

Use of Whole Genome Amplification for Genetic and Epigenetic Analysis of Single Cells

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Types of Single Cell Analysis

- Phenotypic Analysis
 - Genetic Analysis
 - Image Analysis
 - Cytometry
 - Metabolomic Analysis
 - Proteomic Analysis
 - Expression Analysis

 - Genomic Analysis
 - Epigenomic Analysis
- } Rubicon PicoPlex WGA
Rubicon PicoPlex WMA

Opportunities For Single Cell Genetic and Epigenetic Analysis

- Pre-implantation Testing for Embryo Selection in IVF
- Pre-natal Testing
- Cancer Research and Testing
- Stem Cell Research and Therapeutics
- Infectious Disease

Embryo Selection for IVF

- PGD--preimplantation genetic diagnosis of inherited disease
 - Current practice uses family-specific PCR assays
- PGS--screening for developmental chromosomal abnormalities
 - World-wide annual demand for PGS currently 1,000,000 embryos
 - Array analysis of single cells shown to be substantially more accurate for detecting aneuploidy than FISH
 - Prospective trials of array-based PGS will begin in 2011
- PGD/PGS is currently the largest market for single-cell analysis, and therefore a good indicator of state-of-the-art genomic analysis of single cells

SCA for Pre-Natal Molecular Diagnosis

- Huge global market--11 million pre-natal tests in 2009
- High value market--equal to entire cancer MDx market
- Anticipated improvements focus on earlier detection of genetic diseases
- Analysis of single fetal cells in maternal circulation
- Analysis of small amounts of free DNA in maternal plasma

SCA for Cancer

- Study of genomic/epigenomic heterogeneity within tumors
- Reconstruction of cell lineages
- Study of CTCs and DTCs
- Prognostic and predictive tests based on CTCs

SCA for Stem Cells

- Study of stem cell genetic and epigenetic heterogeneity
- Study of instabilities
- Epigenetic study of stem cell reprogramming
- QC for stem cell manufacturing
- QC for regulatory approval

SCA for Infectious Diseases and Other Rare-Cell Applications

(one needle in a haystack is still one needle)

- Microbial detection and characterization from complex samples
 - pan-organism arrays and NGS used to detect very low number of disease microbes in a background of large amounts of human or common bacterial DNA
 - metagenomics
 - biodefense
- Forensics

SCA Toolbox

PROCURE SINGLE CELL	LYSE CELL AND PURIFY DNA	AMPLIFY DNA	ANALYZE	RESULTS
flow sorting	alkaline lysis	single-locus PCR	gels	sequence
dilution	proteinase K	multiplex PCR	qPCR	copy number
capillary pipet	other enzymes or detergents	multiplex pre-amp	microarrays	chromosomal mutations
LCM		DOP and PEP	sequencing	point mutations
enrich		MDA WGA	mass spec.	genotype (SNP, CNV, STR)
		GenomePlex™ WGA		
		PicoPlex™ WGA		

Methods to Amplify DNA for SCA

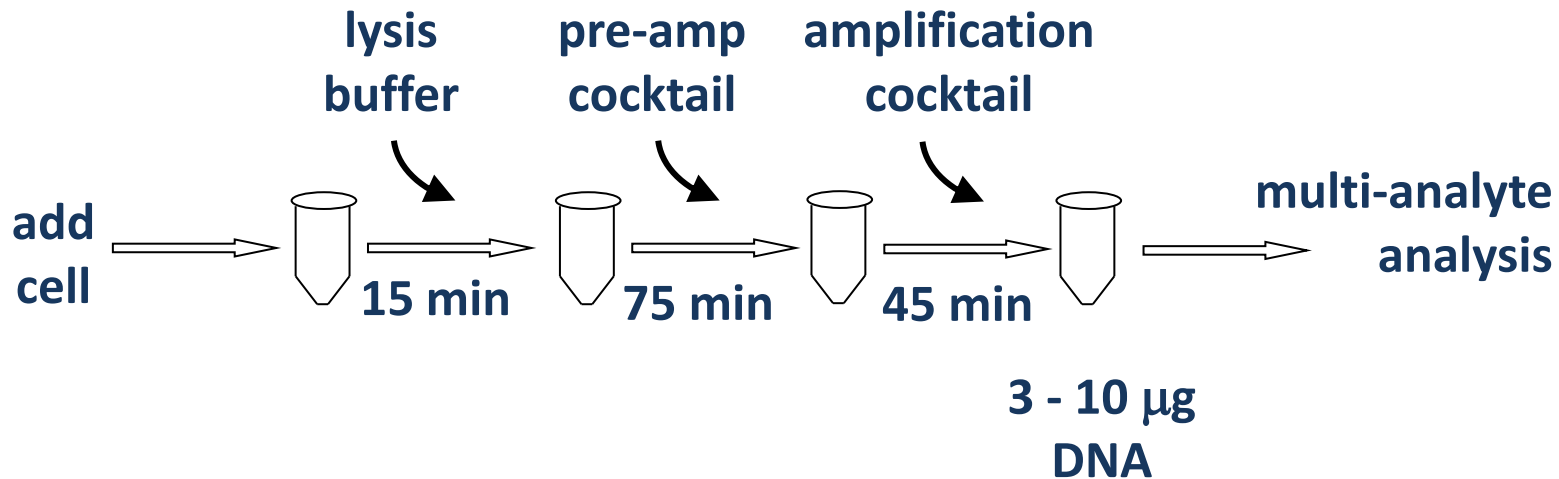
- Single-locus PCR
- Multiplex PCR and pre-amplification of specific loci
- Whole Genome Amplification
 - DOP, PEP
 - Molecular Displacement Amplification, MDA (Qiagen REPLI-g™, and GE GenomiPhi™)--Isothermal amplification with phi29 polymerase
 - Sigma GenomePlex™ WGA--Isothermal library preparation followed by universal-primer PCR
 - Rubicon PicoPlex™ WGA--Thermal cycling library preparation followed by universal-primer PCR, with redundant utilization of template molecules

Common Problems for Single Cell Analysis

- Degradation of DNA
 - apoptosis
 - nuclease activity
- Contamination of DNA
 - extracellular DNA from homologous cells (e.g., carry over from trypsinization)
 - reagents that contain extraneous DNA (e.g., serum, BSA, polymerase)
 - incomplete lysis and removal of cellular proteins and inhibitors
- Imperfect DNA amplification
 - systematic bias (e.g., loss of GC-rich loci)
 - stochastic bias (e.g., random locus or allele drop outs)
 - high or inconsistent background (non-templated DNA amplification)
 - rearrangement of DNA (e.g., chimeras formed during amplification)

