

# Application Note

## *Microarray-Based Comparative Genomic Hybridization (Array CGH): Identifying Chromosomal Abnormalities Associated with Mental Retardation and Developmental Delay*

NimbleGen oligonucleotide array CGH platform offers significant advantages over other available technologies (e.g. fluorescence *in situ* hybridization (FISH) or BAC array CGH) for the identification and characterization of DNA copy number changes associated with mental retardation and developmental delay. Specifically, NimbleGen array CGH provides:

- Ultra-high probe density – including 385,000 long oligonucleotide probes per array (and 2.1 million probes coming soon) – enabling genome-wide screening with a median probe spacing of 6kb.
- Comprehensive whole-genome tiling-path designs – covering genic and intergenic regions – that are not limited to loci represented by SNP arrays.
- Flexibility to analyze specific regions of interest at ultra-high resolution – down to 50 - 100bp resolution using NimbleGen custom fine-tiling arrays.
- Multiplex formats available to enable lower cost analysis of samples.

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### Introduction

Mental retardation (MR) and developmental delay (DD) are estimated to occur at 1 - 3% incidence in the general population, although the specific cause of MR/DD is unknown in the majority of affected individuals (1). Chromosomal abnormalities constitute the most frequently cited cause of MR/DD and have been identified in 15 - 40% of patients with severe MR/DD. Chromosome analysis in patients by conventional cytogenetic techniques (i.e. karyotyping) remains an instructive tool in the diagnosis of MR/DD. However, standard karyotype analysis is limited to a resolution of 5 - 10Mb.

Growing evidence suggests that submicroscopic chromosomal rearrangements not detectable by routine karyotype analysis constitute a significant and under-diagnosed cause of MR/DD (2, 3). Molecular cytogenetic techniques, including FISH, have been used to detect submicroscopic (<5Mb) genomic rearrangements of the subtelomeric regions in ~5% of patients with mental retardation (4, 5, 6). Although FISH offers improved resolution (30 - 200kb) over routine karyotype analysis, its application is limited to a small number of loci. Thus, it is generally useful only when a patient's phenotype suggests a particular disorder and/or genomic alteration.

Microarray-based comparative genomic hybridization (array CGH) has emerged as the method of choice for detecting submicroscopic chromosomal imbalances in patients with disorders such as MR/DD (2, 7). Array CGH enables genome-wide screening at a resolution not achievable using cytogenetic methods and provides the ability to directly correlate chromosomal abnormalities to genomic sequence. In array CGH, genomic DNA isolated from reference (control) and test (patient) samples are differentially labeled with fluorescent dyes, competitively hybridized to glass slides arrayed with either cloned DNA fragments (e.g. BAC clones) or oligonucleotides, and analyzed for copy number variation between the two genomes. It is estimated that BAC array CGH has the potential to detect twice as many chromosomal abnormalities in MR/DD patients compared to routine karyotype analysis (2, 3).

## Array CGH Offers Improved Detection of Submicroscopic Chromosomal Rearrangements

A number of recent studies illustrate the importance of array CGH for the identification of submicroscopic chromosomal abnormalities in MR/DD patients. Using genome-wide 1Mb resolution BAC microarrays, copy number abnormalities were identified in several cohorts of cytogenetically normal individuals with unexplained MR/DD (8, 9, 10, 11, 12). More recently, Veltman and colleagues employed a whole-genome tiling-path microarray comprised of 32,447 BAC clones to screen 100 individuals with normal karyotype and unexplained mental retardation (2). Copy number abnormalities considered to be clinically relevant were present in 10% of evaluated patients.

To date, the majority of array CGH studies designed to identify chromosomal abnormalities in MR/DD patients have utilized BAC microarrays. Without a doubt, BAC array CGH offers significant advantages over conventional cytogenetic techniques. However, this technique also has several major limitations (13, 14, 15):

- The resolution of BAC microarrays is limited to the size of individual genomic clones (~100kb) (2, 16). BAC microarrays have detected chromosomal imbalances from 0.5 - 15Mb in size; however, other techniques have identified smaller deletions and duplications in MR/DD patients (3).
- BAC array CGH is vulnerable to mapping inaccuracies and cross-hybridization of individual BAC clones. Specifically, Bejjani and Shaffer recently reported that 7% of BACs currently used for CGH microarrays map to an incorrect location and 16% hybridize to multiple loci in the genome (17).
- Tiling-path BAC microarrays (~32,000 clones) are not readily available to most researchers, especially those without access to dedicated microarray facilities.

