

Introduction to Molecular Diagnostics

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Disclosures

- I have nothing to disclose



Outline

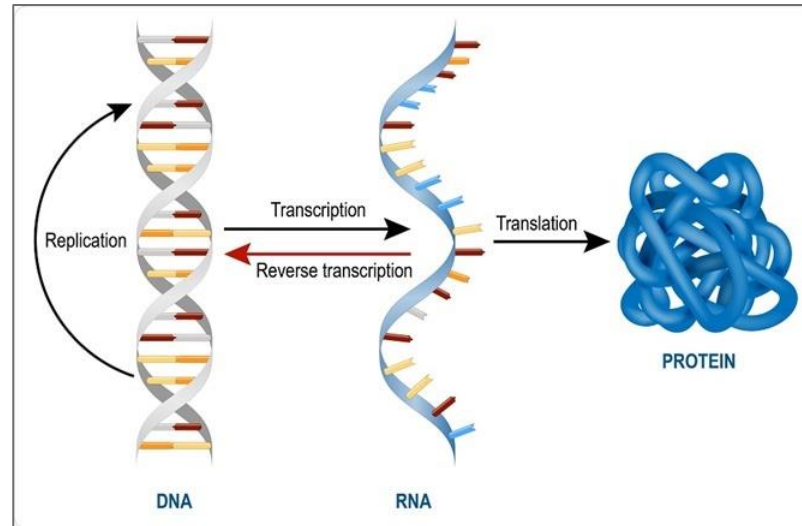
- Back to Basics
- Molecular Diagnostics toolkit
 - Karyotype
 - Chromosomal Microarray
 - Fluorescent in Situ Hybridization (FISH)
 - PCR-based methods



Back to Basics

Molecular Diagnostics

- Application of Molecular biology in patient care



S Cheriyaedath/News-Medical.net



Clinical applications of Molecular Testing

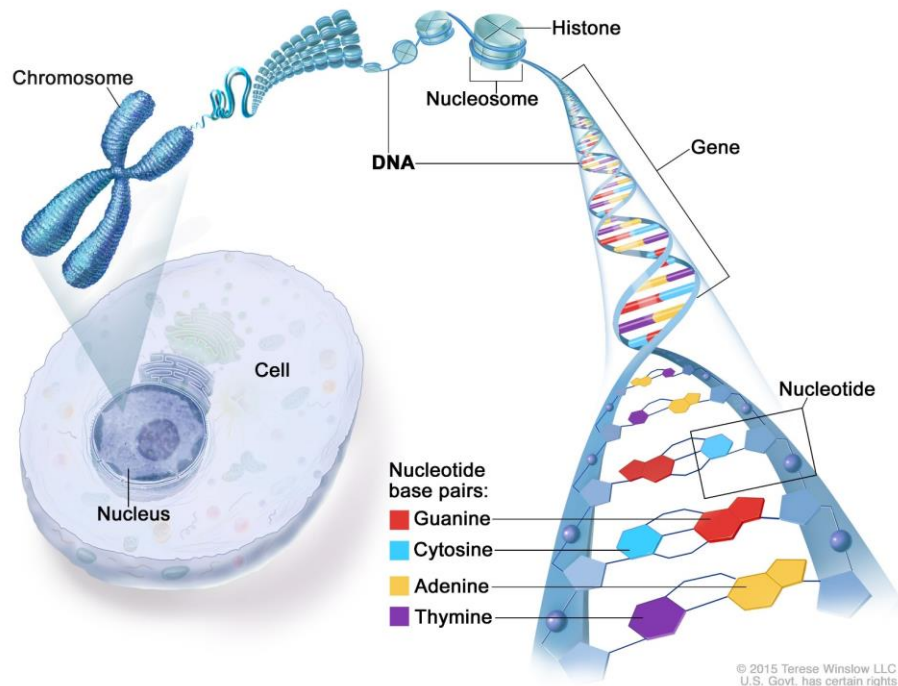
- Oncology
 - Oncogenes and tumor suppressor genes
 - Classification, prognostication, targeted treatments
- Hereditary disorders
 - Germline variants in diseases of Mendelian inheritance
- Microbiology
 - Detection and quantification of micro-organisms
 - Genetic mechanism of drug resistance

Genome

- The entire complement of DNA in an organism
 - Nuclear genome
 - Mitochondrial genome

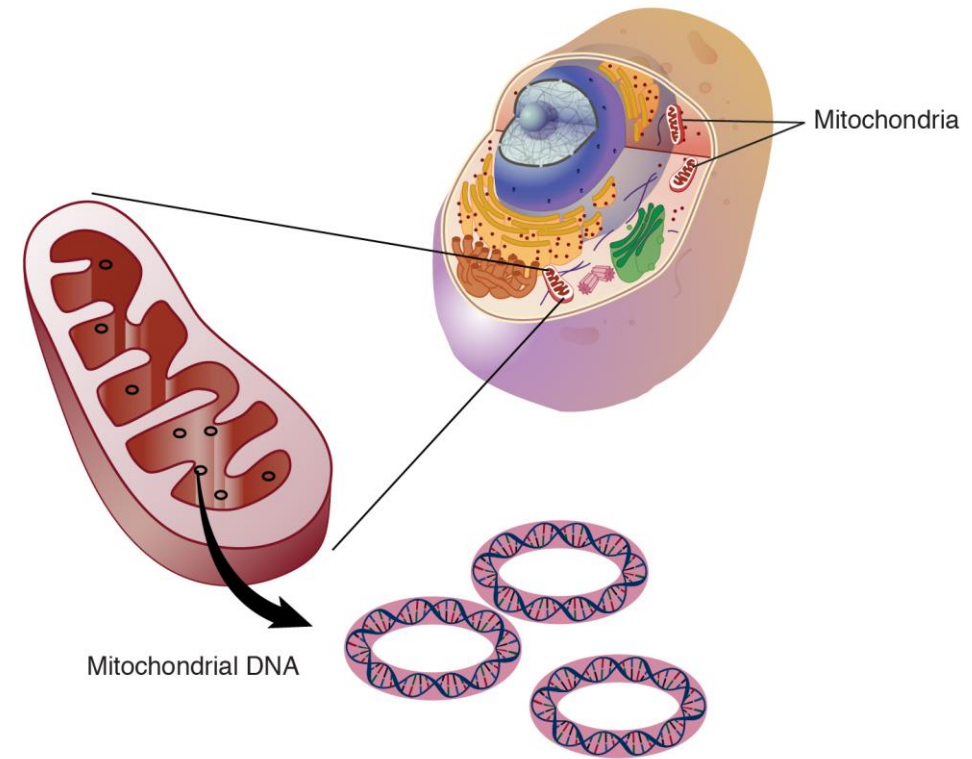
Nuclear genome

- 3.3 billion base pairs (bp)
- Spread out (unevenly) in the form of chromosomes (45-279 Mb)



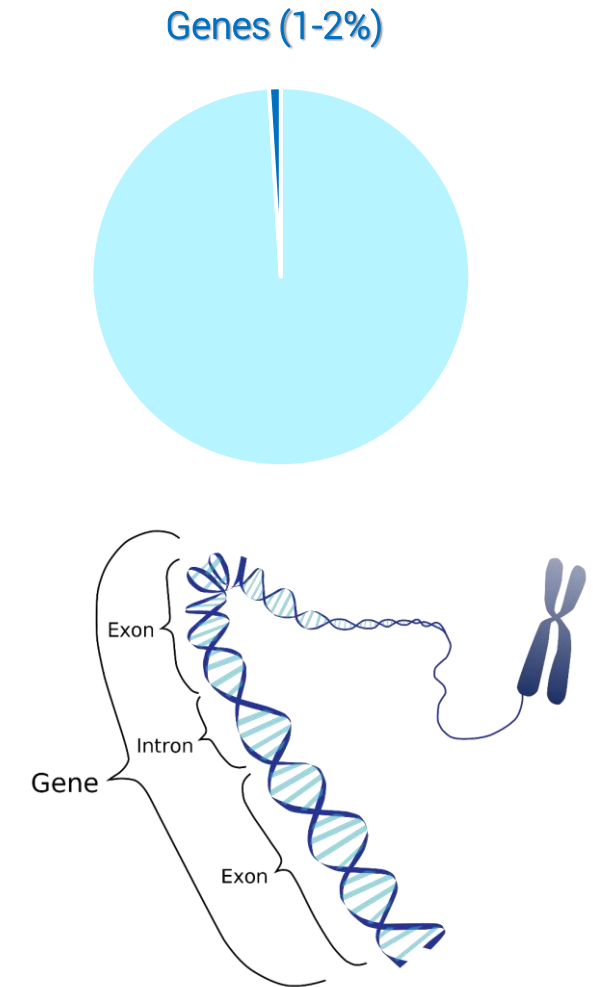
Mitochondrial genome

- 16,500 bp
- Circular double stranded DNA



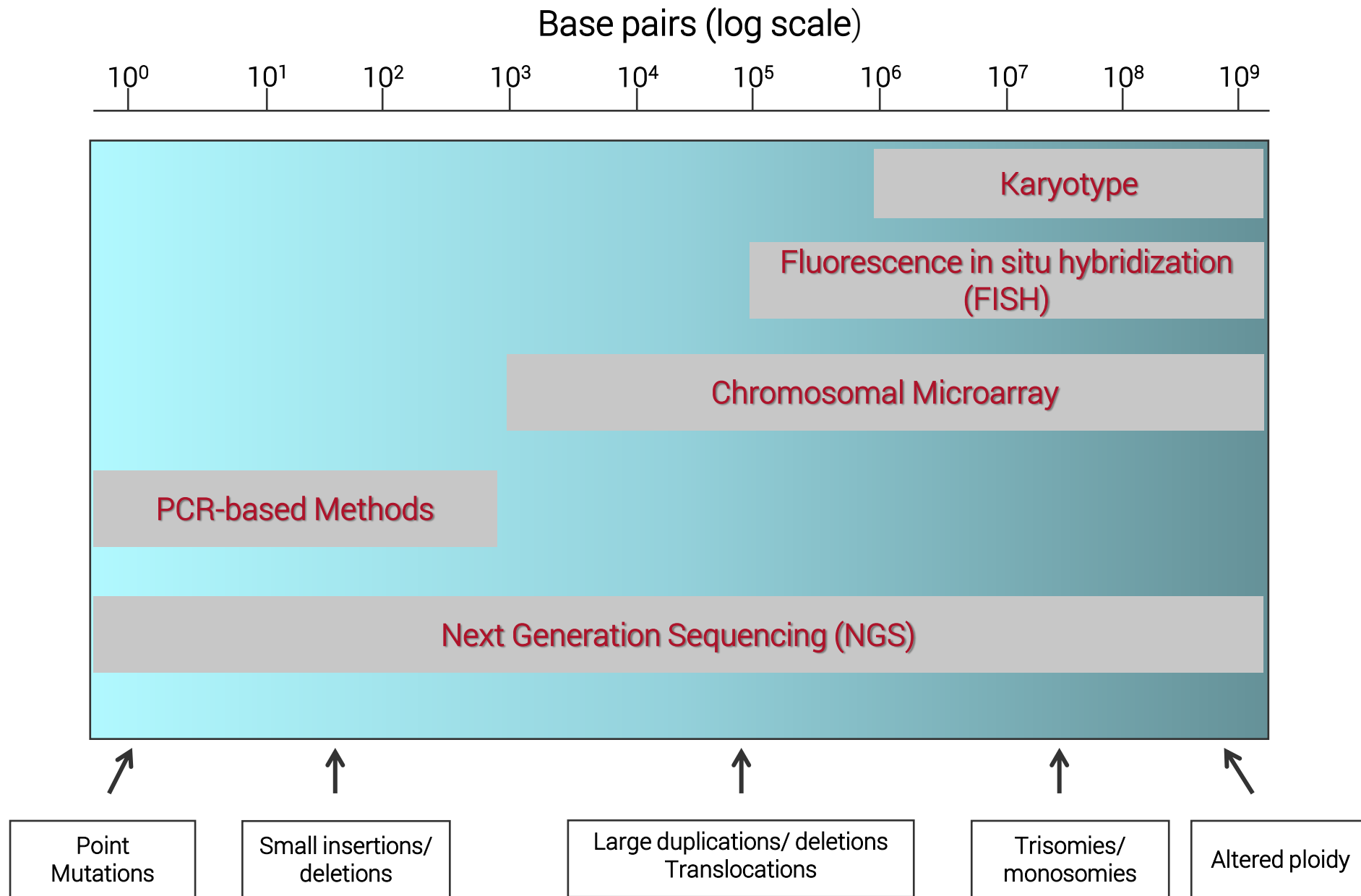
Human genome

- There are 20,000-25,000 genes in the human genome
- Very small amount of the genome contains coding sequences (Genes)
- Vast amount of the genome is made up of non-coding DNA
 - Regulatory elements
 - Repetitive sequences
 - Large duplications



<https://www.creativecommons.org>

Cancer: A Disease of the Genome



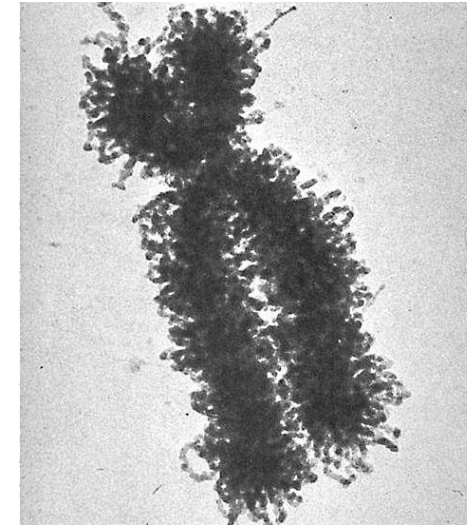
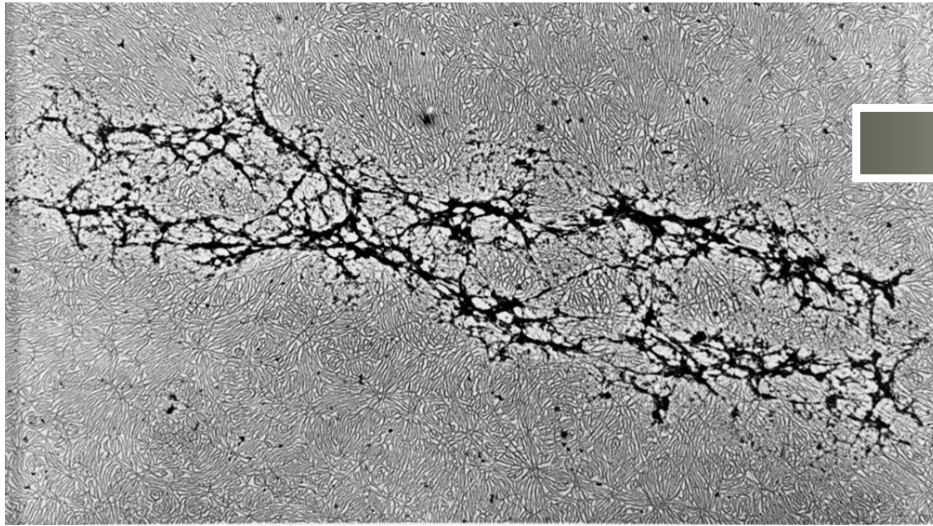


