Optimized Workflow for Single Cell Copy Number Profiling Using High Resolution Oligo CGH Arrays

Proof-of-principle data generated with Agilent's GenetiSure Pre-Screen Amplification and Array CGH Solution

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Introduction

The ability to characterize individual genomes in single cells is very important in pre-implantation genetics research. Traditional FISH and PCR based techniques, and more recently BAC arrays, have been used to provide insights into a single cell's genome, with low resolution due to the limited number of loci that can be analyzed simultaneously. Here we describe GenetiSure Pre-Screen, a same day, cost-effective, analysis workflow (Figure 1) that combines whole genome amplification (WGA) with copy number (CN) profiling using high-resolution oligo CGH microarrays.

Sample Preparation	20 min
Whole Genome Amplification	125 min
Labeling	78 min
Purification	36 min
Preparation for Hybridization	35 min
Hybridization	2-6 hr
Washing, Scanning, Analysis	46 min

Total: 7 h, 40 min – 11 h, 40 min

Figure 1. GenetiSure Pre-Screen workflow to process 16 samples in under 8 and 12 hours. Same day processing time is especially important because of the time sensitive nature of pre-implantation genetics research.

Experimental Workflow

To assess the accuracy of chromosomal aberration detection, a single cell model system was built from a set of normal and aberrant cell lines with varying sizes of known CN changes, obtained from Coriell Biorepository. As references, lymphocytes isolated from normal male and female individuals were used. Due to the minute amounts of genetic material contained within each cell, all samples and references were subjected to a multiple displacement amplification-based WGA to increase the amount of DNA while maintaining its genomic representation. Amplified samples were then differentially labeled and combined in 7 pairs of test vs. test samples and 1 pair of reference vs. reference per 8-pack microarray slide, or 3 pairs of test vs. test samples and 1 pair of reference vs. reference per 4-pack microarray slide (Figure 2). This eliminates the use of a reference for every array and allows the processing of more test samples per slide resulting in a shorter and more cost-effective assay.

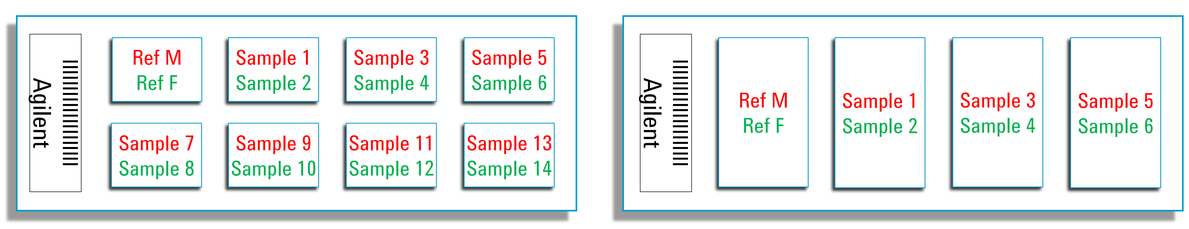

Figure 2. GenetiSure Pre-Screen Arrays in 8x60K and 4x180K formats, showing layout with a single pair of reference samples.

Labeled samples were combined, purified and hybridized for 2 or 6 hours to 8x60K or 4x180K CGH microarrays containing probes optimized for single cell WGA and analysis (Table 1). Following hybridization, microarray slides were washed and scanned. The data were extracted and analyzed for CN alterations using algorithms and a single cell specific analysis method implemented in Agilent CytoGenomics 2.9 software.

Table 1. GenetiSure Pre-Screen CGH array specifications.

Format	8x60K	4x180K
Design ID	067559	067649
Total Features	62,976	180,879
Control Grid Feature Count	3886	6539
Biological Features	55,090	170,340
Distinct Probes	54,911	86,649 present in duplicate
Median Probe Spacing	49,763	30,779
Average Probe Spacing	52,106	33,000
Chromosomes with increased density	13, 18, 20, 21, 22 and X	13, 18, 20, 21, 22 and X

