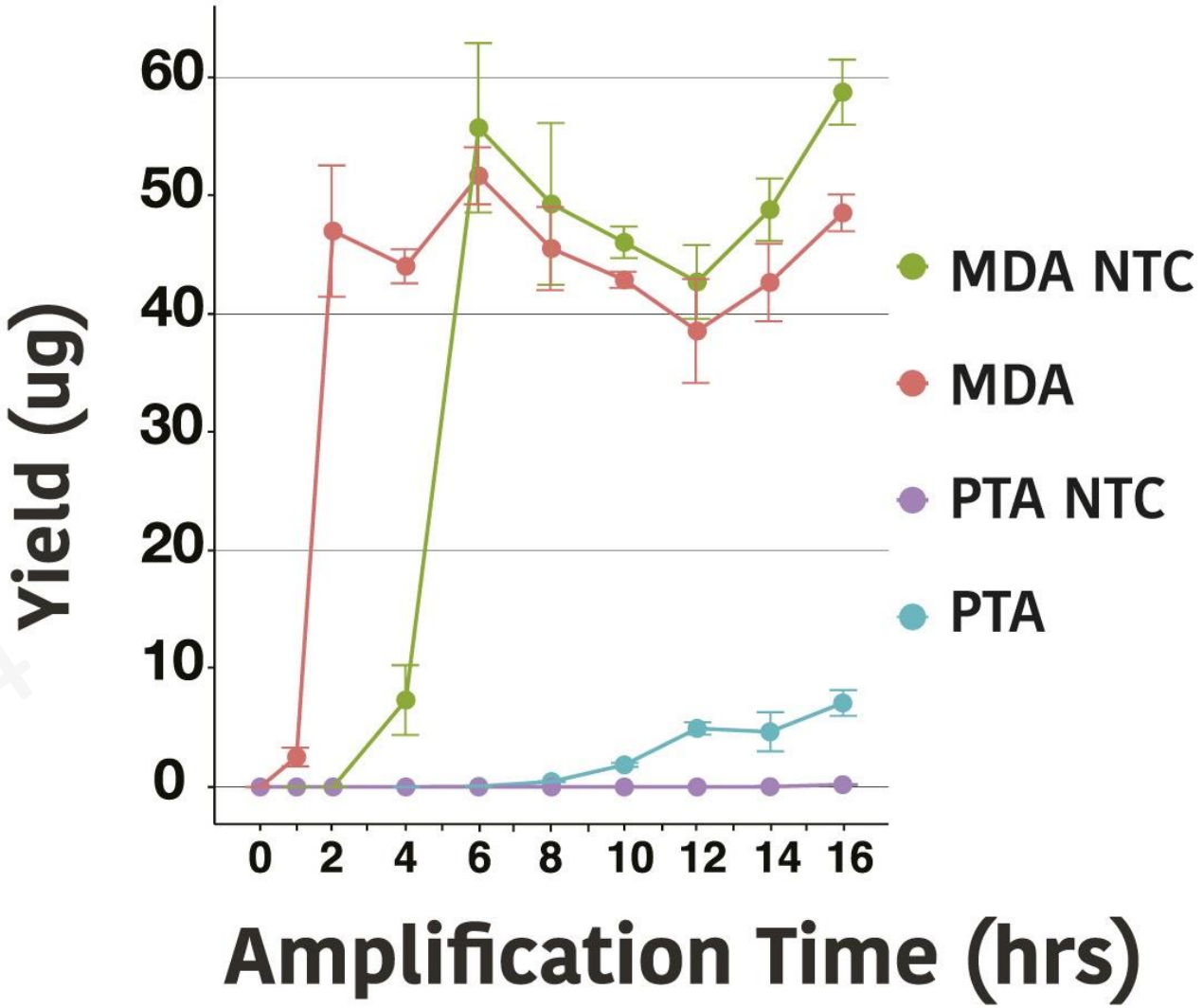
(Gonzalez, V…. Gawad, C *PNAS* 2021)

Primary Template-Directed Amplification (PTA)



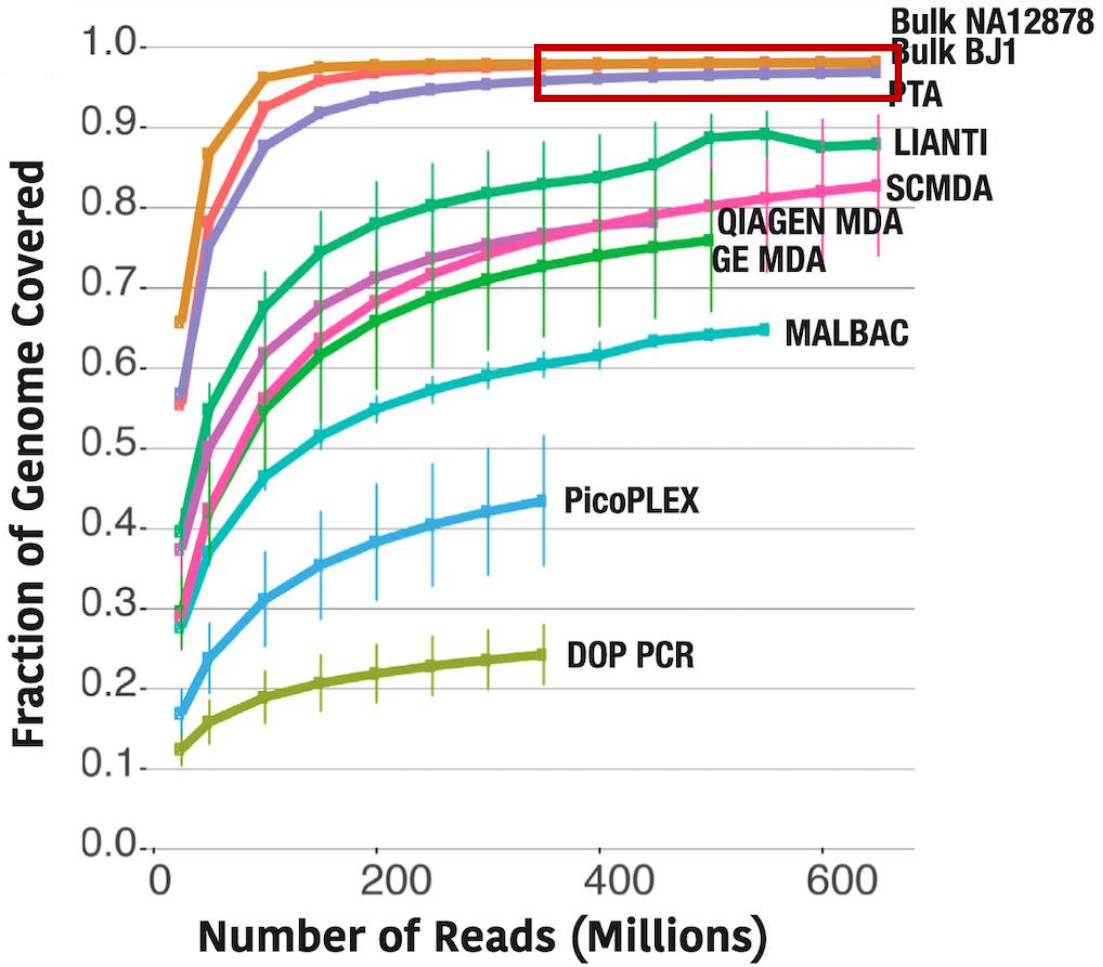
(Gonzalez, V…. Gawad, C *PNAS* 2021)

PTA Kinetics Are Quasilinear



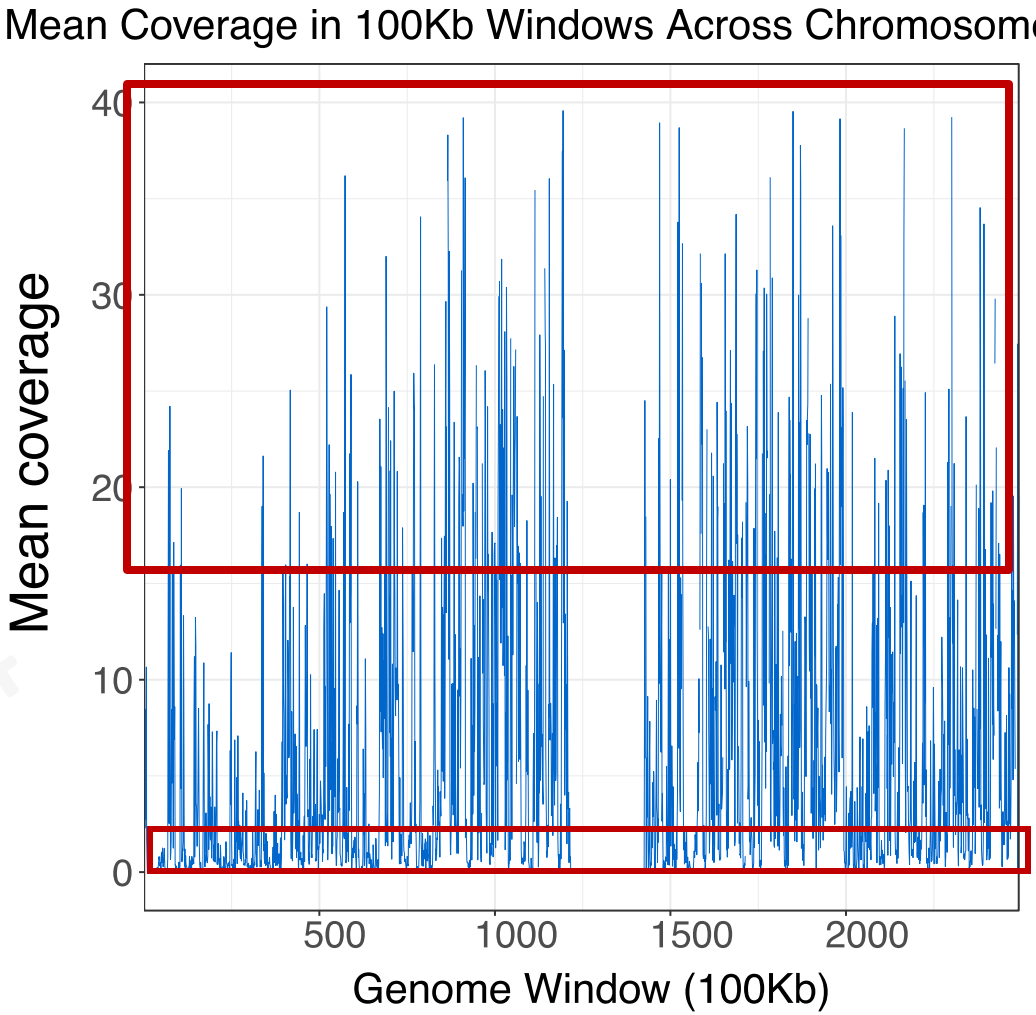
(Gonzalez, V.... Gawad, C *PNAS* 2021)

Genome Coverage at Increasing Sequencing Depth



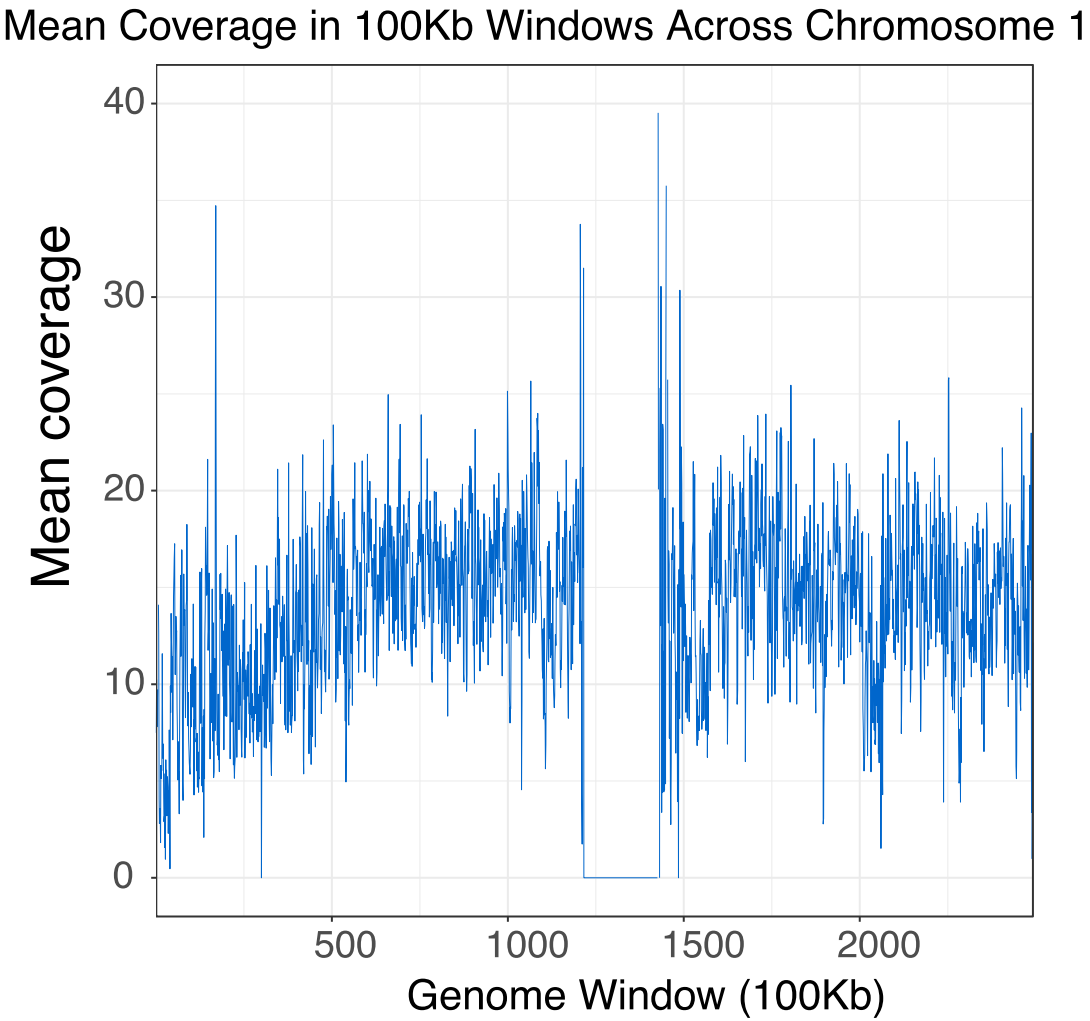
(Gonzalez, V…. Gawad, C *PNAS* 2021)

MDA (the Current Gold Standard)



