



Analyze single cell genomes in under 24 hours with Agilent SurePrint G3 8x60K CGH microarrays

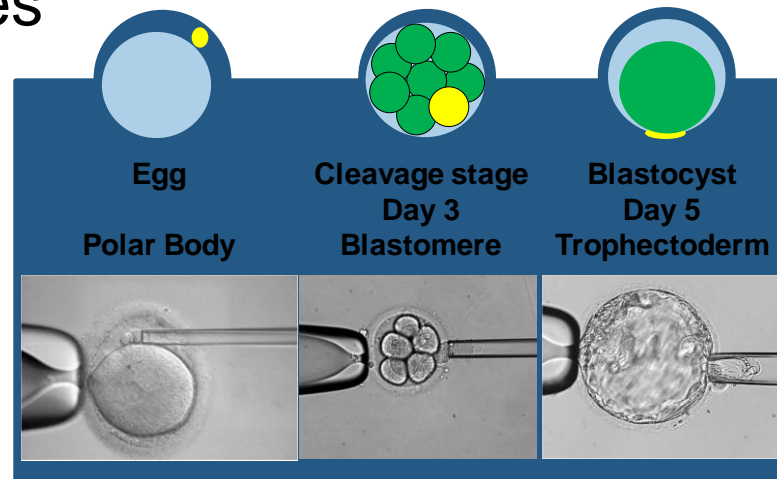
Anniek De Witte
Product Manager, Clinical software (NGS & CGH)

Jan 15, 2013



Single cell analysis is important for specific sample studies

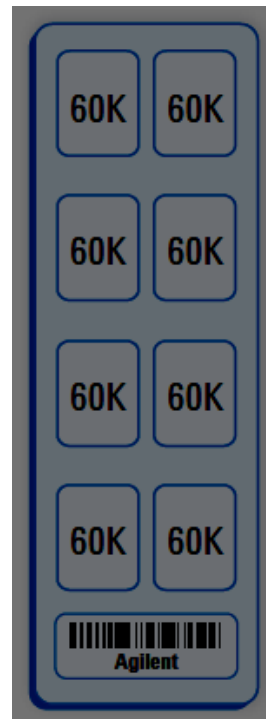
1. Preimplantation samples: Comprehensive chromosome analysis including aneuploidy analysis of all 24 chromosomes



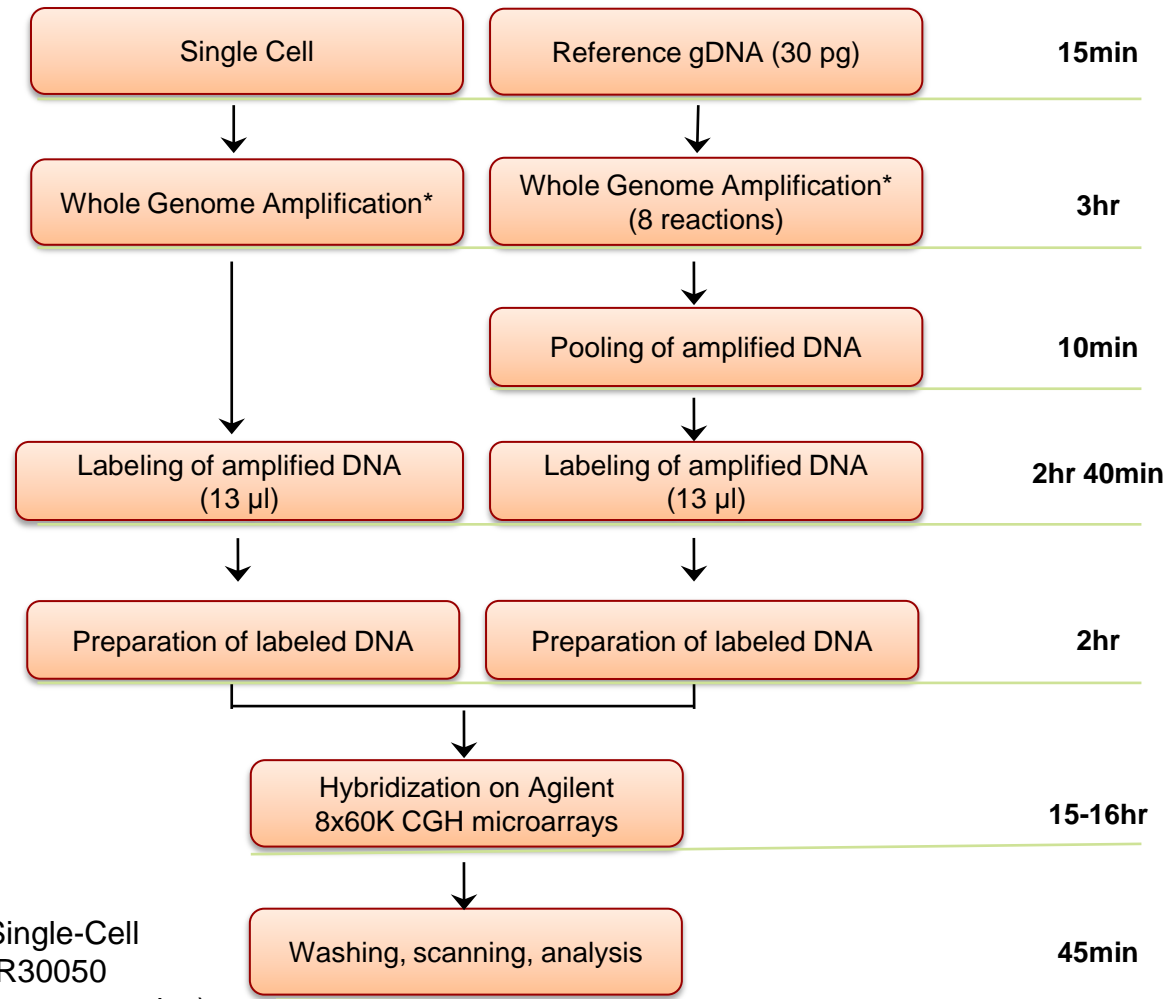
2. Cancer samples: Tumor heterogeneity, micrometastases, minimal residual disease
3. Noninvasive prenatal samples