Results and Discussion

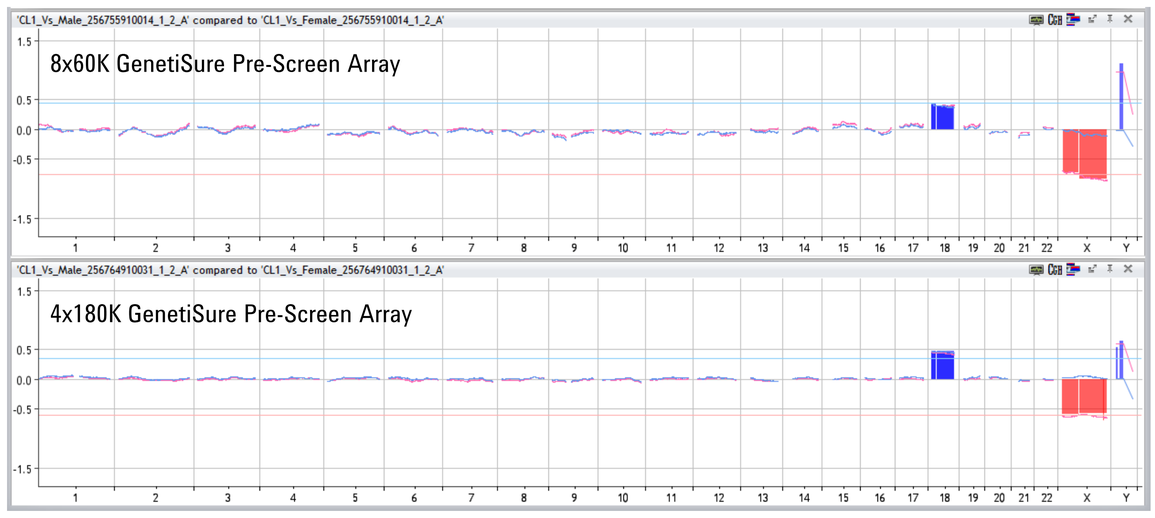

Figure 3. Whole genome CN profiling of amplified single cells using 4x180K and 8x60K GenetiSure Pre-Screen arrays. Trisomy of chromosome 18 in cell line GM02732 is identified in both formats.

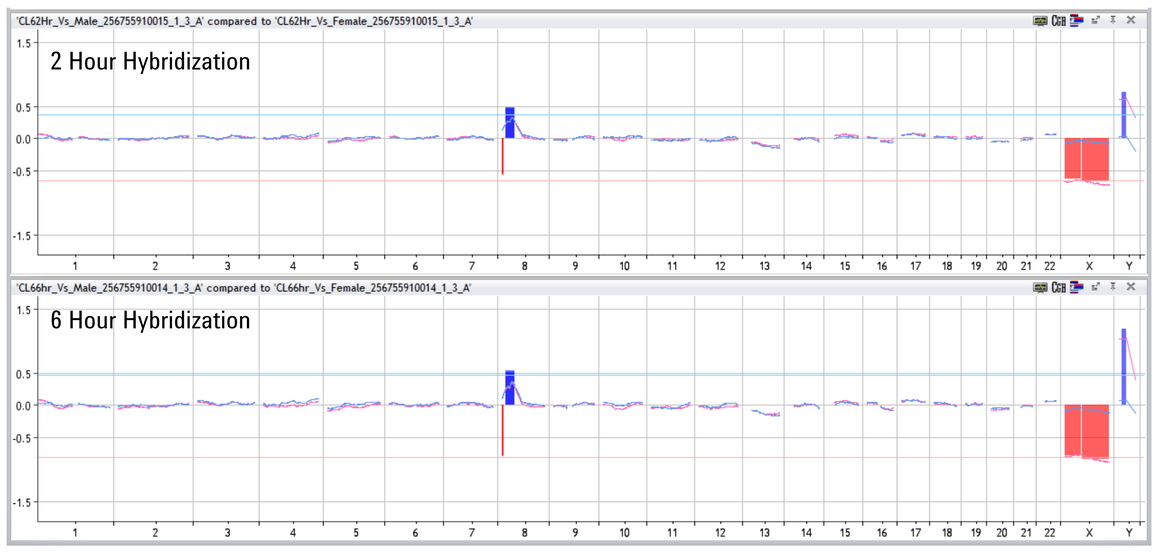

Figure 4. Comparison of 2 hour vs. 6 hour hybridization on 8x60K GenetiSure Pre-Screen microarray. Known aberrations of 7 Mb deletion and 32 Mb amplification in chromosome 8 of cell line GM14485 are identified in both cases.

