

Single Cell Copy Number Screening Using the GenetiSure Pre-Screen Kit

Uncover Aneuploidies in Embryos

Application Note

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Abstract

Genomic profiling of single cells is of great utility in pre-implantation genetics research. To overcome the limited resolution of traditional techniques such as FISH, PCR, and BAC arrays, the power of genome-wide copy number profiling obtained with Agilent's Oligonucleotide array CGH platform was extended to single cell samples. The new GenetiSure Pre-Screen workflow combines whole-genome amplification (using the multiple displacement amplification method) with oligonucleotide microarrays specifically designed for single cell screening. Using cells isolated from cell cultures with known aberrations and cells biopsied from embryos, single cell analysis was carried out with algorithms implemented in the Agilent CytoGenomics v3.0 software. Expected chromosomal copy number changes were accurately assessed in the cell culture data as confirmed by profiling of unamplified genomic DNA. This optimized and cost-effective workflow enables researchers to obtain reliable results at a high resolution in under eight hours from sample preparation to analysis.



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Introduction

Pre-implantation genetics research is a fast growing area where technological advancements are helping researchers investigate aneuploidies in a limited number of embryonic cells. Conventional single cell analysis methods, including fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR), lack genome-wide scalability^{1,2}. In response to this limitation, high-throughput array-based comparative genomic hybridization (CGH) technology has emerged and become increasingly valuable in assessing genome-wide copy number (CN) in a sample relative to a diploid reference. Because of the minute amounts of DNA in a single cell, CGH array platforms require whole genome amplification (WGA) of the samples to generate a sufficient amount of DNA for profiling. Bacterial artificial chromosome (BAC) arrays, adopted to analyze individual cells, suffer from low resolution due to the availability of only a few thousand probes for data collection³. More recently, the power of Agilent's high-resolution and highly sensitive oligonucleotide array CGH technology has been successfully applied to single cell profiling^{4,5}.

This application note describes an extension of our CGH platform to include a new single-cell analysis offering for embryonic pre-implantation screening. The GenetiSure Pre-Screen Kit consists of multiple displacement-based WGA and labeling reagents, hybridization gaskets, and microarrays (8x60K and 4x180K formats) specifically designed for single cell analysis. To address the need for an economical solution, two unknown differentially labeled test samples are hybridized together on each of seven arrays (8x60K format) or three arrays (4x180K format) and compared to male and female reference samples, which are co-hybridized to the remaining array on each slide. The accompanying Agilent CytoGenomics v3.0 software is used for data analysis. Using the GenetiSure Pre-Screen Kit, results are generated in less than eight hours.

Sample Preparation	20min
Whole Genome Amplification	125min
Labeling	78min
Purification	36min
Preparation for Hybridization	35min
Hybridization	2-6hr
Washing, Scanning, Analysis	46min
Total	7hr 40min-11hr 40min

Figure 1. GenetiSure Pre-Screen workflow from sample preparation to data analysis and processing times for 8 arrays.

Methods

Single cell preparation, hybridization, and imaging

To test the CN detection accuracy and resolution limit of the GenetiSure Pre-Screen workflow (Figure 1), cell lines containing genomic aberrations ranging in size from a whole chromosome down to 1.3Mb were obtained from Coriell Biorepository, and used to set up a model system. From each cell line, 2–3 cells were isolated for genome profiling and processed in triplicate. Additionally, cells biopsied from embryos were investigated for CN imbalances. For references, ~280 leukocytes were isolated from both normal male and female fresh blood. To uniformly increase the amount of DNA to levels necessary for downstream microarray analysis, isolated cells were subjected to multiple displacement-based WGA following the protocol in the *Agilent GenetiSure Pre-Screen Kit for Single Cell Analysis - Whole Genome Amplification, Labeling and CGH Microarray Hybridization* manual. For each cell line and reference, a 13 μ L sample of amplified DNA was fluorescently labeled. Differentially labeled samples were then combined in pairs of test vs. test samples and one pair of reference vs. reference samples per microarray slide (Figure 2). To serve as non-amplified controls, genomic DNA was isolated from each cell line and from male and female reference leukocytes and used without amplification. For each non-amplified genomic DNA sample, 500 ng were labeled using the same labeling protocol used on the amplified cell samples. All labeled samples (amplified cells and non-amplified genomic DNA samples) were hybridized for 2–6 hours to either the 8x60K or 4x180K GenetiSure Pre-Screen microarrays. Following hybridization, microarray slides were washed and imaged using the Agilent SureScan Scanner.

