Rubicon PicoPlex™ WGA

Improved Single-Cell WGA for PGD and Other Applications

June 2009

Current Problems in Single-Cell Whole Genome Amplification Methods

- **Technical problems with existing methods**
  - Irreproducible locus and allele representation
  - High background of extraneous DNA
  - High frequency of locus and allele drop out
  - Recombination of sequences during amplification

- **Clinical problems**
  - Single-cell molecular diagnostics very difficult
  - Assays must depend on multiple, redundant analytes
  - Microarray and next-gen assays of single cells (or other small samples like CTCs) problematic

Rubicon Solution:

PicoPlex Single-Cell WGA

- Simple, rapid, reproducible
- Much improved representation
- Highly reproducible for ~ 80% of genome
- High sequence fidelity
- Virtually no background

High Performance

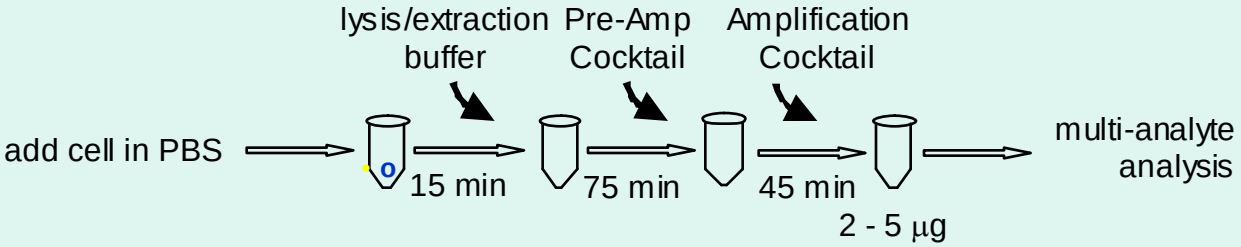
- Excellent single-cell oligo aCGH able to detect deletions of <75 kb
- Excellent array karyotyping
- Reproducible Q-PCR results
- Reproducible SNP profiling
- Performance with single cells equivalent to 1,000+ cells

Customers Benefits:

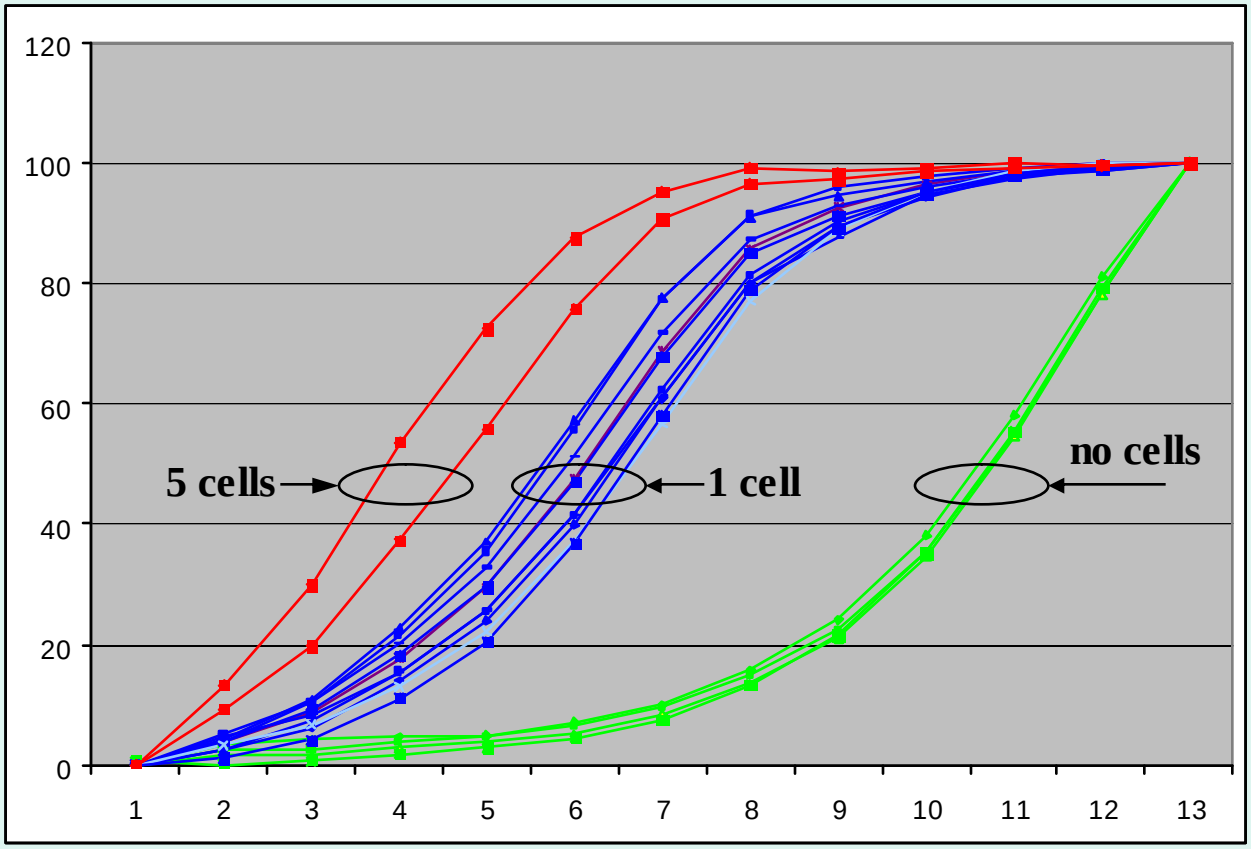
Improved analytical performance for clinical testing of polar bodies, embryo biopsies and single cancer cells

