

Mutation Detection Strategies

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Mutation Detection Strategies

- What type of sample are you using?
 - Paraffin embedded tissues may limit options
- What type of mutation is it?

Common Types of Mutations

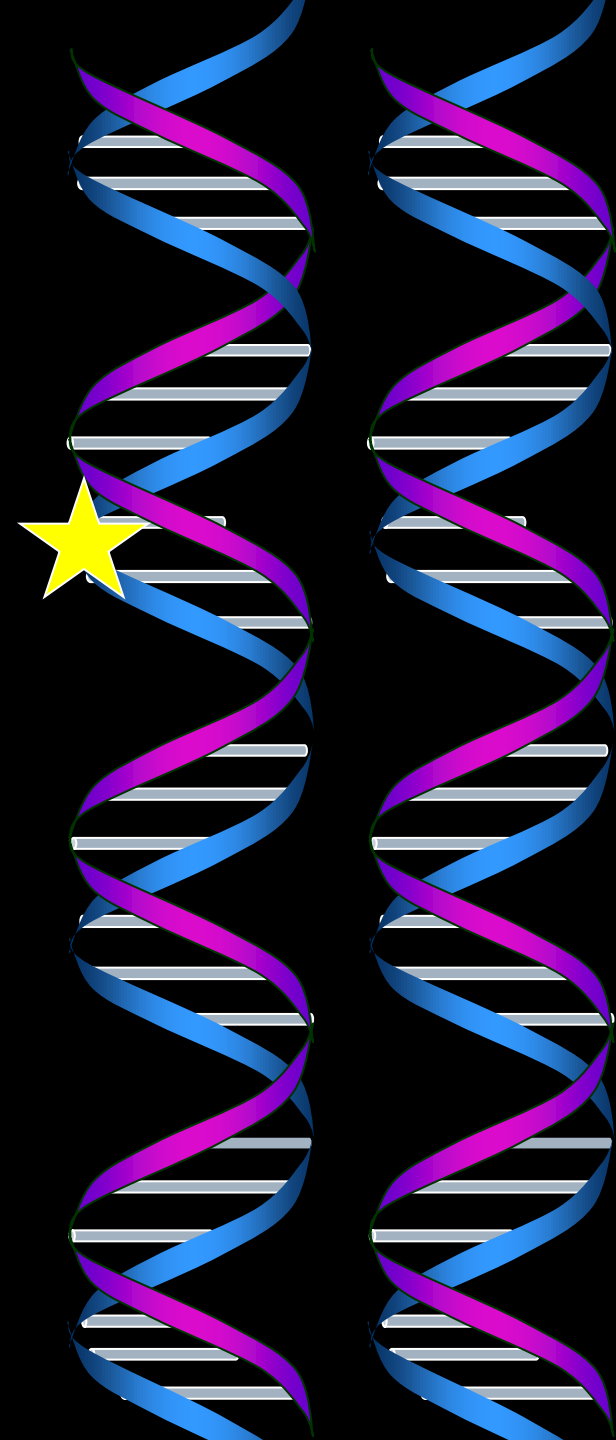
- Point mutation
- Translocation
- Amplification
- Deletion
- Microsatellite instability

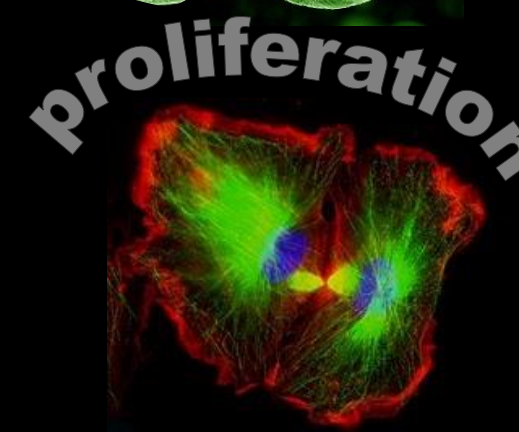
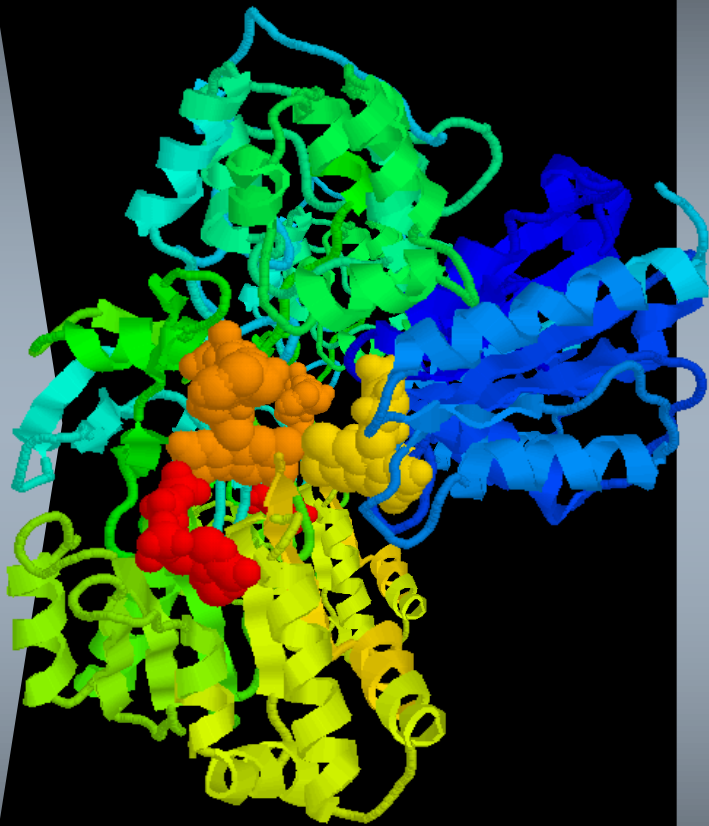
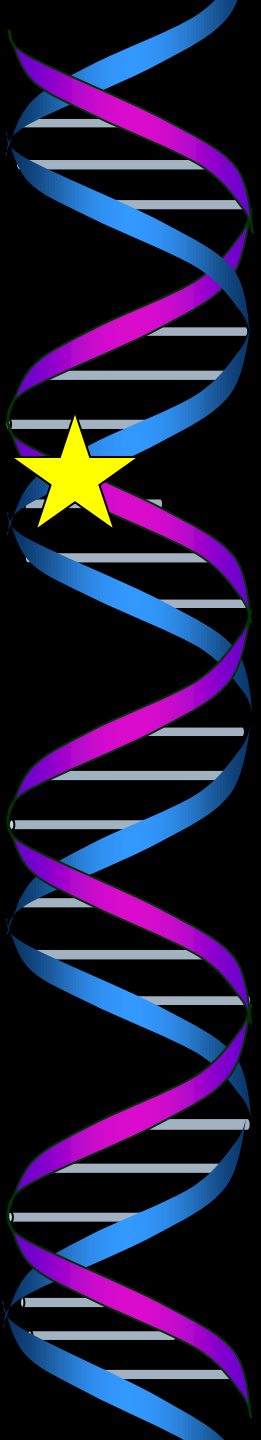
Point Mutations

- Diseases associated with point mutations
 - Hereditary diseases
 - Tumors with somatic mutations in oncogenes

Oncogenes

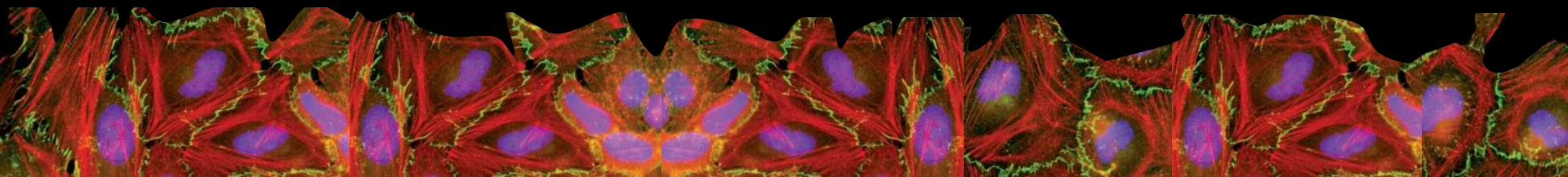
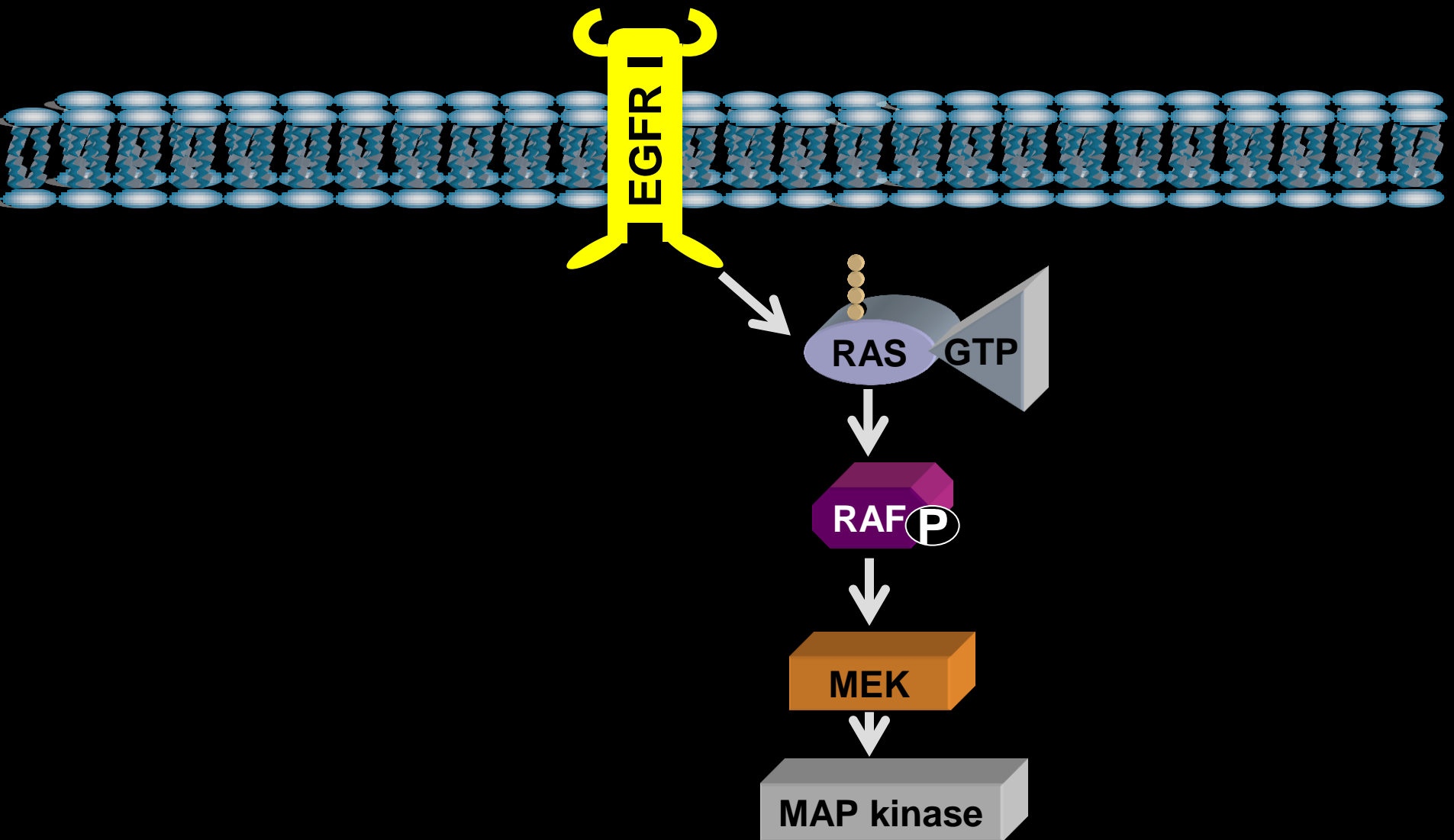
- Dominant genes
 - One copy mutated
 - Activating mutations
 - Point mutation
 - Amplification
 - Translocation
-