GenetiSure Pre-Screen microarrays

As part of Agilent’s single cell solution, two GenetiSure catalog microarrays were developed utilizing the 8x60K and 4x180K formats (Figure 2, Table 1). The 4x180K microarrays are intended to be used as a more cost-effective way to run fewer samples. For both formats, CGH probes were selected from a large pool of 423,000 60-mer oligonucleotides. Following a stringent selection process, the resulting designs include probes empirically optimized for use with the multiple displacement-based WGA method. Both microarrays have uniform genome-wide probe coverage, with increased probe density on chromosomes 13, 18, 20, 21, 22, X, and Y, which are commonly associated with aneuploidy events^{6,7}. Although the probe spacing of the 4x180K microarrays is slightly denser than that of the 8x60K microarrays, the effective biological resolution is the same for both formats.

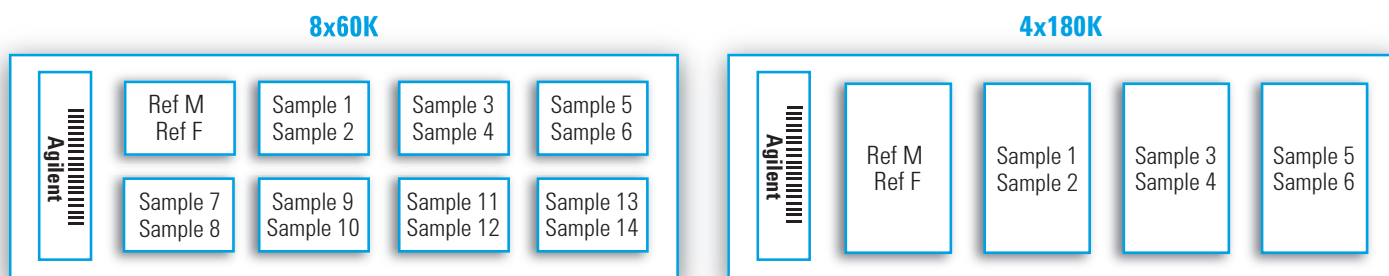


Figure 2. Hybridization of single cell and reference samples to 8x60K and 4x180K GenetiSure Pre-Screen microarrays.

Single cell copy number analysis

For data extraction and CN assessment, microarray images were imported into Agilent CytoGenomics v3.0. This new software version enables comparative genome-wide screening between single cells and references hybridized to different arrays within the same slide. After feature extraction of each array, the processed signals of a single cell sample are compared to the reference array. The resulting \log_2 ratios are computed and the CN changes identified. Aberrations that do not meet specific probe number, size, and \log_2 ratio criteria (Table 2) are then filtered out and not reported. A “Single Cell Recommended Analysis Method” has been integrated in Agilent CytoGenomics v3.0 with an aberration filter that identifies aberrations of 5Mb and larger, and with a minimum average \log_2 ratio of 0.35 for CN gains and -0.45 for CN losses.

For a targeted analysis of loci known *a priori* to be affected, a “Single Cell Small Aberration Analysis Method” is available in the software to detect variations as small as 1Mb. However, the reduced stringency of this filter, combined with the noise inherent to the amplification process, increases the risk for false positives and missed calls for this analysis method. A third single cell-specific analysis method has been implemented in the software to analyze mosaic samples, which are expected to have \log_2 ratio compression due to the admixture of normal and aberrant cells. The “Single Cell Long/Low Aberration Analysis Method” reports 20Mb or larger CN alterations, with a minimum \log_2 ratio of 0.2 for CN gains and -0.25 for CN losses.