Karyotype

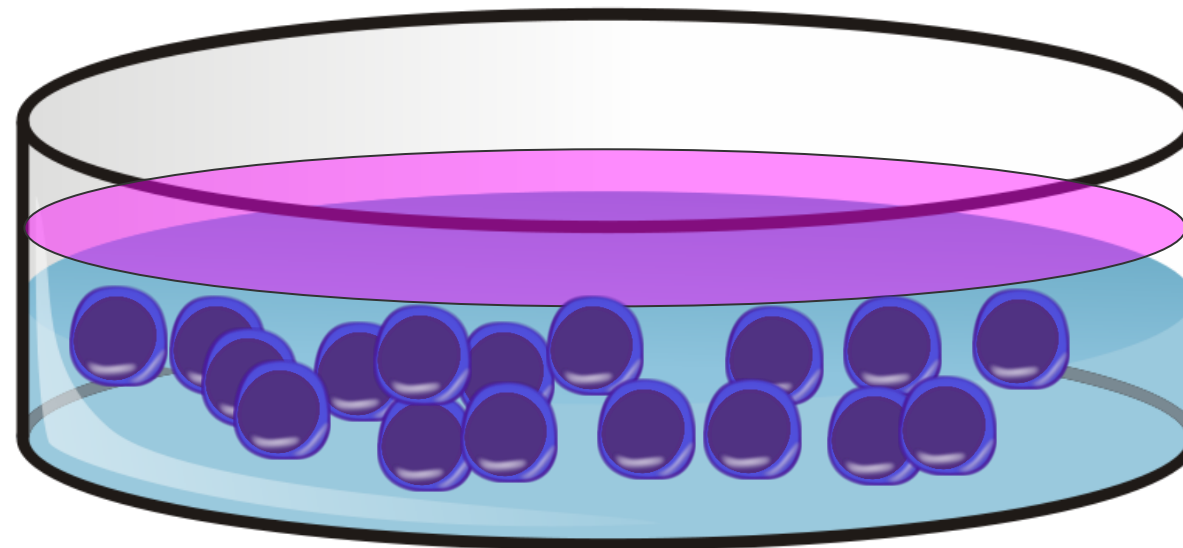
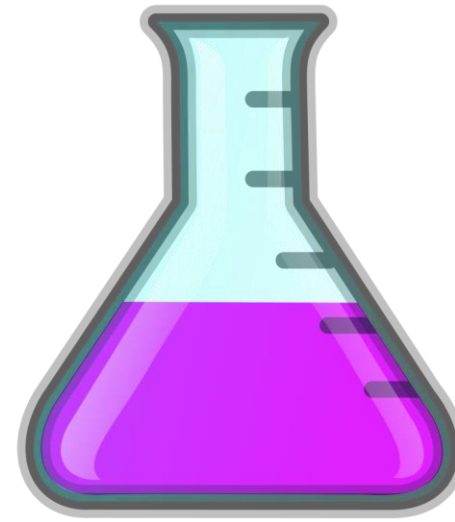
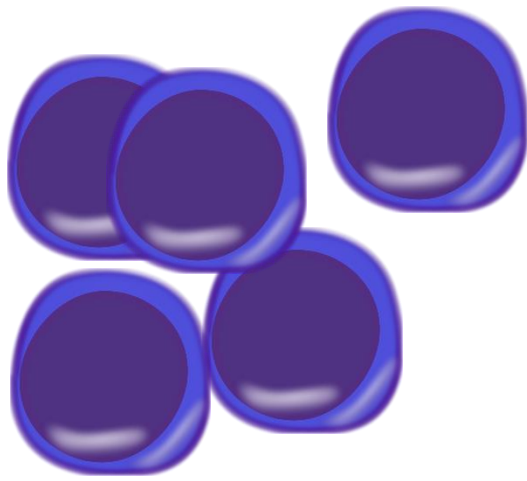
The concept of Karyotype

- Specialized laboratory technique that permits visualization of DNA in its “bundled” form

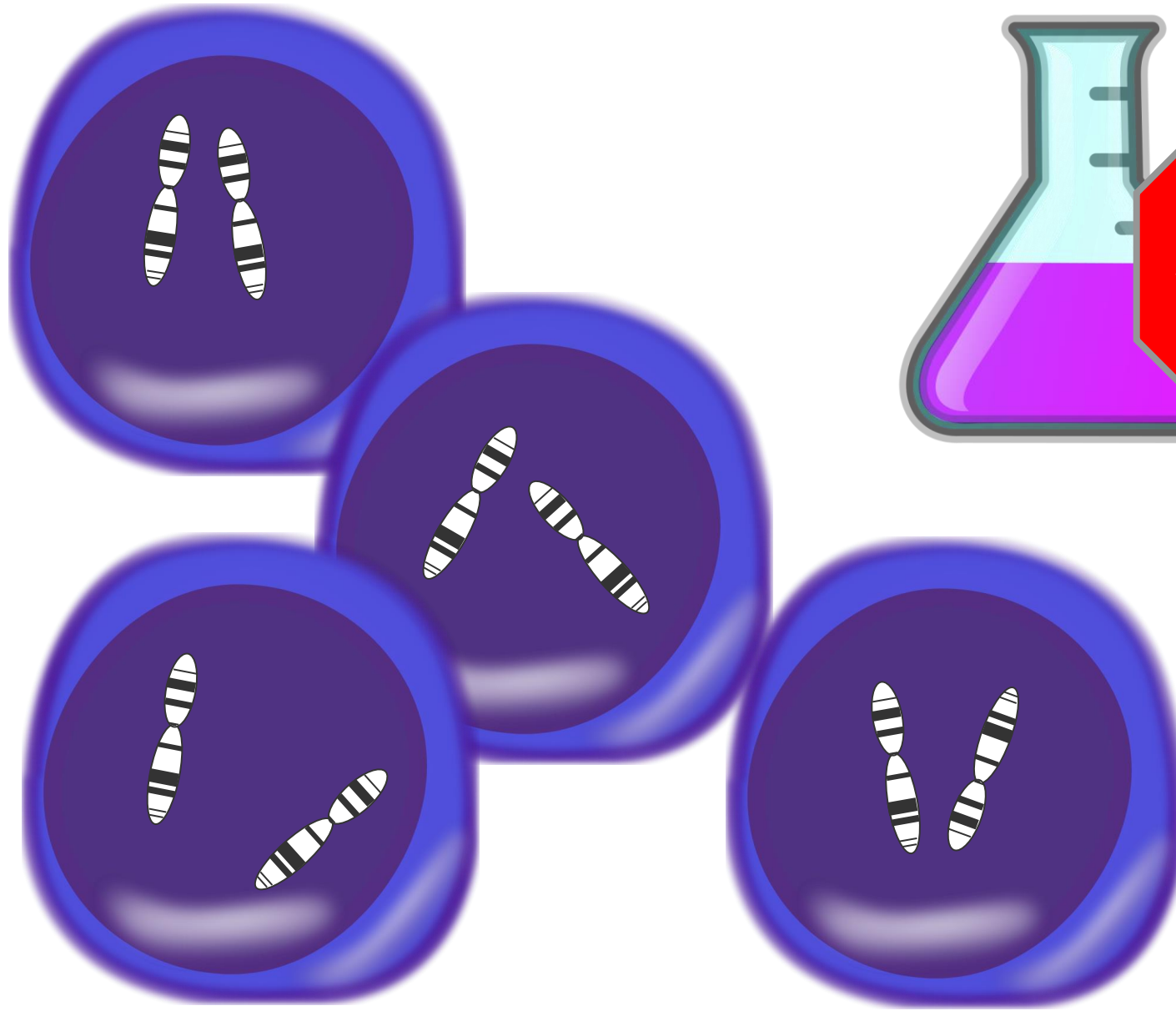




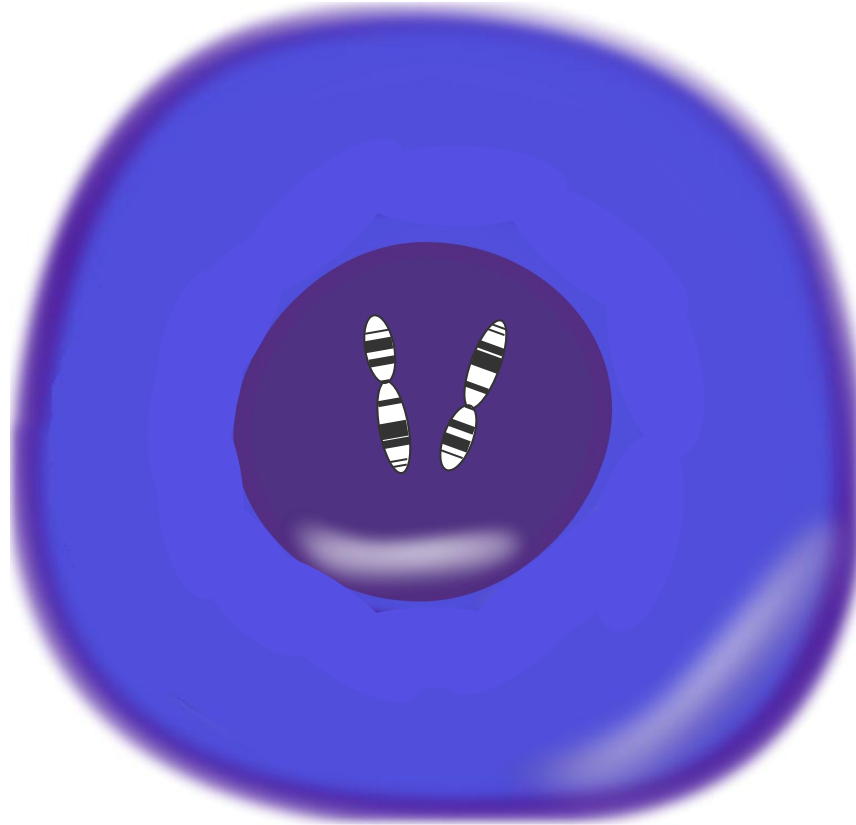
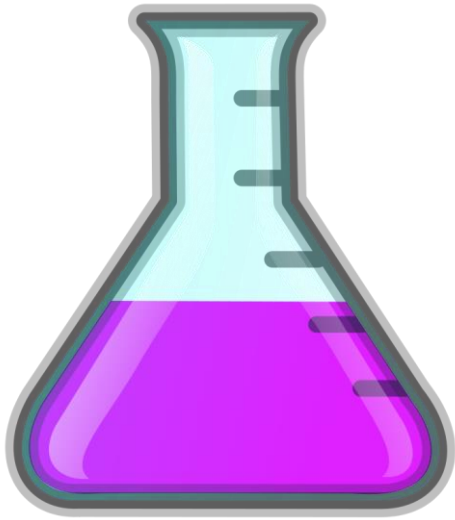
CELL. 1977 NOV;12(3):817-28



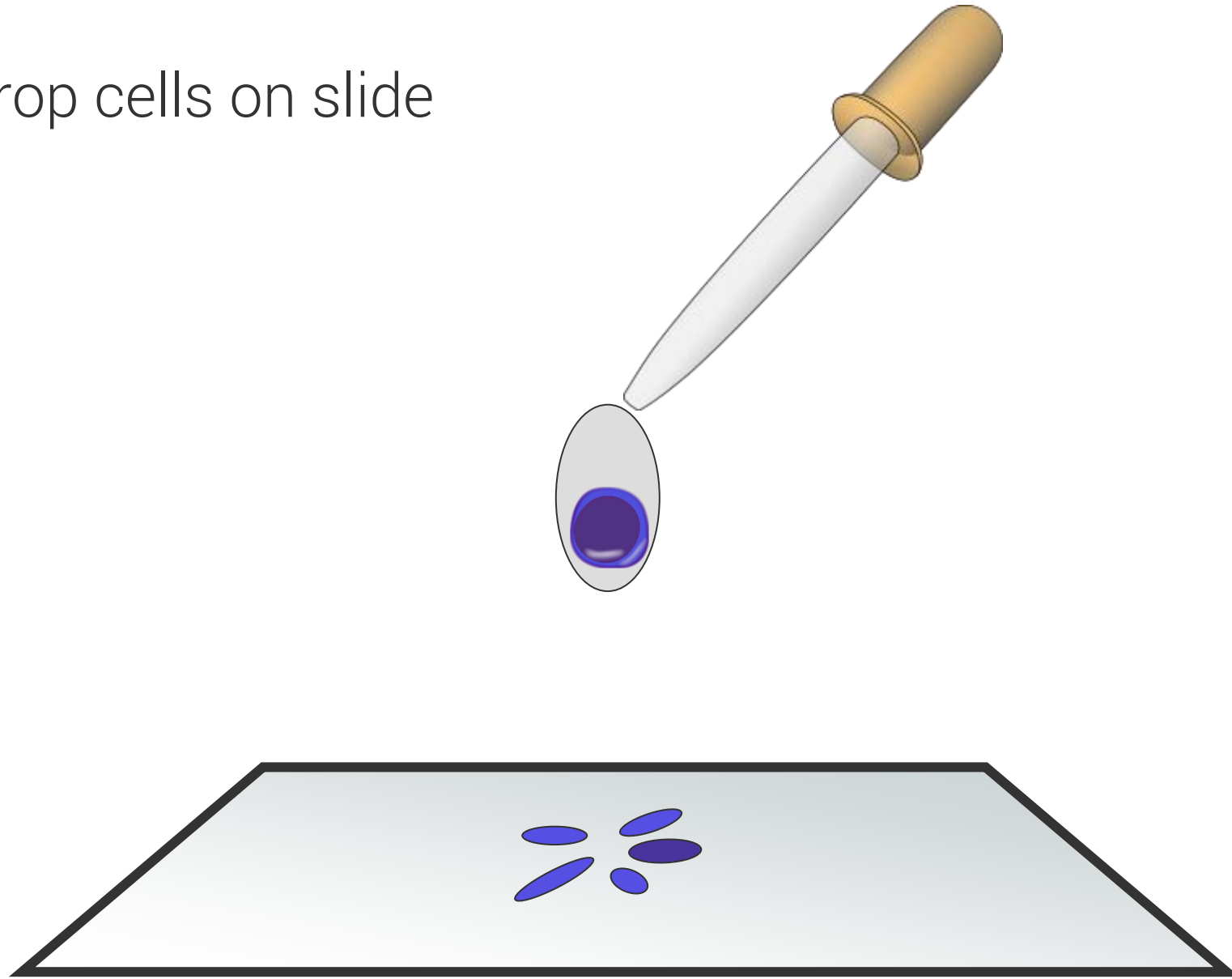
Colcemid



Hypotonic solution



Drop cells on slide



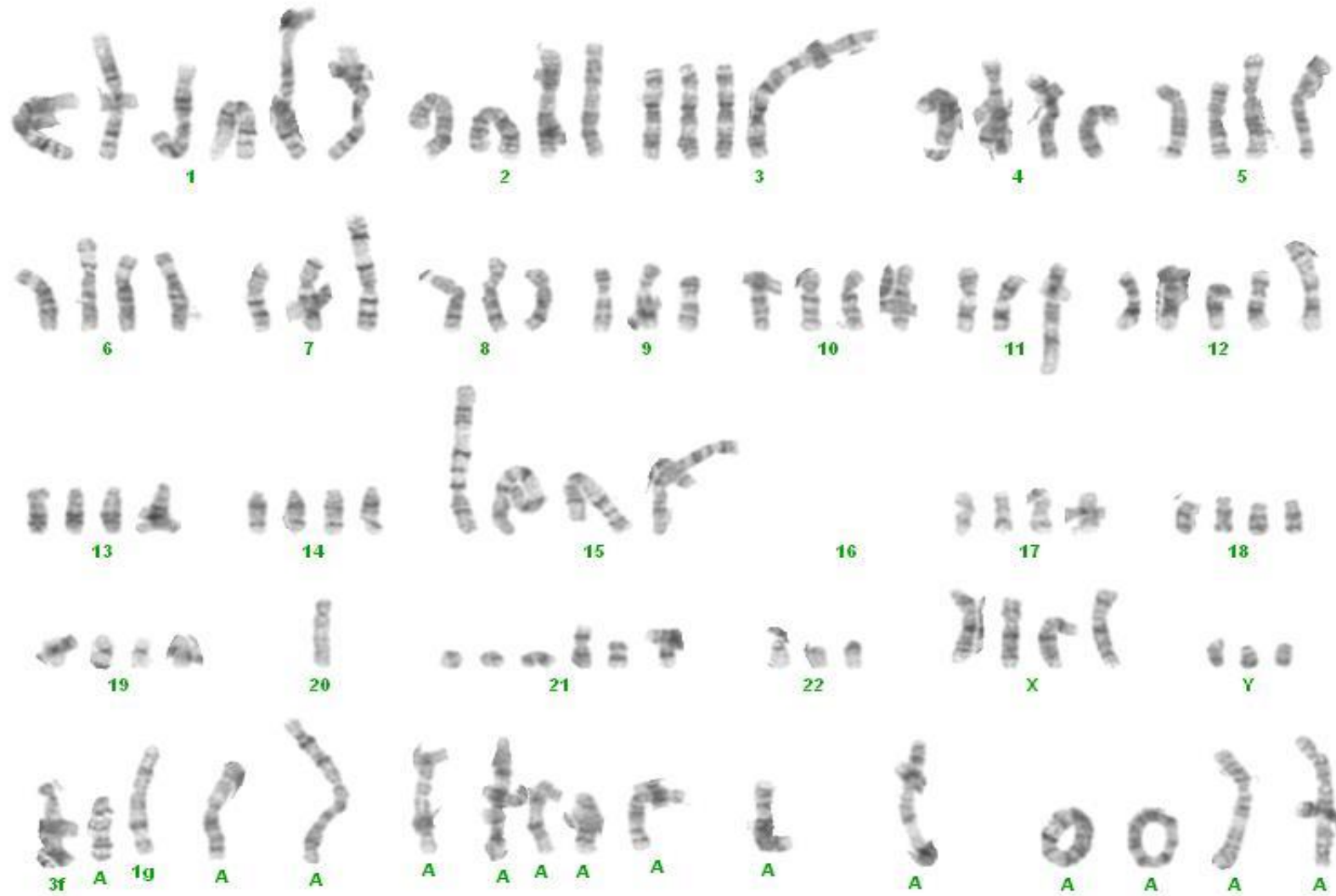


Karyotype (specimen requirements)

