

Comparative Genomic Hybridization of DNA Amplified with GenomePlex® Technology for Detection of Trisomy Chromosomal Abnormalities from Limited Patient Source Material



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Introduction

Comparative Genomic Hybridization (CGH) has been refined to find chromosomal abnormalities at progressively smaller resolution. Initial studies were limited to trisomies and some terminal deletions, but recent work has been successful in finding microdeletions or duplications; for instance, in the telomeric regions. This growing technique, however, is somewhat hampered by the large DNA input requirement—over 75,000 copies of a human genome are needed to perform one CGH assay.

Whole Genome Amplification represents a means to decrease the amount of required DNA for CGH, which would open the technology for analysis of small tissue biopsies, or perhaps individual organisms with smaller genomes. In this study, GenomePlex Whole Genome Amplification (WGA) technology was combined with CGH arrays to detect chromosomal abnormalities in patients with mental and developmental disabilities, such as Down (Trisomy 21) and Edward's (Trisomy 18) syndromes. The use of GenomePlex WGA kit allowed for the identification of genome-wide copy number and chromosomal abnormalities without any bias detected in the array. The technique is simple to use and can be applied to academic and clinical research.

Materials and Methods

Representation Assay

Human genomic DNA was extracted using GenElute™ Mammalian Genomic DNA purification kit (Cat. No. G1N) and subjected to GenomePlex amplification. The samples were then purified using GenElute PCR Cleanup Kit (Cat. No. NA1020). This DNA was aliquotted and subjected to 79 different SYBR® Green quantitative PCR analyses (Cat. No. 54438) using different UniSTS primers. These independently designed primers targeted the majority of human chromosomes. Reactions were run using three replicates and compared to a similar number of reactions on an MJ Opticon 2 instrument. Results were plotted as a ratio of amplified and unamplified hg DNA after qPCR analyses.

Chromosomal Microarray Analysis

DNA was chemically modified and attached to an unmodified glass surface to produce arrays.¹ This method has been validated as a simultaneous screening method for DNA copy number changes in chromosomal regions associated with 41 well-established genetic disorders.⁴ Genomic DNA from Down and Edward's patients and healthy individuals were isolated from peripheral blood using PureGene® DNA-Purification Kit (Gentra Systems, Minneapolis, MN) and used in whole genome amplification with Sigma's WGA kit. DNA from patient samples and controls were differentially labeled with cyanin-3 (Cy3) and Cy5 (PerkinElmer, Boston, MA) as described in the literature.¹ Labeled DNA was hybridized onto array at 37 °C for 24 hours. Fluorescent signals on the slides were scanned into image files using an Axon microarray scanner (Axon Instruments, Union City, CA).

Results

Highly Representative Whole Genome Amplification

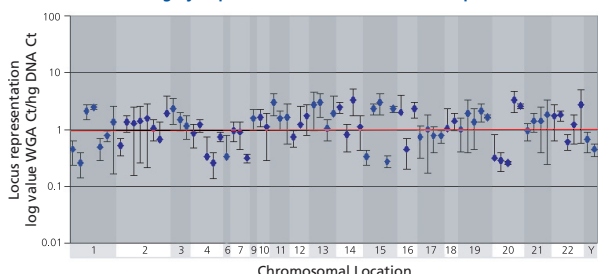


Figure 1. Real-time quantitative PCR was performed targeting 79 loci on human genomic DNA and whole genome amplified DNA. Thirty-six of the 79 loci are represented within two-fold when comparing genomic DNA to amplified DNA. The maximum bias for all samples was less than or equal to four-fold.

CGH Analysis and Karyotype Comparison in Trisomy 21 Male (Down Syndrome)

