Same Day, Cost-Effective Aneuploidy Detection with Agilent Oligonucleotide array CGH and MDA Single Cell Amplification Method

Application Note

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Abstract

The ability to characterize individual genomes in single cells is very important in pre-implantation genetics research. 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. Recently, BAC arrays have been used, but because they typically contain only a few thousand probes, their resolution is low. Agilent's high resolution CGH microarrays with an optimized protocol would be well suited for the detection of aneuploidies in single cells. Because of the time sensitive nature of pre-implantation genetics research and the need for an economical method to screen many samples in a high throughput environment Dr. Hellani developed a same-day, cost-effective protocol. The protocol uses the Agilent SurePrint G3 Human CGH 8x60K or 4x180K Microarrays, and a Multiple Displacement amplification (MDA) method. The total time from amplification to data analysis can be as short as 6.5 hours. Two unknown samples are hybridized on the same array. This eliminates the use of a reference for every array and allows the processing of 14 samples on one 8x60K slide and 6 samples on one 4x180K slide. This approach is more cost-effective compared to the standard analysis method where only 8 samples or 4 samples, respectively, can be processed per slide. Using this short and cost-effective protocol, copy number changes in individual genomes amplified from single cells can be accurately identified.

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Introduction

In pre-implantation genetics research, sample quantity is limited. One to five cells are usually biopsied for multiple applications. There is a need to amplify cells to generate CGH data for copy number variation detection, Sanger sequencing, and possibly Next Generation Sequencing (NGS). In the application note 'High-Resolution Oligonucleotide-Based aCGH Analysis of Single Cells in Under 24 Hours' (Agilent p/n 5991-0643EN) we described a method to analyze single cells on Agilent CGH microarrays. However, this method is based on the PicoPlex single cell Whole Genome Amplification (WGA) kit which is not optimal for NGS-based assays. A different WGA method, Multiple Displacement amplification (MDA), is used in the new protocol and generates high molecular weight DNA that enables researchers to use the MDA generated DNA for both CGH and NGS. Furthermore, the new MDA-based single cell protocol was shortened from 24 hours to 6.5 hours. Finally, to address the need to process multiple samples economically, two unknown samples are hybridized on the same microarray. As an additional advantage, unknown samples can be easily compared to both male and female references. This is especially important when investigating X-linked diseases.

Materials and Methods

Figure 1 shows the new amplification and labeling workflow for single cell analysis. A detailed sample processing protocol is available from Agilent upon request. In short, one cell from day 3 blastomeres or 2–5 cells from day 5 blastocysts are biopsied and amplified using the REPLI-g single cell amplification kit (Qiagen p/n 150345) according to manufacturer's protocol with the exception of a shortened amplification time of 80 minutes. Lymphocytes are isolated from peripheral blood from a male and a female person using a Percoll gradient. 0.1 µl is used as reference and also amplified with the REPLI-g kit. The amplified DNA is purified using the illustra GFX PCR DNA purification kit (GE p/n 28-9034-70) according to manufacturer's protocol. The amplified and purified DNA concentration is measured with a NanoDrop instrument and adjusted to 30 ng/µl. For each sample, 13 µl of amplified and purified DNA is labeled with the Agilent SureTag Complete DNA Labeling Kit (p/n 5190-4240) according to Agilent's protocol without the restriction digestion step and with a shortened labeling time of 45 minutes. Unknown samples are differentially labeled with Cy3 and Cy5, and combined in Cy3–Cy5 pairs. Reference male and female samples are also differentially labeled with Cy3 and Cy5, and combined post-labeling. The combined labeled material is purified with illustra ProbeQuant G-50 micro columns (GE p/n 28-9034-08). The appropriate volume of purified labeled material is combined with Cot-1 DNA, Agilent blocking agent, and Agilent hybridization buffer according to Agilent's protocol. DNA is denatured at 96 °C for 2 minutes and allowed to anneal at 37 °C for 18 minutes. The DNA is then hybridized from 2 to 6 hours to the SurePrint G3 Human Catalog 8x60K (Agilent p/n G4450A) or 4x180K (Agilent p/n G4449A) CGH microarrays according to the layout in Figure 2. After hybridization, the microarrays are washed and scanned according to Agilent's protocol.