- Any type of tissue that can produce dividing cells (bone marrow, peripheral blood, fresh tissue)



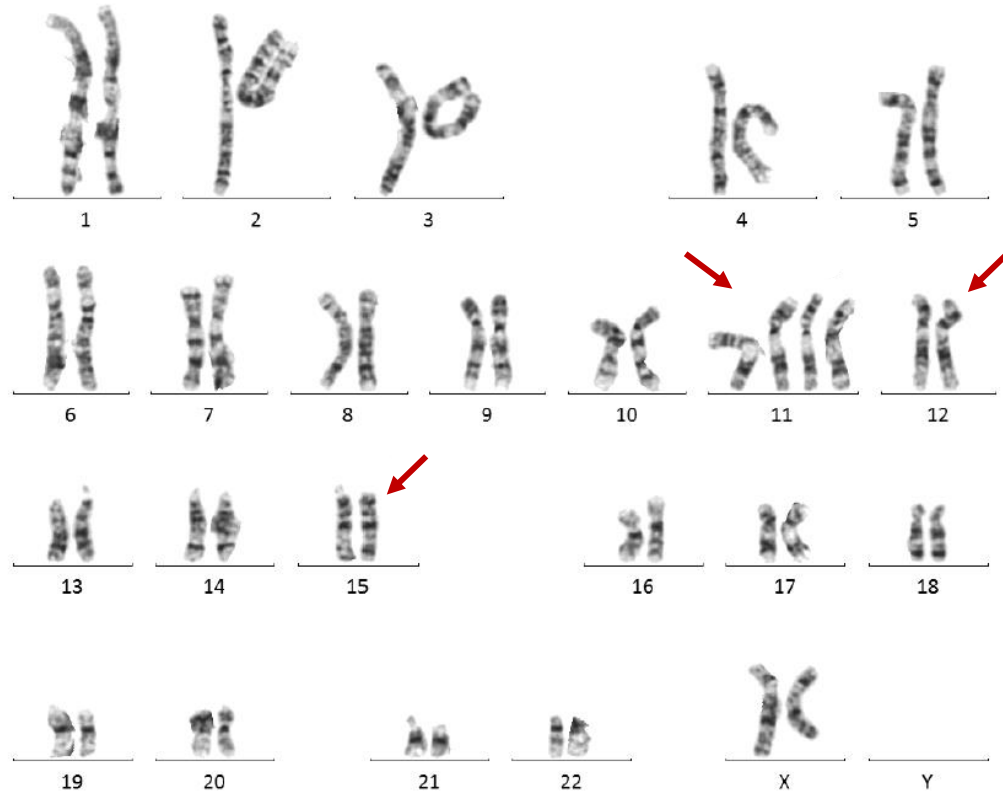
Solid tumors often contain complex abnormalities



10-day-old female with scalp lesion



Chromosome results: 47,XX,+11,t(12;15)(p13;q25)[6]
/48,sl,+11[2]/48,sl,+15[cp4]/48,sl,+18[3]
/48,sl,+20[2]/46,XX[5]

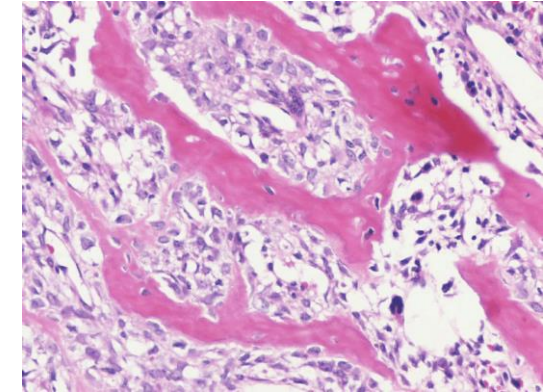


t(12;15)(p13;q25)

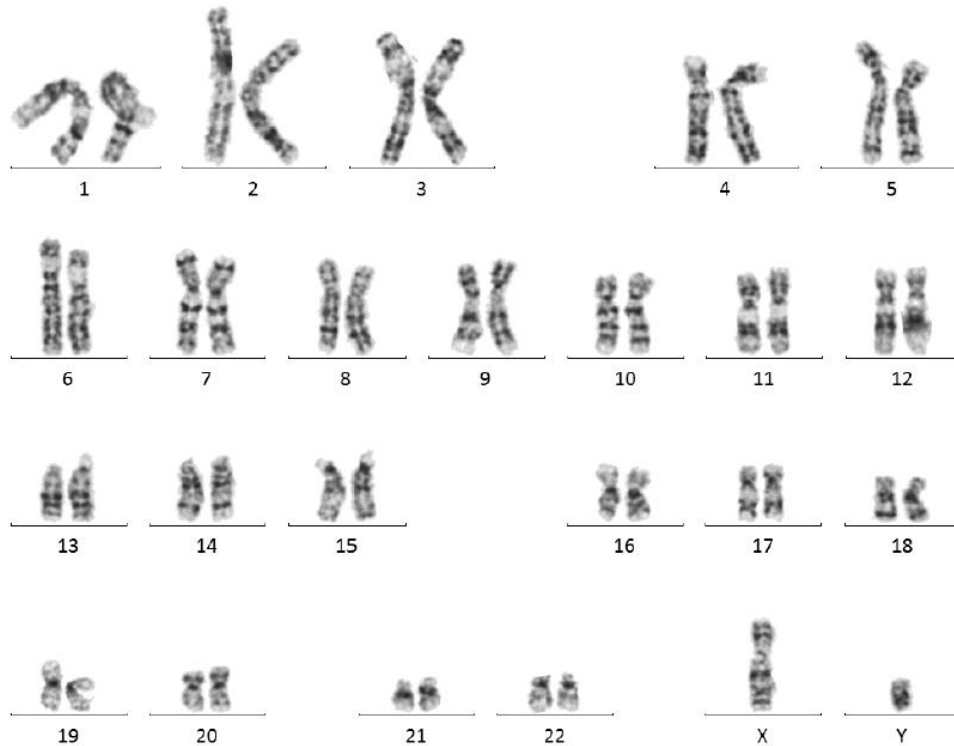


ETV6-NTRK3

15-year-old male with tibial lesion, rule out osteosarcoma



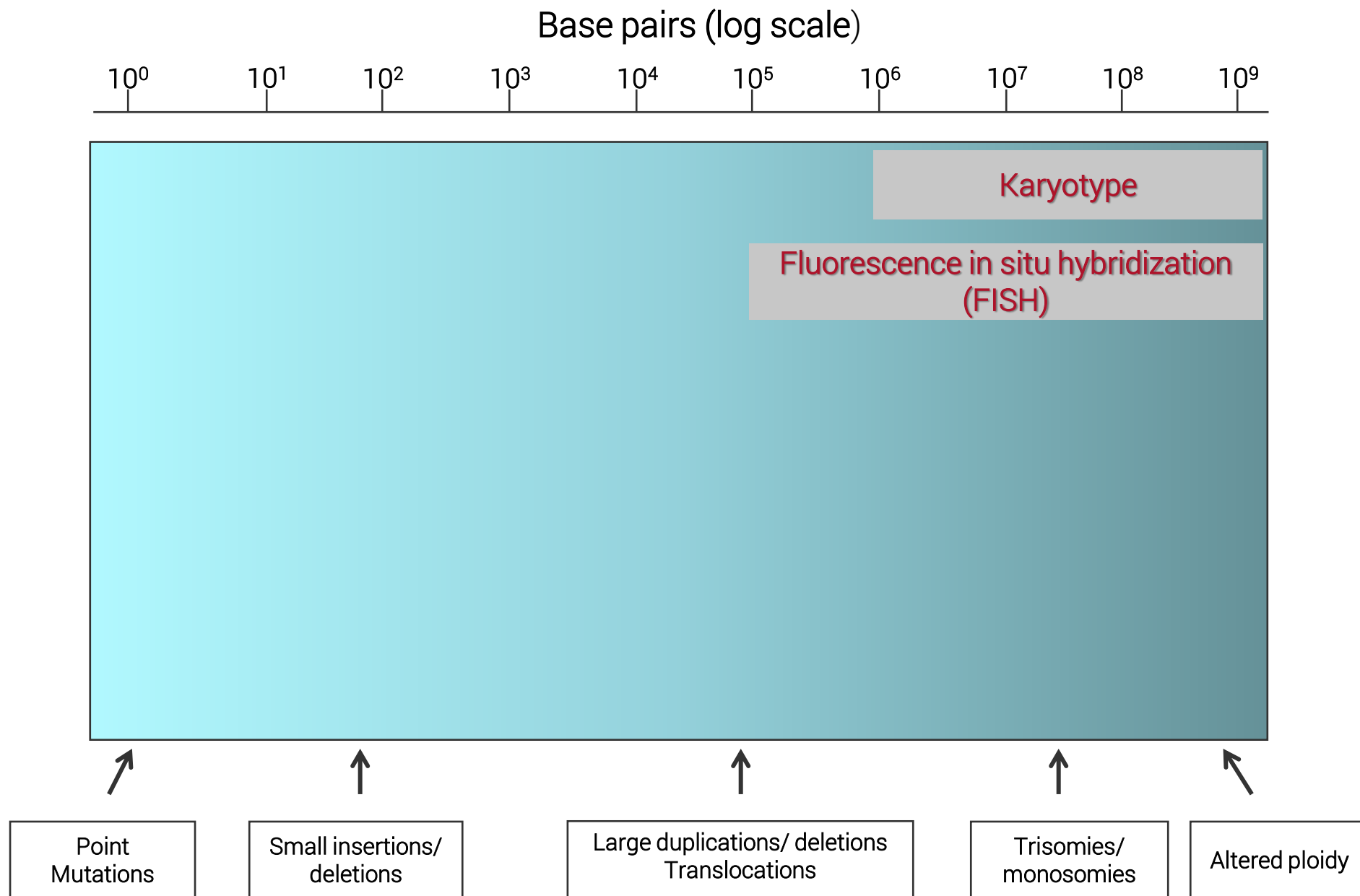
Chromosome results: 46,XY[20]



- A negative study does not necessarily mean genetic abnormalities are not present

Karyotype

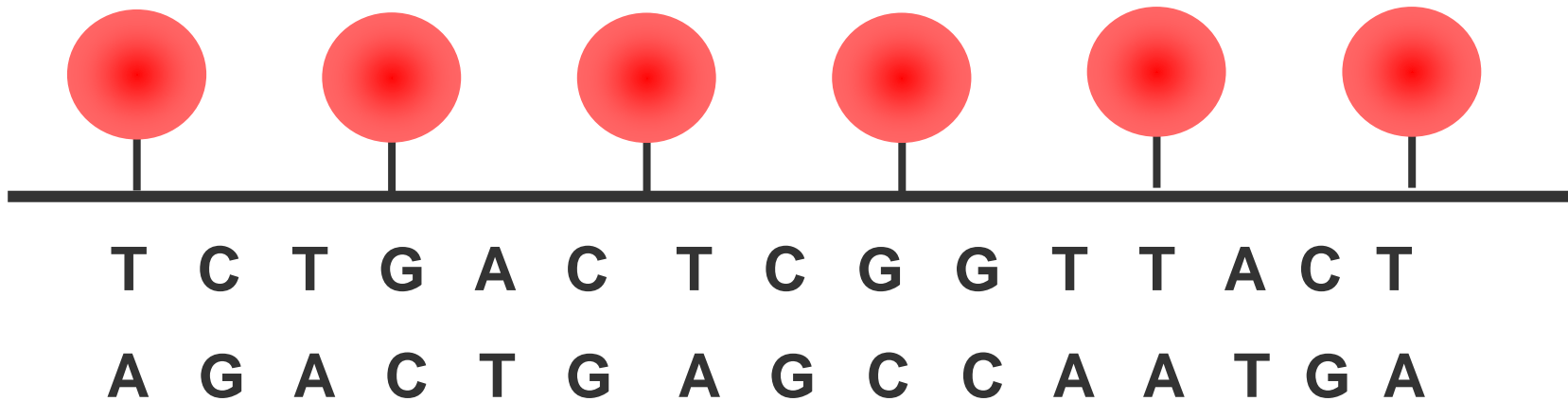
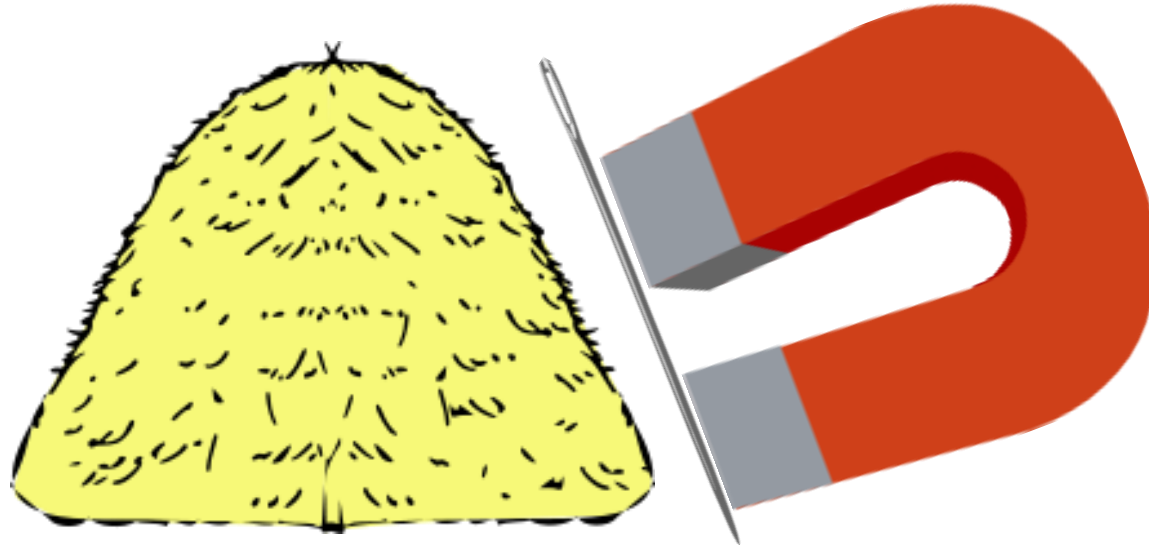
- Provides an overview of the whole genome through evaluation of metaphase chromosomes
- Detects both numerical and structural abnormalities
- Low resolution (5-10 Mb)
- Dependent on proliferation in an artificial environment
- Long and labor intensive



