



SIGMA-ALDRICH

## Amplification of genome-representative DNA from limited sources with GenomePlex<sup>®</sup> WGA technology for use in genetic alterations studies

Modern technologies developed in recent years have prompted new and exciting approaches to support biomedical research. The ability to study the entire human genome using limited sources of genomic material to detect genetic disorders—made possible through Sigma-Aldrich Biotechnology's GenomePlex<sup>®</sup> whole-genome amplification (WGA) system—is a notable achievement.

The development of high-throughput genomics and DNA sequencing has provided researchers the ability to obtain complete and exact DNA sequences of many genomes. Functional genomics researchers use sequencing as an analysis tool for genotyping and microarray-based technologies. These techniques, however, require sufficient amounts of DNA that in some cases may not be available. WGA technology provides the ability to generate adequate amounts of genome-derived DNA to study genetic alterations and their relationship to human diseases. It has become a useful tool for many biomedical applications, such as microsatellite analysis, single nucleotide polymorphism (SNP) detection, comparative genomic hybridization (CGH) microarrays and others.

### GenomePlex WGA kit overview

The GenomePlex WGA technology provides a simple and accurate method for amplifying nanogram quantities of starting material from any source, resulting in microgram amounts of DNA with no detectable allele bias. The GenomePlex WGA technology is time-efficient, allowing the researchers to amplify DNA in less than three hours. The technology is based on random chemical fragmentation of the genome, producing a series of overlapping short templates ranging in the size from 200 to 1,500 base pairs and averaging 400 base pairs.

The resulting DNA fragments are efficiently primed to generate a library of DNA fragments with defined 3' and 5' termini—the OmniPlex<sup>®</sup> library. This library is replicated using linear amplification in the initial stages, followed by a limited round of geometric amplifications.

### Chromosomal imbalances: trisomy 21 and trisomy 18

We combined GenomePlex WGA technology with a CGH array to detect chromosomal abnormalities in patients with mental and devel-

opmental disabilities such as Down and Edwards syndromes. Down syndrome is caused by an extra copy of chromosome 21 (trisomy 21), and Edwards syndrome is caused by an extra copy of chromosome 18 (trisomy 18). It is extremely important to establish methods that allow identification of chromosomal abnormalities without any bias when only small amounts of DNA are available for early diagnosis.

### Chromosomal microarray analysis (CMA)

Combining the GenomePlex WGA kit with a CGH microarray allowed accurate measurement of copy number changes and showed that this technique is simple to use and can be applied in both academic and clinical research. CMA uses CGH with bacterial artificial chromosome (BAC) or phage-derived artificial chromosome (PAC) clones (verified by fluorescence *in situ* hybridization) of known genomic location attached to a glass slide. Three or more different clones were used per region of interest. Genomic DNA was isolated from peripheral blood of Down or Edwards syndrome patients and healthy individuals using the PureGene<sup>®</sup> DNA Purification kit (Gentra Systems). The samples were amplified with the GenomePlex WGA kit. Each sample was hybridized twice, using a dye reversal process. The combined results were analyzed using quantitative imaging methods and analytical software to determine a loss or gain of chromosomal copies.

### Detection of chromosomal alterations with confidence

To perform the CGH assay, we prepared probe samples 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 the other with whole genome-amplified DNA. We used only 10 ng of starting material for WGA. The threshold for the designation of over- or under-representation of genetic material in these experiments was determined to be 0.2 and -0.2 respectively.

