

# Single-cell, genome-wide aneuploidy, CNV, SNP and mutation profiling using microarrays and NGS for pre-implantation genetic screening, and cancer research and diagnostics

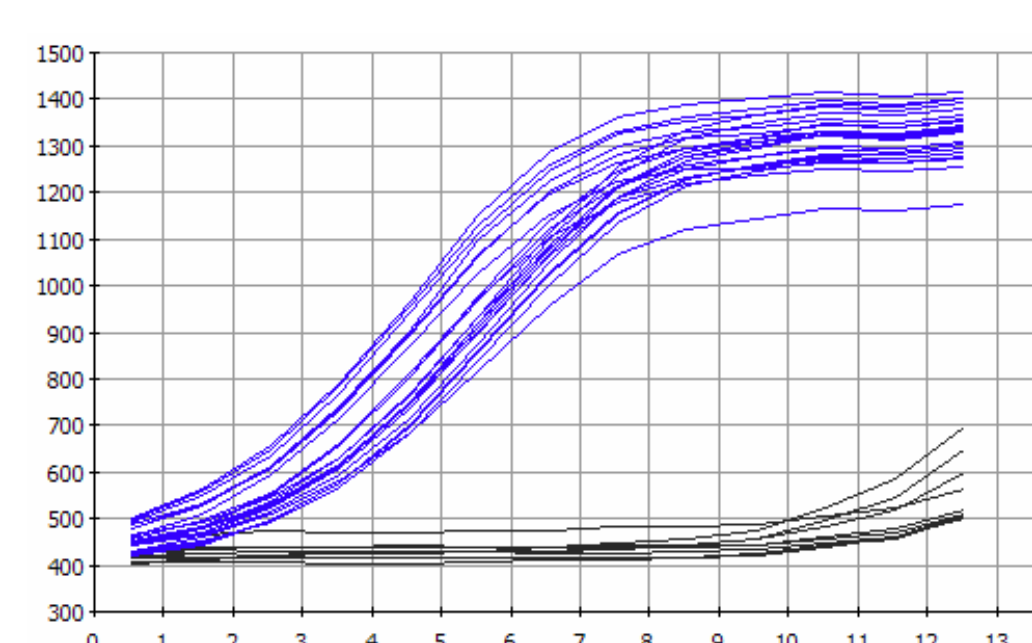


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Accurate single-cell analysis (SCA) of mutations and aneuploidies of polar bodies and blastomeres are critical for successful pre-implantation genetic diagnosis and screening (PGD/PGS). Previously, PCR and FISH SCA have been used for PGD/PGS, but advances in whole genome amplification (WGA) might enable array and NGS SCA. However WGA has never been proven to have low enough background and high enough reproducibility for array or NGS-based embryo selection. We present qPCR, array and NGS results showing that PicoPlex™ WGA enables reproducible profiling of single-gene disorders and mutations, as well as genotyping and copy number variation in single human cells. These results are also applicable to analysis of lineage of cancer tissue and circulating tumor cells (CTC). In testing by third-party reference labs, single-cell qPCR and microarray analysis was possible with >95% of the single embryo and cancer cells tested using BlueGnome, Perkin Elmer, Agilent, OGT, NimbleGen, and Illumina aCGH and SNP arrays. Aneuploidy of blastomeres, polar bodies, sperm, and cancer cells were accurately tested. Deletions >75 kb were reproducibly detected. Although Illumina SNP call rates were only 50-60%, LOH was less than 10%, showing that the SNPs were accurately called. PCR-based SNP and mutation analyses were 95% accurate. A newer version of the technology, PicoPlex-NGS WGA is shown to give reproducible, accurate coverage of single human cells in a single lane of the Illumina GA, These results translate into increased accuracy and reproducibility of single-cell PGD/PGS testing using PCR, microarrays and NGS, and open the doors to successful genetic profiling of cancer and stem cells for research and diagnostics.

