Fluorescence In Situ Hybridization (FISH)

Author Information and Affiliations:

Holli Drendel, PhD, FACMG

Atrium Health, Carolinas Pathology Group

Cytogenetics and Molecular Pathology

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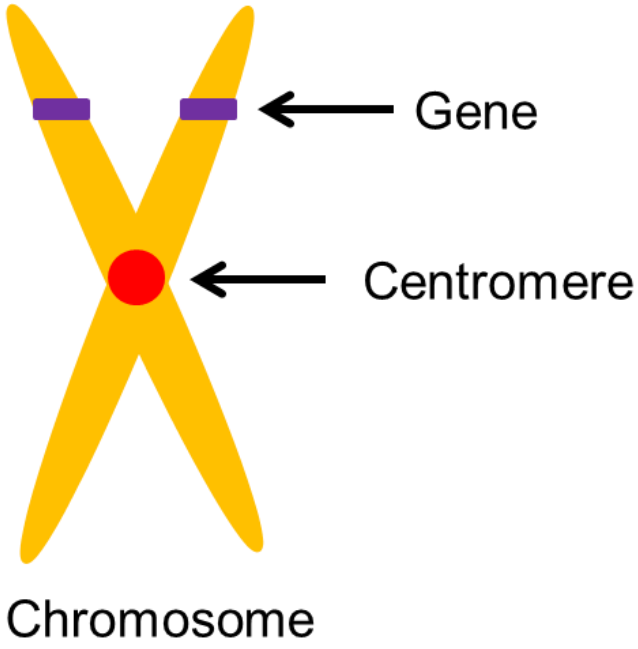
CANCER GENOMICS CONSORTIUM

Fluorescence in situ Hybridization (FISH)

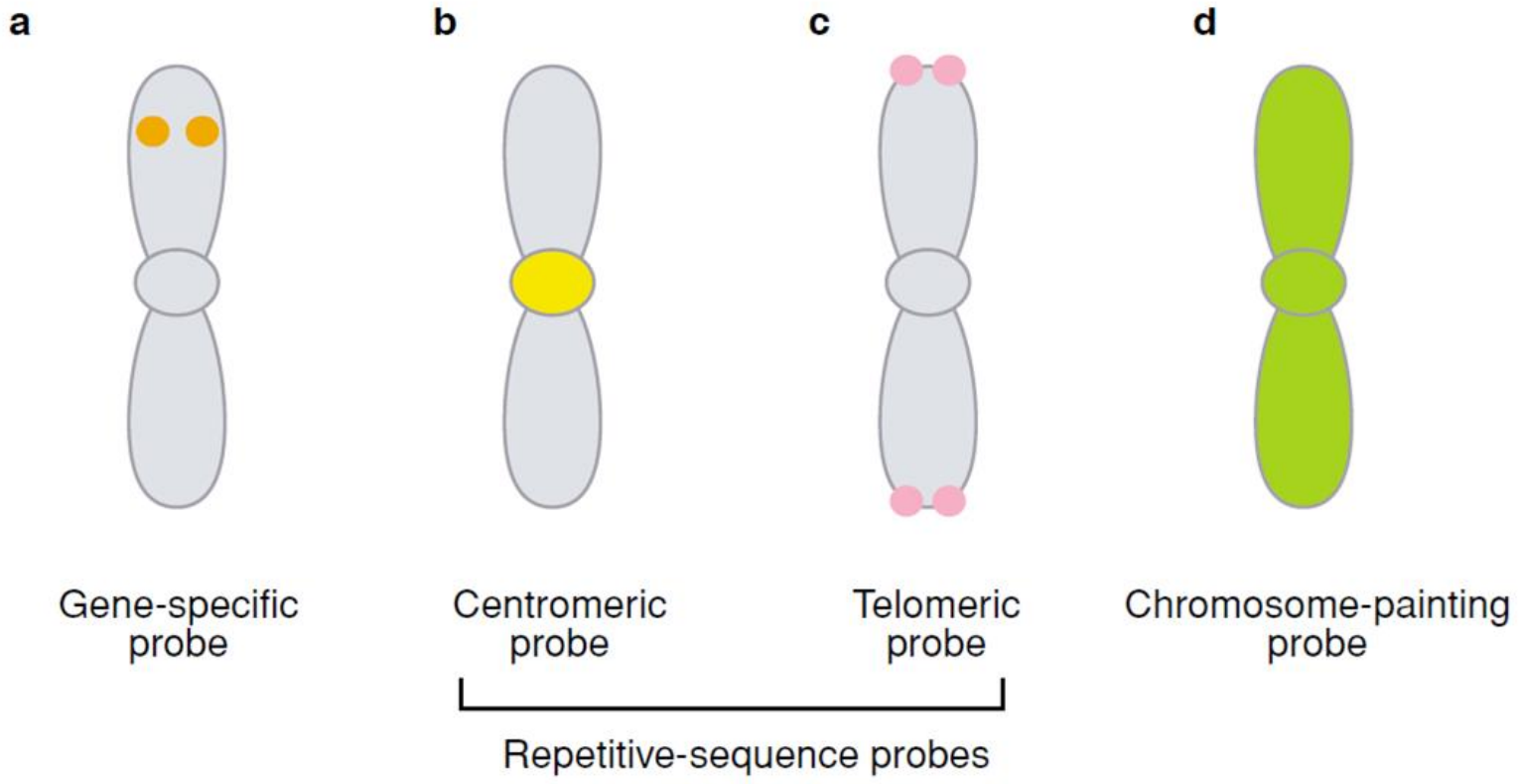
- Fluorescence *In Situ* Hybridization
- FISH is a molecular cytogenetic technique that allows DNA sequences to be detected in cells.
- DNA probes are denatured (made single-stranded) and hybridized to entire chromosomes or specific loci or regions of a chromosome.
- FISH serves as a powerful adjunct to classical cytogenetics.

