Molecular cytogenetics

FISH (fluorescent in situ hybridization)

- detection of target DNA sequences directly on chromosome preparation (in situ) using specific fluorescently labeled DNA probe, the probe is hybridized with complementary segments of the chromosomal DNA
- different modifications according sequences of interest:
  - centromeric probes – rapid counting of particular chromosome number (e.g. sex chromosomes, anuploidies)
  - locus-specific probes – microdeletion syndromes, subtelomeric regions...
  - whole-chromosome (painting) probes – translocations, insertions...
  - M-FISH (multicolor) - all chromosomes labeled by different combination of five fluorochromes to differ from each other, detection of complex rearrangements (e.g. in leukemia cells)
  - M-banding – multicolor combinations of fluorochromes along the chromosome enable to detect breakpoints in specific chromosome rearrangements; method is used especially in haematooncologic patients to help with assessment of prognosis and individual therapy indication according to their tumor subtype

CGH (comparative genomic hybridization)

- detection of quantitative - unbalanced genomic changes (gain or loss), not able to detect balanced rearrangements
- comparison of tested and control DNA (labeled with different fluorochromes, applied as probes on normal chromosomal preparation) used in ratio 1:1
- primarily developed for analysis of solid tumors

Microarrays

- molecular cytogenetic method with much higher resolution level (10-100 kb) then routine cytogenetic methods (karyotyping, 5-10 Mb)
- whole-genome analysis
- main disadvantage – method is targeted only on unbalanced changes, not able to detect balanced rearrangements
- great for detection of submicroscopic microdeletions or microduplications in patients with unexplained mental retardation and/or developmental delay
- reaction is performed on special slides („chips“) with small target region of thousands of pits with short specific chromosomal fragment in each; after reaction the slide is scanned and result from every pit is demonstrated on the chromosome map with the precise location
- data are compared with international databases and genotype-phenotype correlation with prognosis assessment should be commented in clinical report
- two basic modifications: array-CGH x SNP-array
  - **Array-CGH:** based on CGH method (above) but with higher resolution
  - **SNP-array:** based on detection of single nucleotide polymorphisms (SNPs) in the genome