PicoPlex WGA Workflow

1 cell, 1 tube, 3 steps, 2 hr, unlimited assays

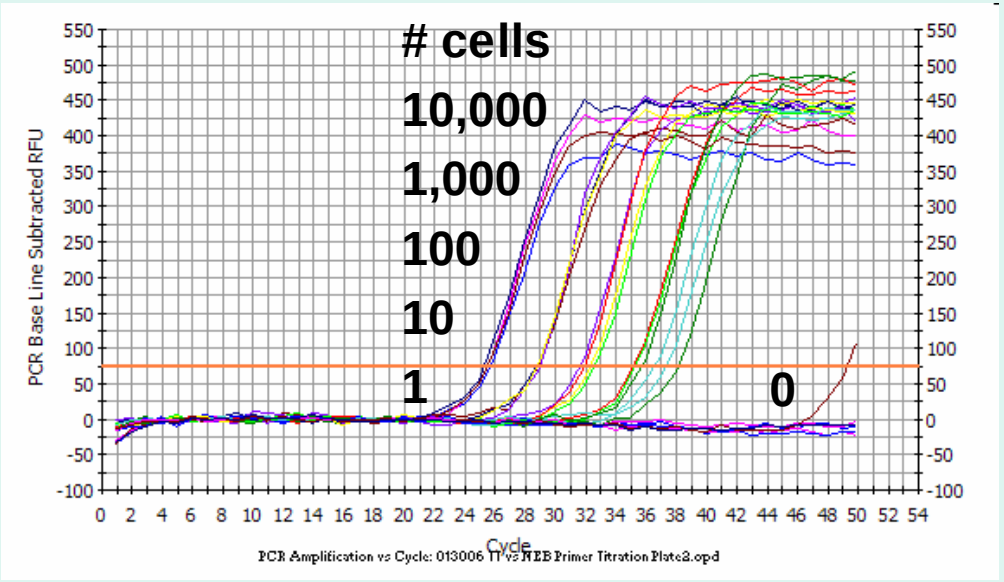


Experimental Real-Time PicoPlex WGA of Samples With 0, 1, and 5 Flow-Sorted Cells



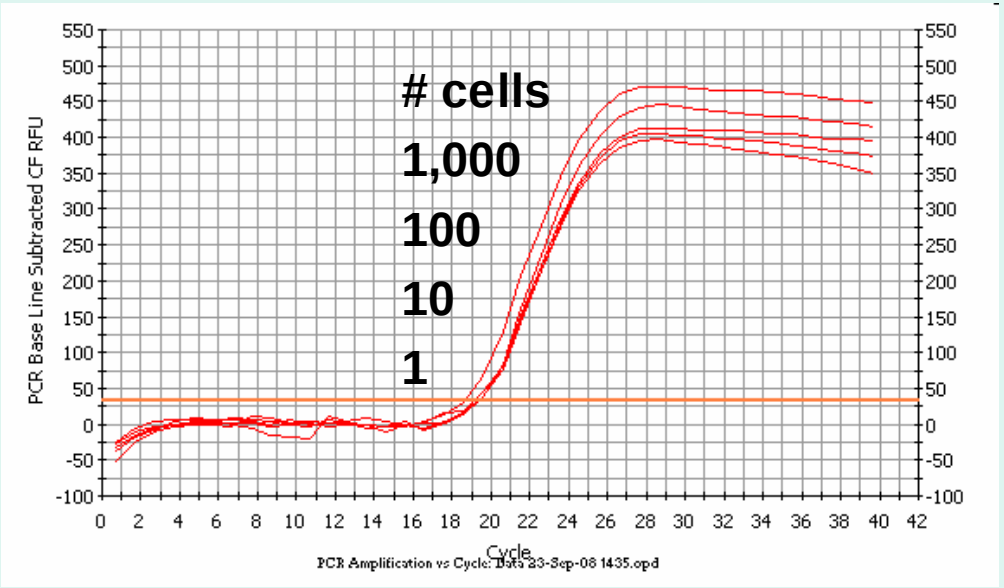
Locus-Specific PCR QC-Testing of PicoPlex DNA

