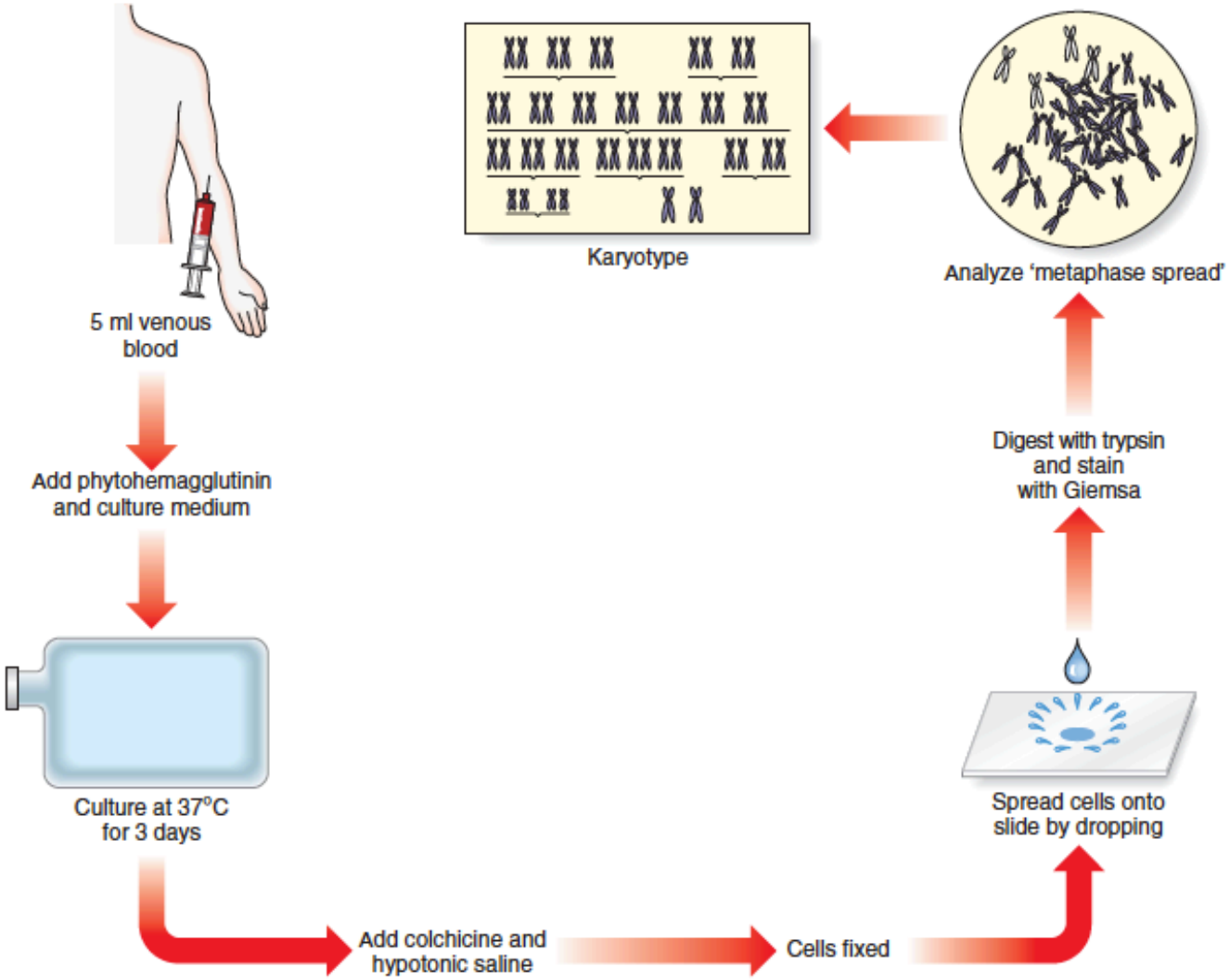
# GENETIC METHODOLOGIES

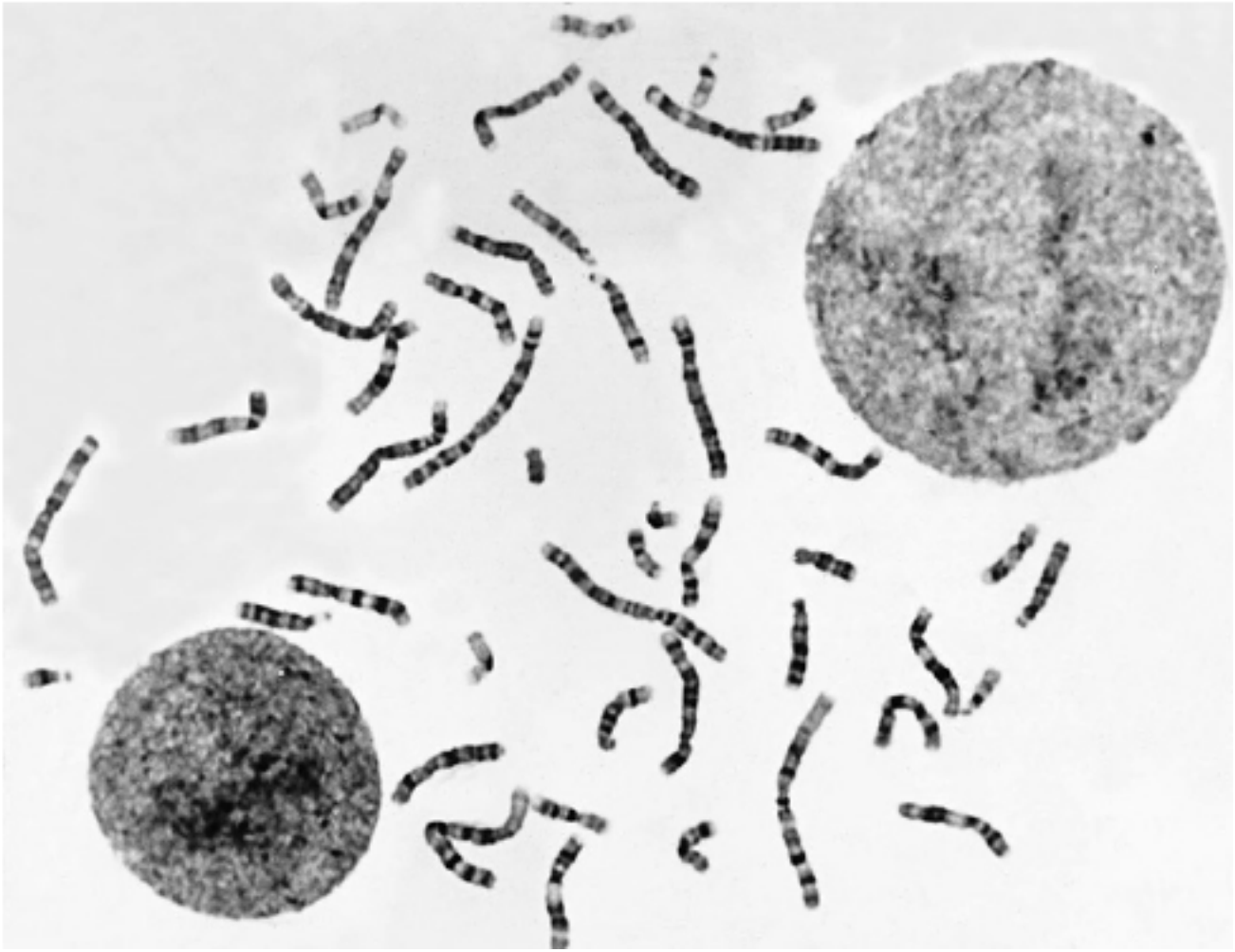
CONVENTIONAL CYTOGENETICS

MOLECULAR CYTOGENETICS

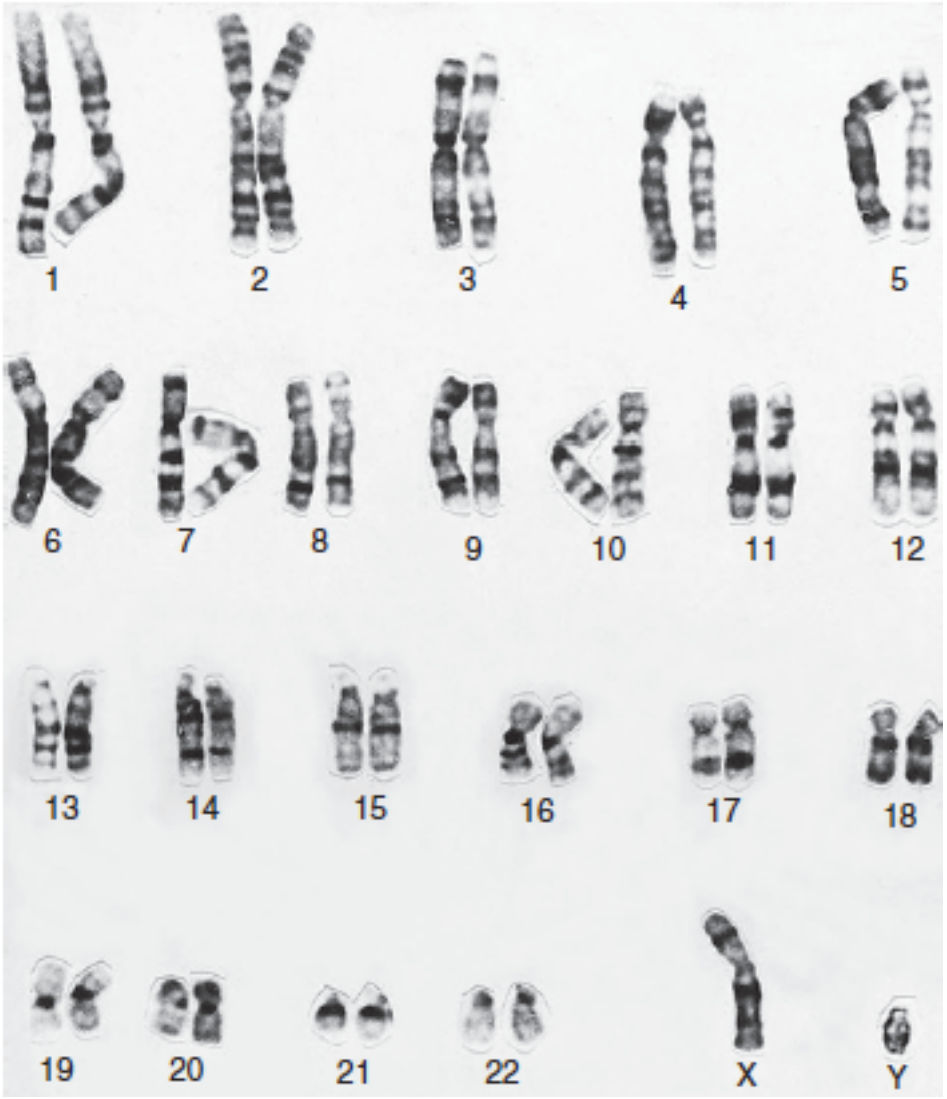
MOLECULAR GENETICS

# Methods of Chromosome Analysis

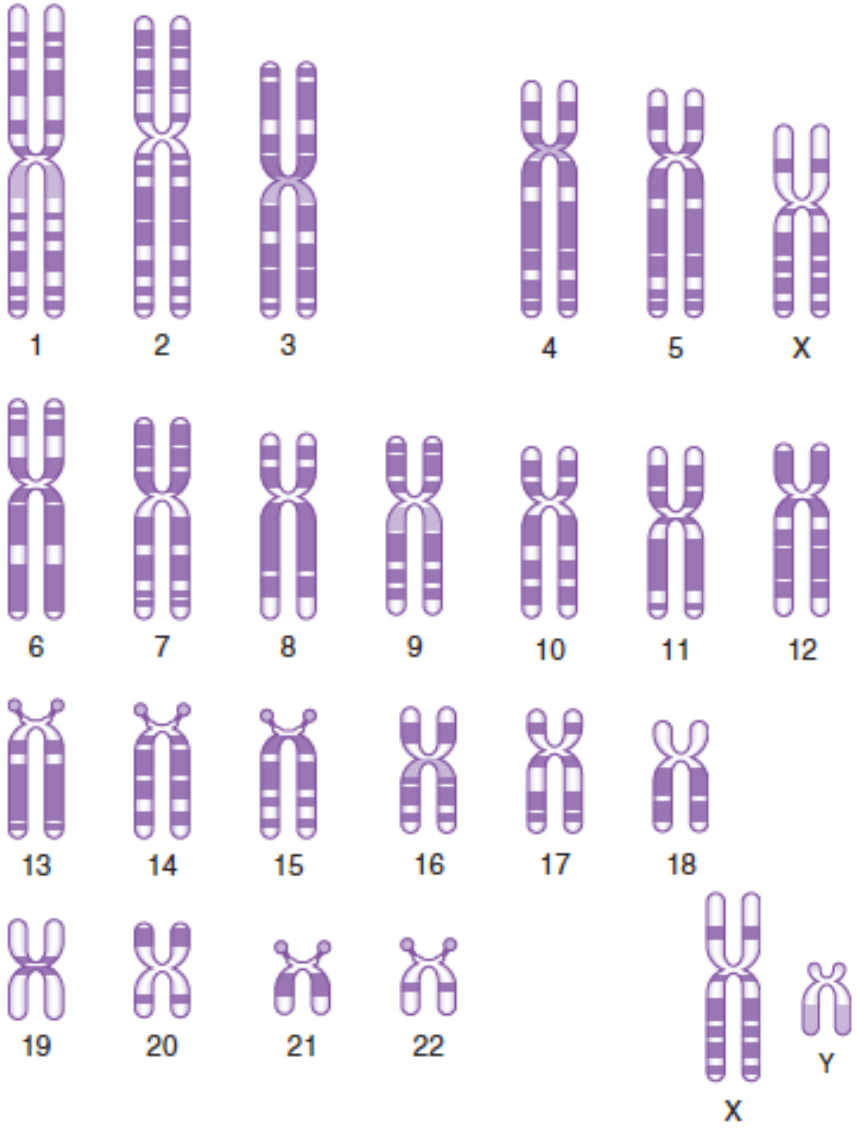




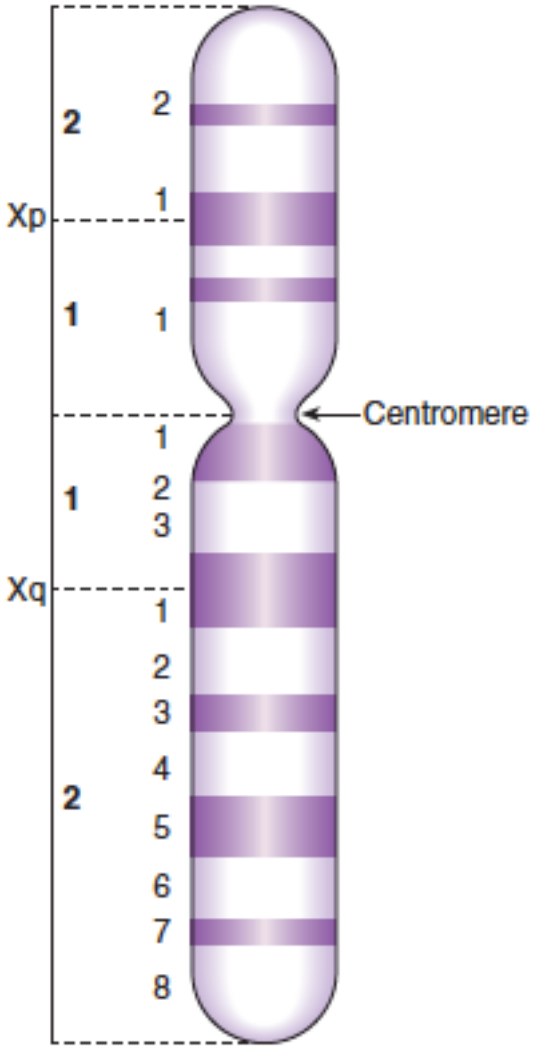
G banded metaphase spread




Normal G banded male karyotype




An idiogram showing G banding patterns

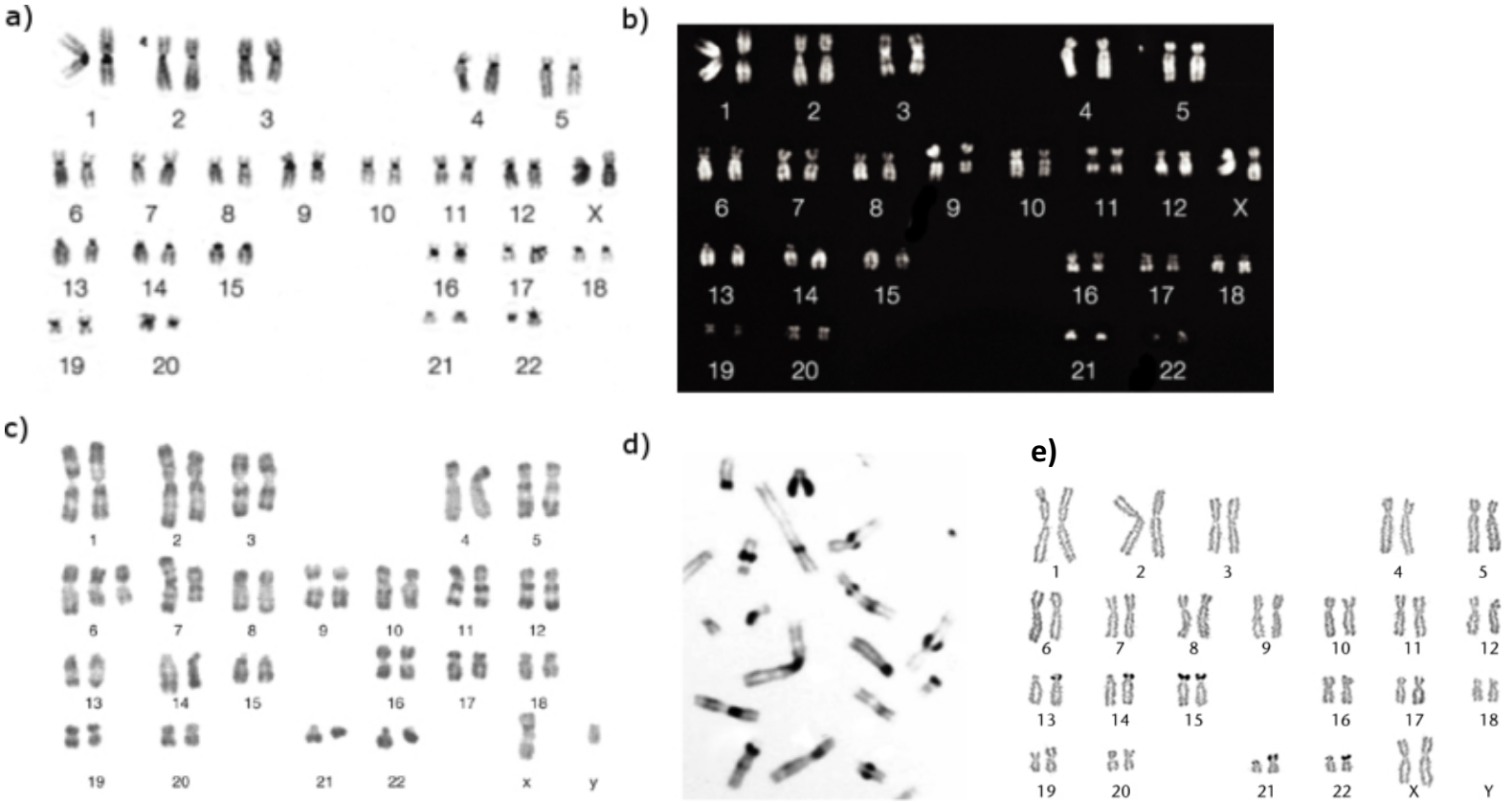



X chromosome idiogram
showing arms regions and bands

## Cytogenetic nomenclature

| Term | Explanation | Example |
|---|---|---|
| p | Short arm | |
| q | Long arm | |
| cen | Centromere | |
| del | Deletion | 46,XX,del(1)(q21) |
| dup | Duplication | 46,XY,dup(13)(q14) |
| fra | Fragile site | |
| i | Isochromosome | 46,X,i(Xq) |
| inv | Inversion | 46,XX,inv(9)(p12q12) |
| ish | In-situ hybridization | |
| r | Ring | 46,XX,r(21) |
| t | Translocation | 46,XY,t(2;4)(q21;q21) |