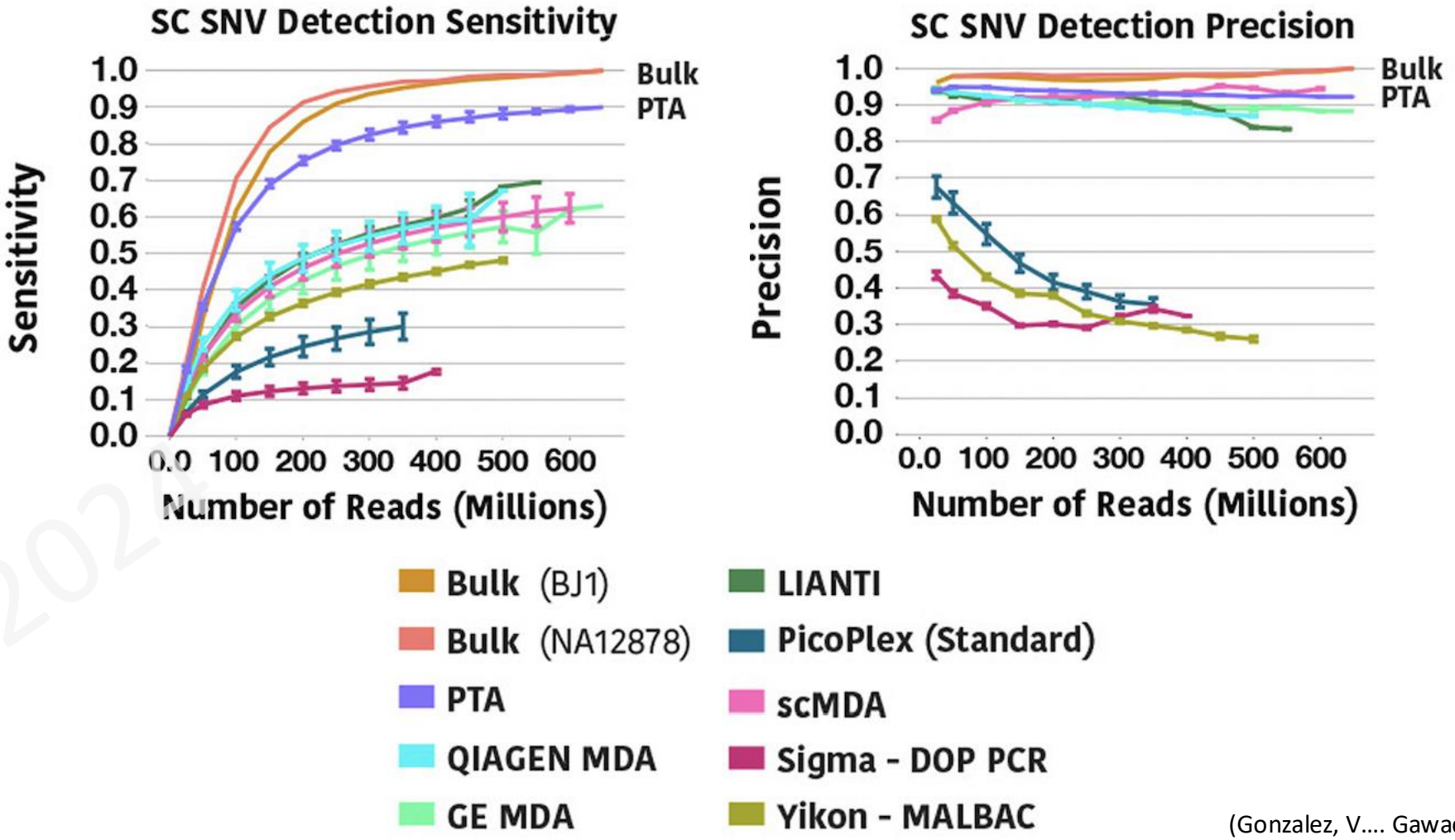
(Gonzalez, V.... Gawad, C *PNAS* 2021)

PTA



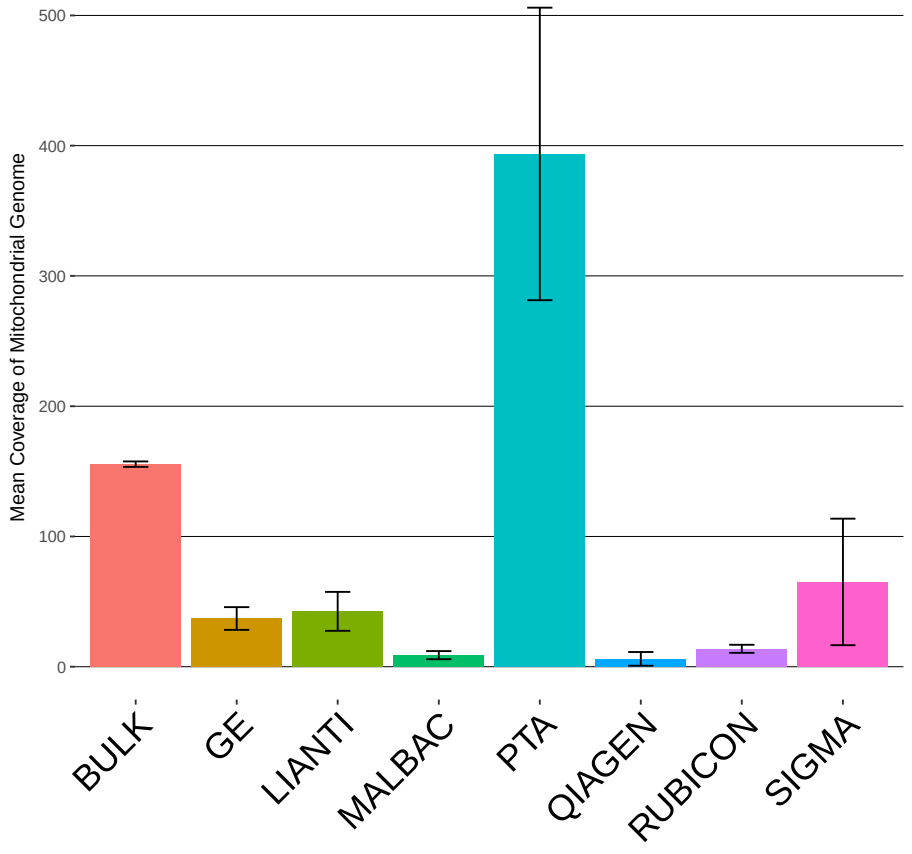
(Gonzalez, V…. Gawad, C *PNAS* 2021)

Accuracy of Single Cell SNV Calling with PTA



(Gonzalez, V…. Gawad, C *PNAS* 2021)

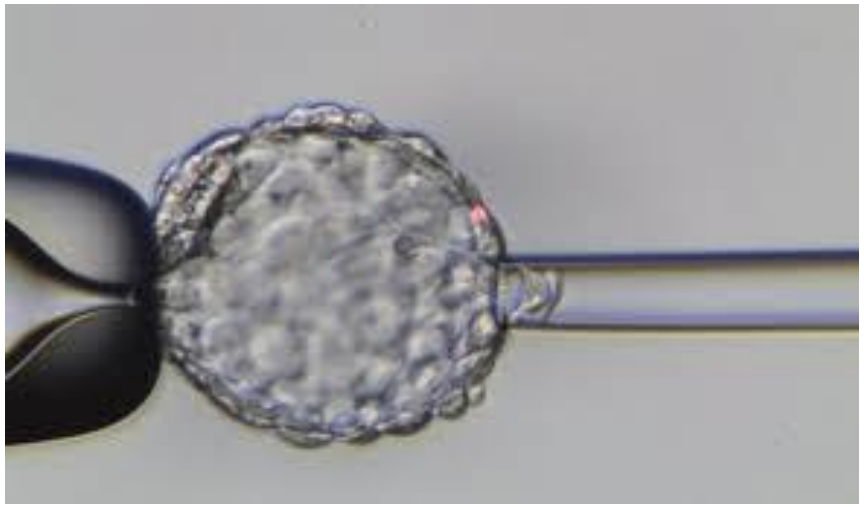
Mean Coverage of Mitochondrial Genome

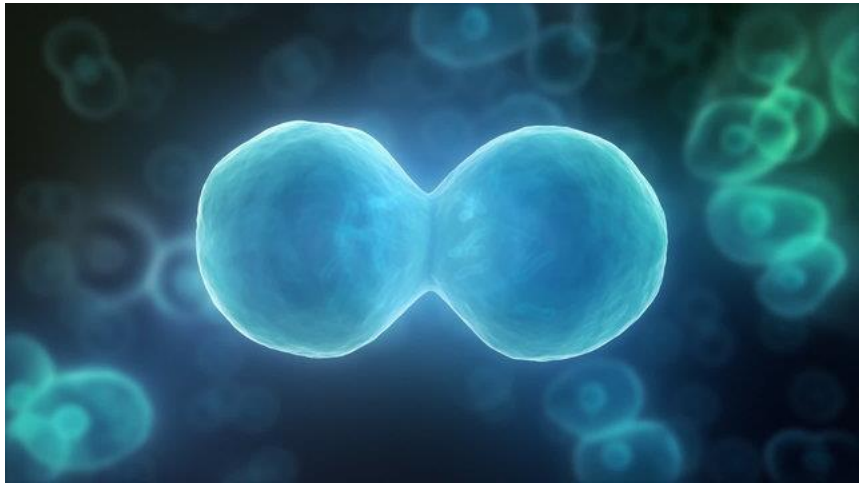


(Gonzalez, V…. Gawad, C *PNAS* 2021)

Can We Establish Improved PTA Performance on the Number of Cells that are Relevant to PGT?

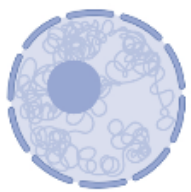
- Need to control for:
  - Phase of cell cycle
  - Number of input cells



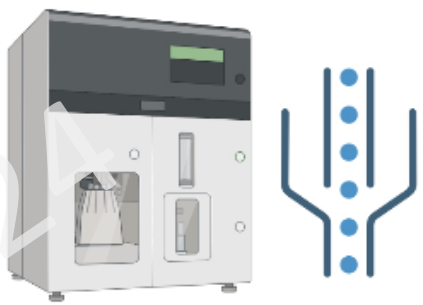


How Does Cell Cycle Alter the Performance of PTA?

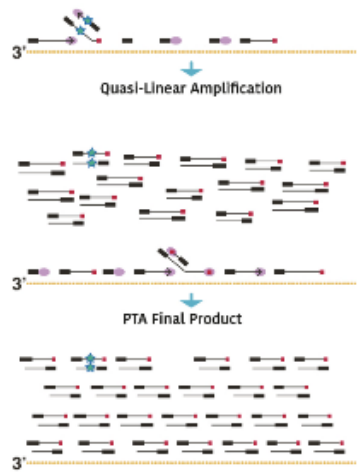
Stain Nuclei for Cell Cycle



Sort Single Nuclei Based on Cell Cycle

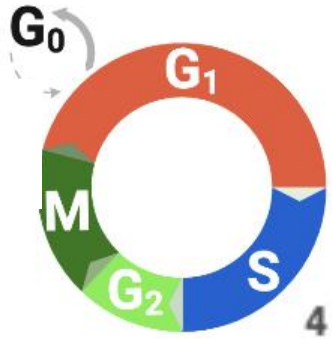


PTA



Compare Data Quality and Variant Calling Metrics

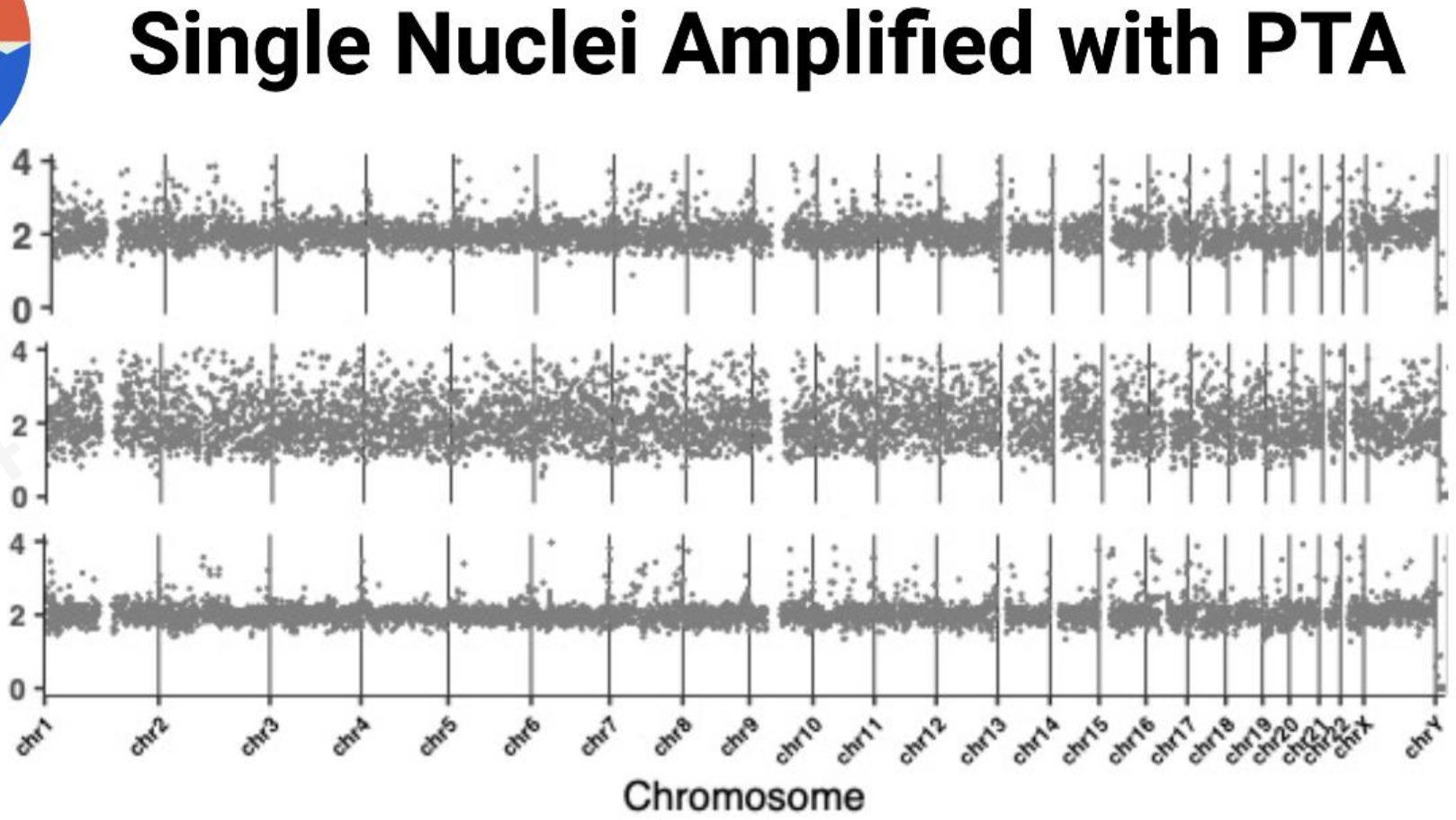
S Phase Does Reduce Amplification Uniformity