Locus-specific PCR of genomic DNA with increasing number cell-equivalents

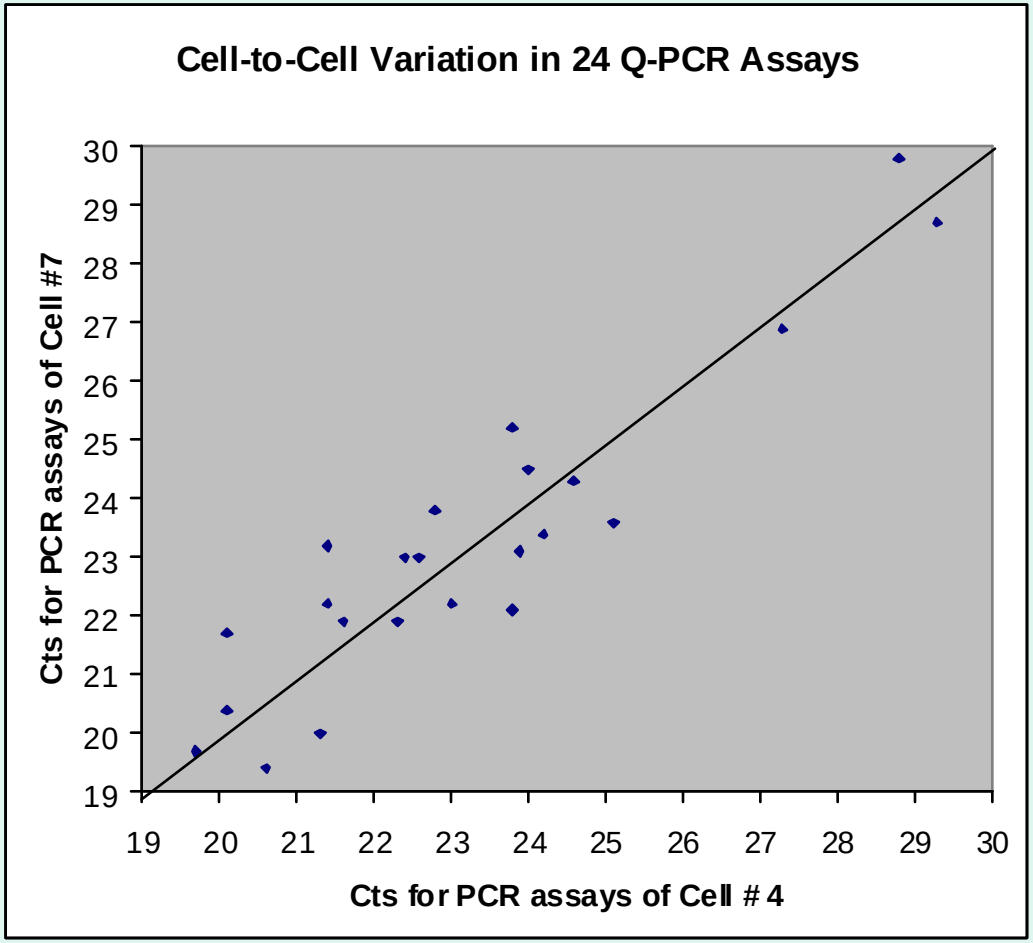


Locus-specific PCR of PicoPlex-amplified DNA from 1, 10, 100, and 1000 cell-equivalents