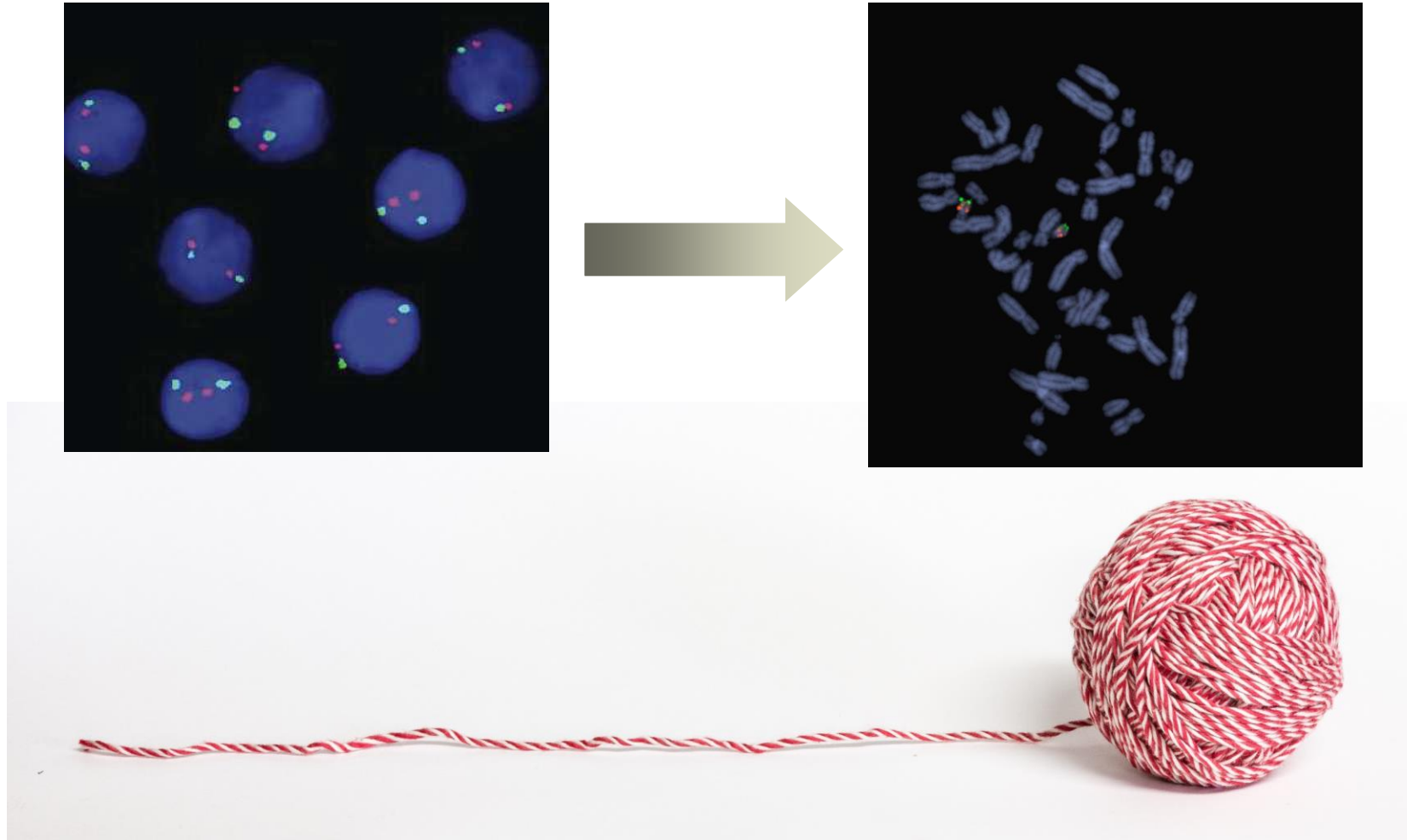


■ Fluorescent in Situ Hybridization (FISH)

The concept of FISH



Metaphase vs Interphase FISH



- Interphase FISH can be performed on:

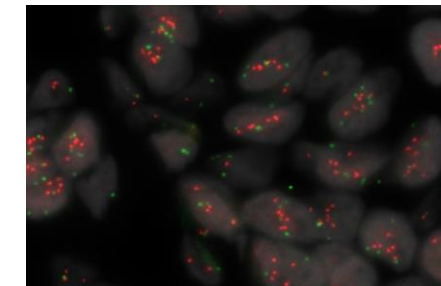
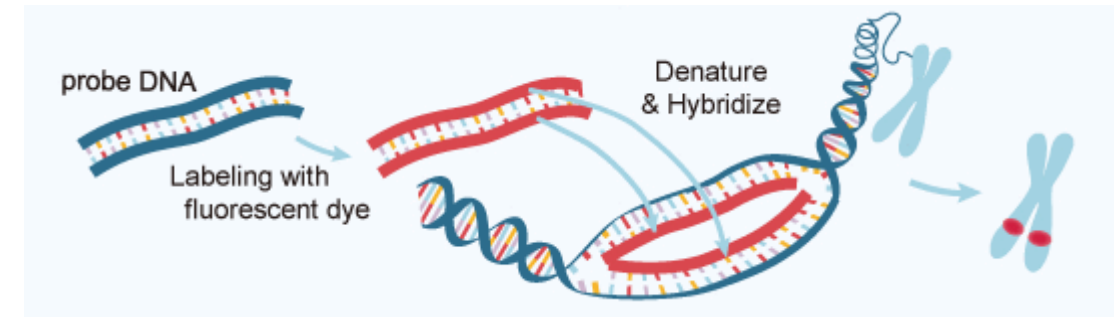
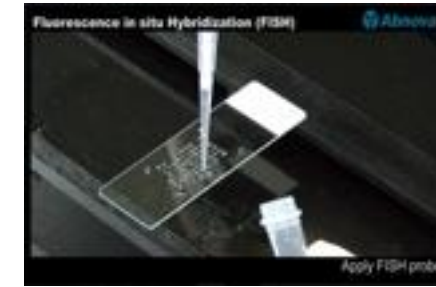
- FFPE tissue/ cell block
 - Smears
 - Touch preps
 - Cytospins
- } Archived material



- Specimen adequacy
 - 50-100 (intact) tumor cells

General FISH procedure

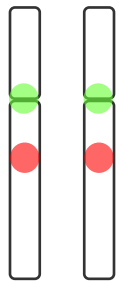
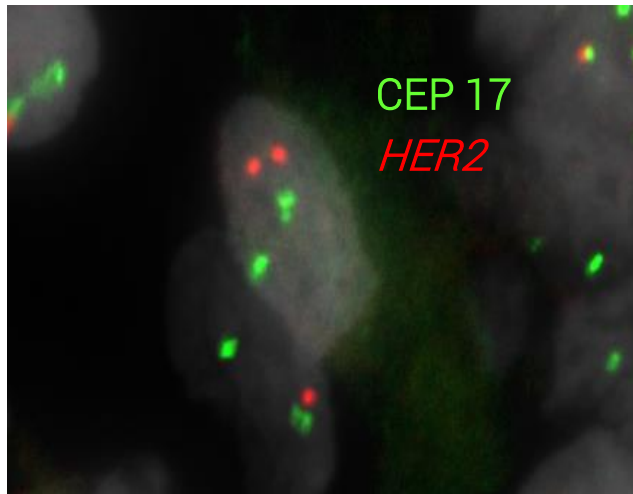
- Obtain specimen and apply to a glass slide
- Denature DNA
- Hybridize overnight with fluorescent probe
- Wash off extra probe
- Add nuclear counterstain (DAPI)
- Perform analysis using a fluorescent microscope



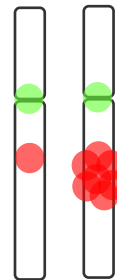
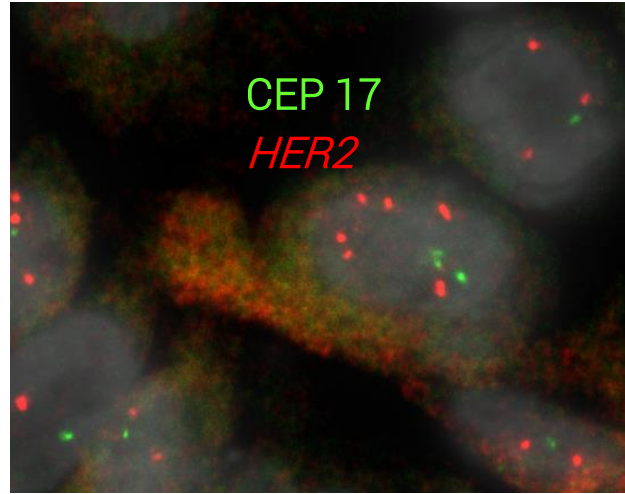
FISH (probe strategies)

- Copy number determination
 - Enumeration probes
- Structural changes
 - Break Apart probes
 - Dual Color, Dual Fusion probes

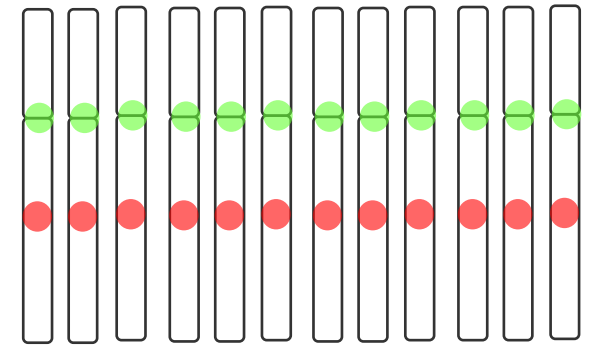
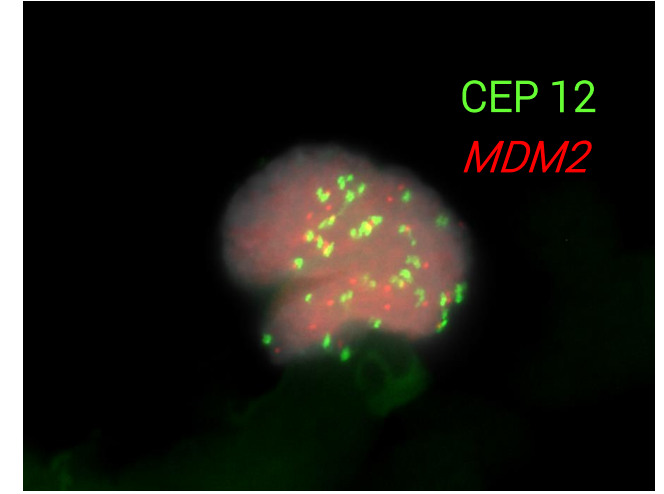
Enumeration probes



17q12

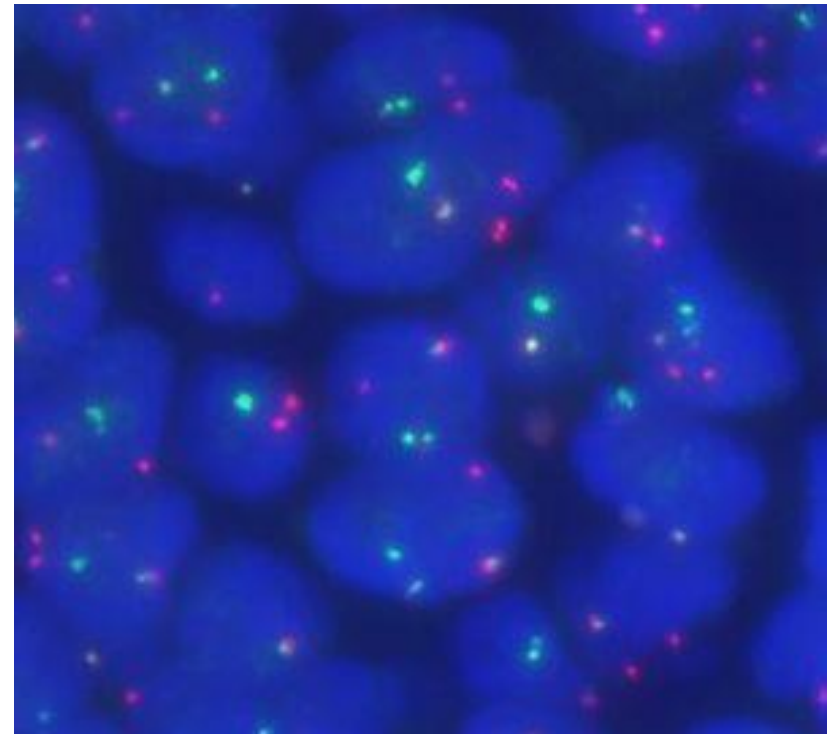
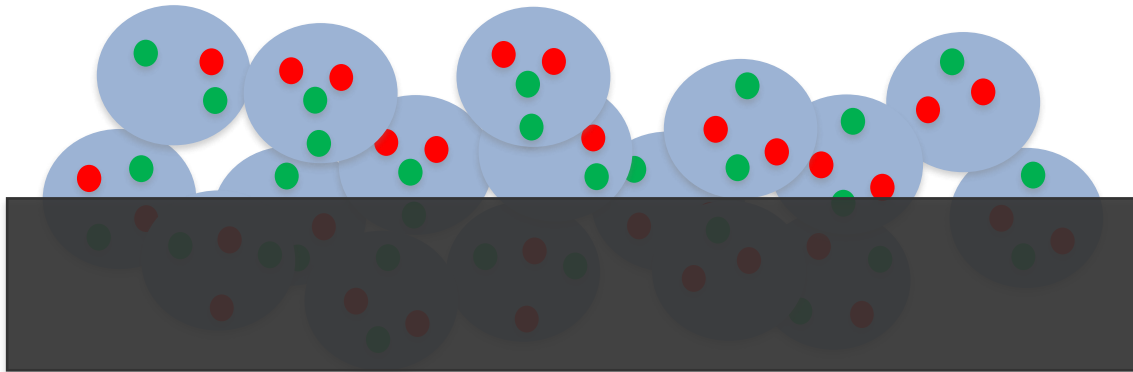


17q12

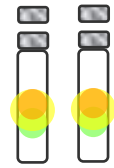
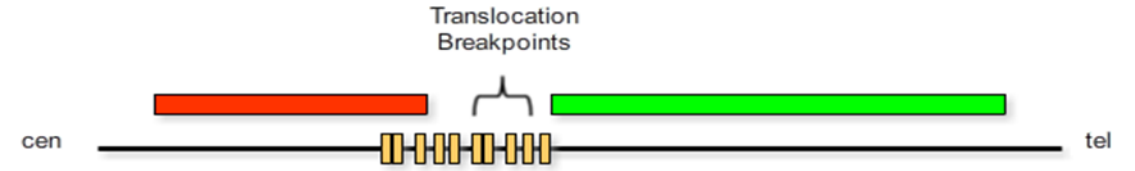


12q15

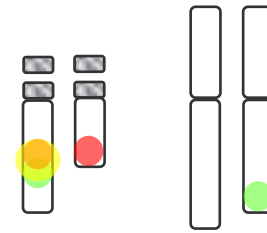
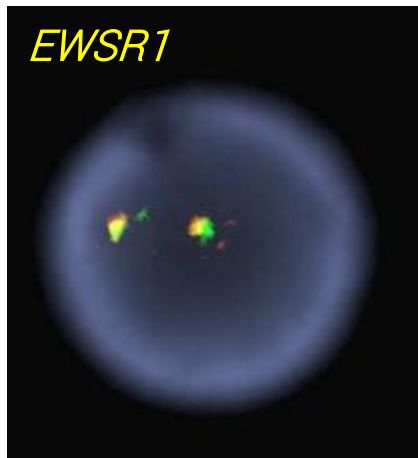
Truncation artifact