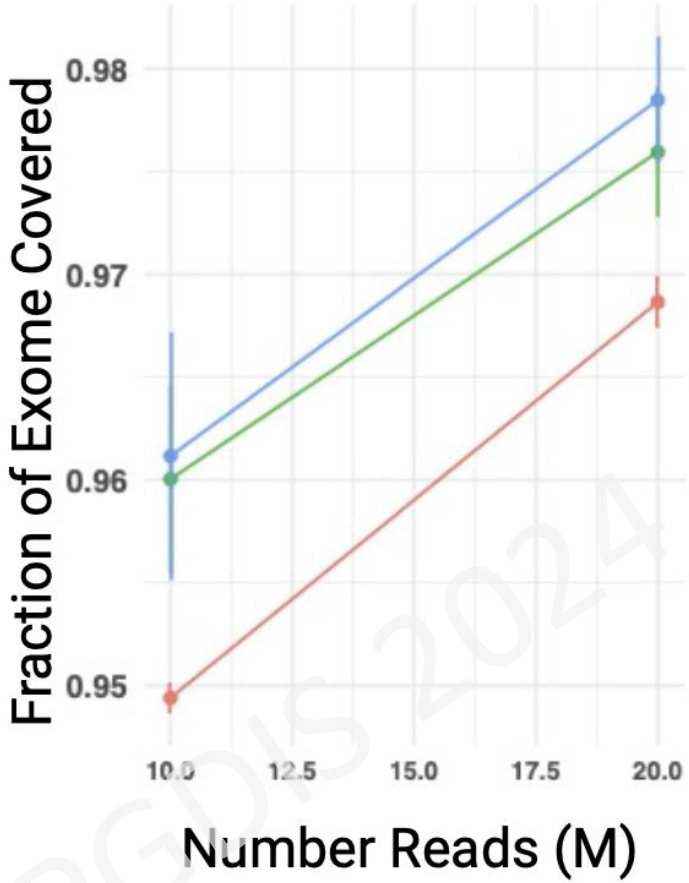
G0/G1

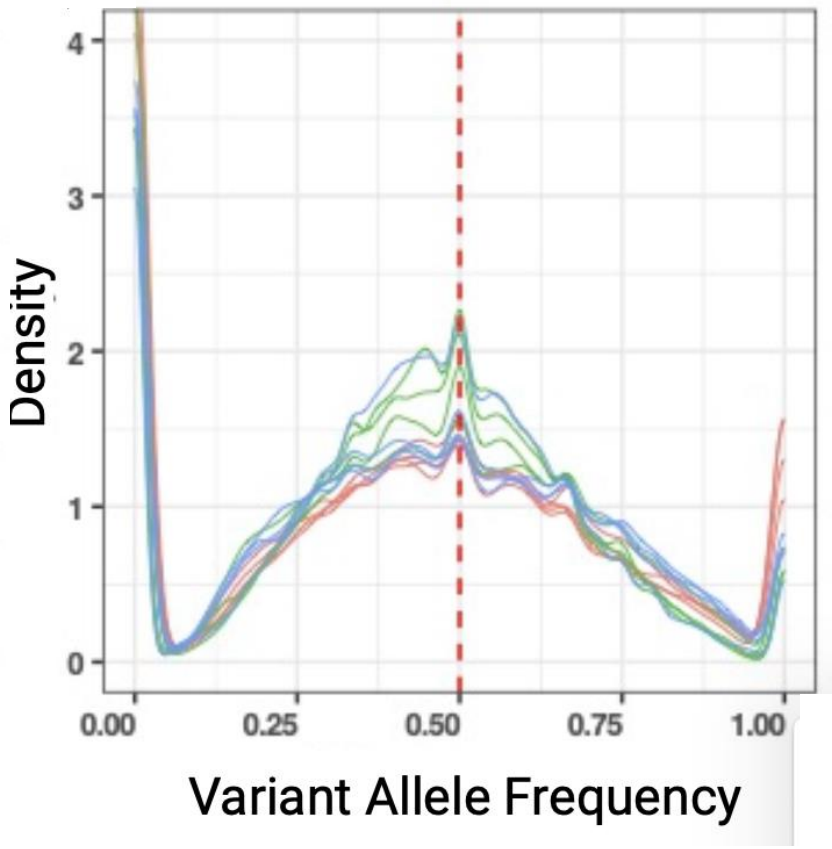
S

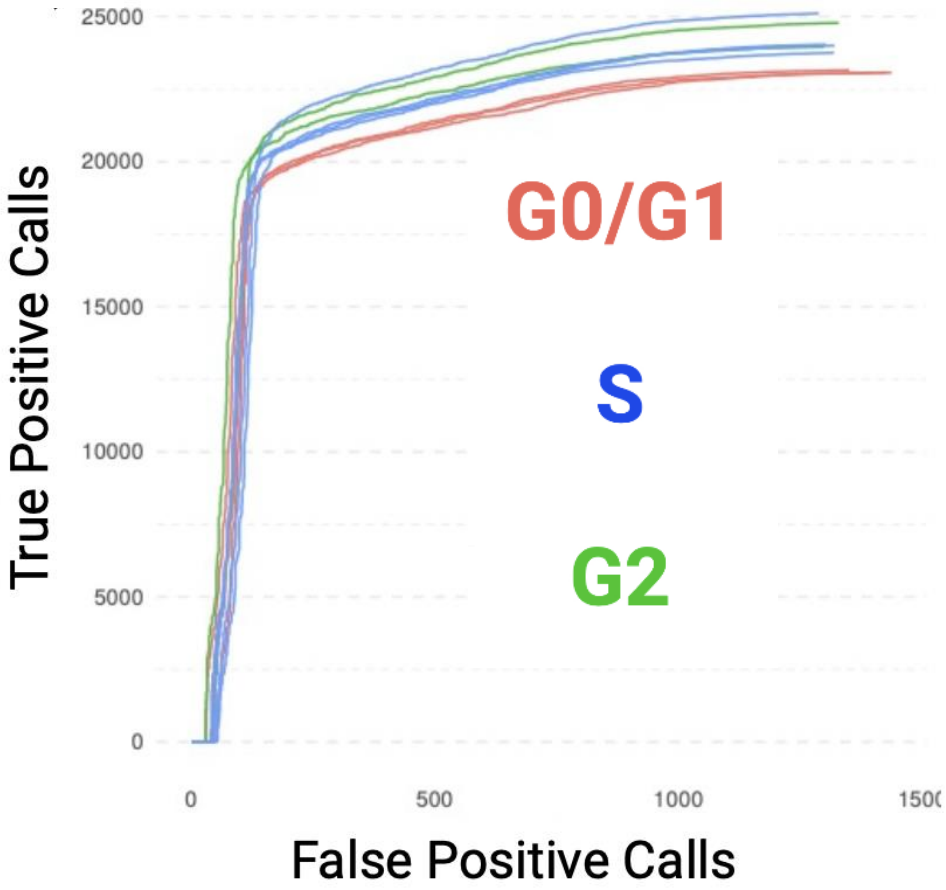
G2



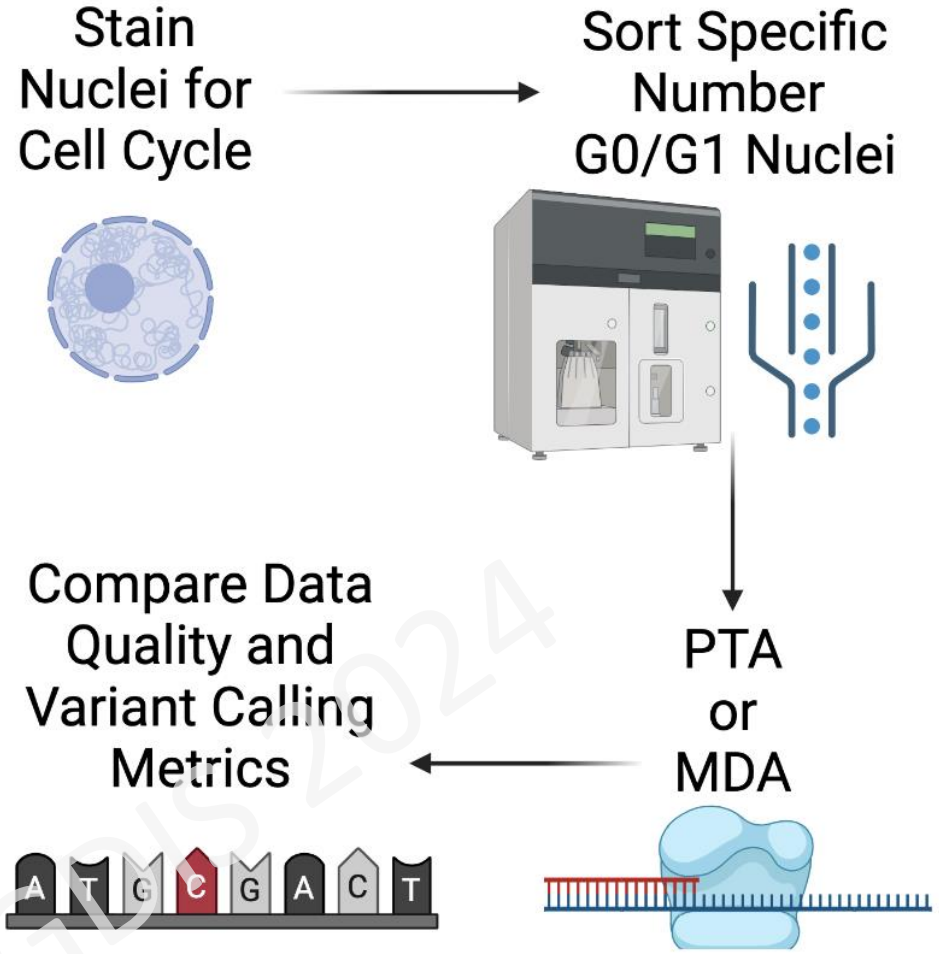
Increasing Number of Input Genomes from S/G2 Phase Slightly Increases Variant Calling Accuracy