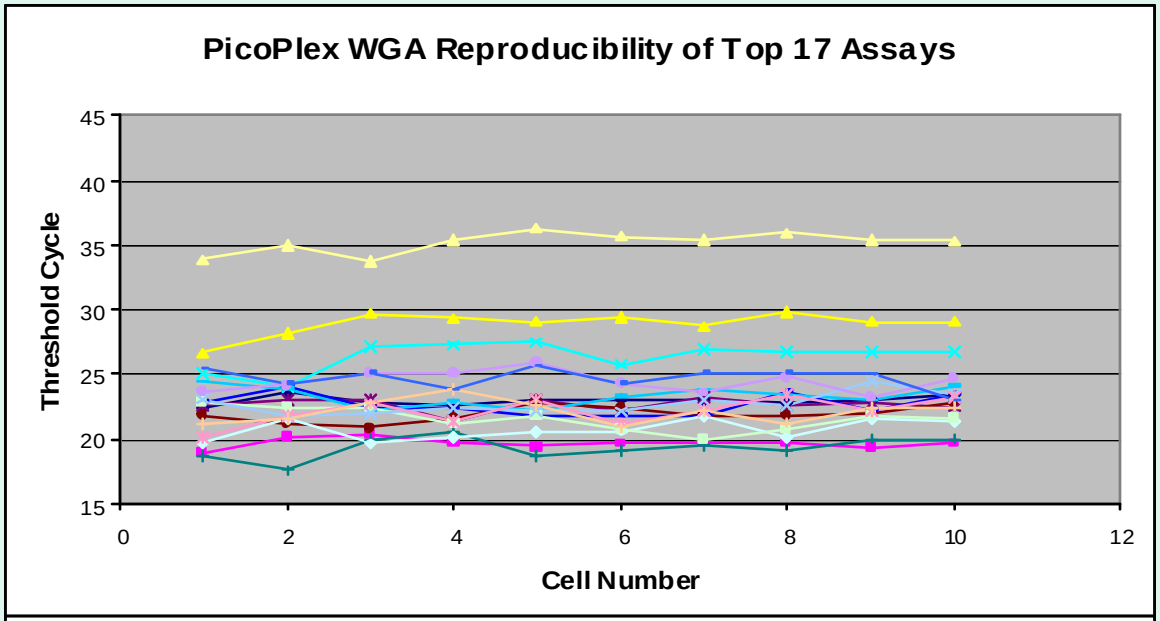
(DNA amplified from a single cell gives the same test result as from 1,000 cells)

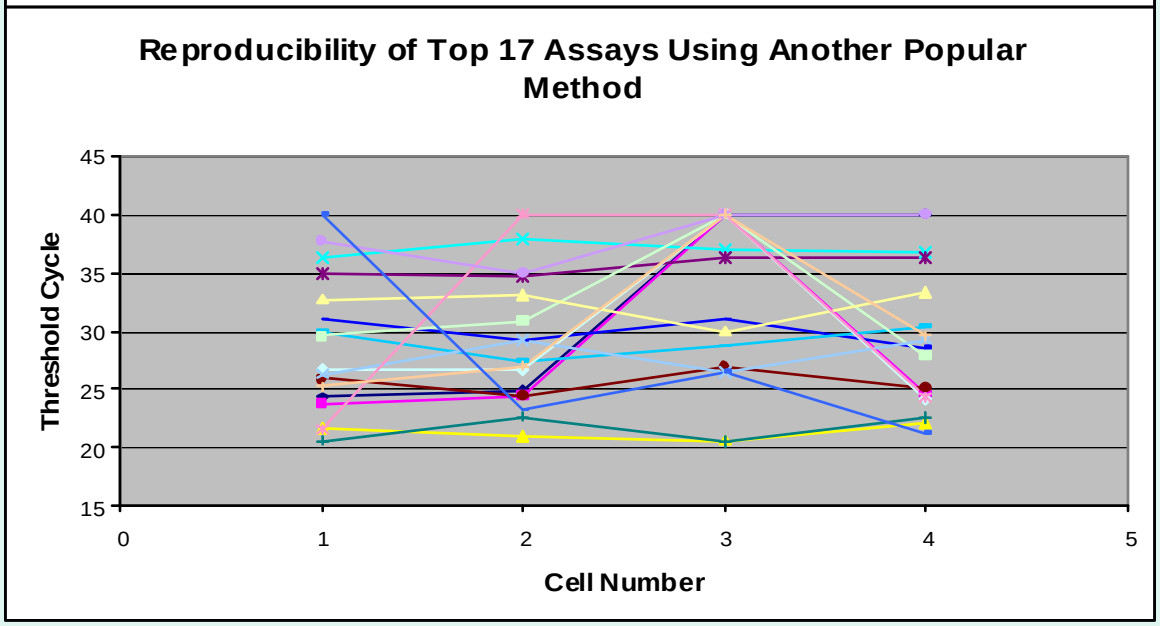


PicoPlex WGA Produces Highly Reproducible Representation of Multiple Loci Tested in Different Single Cells