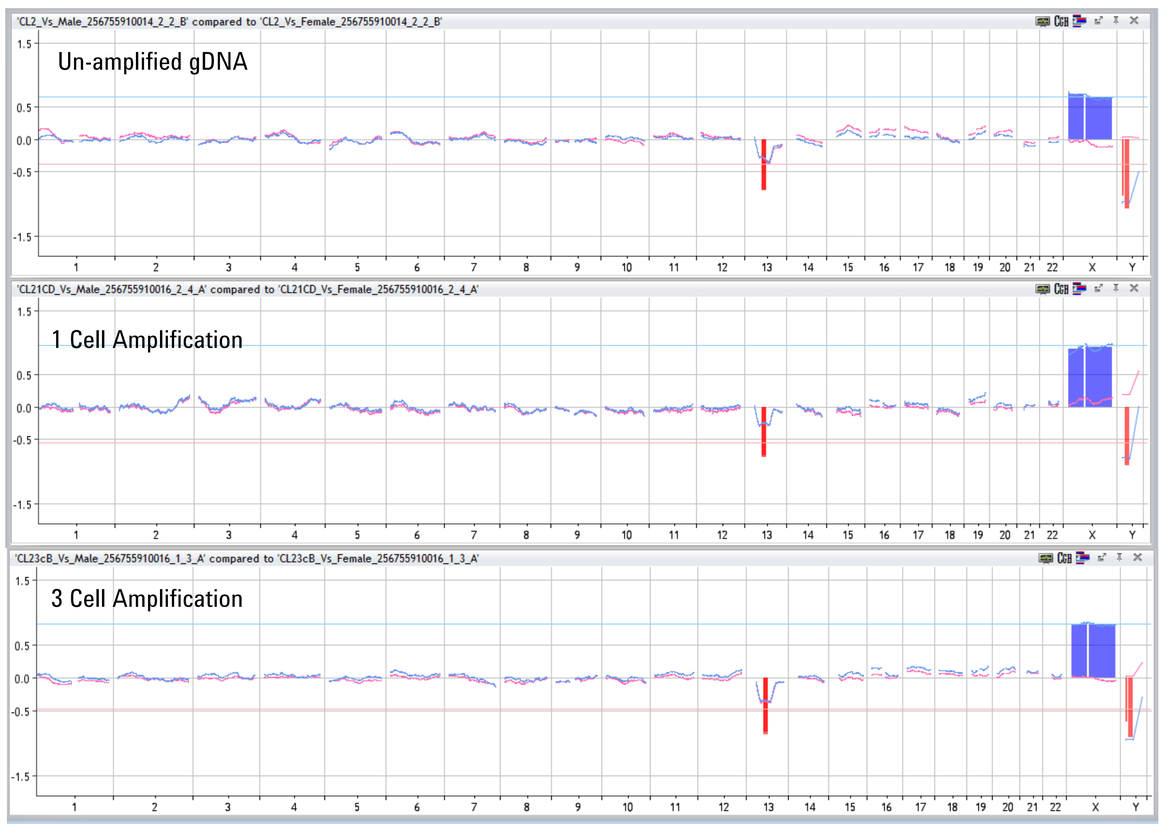

Figure 5. Un-amplified genomic DNA and whole genome amplifications of 1 and 3 cells of cell line GM07312 are compared on a 8x60K GenetiSure Pre-Screen array after a 6 hour hybridization. A 16Mb deletion on chromosome 13 is identified in all profiles.

For all data shown, the Default Single Cell Analysis Method implemented in Agilent CytoGenomics 2.9 was used to report aberrations with a minimum of 5 Mb, and minimum log2 ratios of 0.35 for gains and -0.45 for losses; data are plotted using a 50 Mb moving average.

Conclusions

- The GenetiSure Pre-Screen Kit, comprised of amplification, labeling and array components, provides an optimized workflow for sample processing and genome-wide CN analysis in less than 8 hours.
- Aberrations affecting whole and portions of chromosomes, as small as 7 Mb, were accurately and confidently identified in a model system.
- The GenetiSure Pre-Screen workflow provides an efficient and cost-effective method for researchers to obtain reliable CN results at a high resolution.