Single-cell array comparative genomic hybridization on Agilent 8x60K CGH microarray

- Optimized for time sensitive applications
- More cost effective and higher performance than BAC arrays and FISH



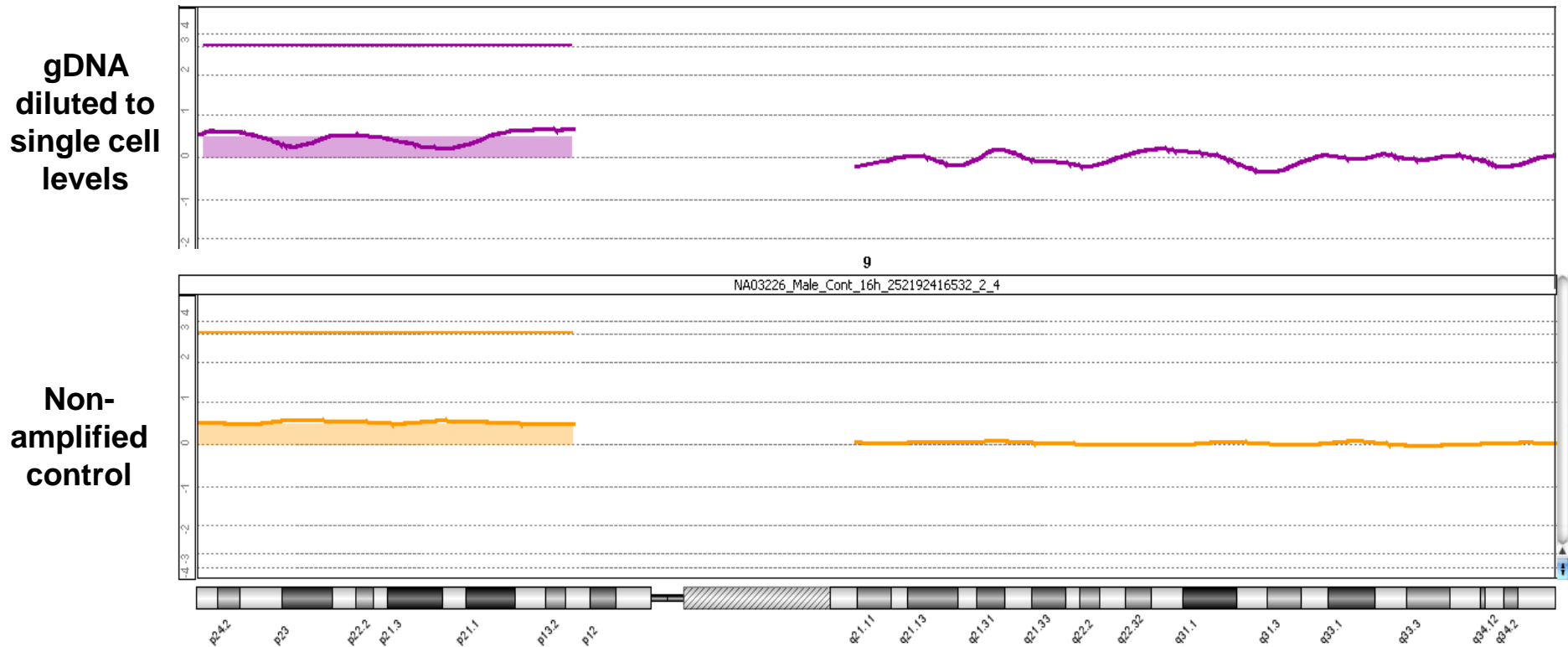
Protocol: Optimized single cell workflow takes less than 24 hours (manual P/N G4410-90012)



