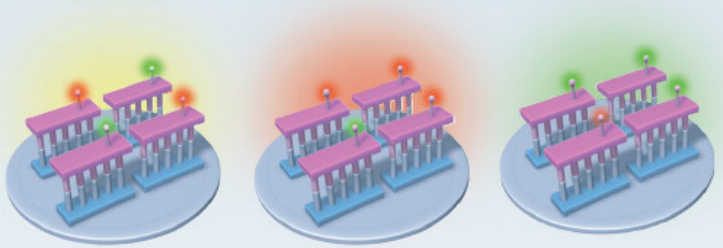
An introduction to
Array CGH



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Debate on the usefulness of PGS

- Steady increase in the number of PGS cycles
- Questions raised about PGS efficacy
- Non-randomised studies reported that PGS increases implantation rate and decreases abortion rate
- Instead, several randomised trials have shown that PGS does not improve ongoing pregnancy and live birth rates
- The debate on the usefulness of PGS is still ongoing
- Further data are required to establish whether PGS results in enhanced live birth rate
- Identification of which patients may benefit from PGS

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Why PGS is not beneficial?

- Current FISH test can effectively analyse up to 12 chromosomes
- Only about 70% of known chromosome abnormalities in spontaneous abortion are detected
- Possible reasons of the limited clinical impact of PGS:
  - Limited no. of chromosomes tested
  - Accuracy of the FISH analysis (FISH error rates, including false negatives and false positives, have been estimated around 7-10%)
  - Presence of a high rate of mosaicism in embryos at cleavage stage, that reduce the diagnostic accuracy

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The new technologies for PGS

- The limitation of the FISH technique has raised an interest towards new technologies for chromosome analysis
  - CGH
  - array-CGH
  - SNP microarrays
- The new techniques enable a complete assessment of the chromosomal constitution of preimplantation embryos
- Several trials actually are ongoing in order to evaluate if an improved pregnancy and take home baby rate outcome can be achieved with the array based technologies over current FISH technique

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CGH

- Comparative genome hybridization (CGH) is a DNA-based method capable of accurately determining total or partial aneuploidy affecting any of the 24 different types of chromosomes.
- The method combines amplification of the entire genome of the biopsied cell(s), followed by fluorescent labeling and hybridization to normal metaphase chromosomes on a microscope slide.
- The use of CGH has enabled comprehensive chromosomal screening of oocytes, polar bodies, and embryos.
- The technique has been successfully tested in human blastomeres, and babies have been born after PGD using CGH
- The main drawback of CGH is that the technique is labor intensive

