Chromosomal microarrays: BREAKING BARRIERS in genomic karyotyping

Classical karyotyping using G-banding is an important technique for pre- and postnatal testing to detect genetic anomalies.

It creates a visible karyotype by staining condensed chromosomes to detect chromosomal abnormalities such as rearrangements, duplications, deletions, and insertions. G-banding only offers limited resolution, and many aberrations associated with congenital diseases cannot be detected using this method.

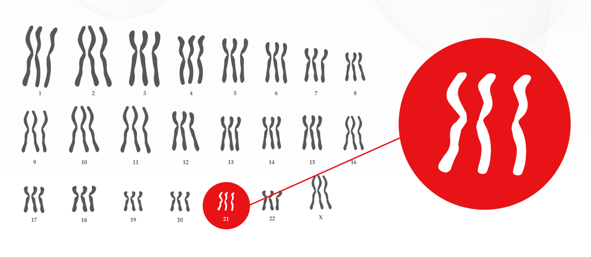
Chromosomal microarray analysis (CMA) is a powerful alternative method to examine genetic material. It is based on having a large assembly of DNA fragments spotted onto a solid surface.

How does CMA work?

In short, CMA works by distributing a test sample of fragmented and fluorescently labeled DNA over an array of immobilized known genetic sequences. If the sequences are complementary, the test DNA fragments will hybridize to the immobilized sequences and become immobilized themselves. The fluorescence signals in specific positions (spots) on the microarray reveal what sequences are present in the test DNA, based on the known sequence identities of the DNA immobilized in each spot.

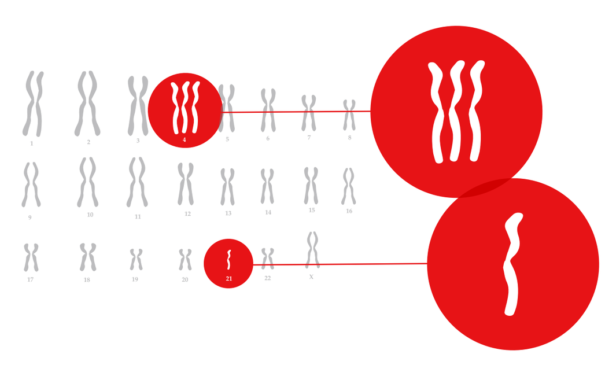
Genetic variations

Among the numerous types of genetic variations and anomalies, some do not cause any disease while others may prove lethal in the early stages of life.



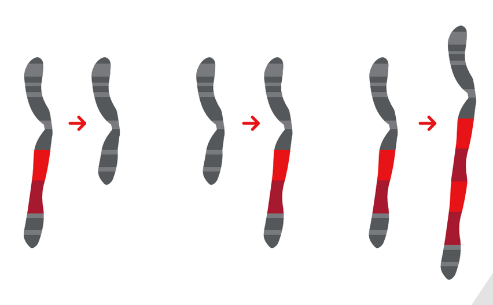
Polyploidy

Cells have more than two sets of chromosomes, a condition that is not compatible with life in humans.



Trisomy or monosomy

The number of copies of a particular chromosome is either one more (trisomy, a total of three) or one less (monosomy, a total of one) than the usual two copies. In some cases, human life is possible with these anomalies. However, they may lead to serious impairments.

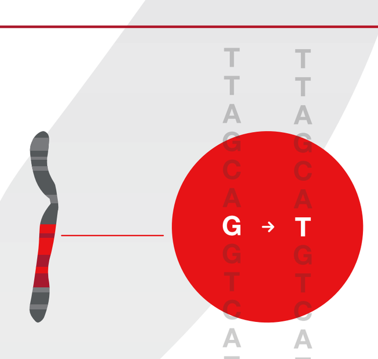


Deletion, insertion, or duplication

Parts of a chromosome are missing, inserted, or duplicated. Gene deletions or duplications lead to copy number variation (CNV).