*PicoPlex WGA Kit for Single-Cell
Rubicon Genomics p/n R30050
(distributed by NEB in some countries)

Expected copy change detected on Agilent 8x60K CGH microarrays in less than 24 hours

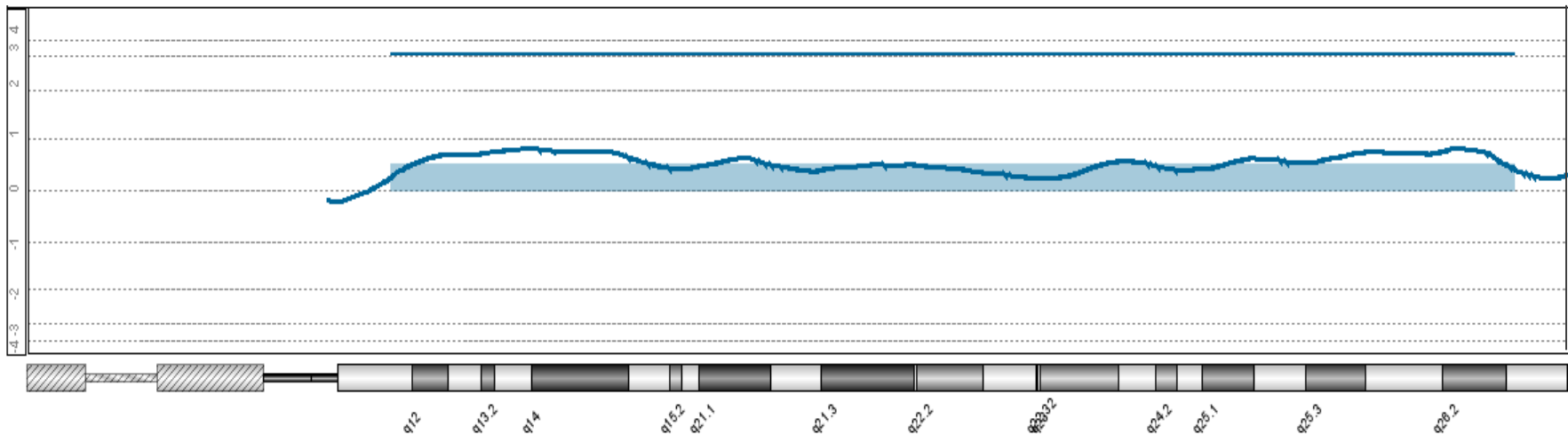
Chr. 9



Expected copy number (CN) change identified in amplified DNA (NA03226) with a known aberration on chromosome 9, co-hybridized with a pooled reference amplified from 30 pg of gDNA. The same aberration could be detected in the non-amplified control hybridization.