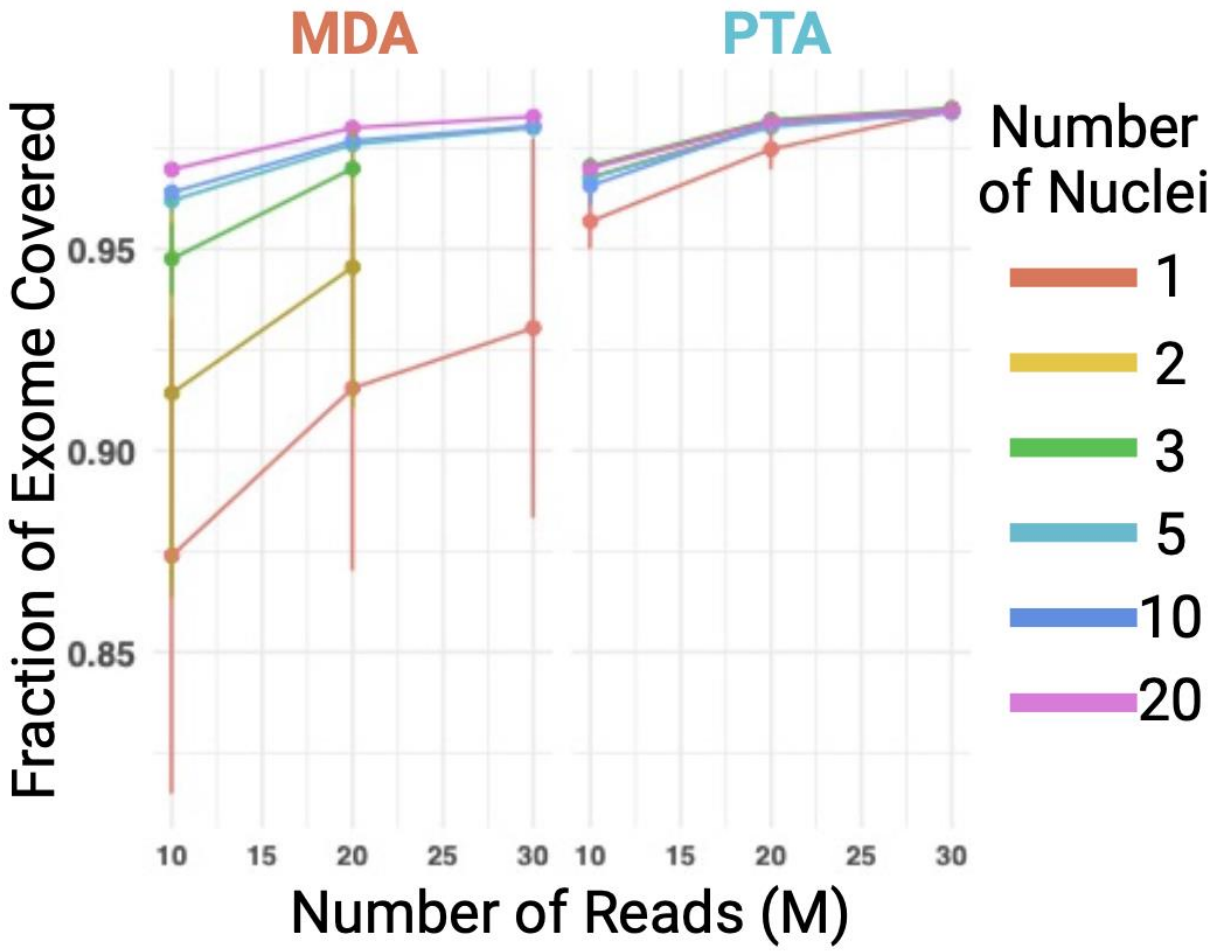




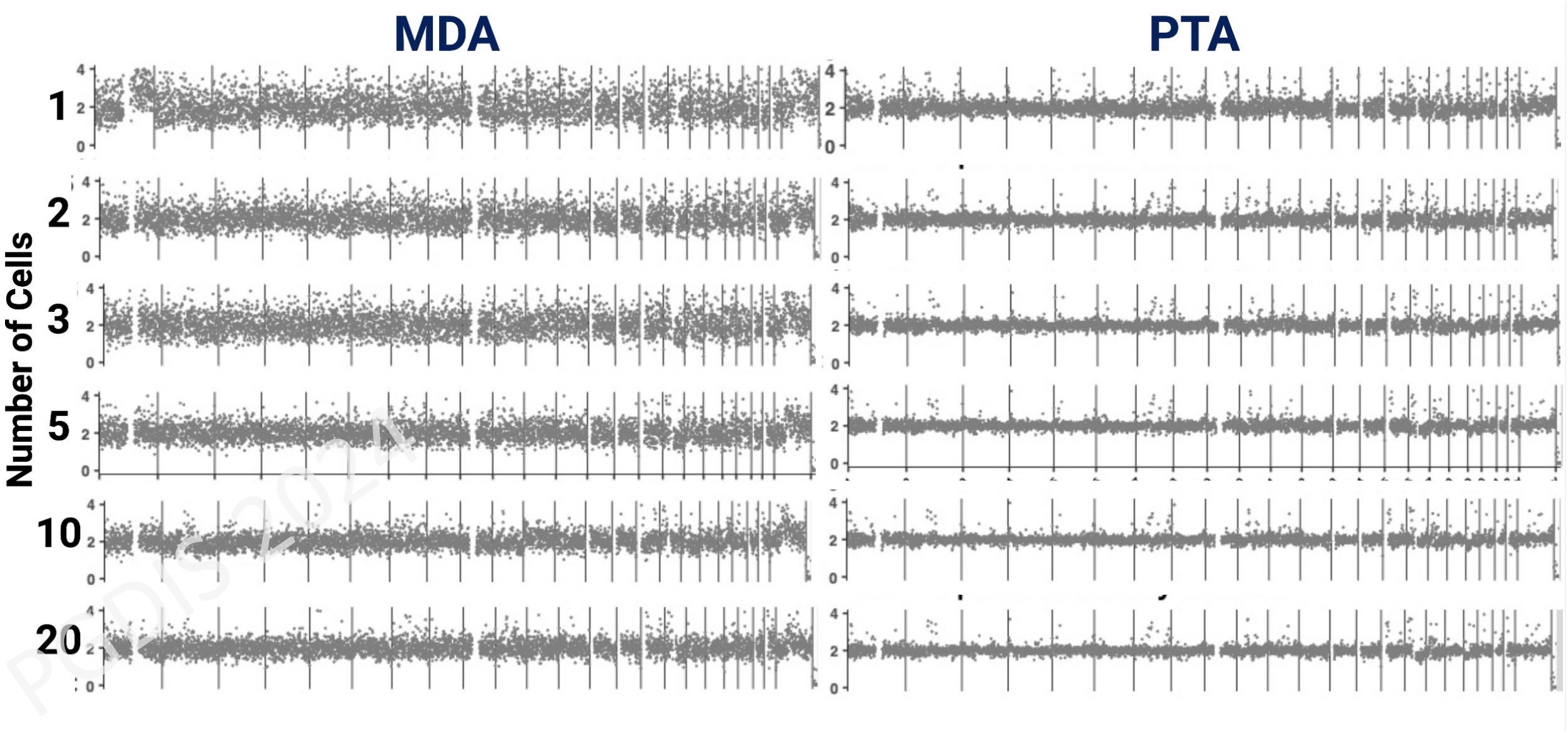


Controlling for Number of Input Cells

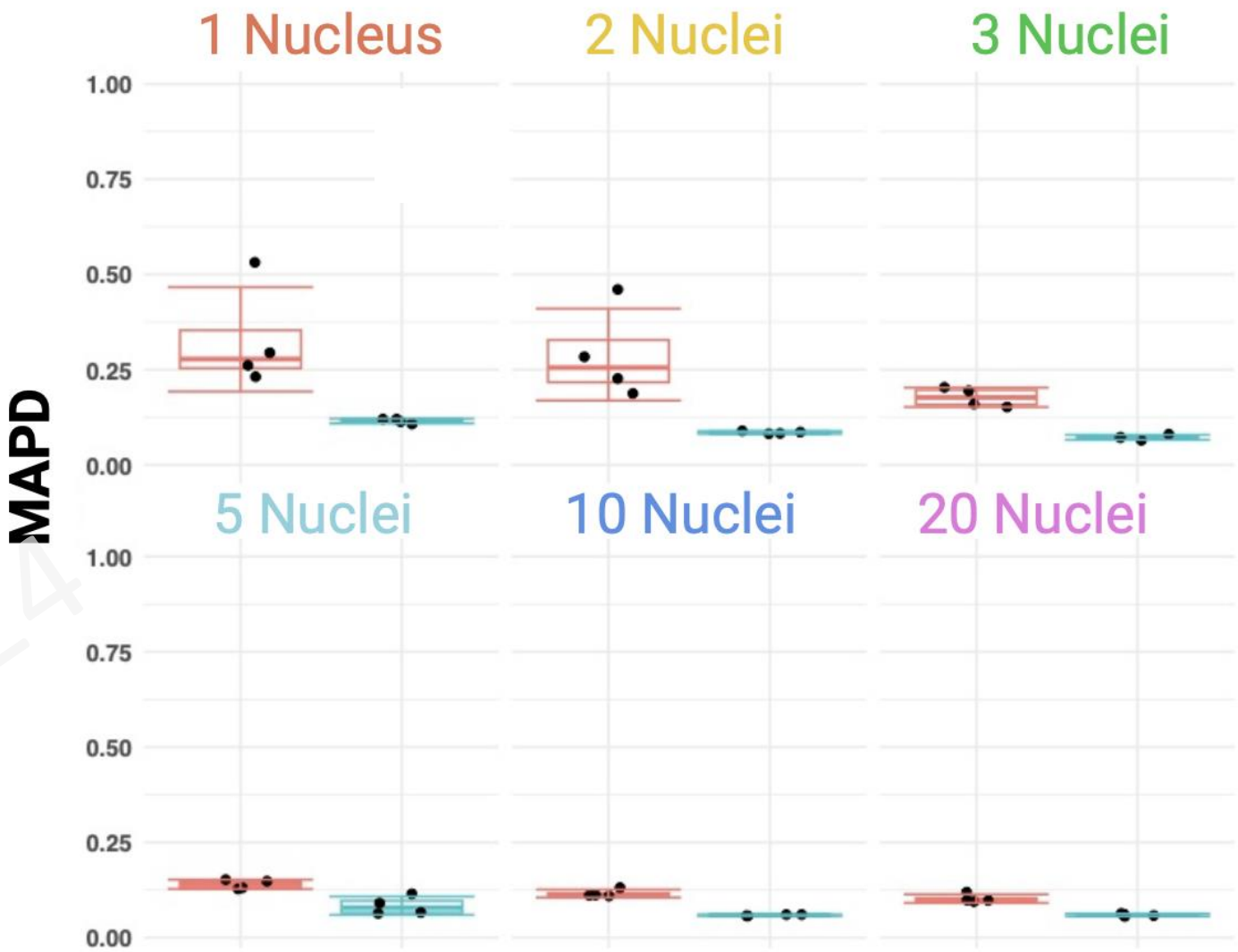




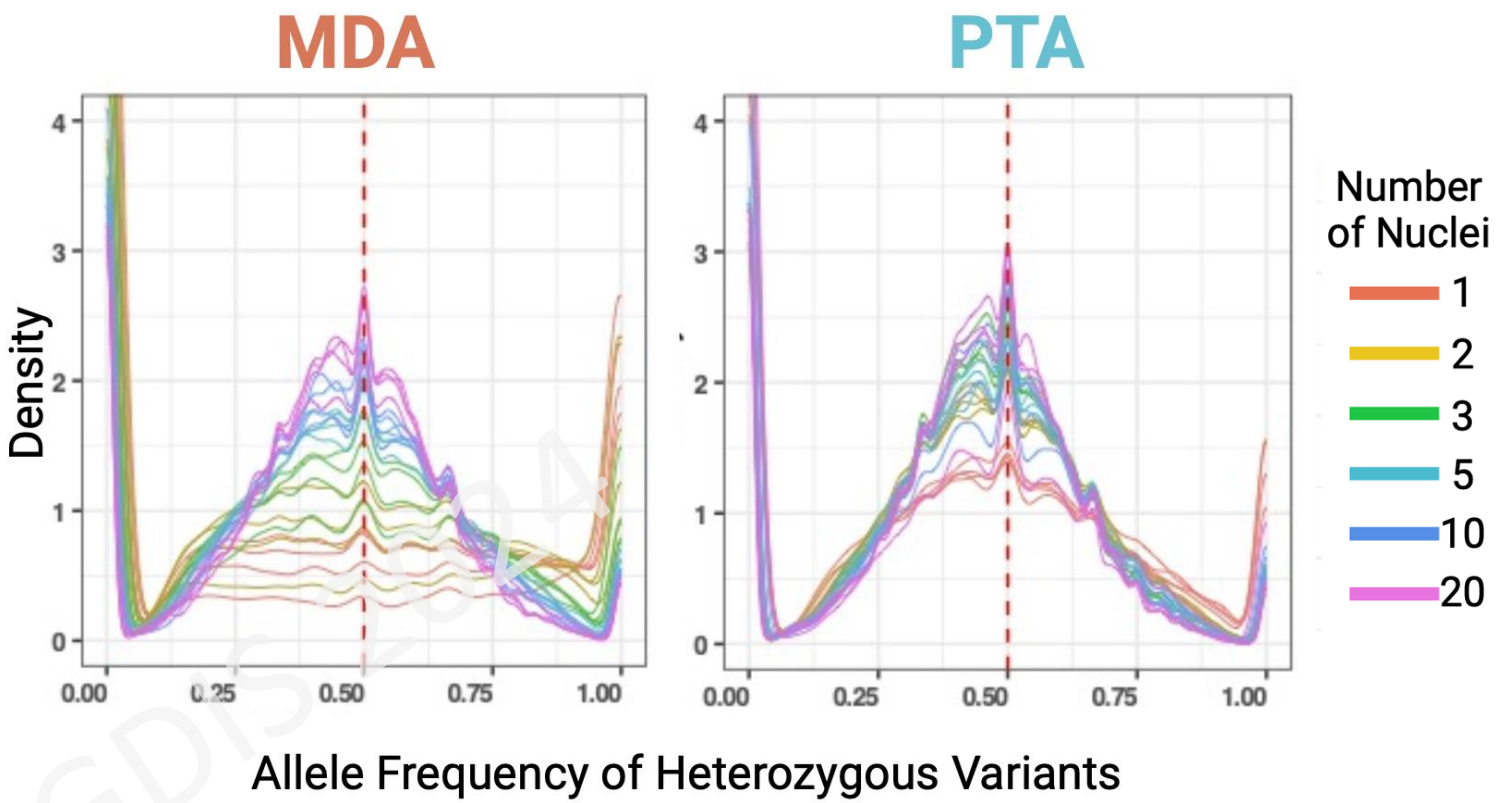
Amplification Uniformity with Increasing Cell Numbers

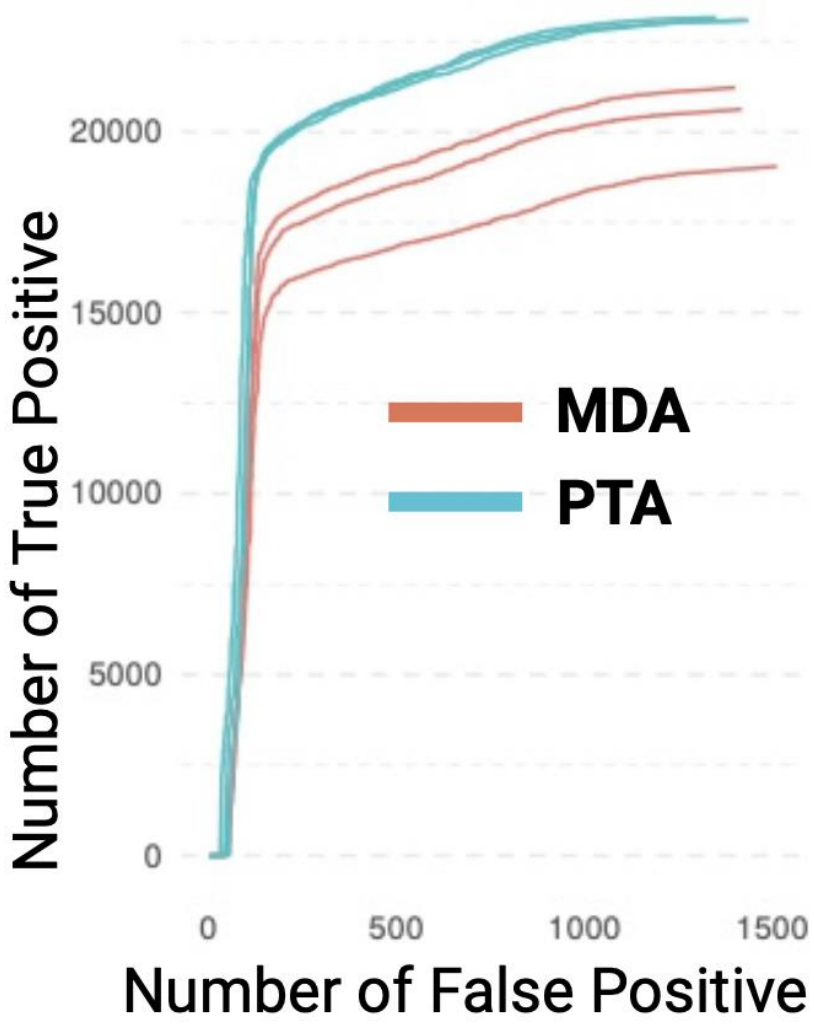


Quantifying Amplification Uniformity

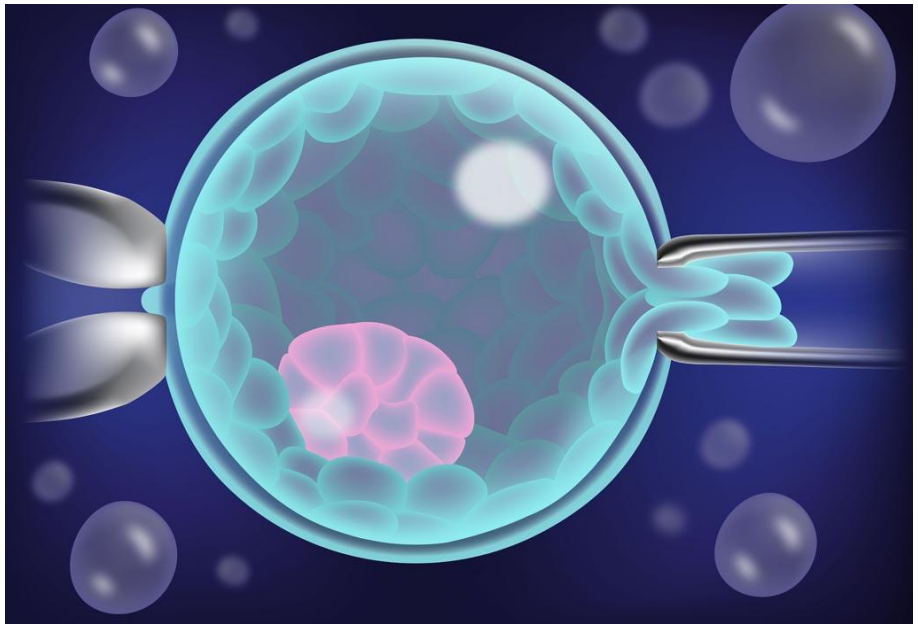


Translating These into Variant Calling Accuracy





Do These Findings Translate to TE Biopsies?

