

Application Note

Array Comparative Genome Hybridization for the Analysis of a Single Cell Aneuploidy

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Introduction

The ability to analyze nucleic acids (DNA) at the level of a single cell is important both for basic and clinical research. Clinical uses could include the ability for aneuploidy screening of preimplantation embryos obtained by in vitro fertilization (IVF)¹. Aneuploidy screening of a single cell could allow for the assessment of the chromosomal constitution of gametes and the mitotic stability of chromosomes during early embryo development. Currently the most common methods used to analyze chromosomes of a single cell, e.g. a blastomere biopsied from an 8-cell embryo², are fluorescent in situ hybridization (FISH) and PCR-based methods. The limitation of these approaches is that they can only analyze a small number of genetic loci in a single cell. We show here the utility of using array comparative genomic hybridization (aCGH) for the detection of chromosomal copy-number change from single amniocyte following whole genome amplification (WGA) by degenerated oligonucleotide primed PCR (DOP-PCR).

Whole Genome Amplification

Whole genome amplification (WGA) is used to greatly increase DNA quantities originating from samples with limited DNA contents. The most common methods currently used for WGA include: Primer Extension Pre-amplification PCR (PEP)³, Multiple Displacement Amplification (MDA)⁴, Linker Adapter PCR (LA)⁵ and Degenerate Oligonucleotide Primed PCR (DOP)⁶. This study uses a modified DOP-PCR methodology currently commercialized by Sigma-Aldrich for research purposes under the name of the GenomePlex[®] Whole Genome Amplification Kit. The kit utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA (gDNA) and conversion of the resulting small fragments to PCR amplifiable OmniPlex[®] Library molecules flanked by universal priming sites. The OmniPlex library is then PCR amplified using universal oligonucleotide primers and a limited number of cycles. The GenomePlex Single Cell WGA kit has been optimized to amplify the genome of a single cell. WGA from a single cell often results in a million-fold amplification yielding microgram quantities of gDNA.



Array Comparative Genomic Hybridization

Array-based comparative genomic hybridization (aCGH) is analogous to traditional karyotyping but with significantly greater throughput, resolution, and ease of use. Traditional karyotyping methods for detecting genomic variation, such as FISH or G-banding are well established for detecting chromosomal aberrations, but they do not always provide the speed, throughput, or resolution required for today's cytogenetic and research laboratories. The Spectral Chip™ 2600 array allows the researcher to perform the equivalent of thousands of FISH experiments simultaneously in a 24 hour protocol. Additionally, the Spectral Chip array provides greater resolution and improved data analysis tools. The 2605 non-overlapping BAC clones on the Spectral Chip array span the entire genome. The BAC clones are spaced at approximately 1 megabase intervals, as compared to traditional karyotyping which has approximately 10 megabase resolution. The clones are covalently coupled onto glass microscope slides spotted in duplicate. Sample and reference DNA are labeled in a two color, ratio metric, experiment. A fluorescent scanner captures data from the array and SpectralWare® aCGH analysis software converts the scanner output data into an intensity ratio profile. The software analyzes copy number changes and displays the location of the changes within the genome. aCGH combines the whole genome perspective of chromosome karyotyping with the increased resolution of FISH and the high throughput of DNA arrays. The Spectral Chip array can generate molecular profiles in a single experiment. Hybridization can be completed in 16 hours, and data are available in 24 hours or less.

Materials and Methods

Two human amniocyte cultures were grown using our collaborators accepted laboratory protocol. Each culture had been previously subjected to cytogenetic analysis and one culture was found to be a normal karyotype and the other carried an aneuploidy on chromosome 18. Upon disassociation of the cells from the culture dish and washing with PBS, single cells were isolated under an inverted microscope using fine hand-drawn microcapillaries and were transferred to 200 µl PCR tubes and immediately frozen at -80°C. Whole genome amplification using Sigma's GenomePlex Single Cell WGA Kit (Cat# WGA 4) was performed on the isolated single cells exactly as outlined in the manufacturer's protocol. The resulting amplified DNA was purified over a column based cleanup kit (Sigma) and immediately used for aCGH analysis.

The aCGH process includes five steps: labeling, hybridization, washing, scanning and data analysis. The labeling was accomplished using the Labeling Reagents kit from PerkinElmer. Approximately 1 microgram (µg) of amplified DNA was labeled along with 1µg of amplified sex-mismatch reference DNA according to the included protocol. Each sample was also labeled in a reverse reaction wherein the test and reference dyes were swapped. After application to the slides and 16 hours of hybridization the slides were washed at high stringency, dried and scanned on a PerkinElmer ScanArray® Gx Plus. The images were quantitated using ScanArray Express microarray analysis software and the resulting data for each direction of the experiment was saved as GPR file for analysis on SpectralWare aCGH analysis software supplied by PerkinElmer.