Single cell hybridized on Agilent 8x60K CGH microarrays: Entire Chr. 15 amplification

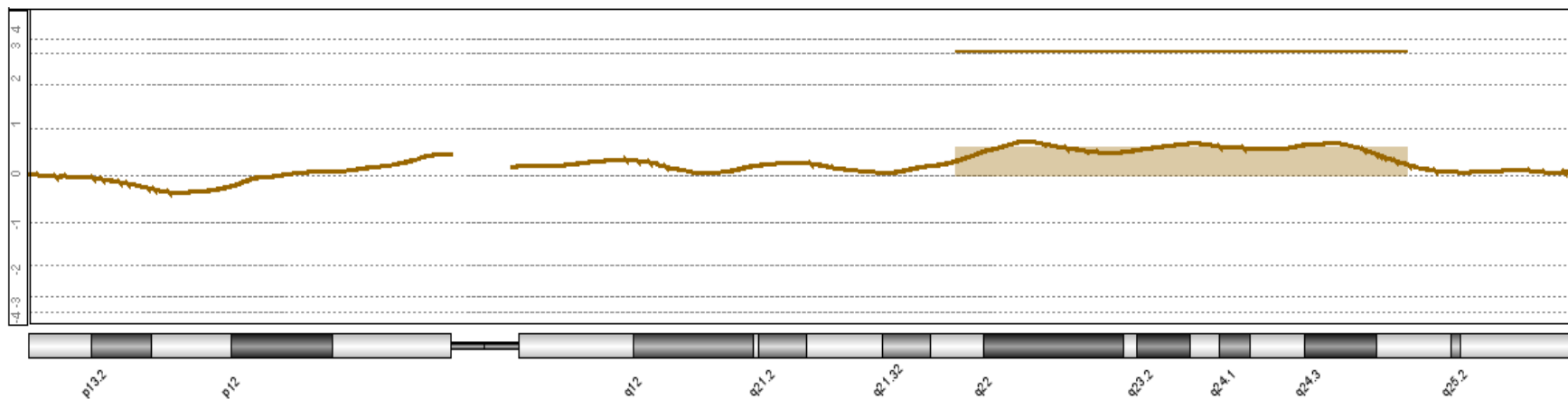
Chr. 15



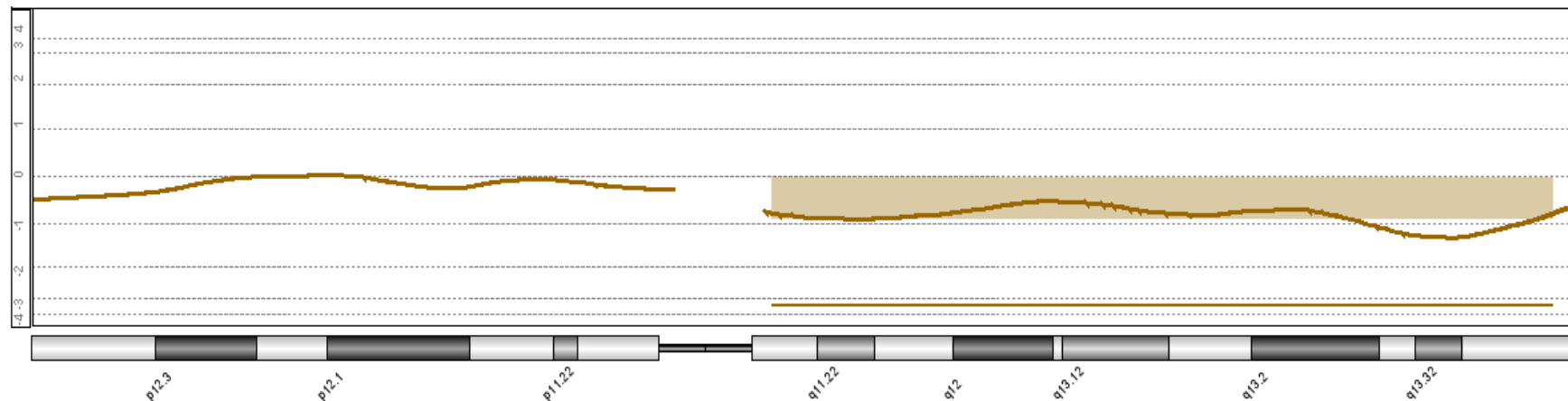
Recommended single cell analysis settings: Log ratio moving average: data points smoothed to 5 Mb windows, Aberration filter: ADM2, threshold 6, minimum of 5 Mb aberrations, ≥ 0.3 log ratio for amplifications and ≥ 0.55 log ratio for deletions

Single cell hybridized on Agilent 8x60K CGH microarrays: Partial chromosome aberrations