Reproducibility of Single-Cell PicoPlex WGA is Superior to Other Current Methods of WGA





Reproducibility of PicoPlex WGA

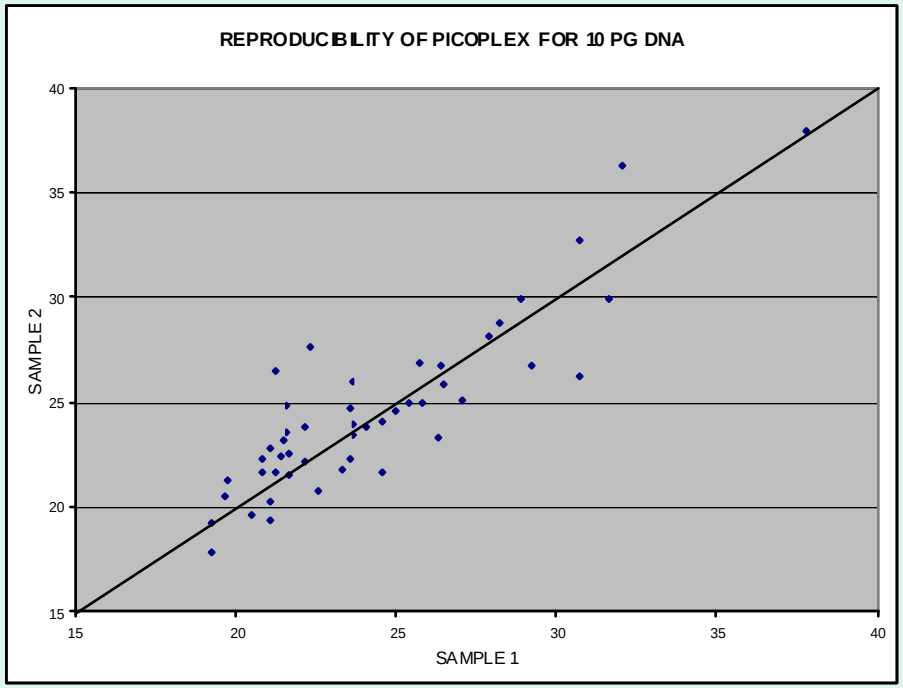
	10 ng DNA	5 cells	single cell
drop out rate*	10%	13%	11%
avg. standard deviation of 48 assays**	-	-	1.6 cycles
ave. standard deviation of top 24 assays***	-	-	0.6 cycles

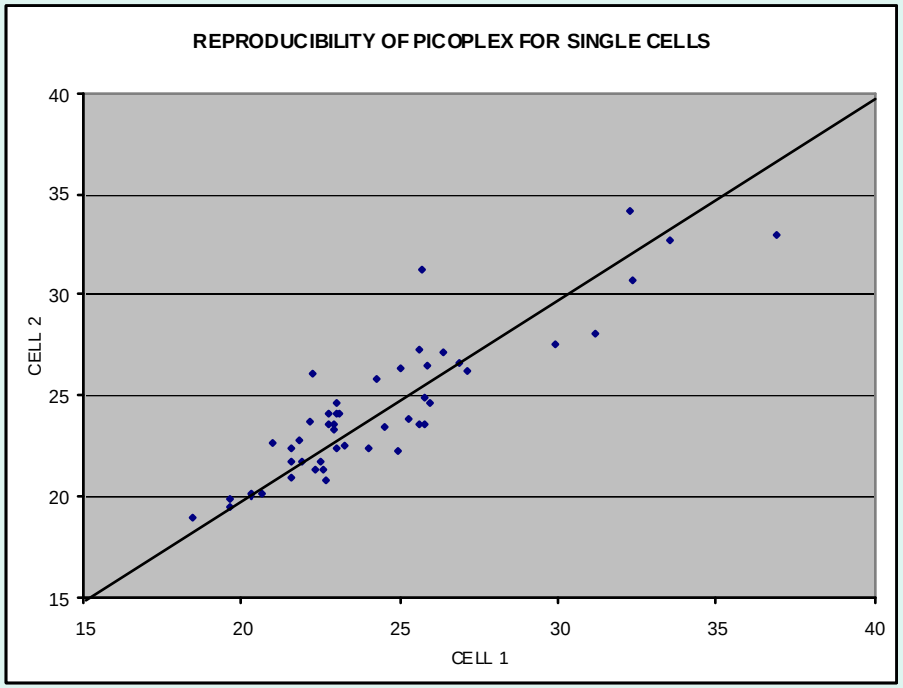
*Drop out rate defined as percentage of 48 locus-specific Q-PCR assays delayed more than 10 cycles relative to unamplified DNA.

