Molecular Diagnostics
(a brief review)

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Overview

- DNA basics and mutations
- Southern Blot
- Northern Blot
- Western Blot
- PCR
- FISH
- DNA Microarray

DNA

- DNA A=T G≡C
- More stable than RNA
- Denaturing depends on G C content
  - Denaturing temperature (T_m)

Mutations

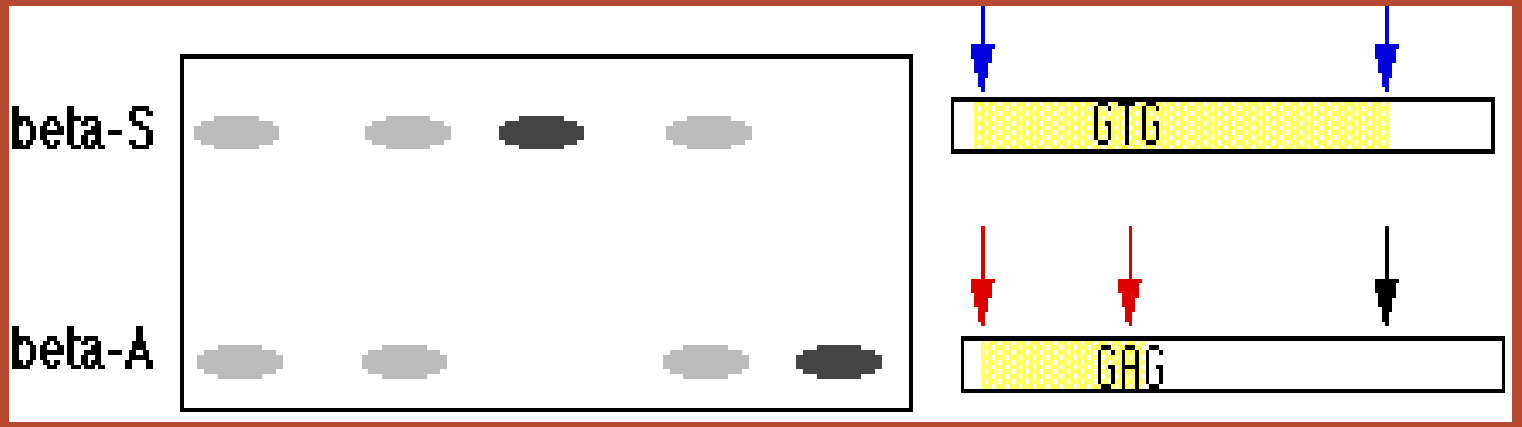
- Any change
- **Silent**- No change in protein
- **Missense**- change in protein
- **Nonsense**- truncation
- **Splice**- change in site = intron not removed
- **Frame Shift**- altered reading frame (+/- 1 or 2 bp)
- **Unstable trinucleotide repeats**
- **Chromosomal translocations**

Restriction Enzymes

- Cut at specific sequences in DNA
- Can be anywhere from 4-8 bp in length
- Usually cut palindromes

  for example: racecar

RFLP (restriction fragment length polymorphisms)



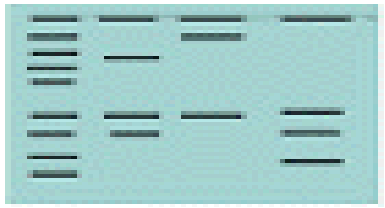
Restriction enzymes recognize a specific “code”

Mutations result in a loss of recognition of that code resulting in a different sized fragment of DNA that can be identified on a gel

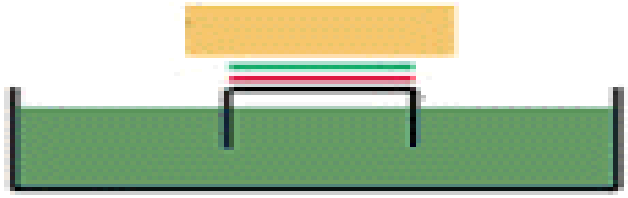
Limitations

- most human genetic diseases are more varied than the single mutation
- diseases which result from several mutant genes working together to produce the disease phenotype
- genetic diseases for which no gene has yet been discovered