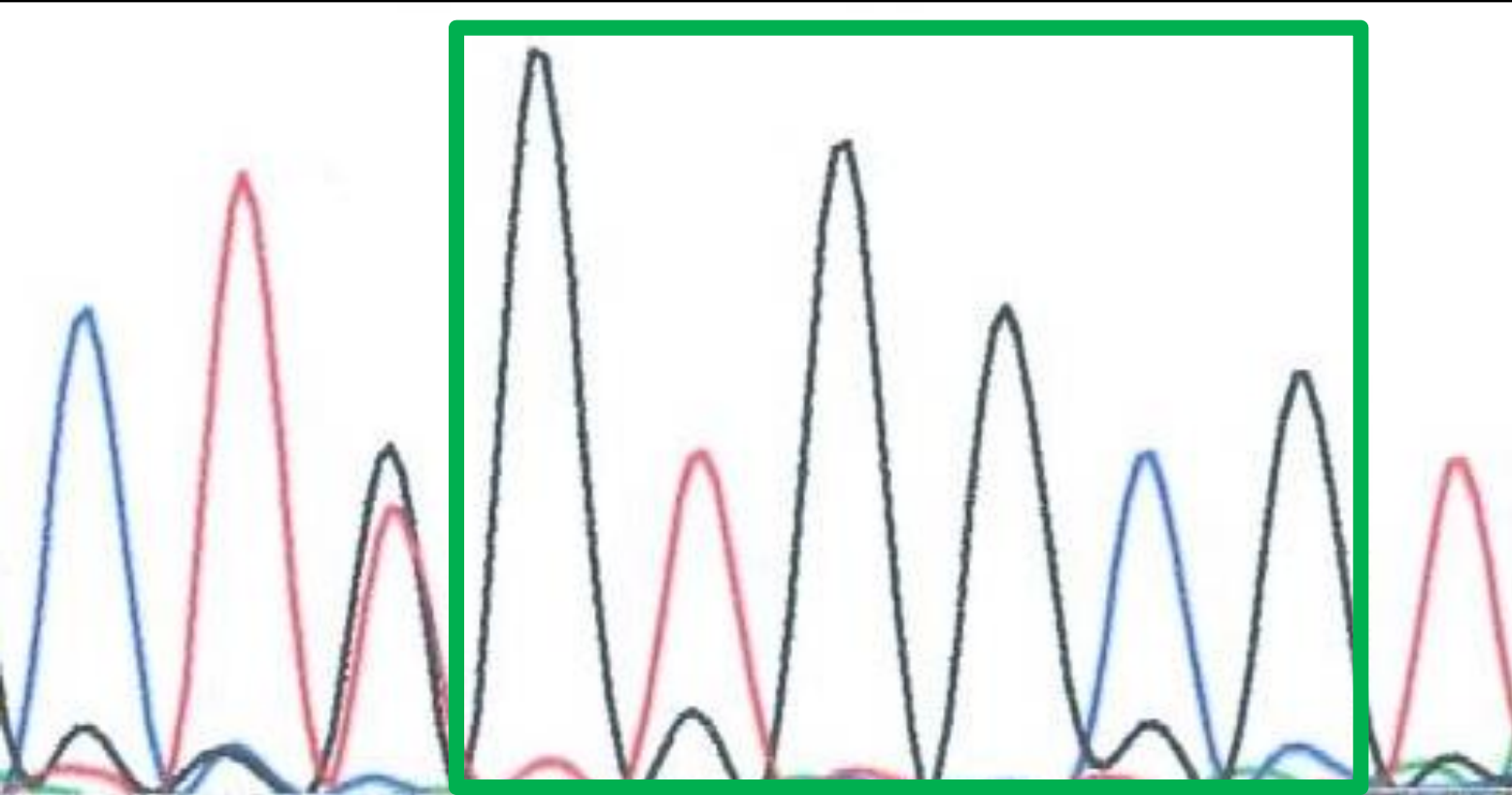


Point Mutation Detection

- Is it in a consistent, small, and reproducible region?
- Is it variable across a larger region?
 - Sanger sequencing
 - Next generation sequencing