PicoPlex WGA

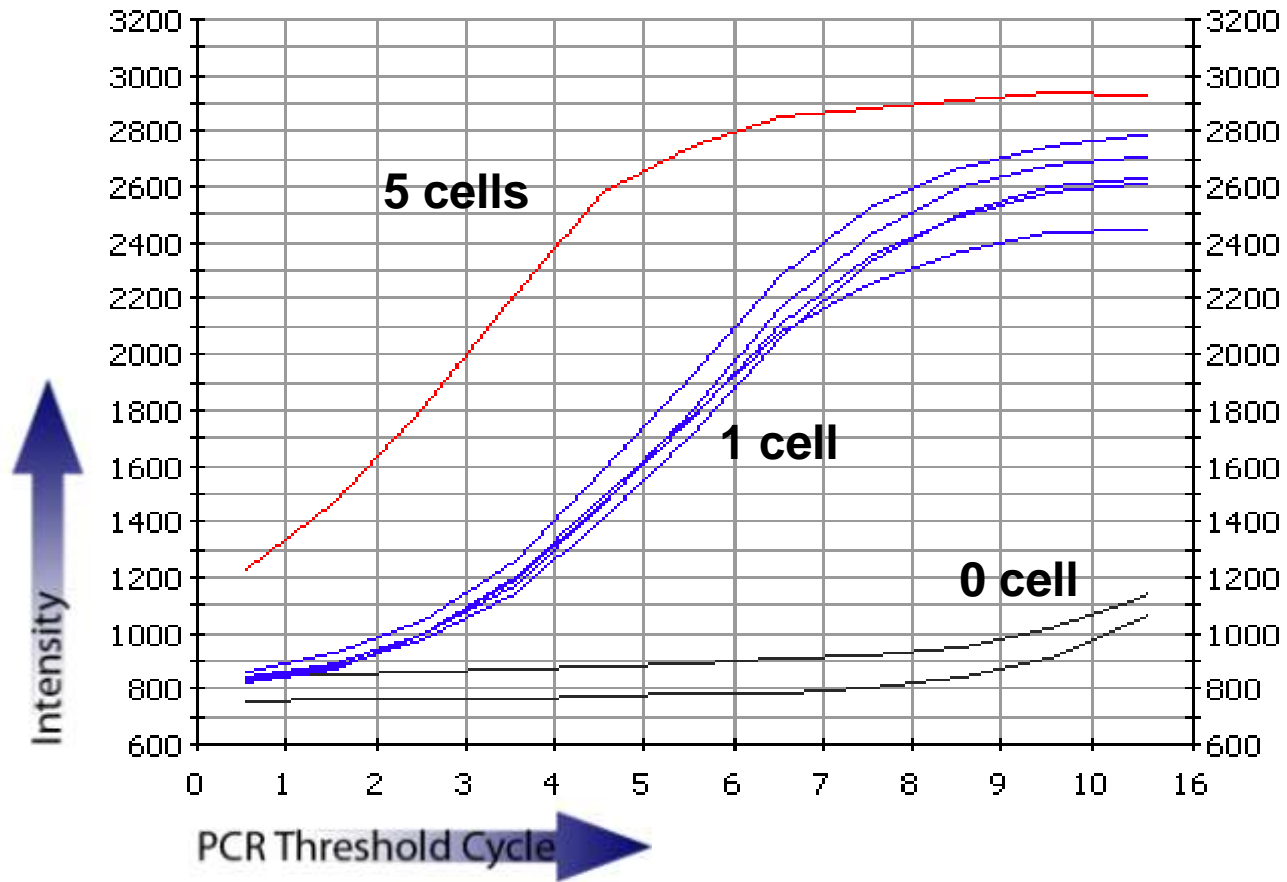
(for PCR and microarray assays)



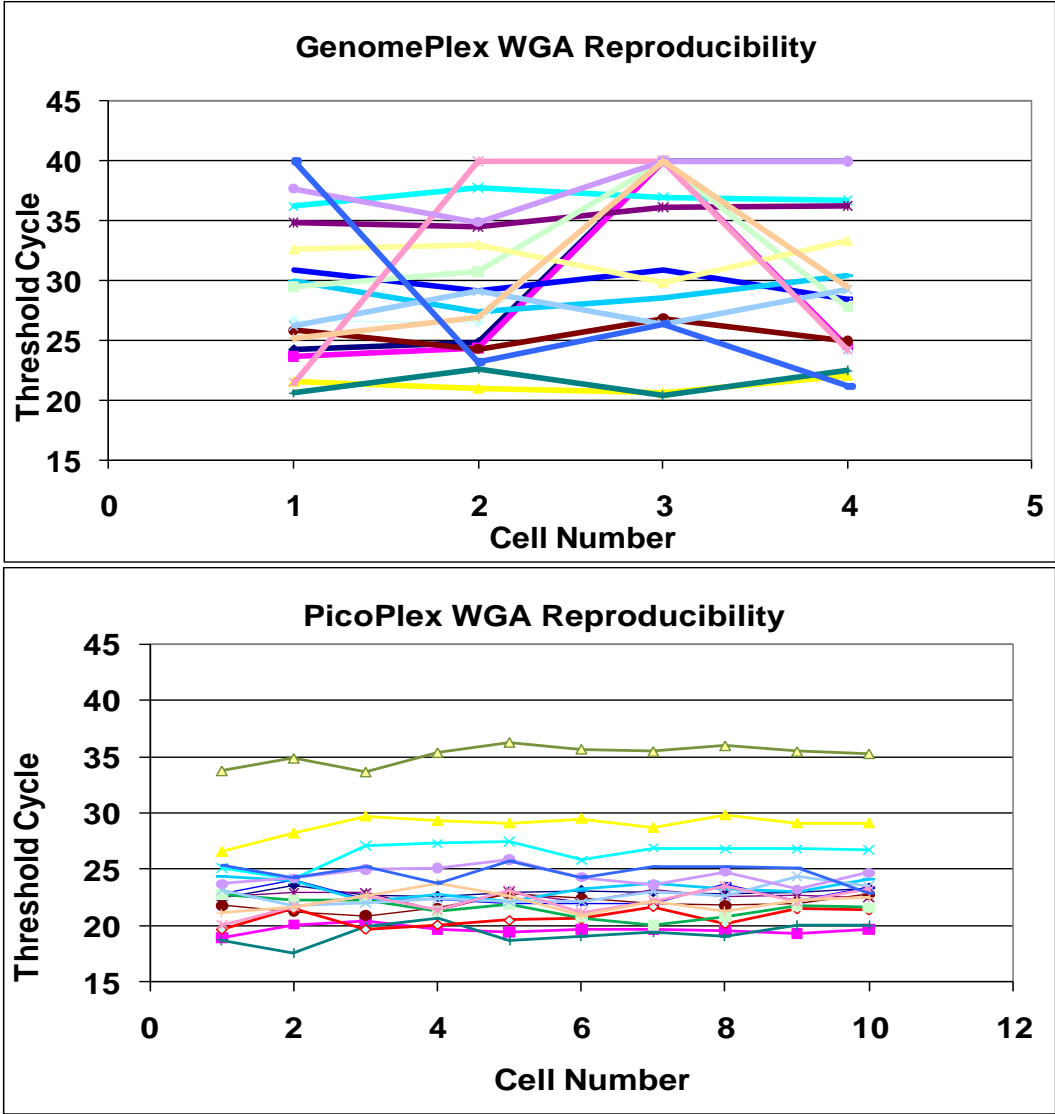
Rubicon Q-PCR Data from PicoPlex-Amplified Flow-Sorted Cells

(Cells Provided by Arul Chinnaiyan, University of Michigan,
Ann Arbor)

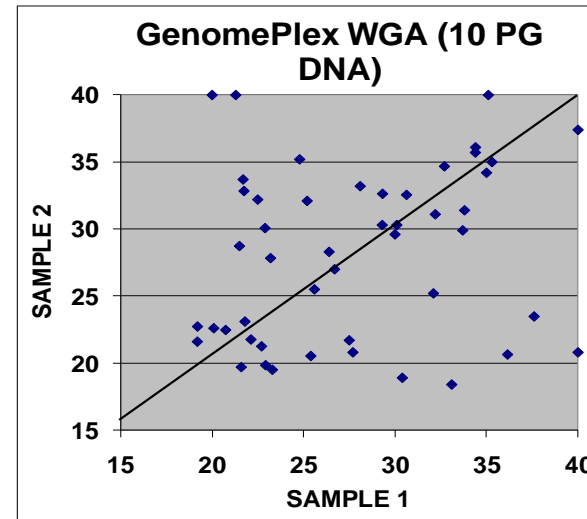
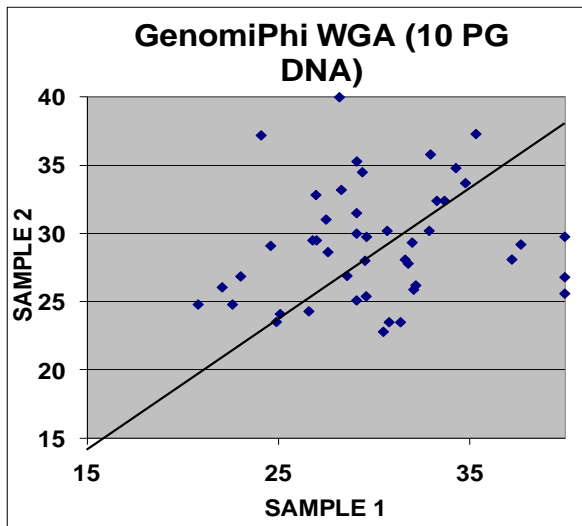
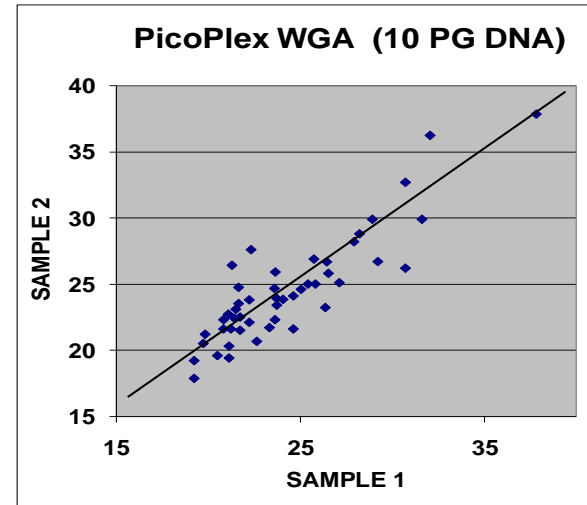
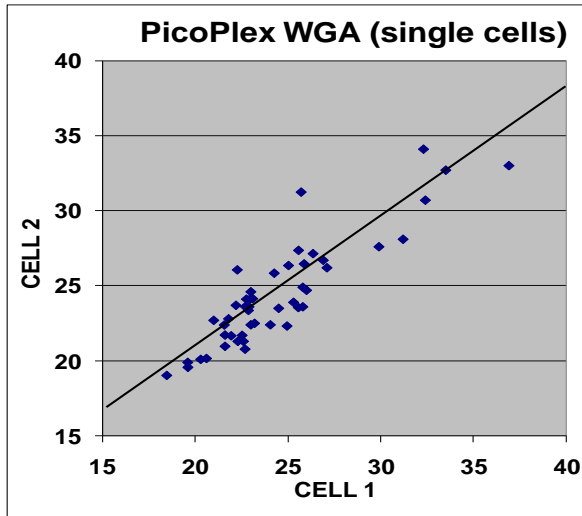
Real-Time PicoPlex Shows Reproducibility of WGA and Low Background