Table 1. GenetiSure Pre-Screen microarray specifications.

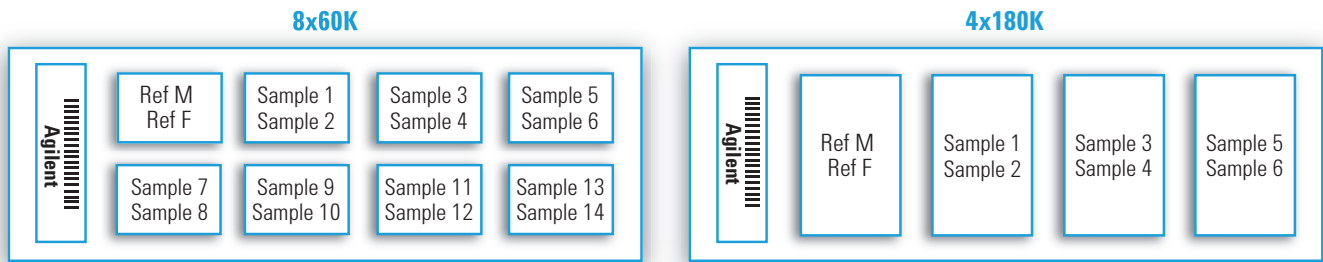


Table 2. Size and \log_2 ratio settings for aberration detection using the single cell-specific analysis methods.

Single Cell-Specific Analysis Methods	Aberration Filter Settings
Single Cell Recommended Analysis Method	Minimum size: 5Mb Minimum \log_2 ratio: 0.35 for amplification/gain -0.45 for deletion/loss
Single Cell Small Aberration Analysis Method	Minimum size: 1Mb Minimum \log_2 ratio: 0.35 for amplification/gain -0.45 for deletion/loss
Single Cell Long/Low Aberration Analysis Method	Minimum size: 20Mb Minimum \log_2 ratio: 0.20 for amplification/gain -0.25 for deletion/loss

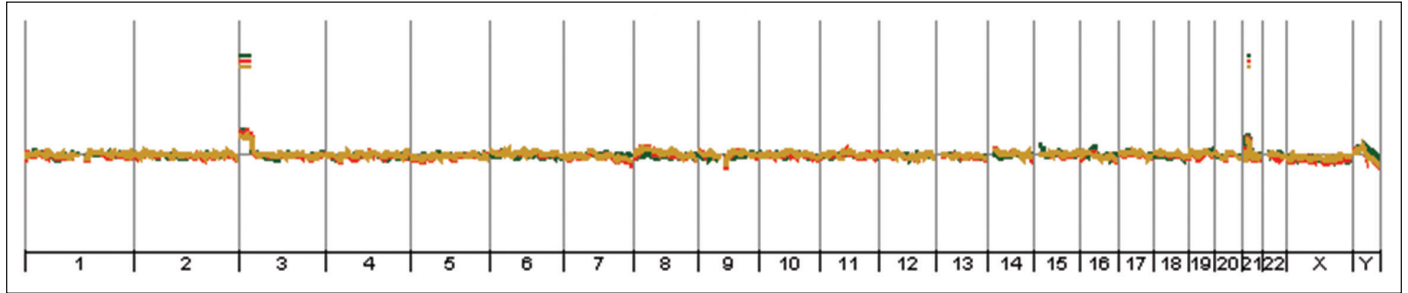
Results and Discussion

Arrays hybridized for two or six hours showed identical whole genome CN profiles in the model system. In addition, CN profiles were similar between the 8x60K and 4x180K microarray formats. Using the Single Cell Recommended Analysis Method implemented in Agilent CytoGenomics v3.0, known aberrations were accurately

