Introduction to Cytogenetic Techniques

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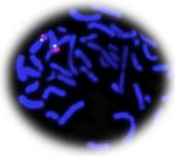
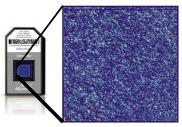
Cytogenetics: the numerical and structural evaluation of the genome from a chromosomal perspective

1. Diagnosis
2. Identify actionable alterations
3. Prognosis
4. Therapeutic management

Karyotyping



FISH



Microarray

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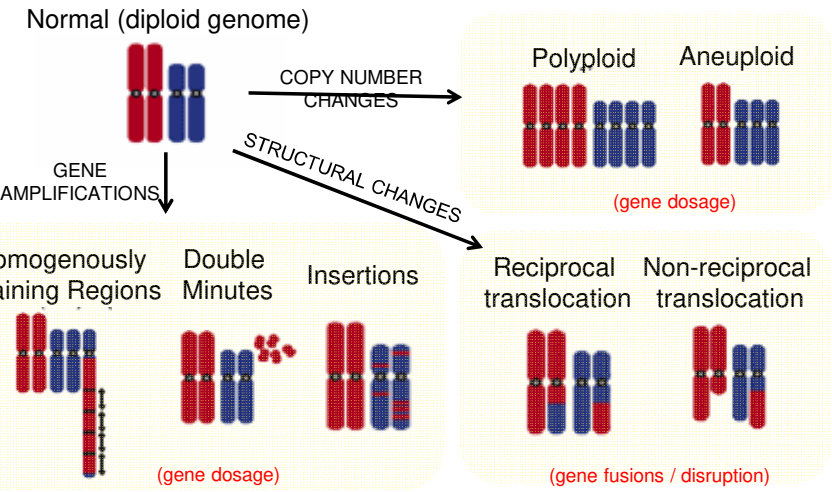
Cancer: A Disease of the Genome



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Modified from Nat Genet. 2003 Aug;34(4):369-76.

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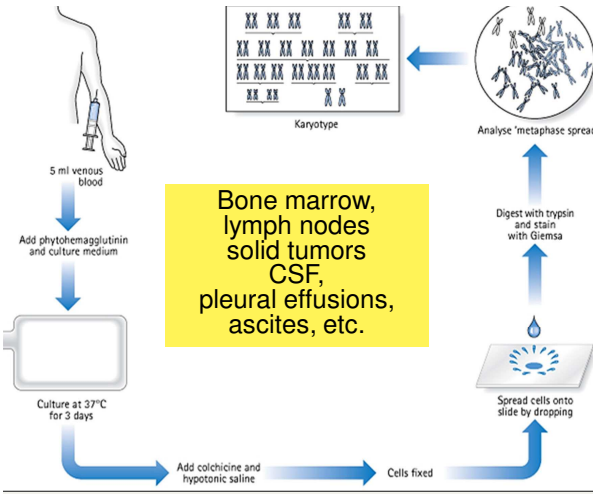
Karyotyping: Metaphase Preparation



Bone marrow,
lymph nodes
solid tumors
CSF,
pleural effusions,
ascites, etc.

Culture
- Peripheral bloods
- Bone marrows
- Solid tumors

Mitogens
- PHA
- Pokeweed
- LPS, CpG etc.