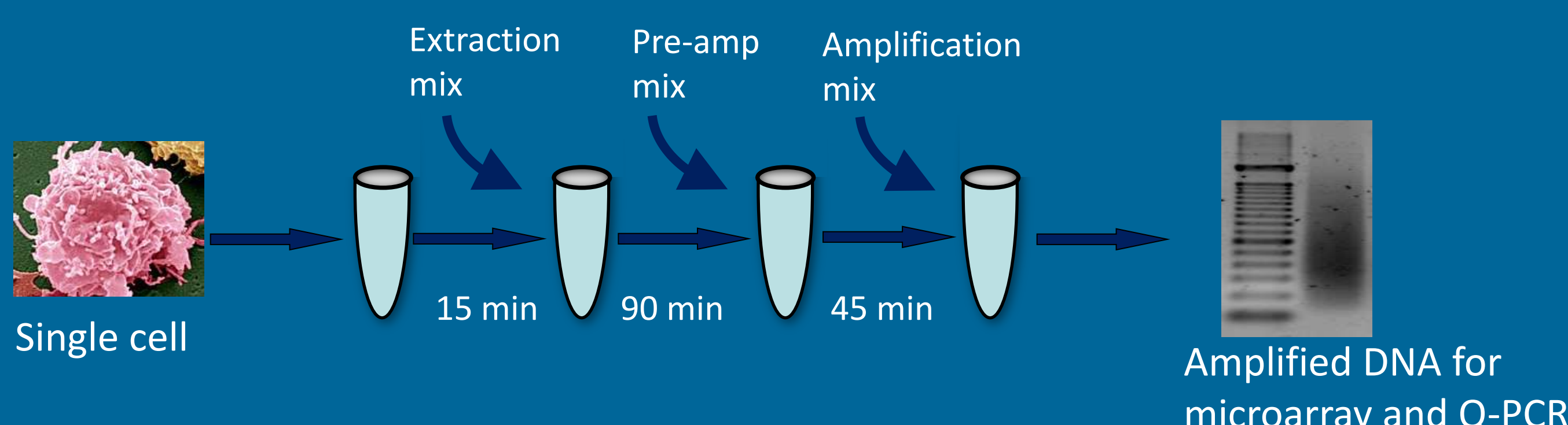
## PicoPlex WGA for Single-Cell Genetic Profiling by Microarrays and Q-PCR

PicoPlex Allows Robust Single-Cell WGA Clearly Distinguishable From Background



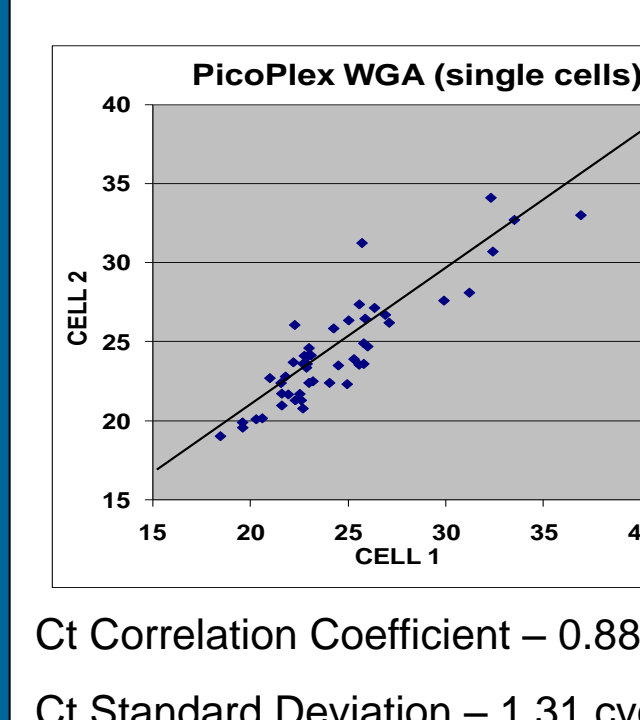
PicoPlex WGA was performed in wells containing single flow-sorted cells (blue curves) or buffer-only controls (black curves). SYBR-Green dye was added to Amplification Mix to allow real-time PCR detection of WGA product accumulation. Amplification curves for single-cell reactions were detected at least 7 PCR cycles prior to buffer control reactions. Over 4 separate experiments, 54 out of 55 (98%) single-cell reaction wells were successfully amplified.