Systematic and Stochastic Bias in GenomePlex and PicoPlex



Reproducibility of Locus Representation After Replicate "Single-Cell" Amplifications

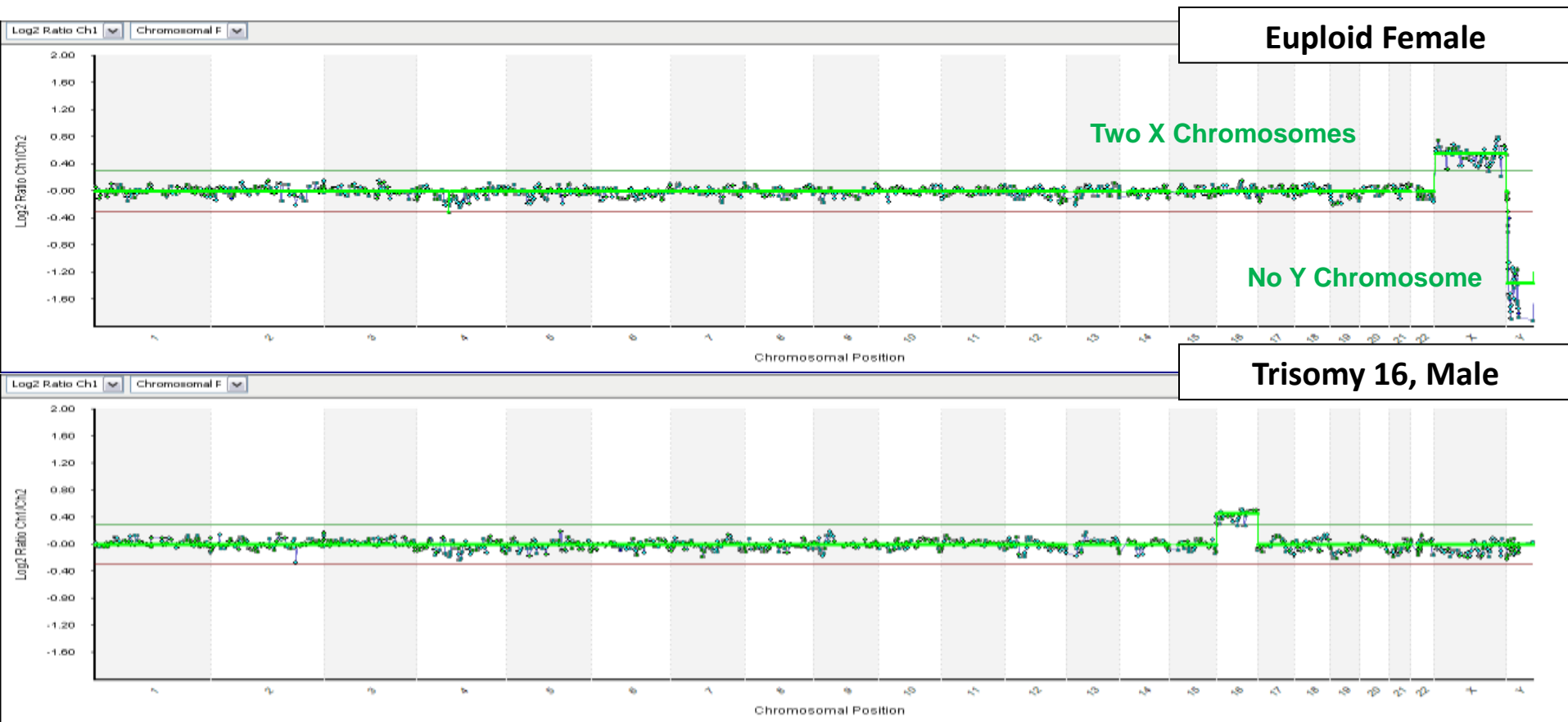


Detection of Aneuploidy in Single Blastomeres and Sperm Amplified with PicoPlex WGA

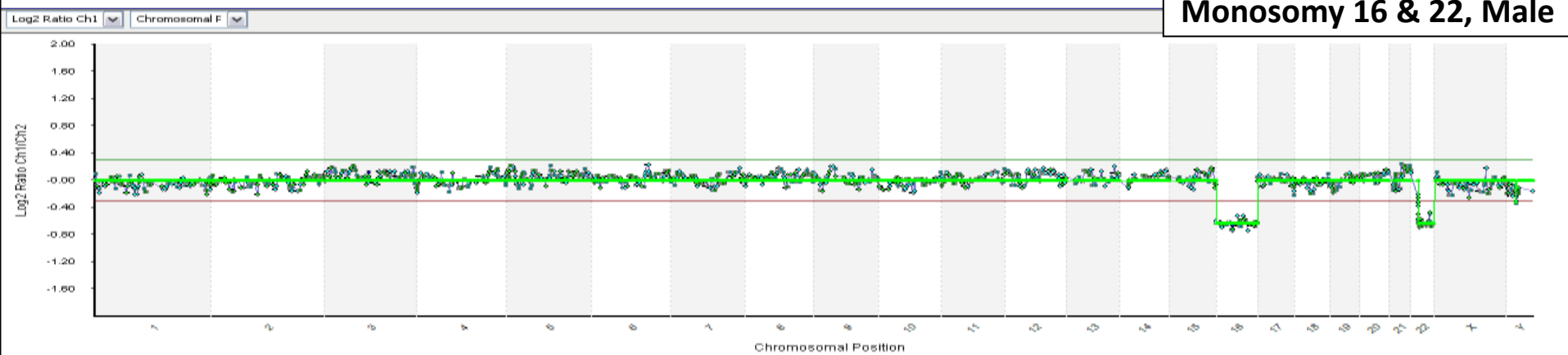
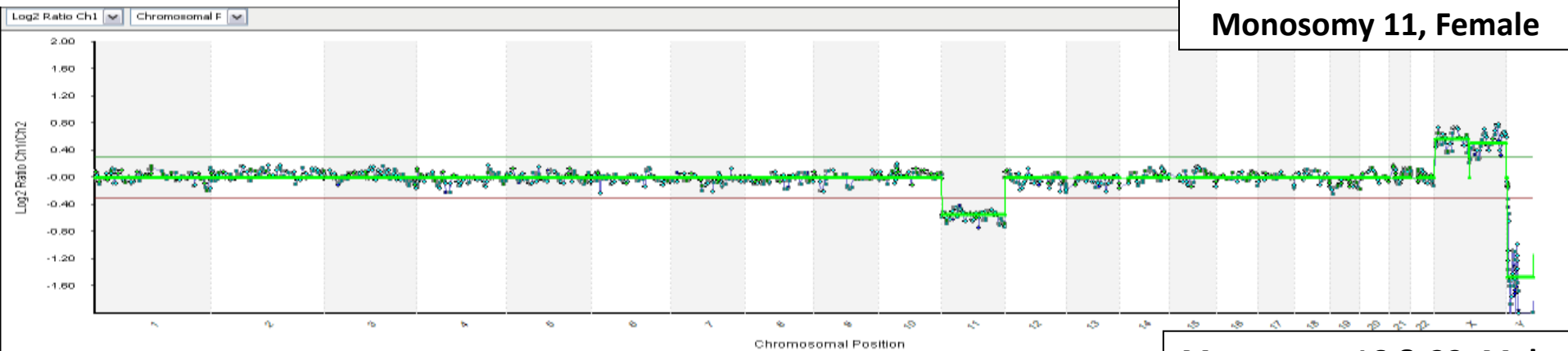
BlueGnome 24Sure BAC Arrays