Colcemid
- Mitotic arrest

Harvest
- Hypotonic solution
- Fixative

Banding
- Trypsin digestion

Staining
- Giemsa

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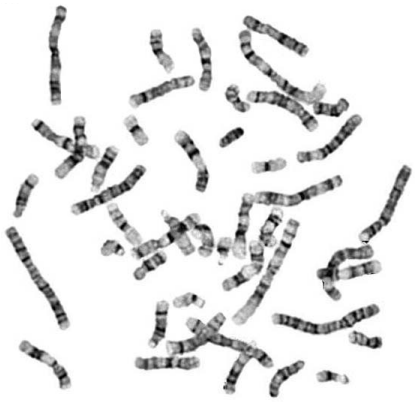
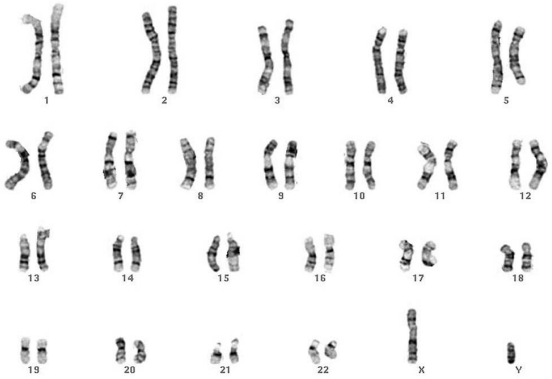
Karyotype Analysis





Evaluation of chromosomal number and structure

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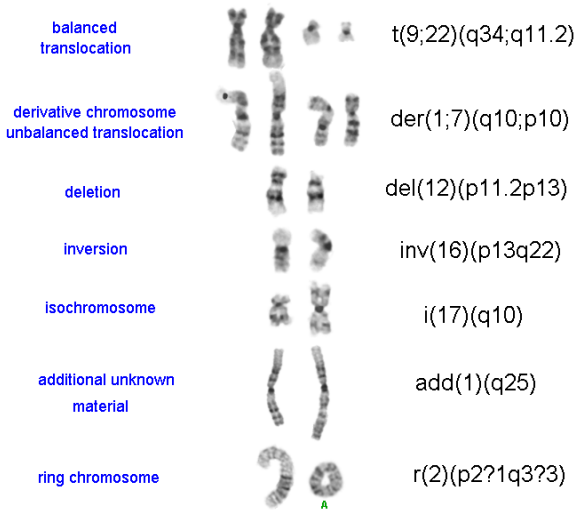
Structural Aberrations: 5-10 Mb resolution



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Overview of Karyotyping

Benefits	Limitations
• Numerical and structural aberrations • Single cell assay • Clonal populations • Requires intensive training	• 5-10 Mb resolution • Requires dividing cells • Requires extensive experience

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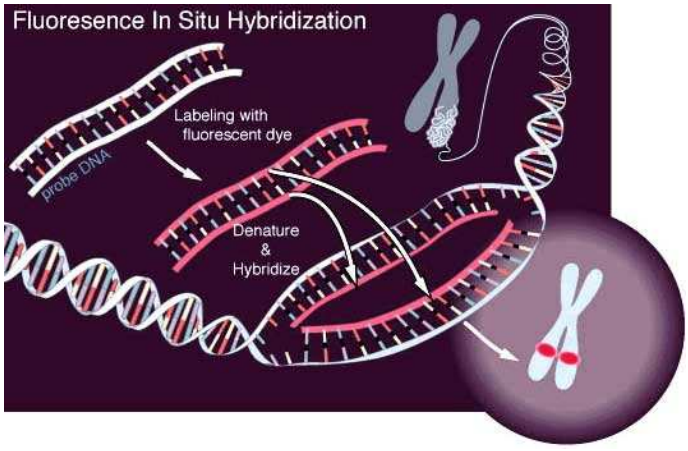
Fluorescence *In Situ* Hybridization (FISH):
~150 kb resolution



Probe Types:

- Enumeration
  - Assess copy number
- Break Apart
  - Is target disrupted?
- Dual Fusion, dual translocation
  - Is specific rearrangement present?