| ter | Terminal or end | Tip of arm; e.g., pter or qter |
| / | Mosaicism | 46,XY/47,XXY |
| + or – | Sometimes used after a chromosome arm in text to indicate gain or loss of part of that chromosome | 46,XX,5p– |

# Chromosome banding techniques




a) G banding
giemsa
AT rich regions

b) Q banding
quinacrine
AT rich regions

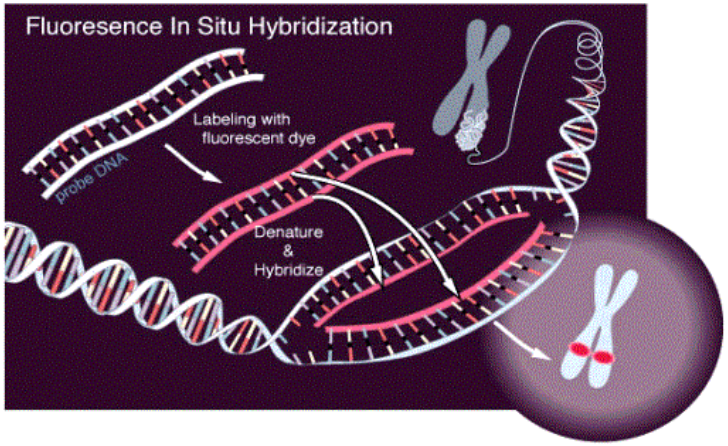
c) R banding
reverse
GC rich regions

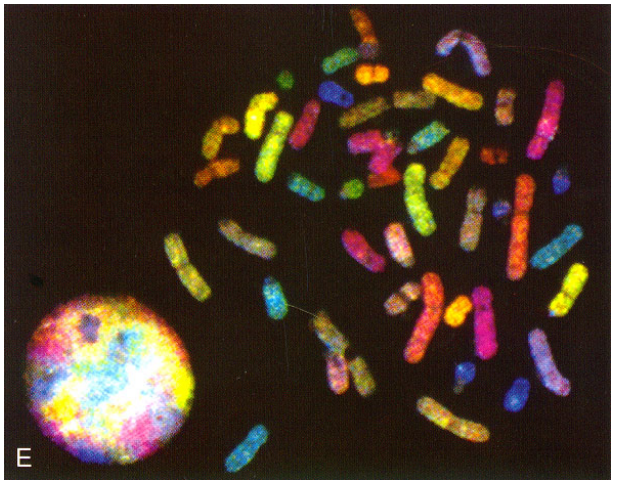
d) C banding
centromere
AT rich satellite regions

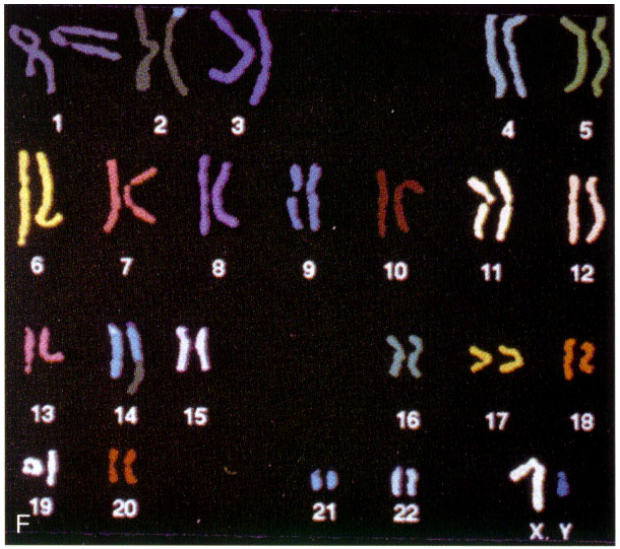
e) NOR banding
Ag-NOR
Satellite regions

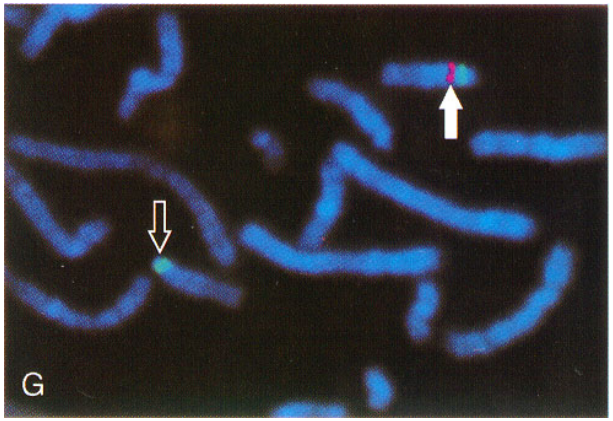
# **Fluorescence in situ** hybridization

(**FISH**) is a molecular cytogenetic technique that uses **fluorescent** probes that bind to only those parts of a nucleic acid sequence with a high degree of sequence complementarity. ... **Fluorescence** microscopy can be used to find out where the **fluorescent** probe is bound to the chromosomes.