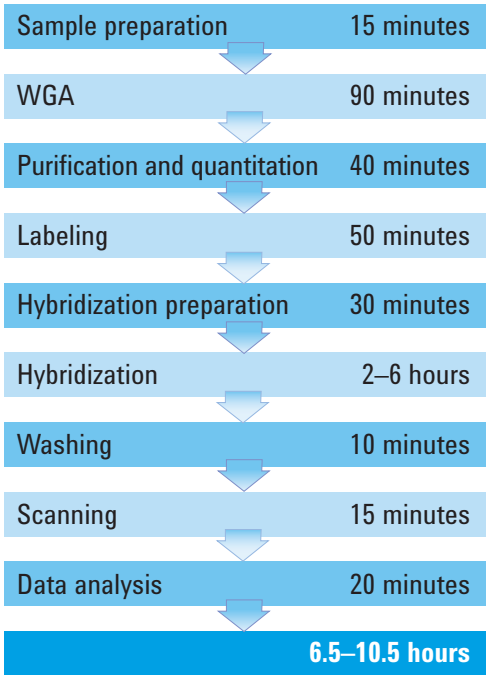

Figure 1. Workflow for single cells analysis and the respective processing times estimated for 8 samples.

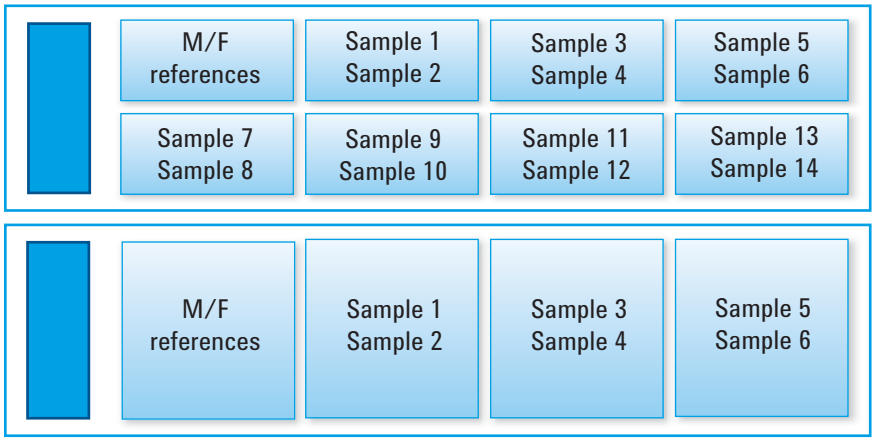

Figure 2. Sample and M/F (male/female) reference layout on the SurePrint G3 Human Catalog 8x60K (top) and 4x180K CGH (bottom) microarrays.

Data analysis

Because it is not possible to process images from two unknown samples co-hybridized on the same array in Agilent CytoGenomics v2.7, data was extracted using an interim external solution. Signal intensities from the unknown sample were compared to signal intensities from both the male and female reference. The resulting log2 ratios were imported in CytoGenomics v2.7. The Default Analysis Method – CGH v2 was used with the exception of raising the ADM-2 threshold to 8 and applying an aberration filter with a minimum 5MB size limit and minimum log2 ratio limit of –0.45 for losses and 0.35 for gains. A future version of CytoGenomics, v2.9, will be released with a single cell analysis method.

Results

In the first experiment, 2 cells were amplified from a day 5 embryo, processed according to the protocol described above, and hybridized to the SurePrint G3 Human Catalog 4x180K CGH microarrays for 2 hours. The signal intensity data from the samples was analyzed against the signal intensity data from the male reference sample and the female reference sample differentially labelled with Cy3 and Cy5. The male and female reference samples were hybridized on a separate array (Figure 2). The data from the autosomal chromosomes was identical irrespective of which sample, male Cy5 or female Cy3, was used as reference sample in the analysis indicating that this method of analysis does not generate dye biases (Figure 3). DLRSD values, a measurement of probe-to-probe noise, were similar and a copy number loss of chromosome 19 could be easily detected in both cases. Analyzing data this way allows processing 6 samples on a 4x180K microarray and 14 samples on an 8x60K microarray. As expected, the X and Y-chromosome show a different profile depending on whether a male or a female sample was used as reference sample. Comparing sample data to both male and female references gives added assurance when investigating X-linked diseases.

To evaluate the impact of longer hybridization time on data quality, cells were isolated from day 5 blastocysts, were processed according to the protocol described above, and hybridized to the SurePrint G3 Human Catalog 8x60K CGH microarrays for 6 hours. High quality copy number data could be achieved and a copy number gain of chromosome 21 could be easily detected (Figure 4).

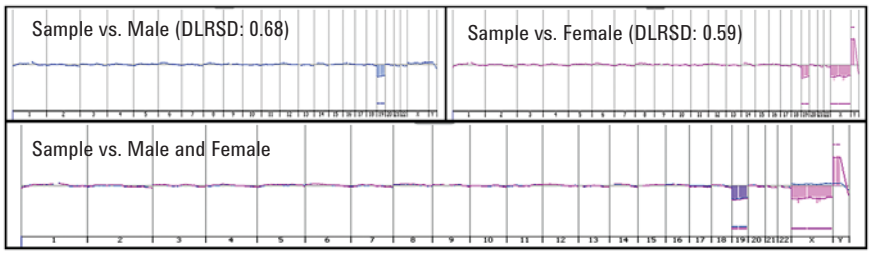

Figure 3. Copy number changes identified by Agilent CytoGenomics software in day 5 blastocysts hybridized for 2 hours on SurePrint G3 Human Catalog 4x180K CGH microarrays. The day 5 blastocysts were analyzed against the male reference sample (blue) and the female reference sample (pink). Data is plotted as a 50 MB moving average line across the entire genome.