**Reproducibility of all assays defined as the standard deviation of the Ct value among all cells, averaged over all 48 assays.

***Reproducibility of top half of assays defined as the standard deviation of the Ct values among all cells, averaged over the most consistent 24 assays

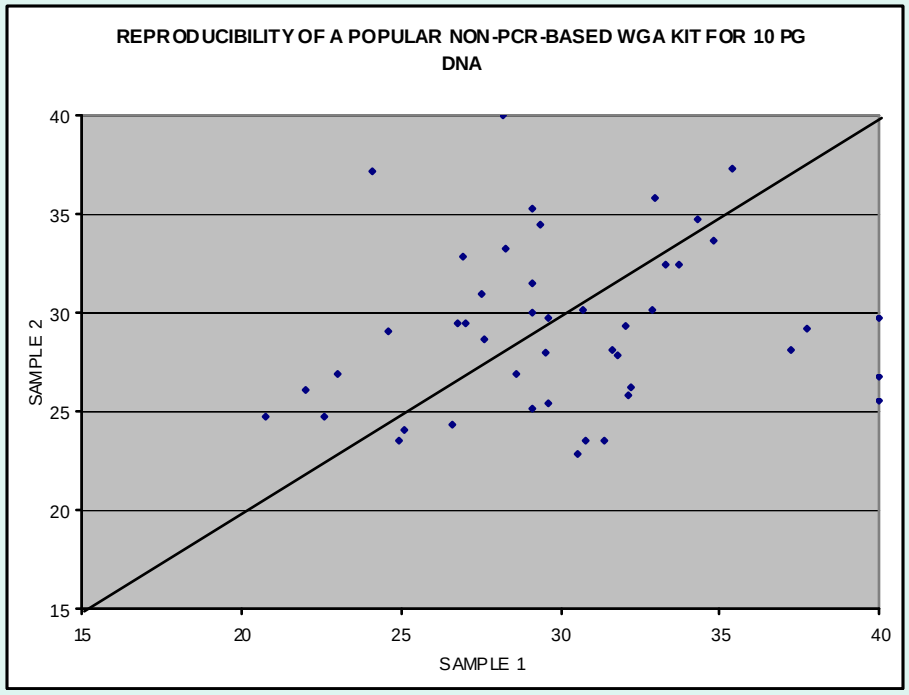
PicoPlex WGA Produces Very Reproducible Q-PCR Results For Multiple Loci Tested in Replicate Isolated DNA or Single Cell Samples