### PicoPlex WGA Kit Workflow



PicoPlex WGA kits contain all reagents necessary to produce >2 ug amplified DNA from a single cell in under 3 hours with 3 simple addition and incubation steps

PicoPlex WGA Produces Reproducible qPCR Results from Single Cells



Single flow-sorted RWPE cells (SV40-transformed normal prostate epithelial cells) were lysed and amplified by PicoPlex WGA.

50 ng aliquots of amplified DNA were tested by 48 human qPCR assays representing a range of GC-content to quantify the stochastic and systematic bias of PicoPlex.

About 50% of the loci were as well represented in amplified DNA as in unamplified DNA. The remaining loci were reproducibly under- or over-represented. There was a high Ct correlation coefficient between replicates and low standard deviation between Ct values in replicates.

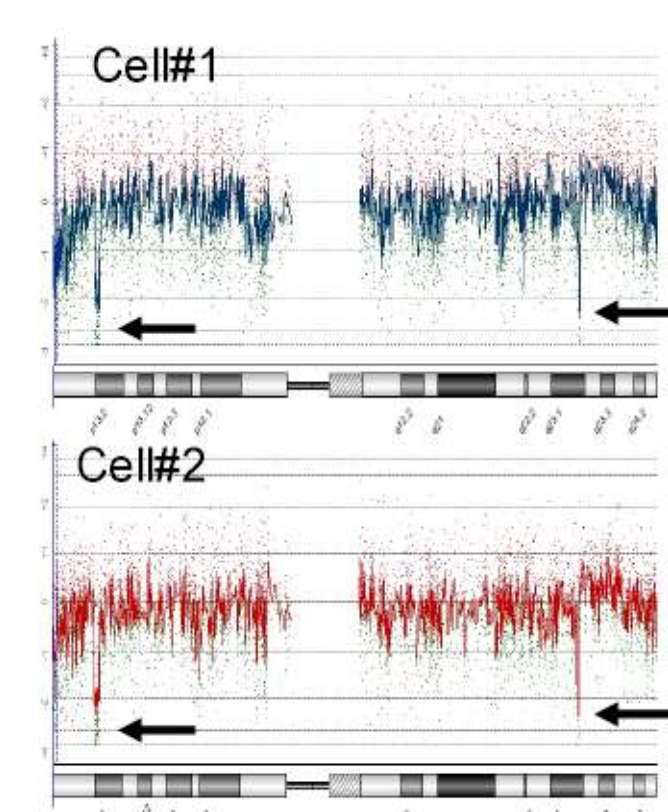
PicoPlex WGA Enables Accurate SNP Genotyping From Single Cells

SNP Genotyping Method	Single-Cell Amplification Success Rate	SNP Call Rates	Loss of Heterozygosity (LOH)
PCR	95% (90% with locus-specific PCR alone)	> 95%	< 10%
Illumina SNP array	95%	50% - 60%	7% - 12%

Locus-specific PCR or Illumina SNP array was used to evaluate PicoPlex amplification success rate and SNP call rates and LOH rates in amplified PicoPlex products. The < 10% LOH rate is much lower than the 40% - 50% LOH rate for other WGA methods and indicates that PicoPlex reproducibly amplifies both alleles, allowing more accurate SNP genotyping than other WGA methods.

Data Provided by Dagan Wells, Nuffield Department of Obstetrics and Gynaecology, University of Oxford, UK

PicoPlex WGA Enables Consistent Detection of Small (75 kb) Deletions using Agilent Oligo aCGH