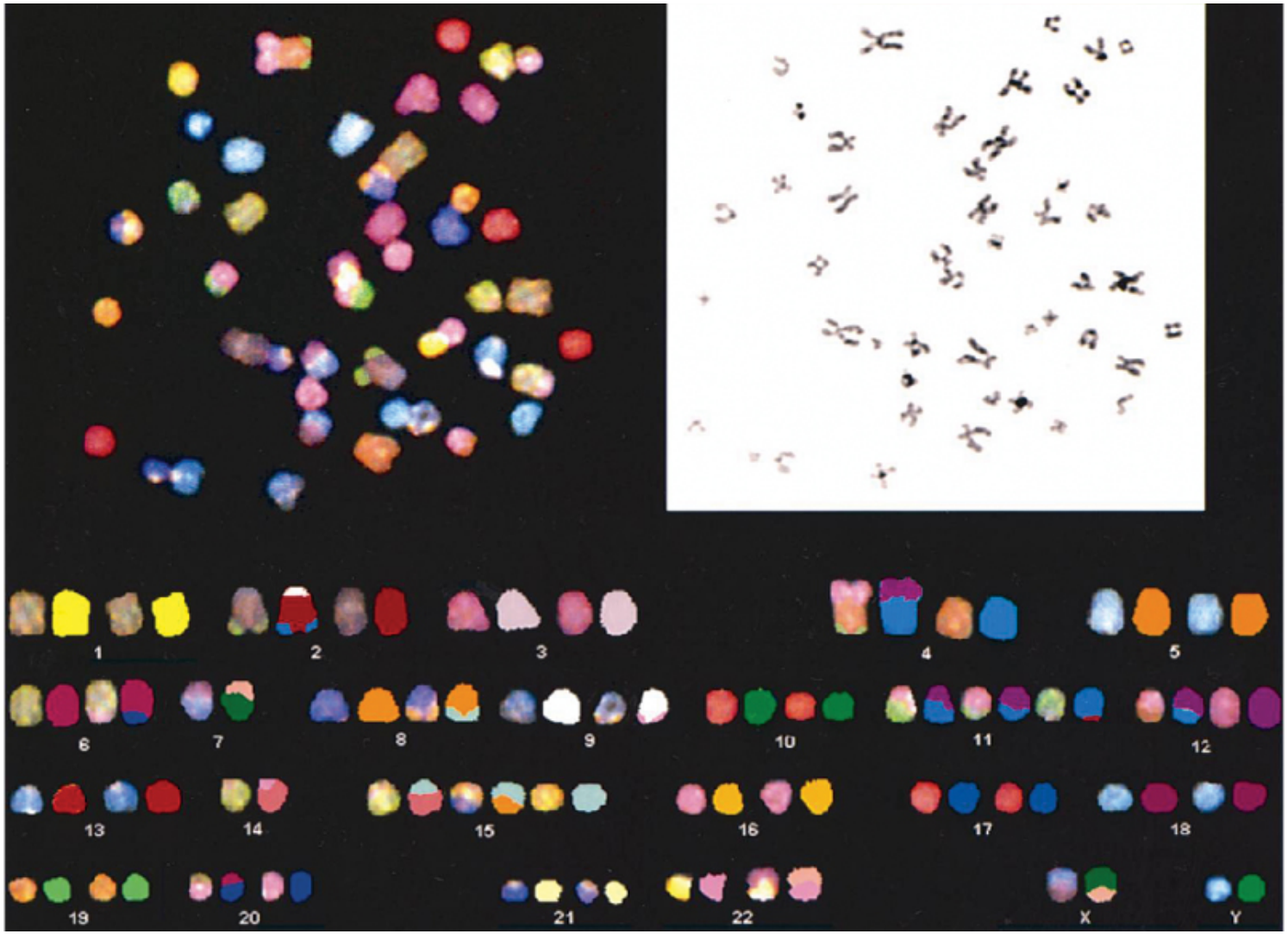


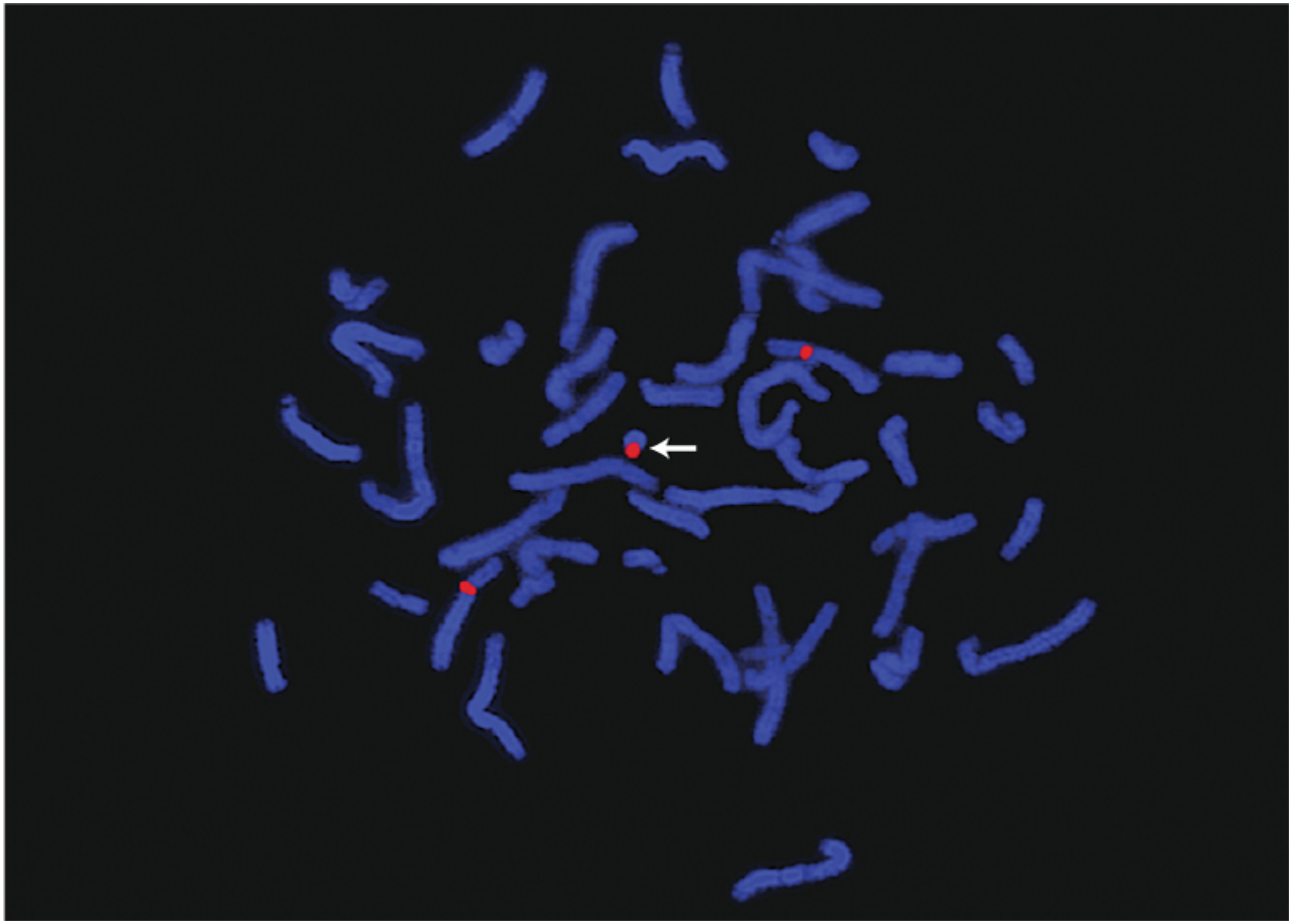



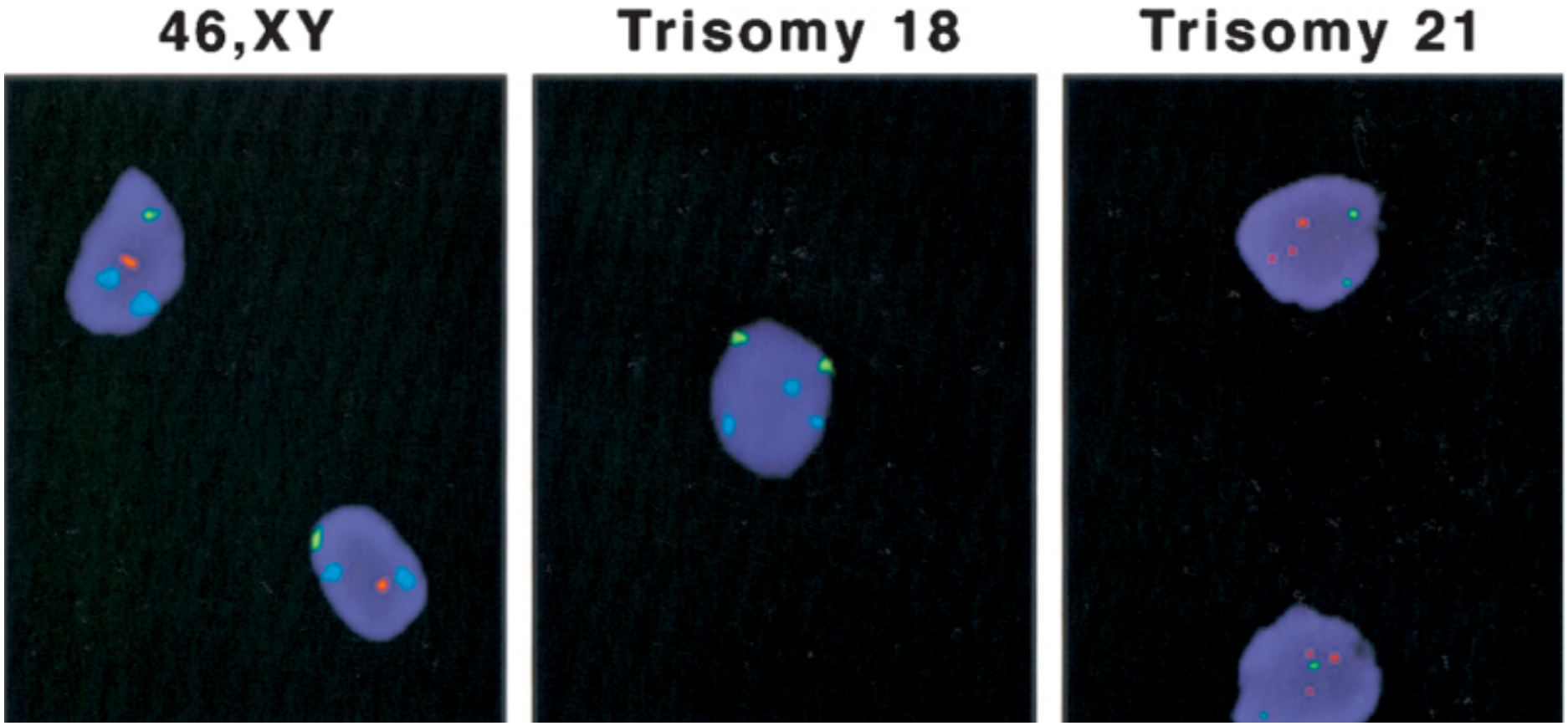



# SPECTRAL KARYOTYPING (SKY) with MULTICOLOR FISH PROBES



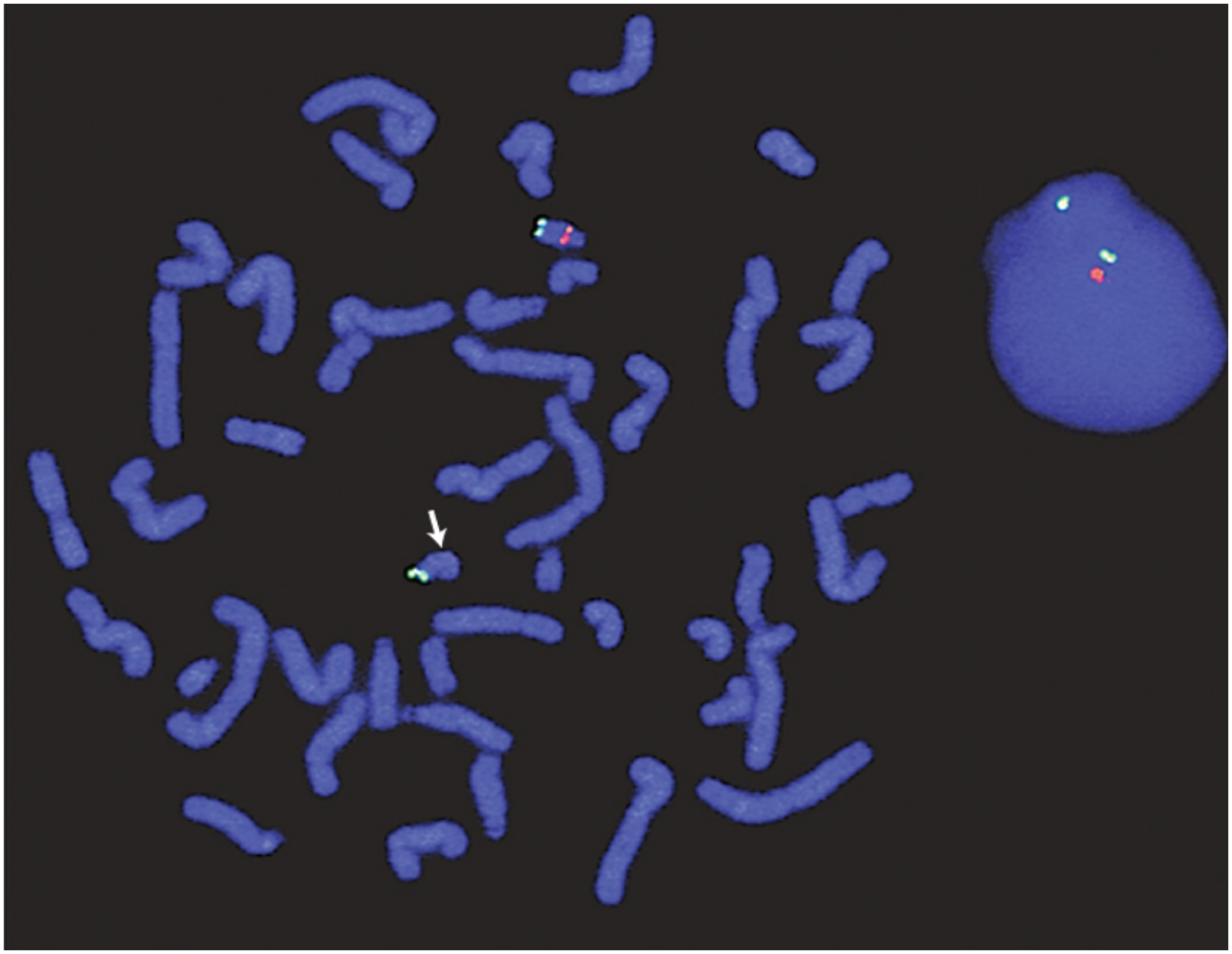


FISH centromere probe for chromosome




Simultaneous use of three FISH probes

1. 18. chr blue , X green, Y red
2. 3 blues one green
3. 3 reds, two greens

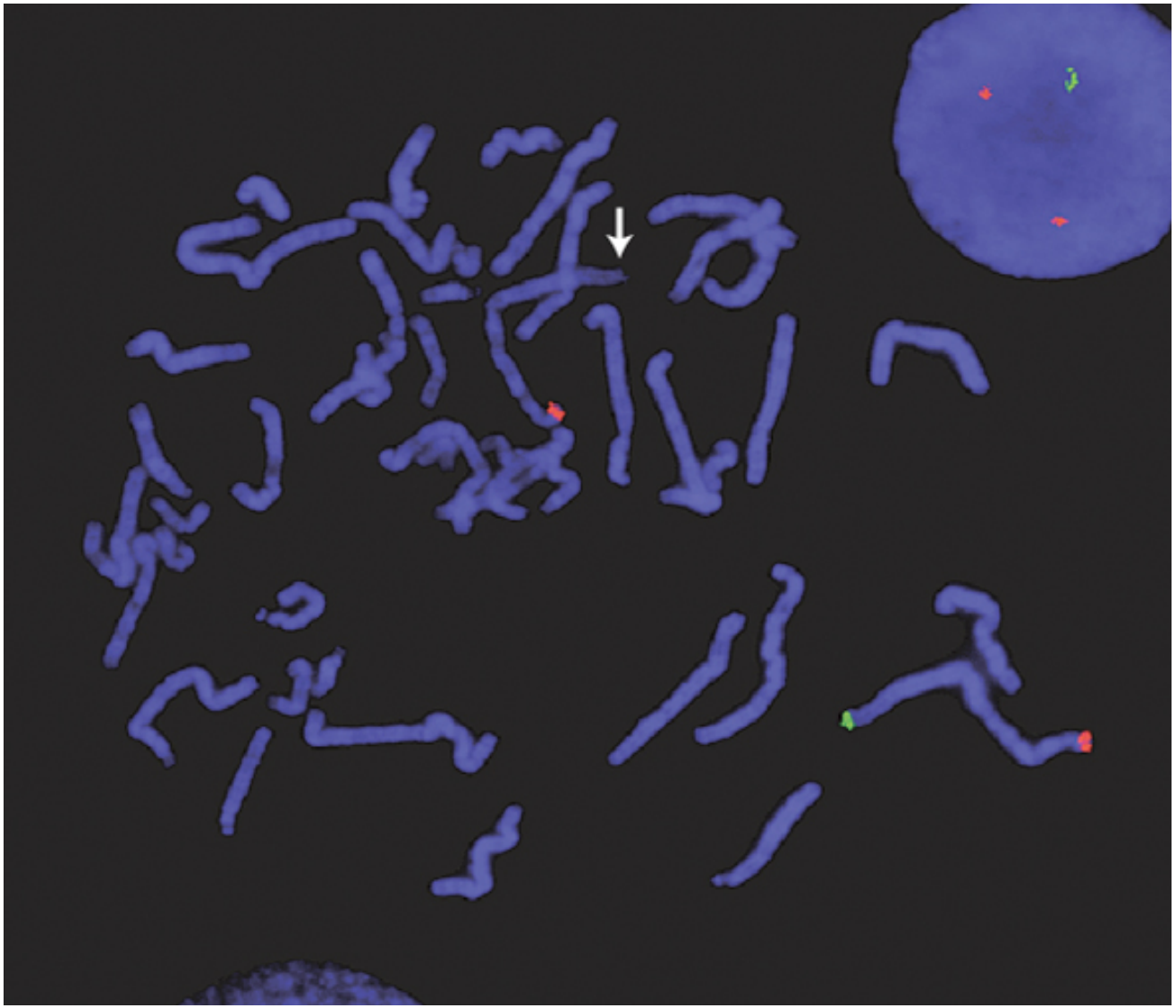


FISH

Two color probe
For Di George
syndrome

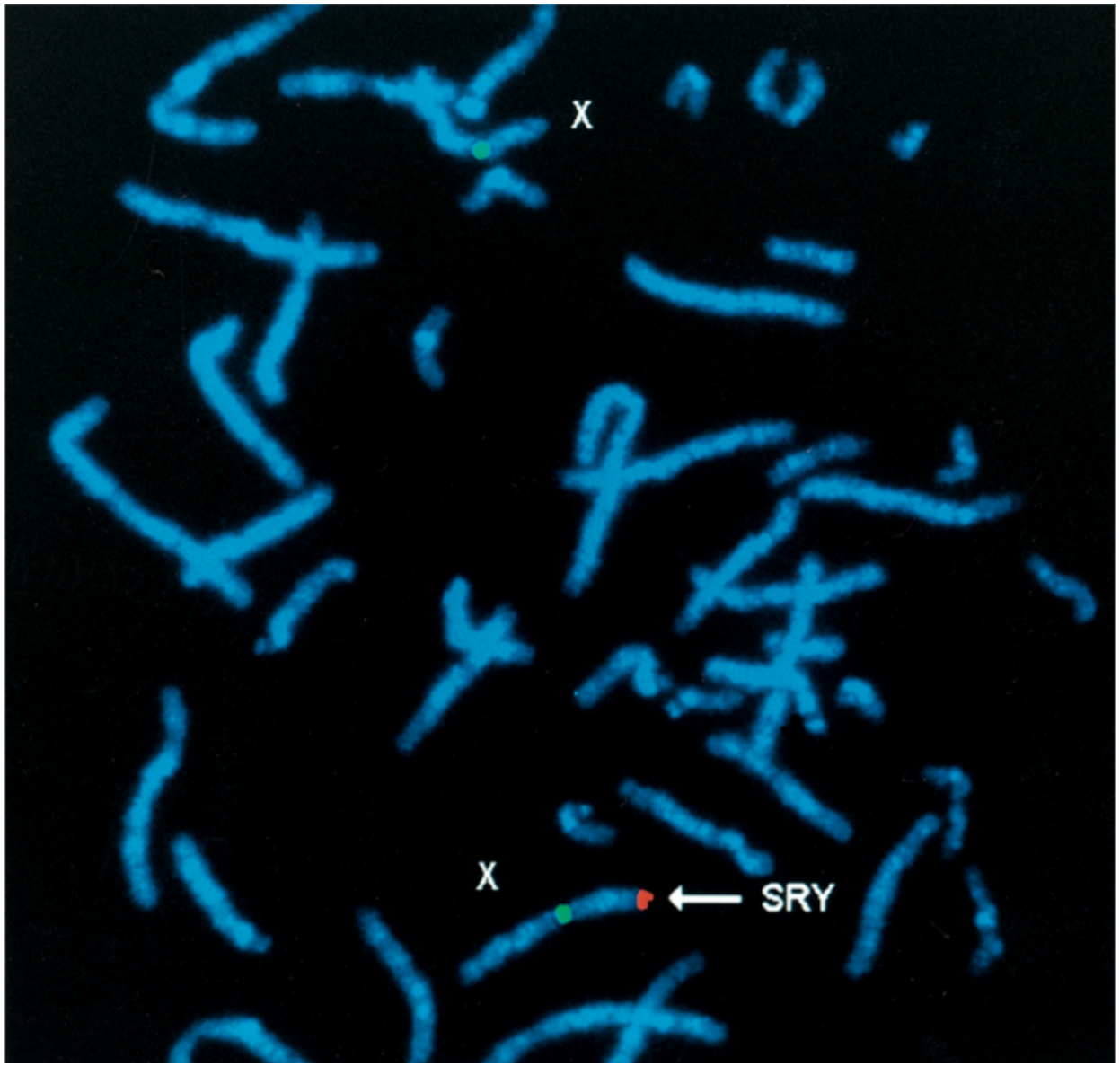
22q11.2 deletion

22q distal green
22q proximal red
locus spesific prob



FISH probes for
Subtelomeric regions
1p deletion

p : green
q : red




# FISH

Locus spesific probe
For SRY locus

XX male is $SRY^{+}$
t(X;Y)

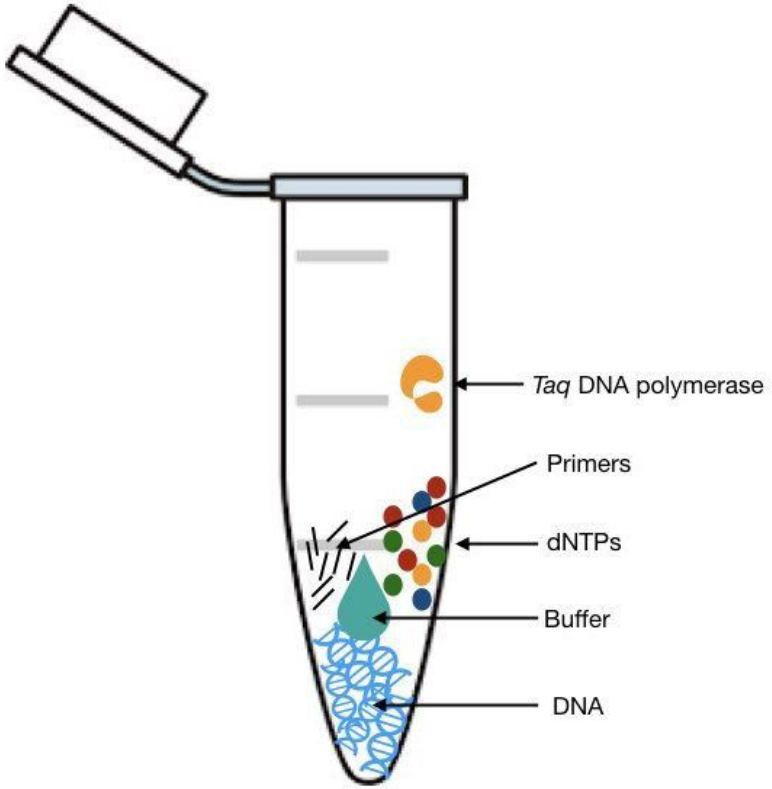
## Applications of DNA technology

Gene structure/mapping/function
Population genetics
Clinical genetics
  Preimplantation genetic diagnosis
  Prenatal diagnosis
  Presymptomatic diagnosis
  Carrier detection
Diagnosis and pathogenesis of disease
  Genetic
  Acquired—infective, malignant
Biosynthesis (e.g., insulin, growth hormone, interferon, immunization)
Treatment of genetic disease
Gene therapy
Agriculture (e.g., nitrogen fixation)

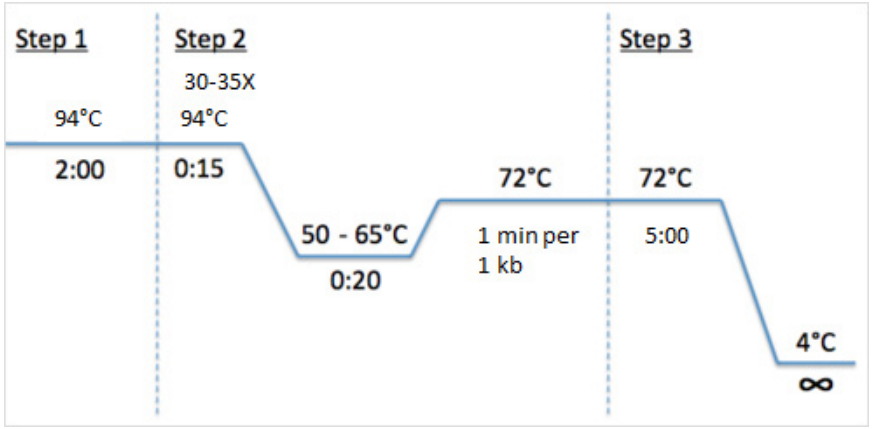
# Development of DNA technology

| Decade | Development | Examples of Application |
|---|---|---|
| 1980s | Recombinant DNA technology, Southern blot, and Sanger sequencing | Recombinant erythropoietin (1987), DNA fingerprinting (1984), and DNA sequence of Epstein–Barr virus genome (1984) |
| 1990s | Polymerase chain reaction (PCR) | Diagnosis of genetic disorders |
| 2000s | Capillary sequencing and microarray technology | Human genome sequence (2003) |
| 2010s | Next-generation sequencing | First acute myeloid leukemia (AML) cancer genome sequenced (2008)<br>Human genome sequenced at a cost of approx. $1000 (2014) |