## NimbleGen Oligonucleotide Array CGH Platform

Oligonucleotide-based array CGH overcomes the limitations inherent in BAC array CGH and is rapidly becoming the preferred platform for identifying submicroscopic chromosomal abnormalities in MR/DD patients (3, 18). NimbleGen oligonucleotide-based array CGH platform offers unprecedented resolution and flexibility in experimental design. NimbleGen whole-genome CGH arrays consist of oligonucleotide probes tiled through both genic and intergenic regions with a median probe spacing of 6kb, providing unbiased analysis of genomic structure down to ~25kb resolution (~5kb genome-wide resolution coming soon with 2.1M feature microarrays). Further, chromosomal aberrations identified by whole-genome array CGH analysis (or FISH and karyotype) can be mapped at even greater resolution (down to 50 - 100bp) using NimbleGen fine-tiling arrays.

The key features of NimbleGen microarrays – long oligo probes and ultra-high density (385,000 features per array now, with 2.1 million features by mid-2007) – are made possible by NimbleGen Maskless Array Synthesis technology (19), which uses digital light processing and rapid, high-yield photochemistry to synthesize DNA. The use of long oligo probes (e.g. 50 - 75mer) provides better signal-to-noise ratios and specificity compared to short 25mer oligo microarrays. NimbleGen probes are designed according to melting temperature rather than nucleotide length, providing enhanced probe performance and greater reliability and reproducibility of data between arrays. Combined with our exclusive, ultra-high density probe format and unbiased sample processing, these characteristics ensure the most comprehensive, high-definition, flexible, and cost-effective array CGH platform currently available for whole-genome analysis.

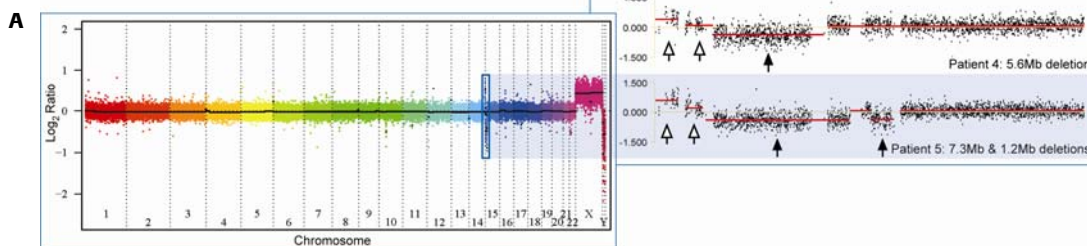
## NimbleGen Ultra-High Tiling-Path Array CGH Identifies Novel Microdeletions

NimbleGen ultra-high resolution tiling-path array CGH has been used to identify novel and clinically relevant microdeletions and duplications associated with MR/DD. In one study, Snyder, Weissman, and colleagues used NimbleGen fine-tiling array CGH (85bp median probe spacing) to fine-map the chromosomal breakpoints in patients with known copy number variants in chromosome 22 (20). These patients included those with Cat-Eye syndrome, Emanuel syndrome, Dup22 syndrome, and DiGeorge syndrome. Importantly, high-resolution array CGH revealed distinct chromosomal breakpoints (affecting at least seven known and seven predicted genes) among patients whose chromosomal abnormalities had been indistinguishable by other methodologies.

In another study, Eichler and colleagues used a custom fine-tiling multiplex microarray format (two patients per microarray) containing 166,000 long oligo probes (131bp median probe spacing) to identify a 478kb region (17q21.31), which contains six genes and was deleted in four individuals with MR/DD of unknown etiology and normal karyotype. Data from this study suggest a frequency of 1% for this deletion in patients with mental retardation. NimbleGen fine-tiling arrays were used to fine-map the 17q21.31 deletion breakpoints, which occurred within large clusters of flanking segmental duplications. In this study, four