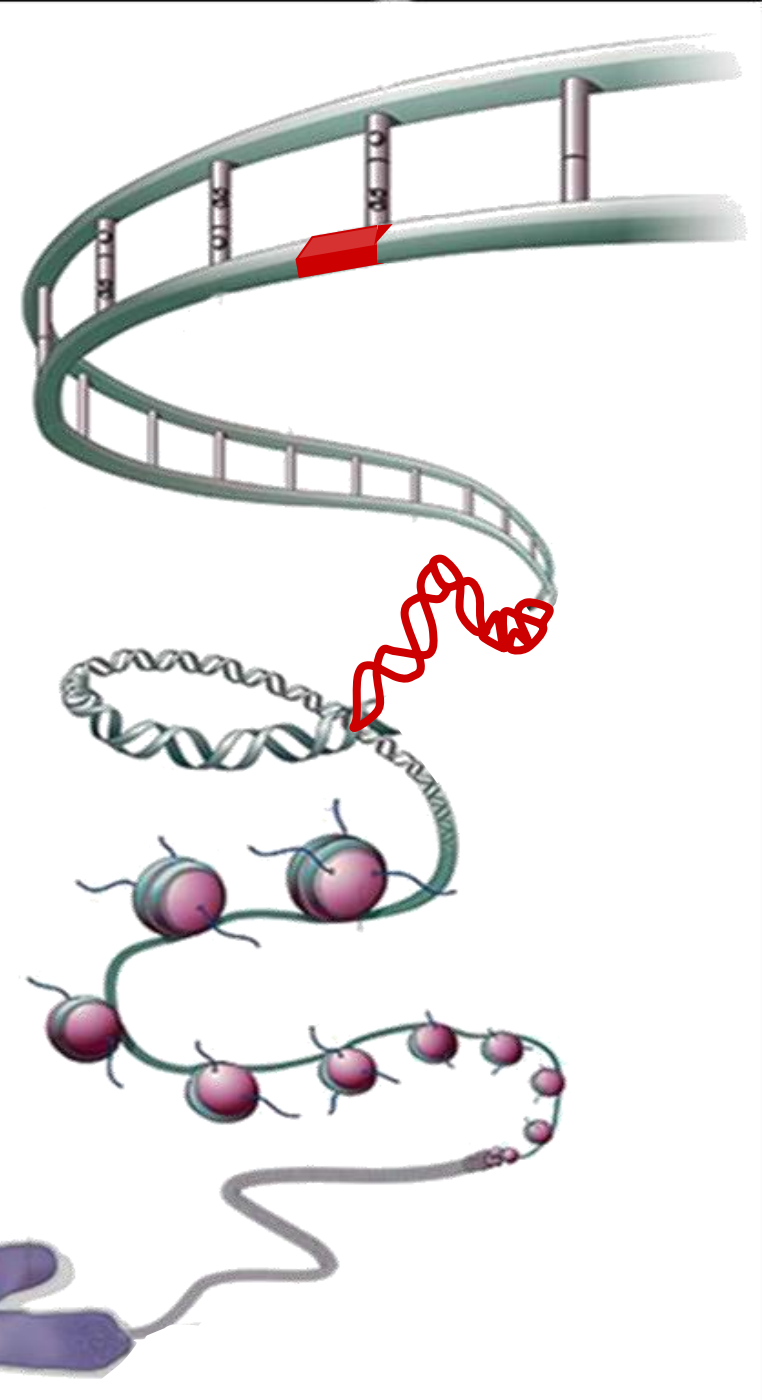
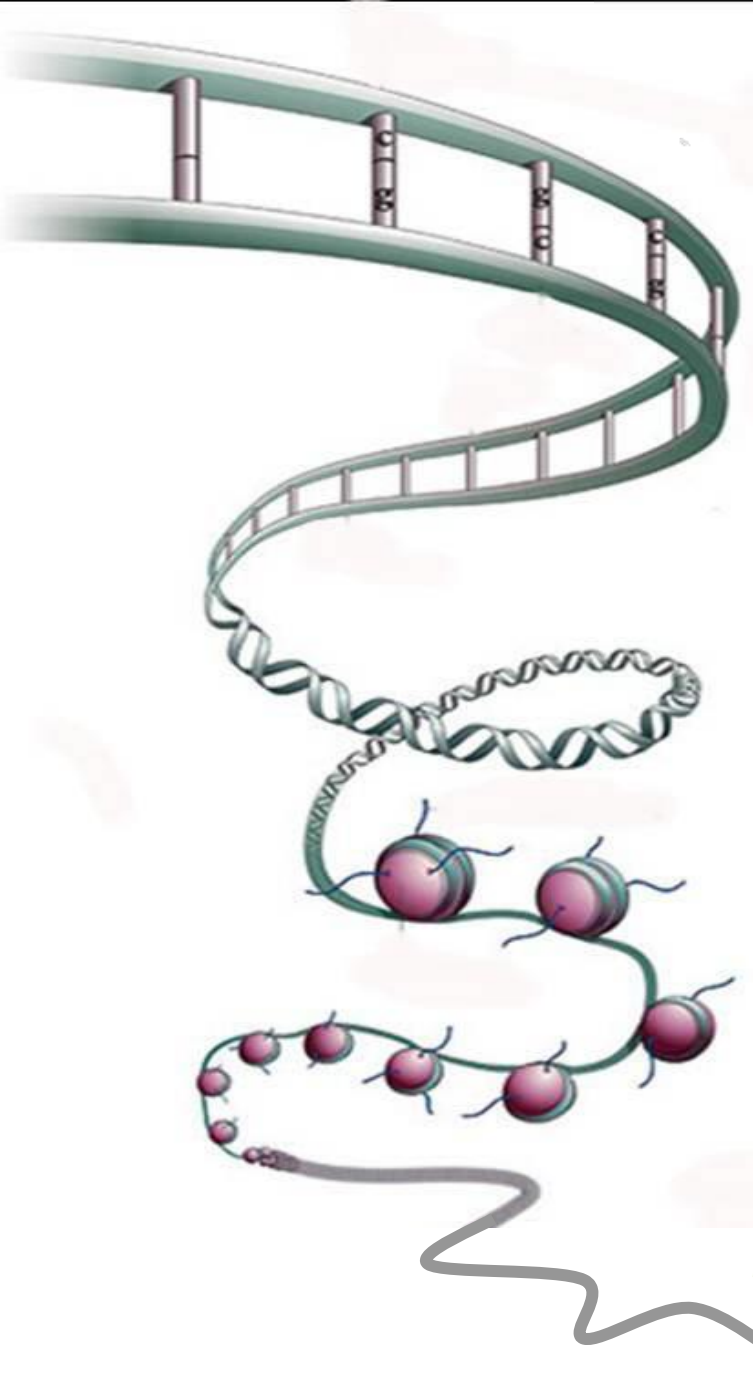


KRAS Codons 12 & 13



Oncogene Detection Techniques

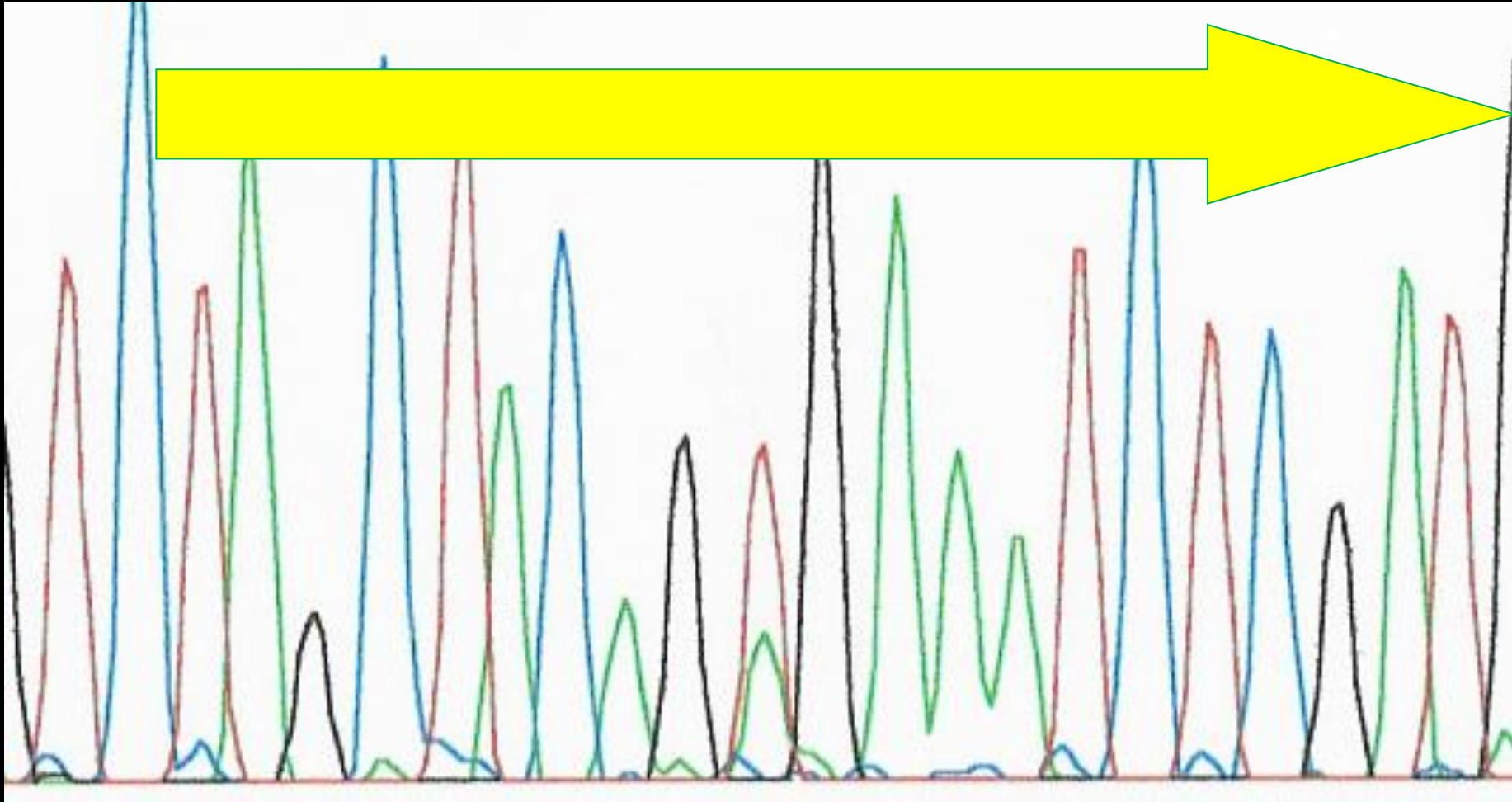
- PCR detection methods
 - Screening techniques
 - Allele specific PCR
- Full sequencing methods
 - Sanger sequencing
 - Single base extension
 - Pyrosequencing
 - Next generation sequencing