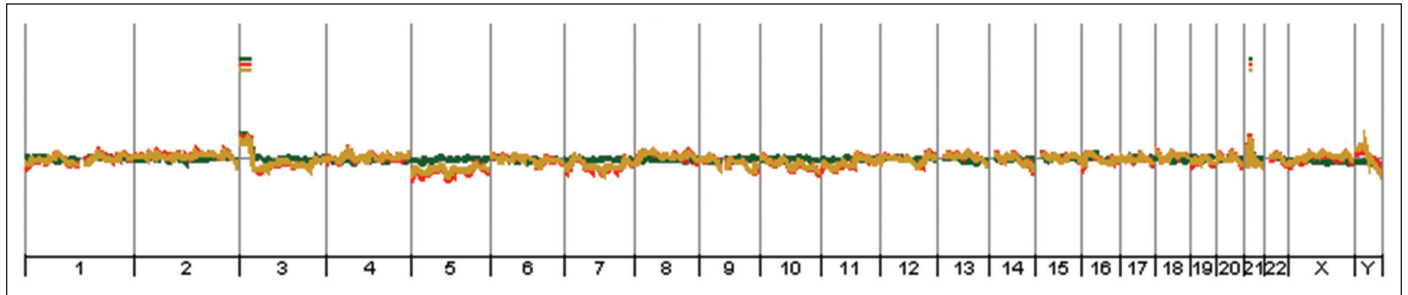
identified in the amplified replicates, and corroborated by the non-amplified genomic DNA controls. A gain of 31Mb and a gain of 7Mb were detected on chromosomes 3 and 21, respectively, in cells isolated from cell line GM09552 (Figure 3A). For cells from line GM14485, a loss of 7Mb and a gain of 31Mb on chromosome 8 were reported (Figure 3B).