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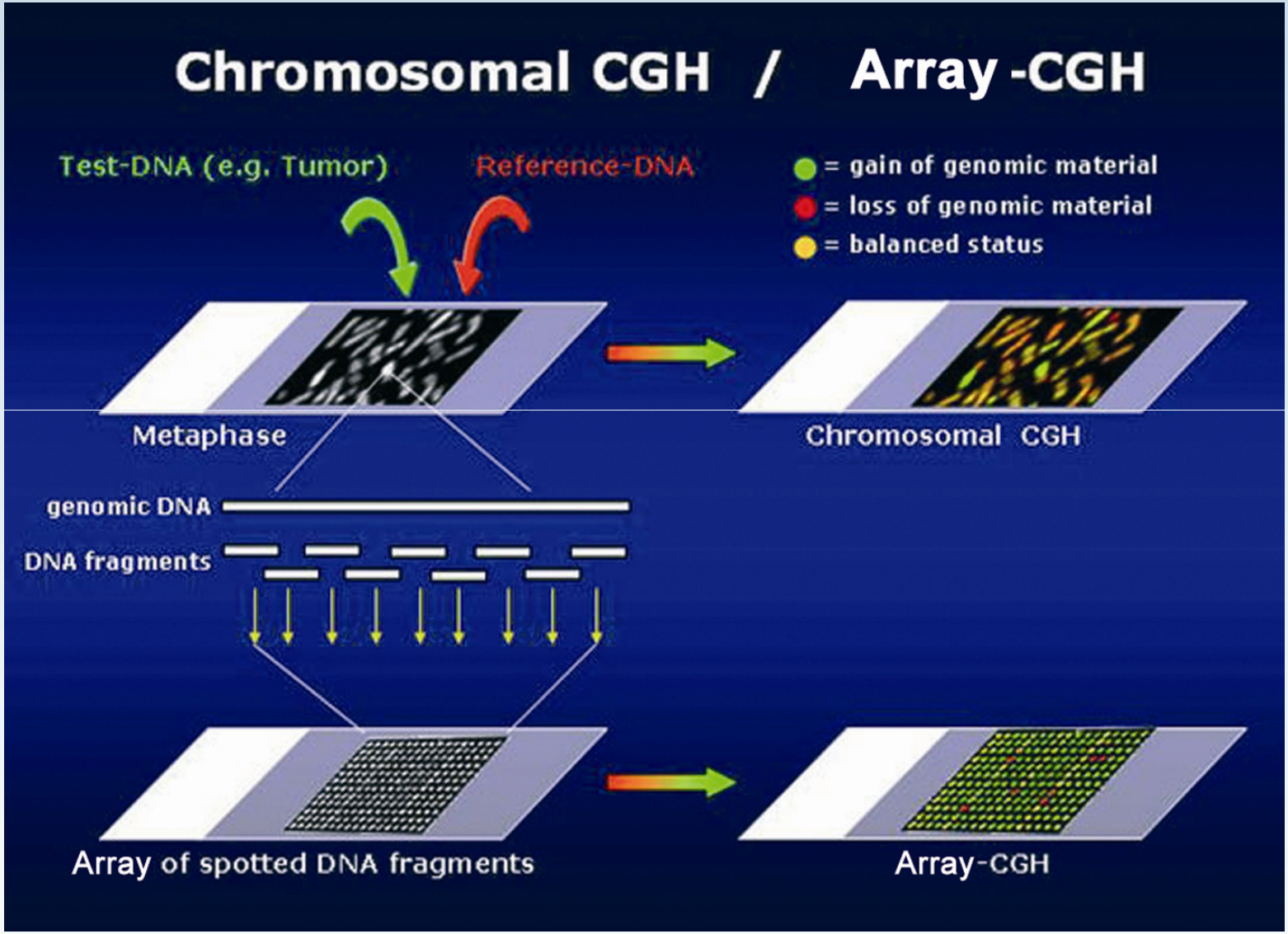
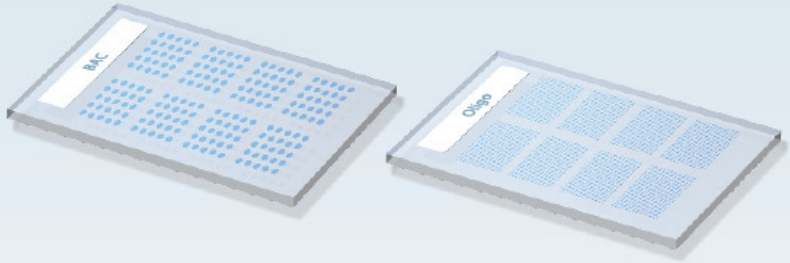
CGH vs array-CGH



Array - CGH

- Array-CGH is the evolution of CGH
- As with conventional CGH, it involves the competitive hybridization of differentially labeled test and reference DNA samples.
- the labeled DNAs are hybridized to DNA probes affixed to a microscope slide rather than metaphase chromosomes.
- Each probe is specific to a different chromosomal region and occupies a discrete spot on the slide.
- Microarrays have an advantage over conventional CGH in that the evaluation of fluorescence ratios is simple and easily automated and that the time required for hybridization is generally less.