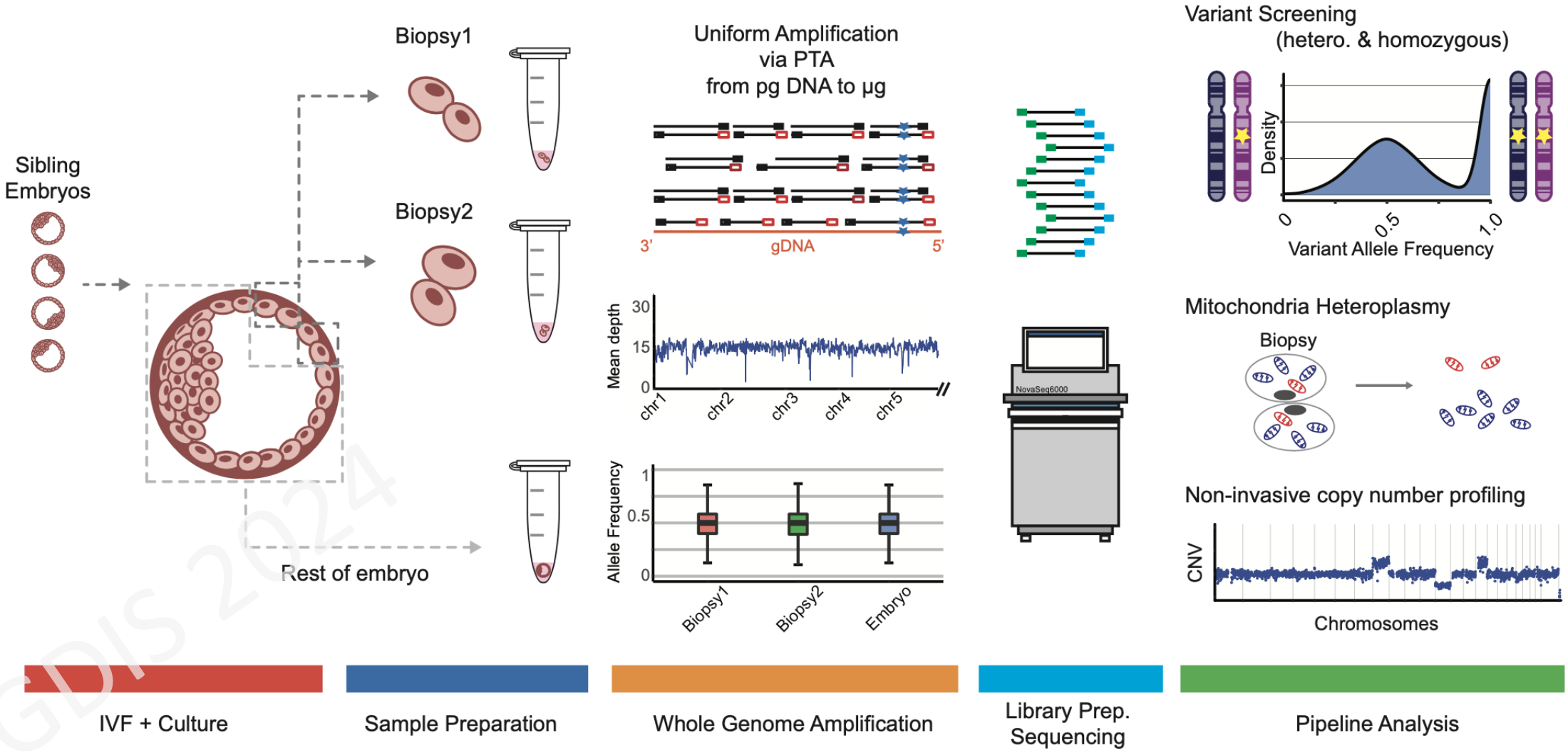


Yuntao Xi, PhD
Postdoc, Gawad lab

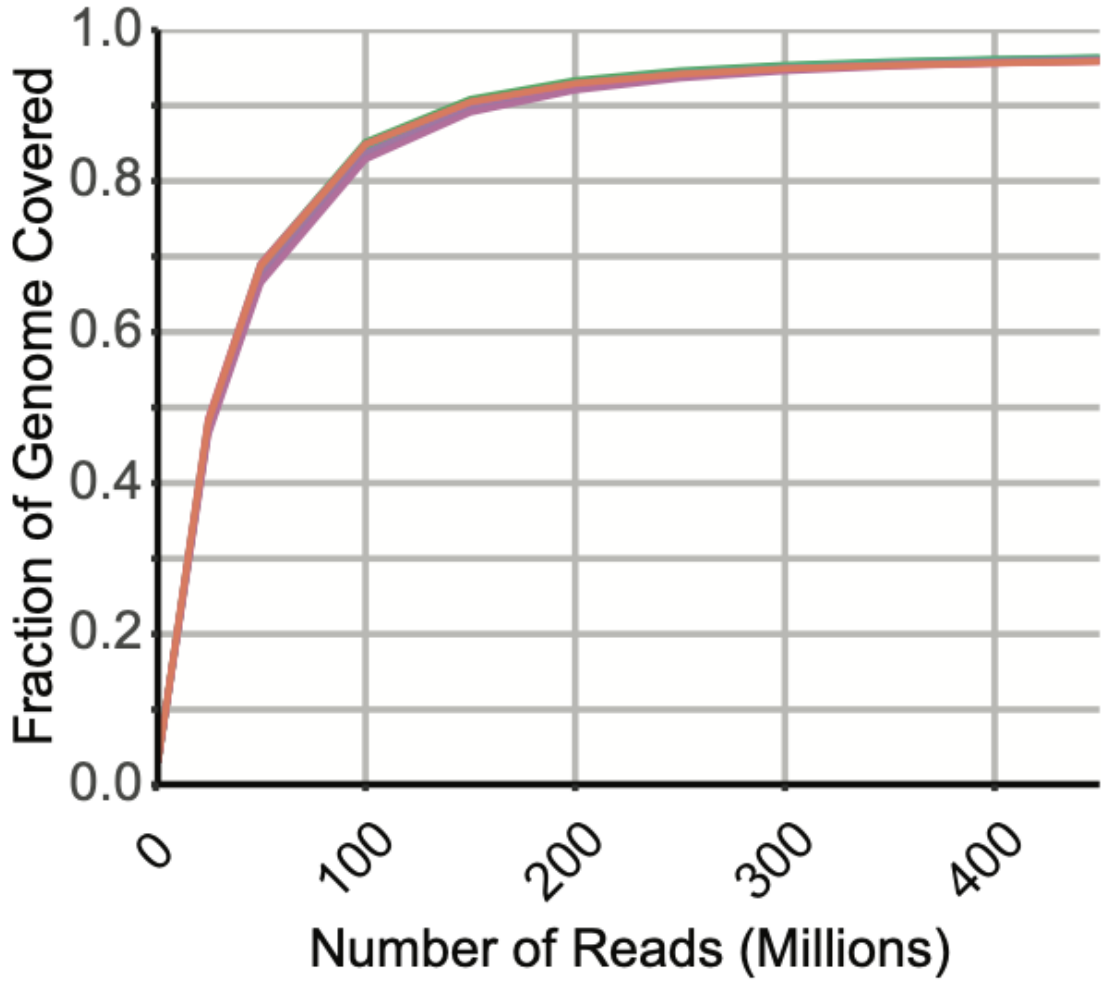
Veronica Gonzalez-Pena, PhD
Staff Scientist, Gawad lab

Barry Behr, PhD
Obstetrics & Gynecology Professor,
Embryology Laboratory Director, Stanford