A. GM09552

8x60K

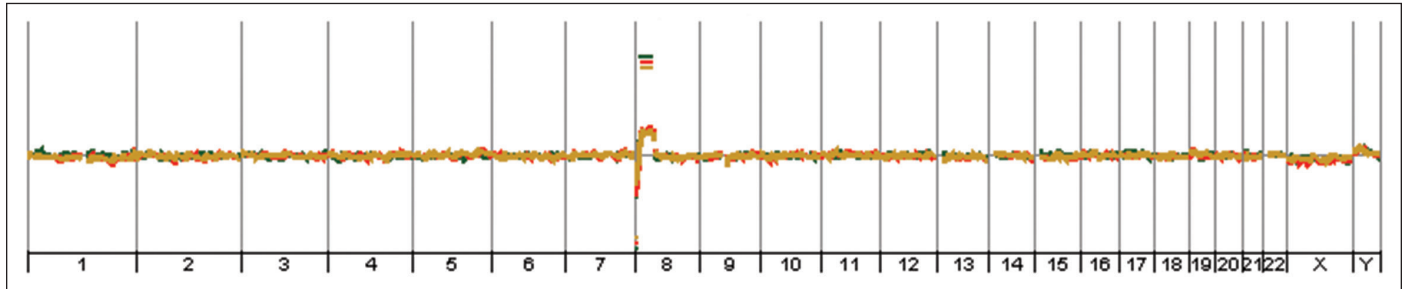


4x180K



B. GM14485

8x60K



4x180K

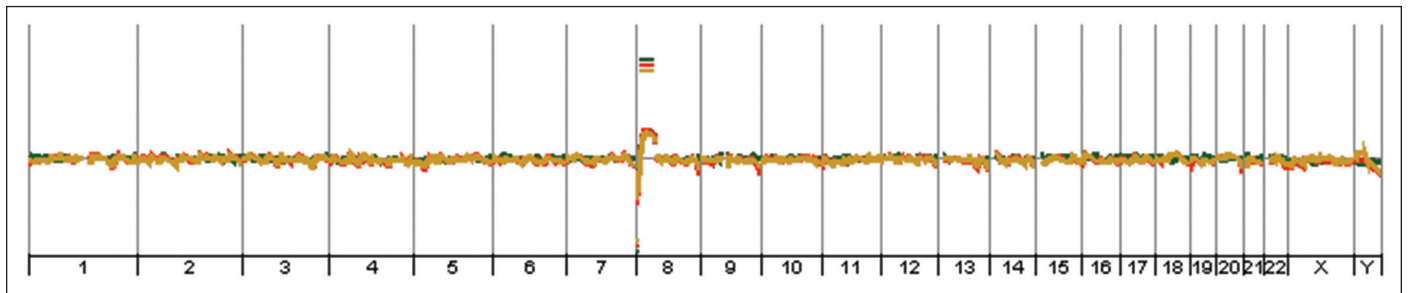


Figure 3. Whole genome CN profiling in the non-amplified genomic DNA control (—) and in cells included in the model system, hybridized for 2 (—) and 6 hours (—) to 8x60K and 4x180K arrays. A gain of 31Mb at 3p and a gain of 7Mb at 21q for cell line GM09552 (A), and a loss of 7Mb and a gain of 31Mb on 8p for cell line GM14485 (B) were accurately identified. The Single Cell Recommended Analysis Method was used to report aberrations with a minimum size of 5Mb, and minimum \log_2 ratios of 0.35 for gains and -0.45 for losses, displayed with a 10Mb moving average.