Array Design and Technologies



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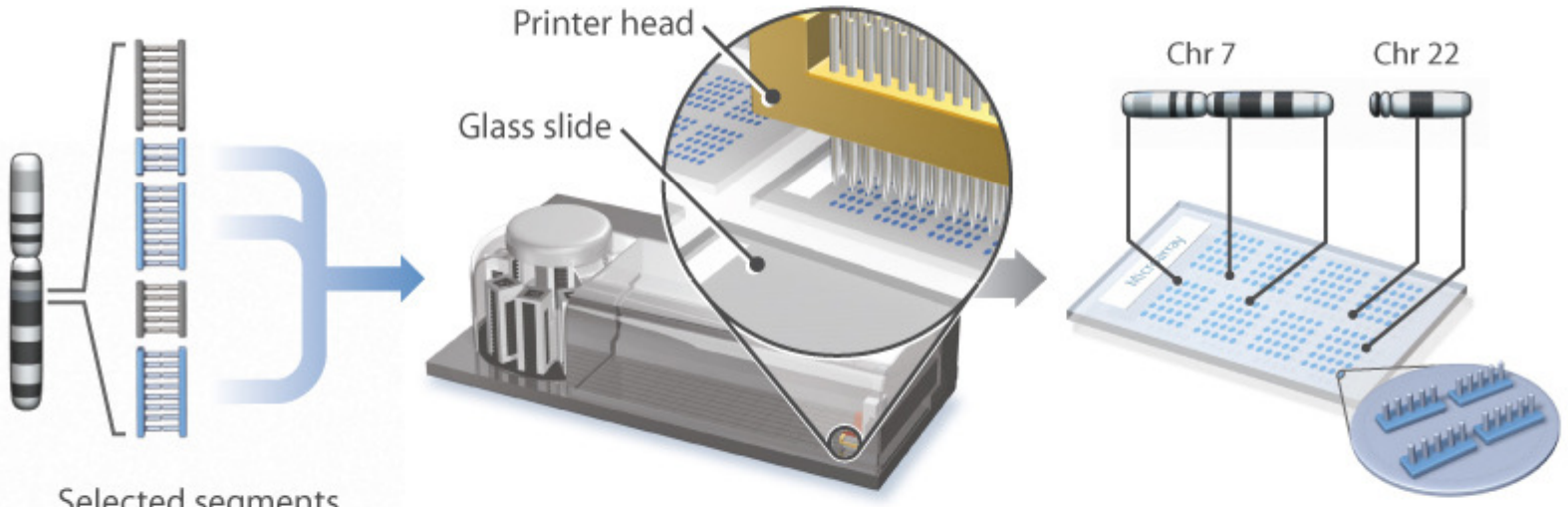
What is a Microarray?

Understanding CGH Technology

Short segments of DNA (such as bacterial artificial chromosomes, BACs) containing regions of interest are printed onto a glass slide.



