Comparative genomic hybridization

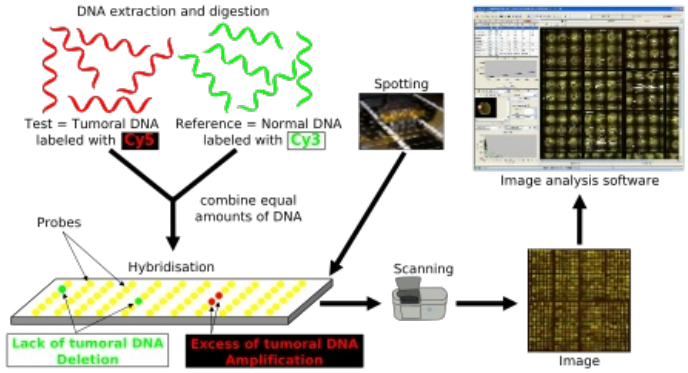
CGH (Comparative Genome Hybridization) is molecular cytogenetic method that allows the analysis of the entire genome is a single experiment. CGH is based on the principles of the FISH, n which we use 2 DNA samples (isolated from the tissue under investigation) labeled differently with fluorochromes. The use of this method is limited to the **detection of quantitative genome changes** (amplification/deletion).

Execution

Isolate **2 DNA samples**, which are subsequently labelled with two different fluorochromes using the nick-translation method.

One of the **DNA samples taken from the tissue under examination** is usually labelled in green. The other, **control (reference) DNA** is usually labelled in red. These probes are then hybridized together with normal chromosomes.

The result of the hybridisation is imaged with a fluorescence microscope, karyotyped and computerised.



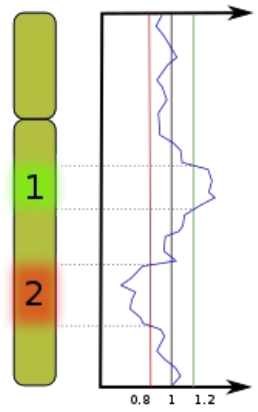
Array-CGH procedure

Evaluation

The DNA of the individual under examination and **the reference DNA** essentially "*compete*" for binding sites on the chromosome. Depending on whether a particular stretch of the DNA under investigation is amplified or deleted, the result is then dominated by the fluorochrome of the DNA under investigation or the reference DNA at different sites on the chromosome.

The computer analysis gives the fluorescence intensities of the test and reference DNA.

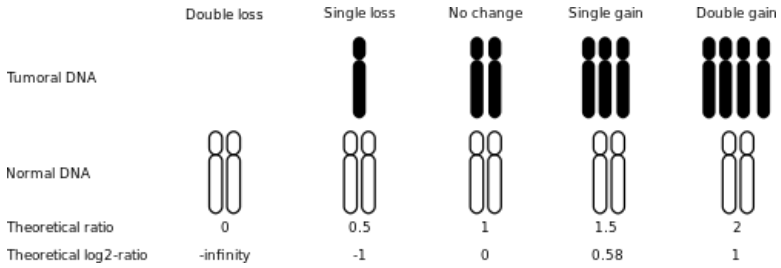
The values are arranged in a curve expressing the ratio of fluorochrome intensities over each chromosome, the so-called **CGH profile**.



CGH profile (curve)

Interpretation of results

- values equal to 1 (**chromosomes without quantitative changes**),
- values less than 0,75 (**deletion**),
- values greater than 1,25 (**amplification or duplication**).



Comparison of sample with reference DNA

Usage

The CGH method is mainly used in **oncocytogenetics** for detection of unbalanced chromosomal aberation in tumours. It allows the identification of sites in the genome where deletions or amplifications have occurred and which may thus be related to the origin or development of cancer.

Another use in assisted reproduction in the context of **preimplantation genetic screening**, kwhere puncturing of several blastocysts is possible (thus bypassing possible mosaicism). Application to all embryos may increase the success rate of assisted reproduction and reduce the number of multiple pregnancies.

In fetuses with morphological changes present on ultrasound and with a normal karyotype, CGH can provide additional diagnostic information.

Modifications of CGH

Although the classical method allows whole-genome screening without the need for culture and detection of aberrant sites without prior knowledge of their location, it encounters complications in many respects. **Limitations of conventional CGH** include the necessary presence of at least 50% aberrant cells in the sample, the inability to detect aberrations without quantitative changes, the differentiation of diploid and tetraploid tumours, and the low resolution of the method (5–10 Mb).

These shortcomings can be addressed by **modification of CGH methods** (HR-CGH, array CGH).

HR-CGH (*High Resolution CGH*)

it differs from classical CGH in the way of software processing of the captured fluorescence image. It uses **dynamic standard reference intervals** (natural variation of fluorescence intensities of chromosome pairs) instead of fixed ones. Dynamic values are obtained by statistically processing the deviations of control profiles in individuals with a normal karyotype.

The advantage of the method is the resolution at the level of **4–5 Mb** and the possibility of detecting aberrations even at **20–30%** clonal representaion.

Array CGH

Hybridization of fluorescently labelled probes of the DNA under investigation and reference DNA **with many specific DNA sequences** (instead of chromosomes). The genome is thus divided into **small sections and arranged in a grid** (*microarray*, biochip). The individual sections have a precise location and can thus represent individual genes or parts of genes.

After hybridisation with the DNA under investigation and the reference DNA, the fluorescence ratio for each grid point is calculated using special software. This makes it possible to analyse amplifications or deletions of several thousand genes at once, with a resolution of less than **100 kb**. This takes *array CGH* to the **level of molecular diagnostics**.

Links

Related articles

- Tumor cytogenetics
- Molecular cytogenetics

External links

- NCBI SKY/M-FISH & CGH Database (https://www.ncbi.nlm.nih.gov/projects/sky/)

References used

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- NEČESALOVÁ, Eva. *Use of HR-CGH method for molecular cytogenetic characterization of brain tumors* [online]. Brno, 2007, Available from <https://is.muni.cz/th/ujhk6/Disertacni___prace.pdf?so=nx>.
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