Break Apart probes

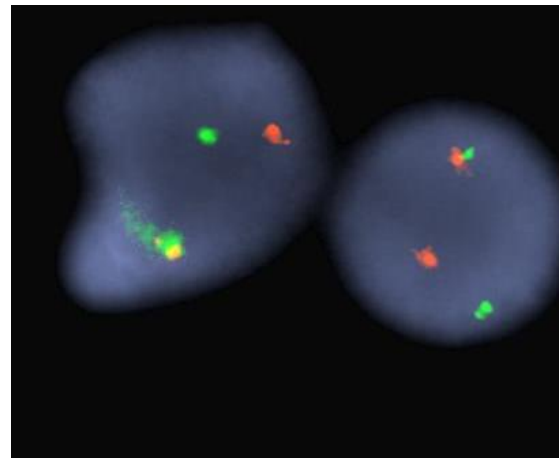


22q12

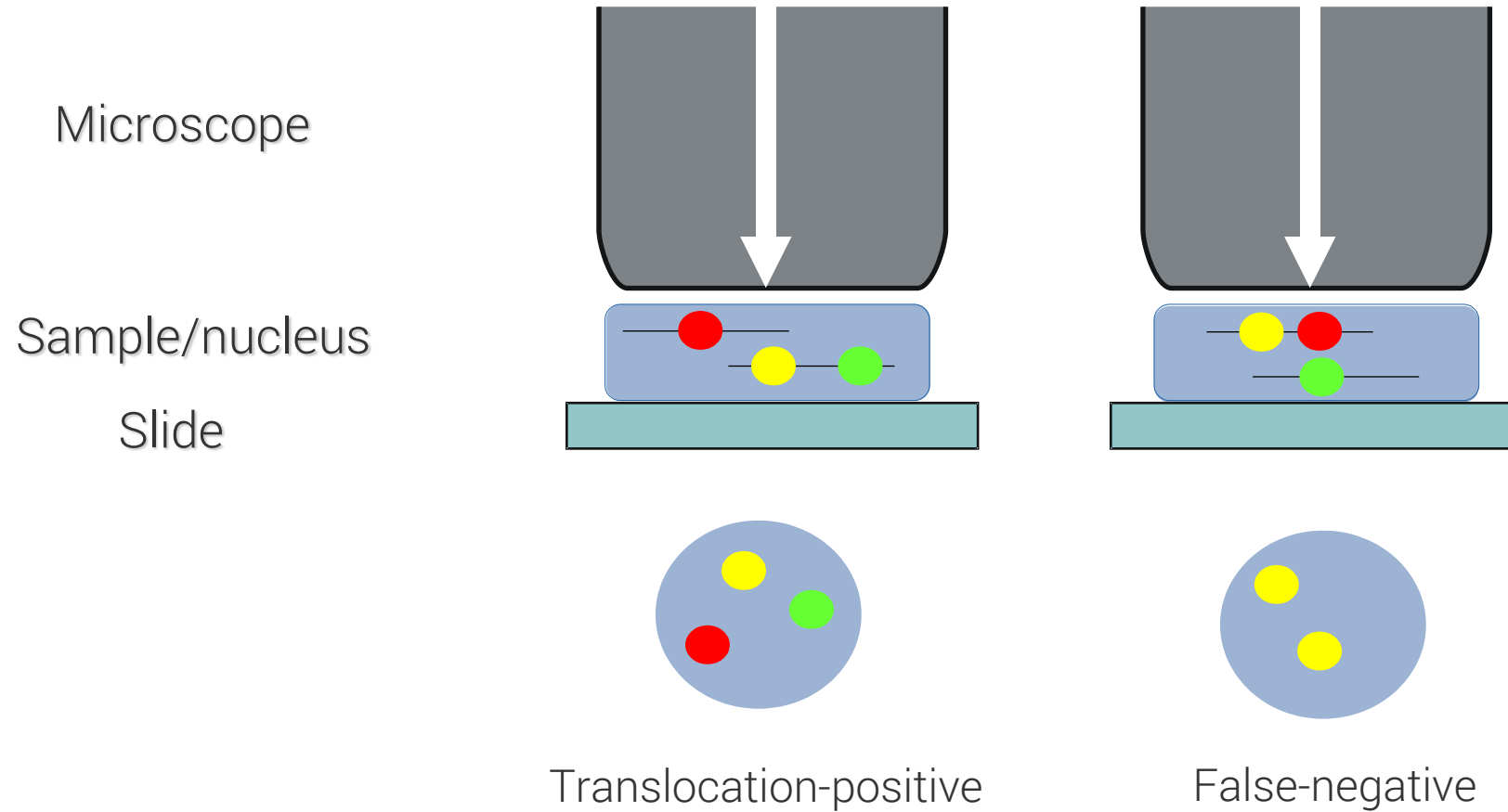


22q12

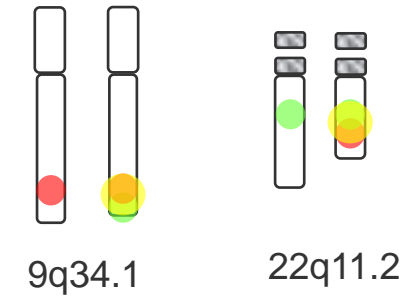
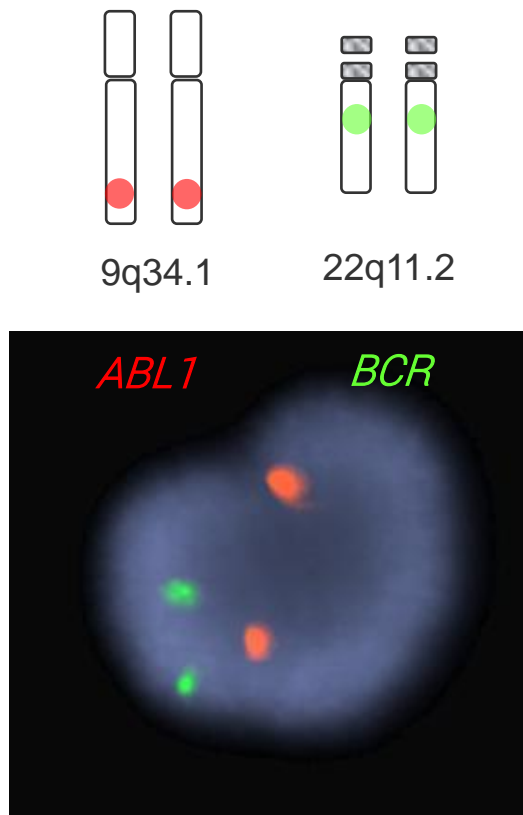
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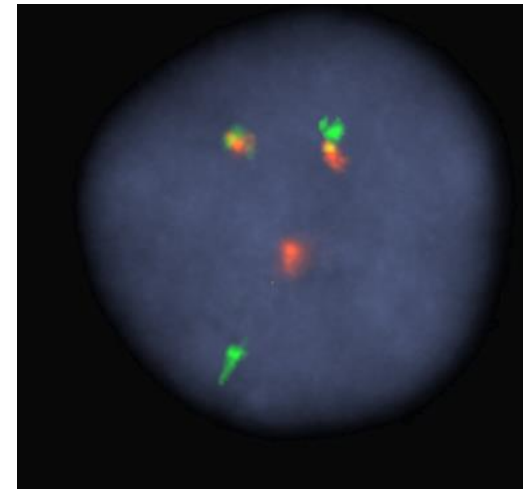
False negativity



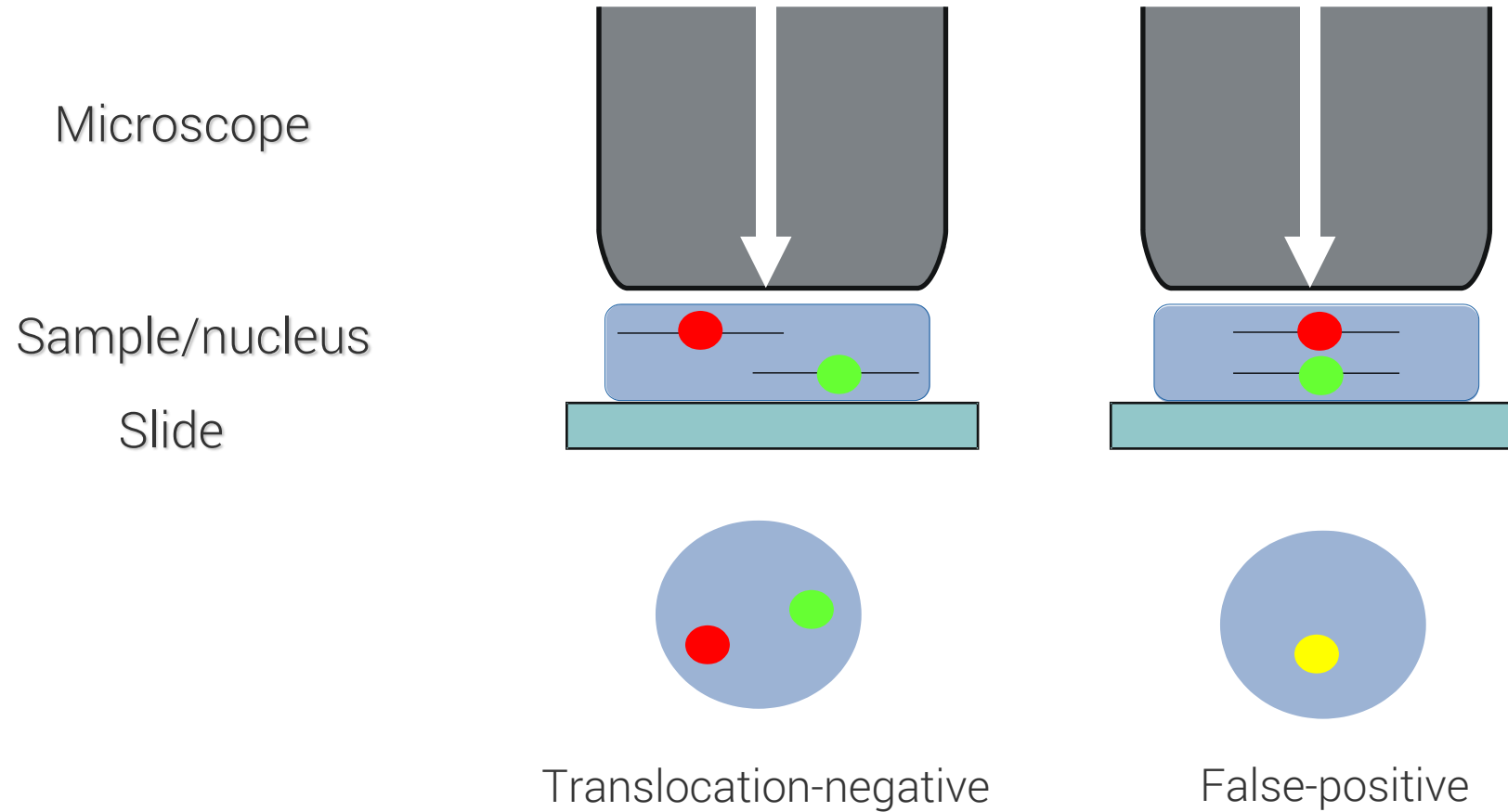
Dual Color, Dual Fusion probes



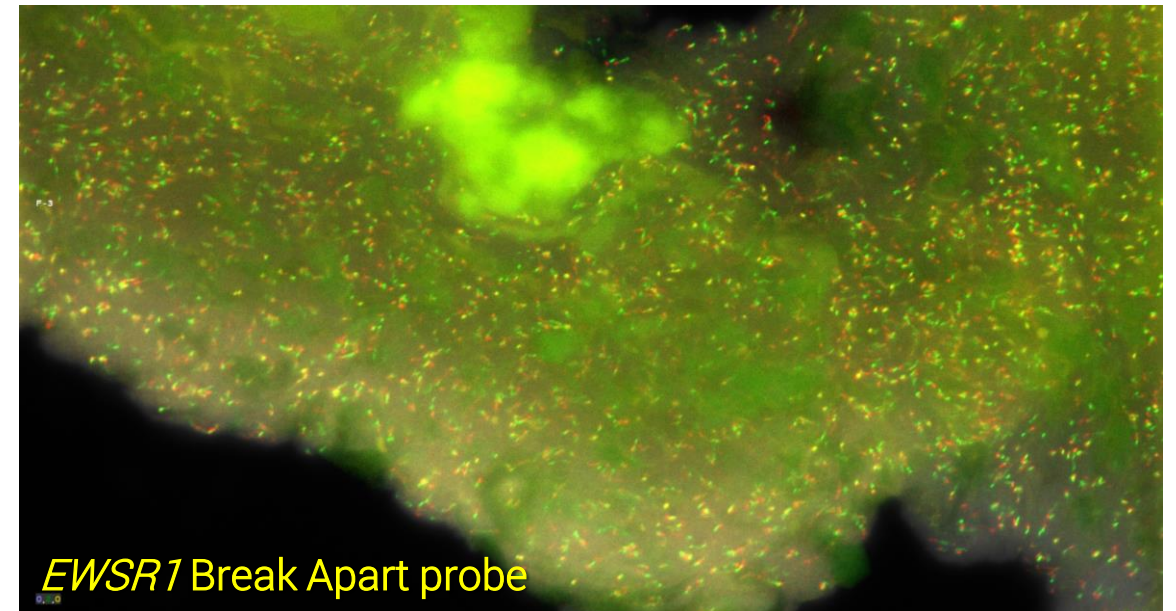
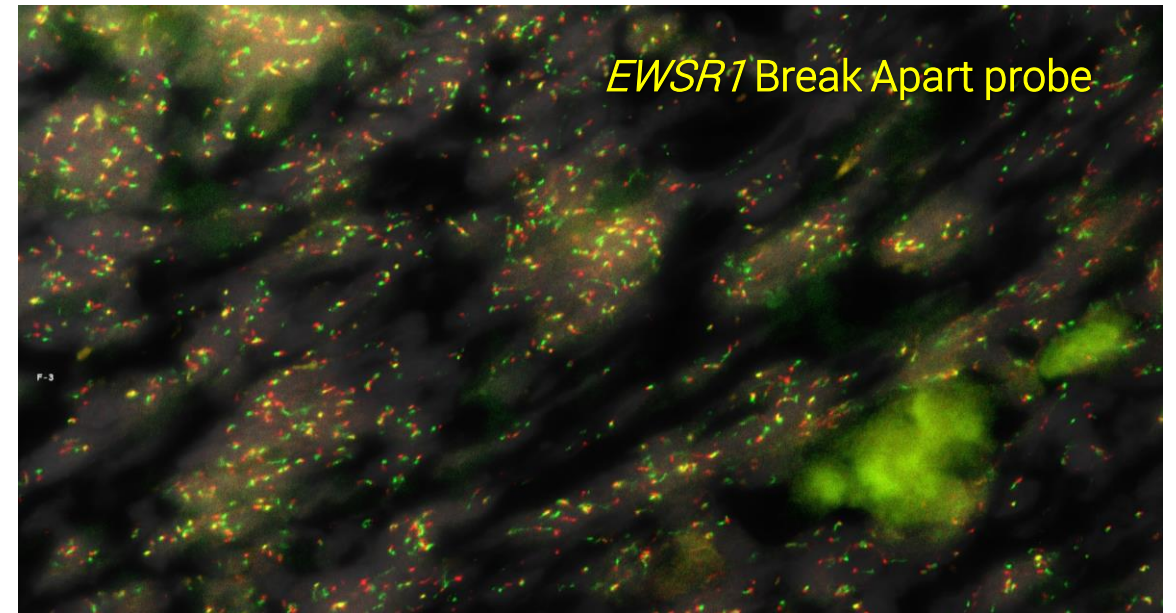
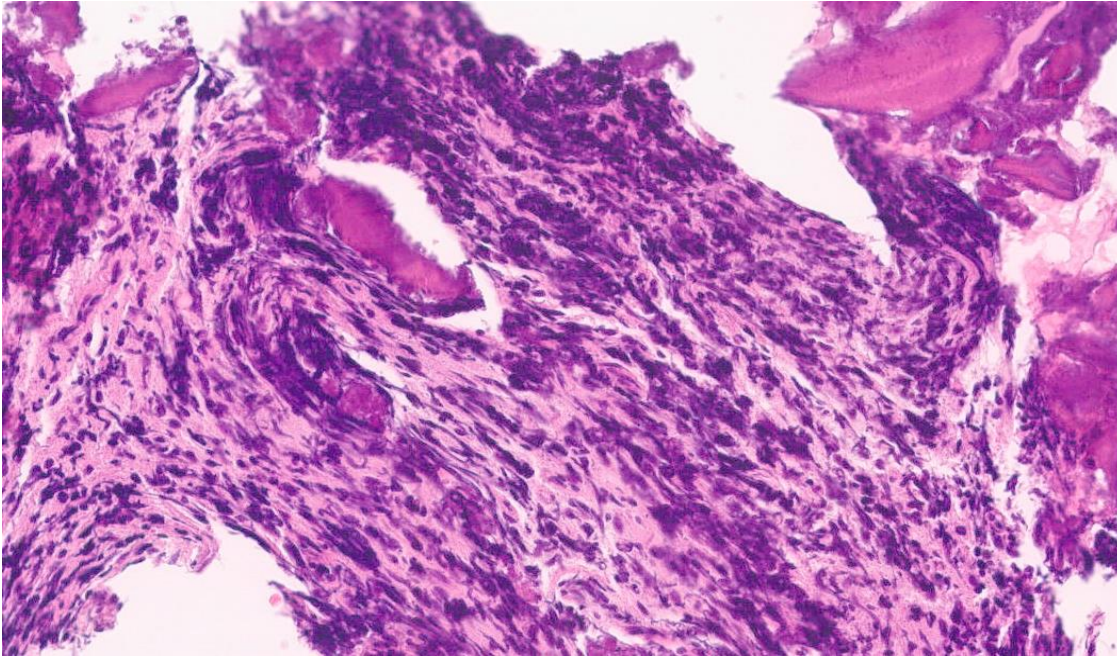
BCR-ABL1
ABL-BCR