Results and Discussion

All samples were run at least two times using the Spectral Chip 2600. When analyzed on SpectralWare software the data showed that ideograms from the normal cell line appeared as normal. The amniocyte that was known to have the aneuploid chromosome 18 clearly showed the aberration with no other significant abnormalities. The results were statistically very sound with minimal

noise. Figures 1 and 2 show the SpectralWare ideogram and chromosome view of the normal cell line and the aneuploid cell line respectively. It is clear from this data that it is possible to analyze chromosomal copy number change from single cells. Using the Spectral Chip 2600 platform this data was collected in 24 hours for each replicate, which represents a substantial increase in speed when compared to conventional cytogenetic methods.

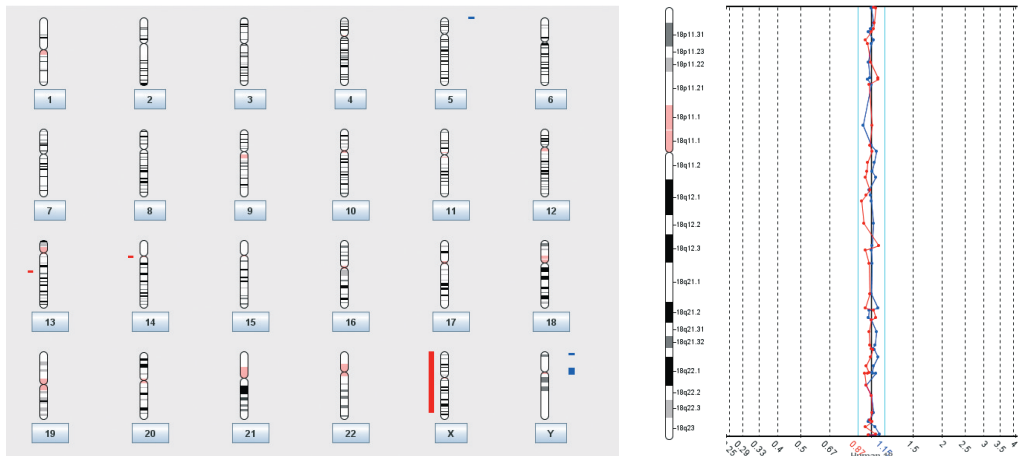


Figure 1. A) SpectralWare® ideogram view of the entire genome and B) chromosome 18 view of DNA derived from normal amniocyte. A single human amniocyte was amplified using the GenomePlex® Single Cell WGA kit from Sigma. The resulting DNA was compared with amplified sex mismatched gDNA and hybridized to the Spectral Chip™ 2600 from PerkinElmer.

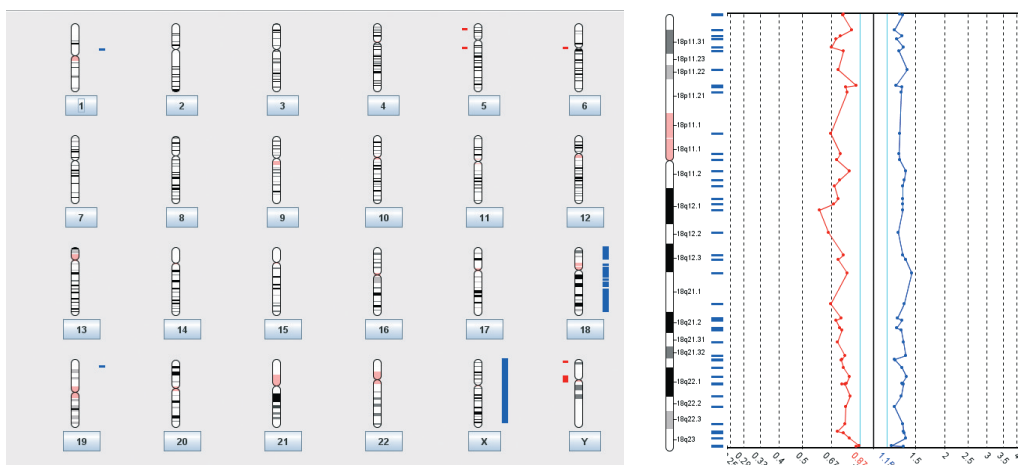


Figure 2. A) SpectralWare® ideogram view of the entire genome and B) chromosome 18 view of DNA derived from aneuploid amniocyte. A single human amniocyte was amplified using the GenomePlex® Single Cell WGA kit from Sigma. The resulting DNA was compared with amplified sex mismatched gDNA and hybridized to the Spectral Chip™ 2600 from PerkinElmer.

Conclusion

The ability to analyze the genetic makeup of a single human cell may be the ultimate in biological goals. Many disciplines have tried to elucidate the causes of disease or constitutional disorders at this level. Unlocking the genetic secrets of single cells may have a profound impact on how we study disease, disorders and human development. These data show that the PerkinElmer aCGH

platform, which includes the Spectral Chip 2600 whole genome BAC array, Labeling Reagents, Hybridization Reagents, the ScanArray Gx plus microarray scanner, and SpectralWare analysis software, provides all the necessary tools for the analysis of the common chromosomal copy number changes seen in therapeutically relevant single cell samples.

References

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