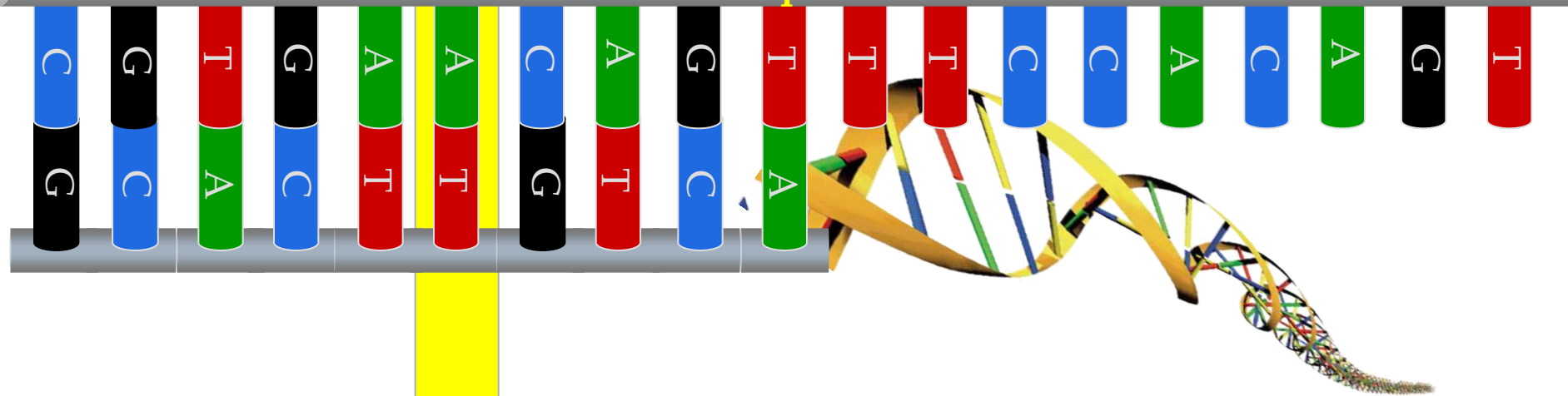
Screening for Mutations



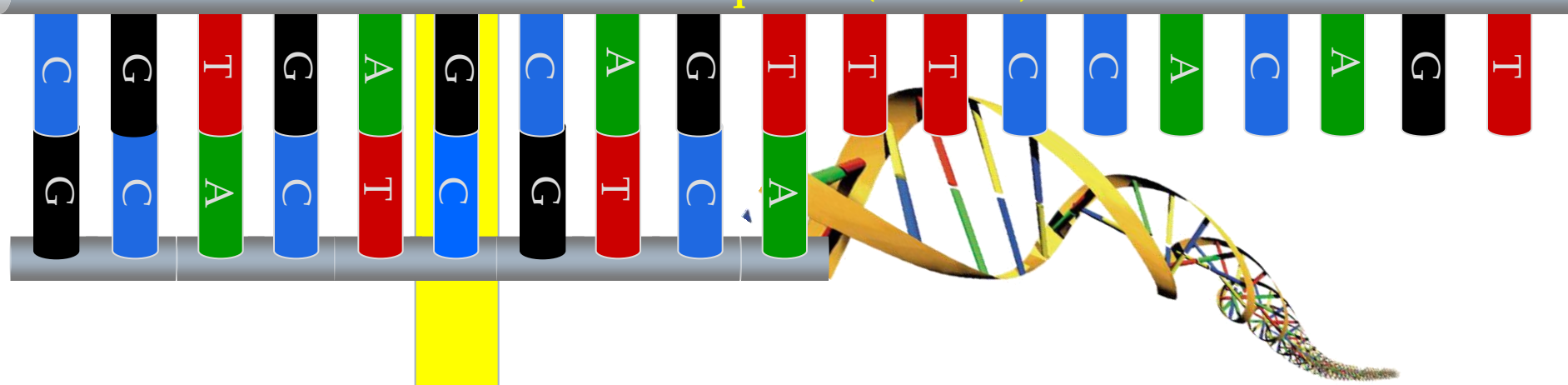
Allele Specific PCR



Normal Sequence (A allele)



Mutant Sequence (G allele)