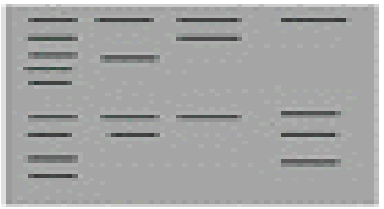
Southern Blot (DNA)



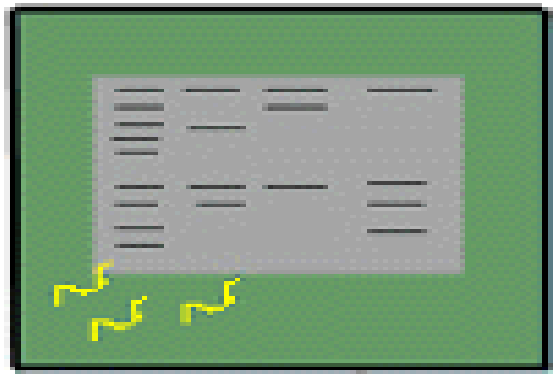
Place DNA fragments on an agarose gel and separate by electrophoresis



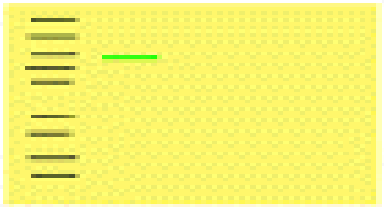
"Blot" DNA fragments from agarose gel onto membrane



Membrane imprinted with DNA bands



Add a labeled probe to the membrane (in buffer solution).



Detection (the method depends on the type of probe you use) reveals a band where your probe bound to the target sequence.

Limitations

- Abundant High quality DNA needed
- Labor intensive

- Requires fresh or frozen tissue

Northern Blot (RNA)

- method for determining transcript size
- for detecting alternatively spliced transcripts.

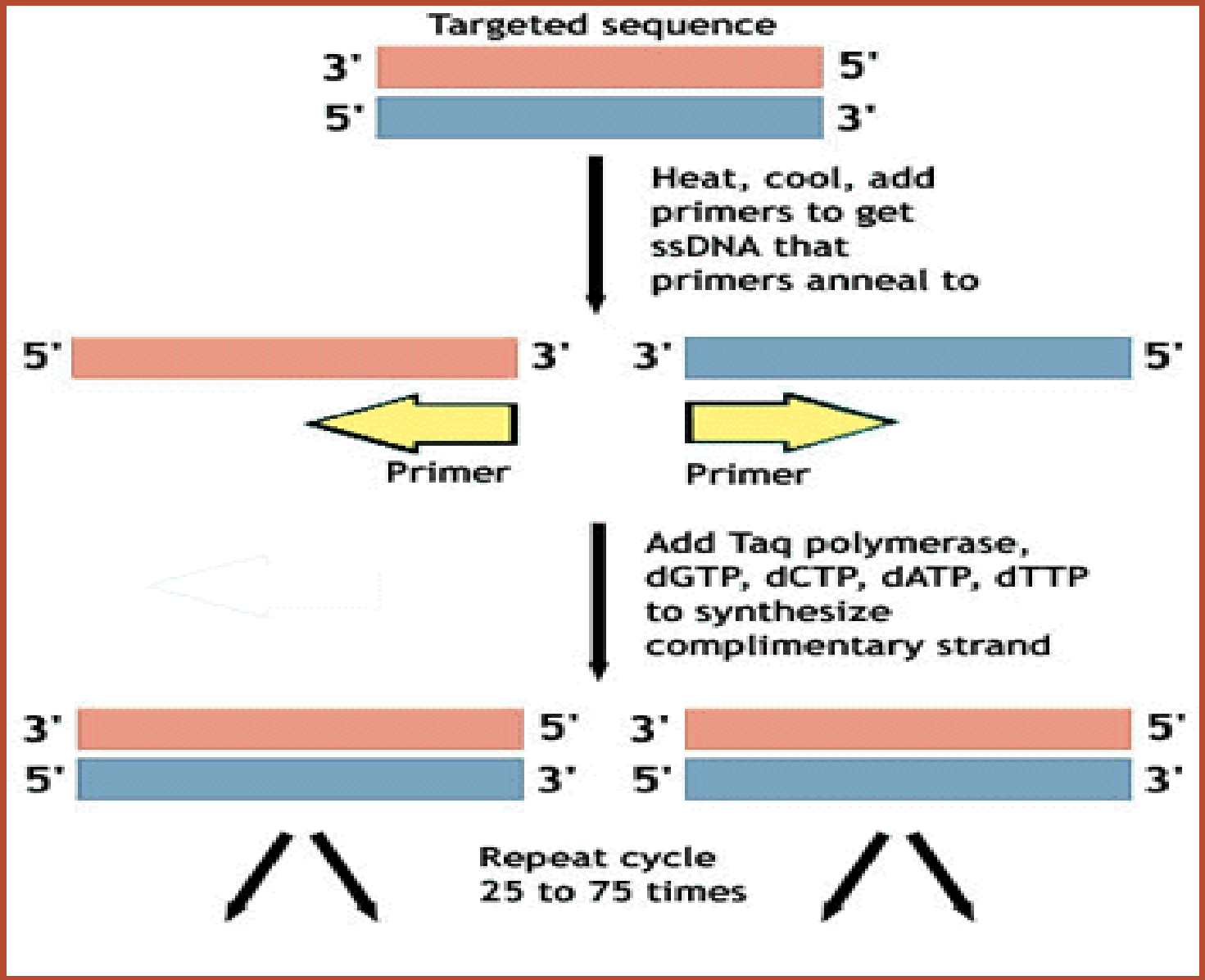
- Same as Southern Blot except the probe is to a specific RNA sequence