# PCR (Polymerase Chain Reaction)


# PCR (Polymerase Chain Reaction)




| | 50µl Reaction | Final Concentration |
|---|---|---|
| **Template DNA** | 2.0µl | - |
| **dNTPs** | 4.0µl | 0.2mM each |
| **Magnesium Chloride** | 6.0µl | 2mM |
| **Primer** | 3.0µl each | 0.6uM each |
| **Taq Polymerase** | 0.2µl | 1.5 units |
| **PCR Buffer II** | 5.0µl | 1X |
| **PCR-quality water** | 30µl | - |

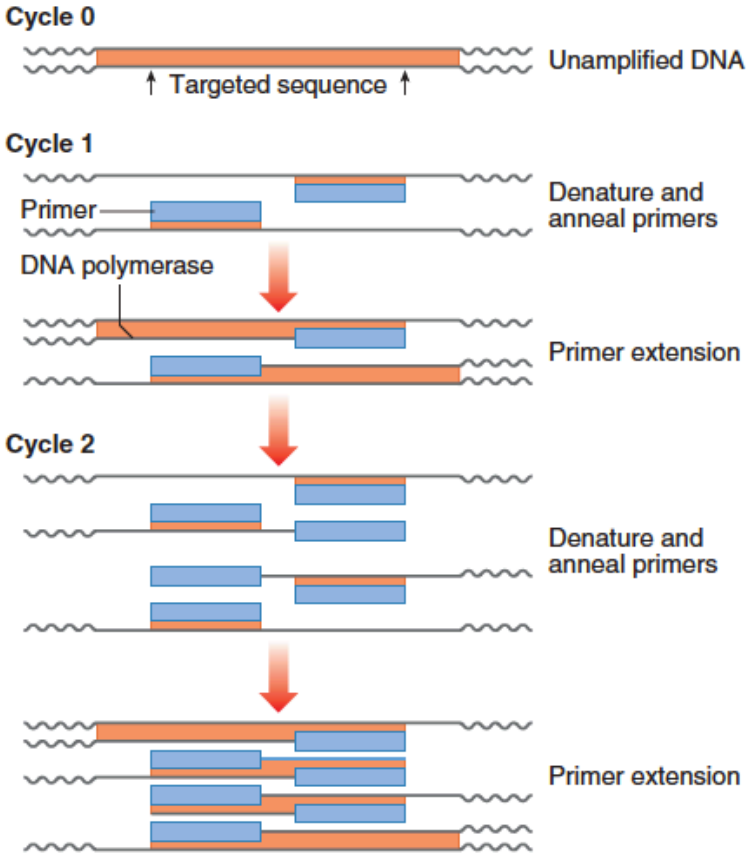
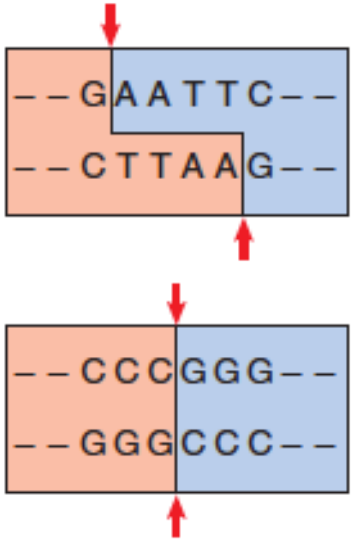


**Table 5.2** Some Examples of Restriction Endonucleases With Their Nucleotide Recognition Sequence and Cleavage Sites

| Enzyme | Organism | Cleavage Site 5′ | Cleavage Site 3′ |
|---|---|---|---|
| BamHI | *Bacillus amyloliquefaciens* H | G · G A T C C | |
| EcoRI | *Escherichia coli* RY 13 | G · A A T T C | |
| HaeIII | *Haemophilus aegyptius* | G G · C C | |
| HindIII | *Haemophilus influenzae* Rd | A · A G C T T | |
| HpaI | *Haemophilus parainfluenzae* | G T T · A A C | |
| PstI | *Providencia stuartii* | C T G C A · G | |
| SmaI | *Serratia marcescens* | C C C · G G G | |
| SalI | *Streptomyces albus* G | G · T C G A C | |



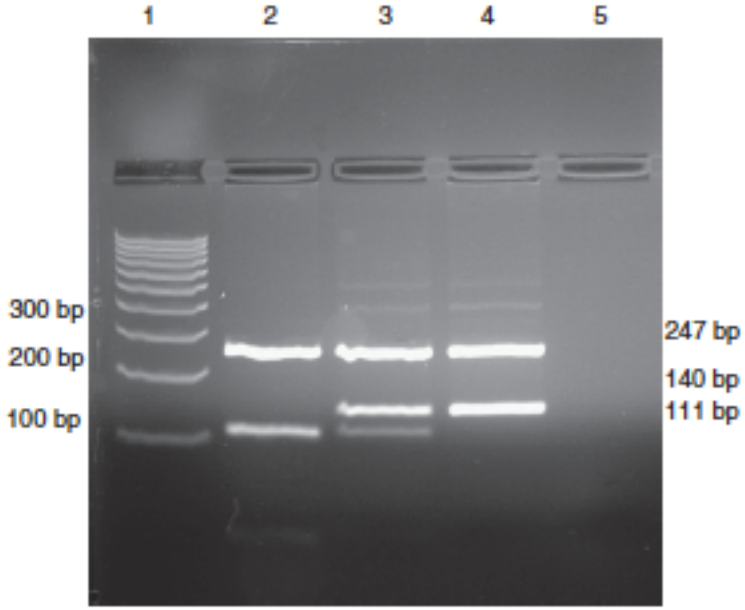



FIGURE 5.9 Detection of the *HFE* gene mutation C282Y by restriction fragment length polymorphisms (RFLP). The normal 387-bp polymerase chain reaction (PCR) product is digested with *RsaI* to give products of 247 bp and 140 bp. The C282Y mutation creates an additional recognition site for *RsaI*, giving products of 247 bp, 111 bp, and 29 bp. Lane 1 shows a 100-bp ladder-size standard. Lanes 2–4 show patients homozygous, heterozygous, and normal for the C282Y mutation, respectively. Lane 5 is the negative control. *(Courtesy N. Goodman, Department of Molecular Genetics, Royal Devon and Exeter Hospital, Exeter, UK.)*