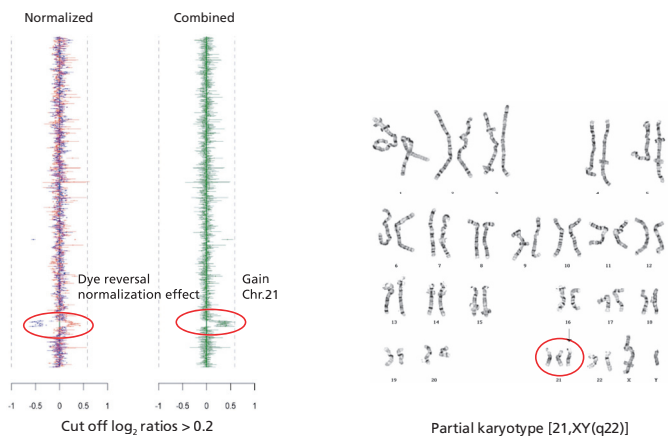


Figure 2. The karyotype shown indicates Trisomy 21. Microarray hybridization was performed simultaneously using patient DNA and a DNA sample from a healthy individual as reference DNA. The effect of normalization is shown by comparing data marked "normalized" with the "raw" data (not shown). The normalized data shows a number of clones from chromosome 21 that were displaced to the left in blue and to the right in the dye reversal, both indicating a gain of chromosomal material in the patient versus the female reference DNA. In the "combined" column, the sign of one of the two reversed hybridizations is changed and the data are averaged with gains to the right and losses to the left. For the combined data of 33 possible clones, there is a strong indication of gain of 14 clones corresponding to the 21q22 region specified in Table 1. The circled regions show the increase in the total genomic data and indicate the presence of an extra chromosome 21.

CGH Analysis and Karyotype Comparison in Trisomy 18 Female (Edward's Syndrome)

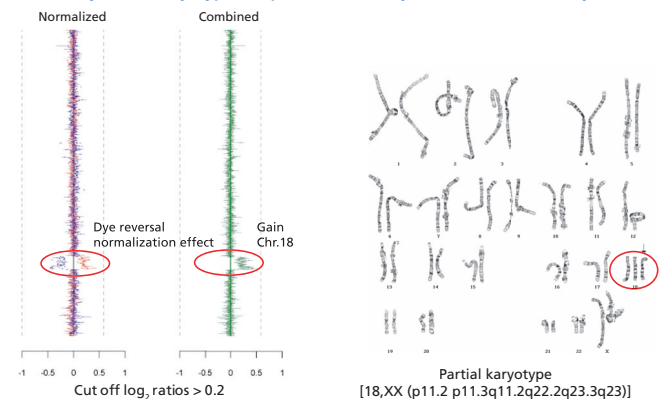


Figure 3. The karyotype shown indicates Trisomy 18 in an Edward's syndrome female DNA sample. The hybridizations and normalization of data were performed in the same manner as indicated in Figure 2. Unamplified genomic DNA showed a gain in 30 clone locations of 33 possible test clones, while whole genome amplified DNA showed a gain in 27 clone locations of 33 possible clones. The combined data represents a strong indication of gain in the 18p11.2p11.3q11.2q22.2q23.3q23 regions as indicated in Table 2. The circled regions show the increase in the total genomic data and indicate the presence of an extra chromosome 18.

Tables of Results Listing Signal Quantities for Loci Showing Major Differences

Clone	Location	Genomic Combined	WGA Combined
RP11-625C23	21:q11.2	0.251	0.278
RP11-840D8	21:q21.1	0.26	0.367
RP11-143A3	21:q21	0.276	0.258
RP11-108H5	21:q21	0.384	0.312
RP11-170Z0	21:q22.1	0.403	0.393
RP11-166F15	21:q22.1	0.36	0.352
RP11-401I23	21:q22.1	0.497	0.356
RP11-35C4	21:q22.3	0.364	0.287
RP11-92D3	21:q22.3	0.259	0.29
RP11-88N2	21:q22.3	0.352	0.34
RP11-190A24	21:q22.3	0.353	0.36
RP11-40L10	21:q22.3	0.31	0.189
RP11-16B19	21:q22.3	0.369	0.276
RP11-640F21	21:q22.3	0.358	0.273
GS-63H24	21:qter	0.31	0.229

Table 1

Tables 1 and 2. Signal values for critical diagnostic clones are listed in the tables above. The threshold for designation of over-representation in these experiments was 0.2.

Table 1 shows quantified data for trisomy 21, where a copy number gain was detected in all possible clones for the unamplified genomic DNA and 14 of 15 clones were detected for the WGA product. All clones were detected in the expected 21q22 region, ranging from 0.251 to 0.497.

Table 2 shows quantified data for trisomy 18, where a copy number gain was detected in 30 of 33 possible clones for the unamplified genomic DNA, while whole genome amplified DNA showed a gain in 27 of 36 possible clones. Clones were detected in expected region, ranging from 0.2 to 0.427 compared to the selected gain threshold of 0.2.