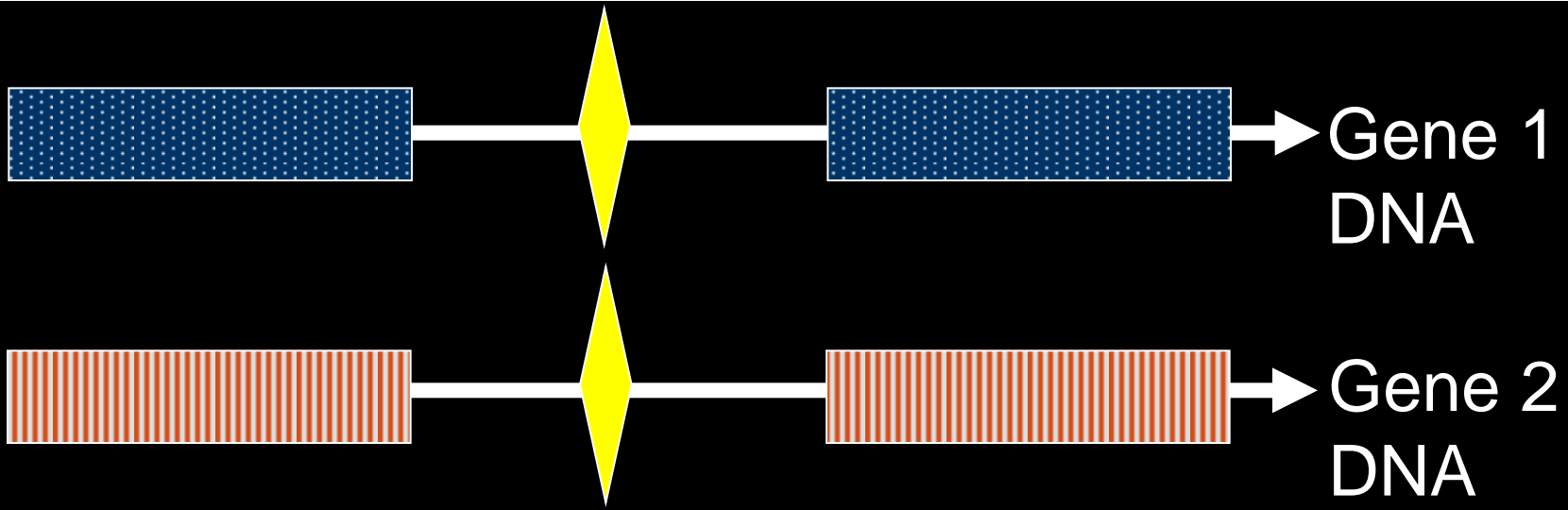
Allele Specific PCR

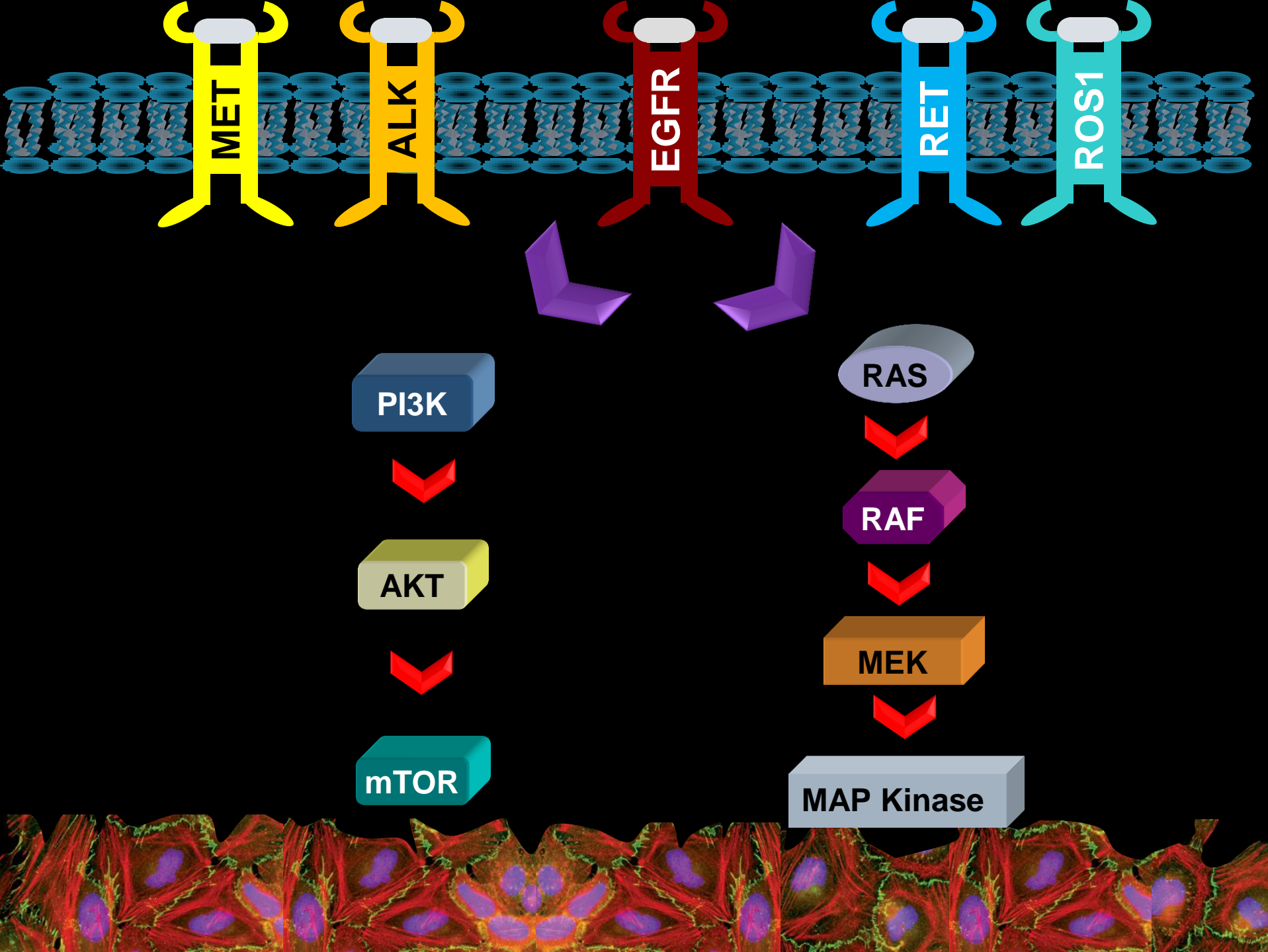
Detecting Point Mutations

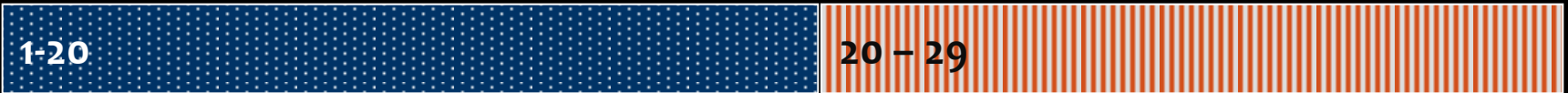
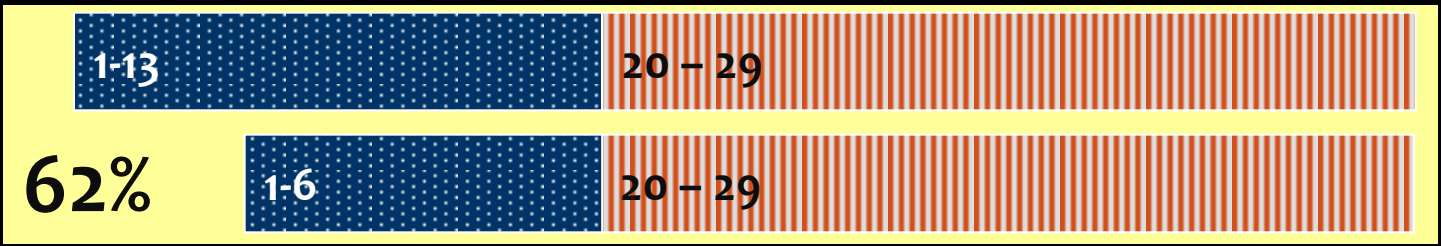
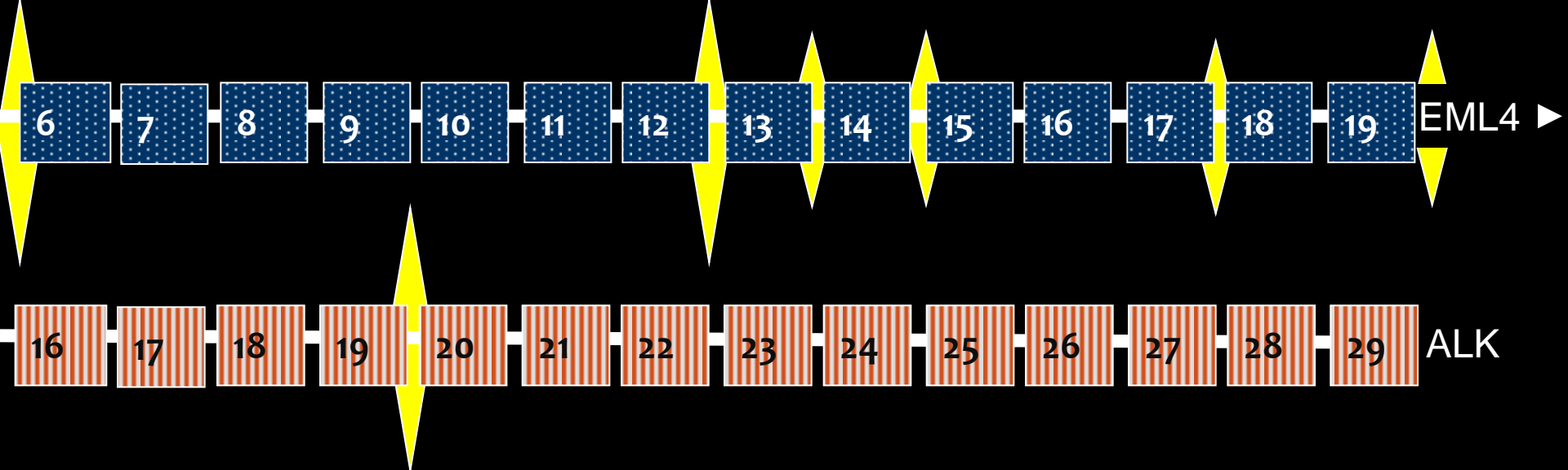
- Gene Sequencing Approaches
 - Dideoxy sequencing (“first generation”)
 - Sanger sequencing
 - Single base extension sequencing (“SNaPshot”)
 - Pyrosequencing
 - Next generation sequencing

Translocation

- Diseases associated with translocations
 - Hereditary diseases
 - Tumors with somatic mutations
- Are both partner genes known and consistent?





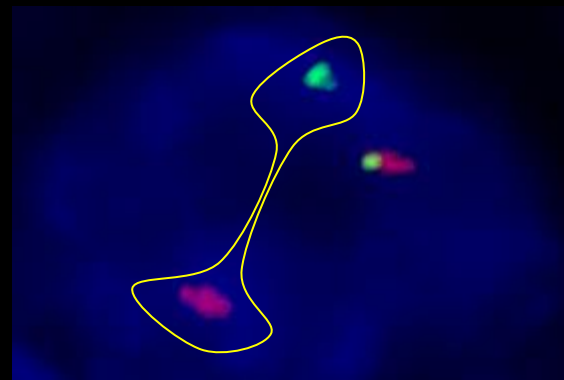
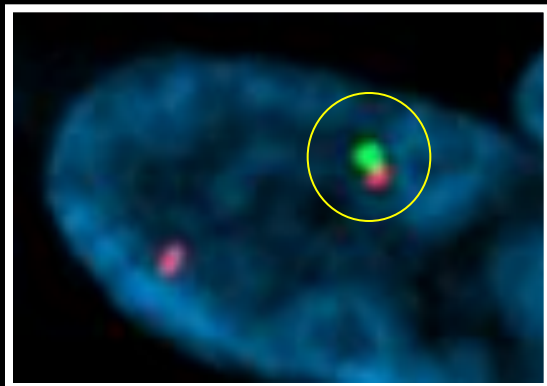


Detecting Translocations

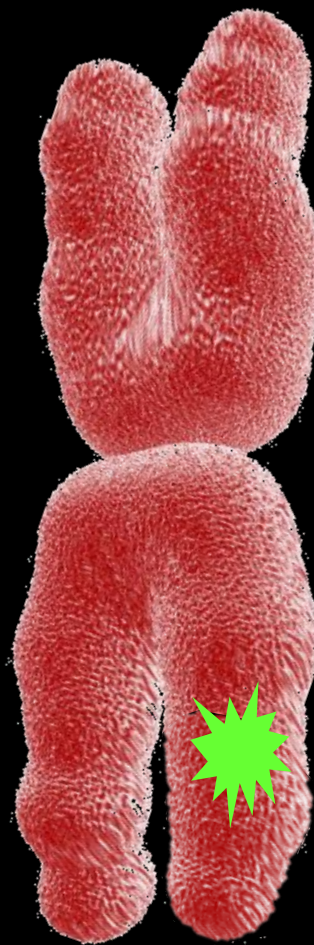
- DNA based PCR testing
- RNA based RT-PCR testing
- Fluorescent in situ hybridization (FISH)

In Situ for Translocations

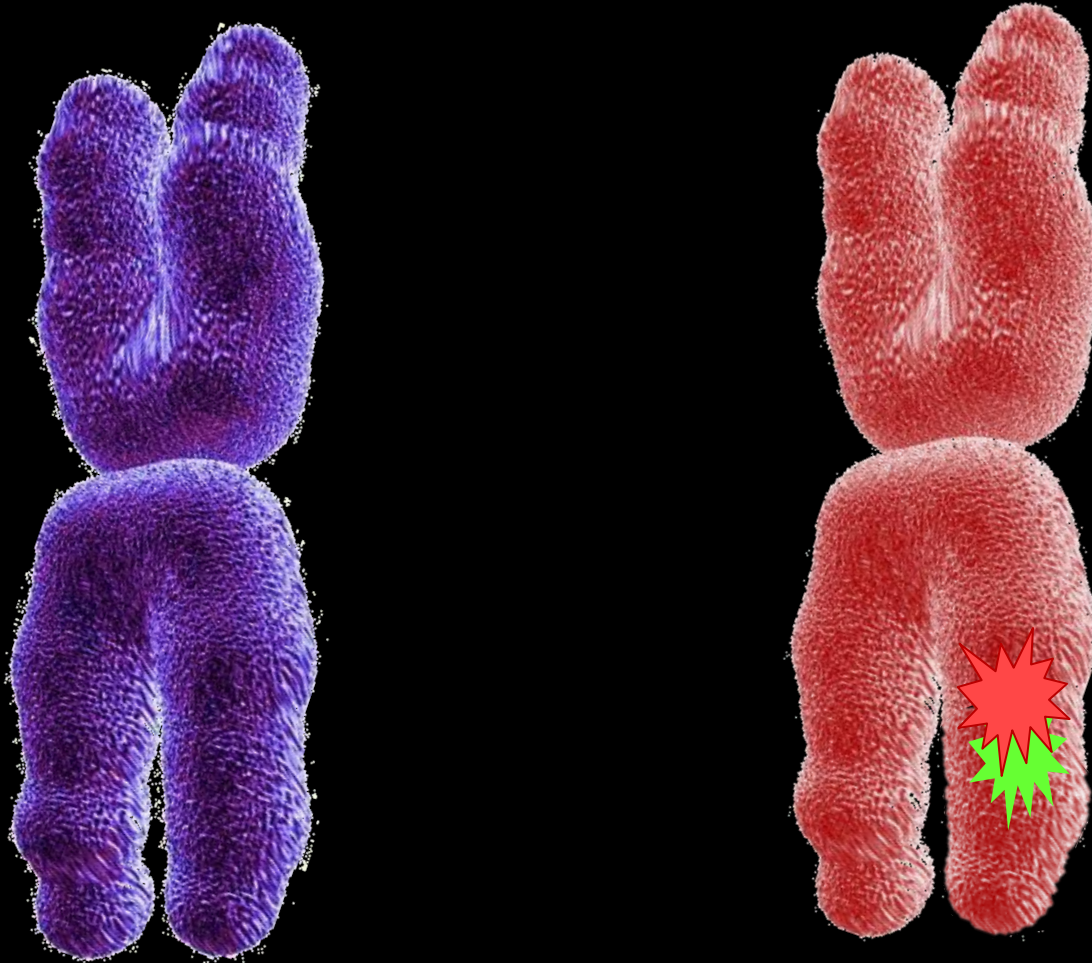
- Fusion probes
 - One probe on each partner
 - Both genes must be known
 - Will only pick up consistent partner genes
- Break-apart probes
 - Probes flank the break point on one partner
 - Only one gene must be known
 - Will pick up variable translocations