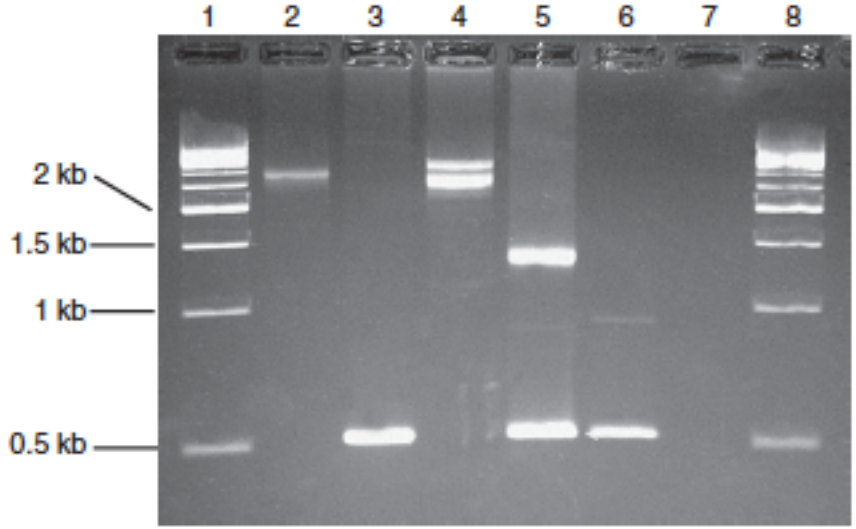
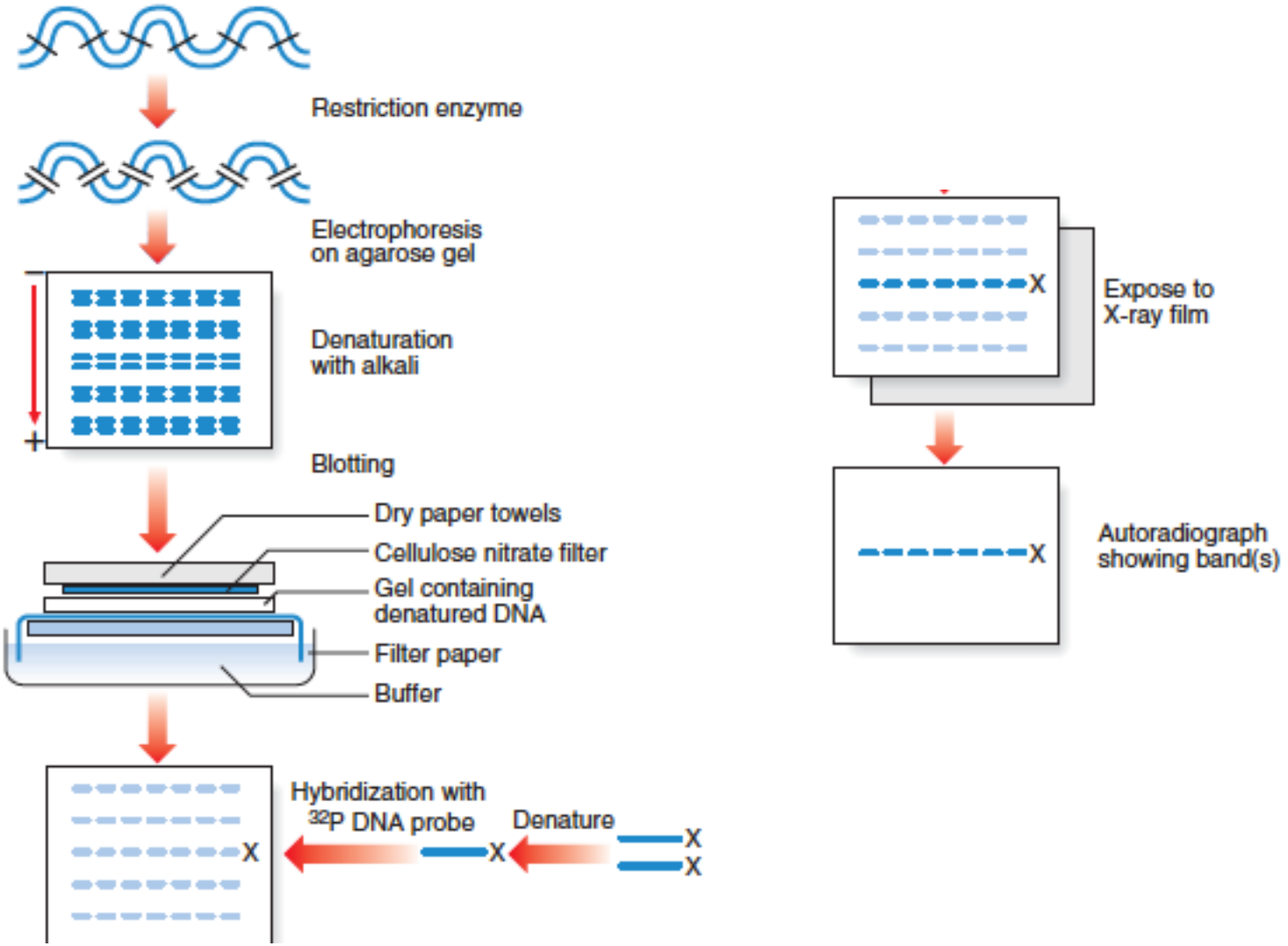
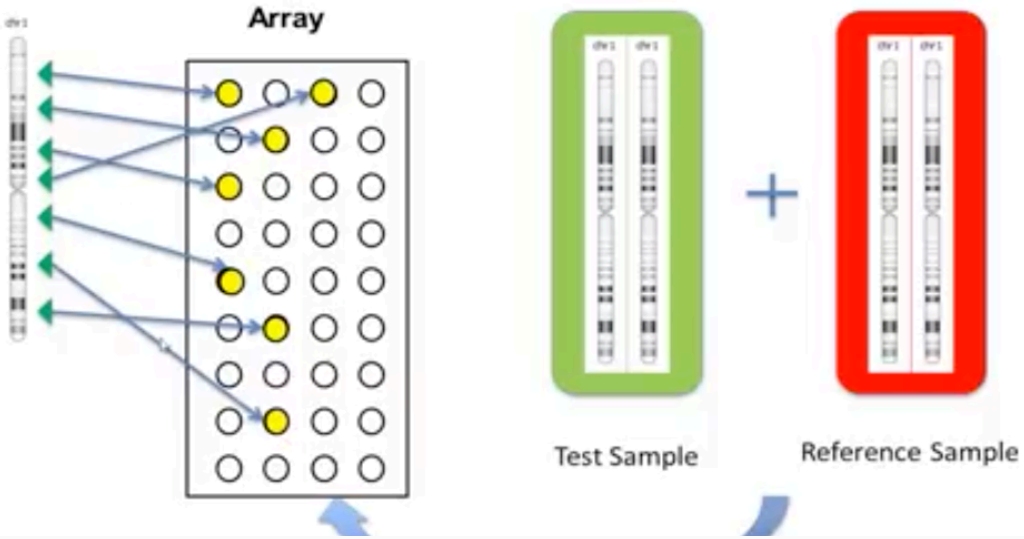



FIGURE 5.8 Amplification of the GAA repeat expansion mutation by polymerase chain reaction (PCR) to test for Friedreich ataxia. Products are stained with ethidium bromide and electrophoresed on a 1.5% agarose gel. Lanes 1 and 8 show 500-bp ladder-size standards, Lanes 2 and 4 show patients with homozygous expansions, Lanes 3 and 6 show unaffected controls, Lane 5 shows a heterozygous expansion carrier, and Lane 7 is the negative control. *(Courtesy K. Thomson, formerly at the Department of Molecular Genetics, Royal Devon and Exeter Hospital, Exeter, UK.)*

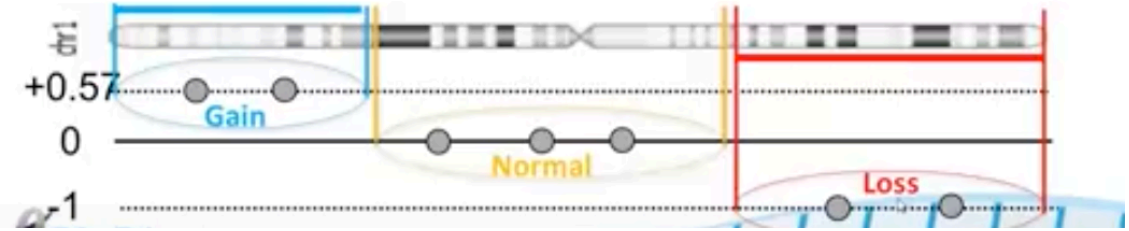
# SOUTHERN BLOT



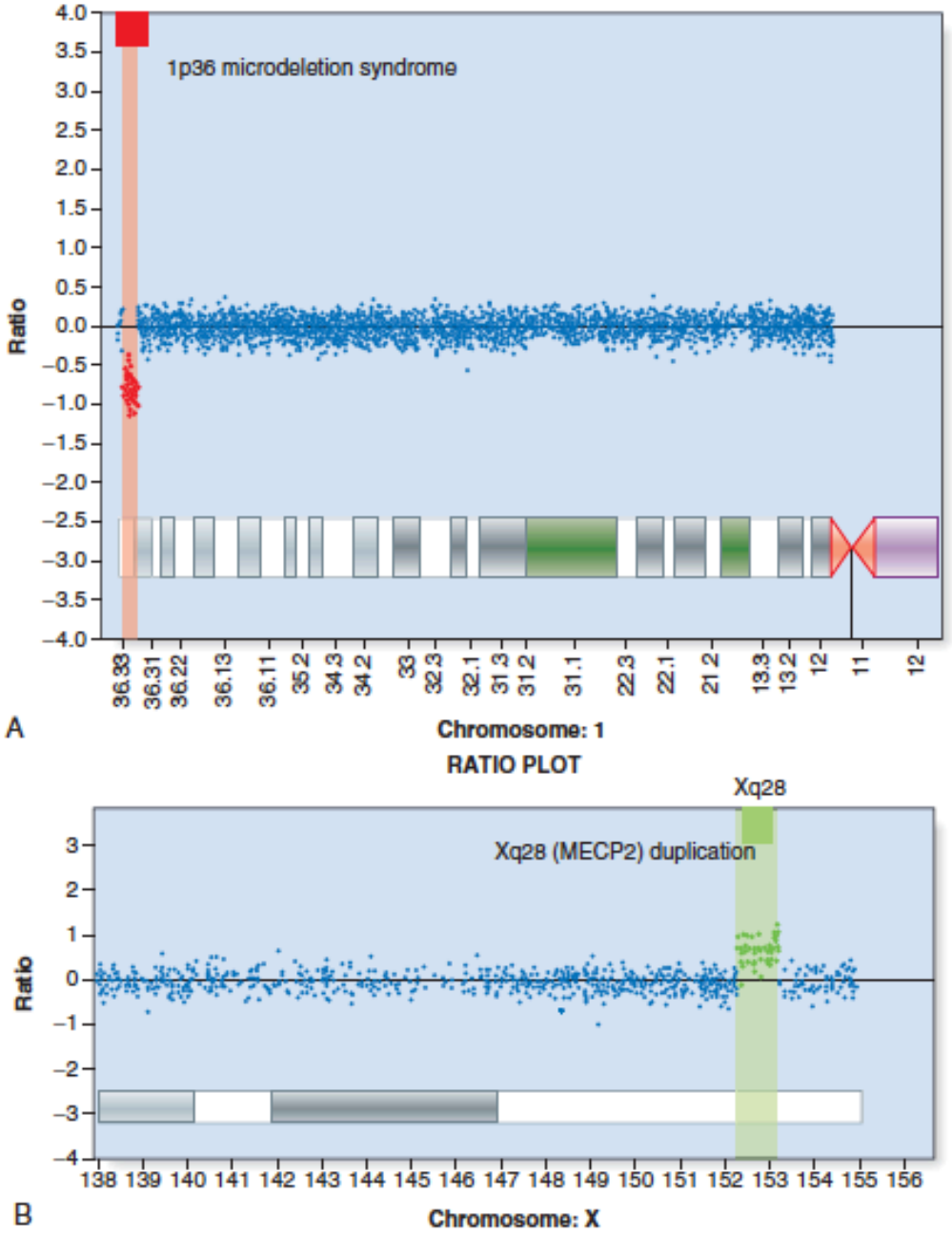
# Basics Comperative Genomic Hybridization (CGH)




| Probe Location | Expr. | Control | Ratio | Log Ratio |
|---|---|---|---|---|
| Chr1:10-20 | 150 | 100 | 3/2 | +0.57 |
| Chr1:50-60 | 300 | 200 | 3/2 | +0.57 |
| Chr1:70-90 | 500 | 500 | 2/2 | 0 |
| Chr1:100-120 | 60 | 60 | 2/2 | 0 |
| Chr1:250-300 | 600 | 600 | 2/2 | 0 |
| Chr1:400-450 | 500 | 1000 | 1/2 | -1 |
| Chr1:500-550 | 80 | 160 | 1/2 | -1 |



# CGH PLOTS FOR CHROMOSOME 1 AND X



# Real Time PCR

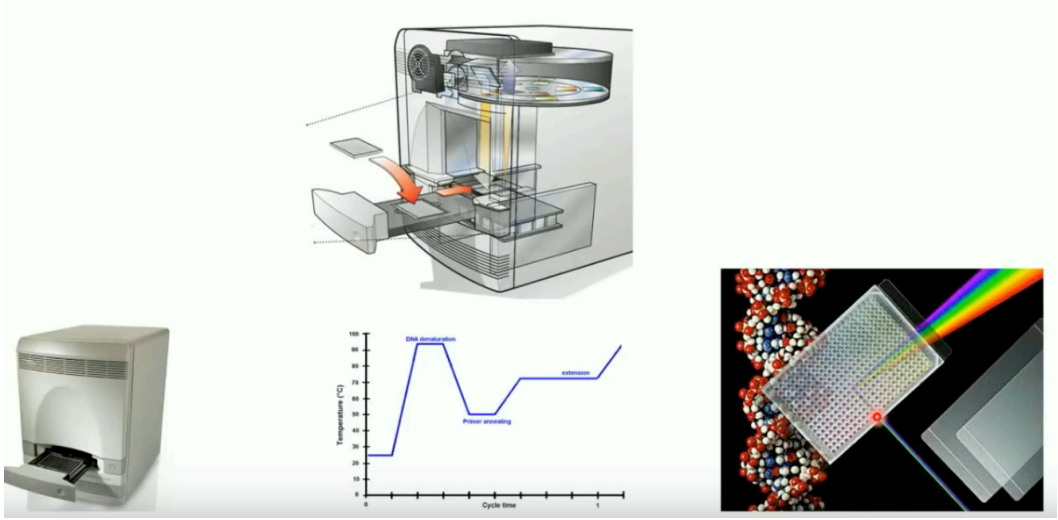
In a real time PCR protocol, a fluorescent reporter molecule is used to monitor the PCR as it progresses. The fluorescence emitted by the reporter molecule manifolds as the PCR product accumulates with each cycle of amplification. Based on the molecule used for the detection, the real time PCR techniques can be categorically placed under two heads:

1. Non-specific Detection using DNA Binding Dyes
2. Specific Detection Target Specific Probes

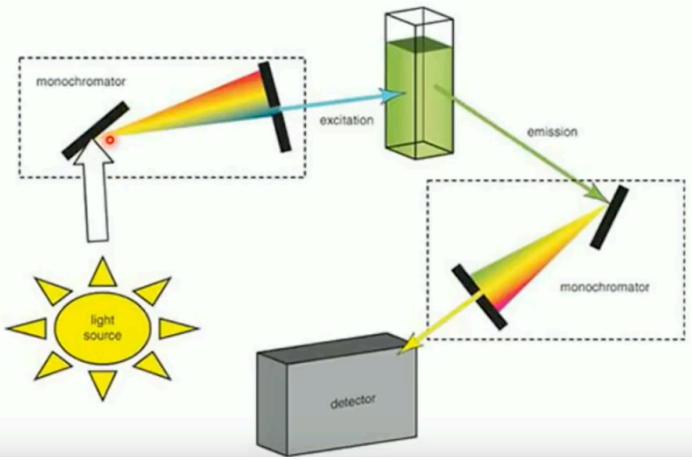
## Real Time PCR Applications Include

1 . Quantitative mRNA expression studies.
2 . DNA copy number measurements in genomic or viral DNAs.
3 . Allelic discrimination assays or SNP genotyping.
4 . Verification of microarray results.
5 . Drug therapy efficacy.
6 . DNA damage measurement.