Data from Mark Hughes,
Genesis Genetics Institute, Detroit, USA
and
BlueGnome, Cambridge, UK

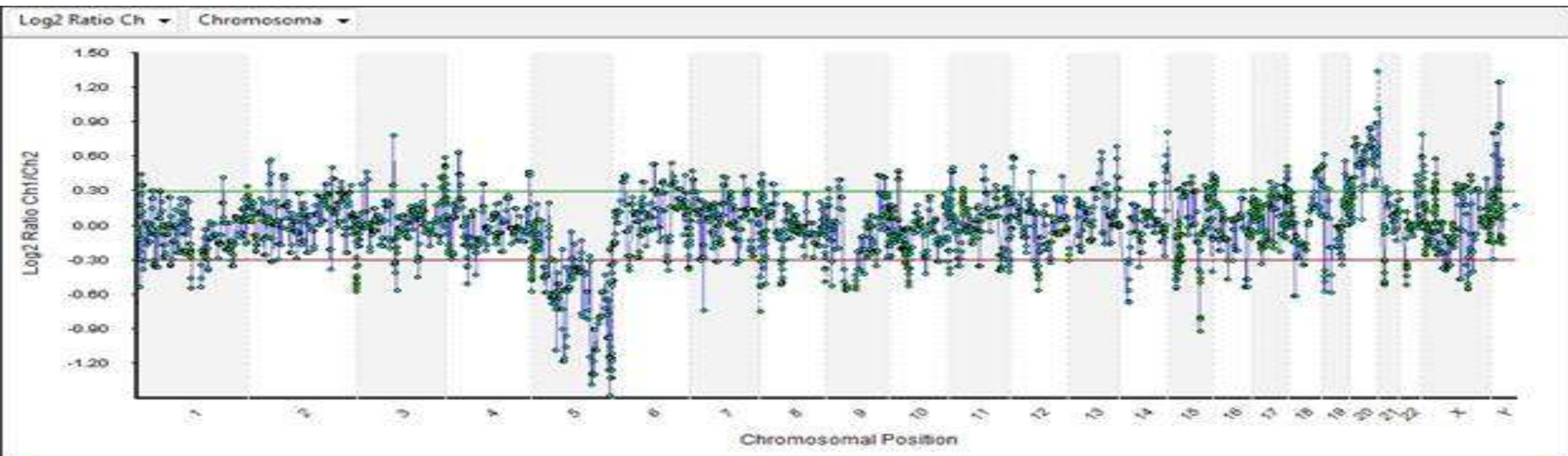
Single Blastomeres (Genesis Genetics Institute)



Single Blastomeres (Genesis Genetics Institute)



Single Male Sperm t(5;20) Translocation (Blue Gnome)

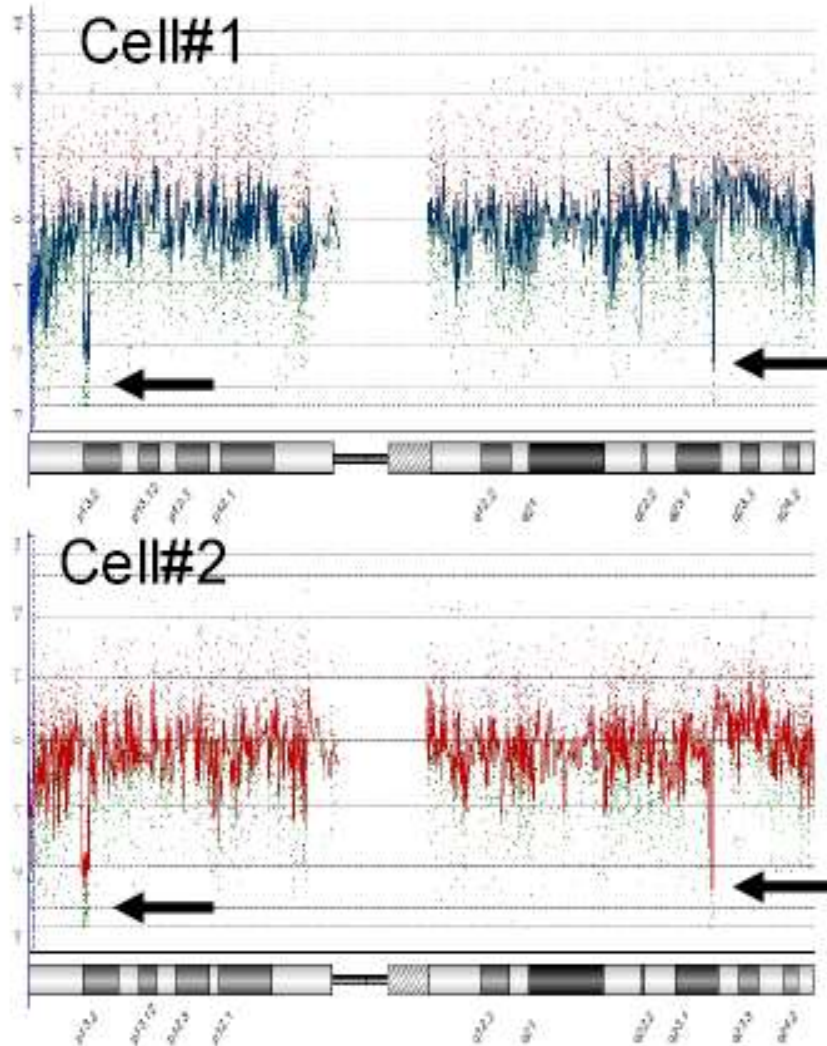


UKS0000548 sperm workup - 156068P - 16-AlkalineLysis - Cytochip Bottom - Fused Chart - 13/05/2010

Detection of Deletions and Aneuploidy in Cancer Cells Amplified with PicoPlex and Analyzed with Agilent Oligonucleotide Arrays

(Alexei Protopopov, Dana-Farber Cancer Institute,
Cambridge, USA)