Limitations

- RNAase!!!
- Less sensitive than RT-PCR
- Can only detect one probe at a time

DNA Amplification

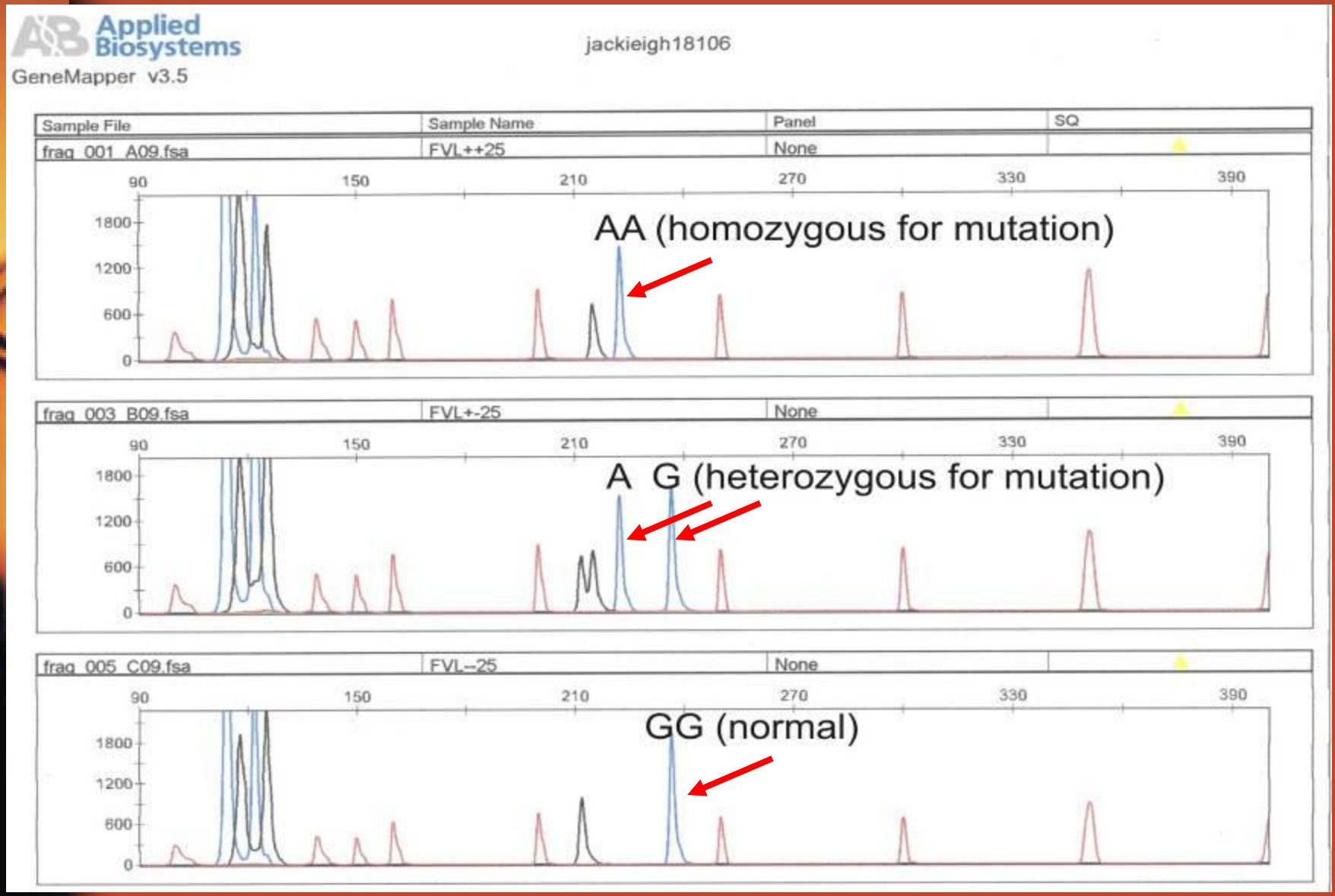
- PCR-



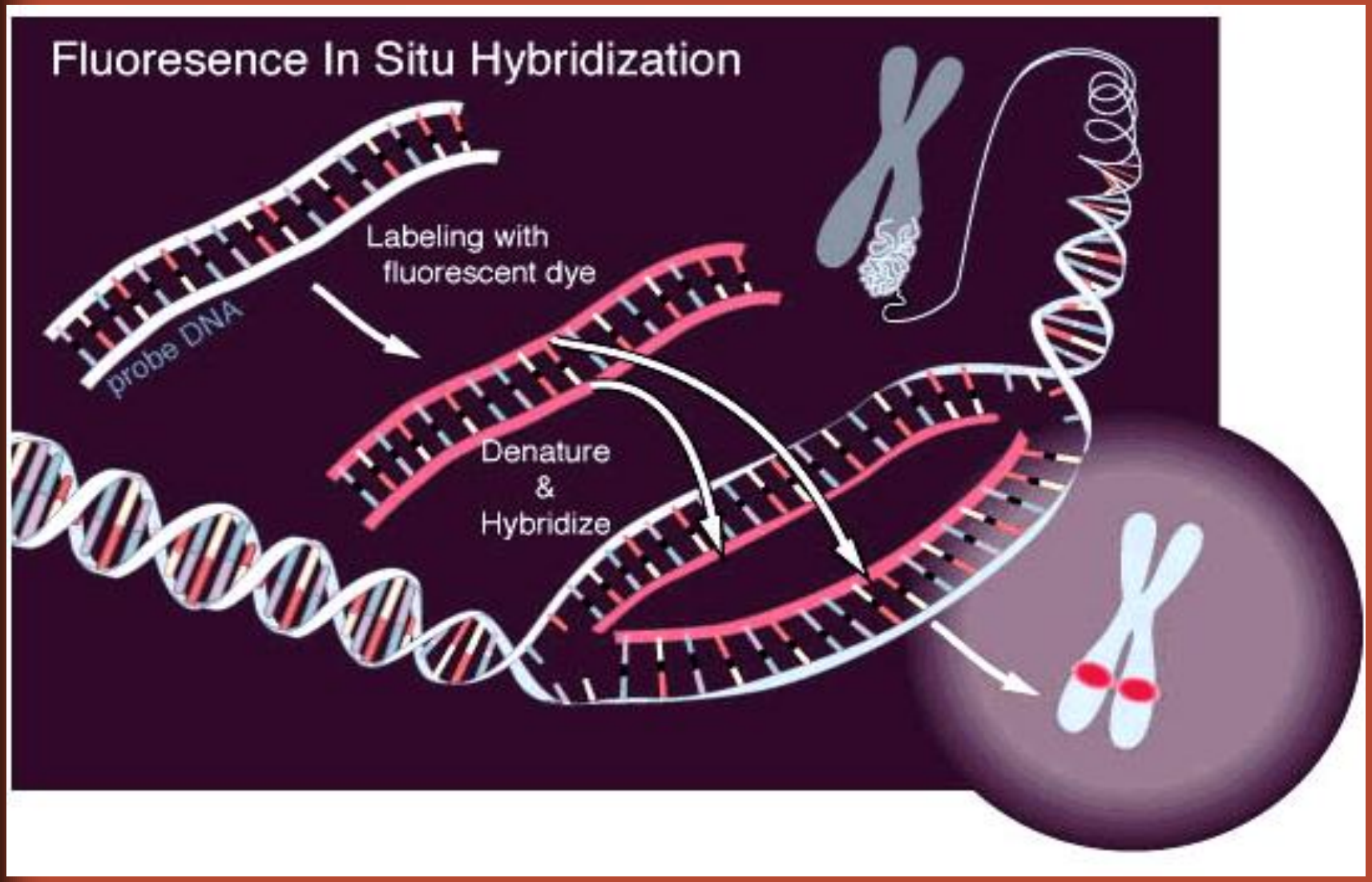
DNA Amplification

- RT-PCR: reverse transcription
  - RNA to DNA (cDNA)
- Multiplex PCR: 2 different targets
- Nested PCR
- Real Time PCR: simultaneous amplification and detection