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Microarrays-CGH platforms

There are two alternative microarray-CGH platforms available for chromosomal screening:

- **Bacterial Artificial Chromosome (BAC) arrays**
- **Oligo-Arrays**

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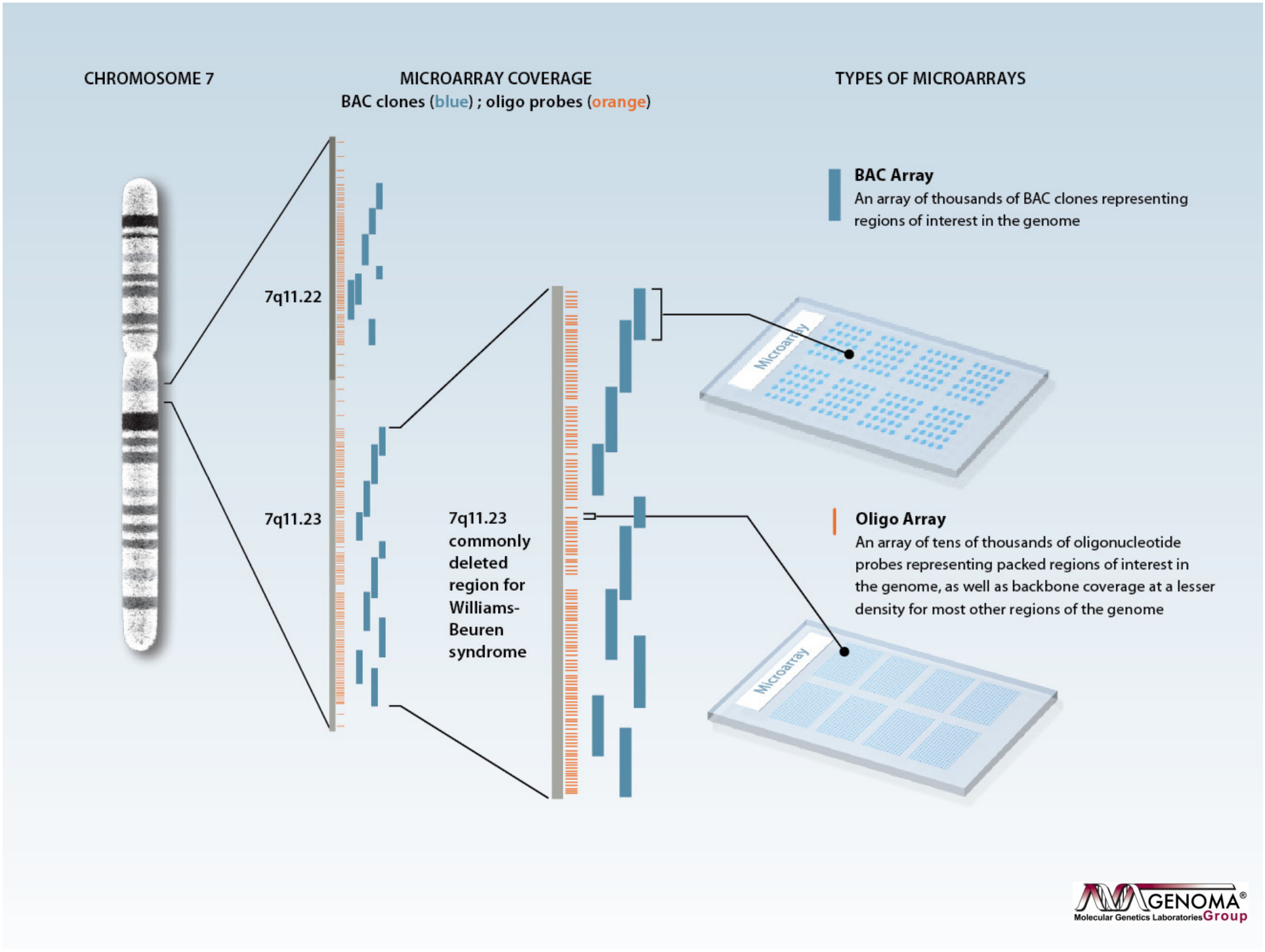
Microarrays-CGH platforms