What is FISH?

- Used to detect abnormalities in patient DNA
- Detection performed with small fragments of DNA (probes)
  - Locus specific: probe is on or near the gene of interest
  - Centromeric: probe identified the centromere of a chromosome
  - Painting probe covers the entire chromosome
  - Telomere probes mark the subtelomeric region of the chromosome
- Each probe has a fluorophore attached to it
  - The fluorescent probe is visualized with a fluorescence microscope equipped with appropriate filters



FISH Probe Types

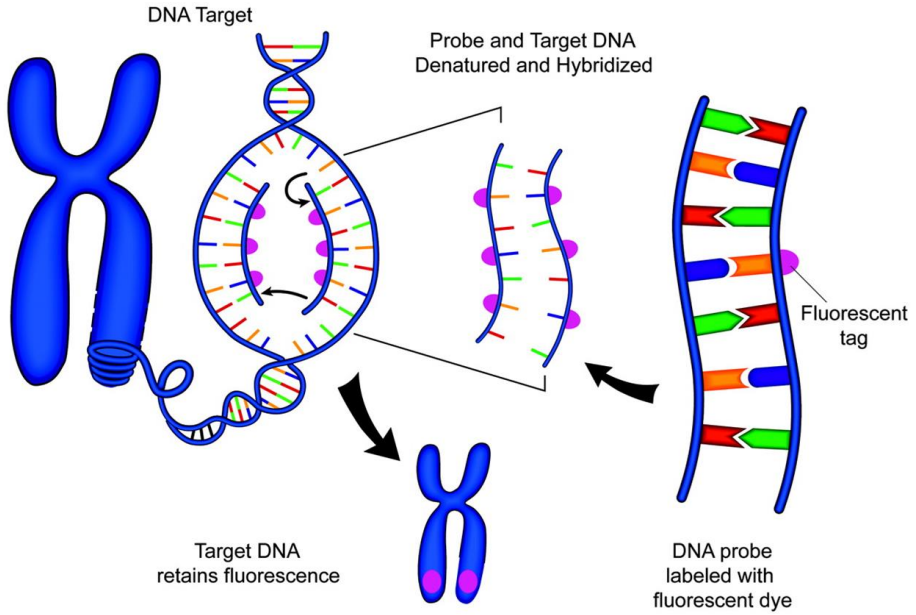


Examples of different types of fluorescence in situ hybridisation (FISH) probes

http://www-ermm.cbcu.cam.ac.uk/00001940a.pdf

Molecular basis of FISH

- DNA probe is labelled with a fluorescent marker
- DNA probe and target DNA are denatured and the probe hybridizes to the target DNA.
- Following hybridization, unbound and non-specifically bound DNA probe is removed by a series of stringent washes and the DNA counterstained for visualization (DAPI).
- Fluorescence microscopy then allows for the visualization of the hybridized probe on the target material.