Inversion or translocation

A part of a chromosome is either inverted or found in a different locus, sometimes even on a different chromosome.



Single-nucleotide polymorphism (SNP)

Incorporation of a change in a single nucleotide leads to a shift to another base, or its deletion or duplication. Many congenital diseases are caused by SNPs.

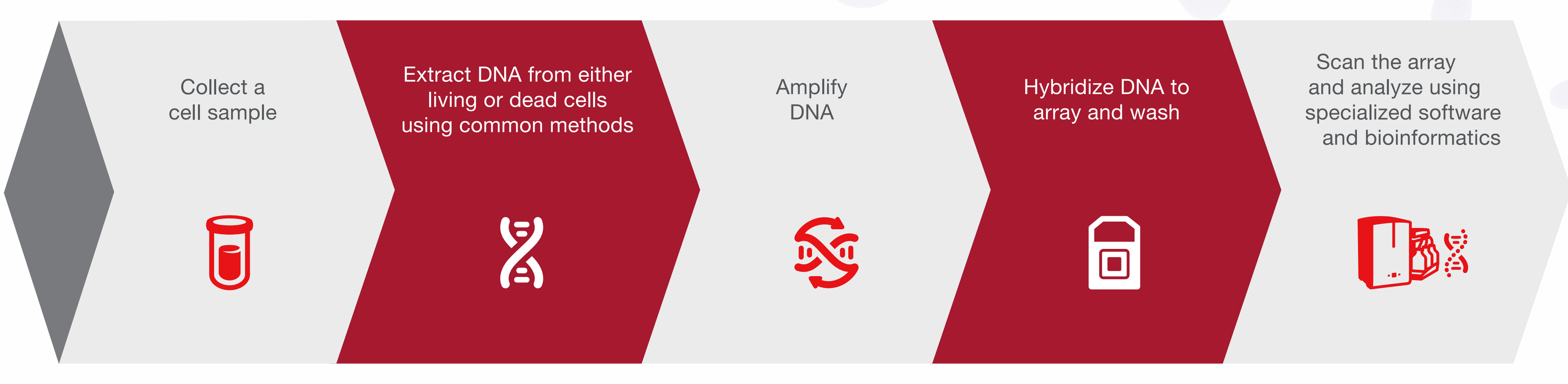
Karyotyping versus CMA

G-banding workflow

Classical G-banding usually follows these steps:

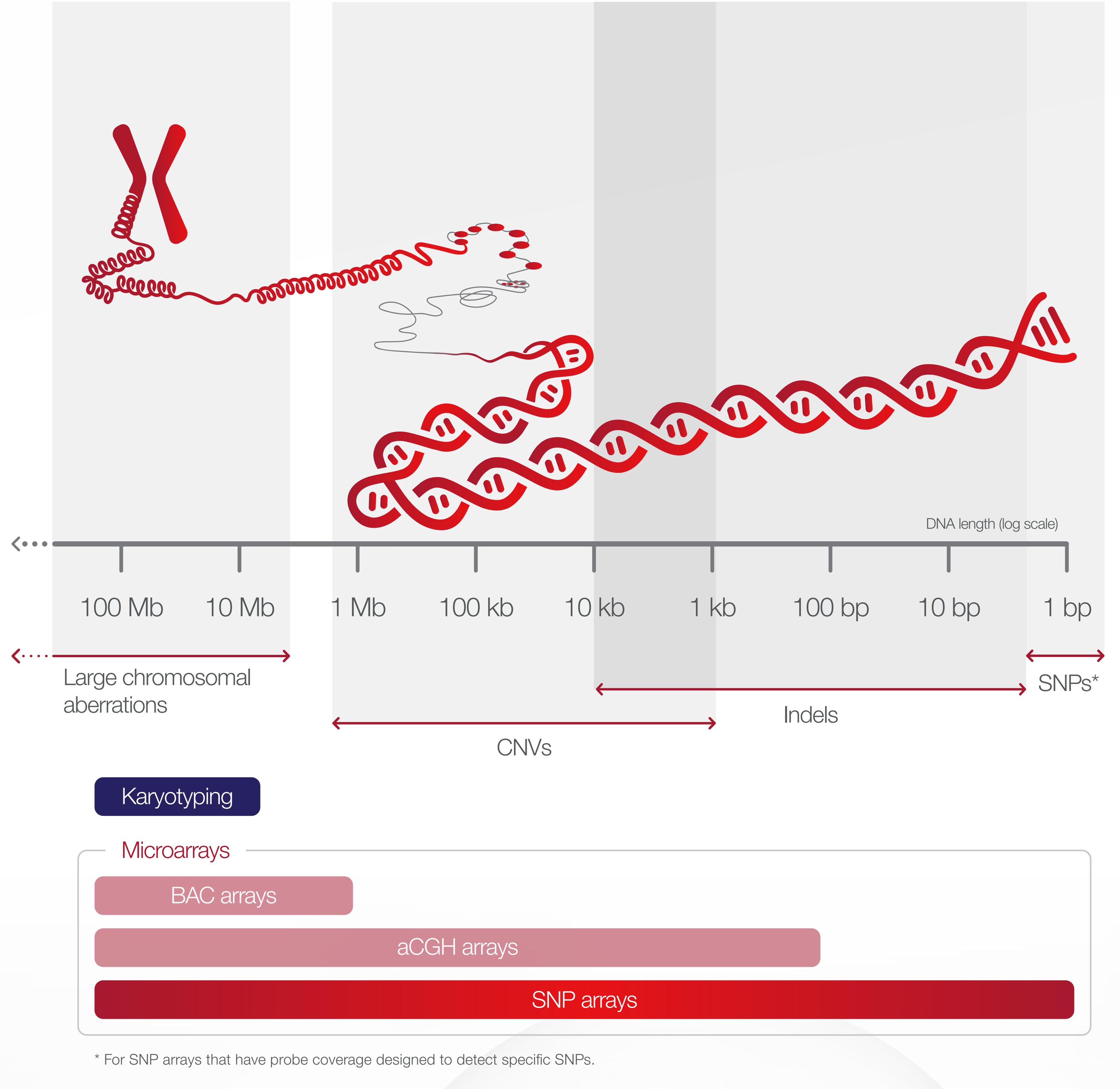
CMA workflow

CMA follows these steps:



Performance comparison

- **G-banding** provides a whole-genome analysis by visual inspection of the number and structure of chromosomes. The resolution is limited to one's visual interpretation under the microscope. Thus, the results reported can be subjective.
- **G-banding** detects aneuploidies, structural rearrangements, large deletions, and large duplications. Chromosomal imbalances smaller than 5–7 Mb are usually considered to be beyond the detection limit (resolution) of traditional karyotyping.
- **CMA** offers much higher resolution with the ability to detect extremely small aberrations, including micro insertions or deletions (indels) and single-nucleotide polymorphisms.
- **CMA**, depending on the array type, can detect either CNVs only or CNVs and SNPs.



* For SNP arrays that have probe coverage designed to detect specific SNPs.

CMA types

- **Bacterial artificial chromosomes (BACs)** were the first type of CMA, introduced in 2003. They do not offer as high resolution as aCGH or SNP arrays.
- **Array comparative genomic hybridization (aCGH)** mixes test and reference DNA, which competitively bind to the array. Most aCGH arrays detect CNVs only.
- **Single-nucleotide polymorphism (SNP) arrays** do not require reference DNA and have the potential to query for higher numbers of SNPs and CNVs at much higher resolution, i.e., down to single-base pair resolution in key genomic regions.

To learn more about the different CMA types, **read our white paper**.