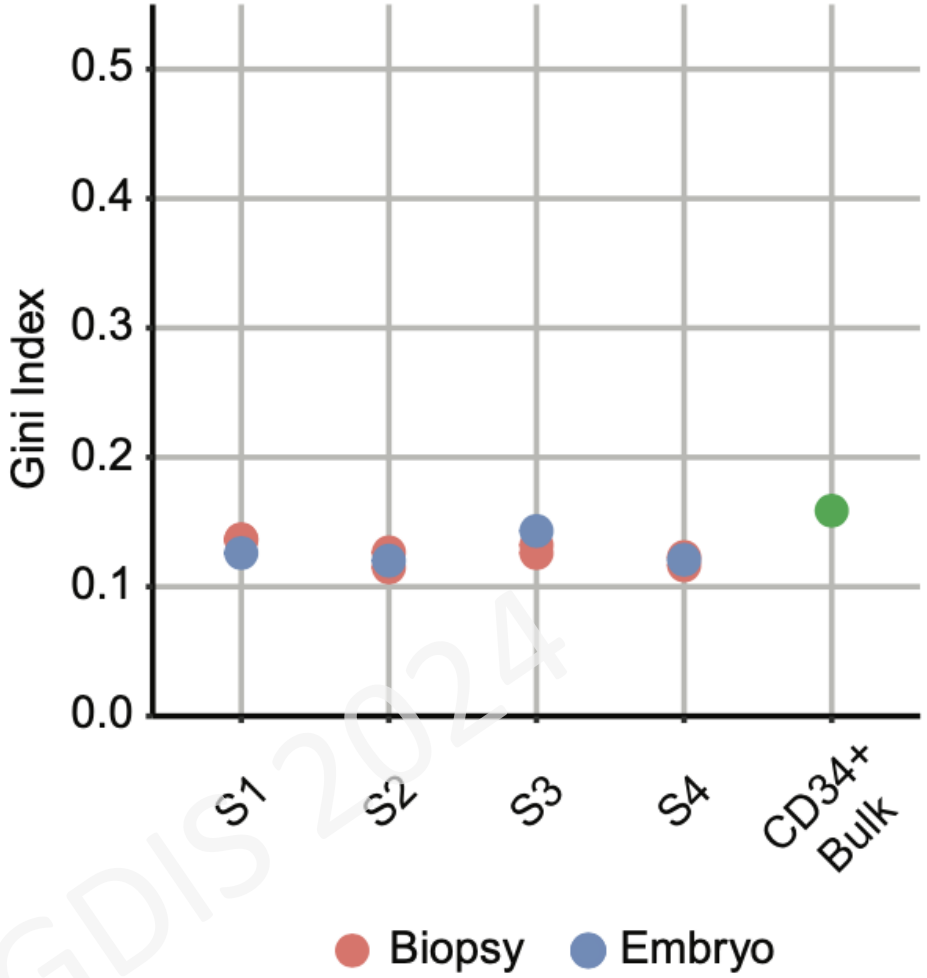
Proof-of-Concept Study Overview

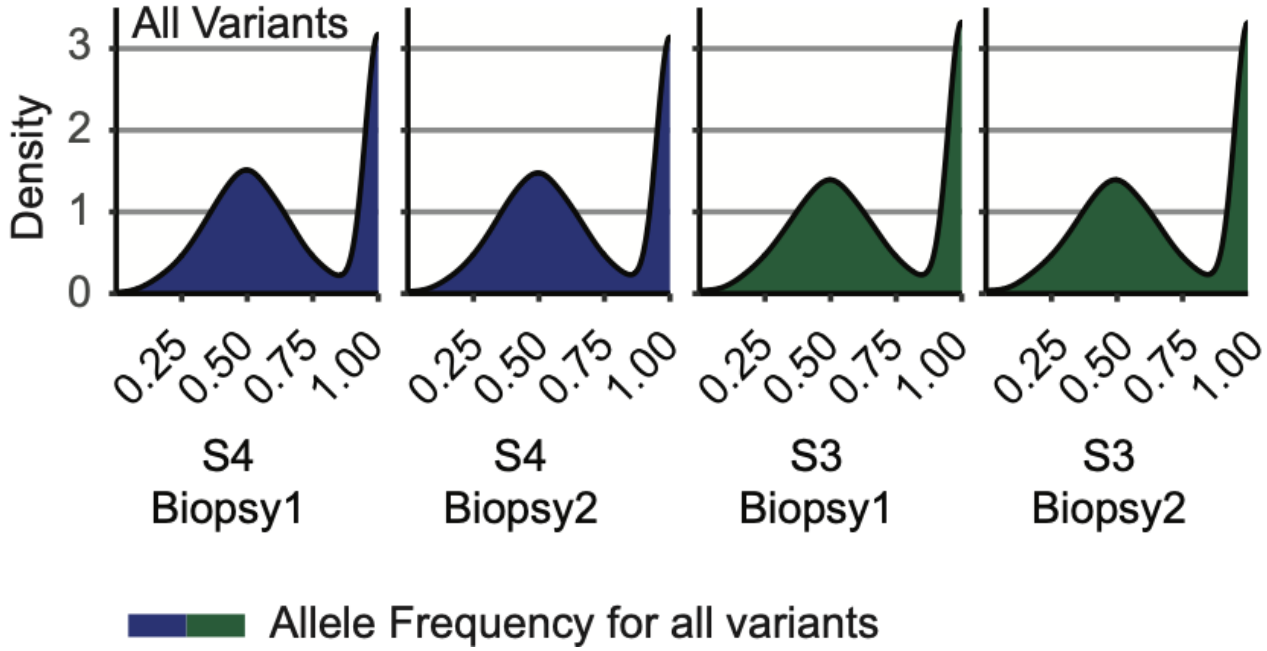


Genome Coverage

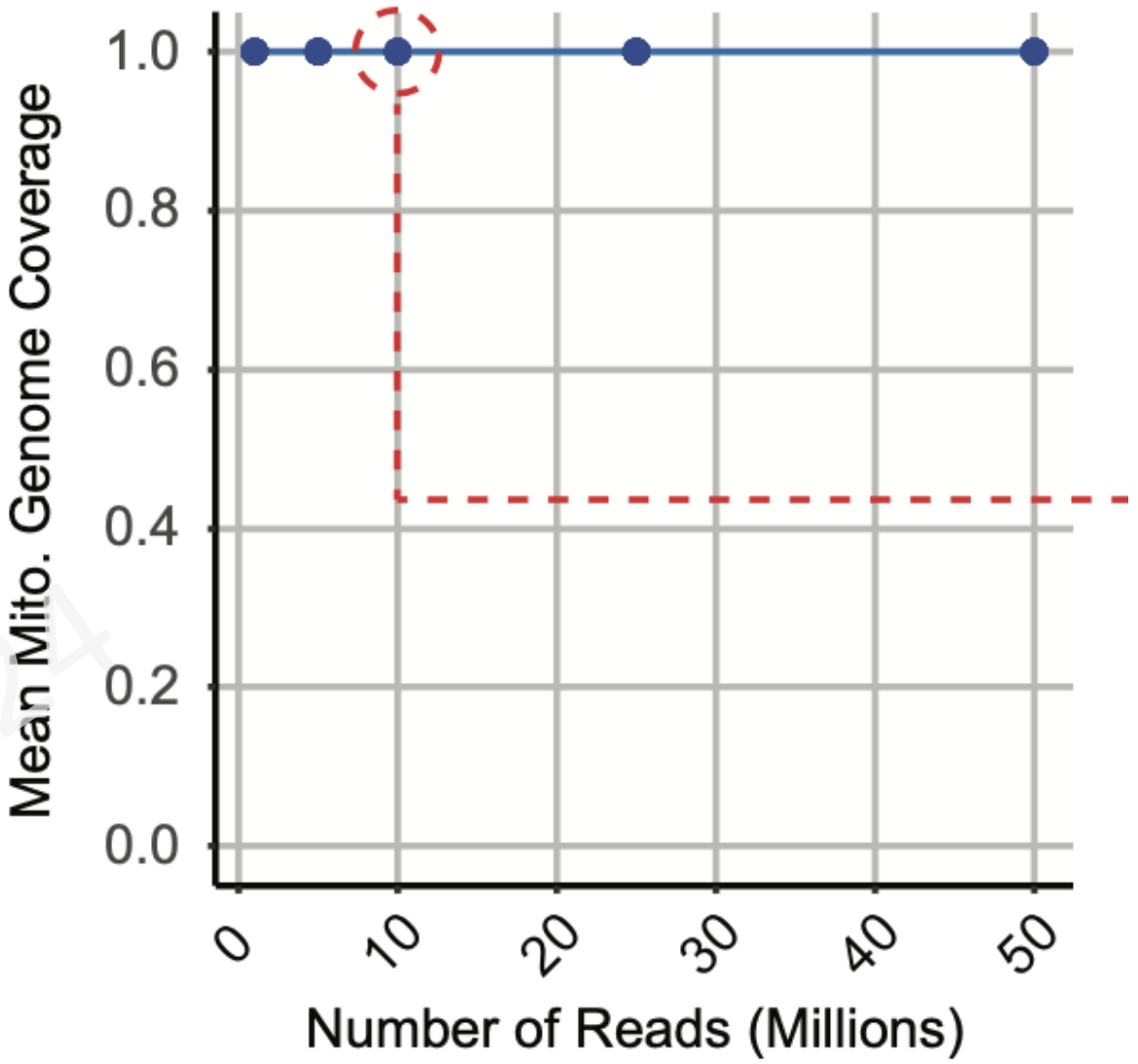


Measurements of Genome Uniformity

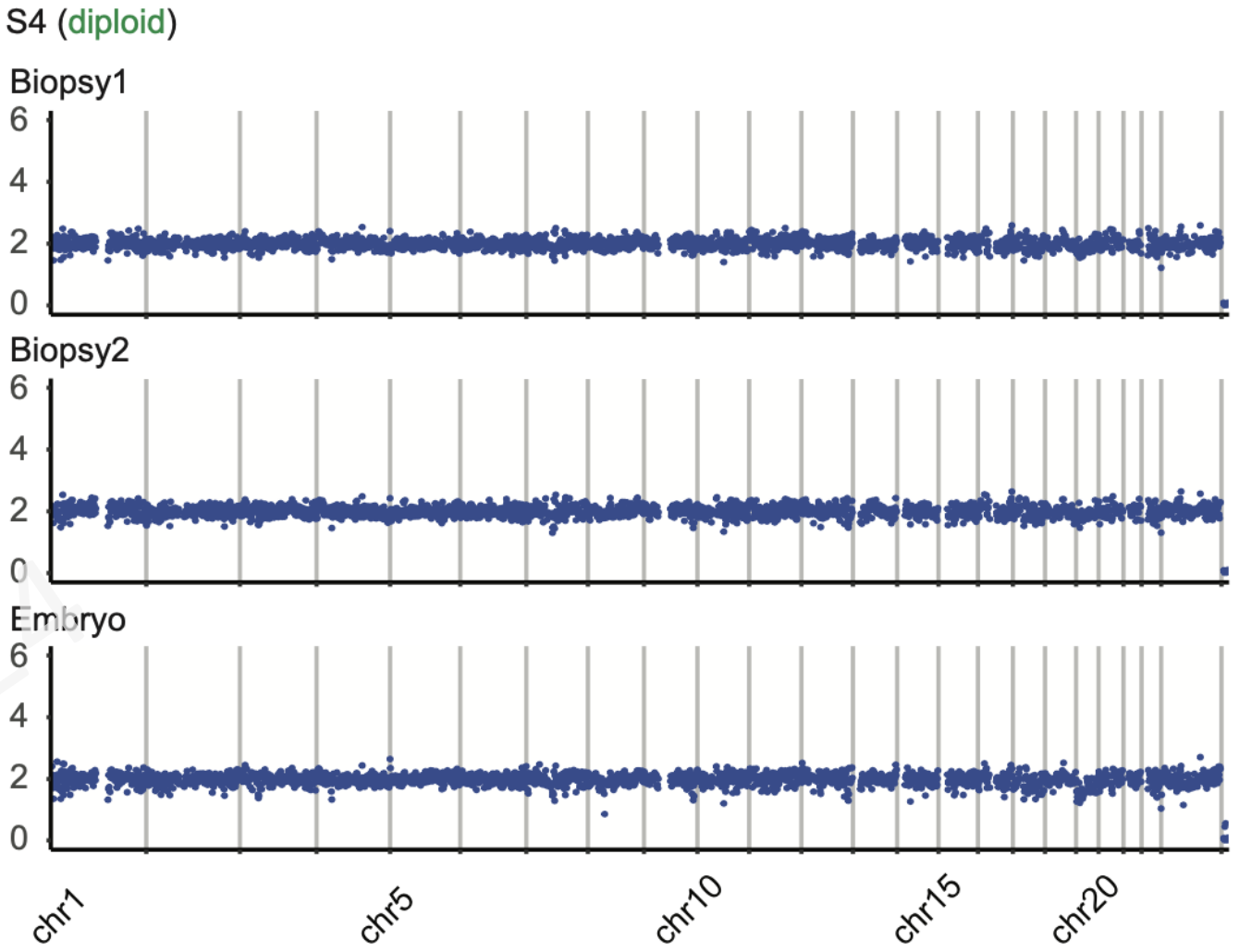




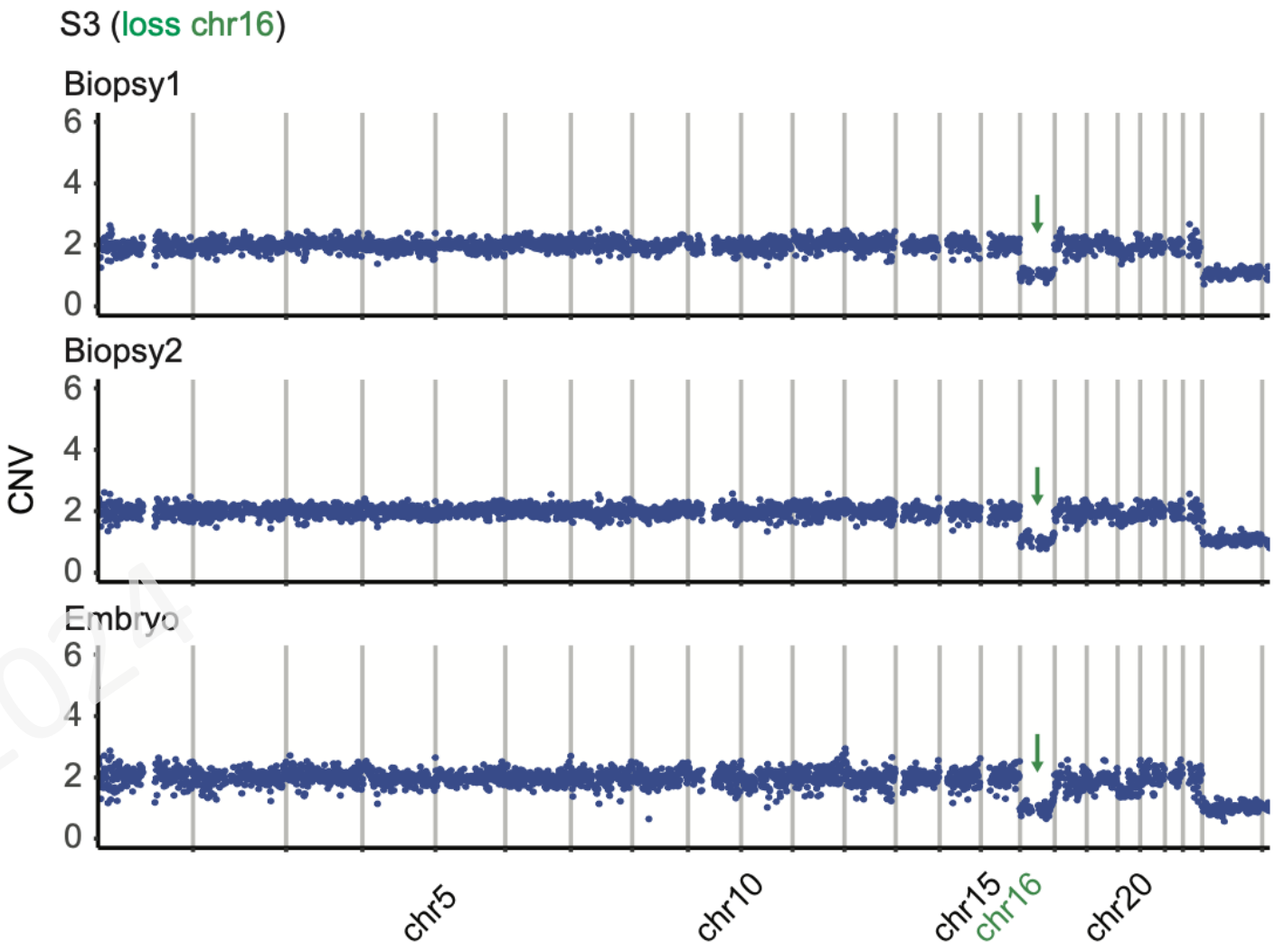
Coverage of the Mitochondrial Genome



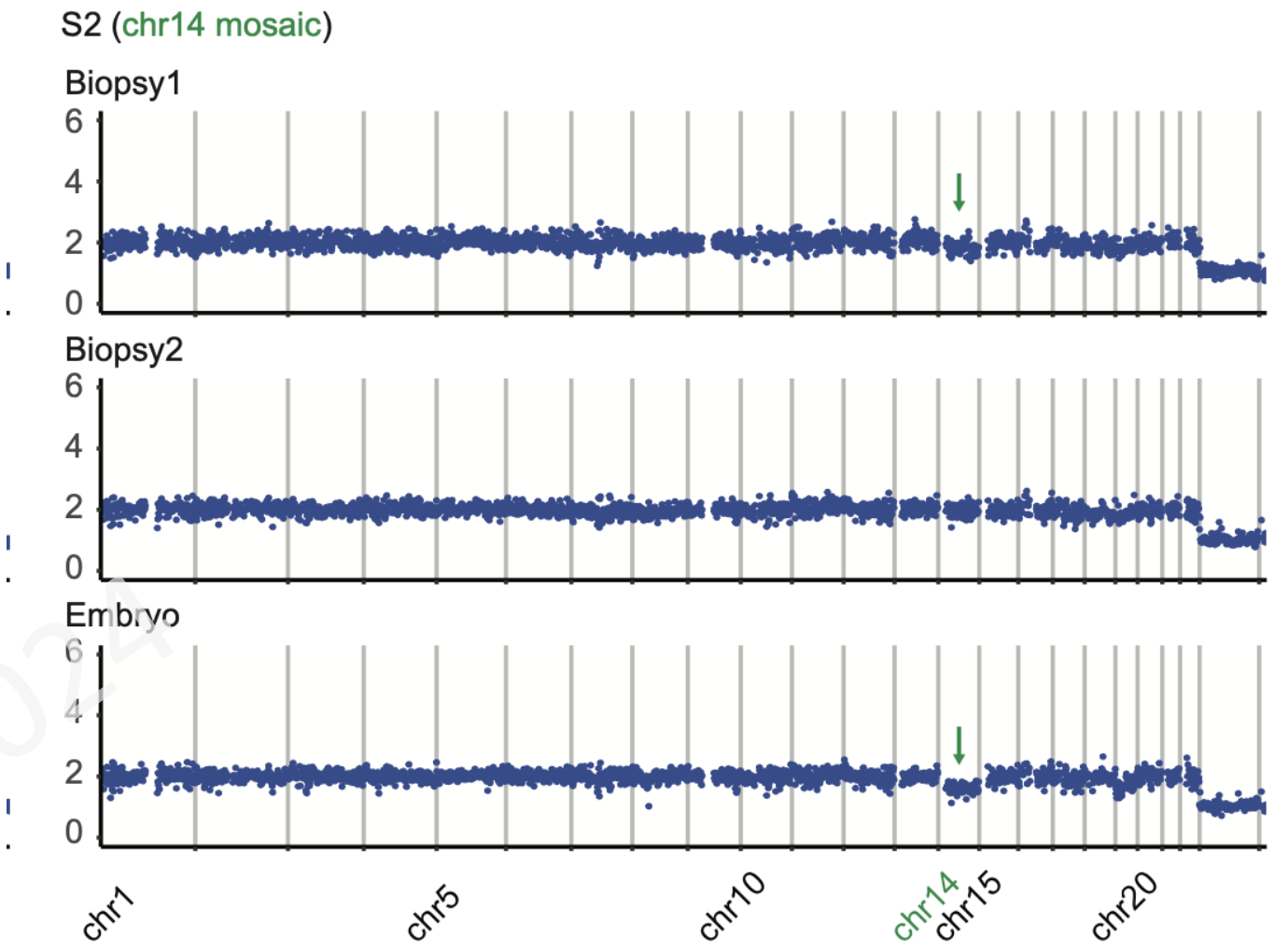