https://www.ajnr.org/content/28/3/406/tab-figures-data

Advantages of FISH

- May be performed on dividing and non-dividing cells
- Will detect cryptic deletions and translocations
- Will detect low level mosaicism
- Faster turn-around-time than G-banded studies
- May be performed on paraffin-embedded tissue (PET) sections
- Clarifies complex/ambiguous cytogenetic results

Sources of DNA for analysis

- Peripheral Blood
- Bone Marrow
- Bone Core
- Amniotic Fluid
- Chorionic Villus tissue
- Neoplastic/Non-neoplastic tissue
- Paraffin Embedded tissue (PET)
- Fixed Cell Pellet
- Others

Classes of Probes

- Probes
  - Derived from gene/location of interest
  - >500 kilobases (kb) long
  - Fluorophore options
    - FITC (green)
    - Texas Red (red)
    - Spectrum Gold (gold)
    - Spectrum Aqua (aqua)
    - Others…

Classes of Probes

- Locus specific probes (LSI)
  - Used to elucidate deletions, rearrangement or gains/amplifications
  - Dual Color Locus Specific / Enumeration
    - Indicates deletions or gains of gene of interest
      - Normal: 2R2G; Abnormal: 1R2G
  - Break apart probes
    - Indicate a rearrangement of gene of interest
      - Normal: 2F; Abnormal: 1R1G1F
      - Can have atypical patterns indicating rearrangement

Classes of Probes

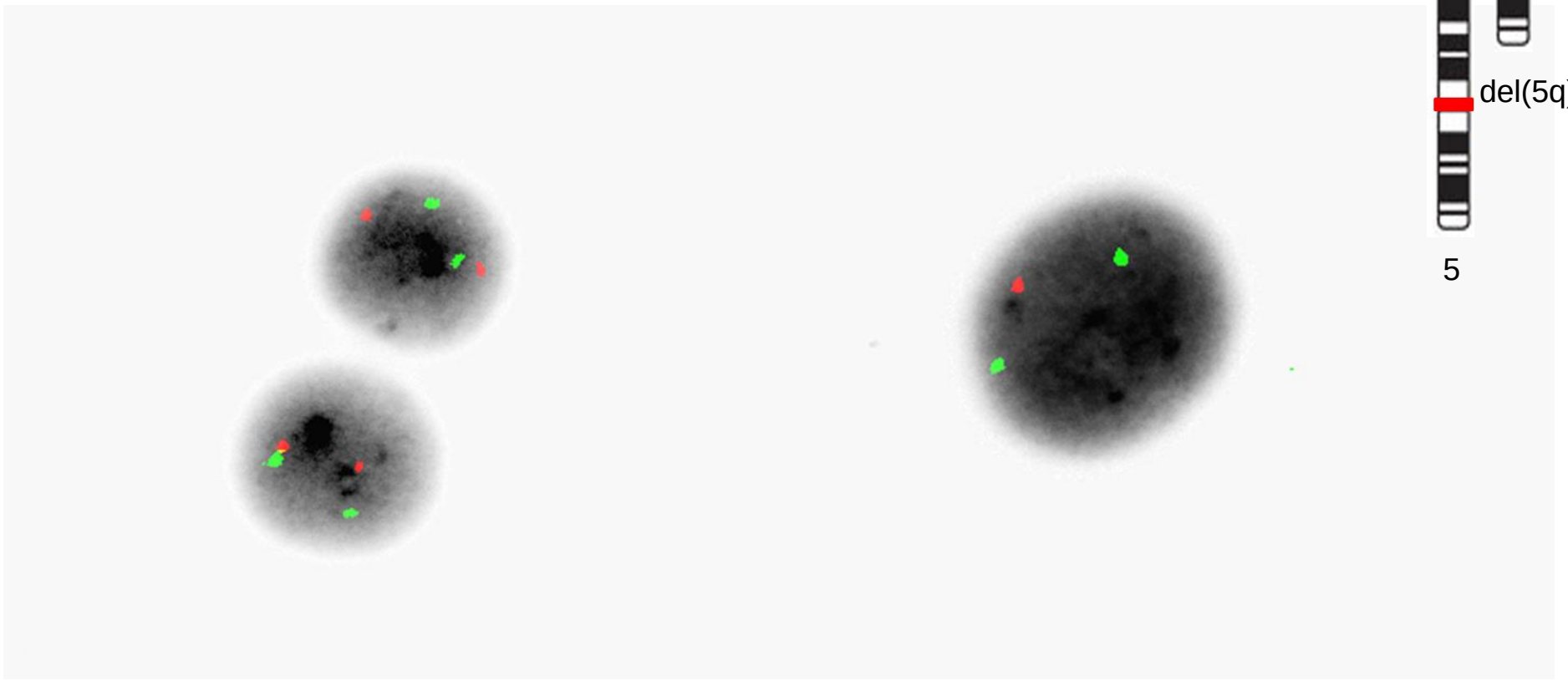
- Dual Color/Dual Fusion or Tri-Color/Dual Fusion
  - Indicates a potential translocation of genes of interest
    - Normal: 2R2G; Abnormal: 1R1G2F