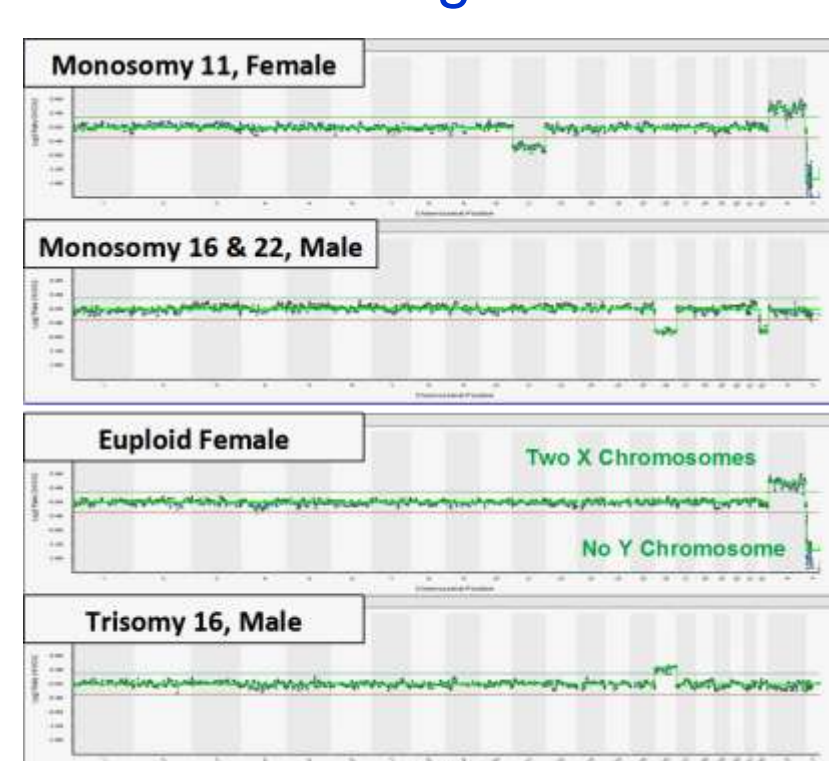
Single human cancer cells were amplified in duplicate, and the amplified PicoPlex products were analyzed by Agilent oligonucleotide aCGH.

Two chr 16 deletions of 350 kb and 75 kb were detected in both cells.

About 70% of probes consistently detected the deletions.

Data Provided by Dr. Alexei Protopopov, Dana Farber Cancer Institute

PicoPlex WGA Enables Reliable Aneuploidy Detection using PGS BAC Arrays



Single blastomeres from different patients were lysed and amplified using PicoPlex WGA kits.

The amplified DNA was labeled and hybridized to 24Sure BAC arrays (BlueGnome, Cambridge, UK). Amplified male DNA was used as the reference

The results show consistently high signal-to-noise ratios and are easily interpretable.

Data Provided by Dr. Mark Hughes, Genesis Genetics Institute

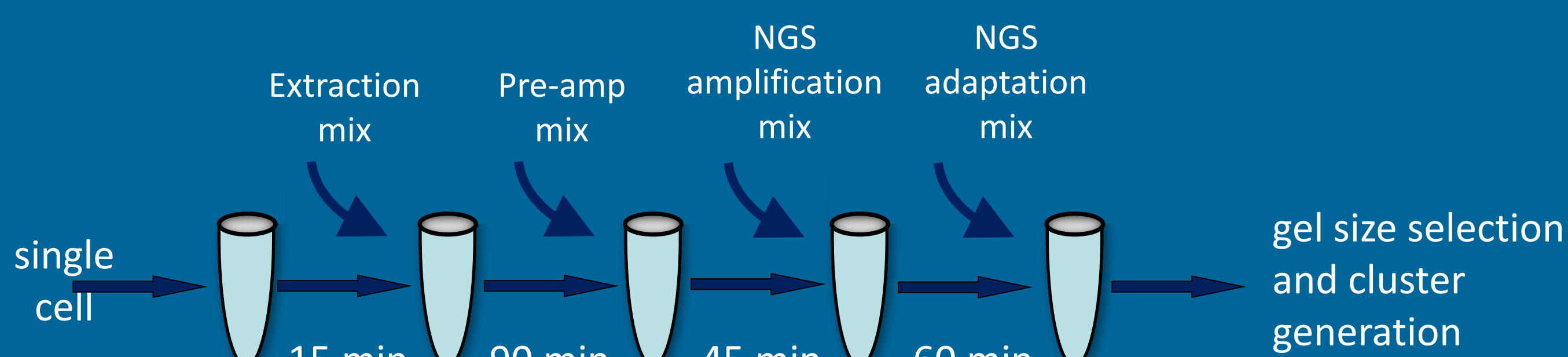
PicoPlex WGA Performance Summary

- The accuracy and reproducibility of PicoPlex single-cell WGA are high enough to enable genome-wide genotyping of SNPs and CNV and detection of mutations and chromosomal abnormalities
- PicoPlex has very high (>95%) single-cell amplification success rate, and successful amplifications are clearly distinguished from background.
- BAC and Oligo array results show that PicoPlex has low noise and reproducible amplification
- qPCR results demonstrate that PicoPlex amplifies genomic loci with reproducible representation from cell-to-cell