- **Bacterial Artificial Chromosome (BAC) arrays:**
  - Consist of thousands of spots, each of which comprises DNA fragments covering relatively large fragments of chromosome (150–200 kb);
  - The main drawbacks of BAC arrays are on the manufacturing side (batch-to-batch variation in performance and problems with reproducibility).
  - Because of WGA from a single cell, some random fluctuations are expected.
    - diagnoses depend on an average fluorescence ratio obtained by pooling the data from several neighboring probes.
    - Increase of the number of probes needed to obtain an accurate result from each chromosome.

Microarrays-CGH platforms

- **Oligo-Arrays:**
  - utilize oligonucleotides, which are synthesized in situ, directly on the surface of the solid support (i.e. the slide) that forms the base for the microarray.
  - the probes typically vary from 25 to 85 nucleotides in length.
  - The main drawback of using oligonucleotide arrays is that the small size of individual probes increases the risk that artifactual losses and gains will be seen, caused by errors introduced during WGA.
  - It is possible to compensate for this problem by analyzing large numbers of probes.



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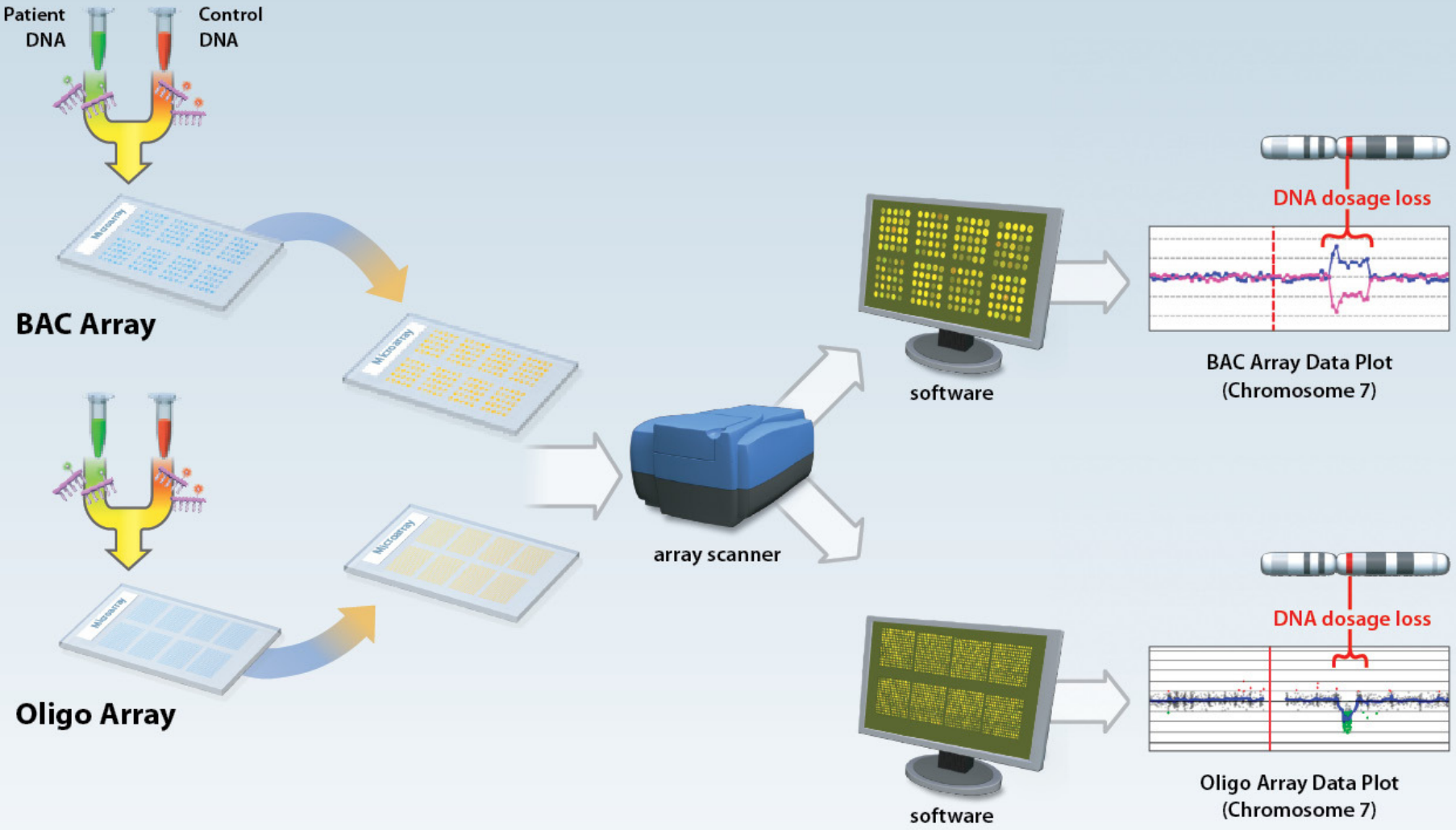

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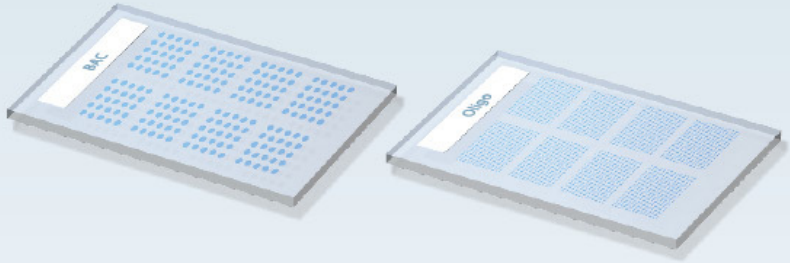
The Array-CGH process