Agilent aCGH chr16 Deletion Detection in Single Cells Using PicoPlex



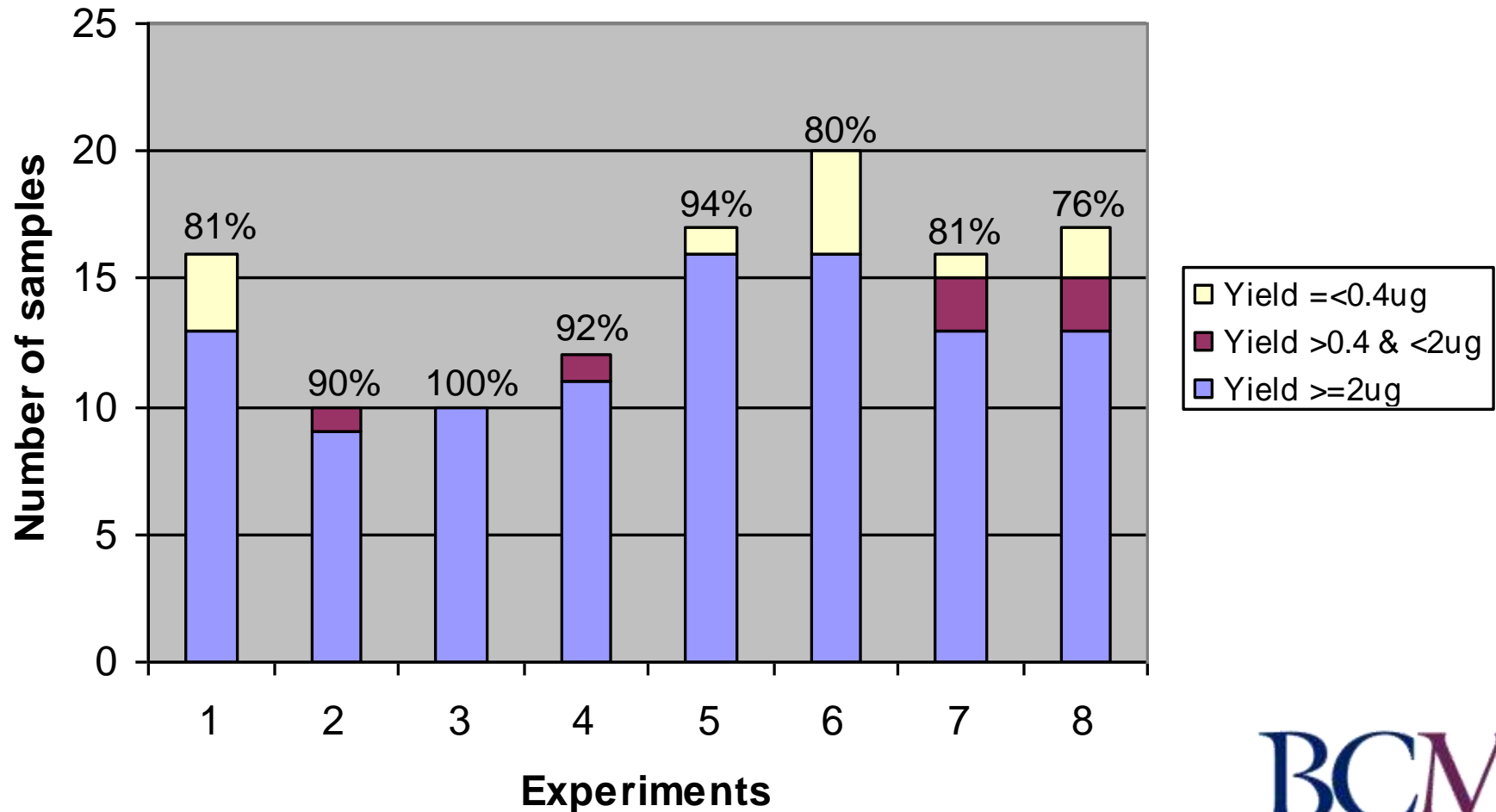
PicoPlex results from two single cells, each clearly showing the 350 kb and 75 kb deletions in chr 16. An average of 70% of the probes detected the deletions. Data provided by Dr. Alexei Protopopov, Dana-Farber Cancer Institute.

Agilent aCGH Analysis of Single Cells Amplified Using PicoPlex WGA

(Dr. Weimin Bi, Dr. Arthur Beaudet, Baylor School of
Medicine, Houston, TX)

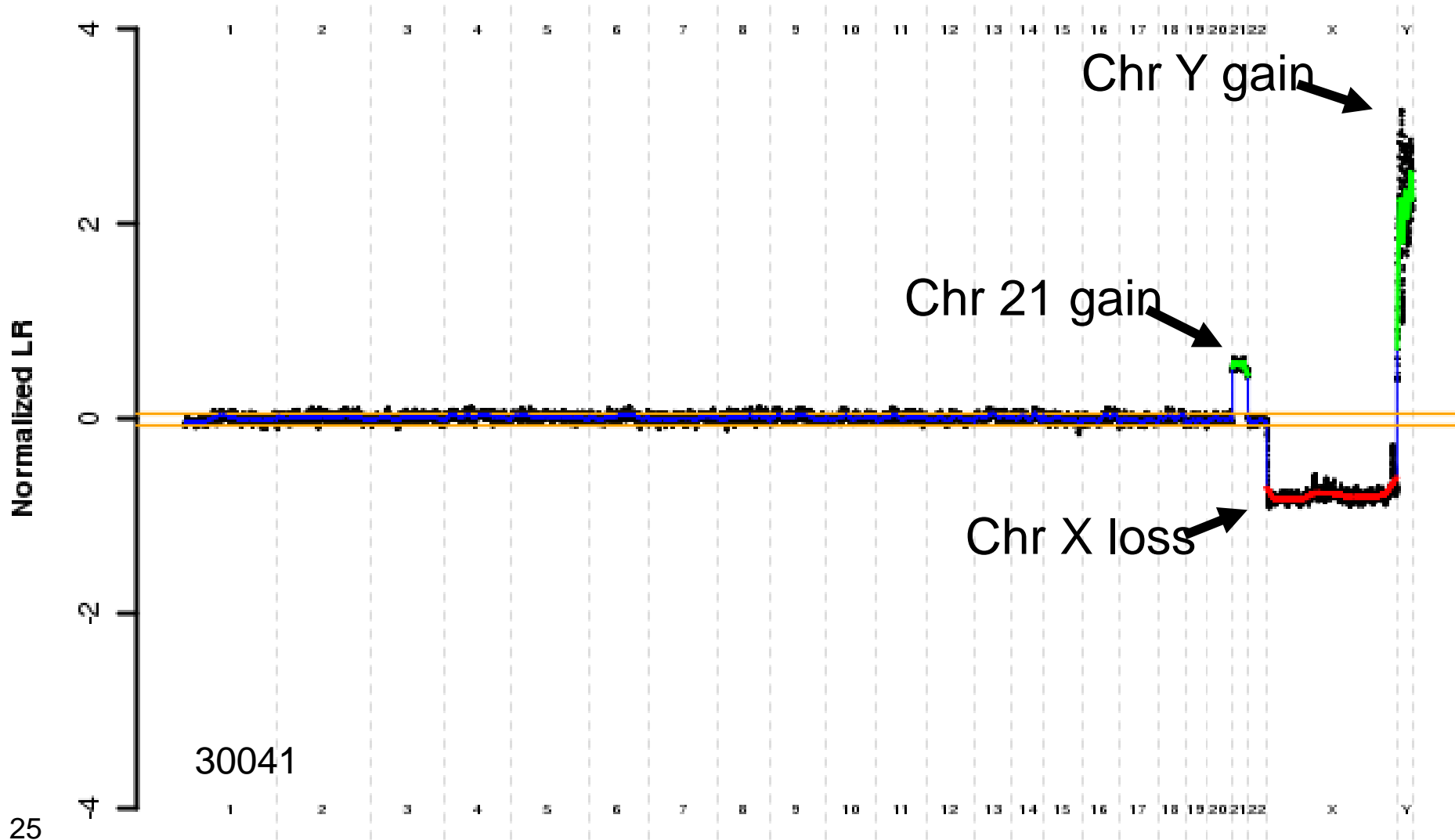
Amplification Success Rates by Experiment

