GPDx
Genomic Precision Diagnostic

Igenomix®
WITH SCIENCE ON YOUR SIDE

Chromosomal MicroArray (CMA)

1. Methodology

Chromosomal microarray analysis (CMA), also known as array CGH is a diagnostic test that can detect clinically significant large (whole chromosome) and sub microscopic (microdeletion/microduplication) copy number changes throughout the genome, which are too small to see under a microscope but may contain multiple genes. Chromosomal microarray analysis is the gold standard for the detection of large deletions and duplications along the whole genome.

CMA 750K: Deletions smaller than 50 kb and duplications smaller than 400 kb may not be reviewed.
Using an Affymetrix CytoScan™ 750K array. This microarray consists of 750K oligonucleotide probes across the genome, including 550K unique non-polymorphic probes, and 200K bi-allelic SNP (single nucleotide polymorphism) probes.

CMA-HD: Deletions smaller than 25 kb and duplications smaller than 200 kb may not be reviewed.
Using an Affymetrix CytoScan™ HD array. This microarray chip consists of 2.6M oligonucleotide probes across the genome, including 1,953K unique non-polymorphic probes, and 750K bi-allelic SNP (single nucleotide polymorphism) probes.

Data was analyzed using Chromosome Analysis Suite (ChAS). The analysis is based on the Human reference genome (GRCh37/hg 19).

Detected copy number variations (CNVs) are reported when found to have clear or suspected clinical relevance; CNVs devoid of relevant gene content or reported as common findings in the general population may not be reported. Regions of homozygosity are reported when a single LCSH is greater than 8-15 Mb (dependent upon chromosomal location and likelihood of imprinting disorder), or when the total autosomal LCSH proportion is greater than 3% (only autosomal LCSH greater than 3 Mb are considered for this estimate). Genomic linear positions are given relative to NCBI build 37 (hg19).

CytoScan HD array	CytoScan 750K
Copy number probes (1.9 million) + SNP (750 K)	Copy number probes only (550K) + SNP (200 K)
Whole genome coverage	Emphasis on clinically relevant regions
Can detect regions of low heterozygosity, uniparental disomy (UPD), low level mosciasim and sample heterogeneity	Identification of regions of excessive homozygosity indicating UPD, may suggest consanguinity and determine candidate genes for further testing.
Highest probe density	

Test results are interpreted based on the recommendations and guidelines of International Standard of Cytogenomics Arrays (ISCA) as described below:

Positive (Pathogenic and likely pathogenic): A positive result indicates that a copy number variant has been identified in association with the disease phenotype under study. This scenario will allow to provide genetic counselling or personal guidance regarding possible medical treatments, disease progression, reproductive-/prevention-strategies and potential implications for other family members.

Negative: A negative result indicates that no disease-causing copy number variation was identified in the test performed. This does not guarantee that the induvial will be healthy or free from other genetic disorders or medical conditions. Additionally, a negative result does not rule out a genetic cause of the disease nor does it eliminate the risk for future offspring. However, if a negative test result is obtained and the variant in question is known to be present in affected family members, this then rules out a diagnosis of that genetic disorder in the proband. A negative result may be explained by several causes, including limited genetic knowledge and limitations associated to the used methodology.

Inconclusive/Variant of Uncertain Significance (VUS): A finding of a variant of uncertain significance indicates that a copy number variation was detected, but it is currently unknown whether that CNV is associated with a genetic disorder or disease. A variant of uncertain significance is not the same as a positive result and does not clarify whether the proband is at an increased risk to develop a genetic disorder or disease. The change could be a normal genetic variant, or it could be disease-causing. Further analysis may be recommended, including testing both parents as well as other affected and unaffected family members. Sometimes, performing ancillary tests is necessary to prove the

phenotype that the proband presents with. Detailed medical records or information from other family members also may be needed to help clarify the result.

Result interpretation is based on currently available information in the medical literature, research, and scientific databases. Because the literature, medical and scientific knowledge are constantly changing, new information that becomes available in the future may replace or add to the information that Igenomix used to interpret the results. Re-analysis of the results in previously issued reports considering new evidence is not routinely performed but is available upon request.

Applications

CMA can detect:
- Small chromosomal microdeletions and duplications
- Copy number variations
- Numerical chromosomal aneuploidy
- Unbalanced rearrangements
- Excessive homozygosity – platform dependent
- Suggestive risk of inheriting recessive disease or imprinting disorders – platform dependent
- Triploidy and tetraploidy – platform dependent
- Mosaicism greater than 20-30%

Limitations

CMA cannot detect:
- Balanced chromosomal rearrangements (balanced translocations, inversions)
- Small changes in the sequence of single genes (point mutations)
- Tiny duplications and deletions of DNA segments within a single gene (Fragile X syndrome, for example)
- Methylation alterations
- Mosaicism less than 20%

2. Sample requirements and TAT

The following sample types are accepted for Igenomix genetic tests. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number.

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	2-3 weeks	EDTA tube	3mL	Room temperature
CVS	2-3 weeks	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	2-3 weeks	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	2-3 weeks	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm3 (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	2-3 weeks	In a sealed eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples