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

Application Note

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Abstract

As some cells have atypical genomic representation, the ability to analyze individual genomes on microarrays represents a great advancement in cancer and reproductive research. Agilent SurePrint G3 Human Catalog 8x60K CGH microarrays provide great power in the detection of genomic imbalances with high resolution and sensitivity, while remaining cost-effective. We empirically optimized the conditions for performing oligonucleotide-based array comparative genomic hybridization (aCGH) on single cells with 8x60K microarrays. Different experimental conditions, including reference sample and length of the hybridization time, were tested. Whole genome amplification (WGA) was performed on genomic DNA (gDNA) with a known aberration that was diluted to single cell levels and on single cells isolated from embryos. Using the optimized 24-hour workflow, genomic aberrations were accurately identified in a sample with a known copy number (CN) gain on chromosome 9 and in individual genomes amplified from single cells.

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Introduction

Since its development, aCGH has revolutionized medical research. The high resolution and sensitivity of this technique has been successfully applied to the detailed investigation of genomic alterations in acquired and constitutional disorders. However, since the genomic background of individual cells may differ, it is important to be able to characterize individual genomes. The aberration assessment in single cells opens new horizons in cancer, stem cell and reproductive research, and forensic applications. While traditional FISH and PCR based techniques have been used for this purpose, their application has been narrowed by the limited number of loci that can be analyzed simultaneously.[1,2] Recently, BAC arrays have been used, but because they typically contain only a few thousand probes, their resolution is low. In addition, BAC arrays are prone to batch-to-batch performance variation. Several groups have successfully used Agilent custom or previous generation HD oligo-based aCGH microarrays for single cell analysis.[3,4,5,6] In this application note, we applied the power of the SurePrint G3 Human Catalog 8x60K CGH microarrays to single-cell analysis for the assessment of genome-wide CN changes in individual genomes.

Experimental

Sample

gDNA from the cell line NA03226, with a known aberration on the short arm of chromosome 9, was obtained from the Coriell Cell Repository.[7] For single cell simulation, 15 pg of gDNA was used to ensure equivalent genomic representation of a single cell. For CN assessment of true individual genomes, single cells were biopsied from embryos. A reference gDNA was prepared by diluting the Agilent Human Reference DNA Male to single cell levels. As controls, 500 ng of gDNA from the aberrant Coriell cell line and Agilent Human Reference DNA (male and female) were also used.

Whole genome amplification

WGA was performed using the PicoPlex Single Cell WGA Kit (Rubicon Genomics, p/n R30050). To minimize the variations introduced both with the extreme dilution of the reference DNA and inherent to the amplification process, multiple reference DNA reactions were combined post-amplification. Following WGA, all samples were subjected to electrophoresis and the absence of contamination and reaction efficiency were confirmed (Figure 1).
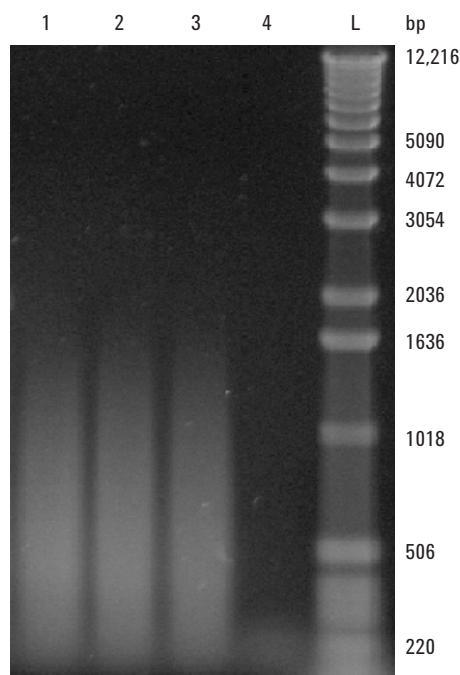

Figure 1. Electrophoresis profiles of amplified products from 30 pg of reference DNA (1), cell line NA03226 diluted to single cell levels (2), a single cell (3), non-template control (4), and a 1 Kb DNA ladder (L).

Microarray processing

For each of the experimental and reference samples, 13 µL of amplified DNA was differentially labeled with fluorescently-coupled nucleotides Cy5 and Cy3 using the Exo-Klenow fragments provided in the Agilent SureTag Complete DNA Labeling Kit (p/n 5190-4240). Experimental samples were combined with the appropriate references and hybridized to SurePrint G3 Human Catalog 8x60K CGH microarrays (Table 1), with uniform backbone coverage and denser gene coverage. Data were extracted from the scanned slides using the Feature Extraction program integrated within Agilent CytoGenomics software. CN changes were assayed from the log ratio of the experimental and reference samples using the aberration algorithm and default filters implemented in the analysis software. For CN visualization, the moving average was applied for each sample, smoothed to 5 Mb windows.