Diploid Copy Number Profile



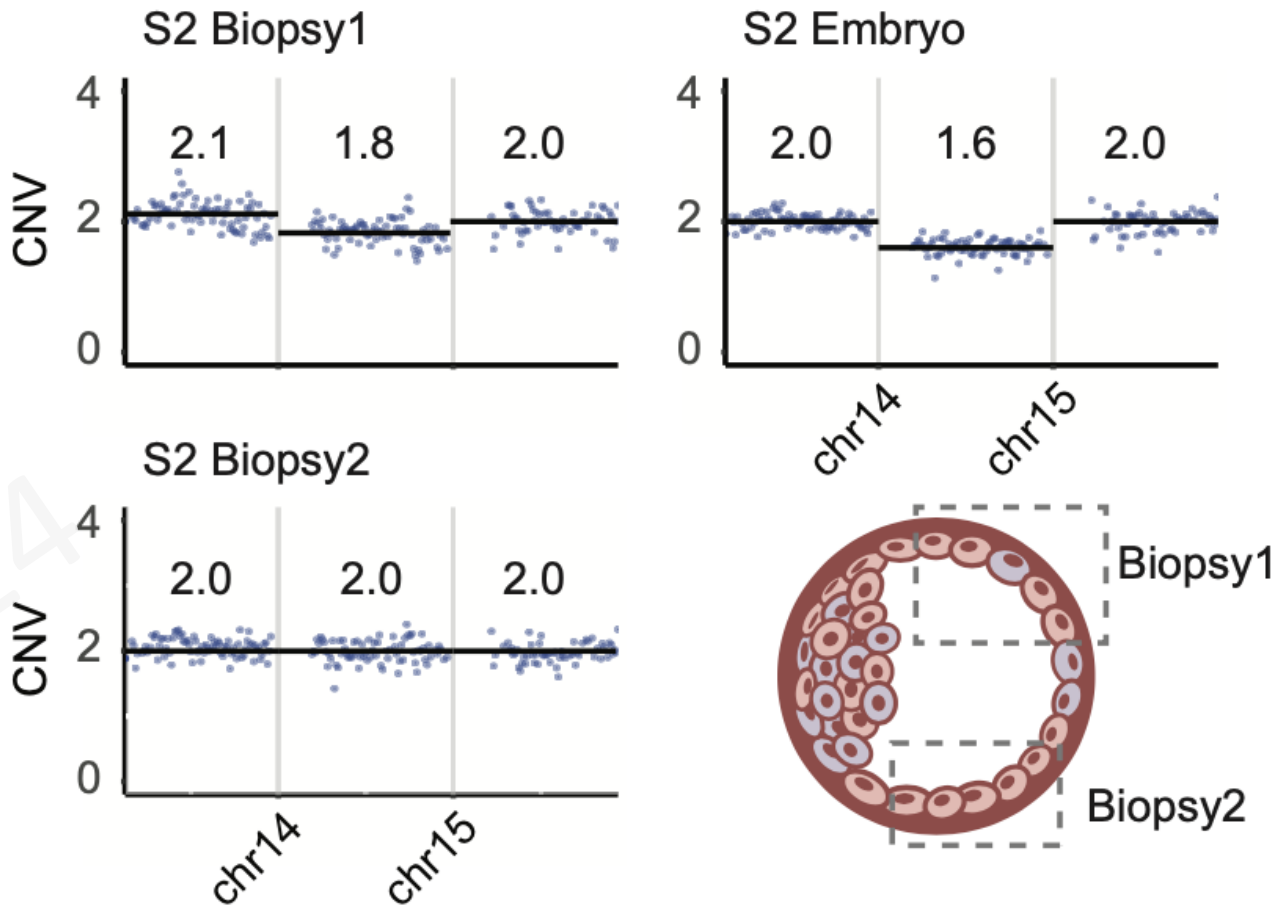
Aneuploid CNV Profile



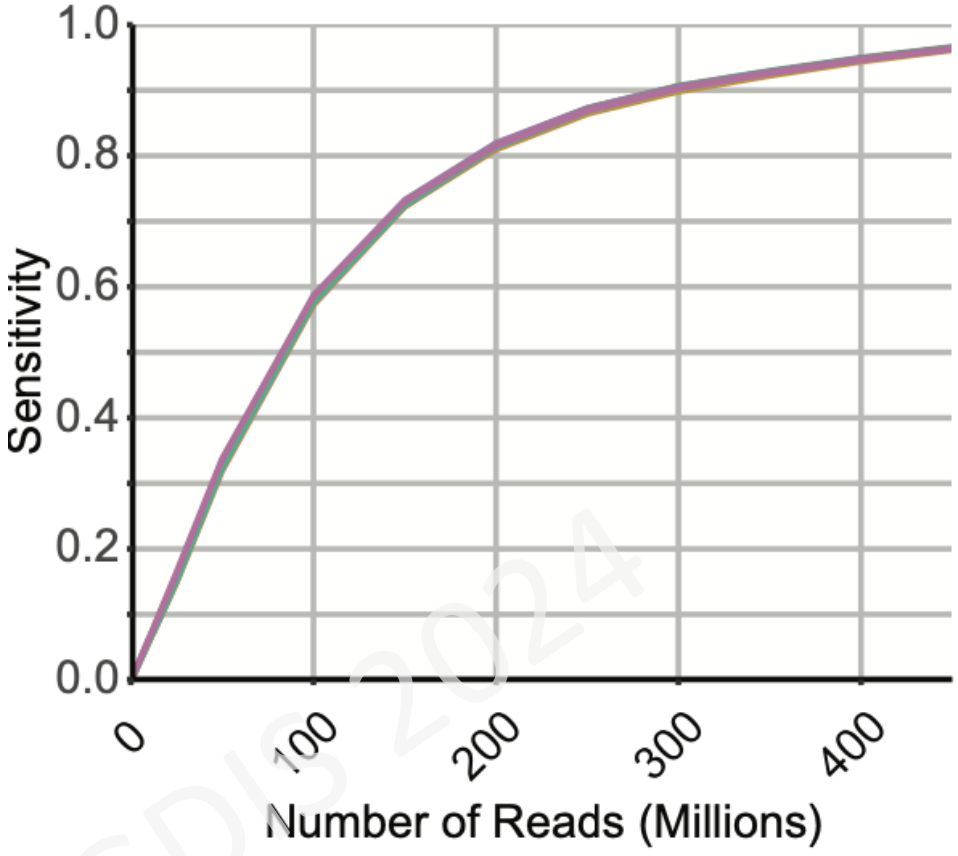
Mosaic CNV Profile

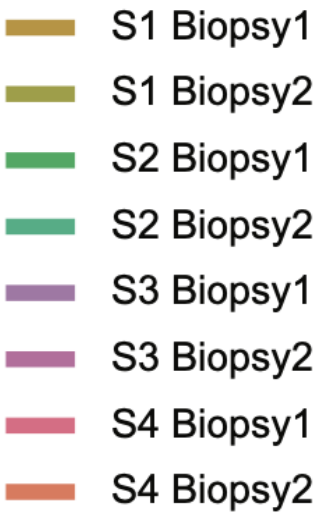


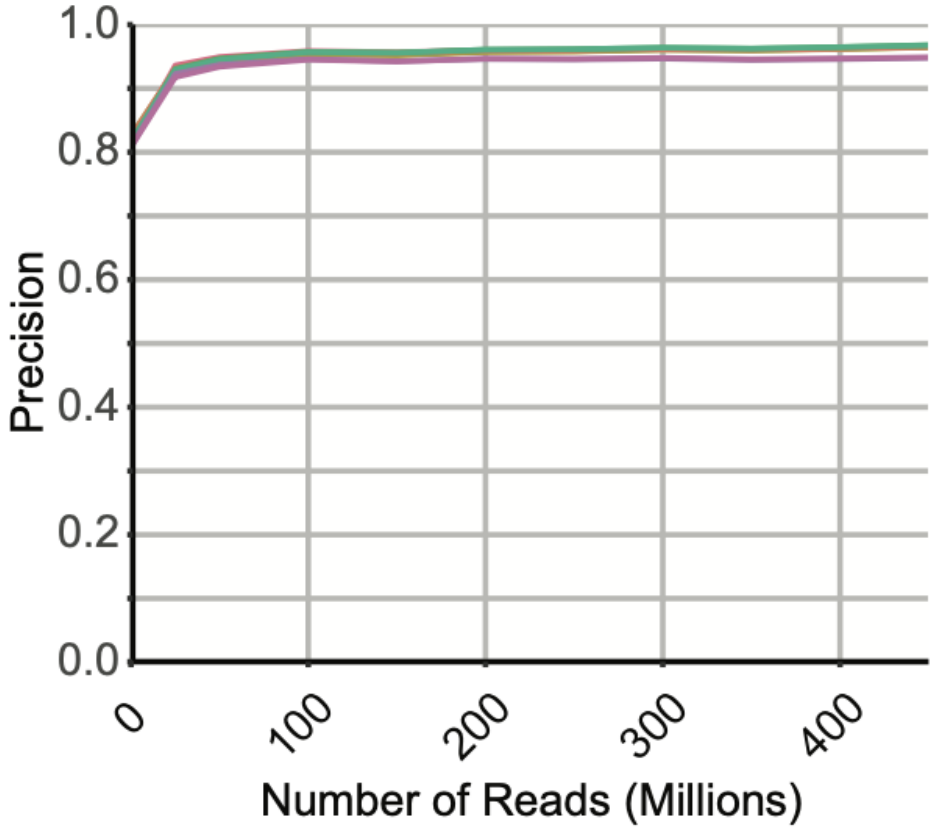
Quantifying Mosaicism with the Improved Uniformity of PTA



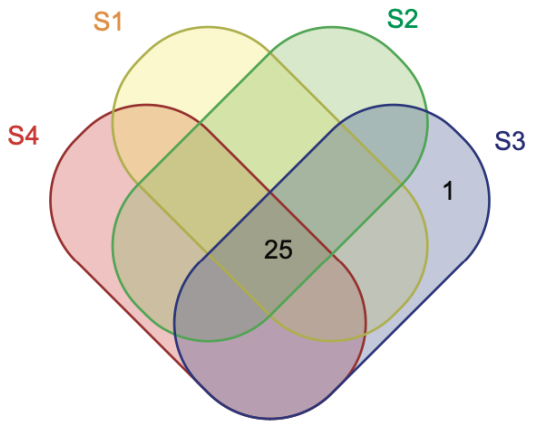
Single Nucleotide Variant Calling Accuracy