Amplification Success Rates Based on Experiment

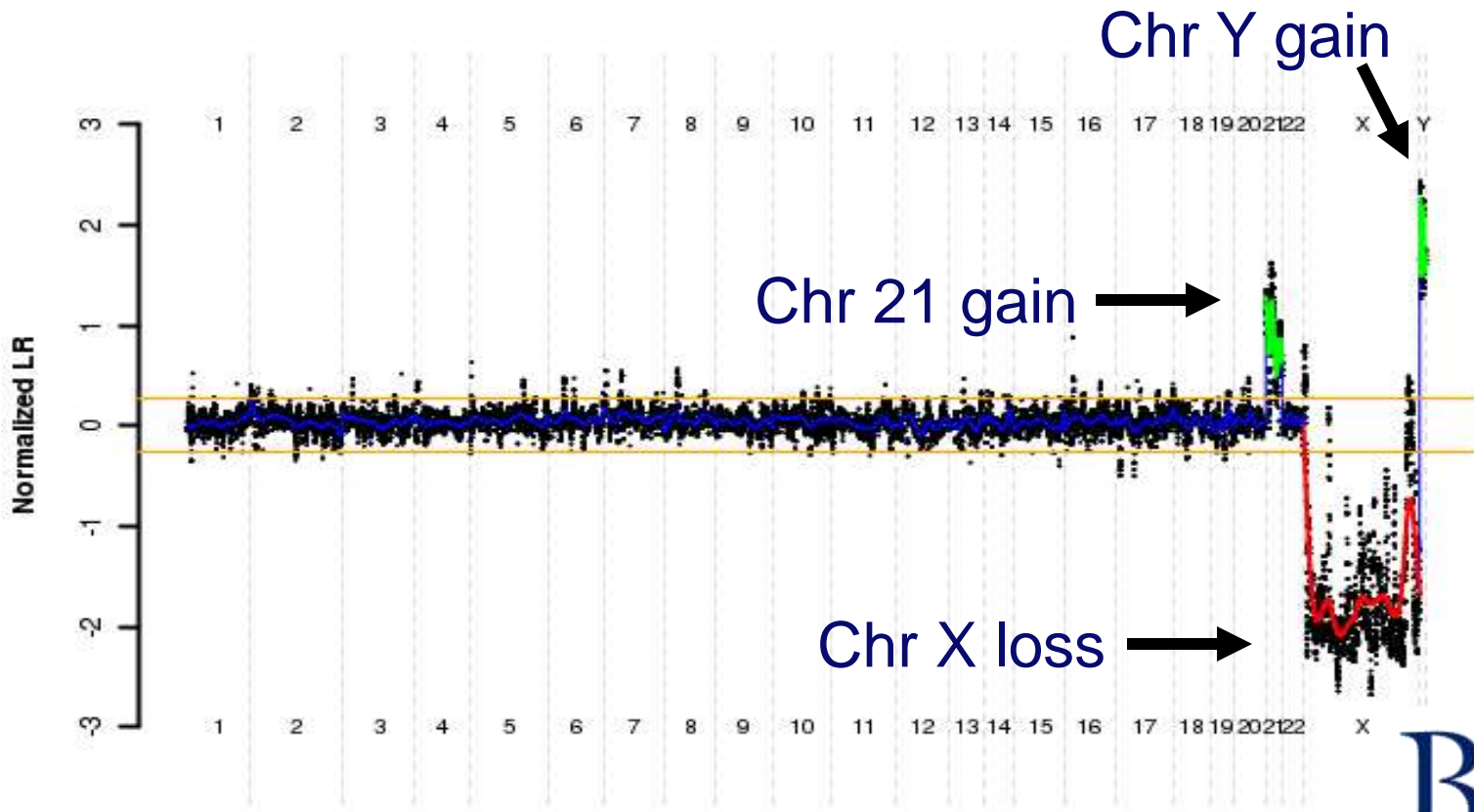


- Note:
1. The percentage is the success rate.
 2. Yield <= 0.4ug is similar to negative control and it could be due to no cells ...

Trisomy 21 No Amplification vs Normal Female DNA No Amplification



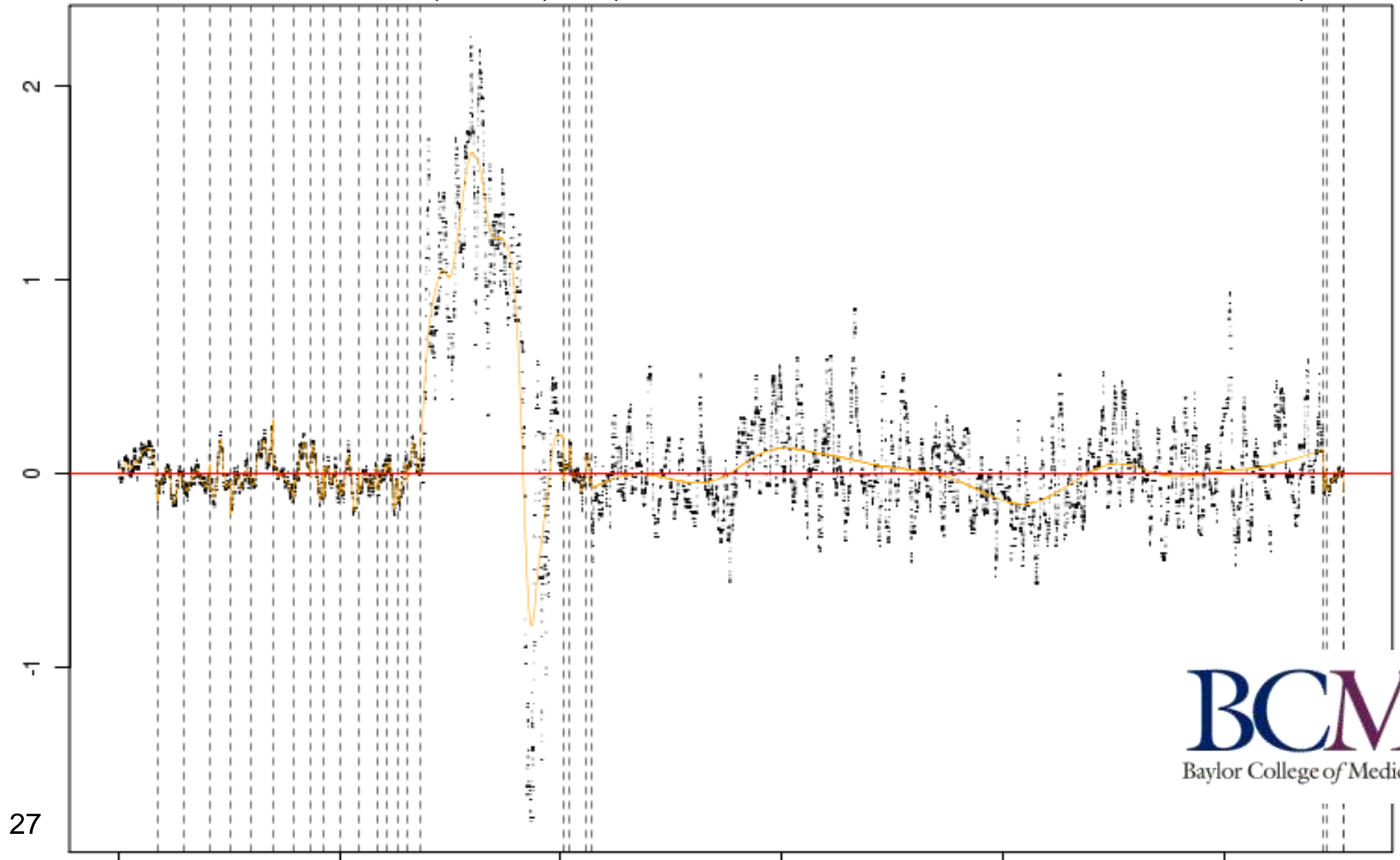
Trisomy 21 Single Cell (Male) vs. Normal Female DNA 5 ng with PicoPlex



Single-Cell Detection of 1.5 MB Gain in CMT1A Region Using PicoPlex

Chr 17

Chr 21



Genotyping and Mutation Detection Using PicoPlex WGA

Dr. Dagan Wells, University of Oxford;