# REAL TIME (QUNATITATIVE) PCR



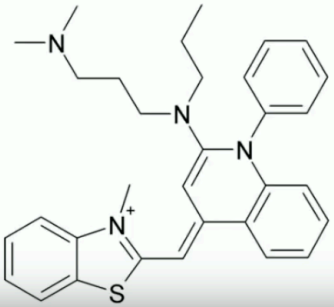

## Real Time PCR/Spectrofluorometer




## Real Time PCR/Detection

**Non-Specific Detection**
**Fluorescent dye**

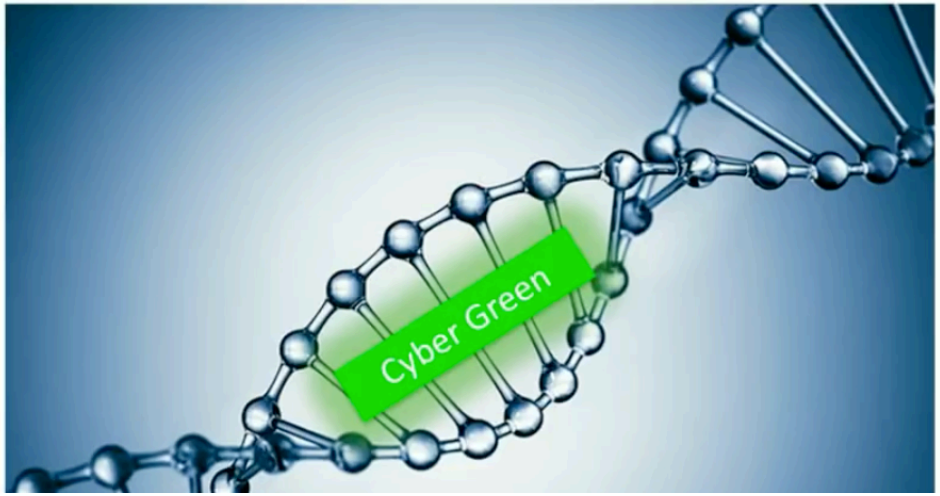
Cyber Green



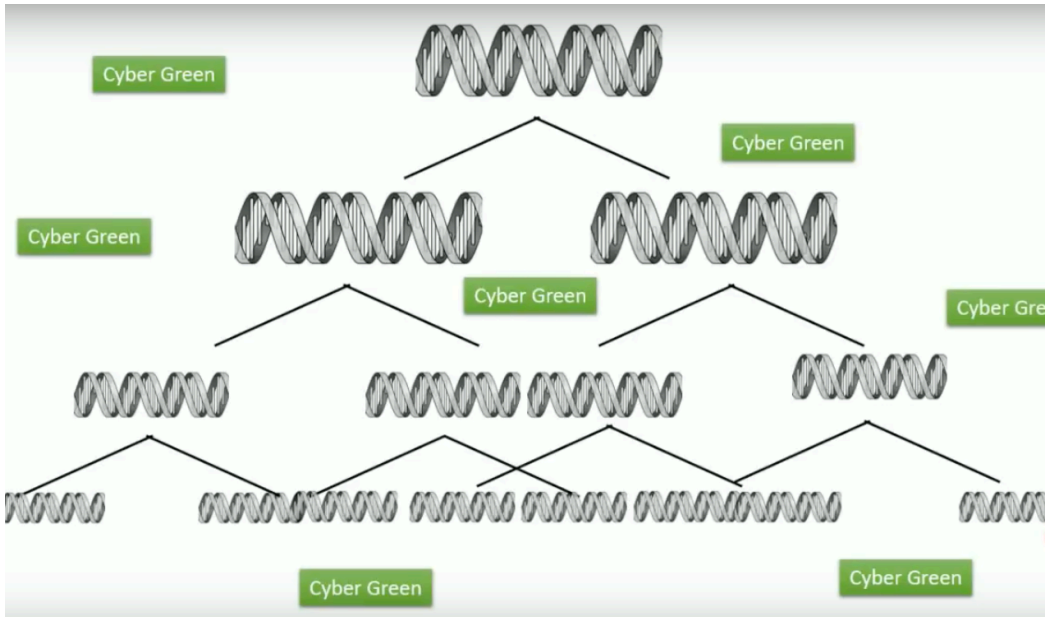
**Specific Detection**
**Detective Probe**

TaqMan

# Real Time PCR/Non-Specific Detection



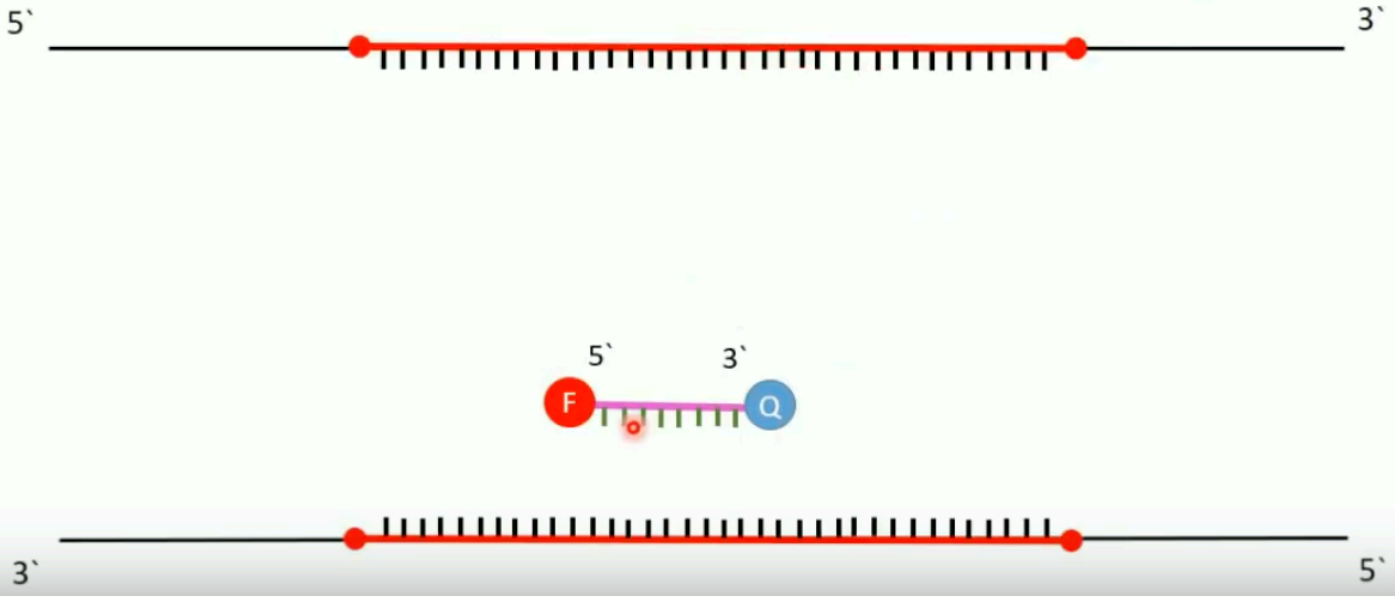

Absorbs 497 nm
Emits 520 nm



# Real Time PCR/Specific Detection

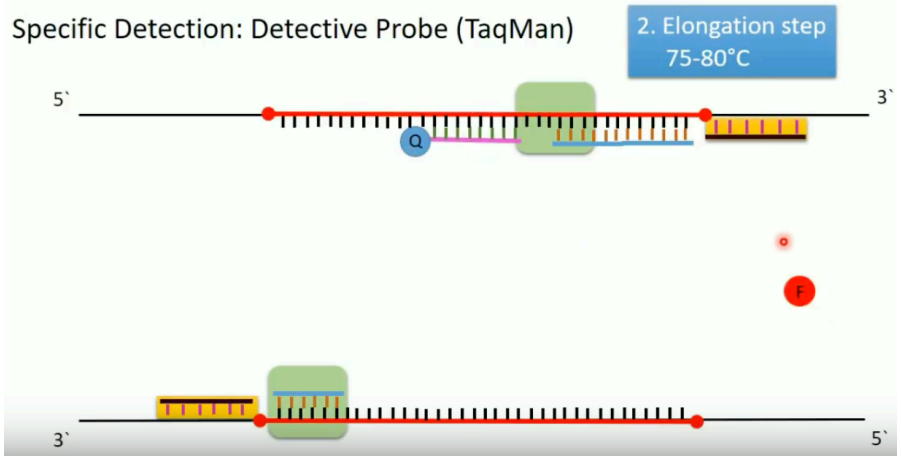
Specific Detection: Detective Probe (TaqMan)

1. Denaturation step 94-98°C

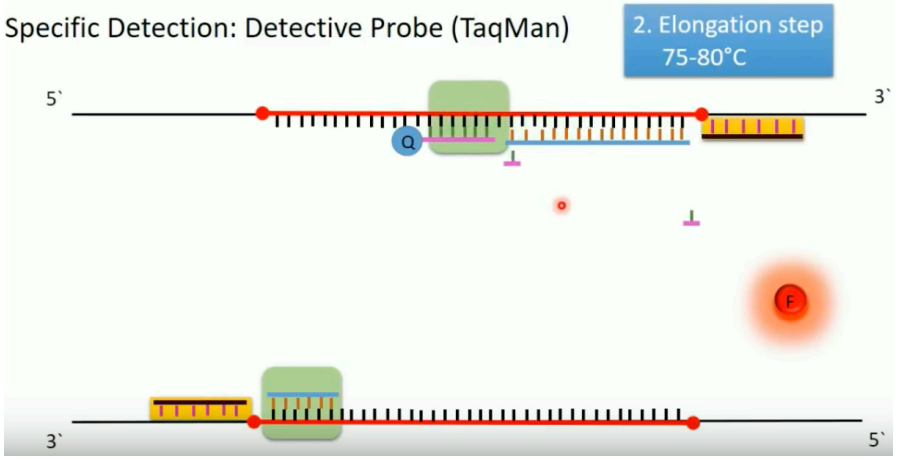


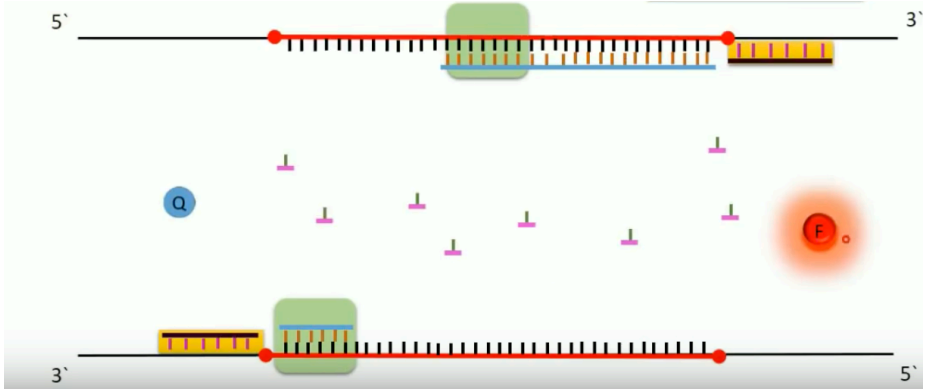
# Real Time PCR/Specific Detection

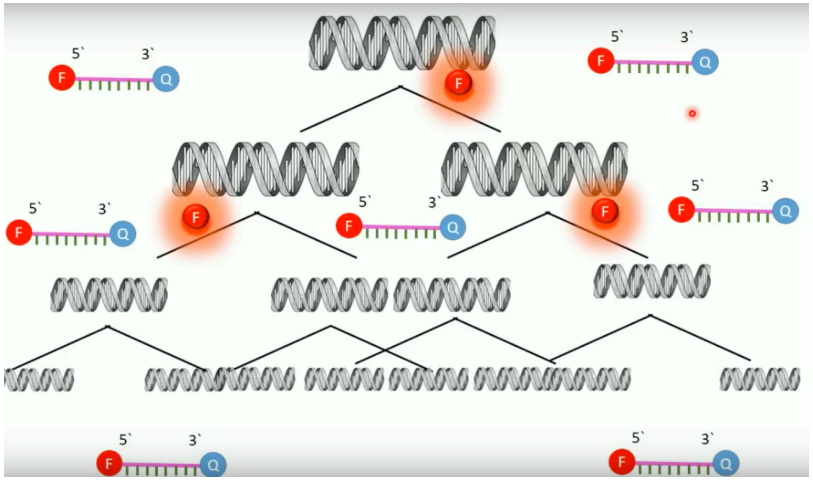
Specific Detection: Detective Probe (TaqMan)



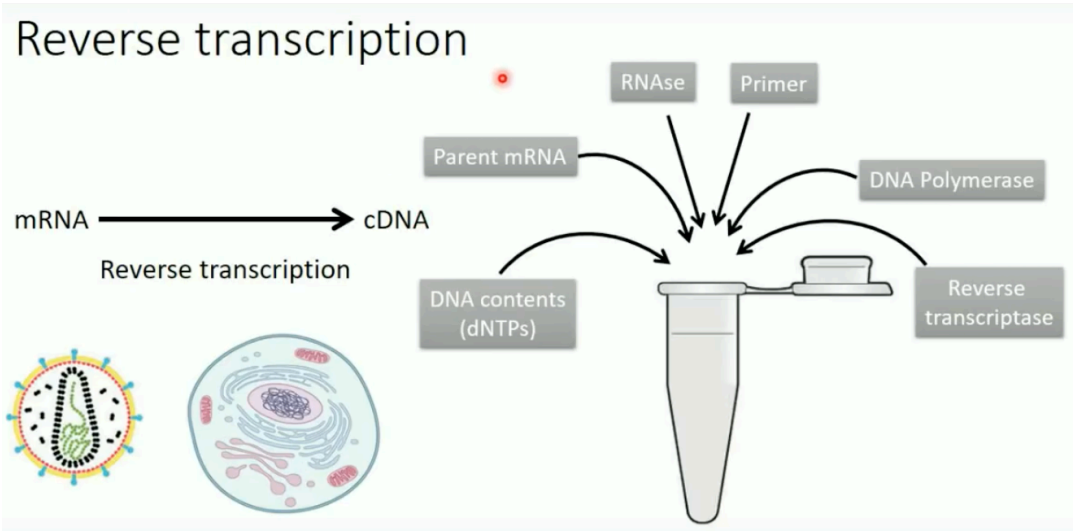

Specific Detection: Detective Probe (TaqMan)