- Chromosome enumeration probes
  - Used to determine how many copies of an individual chromosome are in a cell

  - Centromere probes
    - Indicates the number of chromosomes present
      - i.e. ‘Counts the chromosomes’

Dual color enumeration- 5p and 5q

Interphase (non-dividing) Cells

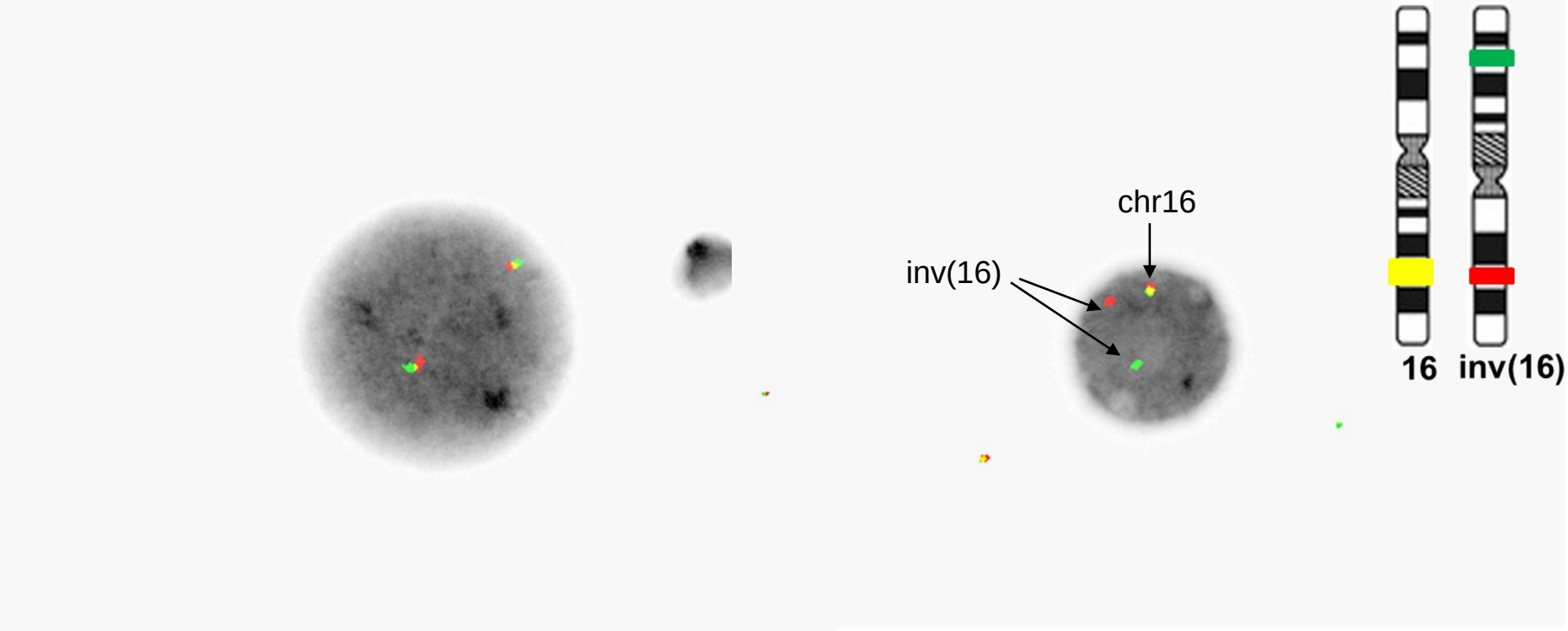


Normal Chromosome 5 signals (2R2G)

