DNA microarray

DNA chip or **DNA microarray** is a technology used to analyze DNA variability and works on the principle of **allele-specific hybridization** . This means that the method is based on the principle of pairing complementary nucleotide bases (connection by hydrogen bridges). It is a plate (mostly glass or silicone) with many (usually tens of thousands, hundreds of thousands, exceptionally even millions) samples of **single-stranded DNA oligonucleotides**.

With this method, we detect changes in the DNA sequence, where it is not enough to monitor the number or length of DNA fragments, because they do not affect any recognition site or have the nature of deletion or insertion .

If we know at least partially the sequences of the normal and mutated allele , directly at the site of the mutation, we can hybridize the studied sample with a short labeled synthetic test oligonucleotide that is complementary to the region of the gene that could carry the mutation . Hybridization occurs under the given conditions (temperature, salt concentration...) only in case of perfect sequence complementarity.

The PCR method made it possible to increase the sensitivity and reliability of allele-specific hybridization. Test oligonucleotides can be hybridized not to complete genomic DNA, but to specifically amplified segments of the gene under investigation, which eliminates the complications of non-specific hybridization. Probes (test oligonucleotides) are attached to the surface of the plate by a strong covalent bond using special methods. A mixture of labeled PCR products of amplified DNA is hybridized to them.

Advantage: *simultaneous examination of a huge number of sequence variants is possible*

Disadvantage: (for now still) *high price*

What does a DNA chip look like?

Nowadays, there are several companies producing DNA chips commertially (e.g Affymetrix, Agilent Technologies, Eppendorf or Illumina). Chips are also created directly in research laboratories for their own use.

We anchor the probes of the known sequence into the glass plate. Amplified fluorescently labeled DNA under investigation is hybridized to the chip, and the hybridization of this DNA to individual positions is detected by a reading device and a computer.

2 Affymetrix chips

Method procedure

Creating DNA chip

By covalently binding the oligonucleotide (probe) to the plate.

Creating a sample

A typical example of a sample is all mRNA present in a particular cell at a particular time. The sample must first be purified ("cleaned") by e.g. electrophoresis, PCR with specific primers, then it is converted into cDNA by reverse transcription. Further, PCR amplification can take place to ensure a sufficient number of molecules of the desired types in the sample and division of cDNA molecules into shorter ones using restriction endonucleases. During or after reverse transcription, PCR amplification, the necessary step of labeling molecules takes place. Several molecules (typically approximately one for every 60 bases) of a fluorescent (or other, e.g. radioactive) substance are attached to each molecule, the presence or quantity of which can be detected later. Typical fluorescent agents used for these experiments are fluorophores. Sample molecules are denatured before application to the chip.

Hybridization of the sample with the chip

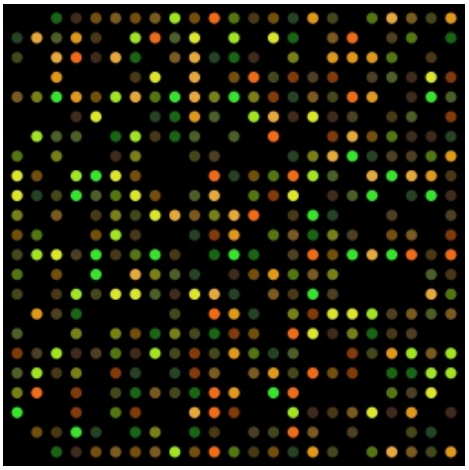
After contact with the DNA chip, the sample molecules hybridize with complementary probes.

Washing the chip

After washing, the probes remain on the chip, firmly attached by covalent bonding to its surface, and the sample molecules attached firmly enough to the probes. Sample molecules attached to the probes with an insufficient number of hydrogen bonds , i.e. insufficiently similar in sequence, are washed away.

Chip scan

Upon laser excitation , the dye molecules present on the sample molecules attached to the probes emit light of a certain wavelength. The light emitted by each property is detected.


DNA chip

Processing results

Using the previous procedure, we obtain information about which sequences attached to the chip had complementary sequences in the sample. The amount of complementary molecules present in the sample can also be determined according to the intensity of the emitted light, but usually only the relative amount is used. Therefore, the amount of light emitted by individual properties is only compared to determine the proportions in which individual sequences were present in the sample. Determining the absolute concentration of individual sequences in a sample is problematic.

Other techniques

- ARMS (amplification refractory mutation system) - if the primer does not hybridize perfectly at its 3' end, no amplification occurs at all
- LCR (ligase chain reaction)
- OLA (oligonucleotide ligation assay)

Links

Related articles

- In situ hybridization

References

- GOETZ, Petr, et al. *Selected Chapters in Medical Biology: Volume 2.* 1st ed. Prague: Karolinum, 2002. 139 pp. ISBN 80-246-0320-9 .
- Wikipedia: The Open Encyclopedia. *DNA chip* [online]. [feeling. 2012-01-22]. < https://cs.wikipedia.org/w/index.php?title=DNA_%C4%8Dip&oldid=7008557 >.
- Wikipedia: The Free Encyclopedia. *DNA microarray* [online]. [feeling. 2012-01-22]. < https://en.wikipedia.org/w/index.php?title=DNA_microarray&oldid=471860388 >