Benefits of CMA

Analyzing genetic material with CMA has key advantages over traditional G-banding:

1. **Faster workflow:** CMA does not require cells to be cultured for 3 days, and they do not have to be in a particular phase of the cell cycle or show good chromosome morphology. Instead, DNA can be extracted from live or dead cells and amplified within a short period of time. CMA also eliminates the potential of maternal cell contamination, which can be a problem with karyotyping.
2. **Additional types of detection:** The higher resolution and probe density of CMA allow for the detection of CNVs and SNPs.
3. **Higher diagnostic yield:** CMA has a higher information yield and is more cost-effective than G-banding.
4. **Easier analysis and better cost efficiency:** G-banding requires highly experienced specialists for visualization and analysis. CMA, on the other hand, uses an imager and bioinformatics software for visualization and data analysis.
5. **Recommended by ACOG and ACMG:** CMA is recommended by the American College of Obstetricians and Gynecologists (ACOG), the American College of Medical Genetics (ACMG), and other clinical organizations.

Case studies

demonstrating CMA benefits

The potential clinical benefits of CMA and how much predictive value it offers compared to conventional G-banding have been analyzed in numerous case studies.

Intellectual disability—Edwards syndrome

Pinto et al. [1] investigated the case of a 4-year old child with several symptoms of Edwards syndrome (trisomy 18). While the G-banded karyotype displayed no numerical or structural karyotype deviations, CMA analysis was able to detect 4 significant genomic imbalances, one of which was a partial trisomy 18 with 40% mosaicism.

Nuchal translucency (NT)

Testing for NT uses a noninvasive method that allows for a quick and painless estimation of possible genetic defects. Submicroscopic chromosomal imbalance is associated with increased NT.

Su et al. [2] investigated the clinical application of CMA in fetuses with increased NT and normal karyotype. The researchers found that CMA improved the diagnostic yield of chromosomal aberrations for fetuses with NT of 2.5–3.4 mm and apparently normal karyotype, regardless of whether other ultrasonic abnormalities were observed.

Heart defect

Song et al. [3] looked at the utility of CMA in analyzing the genomes of fetuses with a congenital heart defect (CHD). They studied fetuses with a normal karyotype as determined by G-banding. CMA detected pathogenic CNVs (pCNVs) in 13/190 (6.84%) fetuses, likely pCNVs in 5/190 (2.63%), and variants of unknown significance (VOUS) in 14/190 (7.37%). Among those with pCNVs, none (0%) yielded a normal live birth. Among those with likely pCNVs, 2/5 (40.0%) yielded a live birth. These results highlight the usefulness of CMA for prenatal genetic diagnosis of fetuses with CHDs and normal karyotype. In fetuses with a CHD, the application of CMA could increase the detection rate of pCNVs causing CHDs.

Conclusion

Many case studies demonstrate that CMA offers much more detailed and differentiated insights into genomic variations, compared to traditional G-banding. CMA is also quicker to perform and more cost-effective. An economic analysis by Harper et al. [4] concluded that CMA, either alone or in cases of a normal karyotype, is cost-effective in the diagnosis of sonographically detected fetal anomalies. From a diagnostic perspective, CMA has the potential to provide insightful information to aid health care practitioners in bringing answers to patients and their families.

Read our white paper "Chromosomal microarrays: next-generation karyotyping assays for detecting inherited chromosomal anomalies" to learn more about CMA technology and additional case studies.

References:

1. Pinto IP et al. (2014) A non-syndromic intellectual disability associated with a *de novo* microdeletion at 7q and 18p, microduplication at Xp, and 18q partial trisomy detected using chromosomal microarray analysis approach. *Mol Cytogenet* 7:44.

2. Su L et al. (2019) Clinical application of chromosomal microarray analysis in fetuses with increased nuchal translucency and normal karyotype. *Mol Genet Genomic Med* 7:e811.

3. Song T et al. (2019) Detection of copy number variants using chromosomal microarray analysis for the prenatal diagnosis of congenital heart defects with normal karyotype. *J Clin Lab Anal* 33:e22630.

4. Harper LM et al. (2014) An economic analysis of prenatal cytogenetic technologies for sonographically detected fetal anomalies. *Am J Med Genet A* 164A(5):1192-1197.