Fusion for Translocation



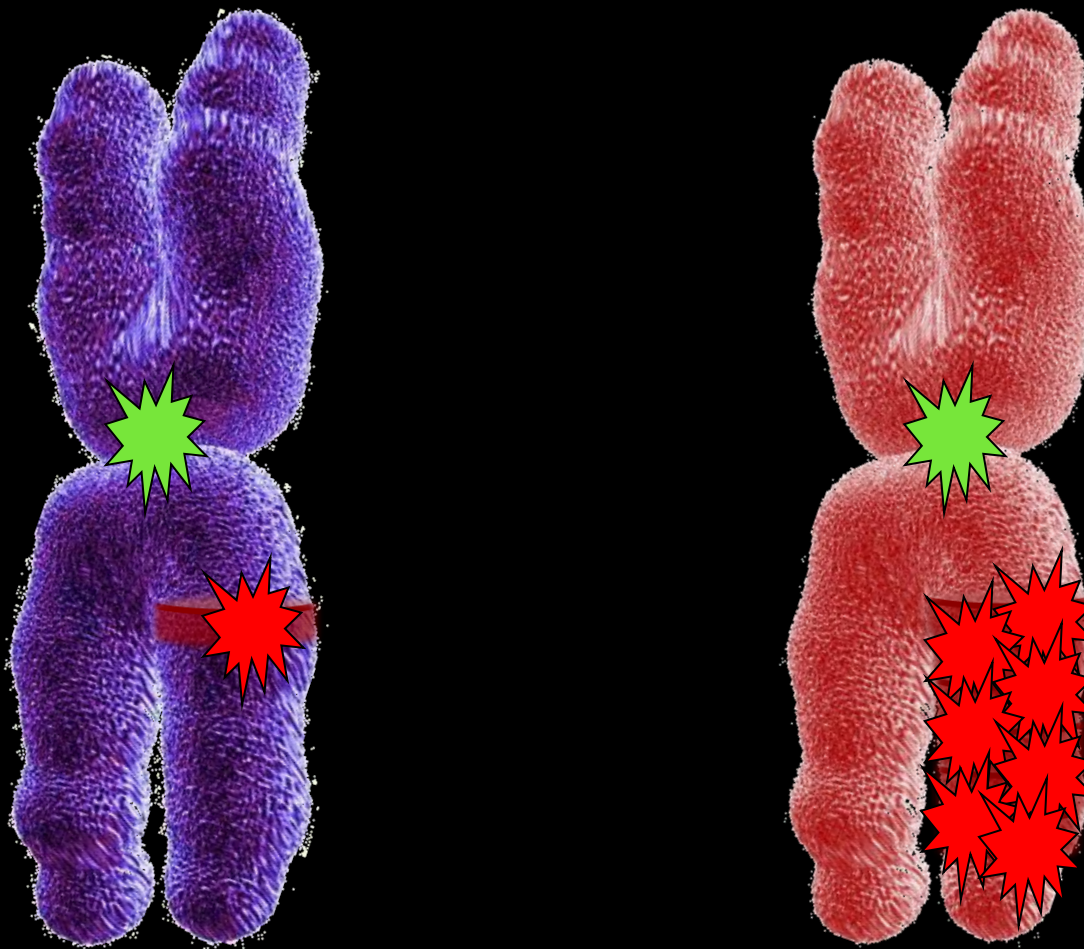
Break-Apart for Translocation



Amplification Mutations

- Diseases associated with amplification mutations
 - Hereditary diseases
 - Tumors with somatic mutations

In Situ for Amplification

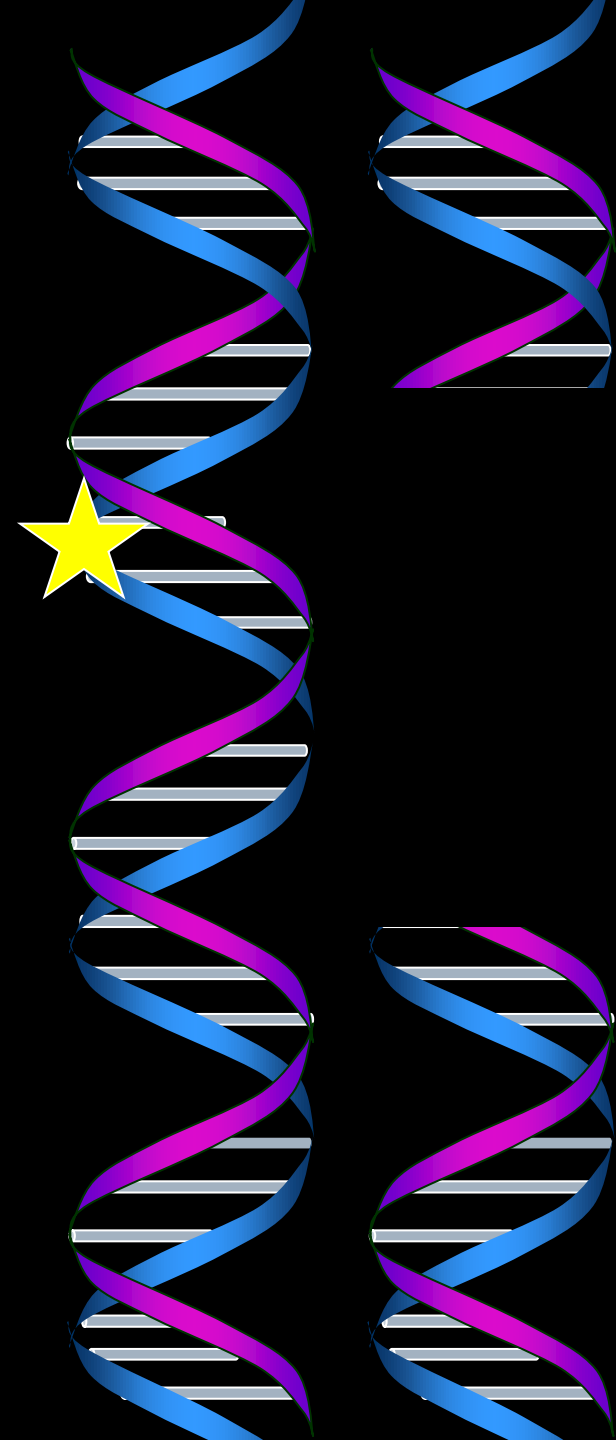


Deletion Mutations

- Diseases associated with deletions
 - Hereditary diseases
 - Tumors with somatic mutations in tumor suppressor genes

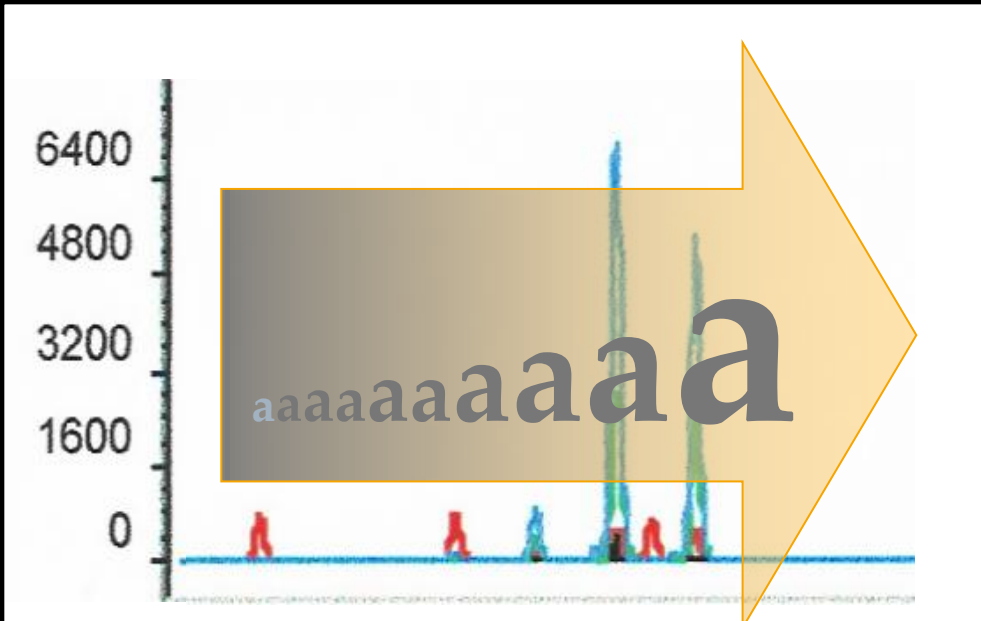
Suppressor Genes

- Recessive genes
 - Both copies mutated
 - Inactivating mutations
 - Point mutation
 - Deletions
 - Methylation
-