Results and Discussion

Protocol optimization

To determine the appropriate reference for single cell aCGH, the use of a reference from an individual amplification reaction was compared with a reference from a pool of multiple amplification reactions generated from either 15 pg or 30 pg of sample. Due to amplification variations introduced with sample dilution to single cell amounts, the highest level of probe-to-probe noise was observed for samples hybridized to individual references amplified from 15 pg of gDNA. An improvement in the noise and CN detection was achieved with references pooled from eight amplification reactions starting with either 15 pg or 30 pg of DNA (Table 2).

To test the effect of reduced hybridization times in the CN detection, a set of samples was incubated for 16 and 24 hours. Data generated at both hybridization times were comparable (data not shown).

The optimized protocol is included in the *Agilent Oligonucleotide Array-Based CGH for Single Cell Analysis — Enzymatic Labeling* manual (p/n G4410-90012).

Table 1. SurePrint G3 Human Catalog 8x60K CGH microarray specifications.

Microarray specifications	
Design ID	21924
Total features	62,976
Control grid feature count	3,886
Distinct biological features	55,077
Replicated probes (5x)	1,000
Additional negative controls	13
Unique probes	54,969 (99.8 %)
Homology filtered probes	149 (0.27 %)
Pseudoautosomal probes	108
Exonic probes	14,259 (25.9 %)
Intragenic probes	36,995 (67.2 %)
Intergenic probes	18,082 (32.8 %)
Median probe spacing	
Intragenic	33,307
Intergenic	78,946
CNV	26,688
Overall	41,448
Average probe spacing overall	54,455
RefSeq gene coverage	
At least 1 probe	15,553 (83.2 %)
≥ 3 probes	4,580 (24.5 %)
Cancer gene coverage	
At least 1 probe	351 (97.0 %)
≥ 3 probes	226 (62.4 %)

Table 2. Microarray performance of single cells hybridized to pooled references showed a decrease in the probe-to-probe noise, derivative log ratio spread (DLRS), as compared to individual references.

Single cell versus	DLRS	Signal intensity		Background noise		Signal-to-noise	
		Green	Red	Green	Red	Green	Red
Individual ref	1.66	224	413	7	9	32	44
Eight pooled ref 15 pg	0.78	318	366	7	10	43	37
Eight pooled ref 30 pg	0.78	324	373	6	8	53	48

CN profiling of diluted gDNA and single cells

The expected CN gain on chromosome 9p of amplified DNA from cell line NA03226 was detected by the assay (Figure 2A). For single cells, the sex chromosomes profile was determined from the log ratio of the sample and the male reference. In Figure 2B, the X chromosome profile displayed confirms that this particular cell is female.