PCR Testing for F V Leiden: Results on Capillary Electrophoresis



FISH


Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize

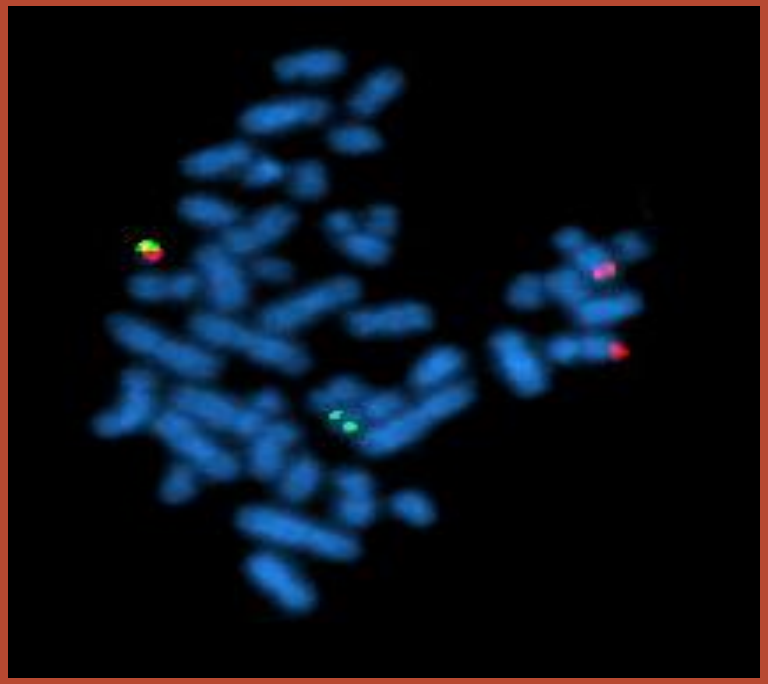
FISH

- Can detect translocations, gene amplifications and deletions

Microdeletion Syndromes Diagnosable with FISH

- Cri-du-Chat
- Steroid Sulfatase Deficiency
- DiGeorge Syndrome
- Kallman Syndrome
- Williams Syndrome
- Prader-Willi/Angelman Syndrome

Detection of bcr/abl mutation by FISH



The chromosomes can be seen in blue. Probes for bcr and abl are normally separated as red spot and green spot. A bcr/abl mutation is seen as a yellow spot (upper left) when the red probe and the green probe are located next to each other.