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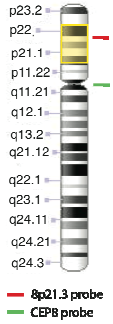
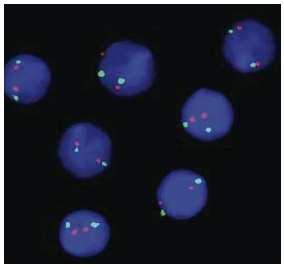
Interphase vs Metaphase FISH

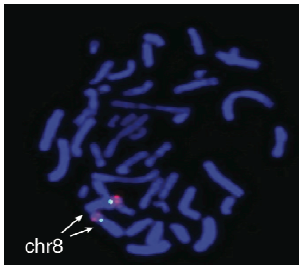


Interphase



- Structural and numerical
- Signals cannot be mapped
- Archival tissue

Metaphase



- Structural and numerical
- Signals can be mapped
- Actively dividing cells

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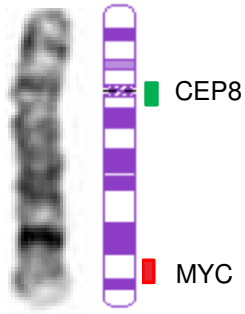
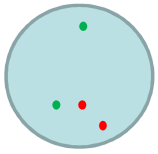
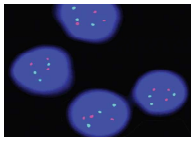
Enumeration Probes: Copy Number Determination

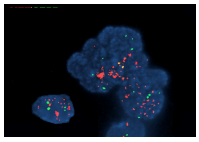


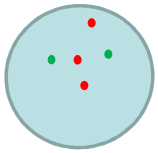
CHROMOSOME 8



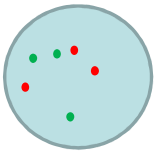
NORMAL



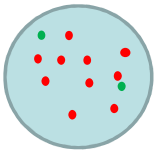




RELATIVE GAIN



POLYSOMY



AMPLIFICATION

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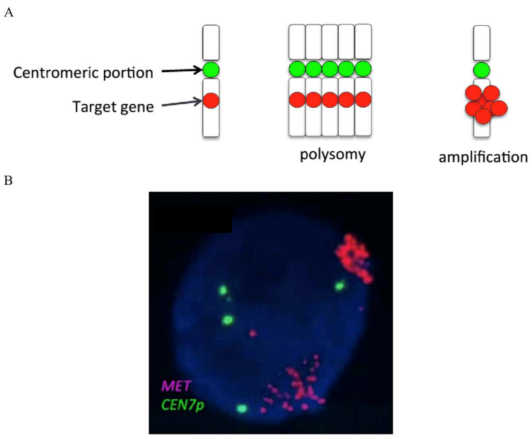
Amplification vs. Polysomy?



Consider:

- Ratio of target to control signals
- Signal pattern

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Excerpted from Kawakami H et al. (2014), PMID: 25055117

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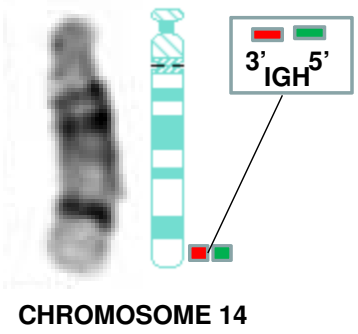
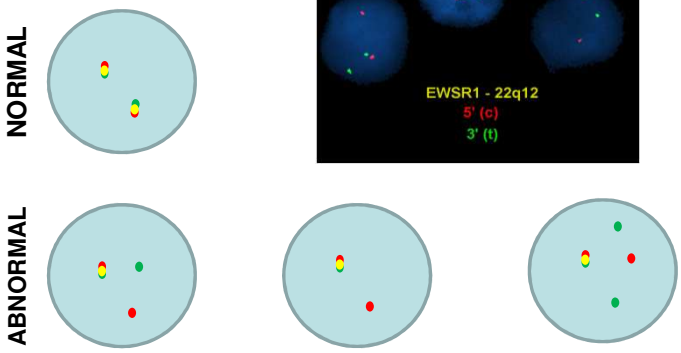
Break Apart Probes