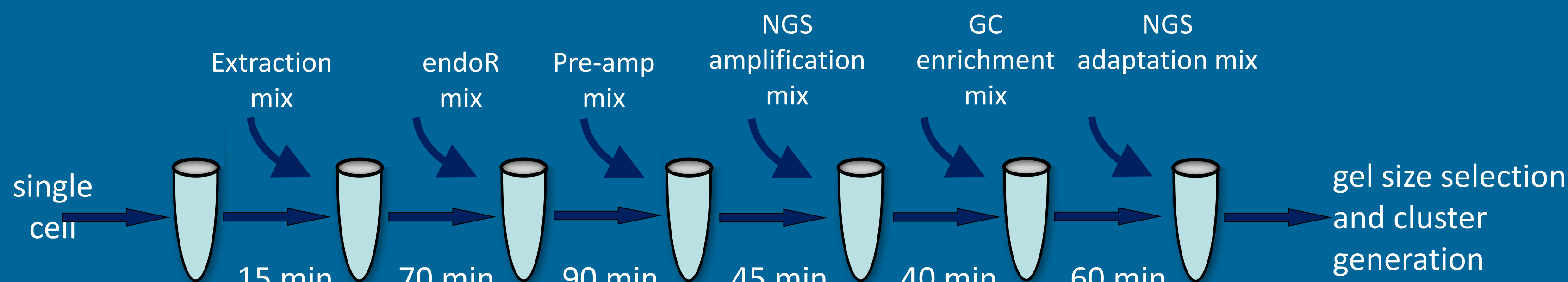
## PicoPlex-NGS WGA/WMA for Single-Cell Genetic and Epigenetic Profiling by Next-Generation Sequencing

Reproducible Sequence Quality and Coverage from PicoPlex-NGS WGA

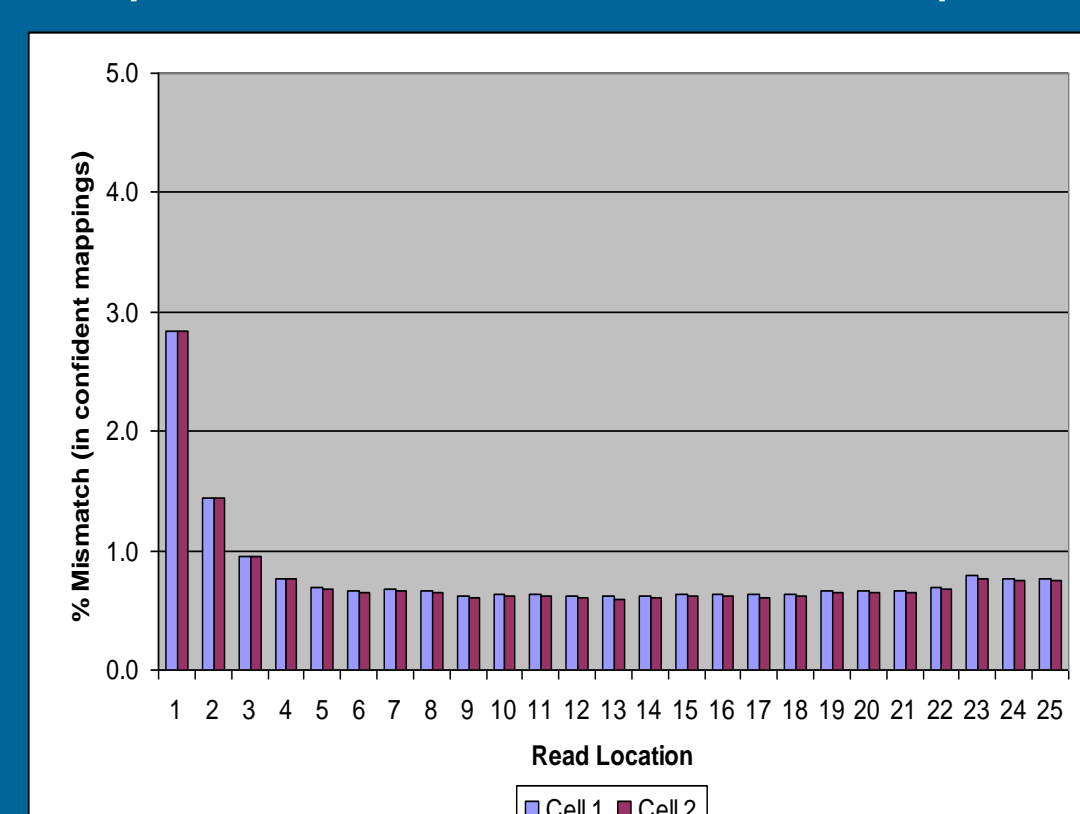
### PicoPlex-NGS WGA Workflow (for Illumina NGS)