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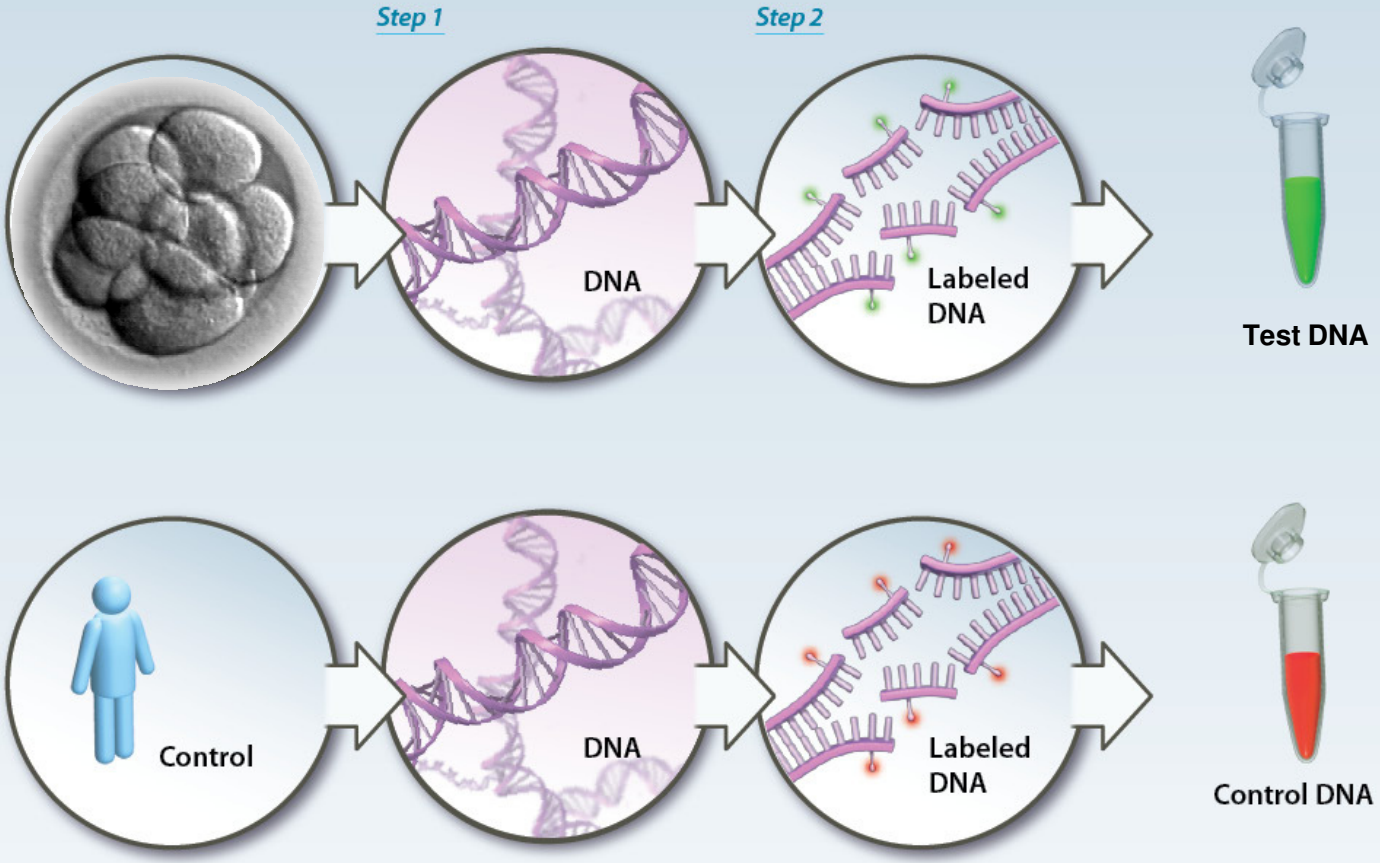
Array CGH: Sample Preparation



Both BAC and Oligo – Arrays involve a competitive hybridization of two differentially labeled samples, one derived from the embryo or polar body (test DNA) and the other from a euploid DNA sample (Control DNA).

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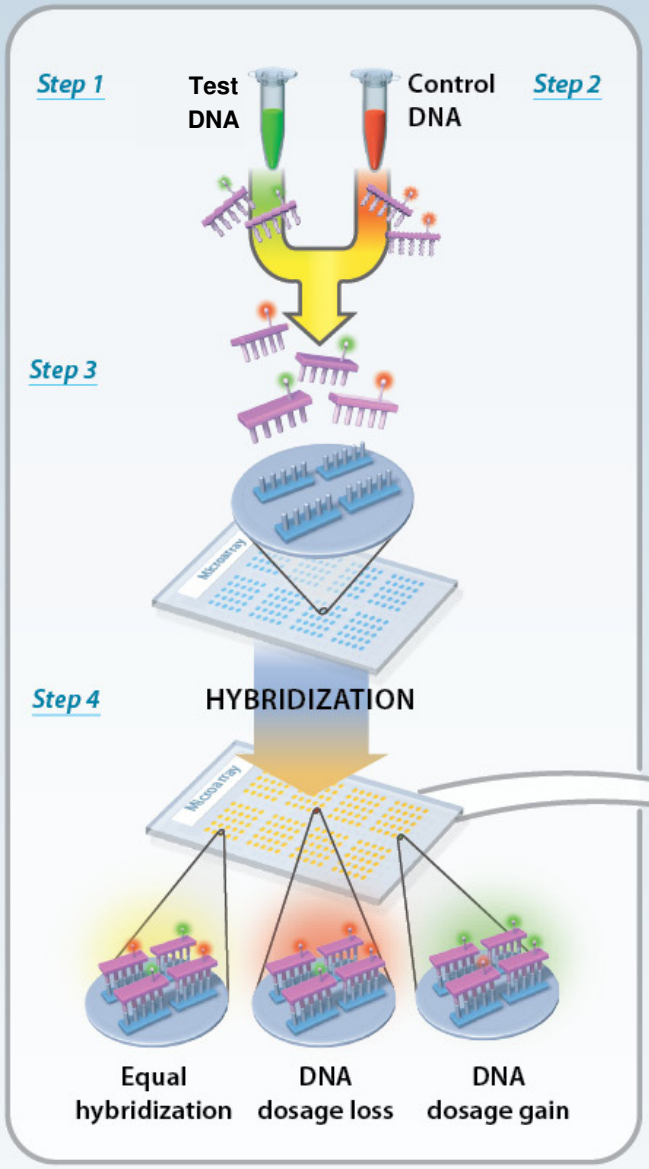
Array CGH: The Complete Process