Chr. 17

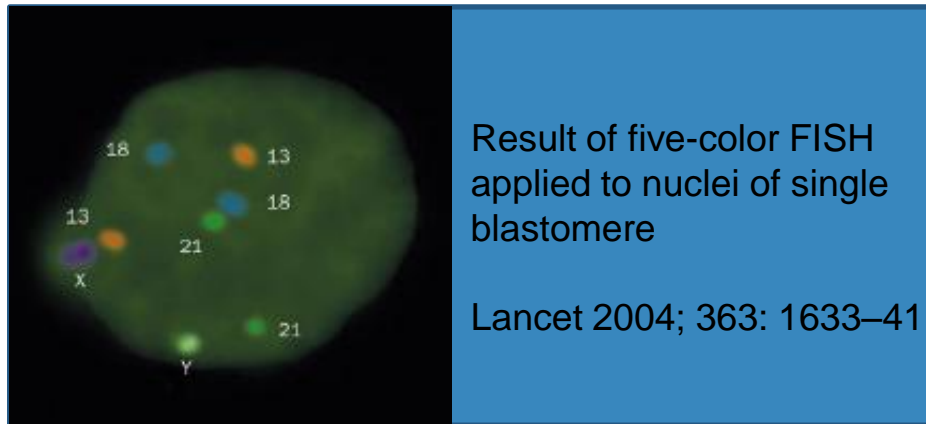


Chr. 20

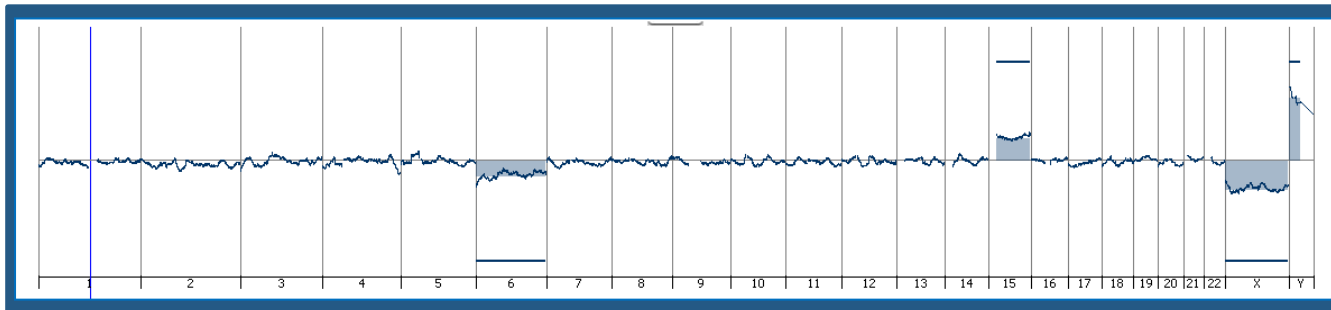


Agilent 8x60K CGH microarrays vs. FISH on single cells

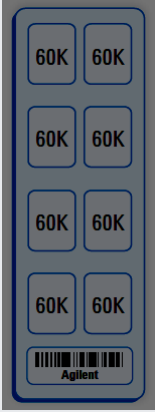
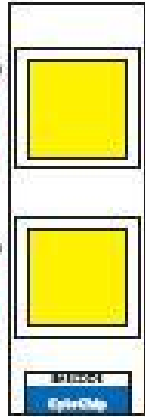
FISH: The number of chromosomes that can be analyzed simultaneously is **limited** by the number of fluorochromes available