Two probes spanning a single locus; partner gene not identified



CHROMOSOME 14



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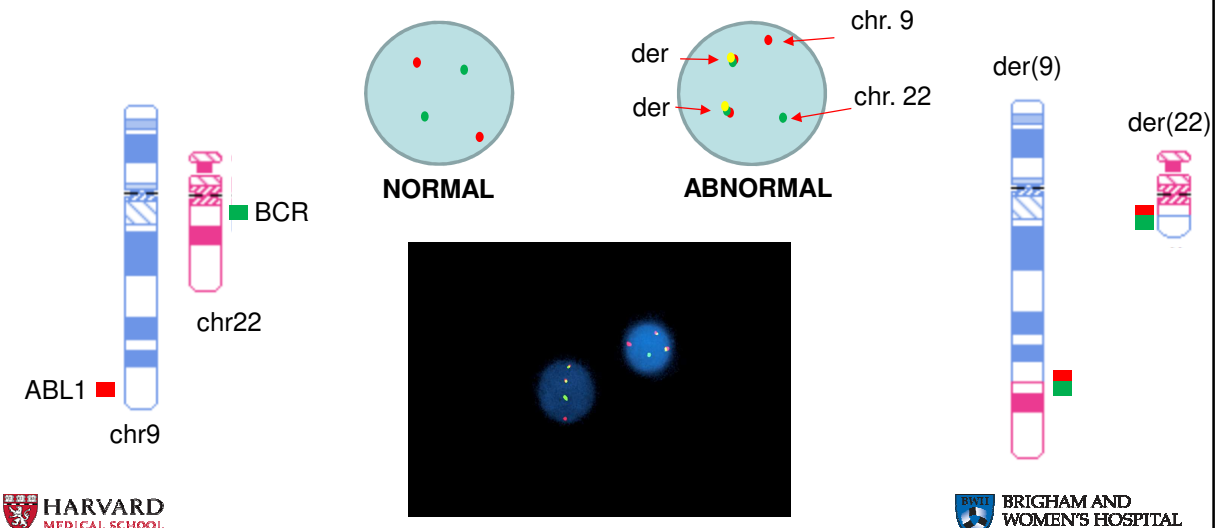
Dual Color, Dual Fusion Probes

Demonstrate rearrangement with probes spanning each partner gene



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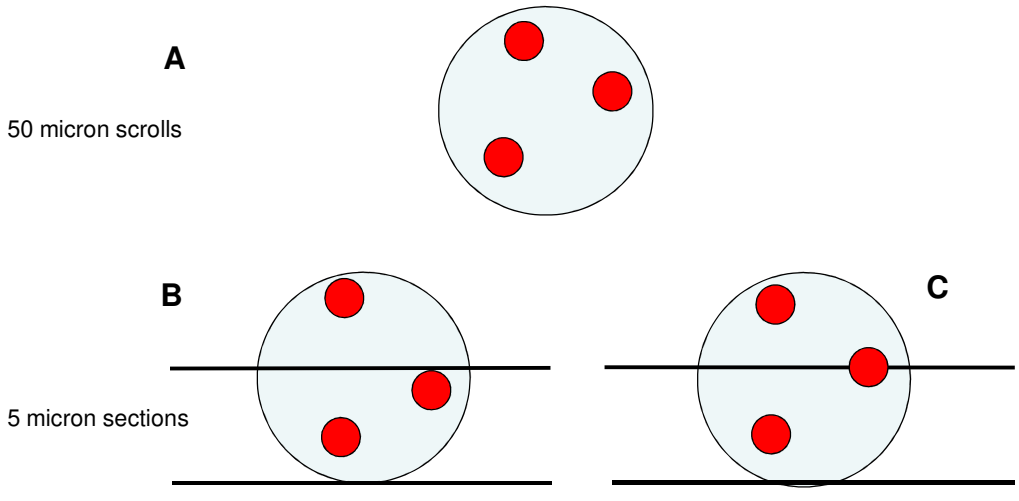
Truncation Artifact: Thin tissue sections