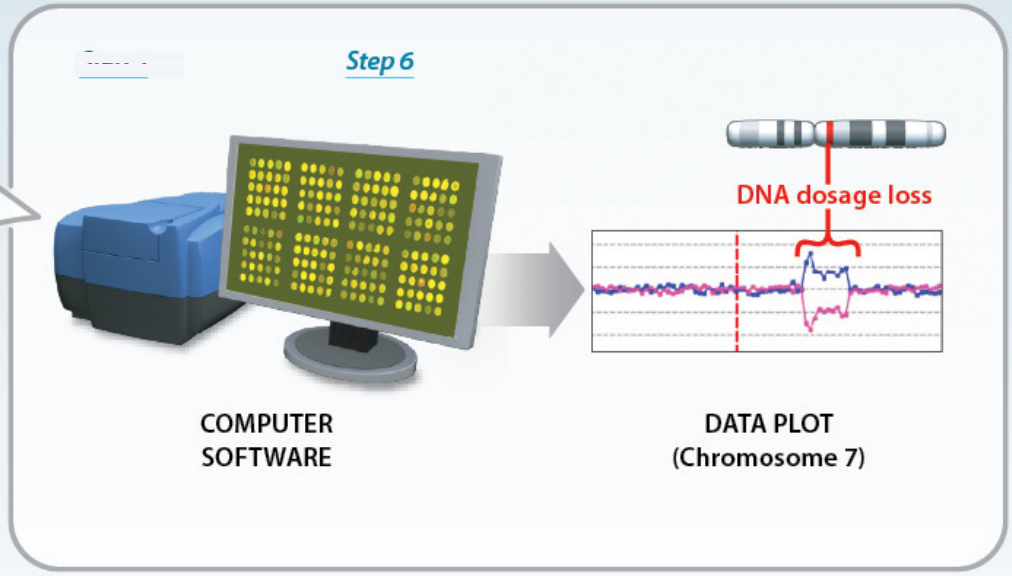


Steps 1-3 *Test DNA* and control DNA are labeled with fluorescent dyes and applied to the microarray.

Step 4 *Test DNA* and control DNA compete to attach, or hybridize, to the microarray.

Step 5 The microarray scanner measures fluorescent signal intensity.

Step 6 Computer software gathers the data and generates a plot.



YELLOW signal: Equal hybridisation of both Test and Control DNA yields a yellow fluorescent signal. There is no DNA gain or loss.

RED signal: presence of less Test DNA compared to control DNA yields a red signal. There is a DNA **loss**

GREEN signal: presence of more Test DNA compared to control DNA yields a green signal. There is a DNA **gain**