Oligo aCGH: The **entire genome** can be interrogated at once

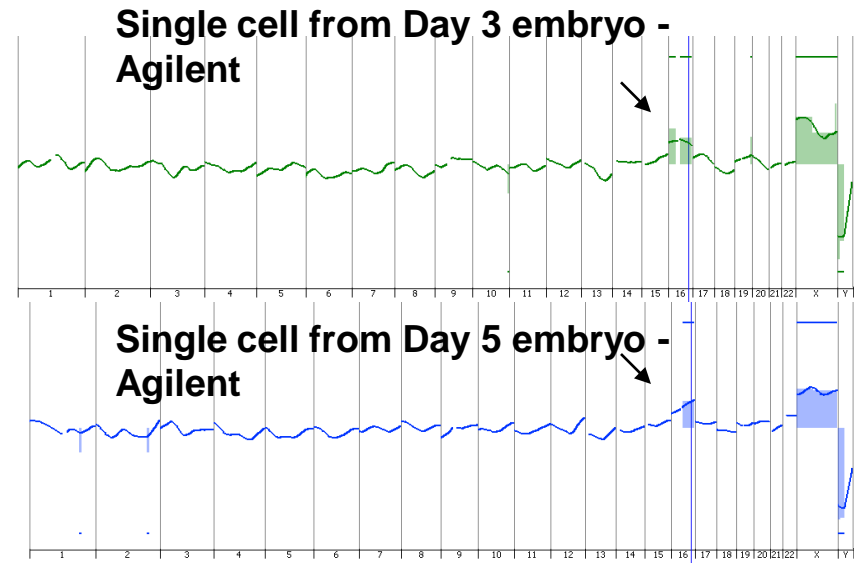
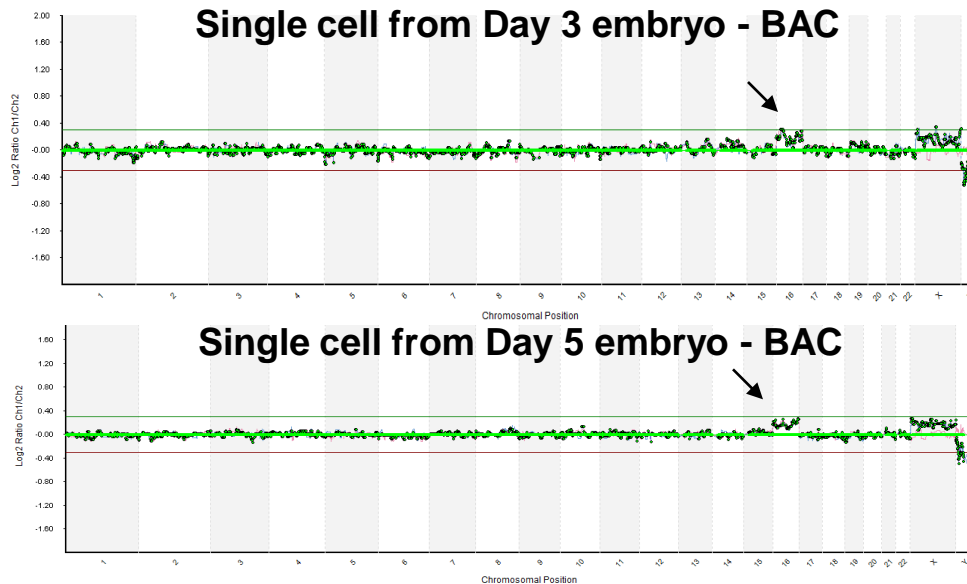


Agilent 8x60K CGH microarrays vs. BAC

Description	Agilent 8x60K CGH microarrays	BAC arrays
Number of probes – higher resolution	60,000	3,000
Number of samples per slide – cost effective	8 	2 
Type of probe – robust	<i>In-situ</i> synthesized 60-mer probes	Spotted BAC probes
Customization possible	Yes (through eArray)	No