Clone	Location	Genomic Combined	WGA Combined
RP11-705O1	18:p11.3	0.191	0.197
RP11-14P20	18:p11.3	0.224	0.243
RP11-607C2	18:p11.3	0.29	0.213
RP11-78H1	18:p11.3	0.294	0.243
RP11-55N14	18:p11.3	0.193	0.174
RP11-193E15	18:p11.3	0.239	0.217
RP11-838N2	18:p11.3	0.208	0.188
RP11-874J12	18:p11.3	0.271	0.15
RP11-183C12	18:p11.3	0.33	0.153
RP11-105C15	18:p11.3	0.339	0.356
RP11-781P6	18:p11.3	0.308	0.257
RP11-931H21	18:p11.2	0.244	0.231
RP11-772F18	18:p11.2	0.213	0.21
RP11-752I5	18:p11.2	0.38	0.272
RP11-807E13	18:p11.2	0.305	0.235
RP11-411B10	18:p11.2	0.203	0.201
RP11-380C8	18:q11.1	0.168	0.169
RP11-459H24	18:q11.2	0.219	0.163
RP11-758N17	18:q11.2	0.336	0.245
RP11-540M4	18:q11.2	0.326	0.256
RP11-90G7	18:q11.2	0.33	0.221
RP11-704G7	18:q22.2	0.246	0.191
RP11-47G4	18:q22.3	0.306	0.204
RP11-669I1	18:q22.3	0.299	0.214
RP11-27C7	18:q22.3	0.373	0.26
RP11-357H3	18:q23	0.402	0.345
RP11-162A12	18:q23	0.2	0.199
RP11-90L3	18:q23	0.311	0.31
RP11-451L19	18:q23	0.354	0.342
RP11-91C19	18:q23	0.322	0.219
RP11-154H12	18:q23	0.325	0.427
GS-964M9	18:qter	0.23	0.241
RP11-89N1	18:q23	0.298	0.34

Table 2

Discussion

To prove unbiased amplification using GenomePlex Whole Genome Amplification Kit, 79 primers were selected across a spectrum of loci in the human chromosome. Quantitative PCR was performed using these primers to amplify human genomic DNA before and after GenomePlex amplification. The cycle number from the qPCR amplification with template prior to WGA amplification was normalized to the dye and the bias for the cycle numbers from the DNA template after WGA treatment was calculated for three replicates. The data demonstrates that all 79 primers were successfully amplified after WGA without any drop-outs, indicating that GenomePlex amplifies the whole genome without any bias.

To perform the CGH assay, probe samples were prepared using 250 ng of control DNA and the same amount of patient DNA combined together. Two microarray slides were run for each trisomy case, one with unamplified genomic DNA and another with whole genome amplified DNA. Only 10 ng of starting material, or 4% of the normal amount, was used for whole genome amplification.² Each pair of patient and control DNA was labeled twice with the dyes reversed, hybridized to the array, and analyzed as previously described.¹ The threshold for the designation of over- or under-representation in these experiments was determined to be 0.2 and -0.2 respectively.⁴

The data (Figure 2) shows that CGH microarray analysis of the Down syndrome patient is consistent with a genomic gain as detected by 15 target clones in the array for unamplified gDNA and the whole genome amplified DNA, reflecting the presence of a third chromosome corresponding to region 21q22. Furthermore, the array for Edward's syndrome (Figure 3) showed a genomic gain in 30 of 33 possible test clones for the unamplified genomic DNA sample and a gain of 27 target clones from 33 in the whole genome amplified DNA sample in 18p11.2 p11.3q11.2q22.2q23.3q23 regions. This represents strong indication of trisomy 18.

The CGH array analysis was accurate in detecting clones displaying a gain for each patient tested and confirms that the GenomePlex Whole Genome Amplification method provides a sufficient amount of DNA without any bias.

Conclusions

The data presented proves that whole genome amplification using the GenomePlex Whole Genome Amplification Kit in conjunction with Comparative Genomic Hybridization array allows for the identification of genome-wide copy number and chromosomal abnormalities amplifying limited DNA without bias.

The results verify that the GenomePlex WGA Kit is able to amplify DNA across >800 loci without detectable bias. When using DNA amplified with the GenomePlex technology four to five CGH arrays were run using a total of 10 ng genomic DNA isolated from the patients. Typical CGH arrays require 500 ng of genomic DNA for a single array. Using the GenomePlex WGA Kit eliminates the need for obtaining a significant quantity of patient samples for CGH and other assay methodologies.

Acknowledgments

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