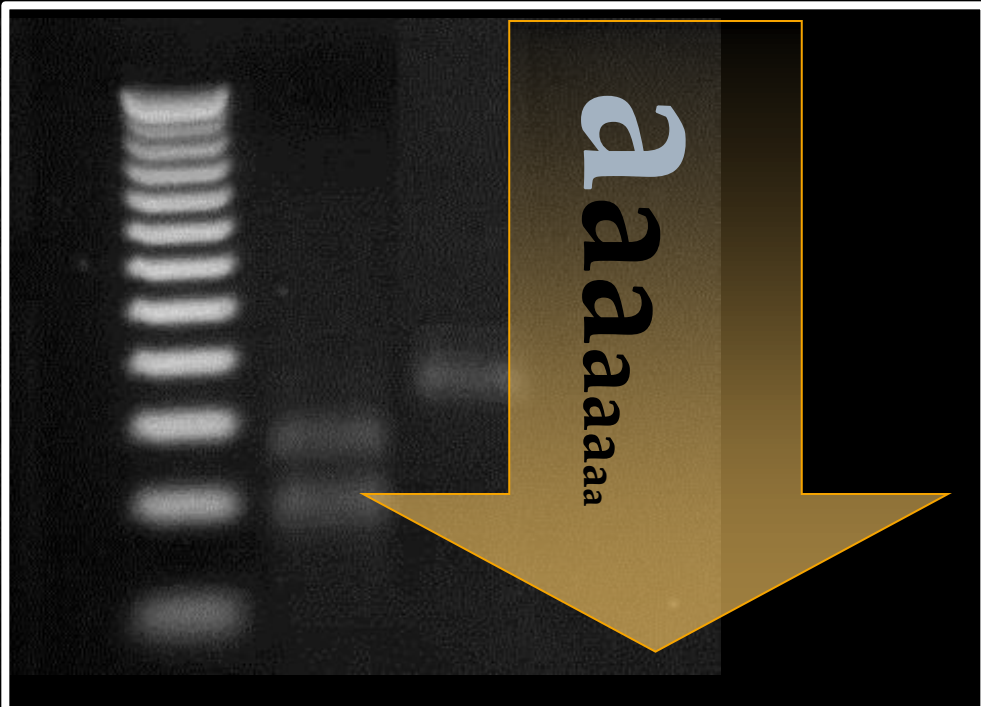


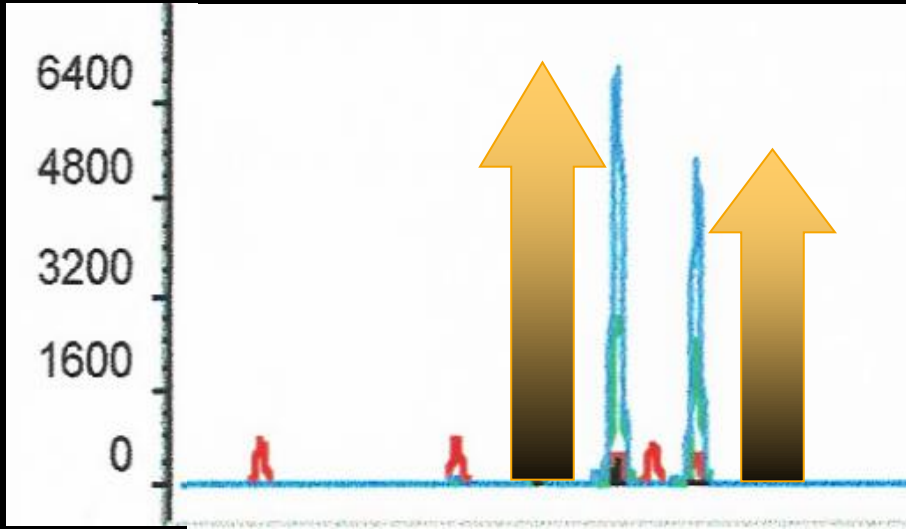
Loss of Genetic Material

- Loss of heterozygosity
- In situ hybridization (FISH or CISH)
- Comparative genomic hybridization

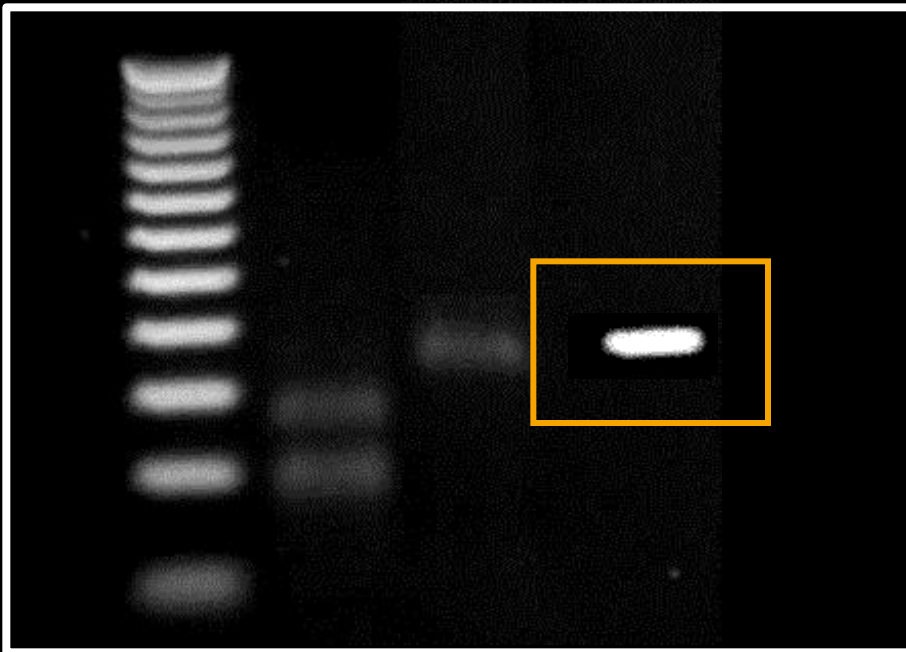


Size of PCR product (basepairs)

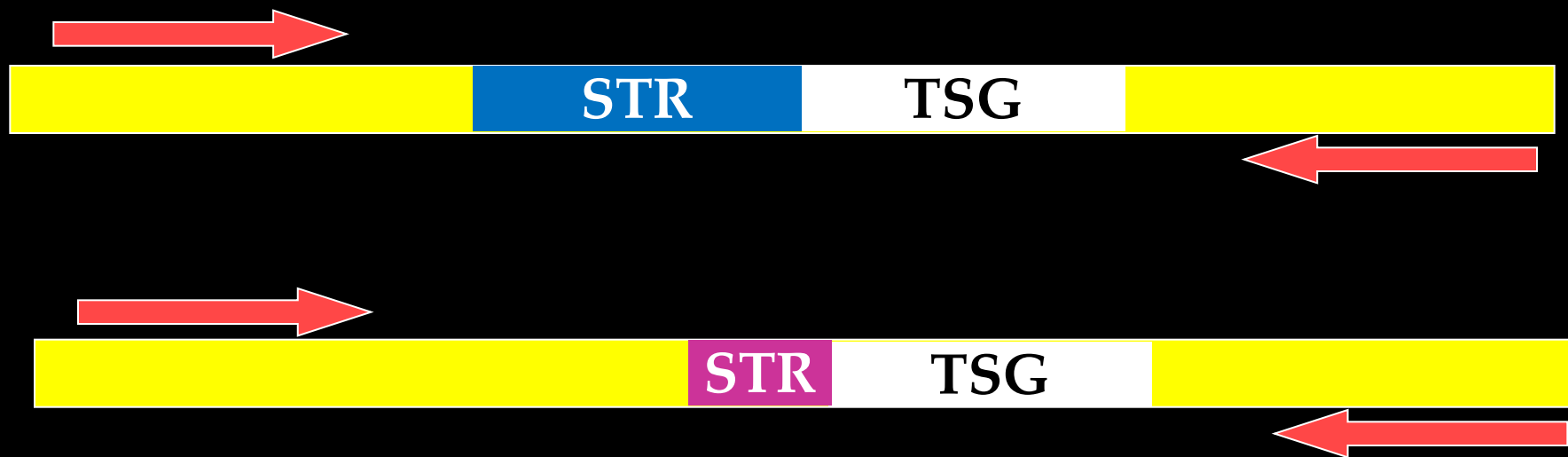




Relative
amount of
PCR product



Tumor suppressor gene

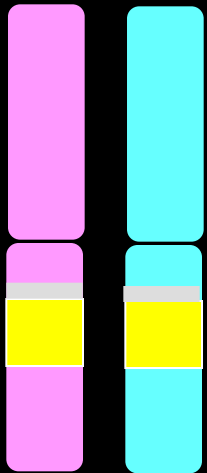
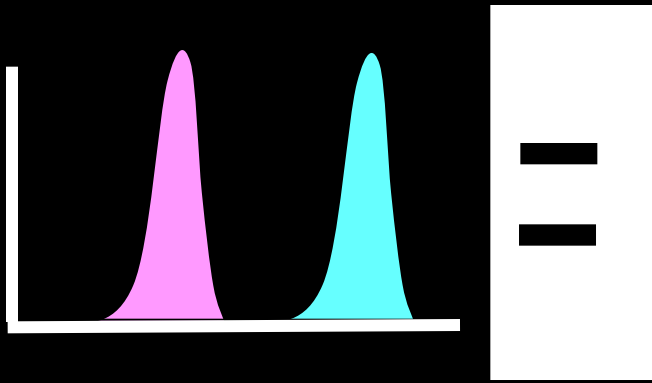


PCR Short Tandem Repeats

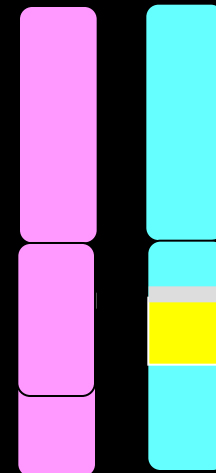
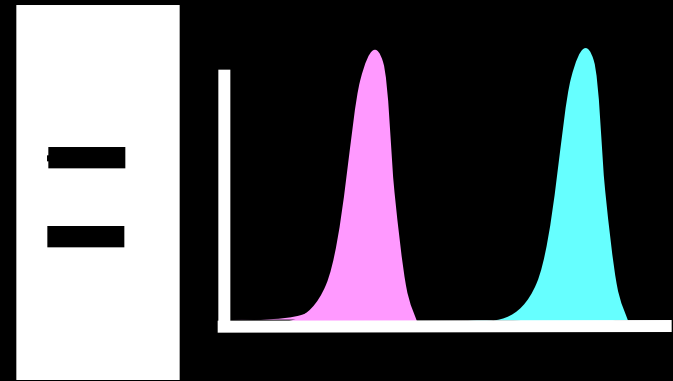