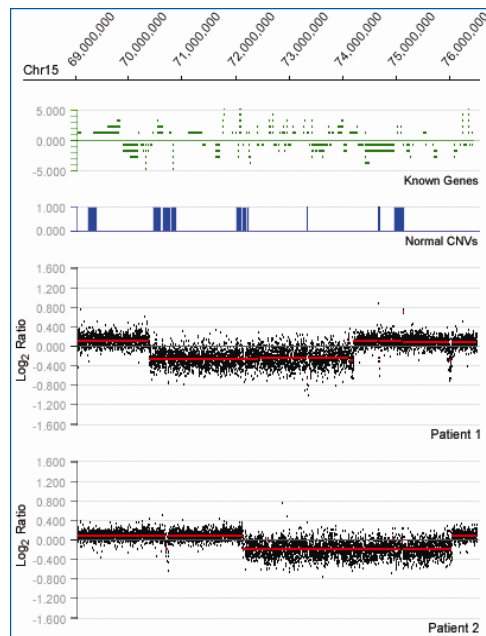
additional pathogenic microdeletions identified in MR/DD patients also mapped to regions of segmental duplication, suggesting that this class of copy number variants may represent sites of frequent genomic rearrangement (18).

**Figure 1. Whole-Genome Array CGH.** (A) Whole-genome array CGH (6kb median probe spacing) was used to identify normal (23, 24) and pathogenic copy number variants (CNVs) in a cohort of patients with mental retardation. Data are displayed as a  $\log_2$  ratio plot of probe intensity versus genomic location. Chromosomal gains and losses are indicated by upward and downward shifts in  $\log_2$  ratio value, respectively (e.g. regions of gain and loss on chromosome 15 are highlighted by the blue box). (B) A region with CNVs in chromosome 15 (blue box in panel A) is displayed in zoom view in Roche NimbleGen SignalMap software. Pathogenic chromosome 15 deletions (Angelman/Prader-Willi region) were identified in Patients 4 and 5 (closed arrows). Interestingly, these deletions are flanked by presumably normal CNVs (open arrows), annotated in the Database of Genomic Variants (<http://projects.tcag.ca/variation/>). Patients 1 - 3 have deleterious chromosome imbalances elsewhere in the genome (data not shown).



**Figure 2. High-Resolution Mapping of Chromosomal Imbalances with Fine-Tiling Array CGH.** Chromosomal abnormalities in two patients with a common developmental disorder were analyzed by ultra-high resolution tiling-path array CGH (18). Two large chromosome 15 deletions, shown in the bottom two tracks, are visualized in Roche NimbleGen SignalMap software with annotation tracks indicating known genes and normal copy number variants (CNVs) in the top two tracks. Regions of deletion were determined by copy number segmentation analysis of the  $\log_2$  ratio values (demarcated by red lines). It is noteworthy that one of the breakpoints in both patients (~74 and ~76Mb) do not coincide with previously reported CNVs (<http://projects.tcag.ca/variation/>) and would have been difficult to detect without the ultra-high resolution breakpoint mapping and CNV detection capabilities unique to NimbleGen array CGH platform.

With high-resolution array designs, DNA breakpoints are validated with ease by the selection of PCR primers that flank the identified breakpoints. Other approaches such as tiling-path BAC arrays or lower density cDNA and oligonucleotide arrays lack the resolution to fine-map breakpoints to this level of resolution. NimbleGen ultra-high resolution array CGH mapping technology can provide further insight into clinical phenotypes by mapping breakpoints down to exon-level resolution.



In fact, fine-tiling array CGH analysis of control individuals (e.g. the parents) revealed extensive copy number variation of the flanking segmental duplications associated with all five pathogenic loci (18), providing further evidence that these regions may be prone to genomic instability. Large-scale DNA copy number variation has recently been discovered (21, 22) and is now recognized as an inherent and important property of the normal human genome (23, 24). Thus, in accordance with other recent findings (25), the authors suggest that the intrinsic genomic architecture of such regions may have predictive value for the frequency of *de novo* pathogenic rearrangements resulting in MR/DD.

## Conclusion

NimbleGen array CGH platform offers several key advantages over other available technologies for analysis of DNA copy number variation, such as the detection of chromosomal rearrangements in patients with MR/DD. Unique capabilities of NimbleGen microarrays enable genome-wide screening at a resolution necessary to

detect small chromosomal imbalances often associated with MR/DD. In addition, NimbleGen array CGH platform offers the opportunity to define the genomic architecture of pathogenic loci at unprecedented resolution. This type of analysis could provide new insights into disease mechanisms, perhaps explaining phenotypic differences between patients with cytogenetically identical abnormalities, and could lead to improved patient management by revealing predispositions to *de novo* genomic rearrangements and/or associated disorders.

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## For More Information

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05227798001 • Reprinted 06/08 • Original Publication 03/07



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