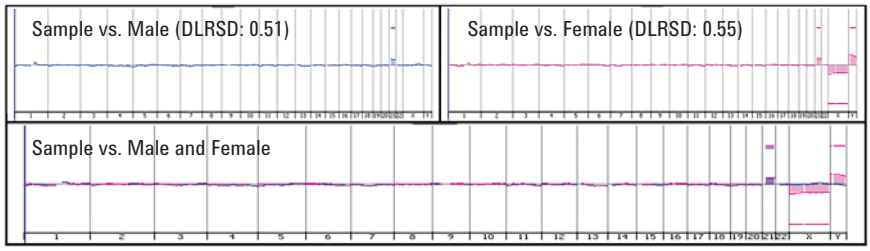

Figure 4. Copy number changes identified by Agilent CytoGenomics software in day 5 blastocysts hybridized for 6 hours on SurePrint G3 Human Catalog 8x60K CGH microarrays. The day 5 blastocysts were analyzed against the male reference sample (blue) and the female reference sample (pink). Data is plotted as a 50 MB moving average line across the entire genome.

Because oligo-based CGH microarrays contain at least 10 times as many probes compared to BAC arrays, it is expected that it will be possible to detect much smaller aberrations and translocations with oligo-based CGH microarrays. Indeed, we were able to detect a 3 MB deletion on chromosome 22 (Figure 5).

Finally, we evaluated the possibility to detect translocations using this protocol. Approximately 1:500–1:600 or about 12 million of the world population are translocation carriers. Translocation carriers typically show no physical symptoms. However, when one parent is a translocation carrier there is a risk that embryos are formed with unbalanced translocations. Figure 6 shows an example of an unbalanced translocation of chromosome 8 and 13.

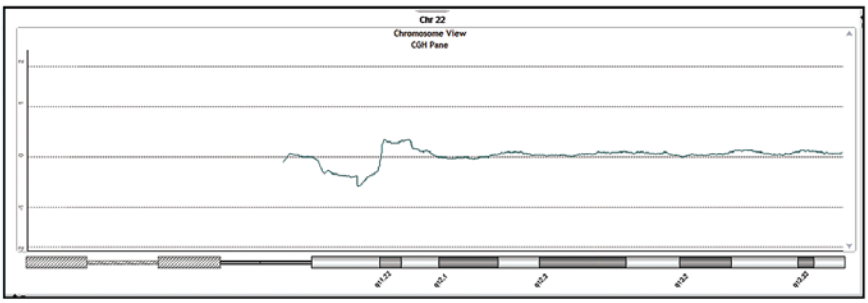

Figure 5. A 3 MB deletion on chromosome 22 identified by Agilent CytoGenomics software in about 10 cells isolated from a day 5 blastocysts hybridized for 6 hours on SurePrint G3 Human Catalog 8x60K CGH microarrays.

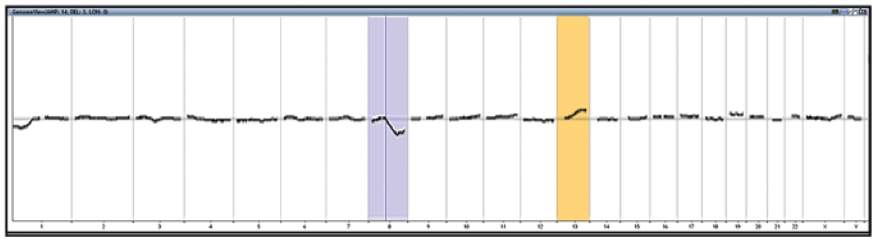

Figure 6. Translocation on chromosome 8 and 13 identified by Agilent CytoGenomics software in day 5 blastocysts hybridized for 6 hours on SurePrint G3 Human Catalog 8x60K CGH microarrays.

Conclusion

Using this short and cost-effective protocol developed by Dr. Hellani, genome-wide copy number changes from blastomeres and day 5 blastocysts can be successfully profiled with oligo-based CGH microarrays. The advantage of the MDA method is that it generates high molecular weight DNA suitable for CGH, and allows the same amplification product to be used for other applications such as next generation sequencing. The ability to hybridize for as short as 2 hours makes this method desirable for labs that require time-sensitive data. The ability to hybridize two unknown samples on the same microarray enables researchers to screen many samples economically in a high throughput environment.

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