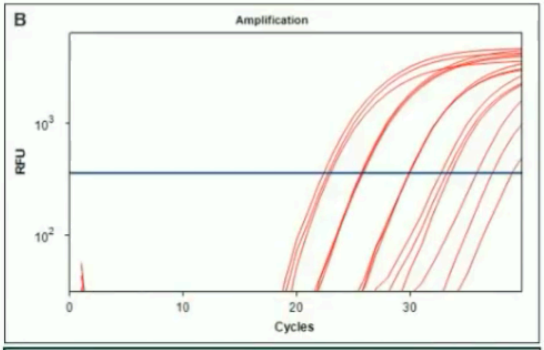



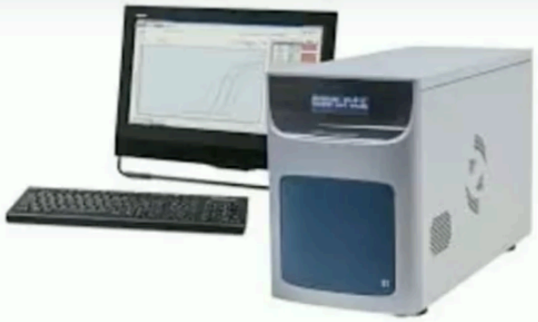



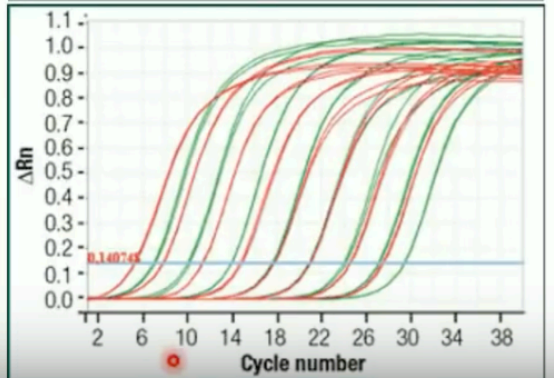
# Reverse transcription

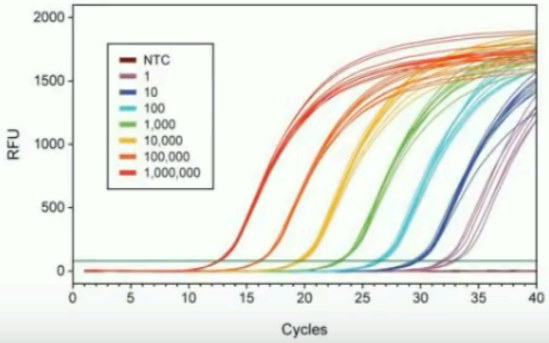


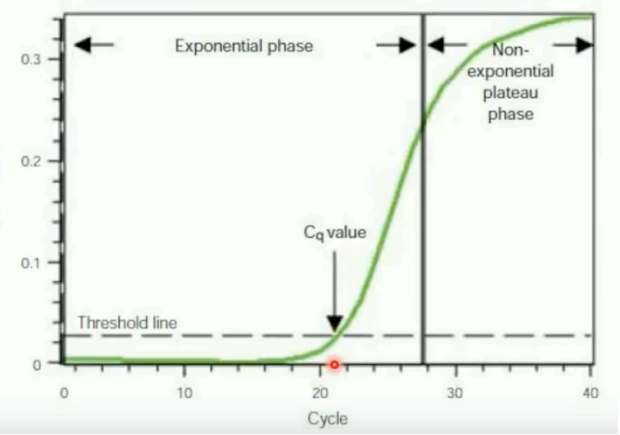
# Quantitative rt-PCR



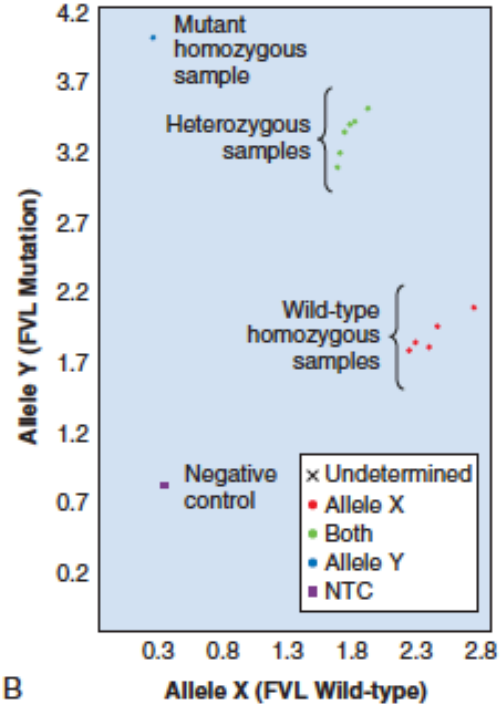





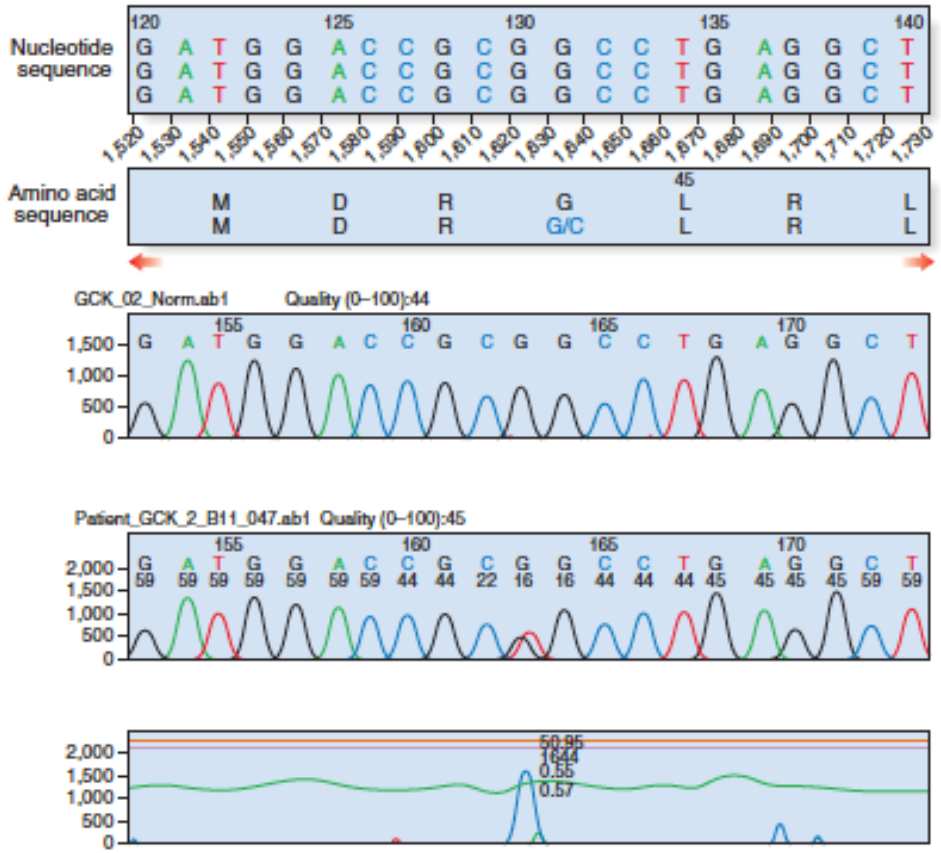



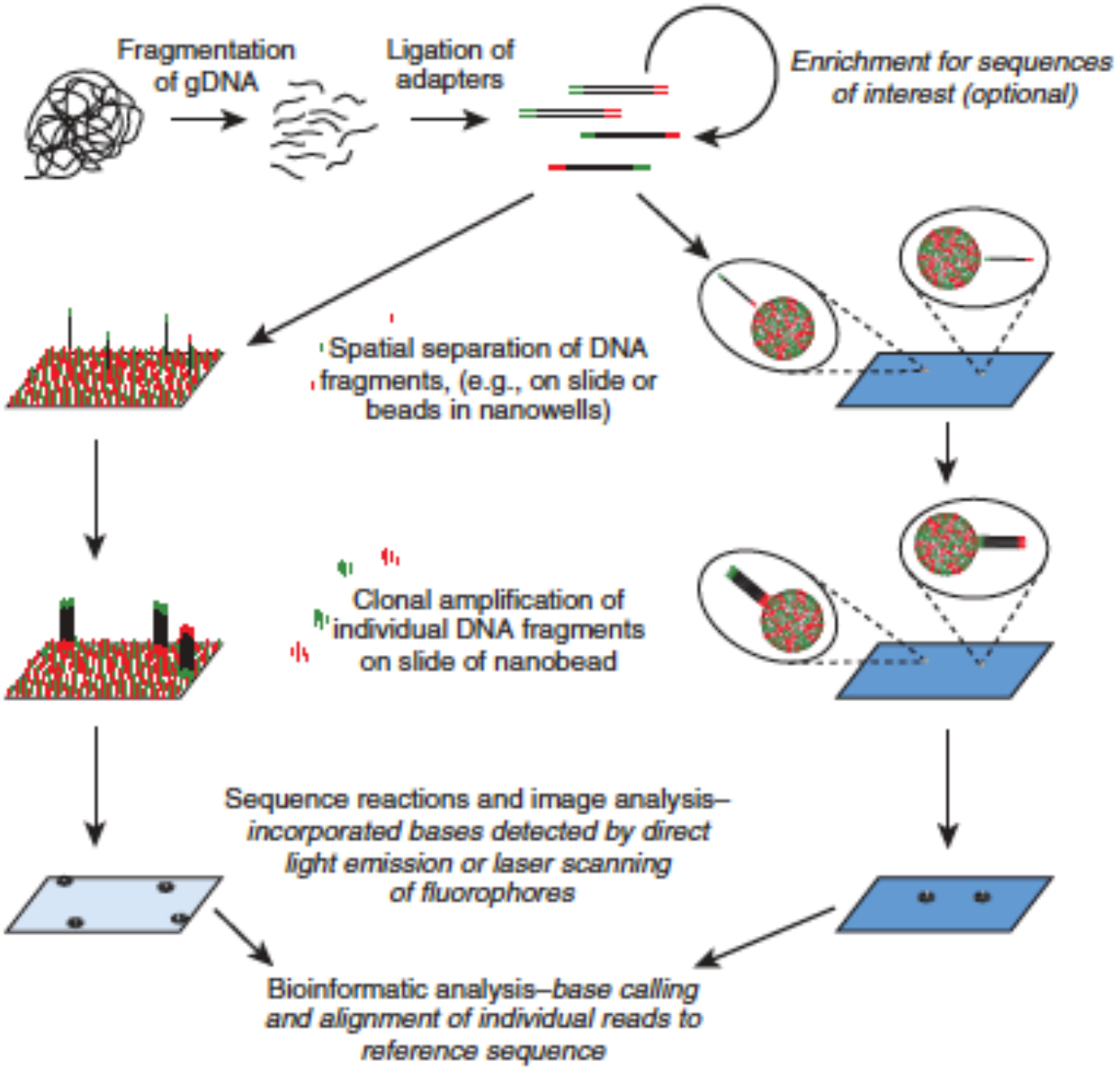

## Allelic Discrimination Plot



# SANGER SEQUENCING (Dideoxy Sequencing / chain termination)

# NEXT GENERATION (CLONAL) SEQUENCING
## *MASSIVELY PARALLEL SEQUENCING*



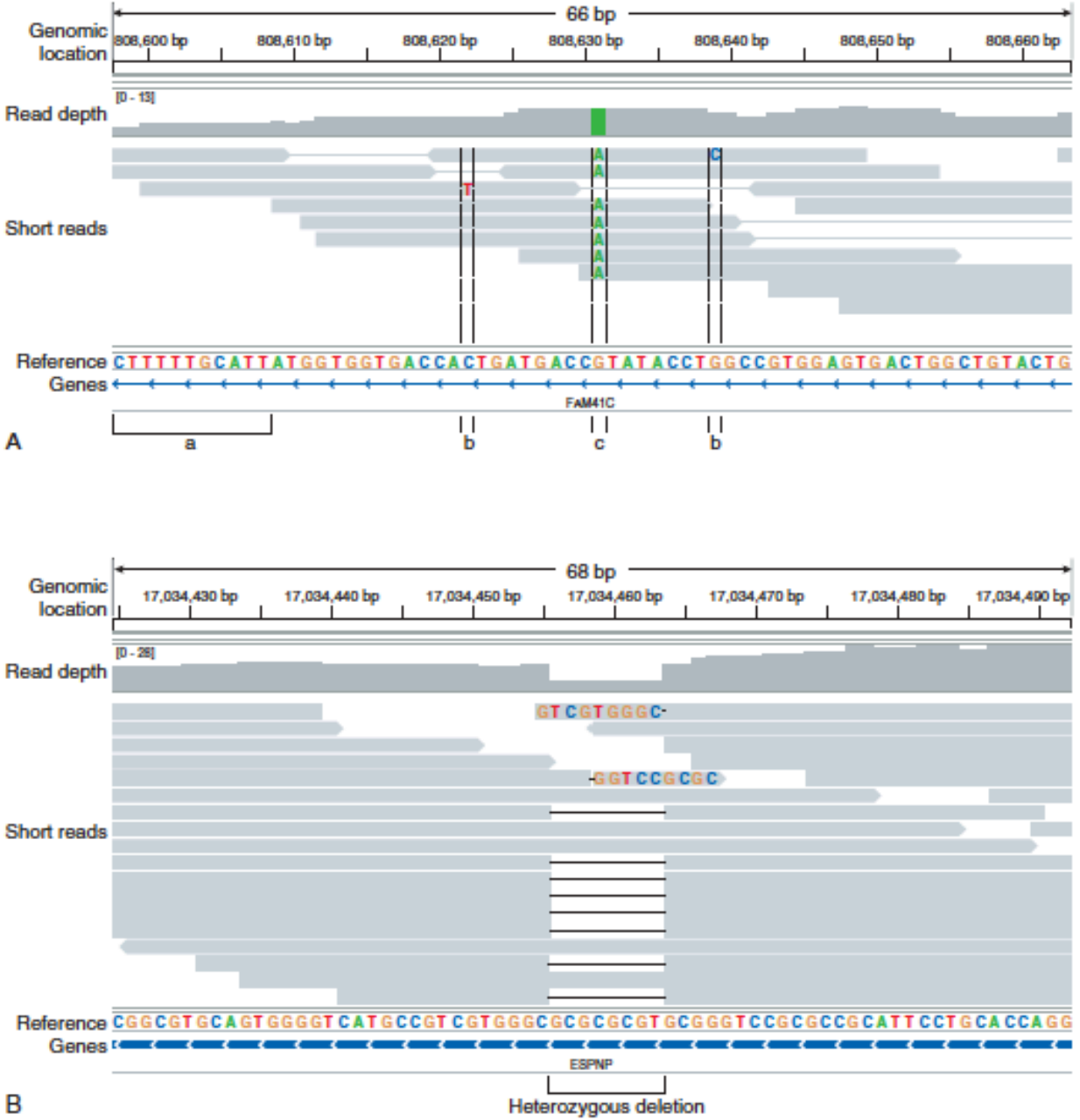
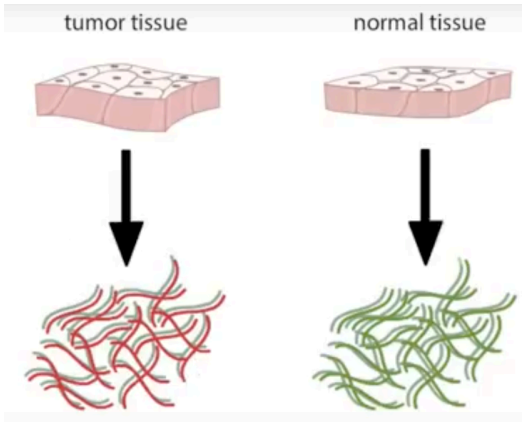


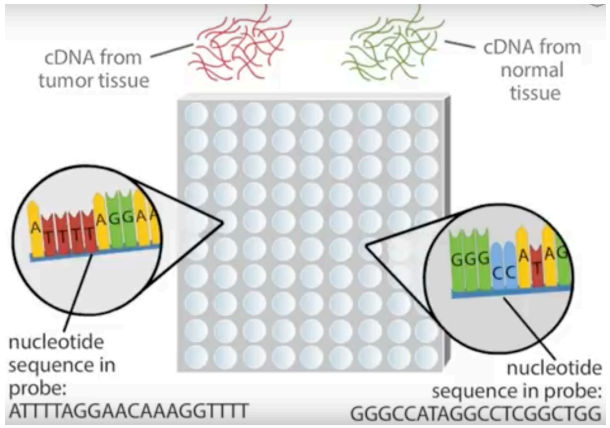

FIGURE 5.16 **A**, Aligning individual paired-end reads to the reference genome. Nucleotides in the reads that differ from the reference sequence are marked. (a) A region with poor coverage. (b) The variants at these positions are most likely sequencing errors. (c) At this position the subject is homozygous for the A alleles. A real example would have longer reads and greater read depth. **B**, Aligned reads with a heterozygous deletion. Reads with an 8-bp deletion identified are marked with a black bar. Images produced using the IGV software package. *(Courtesy Dr M. Wakeling, University of Exeter Medical School, UK.)*