Dr. Mark Hughes, Genesis Genetics Institute

Single-Cell Genotyping Using PicoPlex

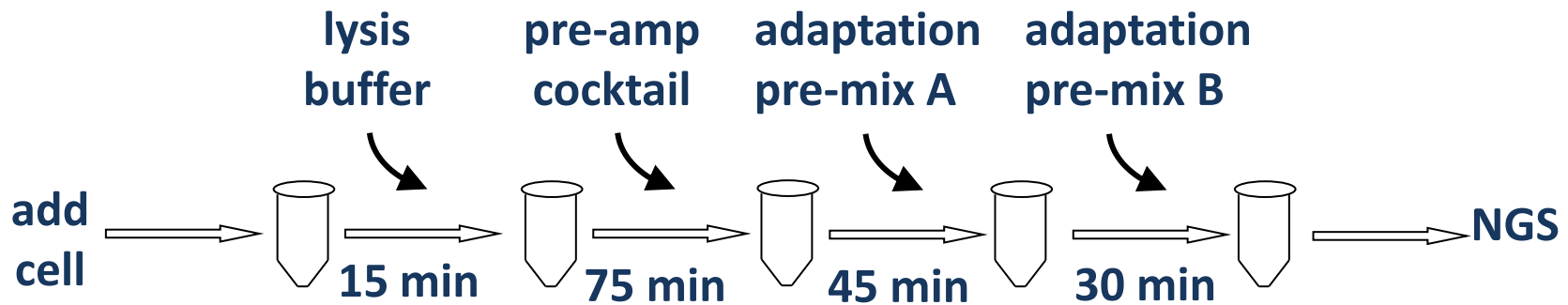
(Dagan Wells, Nuffield Department of Obstetrics and Gynaecology, University of Oxford, UK)

SNP GENOTYPING METHOD	SINGLE-CELL AMPLIFICATION SUCCESS RATE	SNP CALL RATES	LOSS OF HETEROZYGOSITY
PCR	95% (90% with locus-specific PCR alone)	>95%	<10%
Illumina SNP array	95%	50% - 60%	7% - 12%

"Using PicoPlex we can combine highly accurate chromosome screening, using array-CGH or SNP microarrays, with diagnosis of single gene disorders, via PCR amplification plus mutation detection or again using SNP array. This distinguishes the approach from previous methods..."

PicoPlex-NGS WGA

(for Illumina, Roche, and Life Tech NGS)



PicoPlex-NGS WGA Evaluation

FACS-sorted PC3 Cells:

Drs. Ken Pienta, Lalit Patel, Univ. Michigan

Illumina Genome Analyzer Sequencing:

Dr. Heather Koshinsky, Eureka Genomics

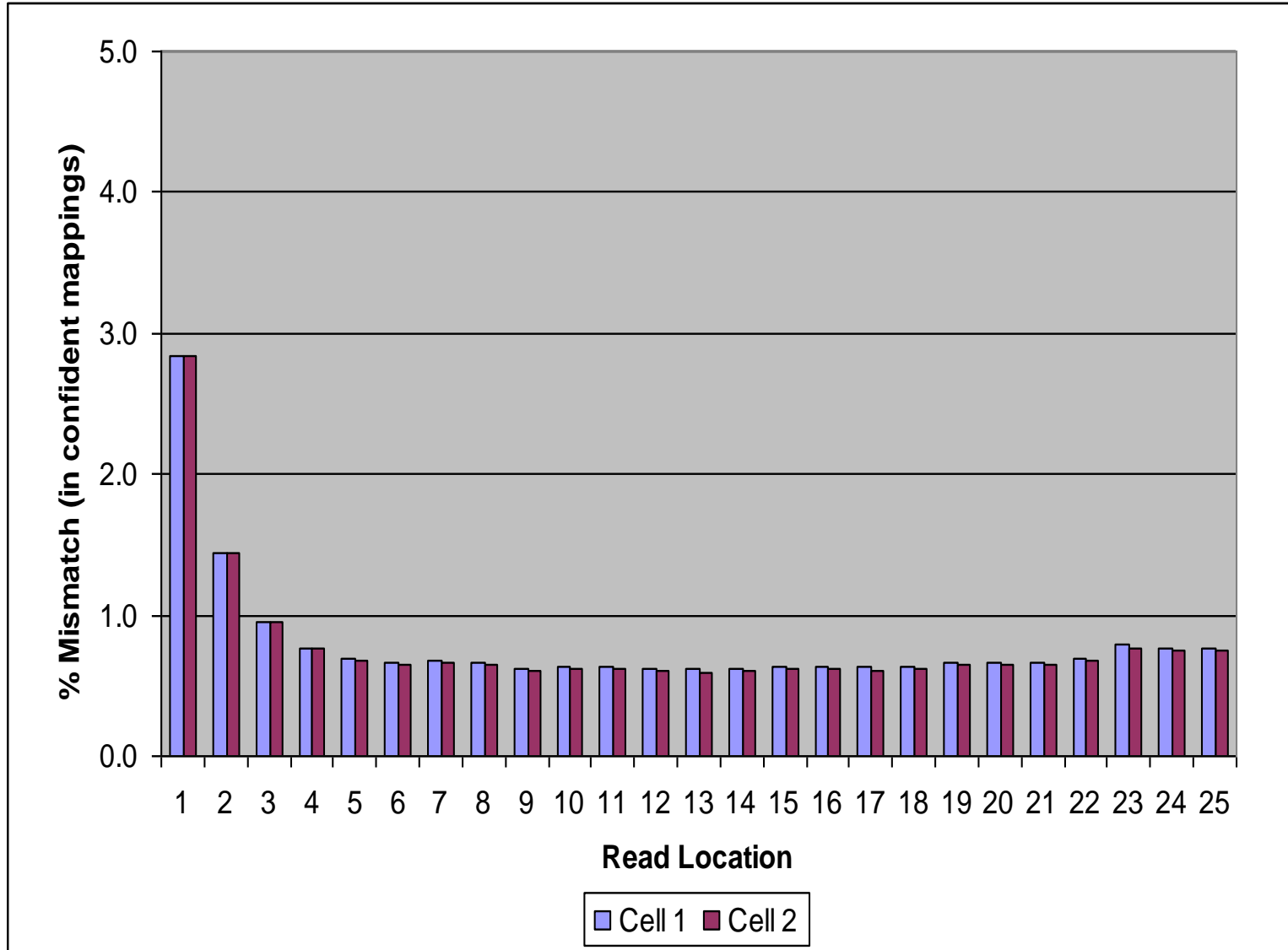
Informatics:

Dr. Andreas Sundquist, DNAnexus

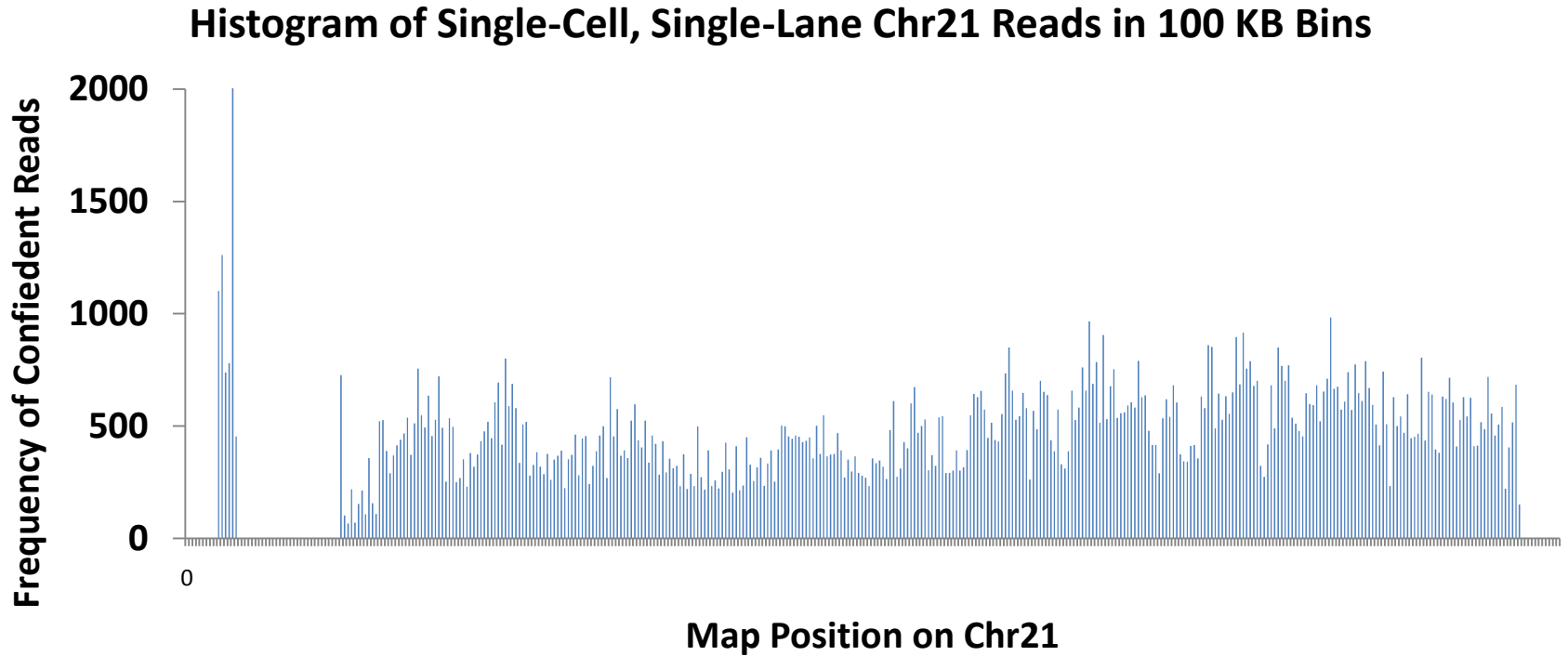
Single-Cell, Single Lane Statistics From Replicate Cells