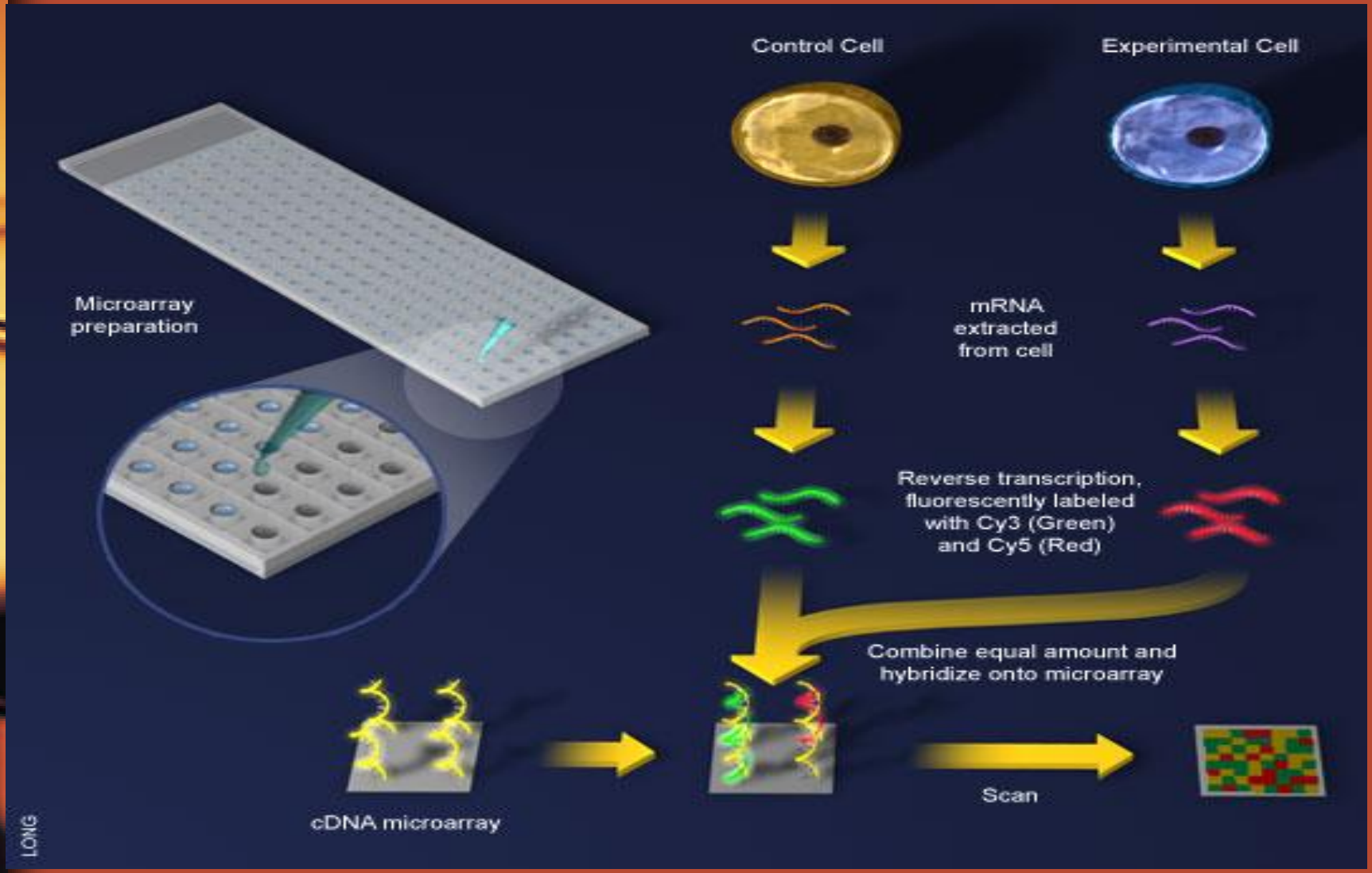
(CISH) Chromogenic In-situ Hybridization

- Similar to FISH
- enzymatic reactions under the brightfield microscope on formalin-fixed, paraffin-embedded (FFPE) tissues.