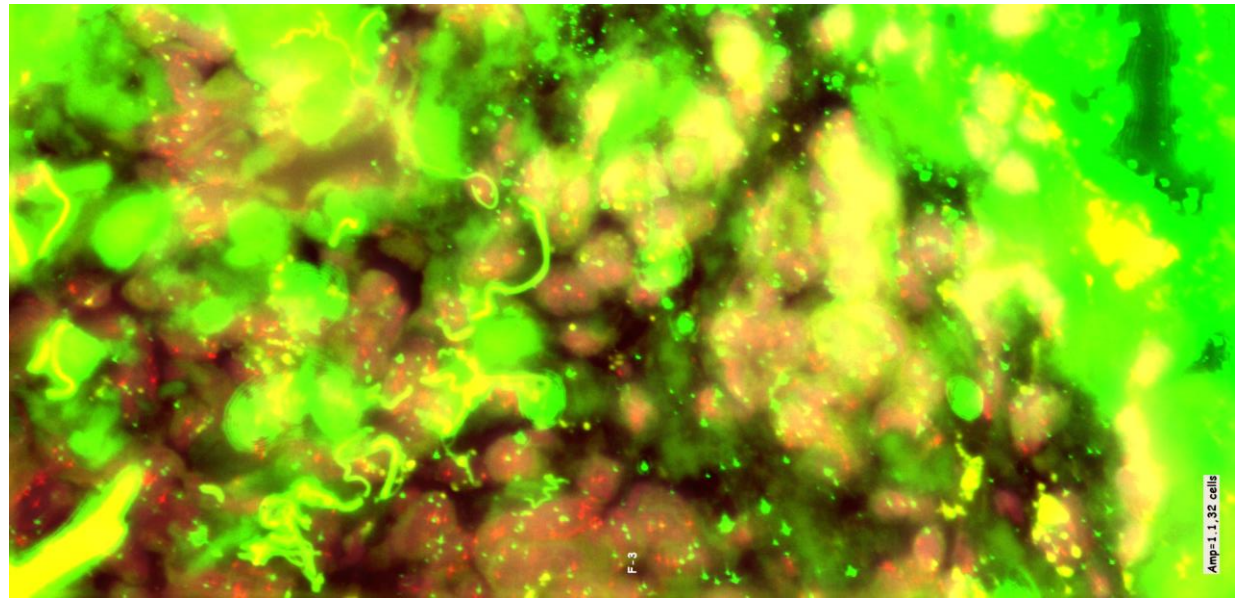
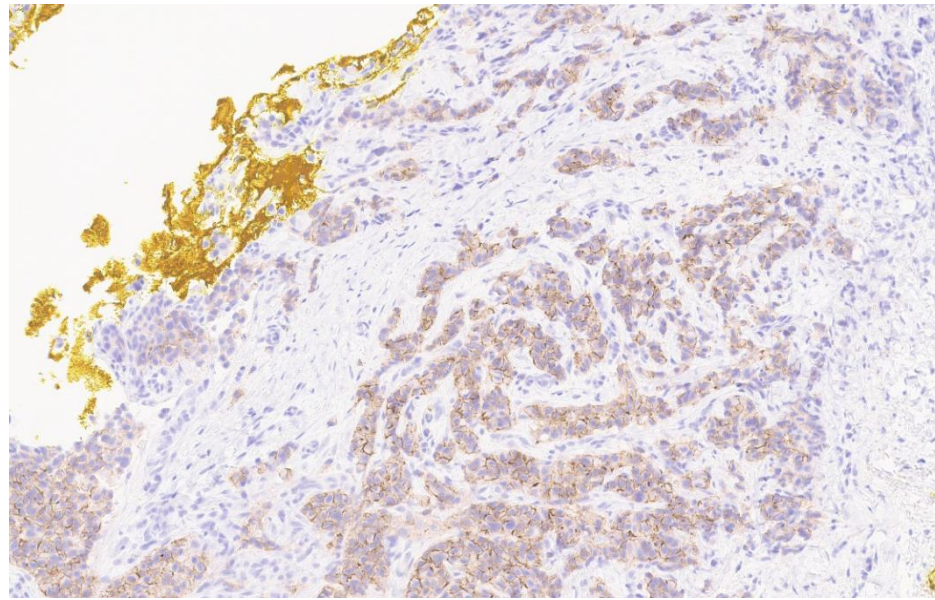
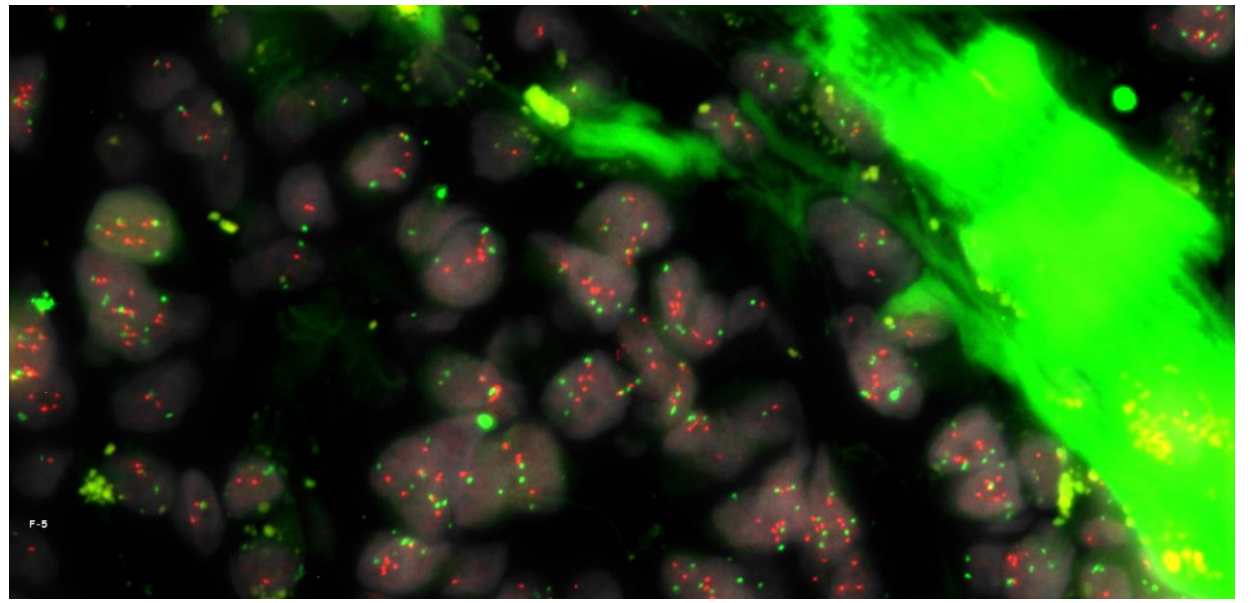
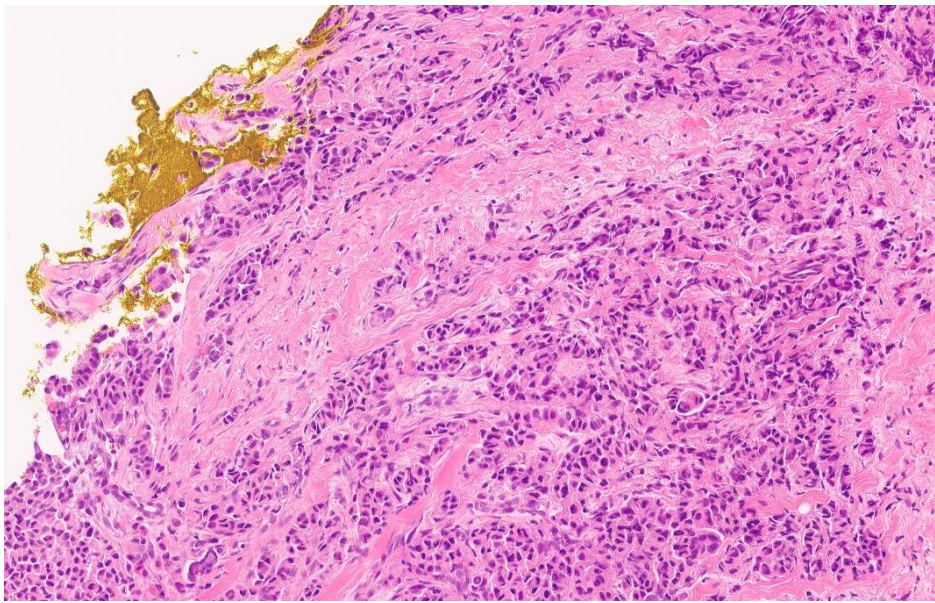


False positivity



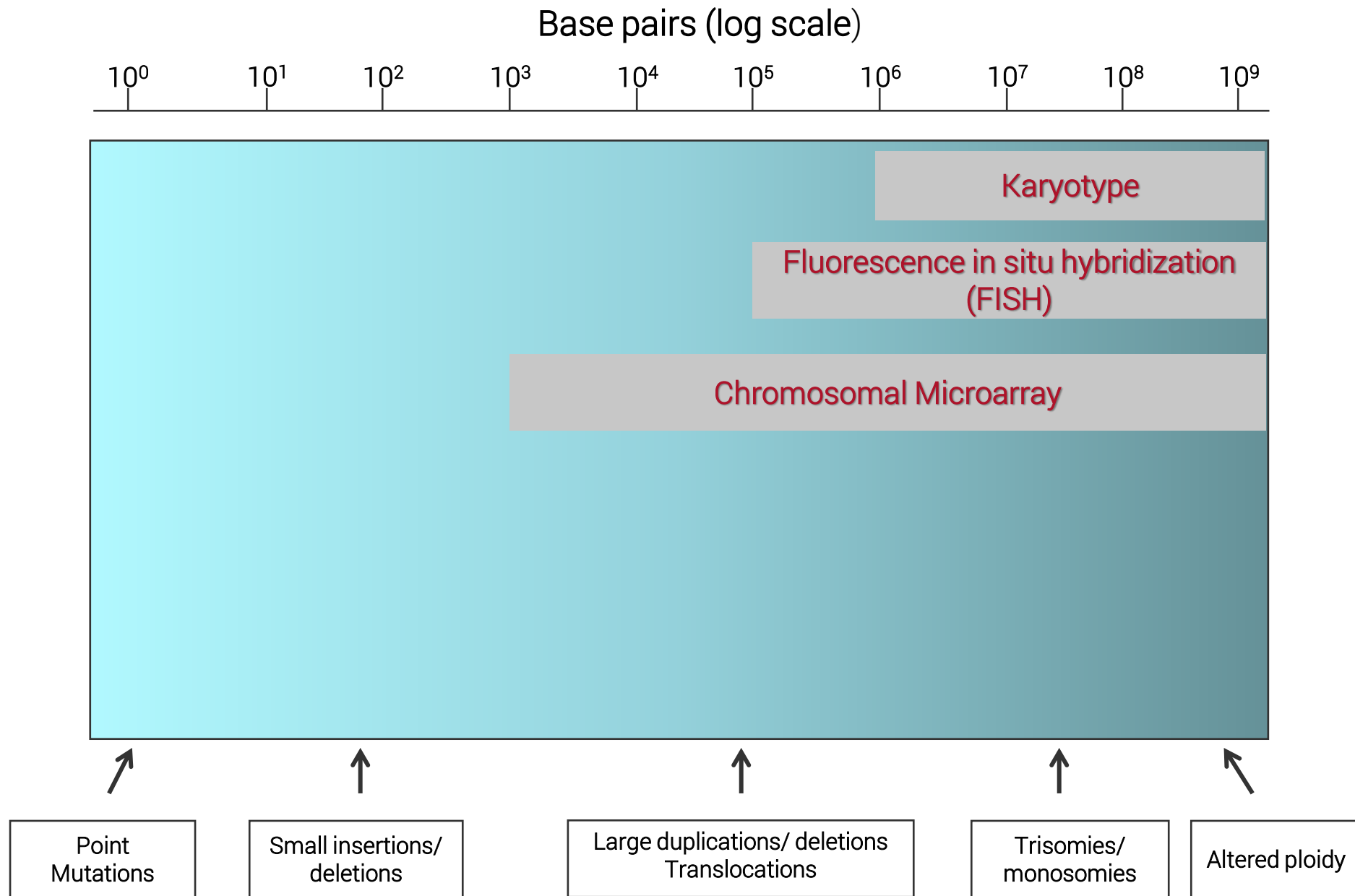
Crush artifact





FISH

- Permits detection of small cytogenetic changes (> 200 kb) that are not visible on karyotype
- No requirement for dividing cells
- Many cells can be analyzed (relatively) quickly
- Targeted assay
- Limited resolution (~200 kb)