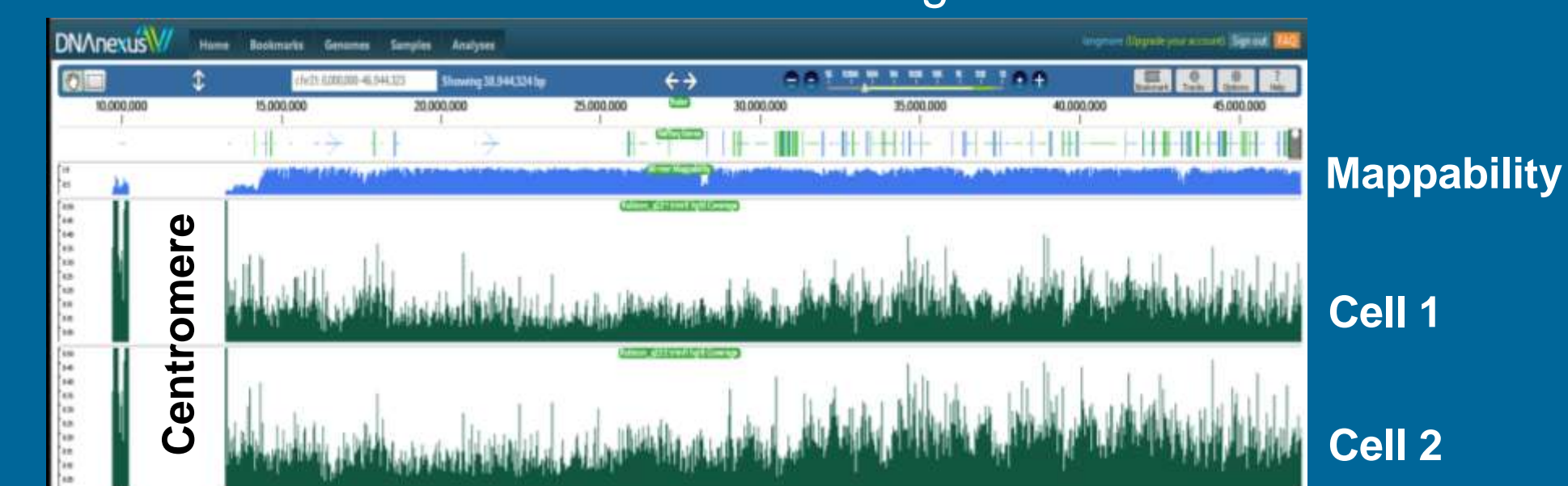
### PicoPlex-NGS WMA Workflow (for Illumina NGS)