Abnormal Chromosome 5 signal Deleted 5q (1R2G)

Break Apart Probe- inv(16)

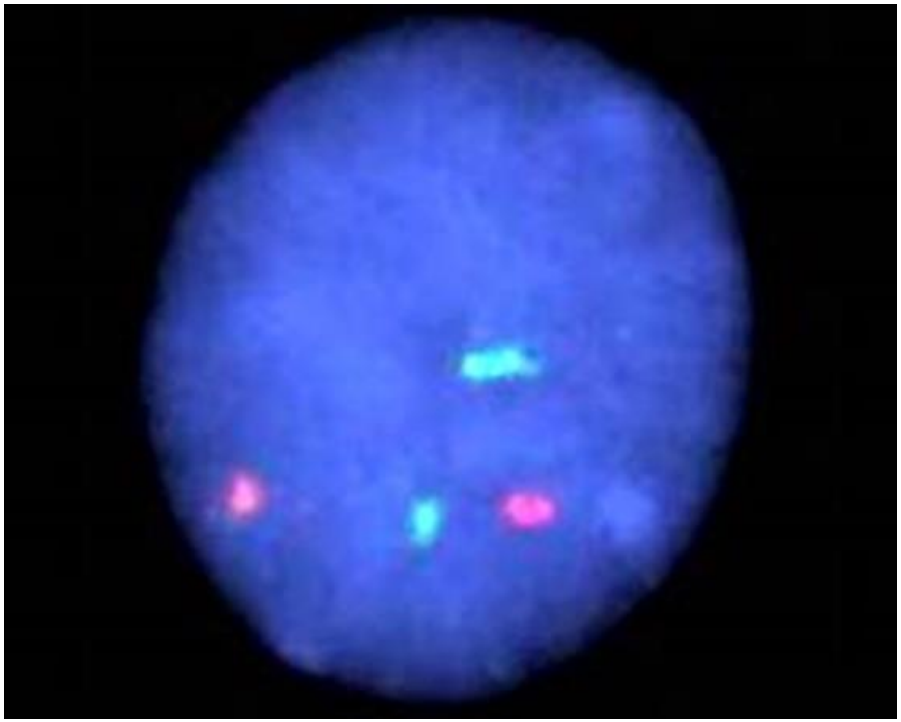
Interphase (non-dividing) Cells



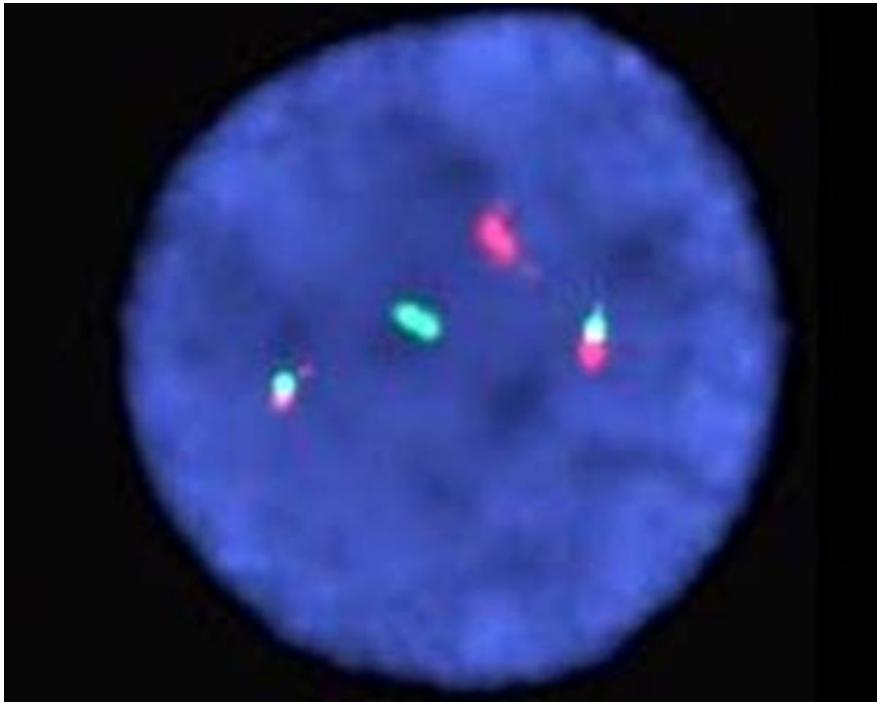
Normal Chromosome 16 signals (2F)