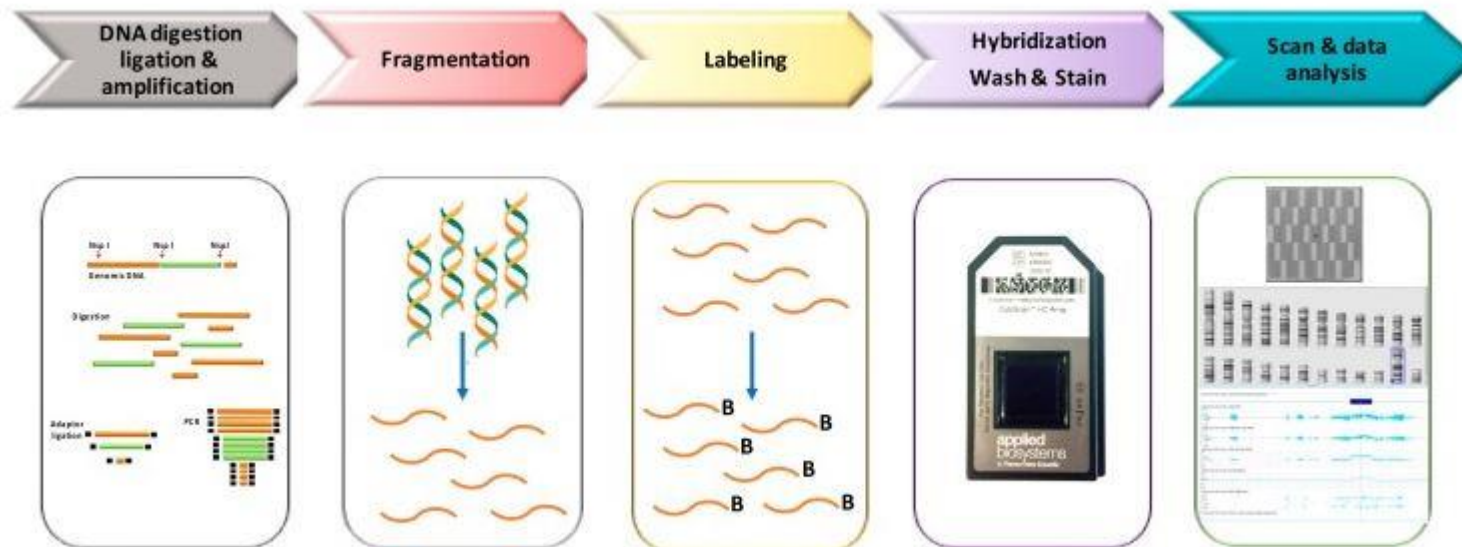




Chromosomal Microarray

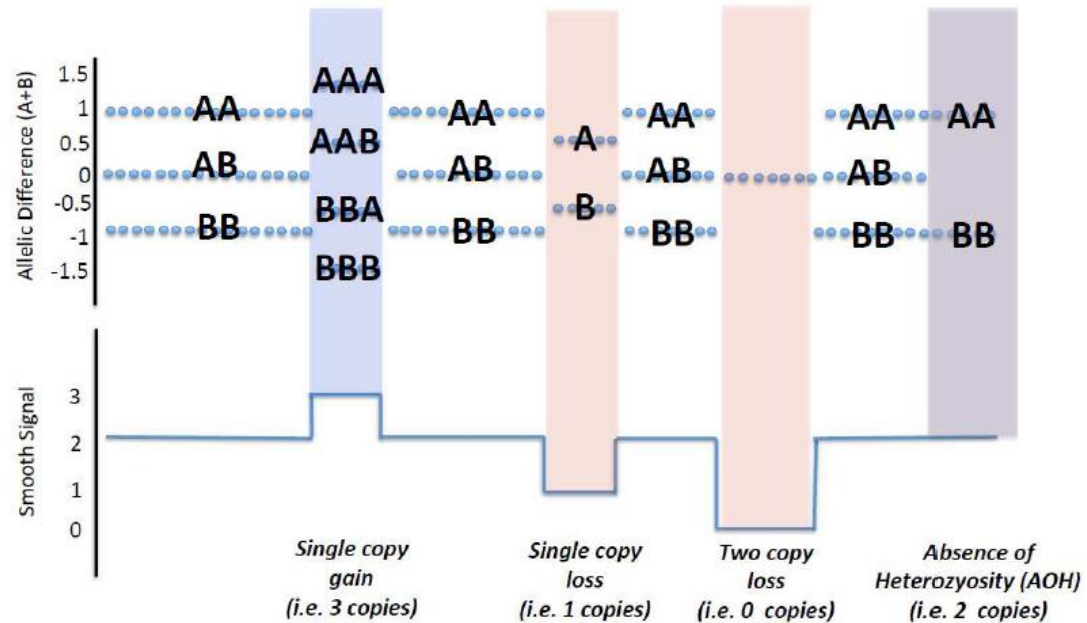
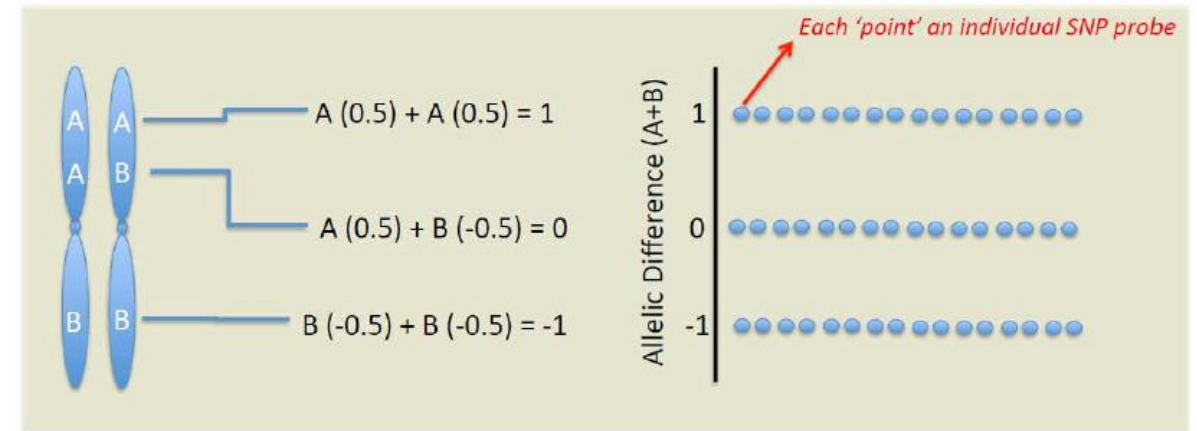
Chromosomal Microarray

- A genome-wide analysis technology used to assess DNA copy number, and in some cases genotype, in a sample



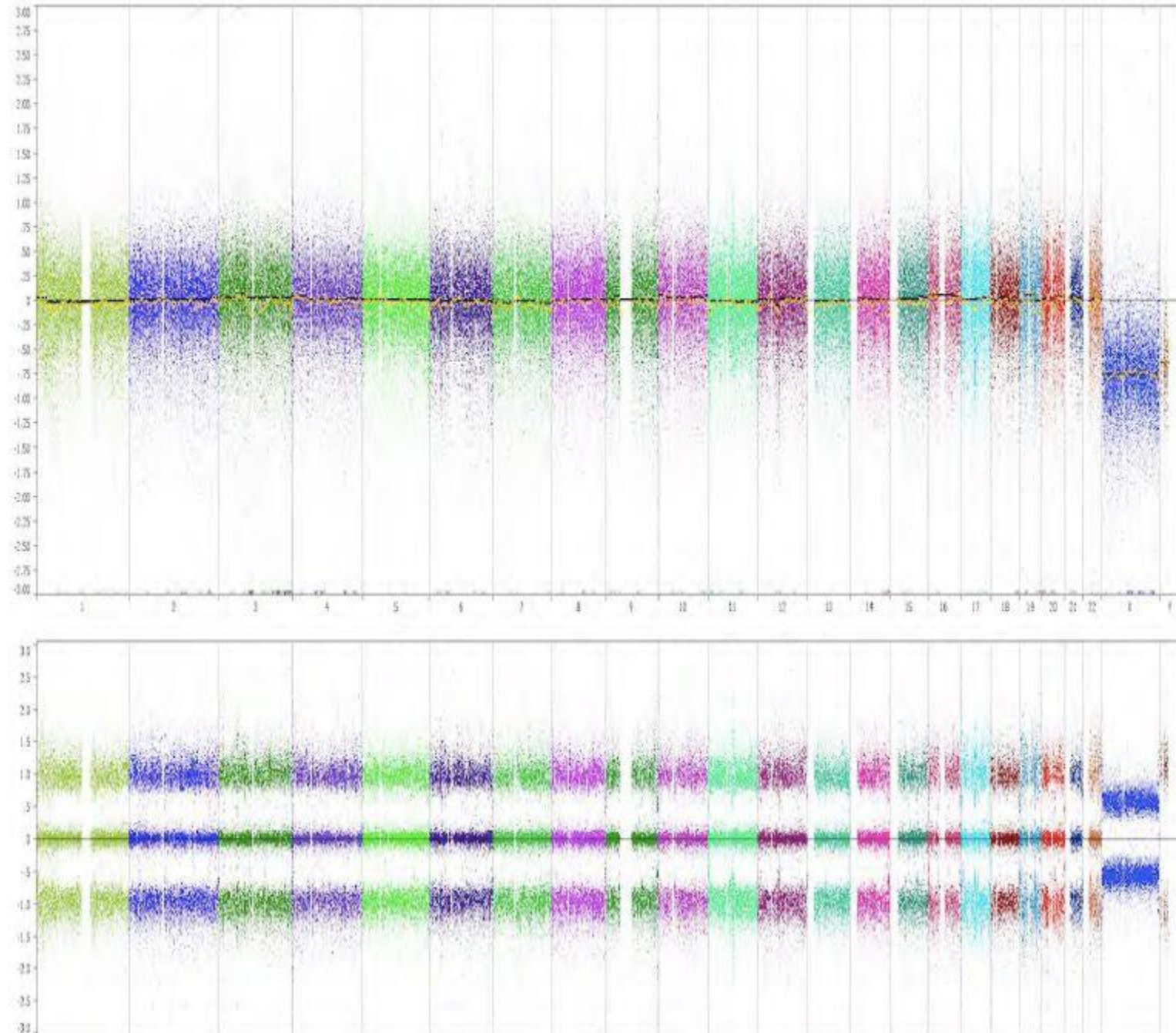
High Throughput. 2018 Sep 14;7(3):28

SNP Microarray



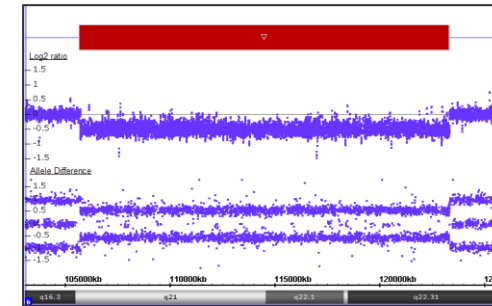
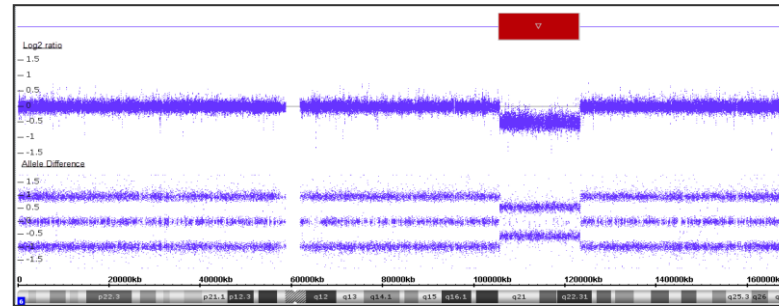
Modified from "Introduction to Cytogenetic Techniques", Azra H. Ligon, Ph.D.

Whole Genome View

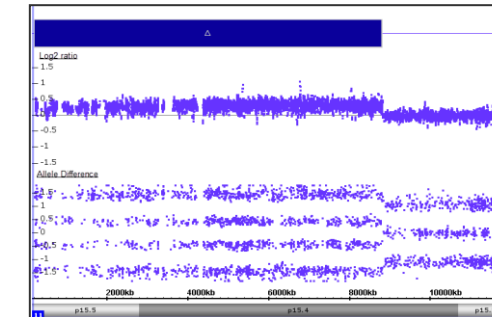
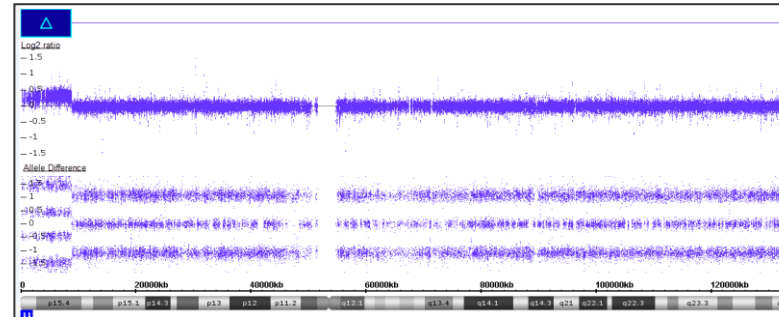


Segmental view

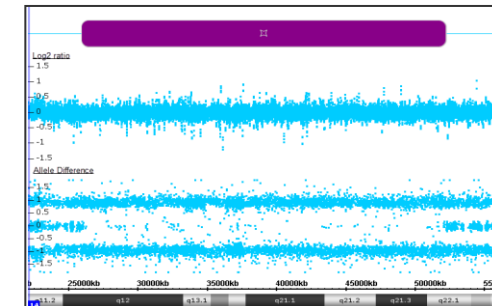
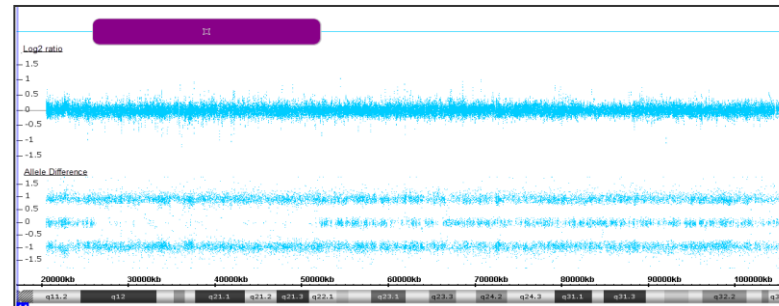
Deletion



Duplication



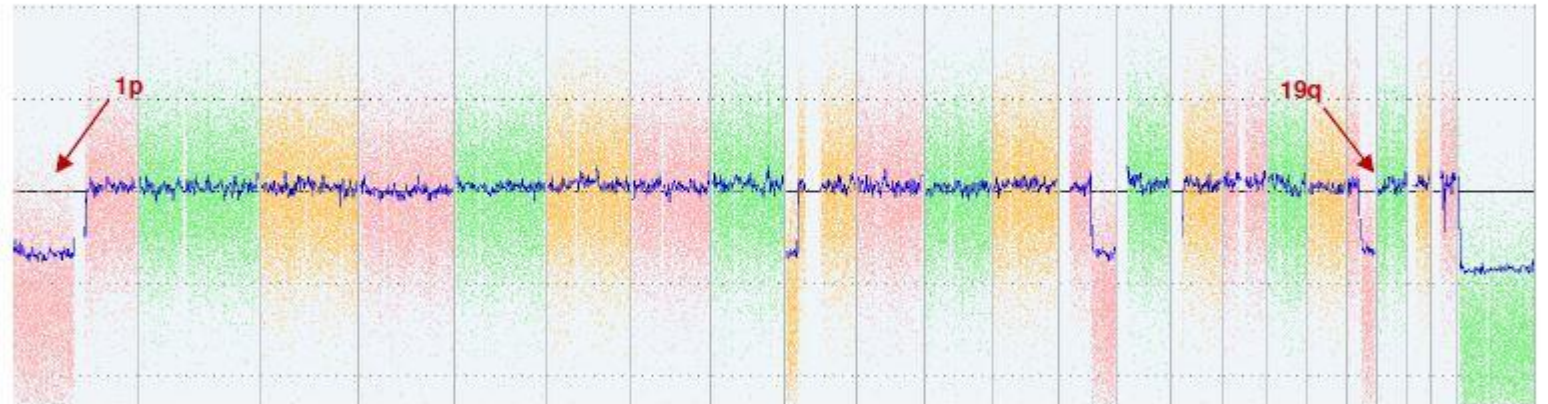
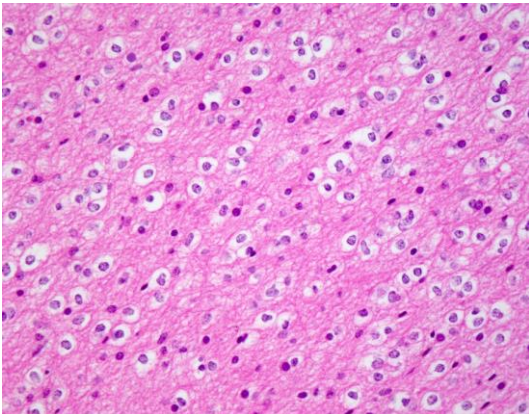
Region of Homozygosity (ROH)



Courtesy: Dr. E. Andersen

Which cancers should be studied by SNP CMA?