Additional partial and whole chromosomal aberrations were detected across the genome of amplified single cells (Figure 3).
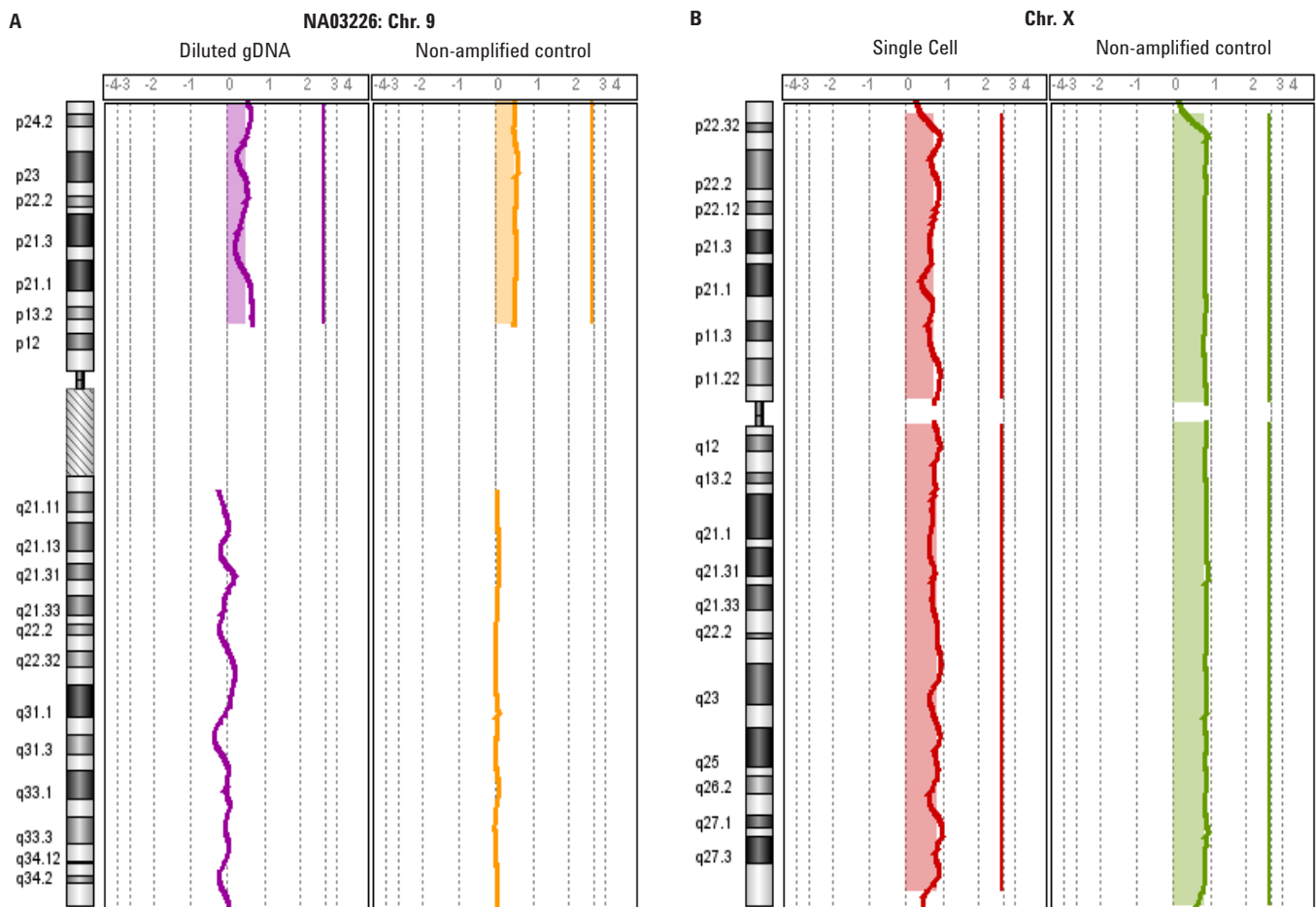