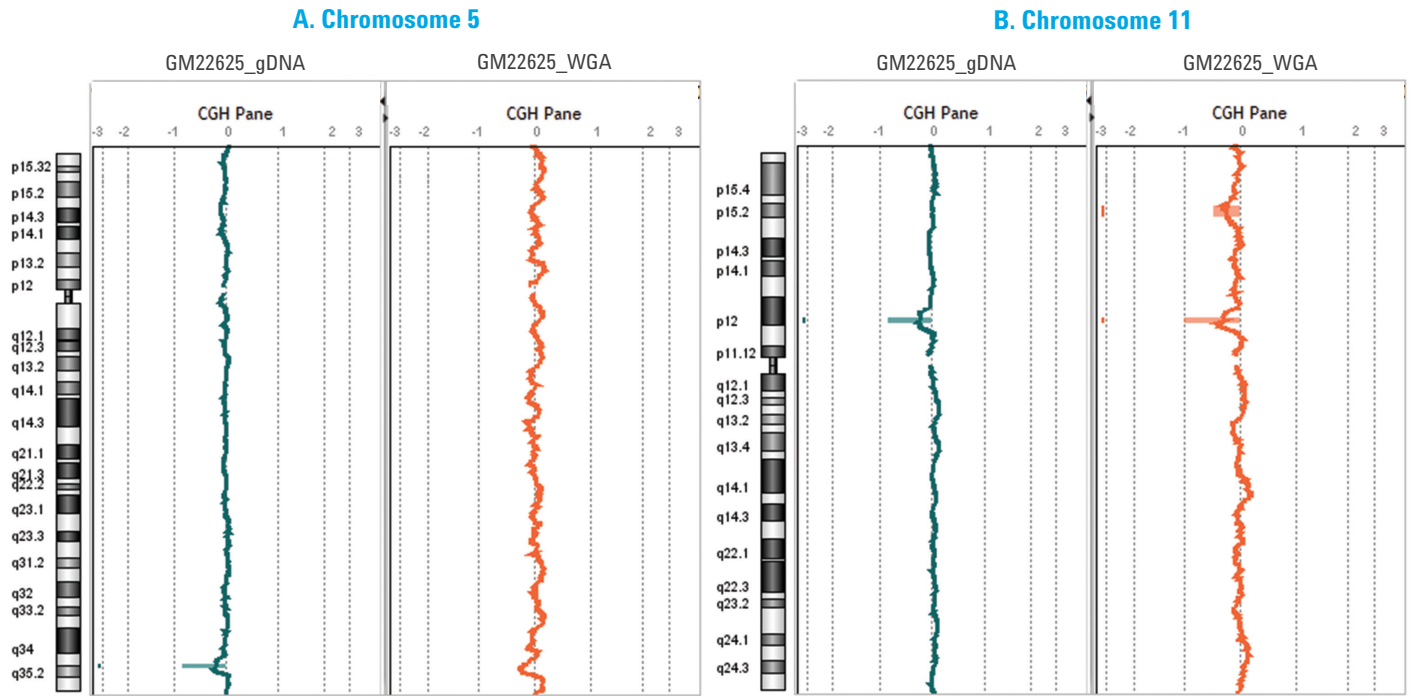


Figure 4. Chromosomal screening of amplified GM22625 cells and non-amplified genomic DNA, known to have a loss of 1.3Mb at 5q (A) and a loss of 1.5Mb at 11p (B), hybridized for 6 hours to 8x60K microarrays. Analysis was performed using the Single Cell Small Aberration Analysis Method (minimum size of 1Mb, and minimum \log_2 ratios of 0.35 for gains and -0.45 for losses), displayed with a 5Mb moving average.

Additional imbalances, including Trisomy 18 and a loss of 16Mb on chromosome 13, were confirmed in cells from cultures GM02732 and GM07312, respectively (data not shown). For the detection of known 1Mb CN changes in aberrant cell line GM22625, the Single Cell Small Aberration Analysis Method was applied. The expected 1.3Mb CN loss at 5q was detected in the non-amplified genomic DNA control but missed in the cells (Figure 4A). In this case, the \log_2 ratio change, visible in the chromosome profile, was below the threshold and therefore filtered out. The known 1.5Mb loss at 11p was identified in both the cells and the non-amplified genomic DNA sample. An additional aberration not present in the genomic DNA, and therefore likely not present in the cells, was also reported in the whole genome amplified cells (Figure 4B).

Due to the noise resulting from the WGA, the investigation of 1Mb aberrations should be limited to confirmation of expected imbalances. The study of cells biopsied from embryos also revealed CN changes affecting distinct chromosomes (Figure 5). In one of the day 5 samples, losses of the entire chromosomes 2, 5, and 9 and a gain of chromosome 16 were detected. Additionally, comparison of the sex chromosome profiles to both male and female references revealed that the test sample is male. In another day 5 sample, cells biopsied from a female embryo carry a CN loss of chromosome 22.