Abnormal Chromosome 16 signal inverted 16 (1F1R1G)

Dual color/Dual fusion probes- t(15;17)

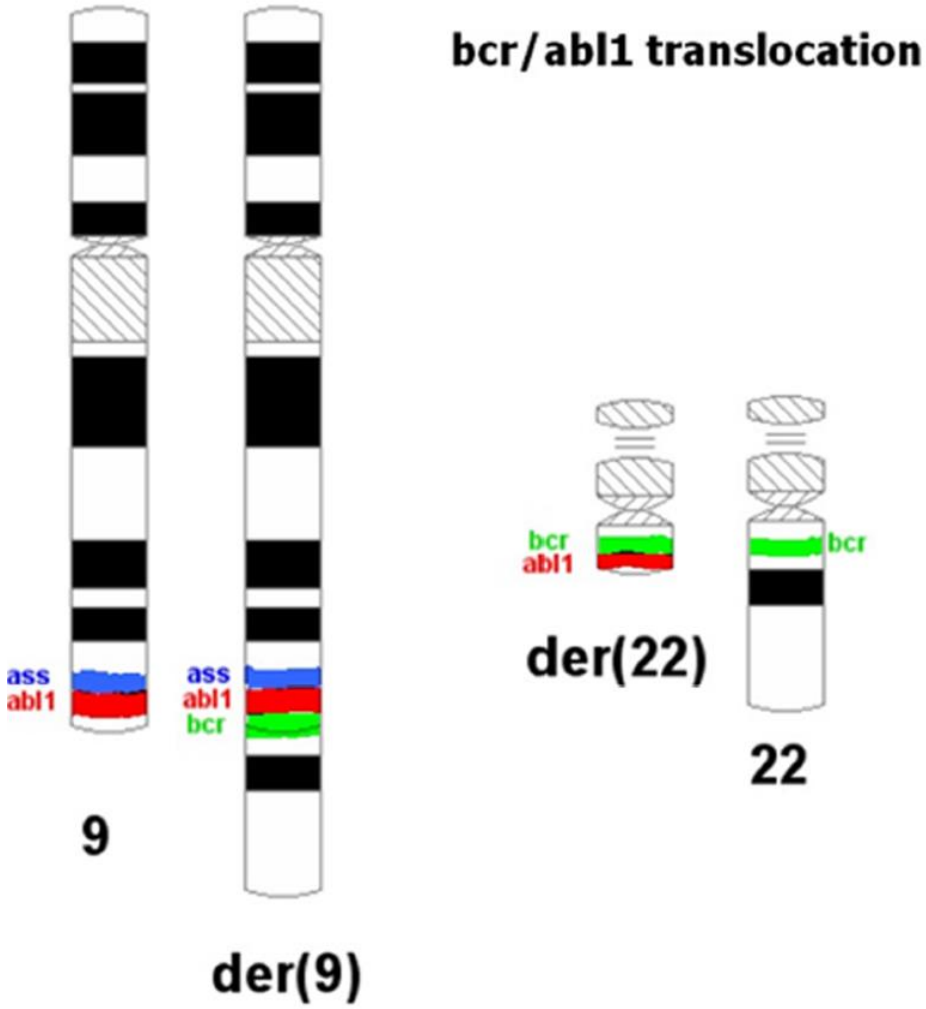


Normal pattern: 2R2G



Abnormal pattern: 2F1R1G

Tri-Color/Dual Fusion-BCR/ABL1/ASS



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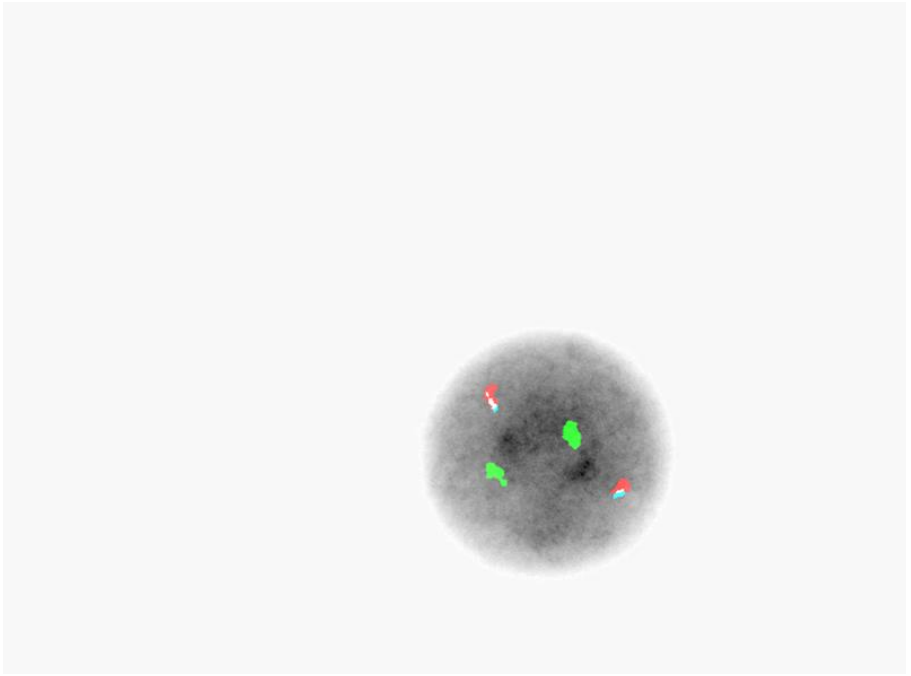