DNA Microarrays

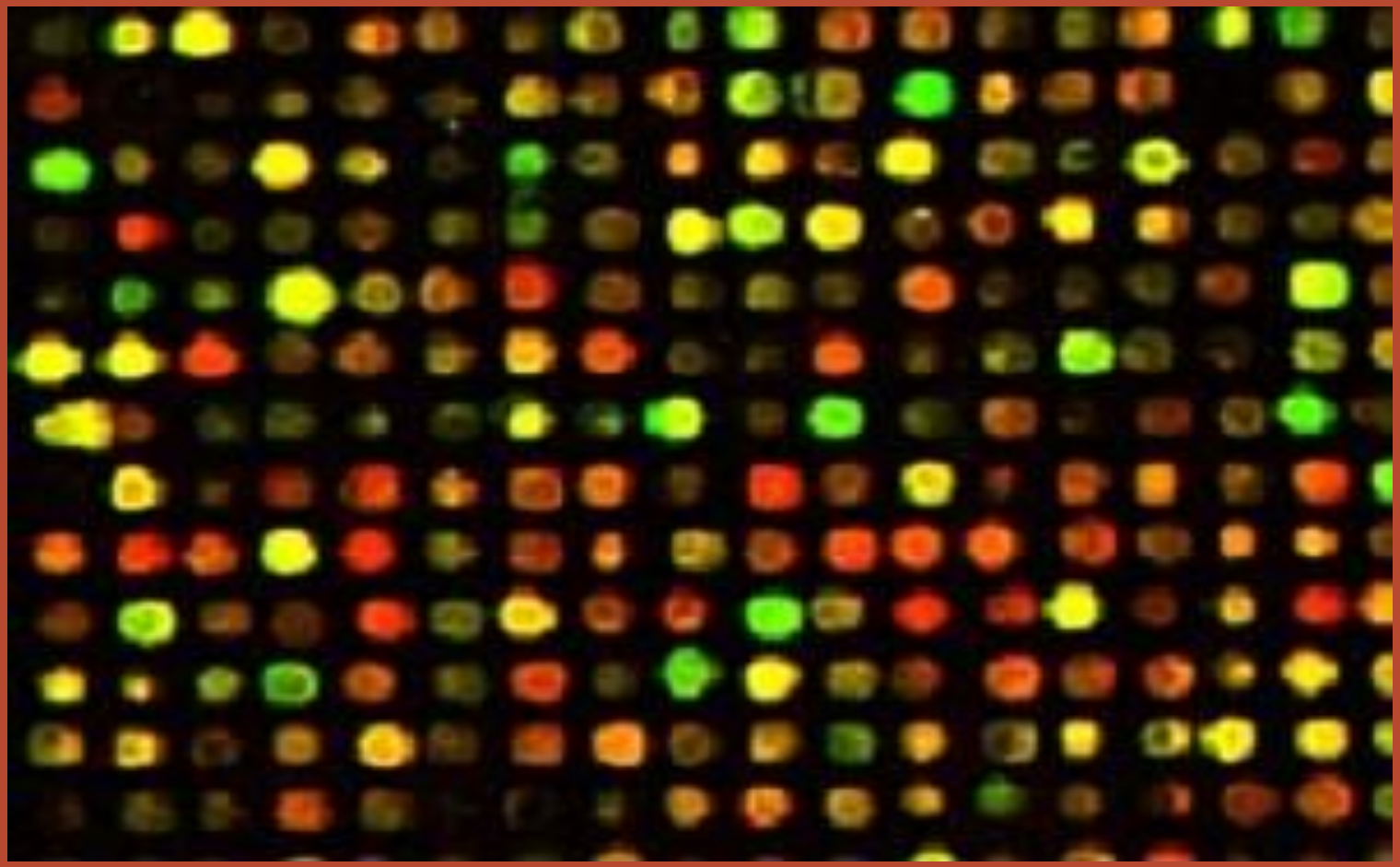
- Genome wide evaluation
- Gene expression profiling
- Genes from 1 sample compared to another (patient and control)

DNA Microarray



http://plasticdog.cheme.columbia.edu/undergraduate_research/projects/sahil_mehta_project/work.htm

DNA Microarray



http://radio.weblogs.com/0105910/images/ecoli_dna.jpg

References

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- Mais, D. Quick Compendium Clinical Pathology