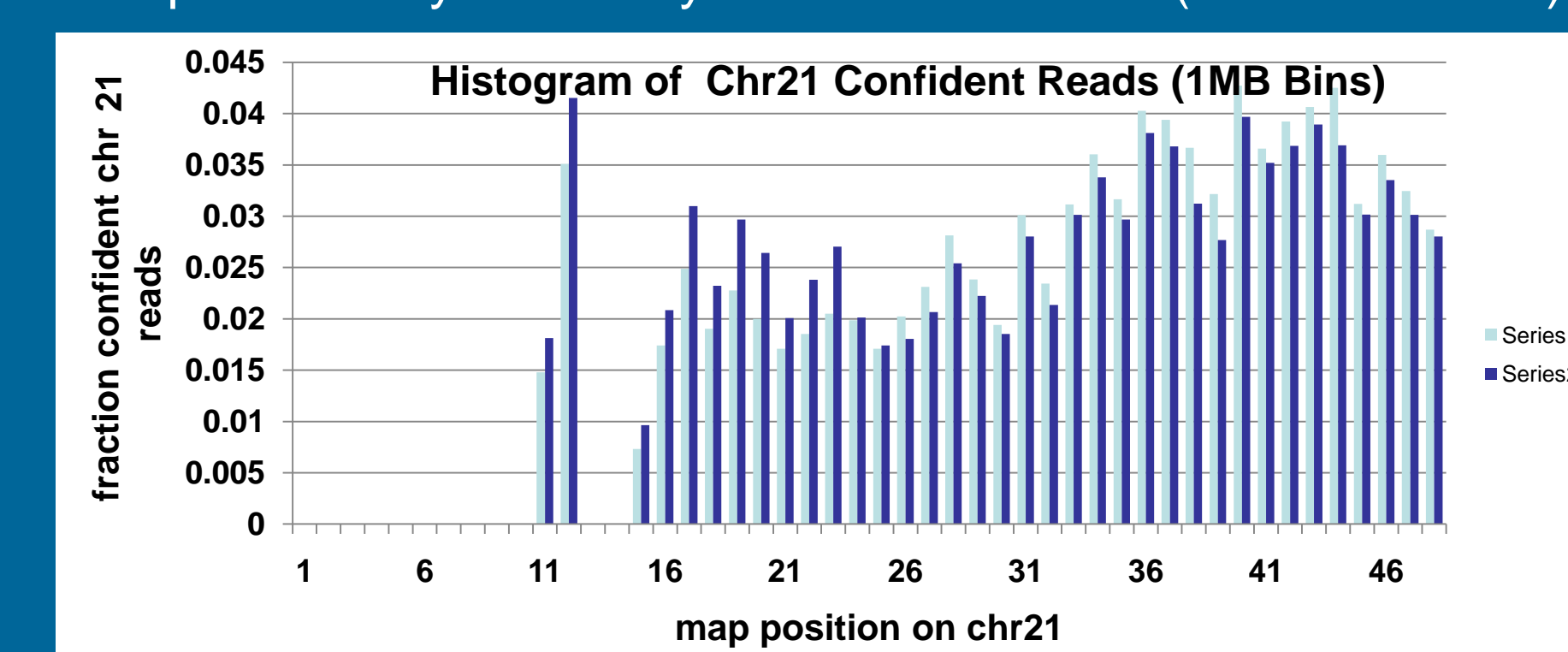
% Mismatch of cell 1 and cell 2 reads compared to reference human sequence



Human Chromosome 21 Coverage Plot



Reproducibility of density of confident reads (cell 1 vs. cell 2)



PicoPlex-NGS WGA/WMA kits are under development and will contain all reagents required to produce amplified DNA ready for gel fractionation and cluster generation in less than four or six hours. Custom primers will allow both single- and paired-end sequencing. The PicoPlex-NGS WGA/WMA kits are designed to have the robust, reproducible, low background features of PicoPlex WGA with the added capabilities of:

- Aneuploidy and CNV analysis in a single lane
- SNP genotyping and mutation detection using deep sequencing
- Profiling methylated genomic regions in a single lane

Flow-sorted single PC3 prostate cancer cells were amplified using PicoPlex-NGS WGA, and 4 pmol of ~450 bp library molecules were used for cluster generation and single-ended (36 bp) sequencing using Illumina Genome Analyzer. The first 11 bases of sequence were trimmed prior to analysis to eliminate PicoPlex-NGS-specific adaptor sequences. High-resolution accuracy results and lower-resolution chromosome coverage results from replicate single-cell samples are shown. PicoPlex-NGS enables aneuploidy and CNV studies of single human cells.

Acknowledgements: Flow sorted cancer cells were provided by the Pienta lab (University of Michigan), Illumina sequencing was performed by Eureka Genomics (Hercules, CA), and sequence analyses were performed using DNAnexus (Palo Alto, CA) Web-based service.