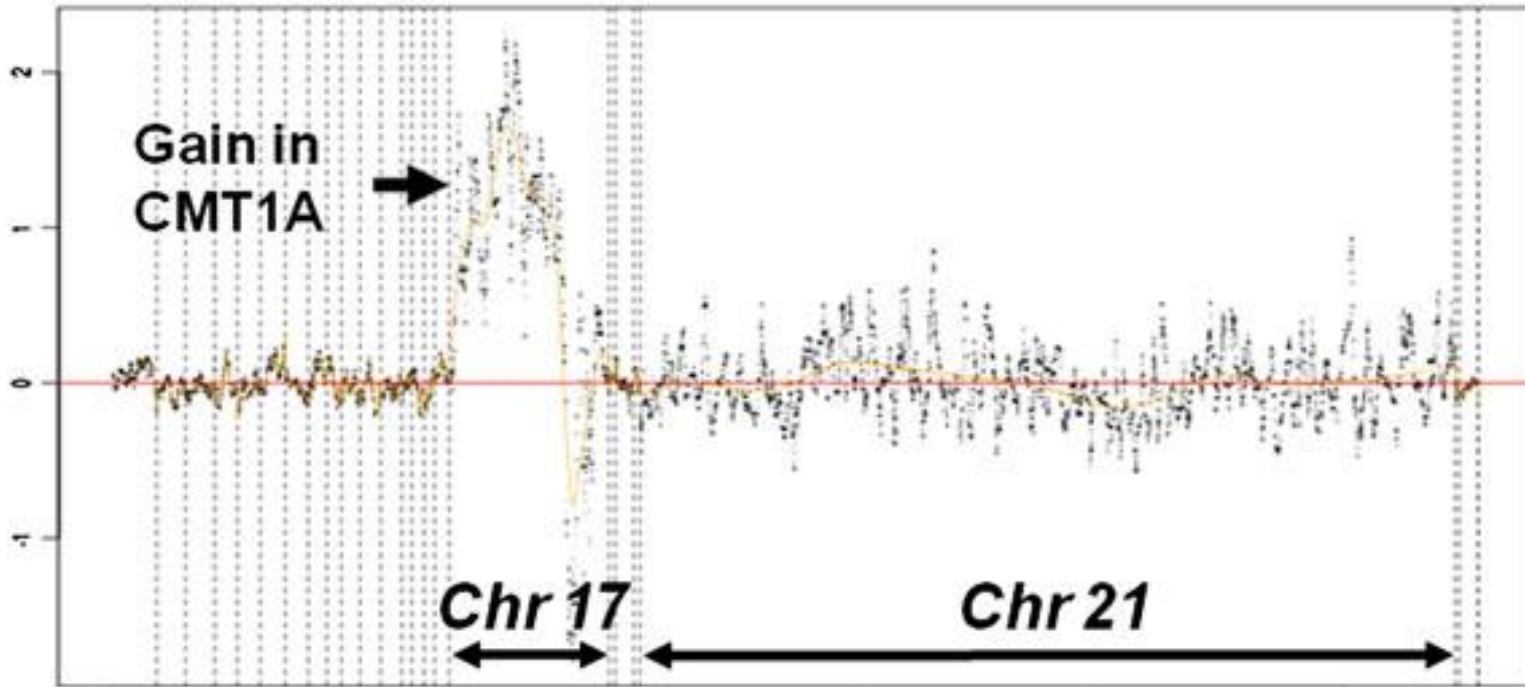
Data comparison Agilent CGH arrays vs. BAC (data generated by Dr. Richard Choy CUHK)

Aneuploid embryo (47,XX,+16)



Consistency of the results is demonstrated across the platforms, as well as among the cells derived from the same embryo.

Example of detection of smaller aberration compared to BAC arrays: 1.3Mb dup in CMT1A




Array CGH of a single cell with a 1.3Mb CMT1A duplication on the customized NIP2 array that has 20,000 probes in this region. The detection of the 1.3Mb copy number gain in a single CMT1A cell was clearly visualized after customized data analysis. The X-axis represents alignment of chromosomes 1 to 22; X and Y are not shown on this rendering. The Y-axis shows the normalized log ratios of the hybridization signals. Note that chromosome 21 takes up a disproportionately large area on the graph, because the array has ~100 K probes covering this chromosome. Data generated by Bi *et al.* Baylor College of Medicine.

Conclusions

- Genome-wide copy-number changes of **single cells** can be successfully profiled **within 24 hours** with Agilent oligo-based SurePrint G3 8x60K CGH microarrays
- The high-resolution genome-wide profiling allows for the cost-effective analysis of single cell whole genomes, overcoming the limitations associated with FISH and BAC arrays

Additional Information



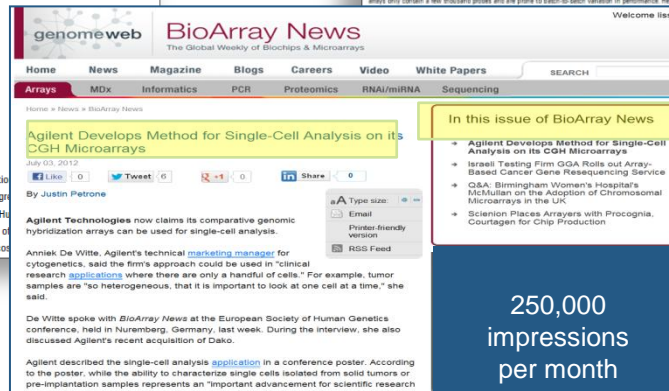
High-Resolution Oligonucleotide-Based aCGH Analysis of Single Cells in Under 24 Hours

Application Note

Authors
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Abstract
As some cells have atypical genomic representation individual genomes on microarrays represents a great and reproductive research. Agilent SurePrint G3 Hi microarrays provide great power in the detection of high resolution and sensitivity, while remaining cost-effective.