	Cell #1	Cell #2
total number of reads	19,170,105	19,720,368
read length	25	25
CG content of mapped reads	41.67%	41.65%
number of reads mapped	17,534,059 (91.47%)	18,056,419 (91.56%)
number of reads mapped confidently	12,618,061 (65.82%)	12,962,748 (65.73%)
number of reads mapped repetitively	4,915,998 (25.64%)	5,093,671 (25.83%)
number of reads discarded or without mapping	1,636,046 (8.53%)	1,663,949 (8.44%)
number of reads of low quality	128,216 (0.67%)	122,346 (0.62%)
bottlenecking	none	none
bottleneck score	13.79	13.12

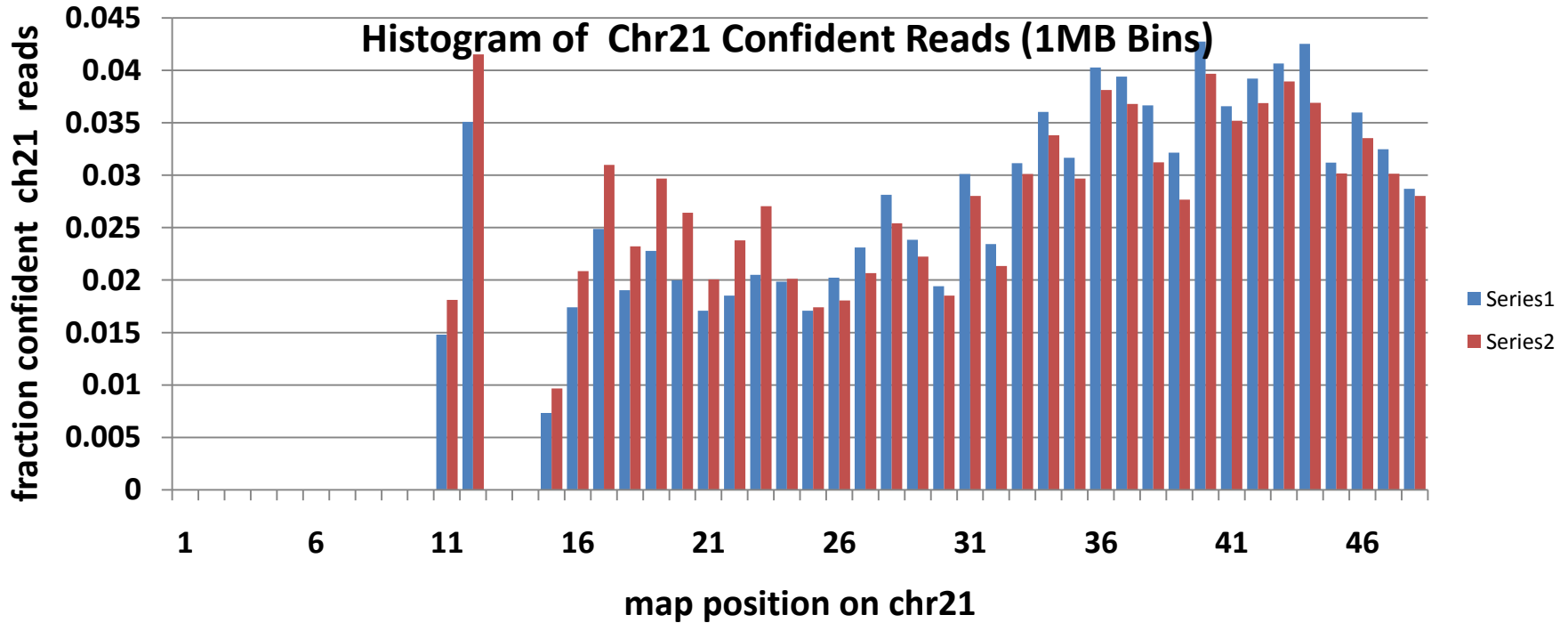
Mismatches of Mapped Reads (cell 1 & cell 2 vs. human reference sequence)



Uniformity of Single-Lane Coverage



Reproducibility of Single-Lane Coverage (cell #1 vs. cell #2)

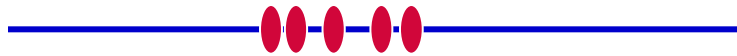


Status of PicoPlex-NGS

- PicoPlex-NGS has been designed for and tested on the Illumina Genome Analyzer using flow-sorted cancer cells
- PicoPlex-NGS sequencing reads are of sufficient number and quality to perform aneuploidy and CNV analyses
- PicoPlex-NGS is currently being tested at five sites to sequence single human cells, and single-copy sorted chromosomes
- PicoPlex-NGS beta kits for the Illumina GA will be released in November

PicoPlex WMA Is "Methylation-Specific WGA"

methyated CpG sites



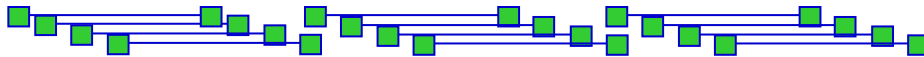
non-methylated CpG sites



MSRE digestion



amplified methylPlex library



no gap

amplified methylPlex library

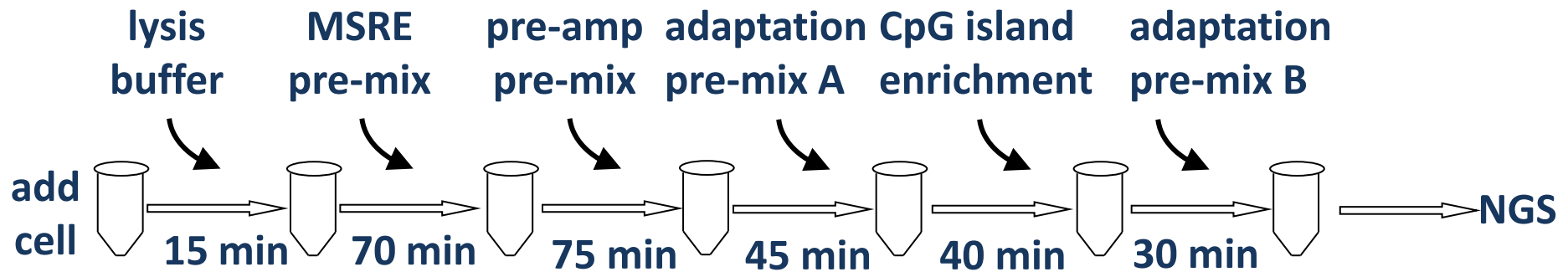


gap



copy number assays using qPCR, microarrays or NGS

PicoPlex-NGS WMA (Single-Cell Methylation NGS Analysis)



- **PicoPlex-NGS WMA beta kits for the Illumina GA will be released in January 2011**

Contributors

- BlueGnome
- Genesis Genetics Inst.
- Eureka Genetics
- DNAnexus
- Univ. Michigan
 - Arul Chinnaiyan
 - John Prensner
 - Ken Pienta
 - Lalit Patel
- Baylor Medical School
 - Weimin Bi
 - Aurthur Beaudet
 - Chad Shaw
 - Pawel Stankiewicz
- University of Oxford
 - Dagan Wells
- Dana-Farber Cancer Inst.
 - Alexei Protopopov
 - Lynda Chin