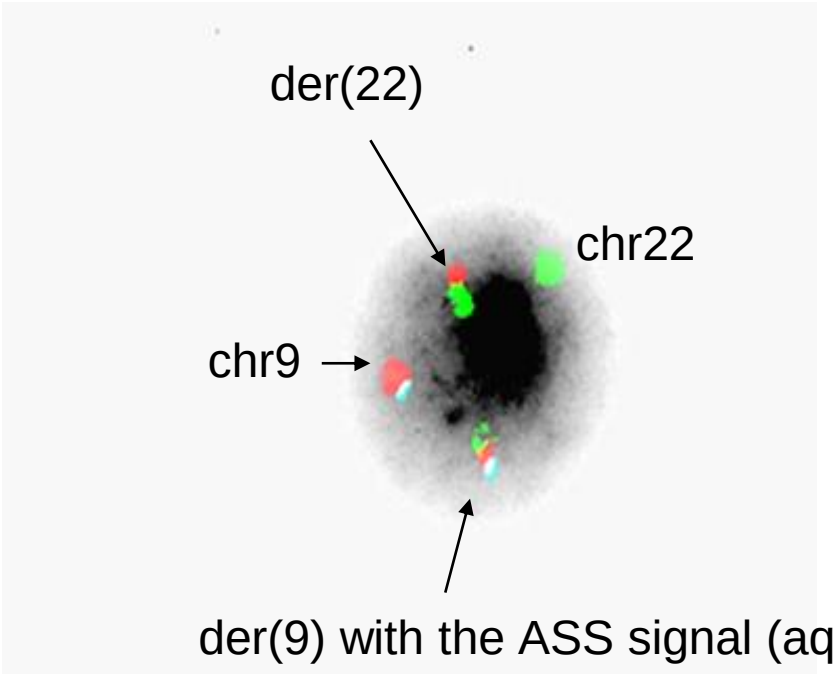

Normal *BCR/ABL1*/ASS signal (2R2A2G)

Abnormal *BCR/ABL1/ASS* signal (2F1R1G)

Centromere Probes- Trisomy 18

Aqua: Centromere 18
Green: Centromere X

Normal Chromosome 18 signal (2G2A)

Abnormal Chromosome 18 signal (2G3A) Trisomy 18