Figure 2. CN changes identified for samples hybridized to SurePrint G3 8x60K CGH microarrays for 16 hours.
(A) Detection of the expected CN gain on chromosome 9 of the amplified DNA from the cell line NA03226 (left) and in a non-amplified control gDNA (right).
(B) Sex determination from the X chromosome profile for a single cell (left) and in a non-amplified control female versus male hybridization (right). The extra copy of the X chromosome indicates that the single cell is female.
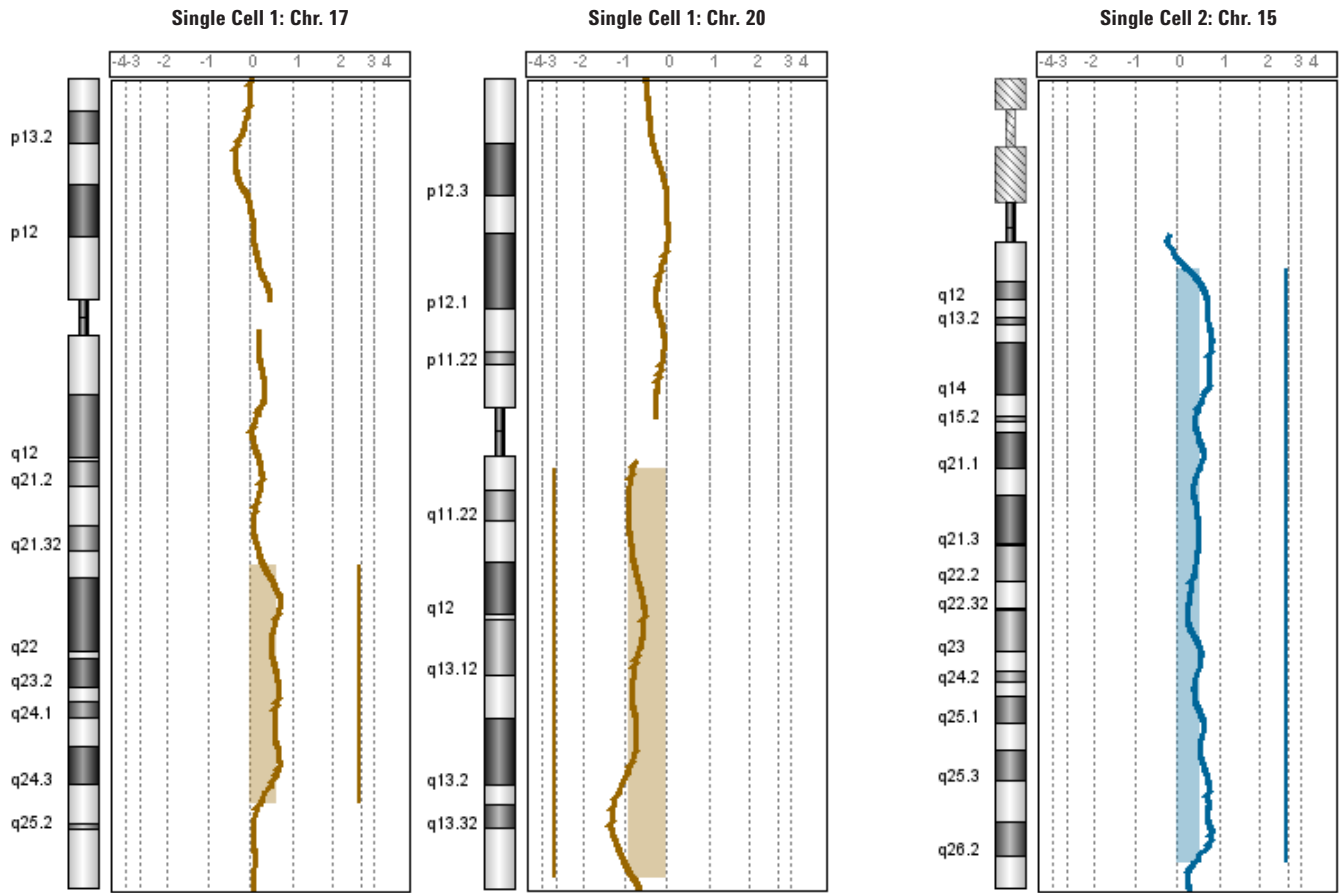
Figure 3. Partial and whole chromosome aberrations identified in single cells genomes.