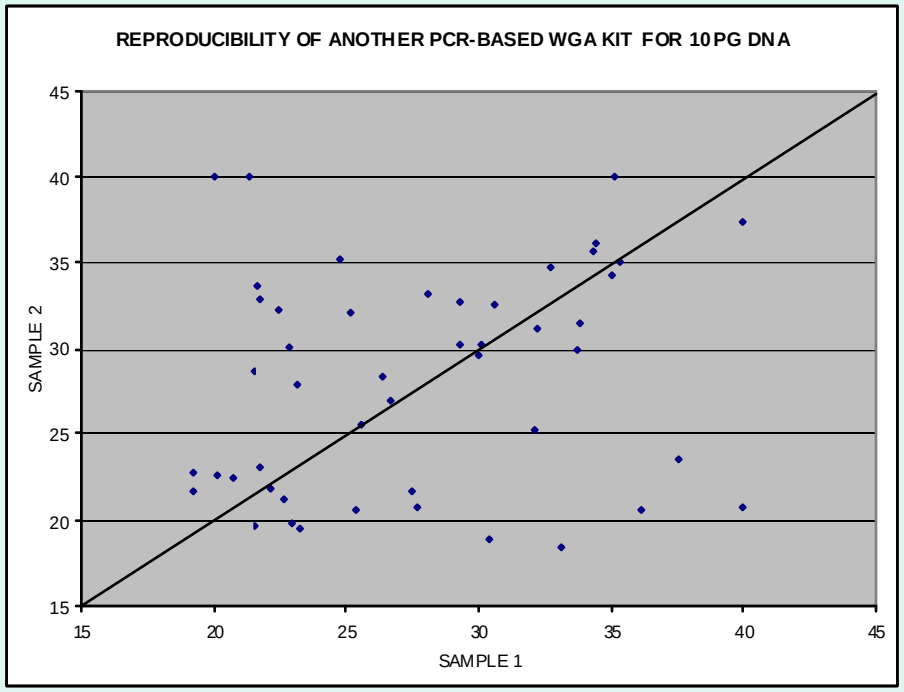




Other Single-Cell WGA Kits Produce Less Reproducible Q-PCR Results For Multiple Loci Tested in Replicate Samples of Isolated DNA

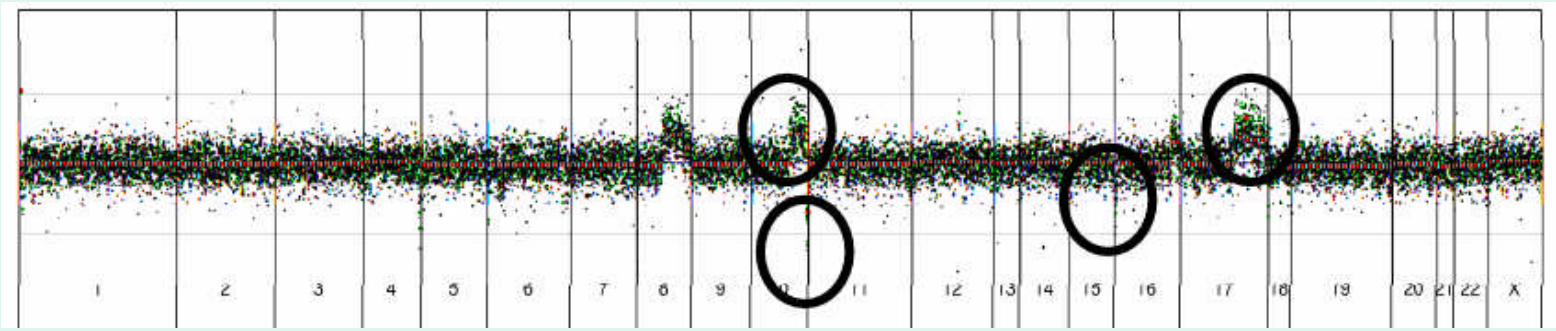
(results from same experiment as shown in previous slide)