PCR Analysis

Normal

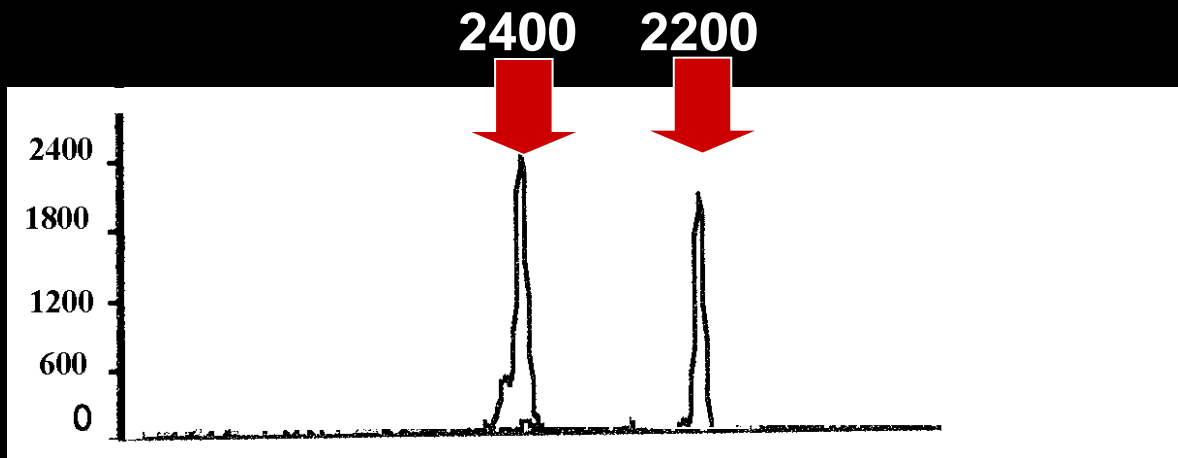


Loss of heterozygosity

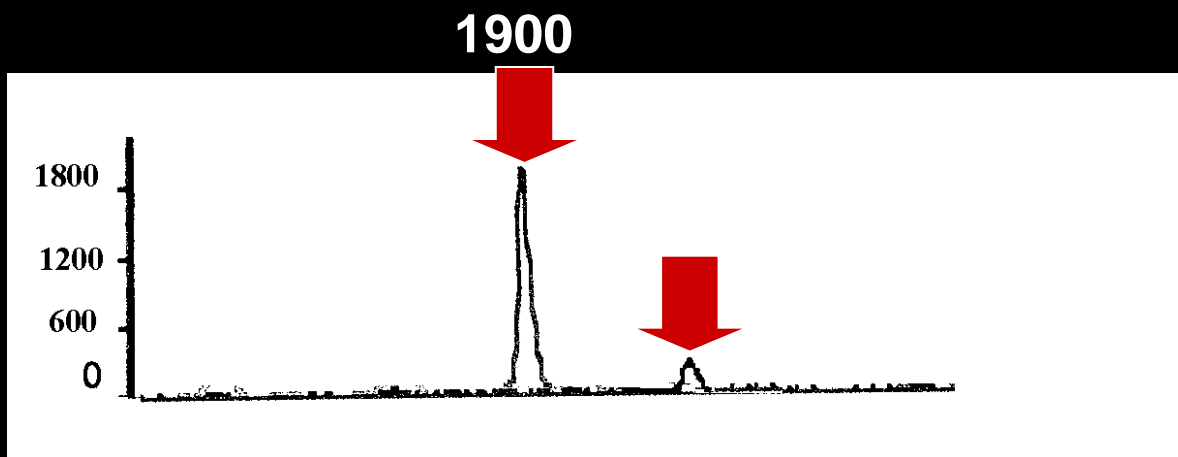


Capillary electrophoresis

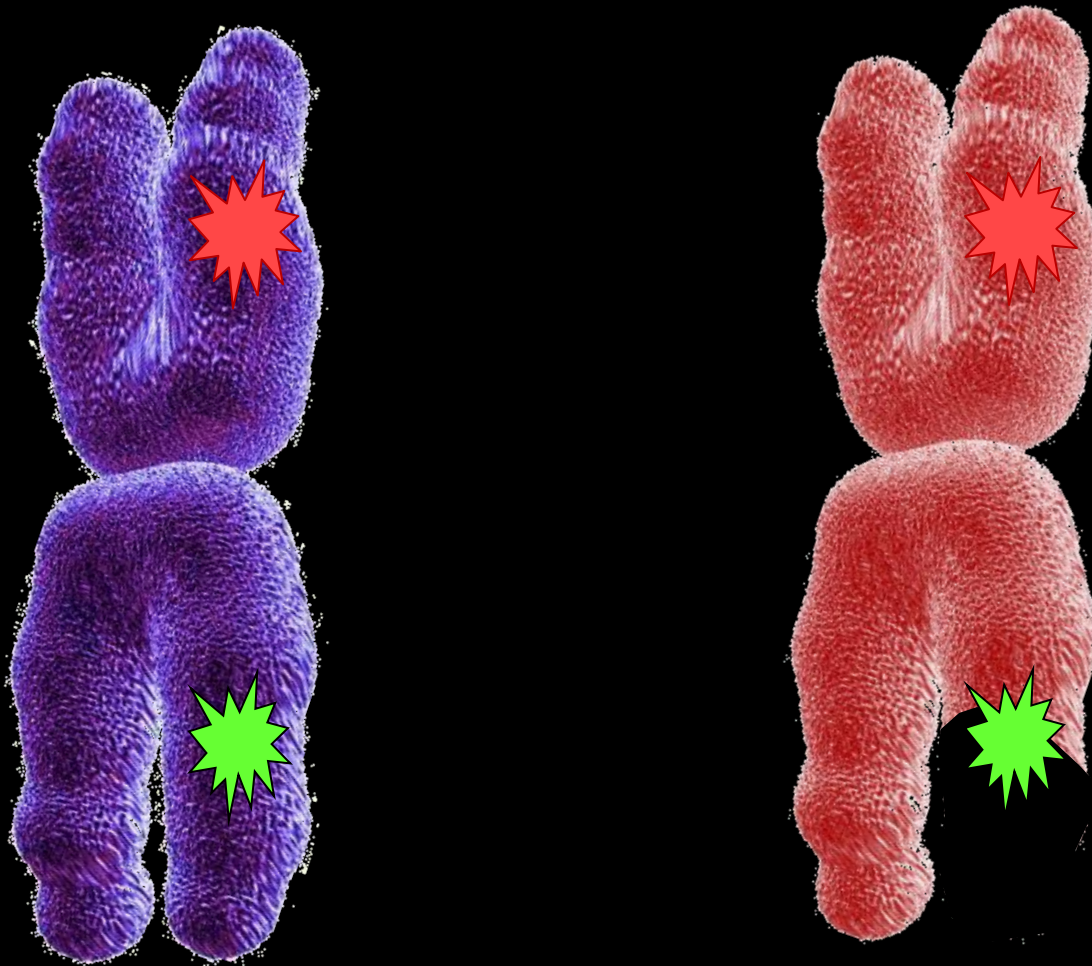
Normal



Tumor



In Situ for Deletion



Microsatellite Instability

- Diseases associated with expansion or contractions of microsatellites
 - Hereditary diseases
 - Lynch Syndrome tumors

Microsatellites

- Short tandem repeats
 - 2 to 7 basepairs in length
 - Dinucleotide, Trinucleotide, Tetranucleotide...

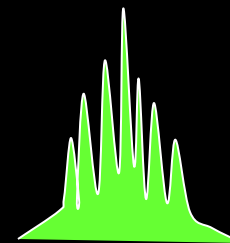
ATCG

- Repeated a variable number of times

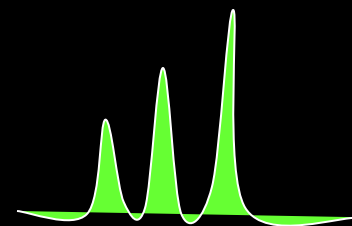
ATCG	ATCG	ATCG	ATCG	ATCG	ATCG
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Short Tandem Repeats

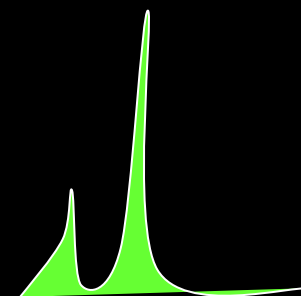
Mononucleotide TTTT TTTT TTTT
(10 repeat allele)



Dinucleotide CACACACA
(4 repeat allele)

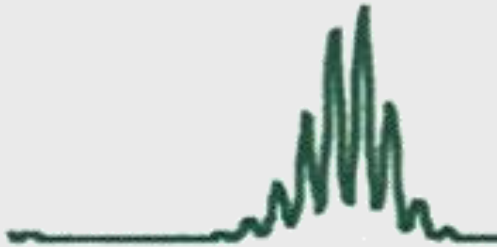


Tetranucleotide GACTGACTGACT
(3 repeat allele)



Microsatellite Instability Testing

NR21



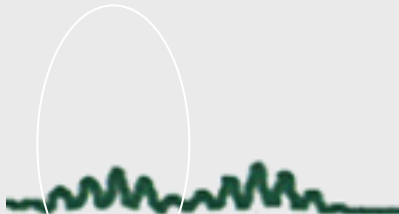
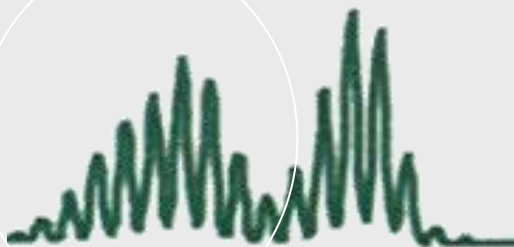
BAT25



Mono27



Normal Tissue



Tumor Tissue



GG CTTT CGG AGATGT TTTGATAG CGACGGG AATTTTAACTTTTCTCACCTT CTGGG ATC

GG CTTT CGG AGATGT TTTGATAG CGACGGG AATTTTAACTTTTCTCACCTT CTGGG ATC

GG CTTT CGG AGATGT TTTGATAG CGACGGG AATTTTAACTTTTCTCACCTT CTGGG ATC