Conclusions

Using a straightforward and simple process (Figure 4), genome-wide CN changes of single cells can be successfully profiled with oligo-based aCGH. Isolated single cells and reference DNA can be processed and analyzed in under 24 hours. The high-resolution, reproducibility, and shorter workflow attained with SurePrint G3 8x60K CGH microarrays allow for the cost-effective analysis of single cell whole genomes for research, overcoming the limitations associated with FISH, PCR-based methods, and BAC arrays.
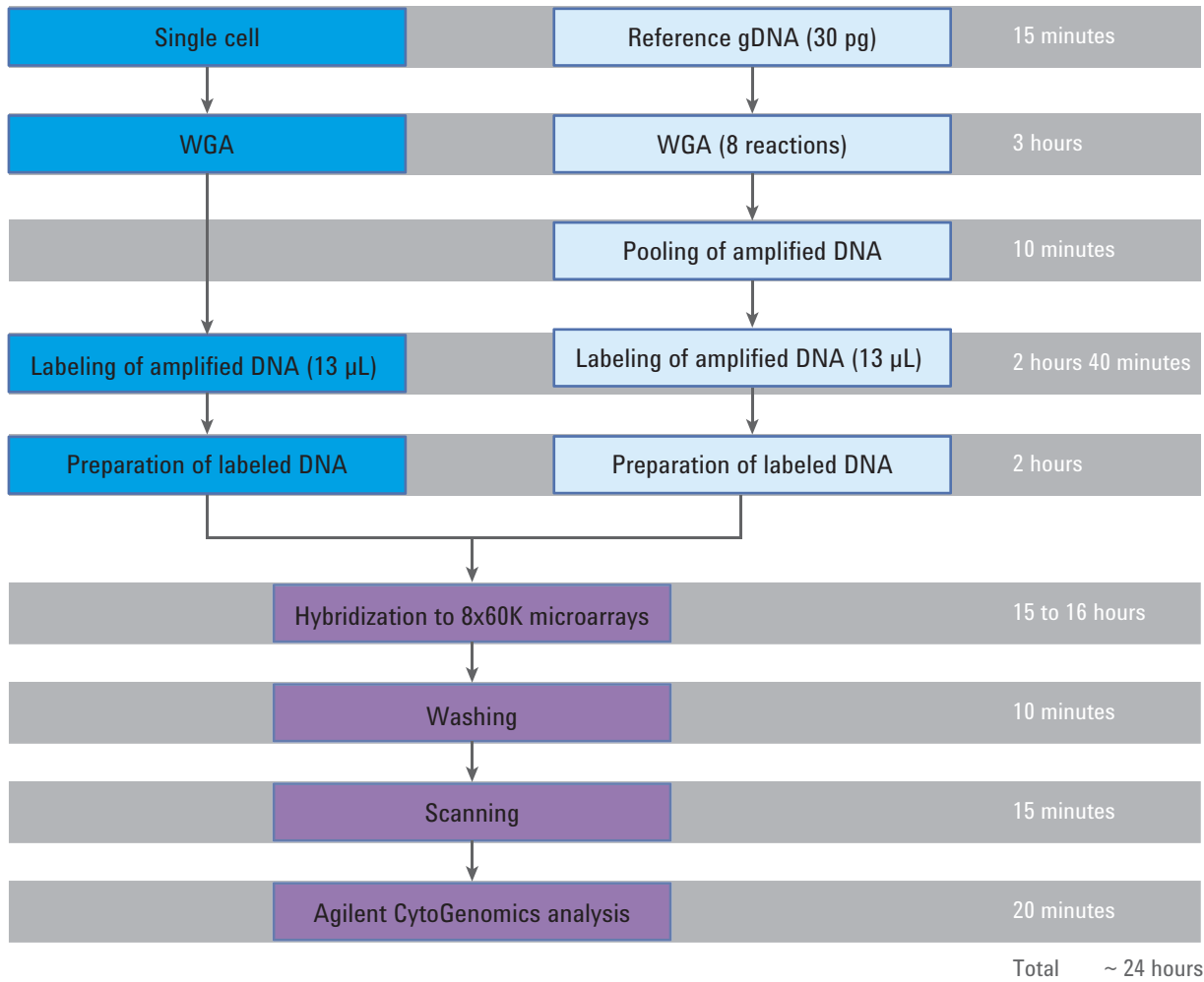
Single cell	Reference gDNA (30 pg)	15 minutes
WGA	WGA (8 reactions)	3 hours
	Pooling of amplified DNA	10 minutes
Labeling of amplified DNA (13 µL)	Labeling of amplified DNA (13 µL)	2 hours 40 minutes
Preparation of labeled DNA	Preparation of labeled DNA	2 hours
Hybridization to 8x60K microarrays		15 to 16 hours
Washing		10 minutes
Scanning		15 minutes
Agilent CytoGenomics analysis		20 minutes

Total   ~ 24 hours

Figure 4. Workflow for single cells analysis and the respective processing times estimated for 8 samples.

References

1. Munné *et al*. Improved detection of aneuploid blastocysts using a new 12-chromosome FISH test. *Reproductive Biomedicine Online*, **2010**, 20(1):92-97.

2. Barat-Houari *et al*. New multiplex PCR-based protocol allowing indirect diagnosis of FSHD on single cells: can PGD be offered despite high risk of recombination? *European Journal of Human Genetics*, **2010**, 18(5):533-538.

3. Bi *et al*. Detection of ≥1 Mb microdeletions and microduplications in a single cell using custom oligonucleotide arrays. *Prenatal Diagnosis*, **2012**, 32(1):10–20.

4. Cheng *et al.* Single-cell copy number variation detection. *Genome Biology*, **2011**, 12(8):R80.

5. Hellani *et al*. Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening. *Reproductive BioMedicine Online*, **2008**, 17(6):841-847.

6. Traversa *et al*. The genetic screening of preimplantation embryos by comparative genomic hybridization. *Reproductive Biology*, **2011**, 11(3):51-60.

7. http://ccr.coriell.org/

Appendix: Agilent CGH processing components

Description	Part number
SurePrint G3 Human CGH Bundle, 8x60K	G5923A, Option 1
Hybridization Chamber, stainless	G2534A
Hybridization Oven	G2545A
Hybridization Oven Rotator Rack	G2530-60029
SureScan Microarray Scanner Bundle	G4900DA
Agilent CytoGenomics Software	G1662AA

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