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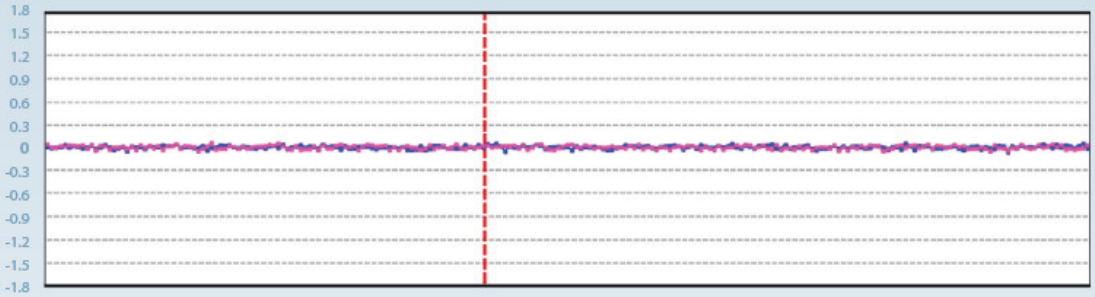
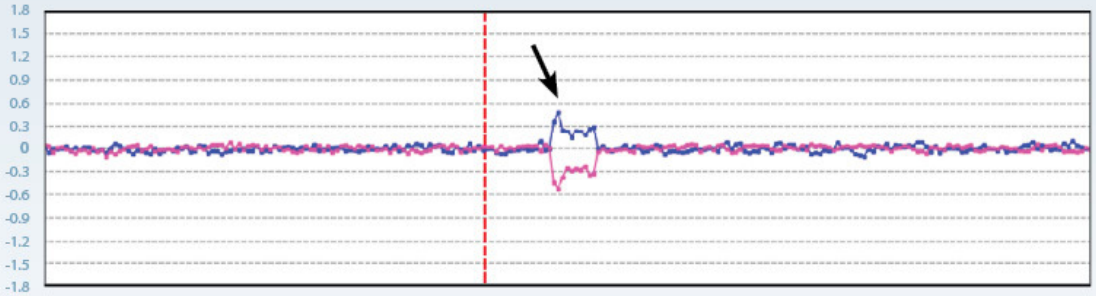
Array CGH: Possible Results

NORMAL



ABNORMAL



Normal array CGH results: For this chromosome, the plot generated shows no significant deviation of the line from the zero mark for all regions analyzed on the microarray.

Abnormal array CGH results: For this chromosome, the plot generated shows a significant deviation of the line from the zero mark for a specific region analyzed on the microarray.

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Limitations of Array-CGH

Array-CGH *cannot* detect:

- **Balanced chromosome rearrangements**
  - **Balanced translocations**
  - **Reciprocal insertions**
  - **Inversions**
- **Some polyploidies**
- **Changes in gene DNA sequences (point mutations, intragenic insertions or deletions, triplet repeat expansion, etc.)**
- **Gains or losses in regions of the genome not covered by the array**
- **Parental origin of aneuploidies**

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SNP-Arrays

- Another form of oligonucleotide array based upon the analysis of SNPs (from tens of thousands to a few hundred thousand).
- SNP-microarrays employ a different approach compared with BAC and Oligo-arrays:
  - Rather than being combined with a differentially labeled normal reference DNA (as with array-CGH), the DNA from the test sample is hybridized separately, with reference DNA samples assessed in parallel.
  - Aneuploidy is revealed by differences in the intensities obtained for test and reference hybridizations.
  - Brighter signals for the test DNA than the reference DNA are indicative of an excess of chromosomal material (e.g., trisomy), while reduced fluorescence for the test sample is associated with chromosome loss.
  - Chromosomal copy number is also obtained through the evaluation of the inheritance of the SNP haplotypes (3 for trisomies and 1 for monosomies).

Advantages of the SNP-arrays

SNP-arrays *allow* :

- **Track of parental origin of aneuploidies;**
- **The achievement of a unique DNA fingerprint for each embryo tested.**
- **To obtain data on the inheritance of specific genetic loci, including those associated with disease.**
- **The linkage-based detection of disease causing mutation by the genotyping of SNPs located in close proximity to the mutation site. This potentially opens up the possibility of using SNP-microarray platforms for the concomitant detection of chromosome anomalies and single gene disorders.**

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Which array to use?

- Each array has advantages and disadvantages
- The choice of one platform rather the other should be evaluated after extensive pre-clinical trials and pilot studies
- It remains to see which microarray approach will ultimately provide the optimal combination of accuracy, speed and cost.

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Which cell should be tested?

- Testing of single blastomeres biopsied from embryos at cleavage stage should be excluded because of the obscuring effects of mosaicism
- 1PB and 2PB analysis are possibly the least invasive technique, but some limitation should be considered:
  - Analysis of single cells (lower quality of the results)
  - Analysis of a huge amount of PBs to be analysed (a lot of unnecessary work, at which cost?)
  - Do not provide information on the paternal contribution (~30% of aneuploidies of paternal origin)
- Analysis of trophectoderm cells at the blastocyst stage coupled with vitrification:
  - Analysis of more cells (better quality of the results)
  - Obviate to the mosaicism issue
  - Information on the paternal contribution
  - May arise concerns because of the vitrification step

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Thank you……..

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