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Agilent Develops Method for Single-Cell Analysis on its aCGH Microarrays

July 03, 2012

By Justin Petrone

Agilent Technologies now claims its comparative genomic hybridization arrays can be used for single-cell analysis.

Annie De Witte, Agilent's technical marketing manager for cytogenetics, said the firm's approach could be used in "clinical research applications where there are only a handful of cells." For example, tumor samples are "so heterogeneous, that it is important to look at one cell at a time," she said.

De Witte spoke with *BioArray News* at the European Society of Human Genetics conference, held in Nuremberg, Germany, last week. During the interview, she also discussed Agilent's recent acquisition of Dako.

Agilent described the single-cell analysis application in a conference poster. According to the poster, while the ability to characterize single cells isolated from solid tumors or pre-implantation samples represents an "important advancement for scientific research

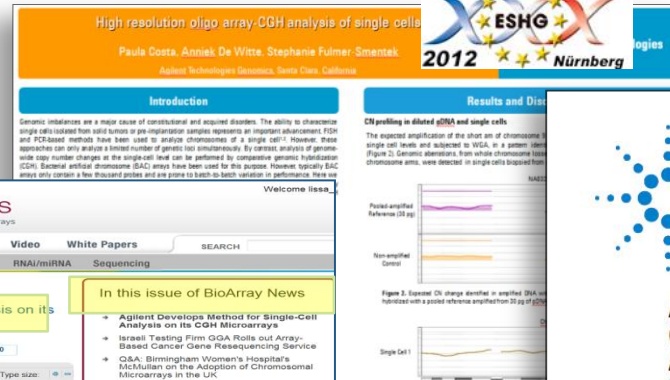
In this issue of BioArray News

- Agilent Develops Method for Single-Cell Analysis on its aCGH Microarrays
- Israeli Testing Firm GGA Rolls out Array-Based Cancer Gene Resequencing Service
- CSA: Birmingham Women's Hospital's McMullan on the Adoption of Chromosomal Microarrays in the UK
- Sciencion Places Arrays with Prologica, Courtagen for Chip Production

250,000 impressions per month

GenomeWeb article

Poster



High resolution oligo array-CGH analysis of single cells

Paula Costa, Annie De Witte, Stephanie Fulmer-Samentek
Agilent Technologies Genomics, Santa Clara, California


ESHG 2012 Nürnberg

Introduction
Genomic imbalances are a major cause of constitutional and acquired disorders. The ability to characterize single cells isolated from solid tumors or pre-implantation samples represents an important advancement. FISH and PCR-based methods have been used to analyze chromosomes of a single cell. However, these approaches can only analyze a limited number of genes for simultaneous results. For contrast, analysis of genome-wide copy number changes at the single cell level can be performed by comparative genomic hybridization (CGH). Commercial artificial chromosome (aC) arrays have been used for this purpose. However, typically aC arrays only contain a few thousand probes and are prone to batch-to-batch variation in performance. Here we

Results and Discussion
CGH profiling in diluted aCGH and single cells
The expected amplification of the short arm of chromosome 9 in single cell levels and subjected to WGA, in a patient (data not shown). Genomic aberrations from whole chromosome loss chromosome arms, were detected in single cells (data not shown).

Figure 2: Genomic aberrations from whole chromosome loss chromosome arms, were detected in single cells (data not shown).

Figure 3: Expected CGH change identified in amplified DNA cell hybridized with a pooled reference amplified from 20 pg of aCGH.



Agilent Oligonucleotide Array-Based CGH for Single Cell Analysis

Enzymatic Labeling

Protocol

Revision A0, June 2012

P/N G4410-90012

Peer Reviewed Papers

- Bi *et al.* Prenatal Diagnosis 2012;32:10–20.
- Cheng *et al.* Genome Biology 2011;12:R80.
- Hellani *et al.* Reproductive BioMedicine 2008;17(6):841-847.
- Traversa *et al.* Reproductive Biology 2011;11(3):51

Example data available on request

Live demo of analysis of single cell data in Agilent CytoGenomics 2.5 software



Questions