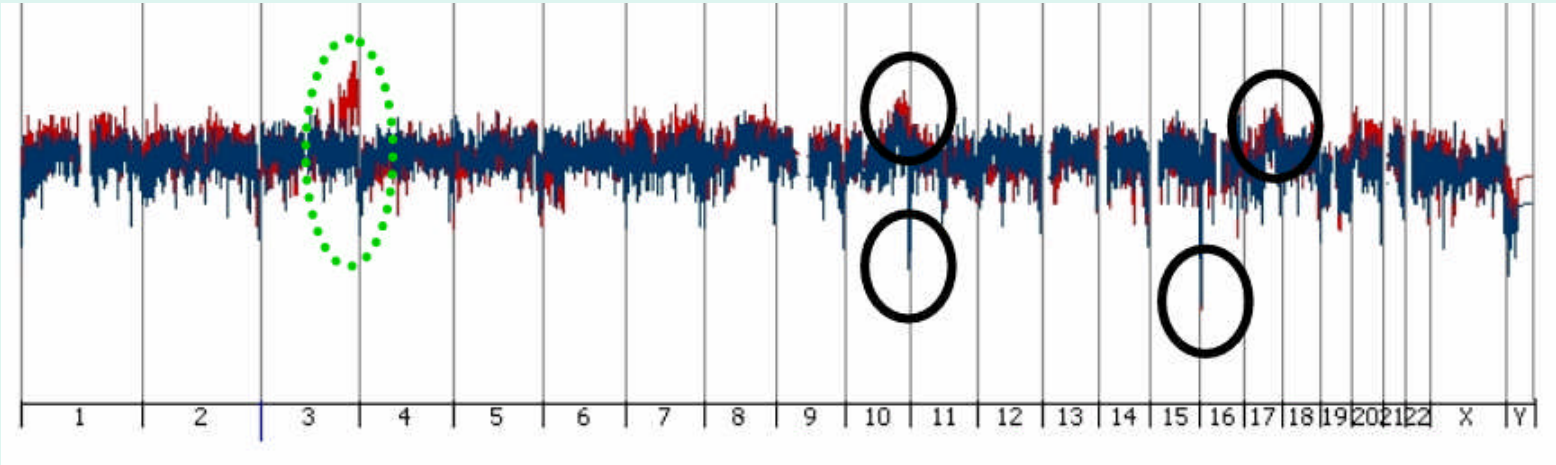


Concordance of Agilent CGH Results From Two Single Cells Amplified using PicoPlex WGA, Compared With Results From 5 micrograms of unamplified DNA

- **Agilent 44K array CGH of ~5 micrograms of unamplified human DNA from tissue culture. Note amplification of chr 8q, 10q and 17q, and deletions in the telomeric regions of chr 10q and 15q**

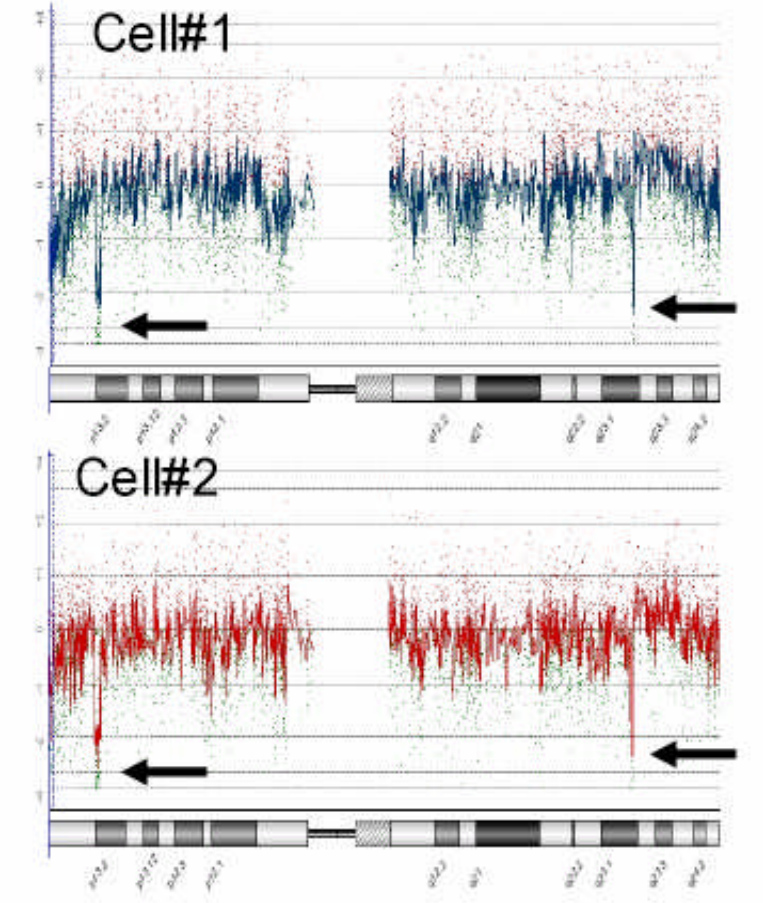


- **Agilent 244K array CGH hybridized to single cells from same tissue culture. Note amplification of chr 10q and 17q, and deletions in the telomeric regions of chr 10q and 15q. Cell #1 (blue trace) has common amplifications and deletions. Cell #2 (red trace) has a rare (but known) amplification of chr 3q.**



Chromosome 16 CGH Results Using Rubicon WGA on Single Cells

PicoPlex WGA results from two single cells, each clearly showing expected 350 kb and 75 kb deletions in chr 16. An average of 70% of the probes detected the deletions



Status of Rubicon PicoPlex WGA III

- **Technical validation**
  - PicoPlex reproducibly amplifies DNA from single cells.
  - QPCR shows that PicoPlex is more representative and reproducible than other methods for single-cell samples
  - Oligonucleotide aCGH analysis shows that ~70% of the probe sequences are highly represented.
  - PicoPlex-enabled BAC aCGH and genotyping array profiling show very significant results for blastomeres, polar bodies and other single cells (data to be released with permission of collaborators).

- **Commercialization**
  - PicoPlex WGA is a new amplification technology, developed and commercialized exclusively by Rubicon.
  - Rubicon is providing PicoPlex WGA kits to the PGD market in July 2009.