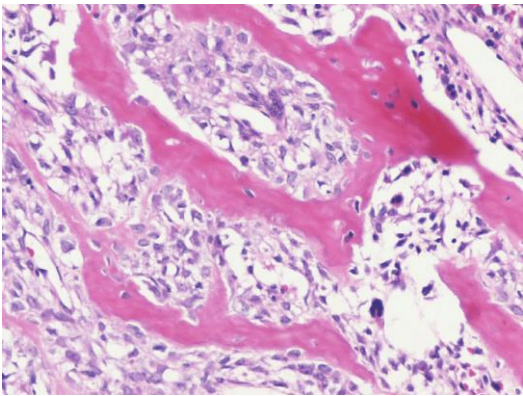
- Those characterized by recurrent copy number changes (whole/segmental aneuploidy and microdeletions/duplications) and/or loss of heterozygosity



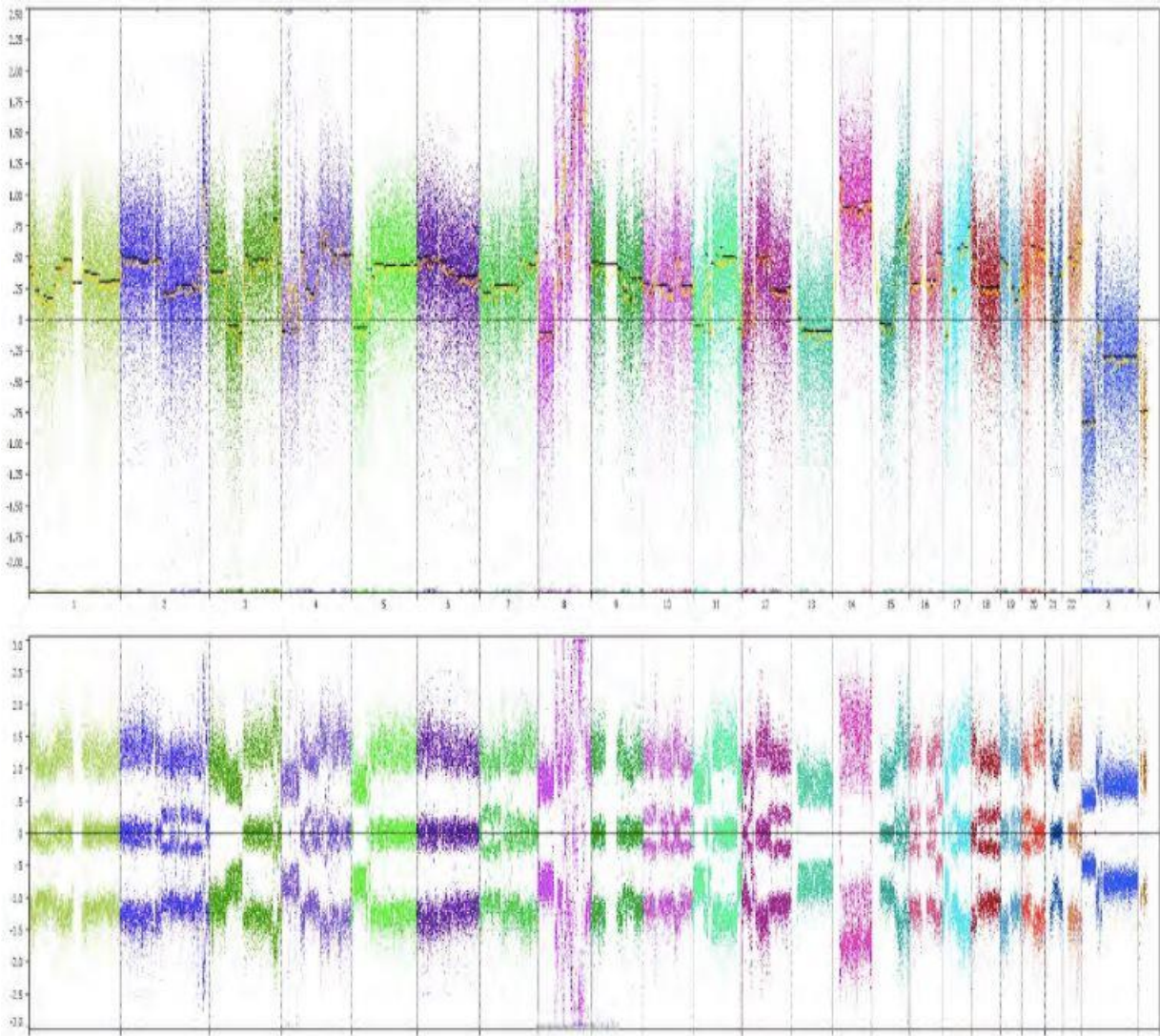
- Those that do not grow well in culture or have poor mitotic activity compared to nonmalignant cells

Modified from "Introduction to Cytogenetic Techniques", Azra H. Ligon, Ph.D.

15-year-old male with tibial lesion, rule out osteosarcoma

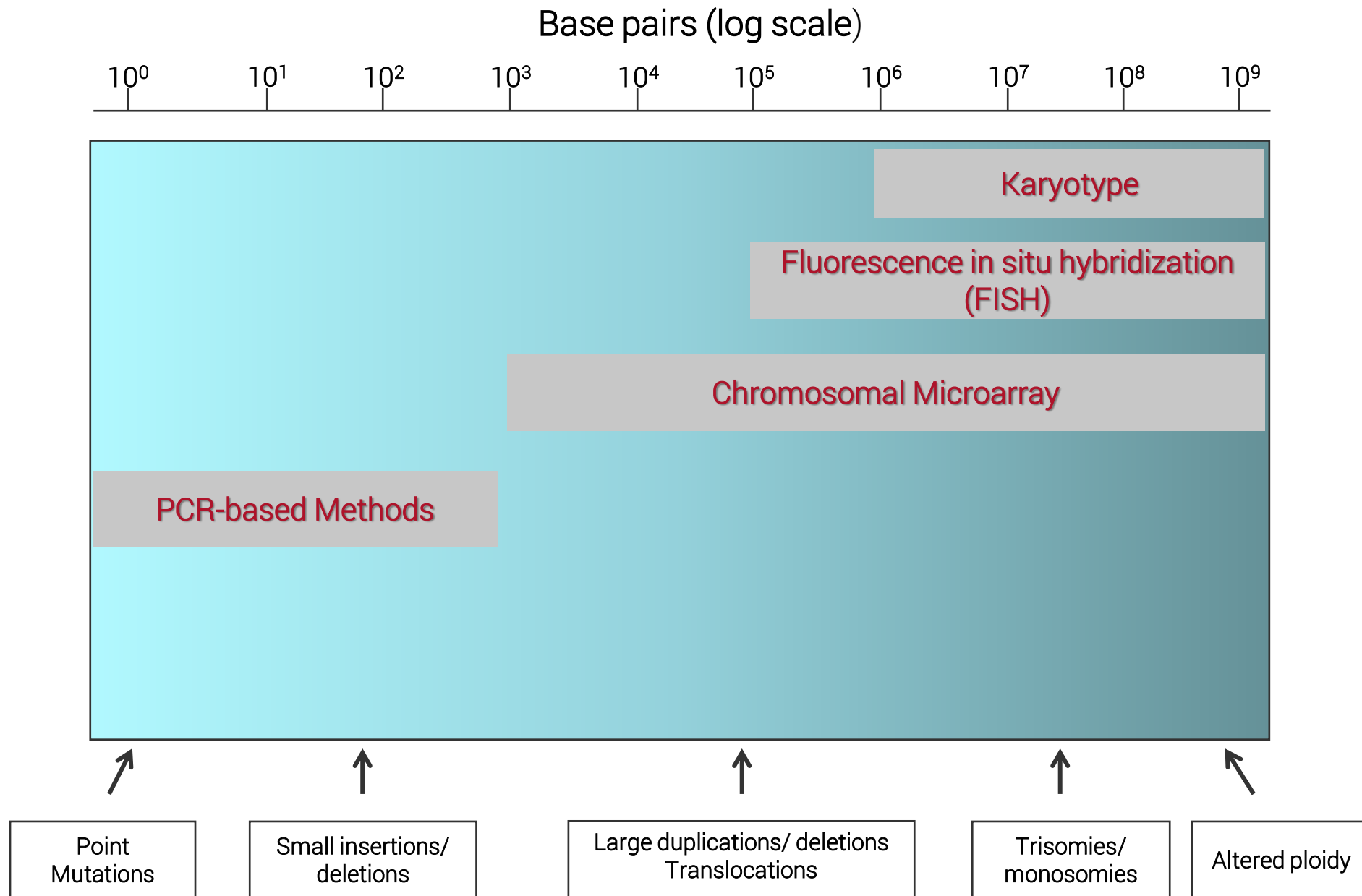


Chromosome results: 46,XY[20]



Chromosomal Microarray

- No cell culturing or cell preparation is required
- Can detect copy number variations in 10's of kb range (compared to 5-10 Mb by karyotype, 100's kb by FISH)
- Can detect copy neutral changes (loss of heterozygosity, LOH) if SNP genotyping is incorporated
- Cannot detect balanced structural abnormalities (balanced translocations, inversions)
- Cannot interrogate repetitive DNA sequence

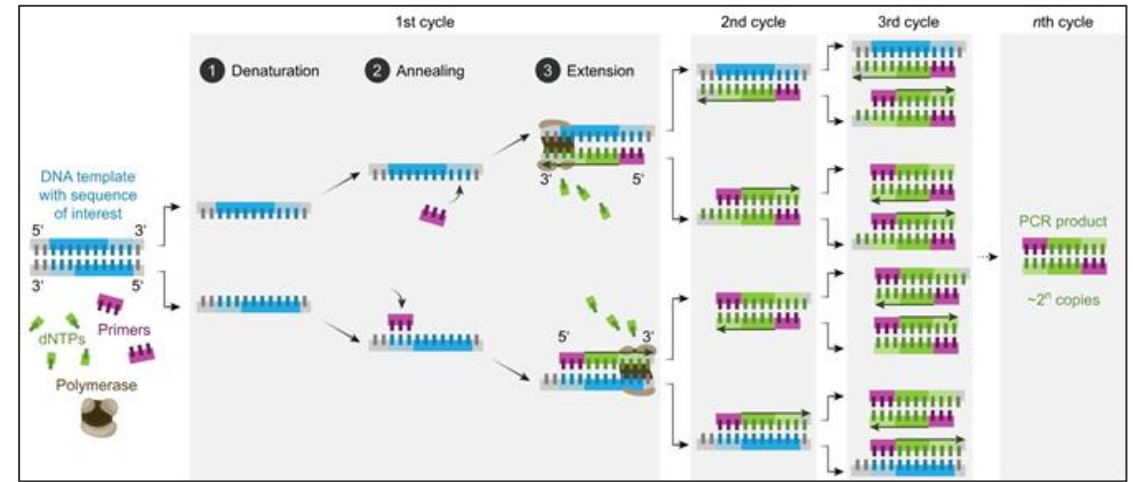
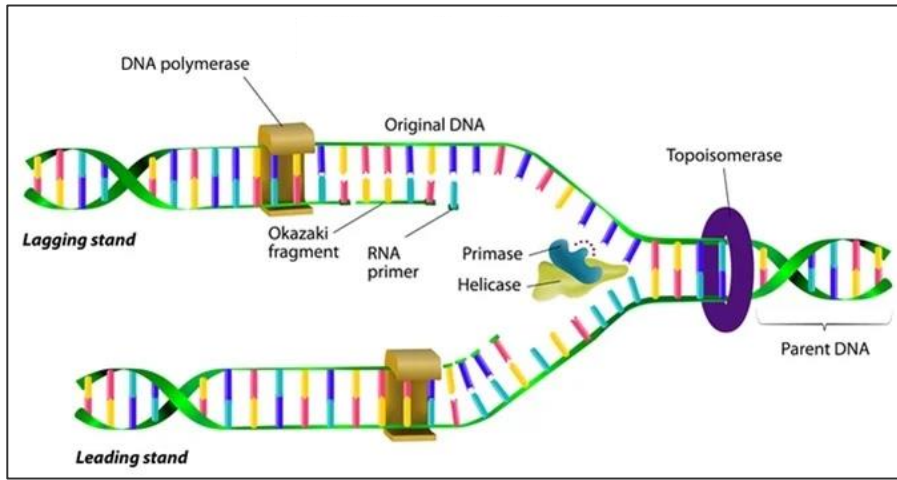




■ PCR-based assays

Polymerase Chain Reaction (PCR)

- Invented in 1983 by Kary Bank Mullins
- Mimics physiological process of DNA replication *in vitro*
- Rapidly makes millions of copies of a specific DNA sample
- Amplify a small amount of DNA to a large enough amount to study in detail



Helicase

DNA denaturation

Heat

Primase

Initial synthesis

Primers

DNA polymerase

DNA extension

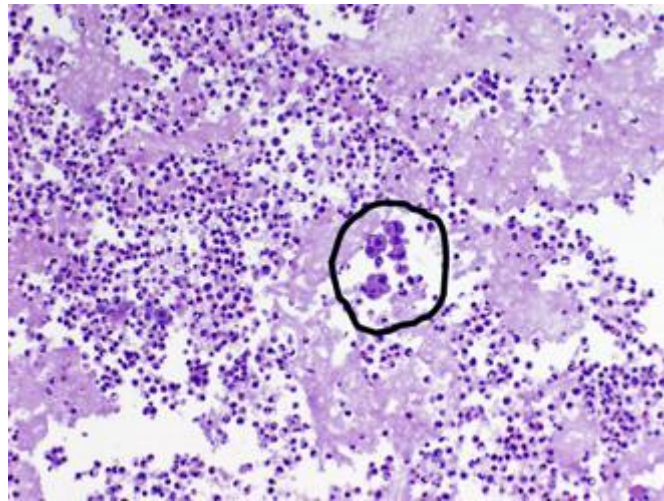
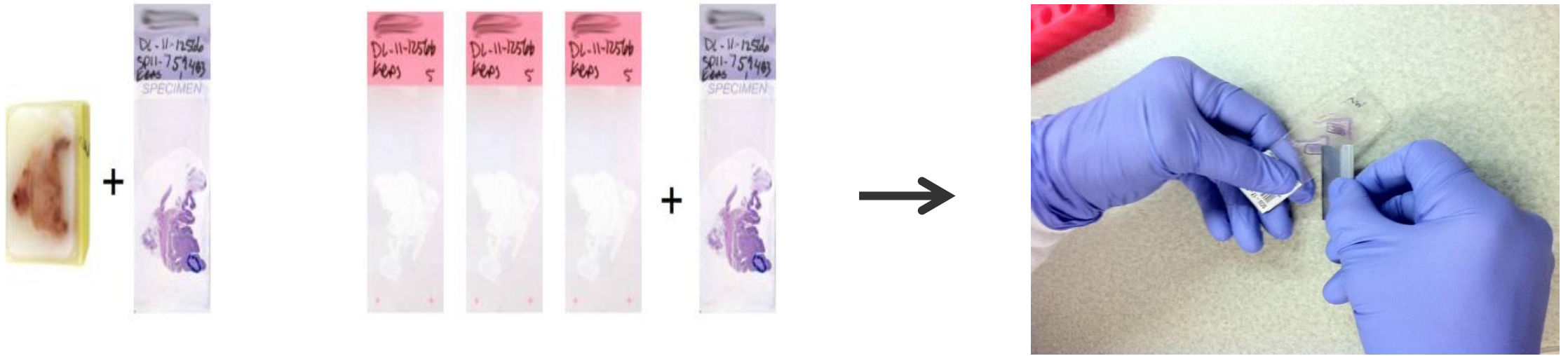
DNA polymerase

PCR variations

- Allele-Specific PCR (ARMS): detection of point mutations
- Real Time PCR: Template quantification
- Reverse Transcription PCR: RNA template
- Multiplex PCR: Simultaneous amplification of multiple DNA sequences

PCR is the initial step in almost all current molecular assays

- Pre-analytical considerations
 - Template quantity
 - How much tumor DNA does the assay require?
 - Assay complexity
 - Assay sensitivity
 - Template quality
- Inhibitors of PCR reaction

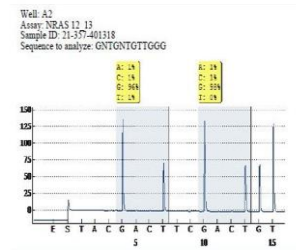


< 10%



Single gene assay

NGS panels



Nucleic Acid Extraction

Courtesy: Dr. M. Vasef

- Pre-analytical considerations

- Template quality

- DNA integrity

- Inhibitors of PCR reaction

- Melanin, heme, etc



Take-home messages

- Select appropriate methodology for the question raised and for the material available.
- Be mindful of pre-analytic factors while processing or selecting specimen for molecular assays.
- Molecular assays should never be interpreted as stand-alone diagnostic tests. Results must be correlated with all other available information, including clinical features, morphology, and immunophenotyping.

Acknowledgments

- Kristina Moore
- Dr. Erika Andersen
- Dr. David Czuchlewski
- Dr. William Bellamy



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