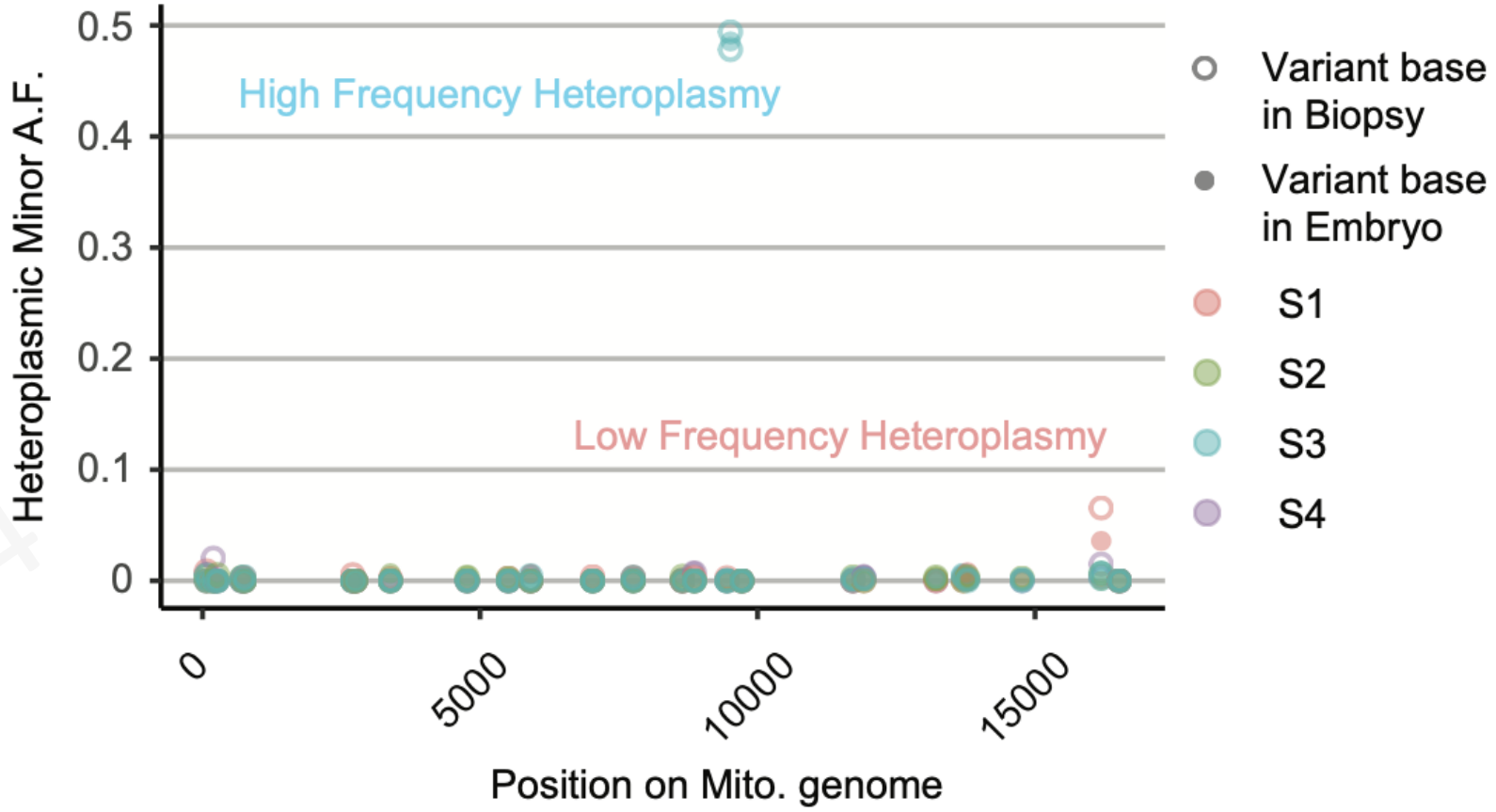




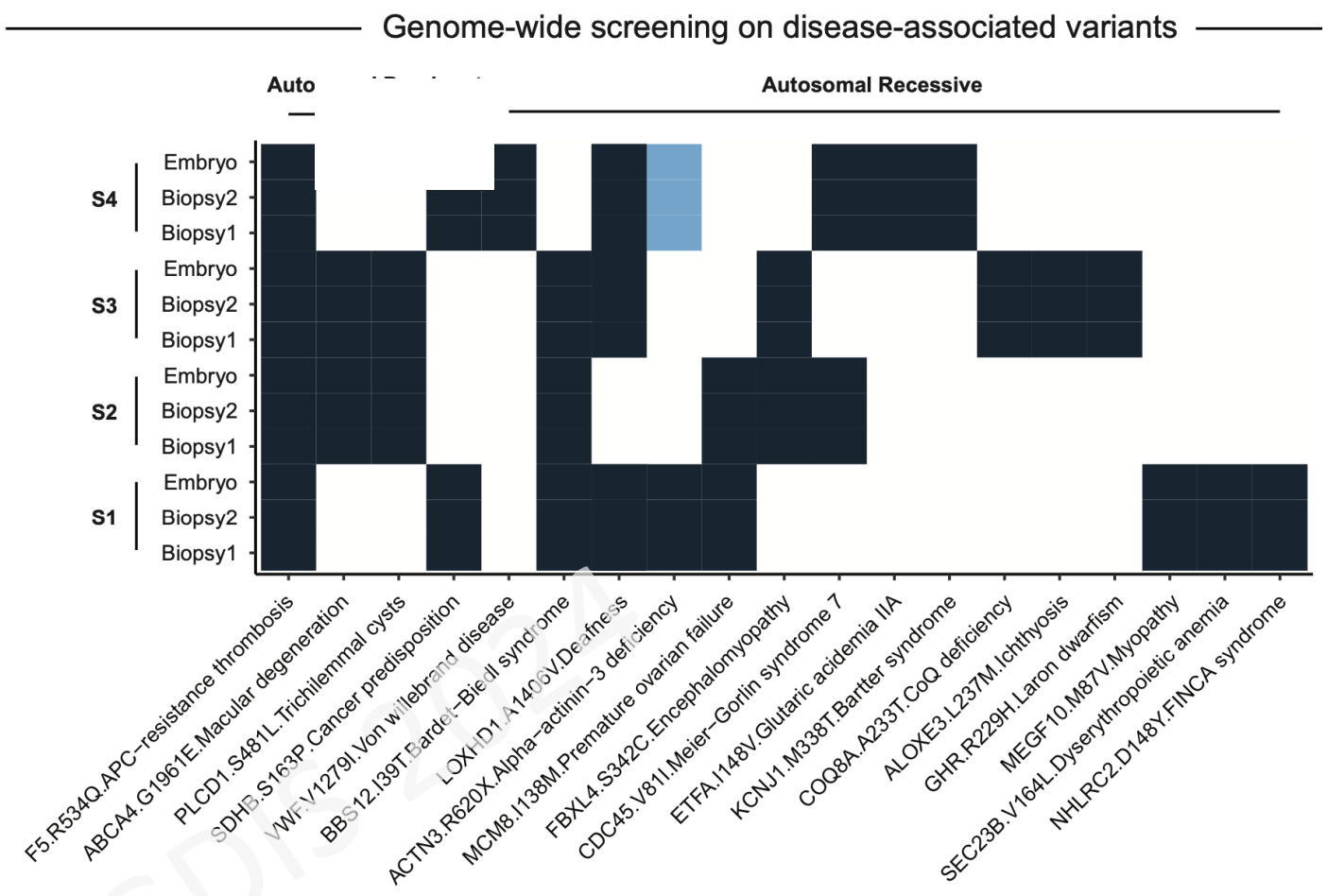


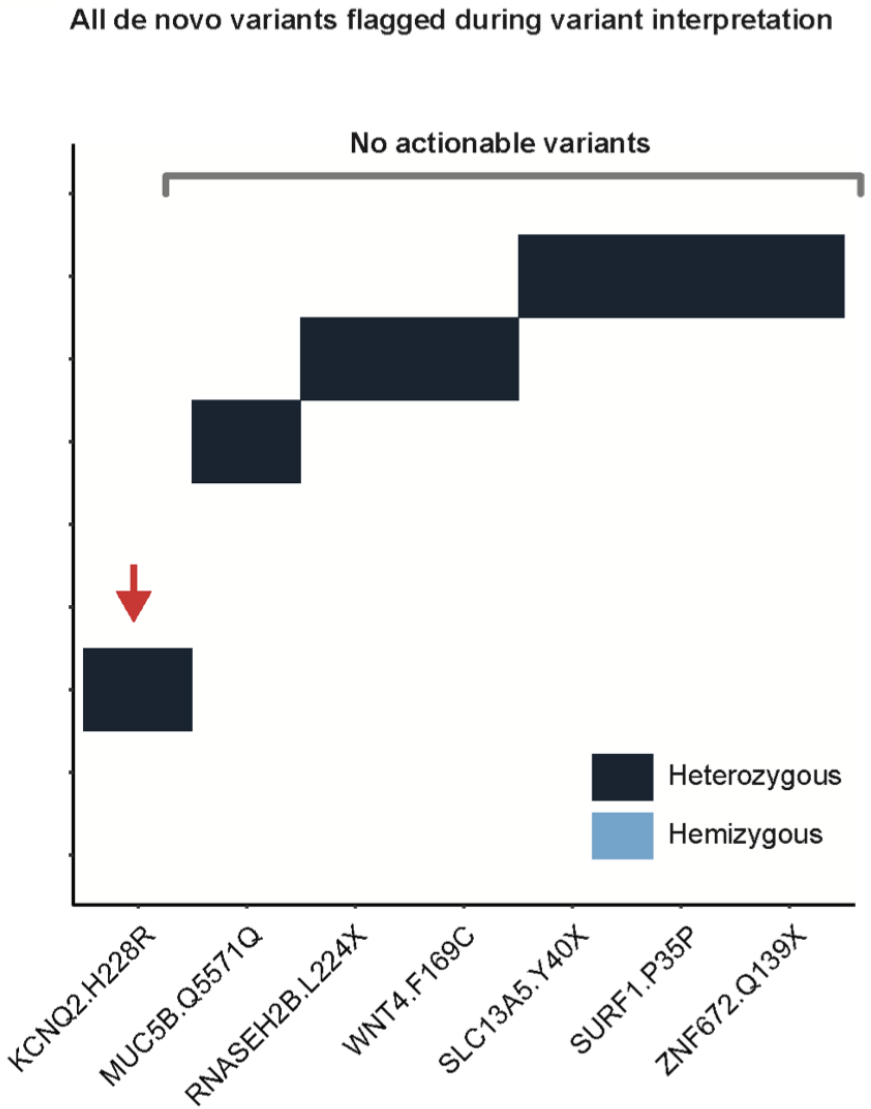
Identification of Low and High Frequency Heteroplasmy





Screening for Known Mendelian Diseases and *de novo* Variants





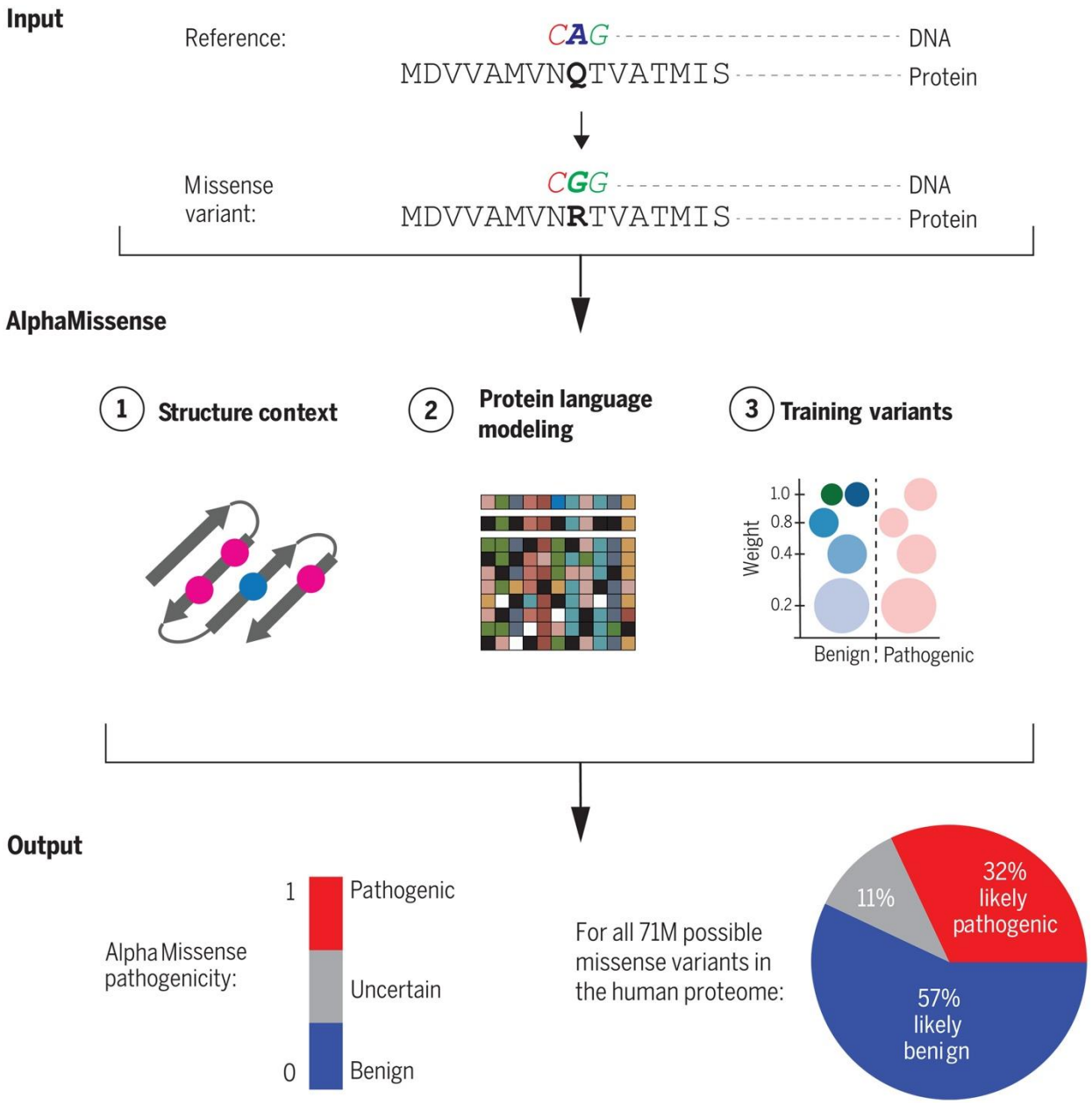
AI to Help Interpret Variants of Uncertain Significance

RESEARCH ARTICLE SUMMARY

MACHINE LEARNING

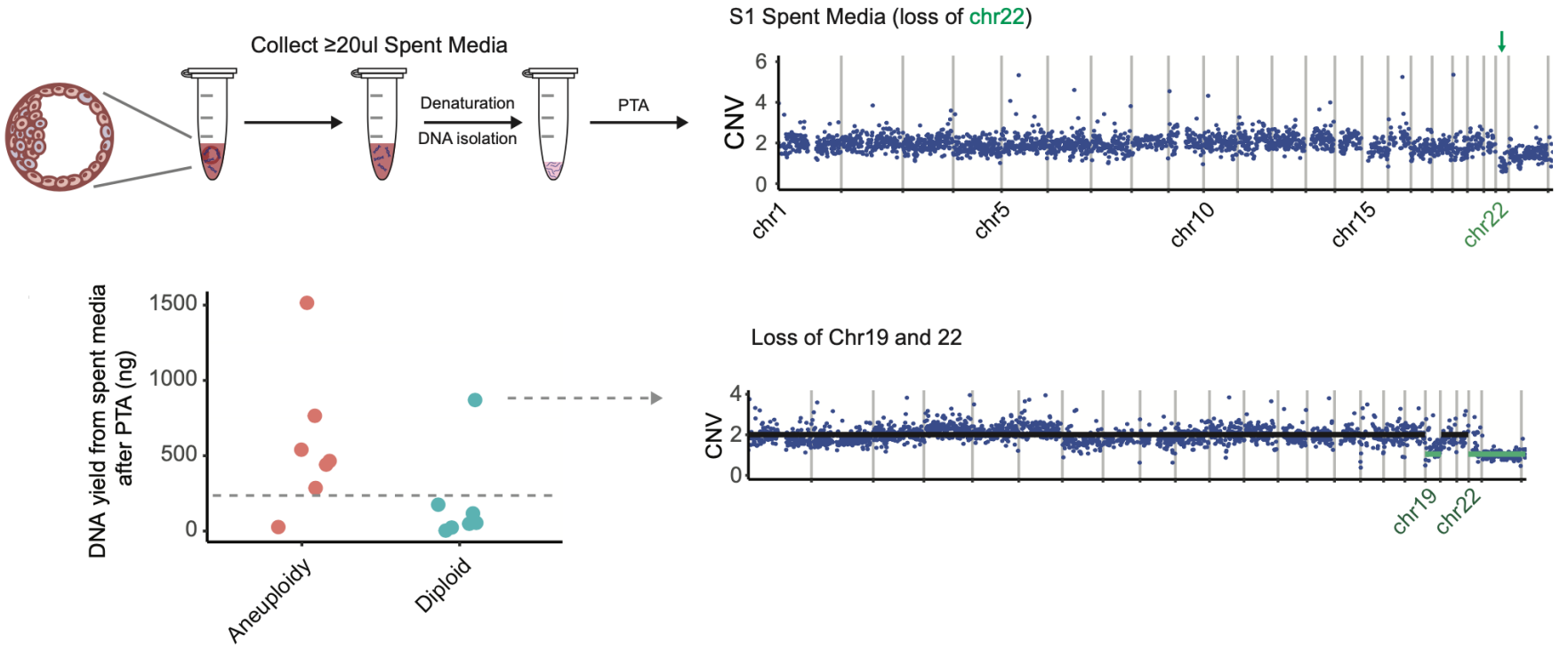
Accurate proteome-wide missense variant effect prediction with AlphaMissense

Jun Cheng*, Guido Novati, Joshua Pan†, Clare Bycroft†, Akvilė Žemgulytė†, Taylor Applebaum†, Alexander Pritzel, Lai Hong Wong, Michal Zielinski, Tobias Sargeant, Rosalia G. Schneider, Andrew W. Senior, John Jumper, Demis Hassabis, Pushmeet Kohli*, Žiga Avsec*



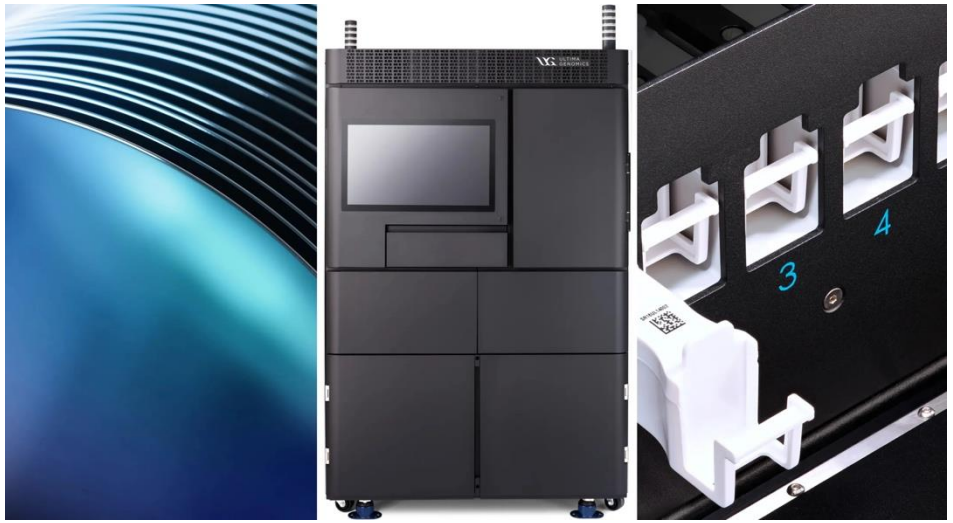
What About niPGT with PTA?

- The Challenge
  - Low and variable quantity of DNA
  - cfDNA is fragmented
- Need to rethink general approach to get more robust results
- We hope to have more to report soon



Lower Sequencing Costs Will Make Whole Genome PGT Financially Feasible

- Improved data quality reduces amount of sequencing required
  - Lower error rates
  - Longer reads
  - Shorter run times
- Lower consumable costs
  - Currently $100 per genome
  - Plans to go much lower in the near future



Conclusions

- PTA shows improved amplification performance and variant calling accuracy at all cell input numbers relevant to PGT
- PTA has exceptionally accurate variant calling of mitochondrial variants
- Whole genome PGT is feasible with PTA, including screening for *de novo* variants
- niPGT with PTA is possible, but additional technical barriers need to be overcome