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Overview of FISH

Benefits

- FFPE or fresh material
- Single cell assay
- Clonal populations identified
- Numerical + structural aberrations
- Rapid TAT

Limitations

- 100-500 kb resolution
- Targeted assay

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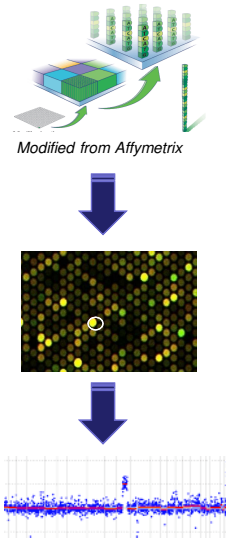
Chromosomal Microarray (CMA)

Array CGH	SNP arrays
Single-sequence oligonucleotides of ~60 bp	Two 20–60-bp oligonucleotides of different sequence
Two labeled DNAs (patient and control) per hybridization	Only patient DNA labeled and hybridized
Resolution down to size of oligonucleotides; exon by exon	Resolution limited by SNP distribution and signal to background
No detection of UPD or consanguinity	Able to detect consanguinity and most UPD
Limited SNP addition possible recently	Detection of most known clinically relevant CNVs but not exon by exon

Abbreviations: CNV, copy number variant; UPD, uniparental disomy.



Modified from Affymetrix

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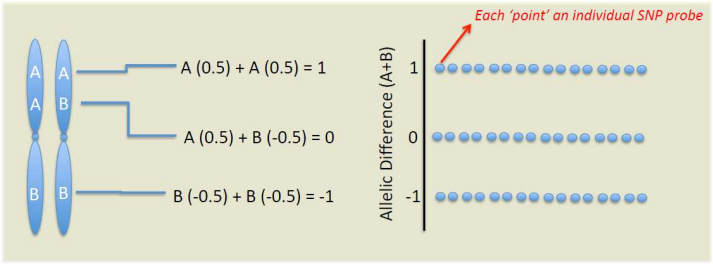
Evaluating Allele Peaks

Consider a diploid genome…



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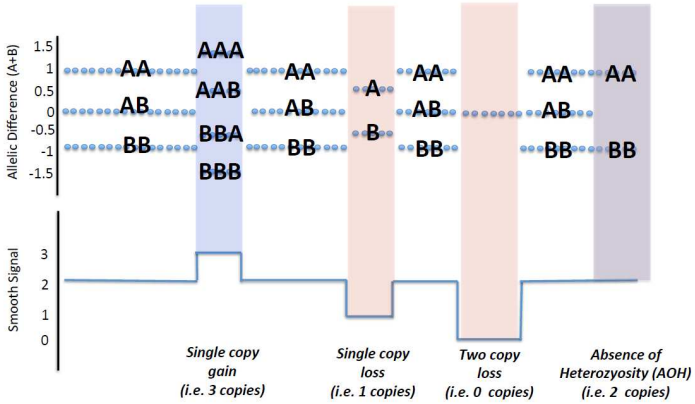
Courtesy: A. Dubuc

Examples of Allele Pattern

Consider the following SNP patterns across a given chromosome



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Courtesy: A. Dubuc

SNP Microarray Analysis of Cerebellar Low-Grade Glioma with Piloid Features

1.9 Mb single copy gain characteristic of *BRAF-KIAA1549* fusion

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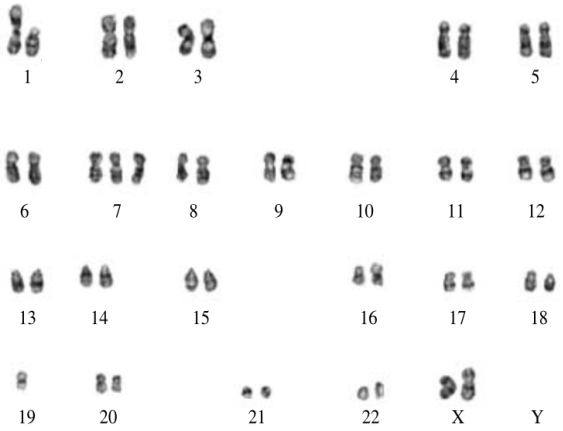
Oligodendroglioma: 1p/19q Whole Arm Co-Deletion