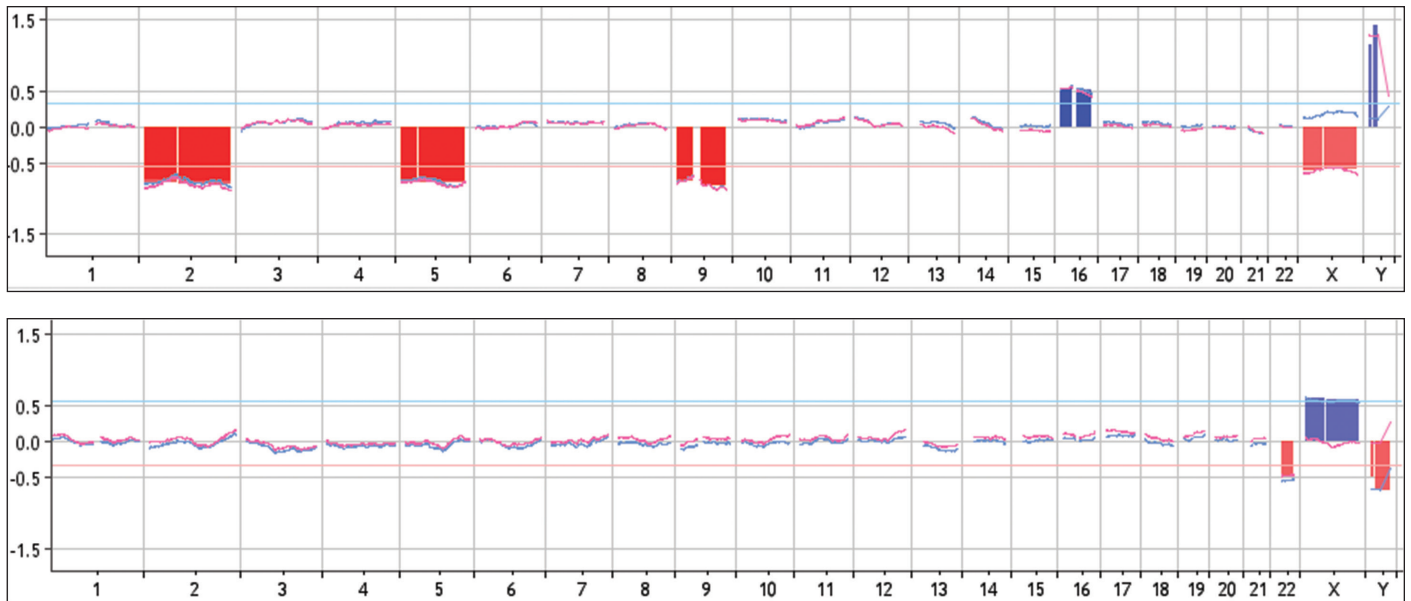


Figure 5. CN imbalances in amplified embryonic cells compared to both male (blue moving average line) and female (pink moving average line) references, hybridized to 8x60K microarrays for 3-4 hours, using the Single Cell Recommended Analysis Method (minimum size of 5Mb, and minimum \log_2 ratios of 0.35 for gains and -0.45 for losses), displayed with a 50Mb moving average. The top image depicts a male sample with CN losses of chromosomes 2, 5, and 9 and a gain of chromosome 16. The bottom image portrays a female sample with a CN loss of chromosome 22. Data for the top image was contributed by Dr. Richard Choy, Department of Obstetrics & Gynecology, The Chinese University of Hong Kong; and data for the bottom image was contributed by PacGenomics, CA, USA.

Conclusion

The GenetiSure Pre-Screen Kit, empowered with high-resolution oligo microarrays, offers researchers a solution that generates reliable data under time sensitive considerations. The GenetiSure Pre-Screen workflow, optimized for multiple displacement-based WGA, allows for cost-effective processing of up to 14 unknown samples on one microarray slide. Sample preparation to analysis can be completed in a typical workday of less than 8 hours.

Using this workflow, whole chromosome to 7Mb aberrations were correctly assessed in the single cell model system, and confirmed by genomic DNA CN profiling. Detection of CN changes in the 1Mb range were possible, but challenging, using a filter with reduced stringency. The accuracy and reproducibility of the GenetiSure Pre-Screen platform is suitable for scientists seeking genome-wide CN comprehension of individual cells biopsied from embryos.

Acknowledgment

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References

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GenetiSure Pre-Screen Kit (4x180K), P/N G5964A

GenetiSure Pre-Screen Amplification and Labeling Kit with sufficient reagents for 36 samples and 12 reference samples

GenetiSure Pre-Screen Array Kit 4x180K, 6 slides

Hybridization Chamber Gasket Slide Kit 4-pack, 6 gasket slides

GenetiSure Pre-Screen Kit (8x60K), P/N G5965A

GenetiSure Pre-Screen Amplification and Labeling Kit with sufficient reagents for 42 samples and 6 reference samples

GenetiSure Pre-Screen Array Kit 8x60K, 3 slides

Hybridization Chamber Gasket Slide Kit 8-pack, 3 gasket slides

Additional Components

Human Cot-1 DNA	5190-3393
Agilent Oligo aCGH Hybridization Kit (25) or (100)	5188-5220 or 5188-5380
Agilent Oligo aCGH Wash Buffer 1 and 2 Set	5188-5226
Hybridization Chamber, stainless	G2534A
Hybridization Oven	G2545A
Hybridization Oven Rotator Rack	G2530-60029
SureScan Microarray Scanner	G4900DA
Agilent CytoGenomics 3.0 License (free)	

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