The CMA data (Fig. 1) shows that CGH microarray analysis of the Down syndrome patient is consistent with a genomic gain, as detected by 14 out of 15 determined target clones in the array for both unamplified and amplified DNA. It reflects the presence of a third chromo-

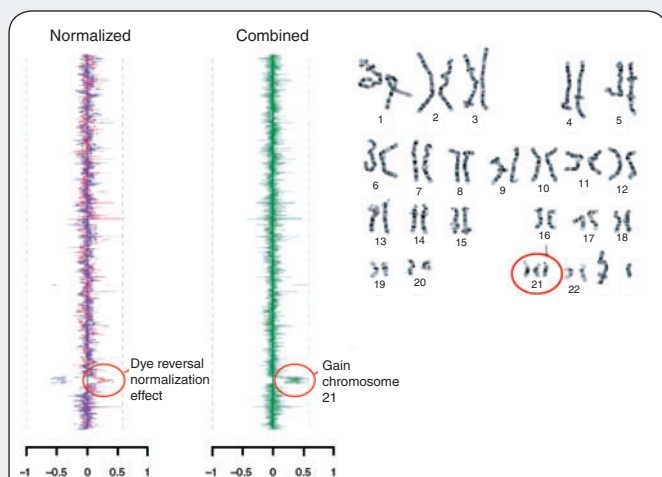
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## APPLICATION NOTES



**Figure 1** | The karyotype shown indicates trisomy 21. Microarray hybridization was performed simultaneously using patient DNA and a sample from a healthy individual as reference DNA. The effect of normalization is shown by comparing the 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 (blue) and to the right (red) in the dye reversal, both indicating a gain of chromosomal material in the female patient DNA 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. The circled regions show the increase in the total genomic data and indicate the presence of an extra chromosome 21.

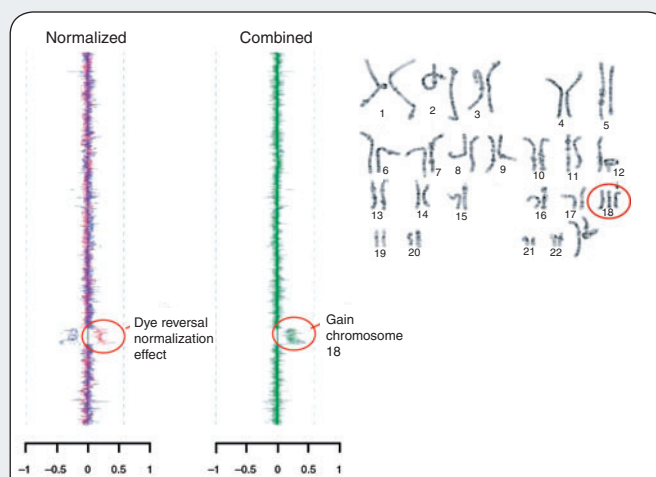
**Table 1** Quantified data for trisomy 21<sup>a</sup>

Clone	Location	Combined genomic data	Combined WGA data
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
RP11-17020	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
RP11-16B19	21:q22.3	0.369	0.276
RP11-640F21	21:q22.3	0.358	0.273
GS-63H24	21:qter	0.31	0.229

<sup>a</sup>Results for signal values after hybridization for critical diagnostic clones above the designated threshold. The threshold value for determination of loci overrepresentation in these experiments was set to 0.2 (cutoff  $\log_2$  ratios > 0.2). The targets whose signals did not meet those criteria were omitted and not presented in the tables.

some 21 (Table 1). Furthermore, the array for Edwards syndrome (Fig. 2) 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 possible test clones in the whole genome-amplified DNA sample in the region tested for chromosome (Table 2). This represents a strong indication of trisomy 18.

The data presented here demonstrate that the GenomePlex WGA kit amplifies limited amounts of DNA without bias, producing material that is suitable for use with the CMA assay, allowing for the identification of genome-wide copy number and chromosomal abnormalities.



**Figure 2** | The karyotype shown indicates trisomy 18 in an Edwards syndrome female patient DNA sample. The hybridizations and normalization of data were performed as indicated in Figure 1. The circled regions show the increase in the total genomic data and indicate the presence of an extra chromosome 18.

**Table 2** Quantified data for trisomy 18<sup>a</sup>

Clone	Location	Combined genomic data	Combined WGA data
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
RP11-193E15	18:p11.3	0.239	0.217
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
RP11-758N17	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
RP11-47G4	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
RP11-90L3	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

<sup>a</sup>See Table 1 footnote.

Additional information is available online (<http://www.sigma.com/wga>).

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