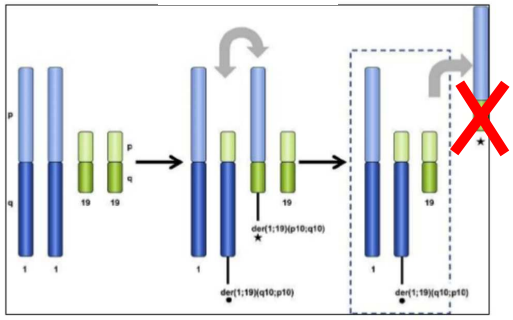
der(1;19)(q10;p10)

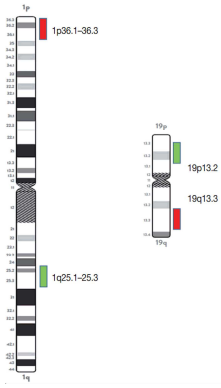
- 1p/19q co-deletion is mediated by **whole arm translocation**

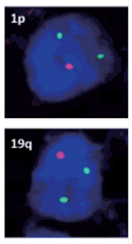
Modified from Jenkins et al, Cancer Res 2006 Oct 15;66(20):9852-61. PMID: 17047046

Modified from Griffin et al, J Neuropathol Exp Neurol. 2006 Oct;65(10):988-94. PMID: 17021403

1p/19q Co-Deletion Detected by FISH







Modified from Ahmed I, Molecular Pathogenesis of CNS Tumors (2016), https://www.slideshare.net/imtiazamc/molecular-pathogenesis-of-cns-tumors
Modified from Park S-H, et al., J Pathol Transl Med (2017) PMID: 28535583

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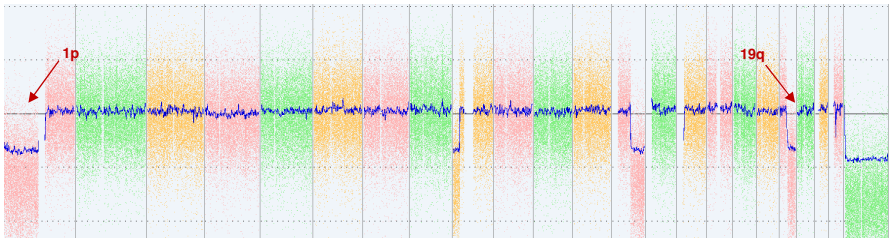
Oligodendroglioma: Analysis by SNP CMA



1p and 19q deletions should always:
(a) have the same amplitude (i.e. log ratio)
(b) be whole-arm losses

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Overview of SNP CMA for Tumors

Advantages	Disadvantages
• Can confirm, refine, or refute histologic diagnosis • 7-14 day TAT • FFPE or fresh material • LOH • High resolution (~ 100 bp)	• Cannot identify balanced structural changes • May also identify incidental findings

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Summary:

- Karyotyping
  - Broad chromosome aberrations can be visualized easily
  - Whole genome assay (~ 5-10 Mb)
  - Balanced and unbalanced rearrangements
- FISH
  - Targeted assay (~ 150 kb)
  - FNA, touch preparations, FFPE material
  - Integration of genomic and morphologic/cytologic studies
- SNP microarray
  - Whole genome assay (~ 100 bp)
  - Unbalanced genomes
  - Some ploidy changes, loss of heterozygosity

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