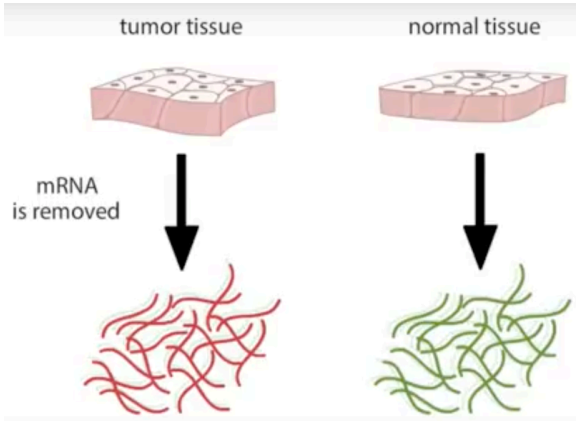
**Table 5.4** Sanger Sequencing Compared to Next-Generation 'Clonal' Sequencing

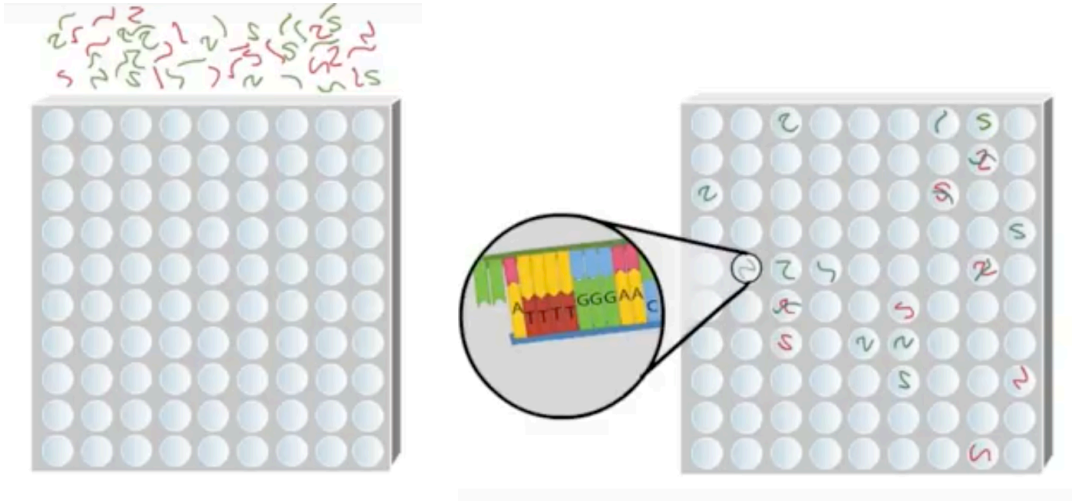
| Sanger Sequencing | Next-Generation 'Clonal' Sequencing |
|---|---|
| One sequence read per sample | Massively parallel sequencing |
| 500–1000 bases per read | 100–400 bases per read |
| Approx. 1 million bases per day per machine | Approx. 2 billion bases per day per machine |
| Approx. $1 per 1000 bases | Approx. $1 per 5,000,000 bases |

# DNA MICROARRAY

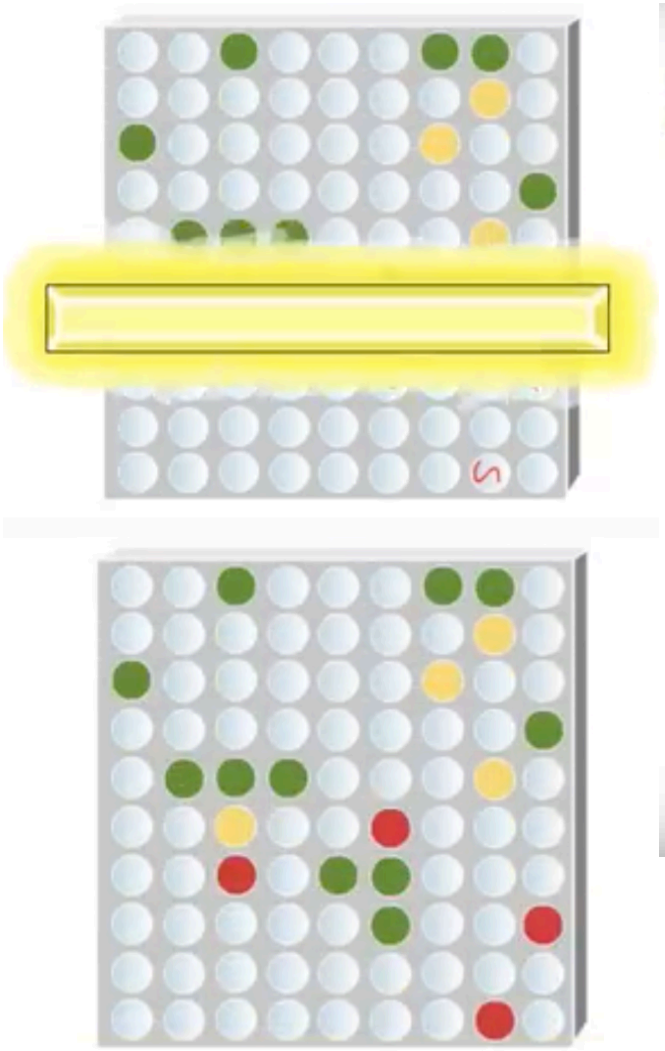


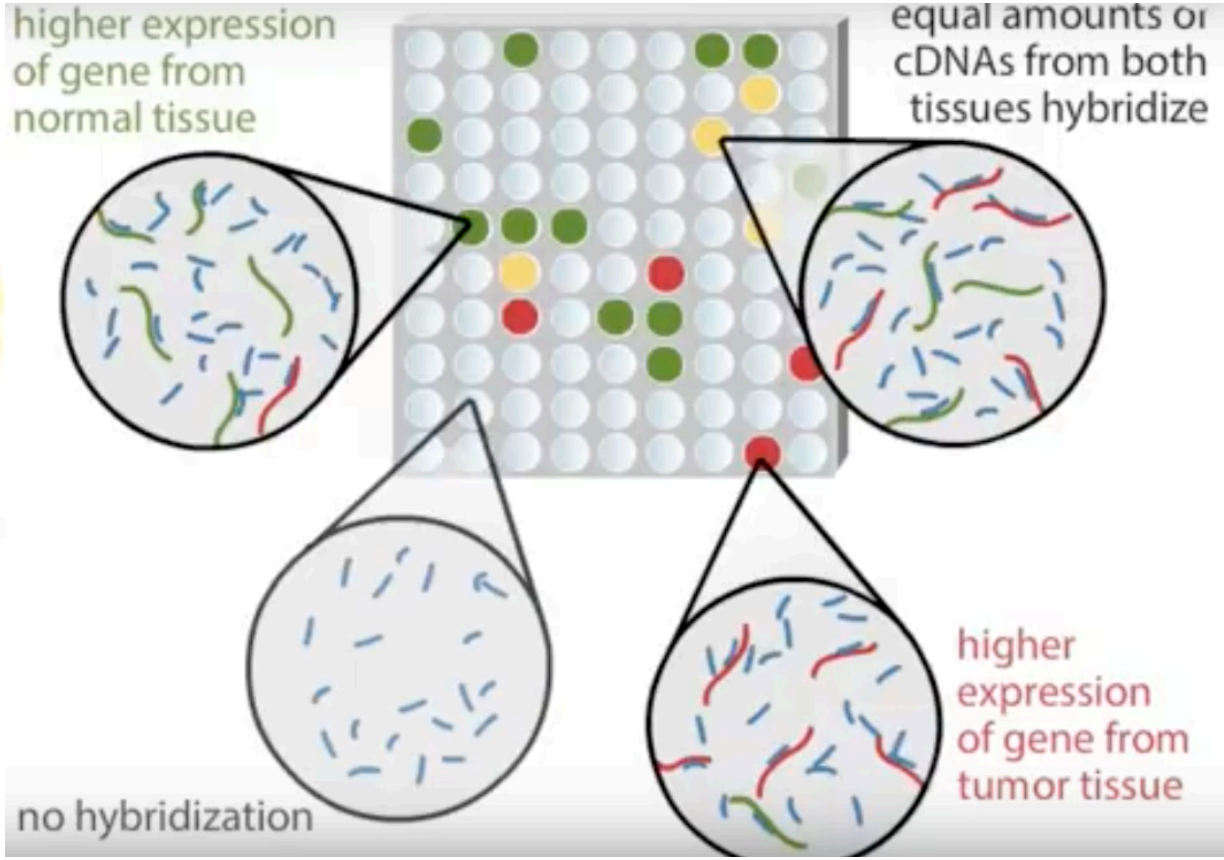



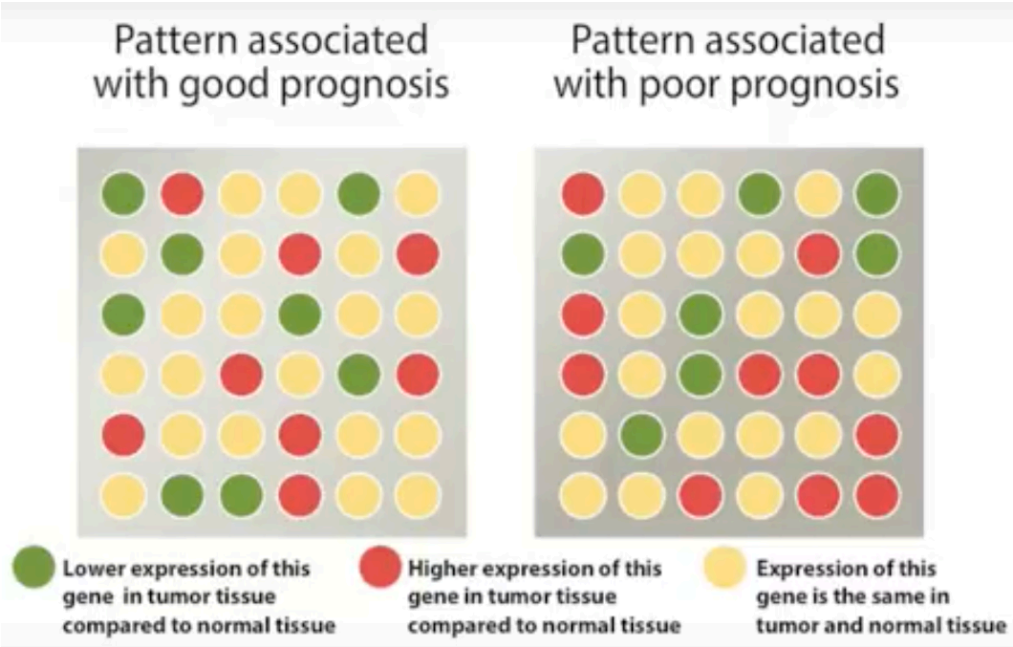



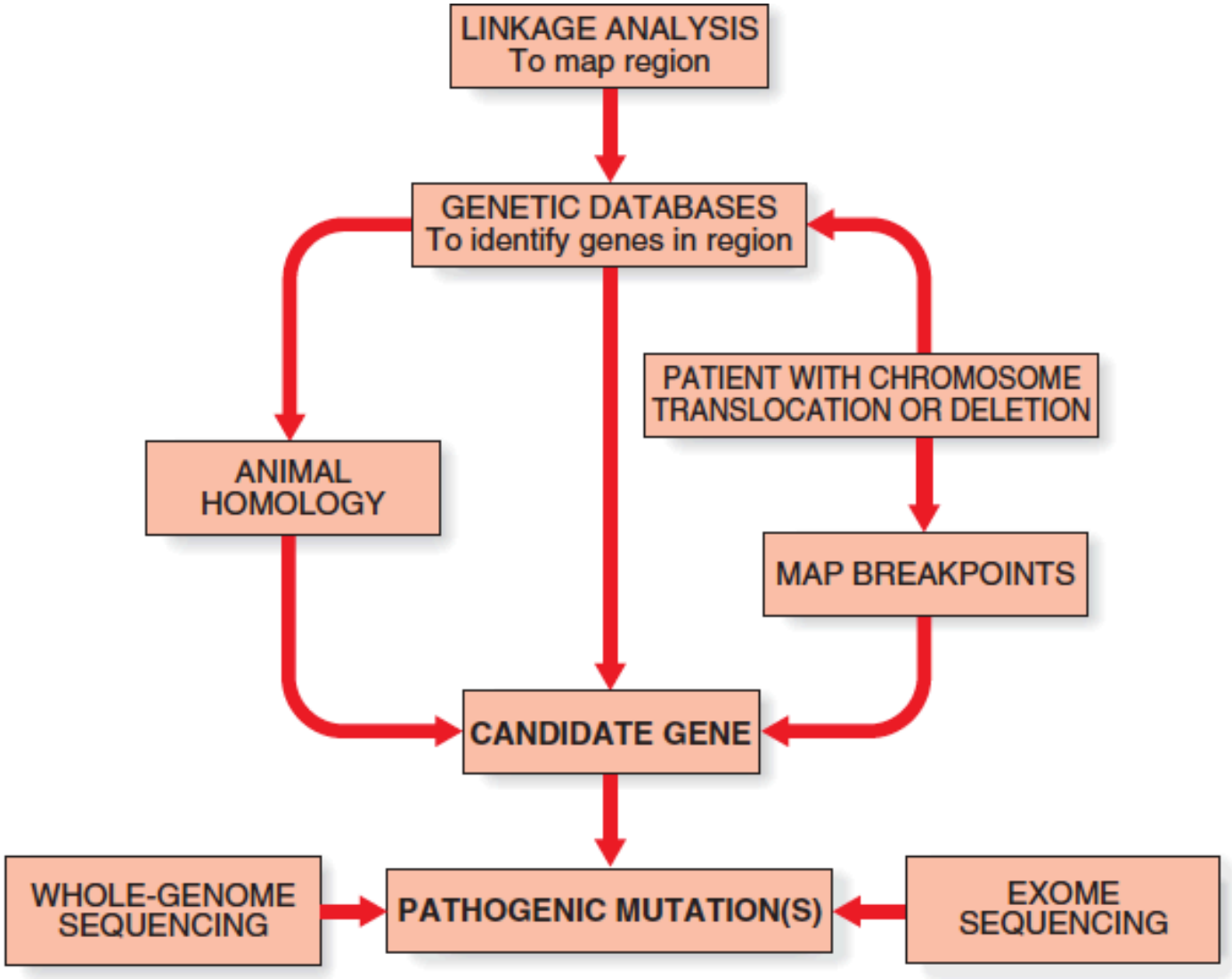



# DNA MICROARRAY



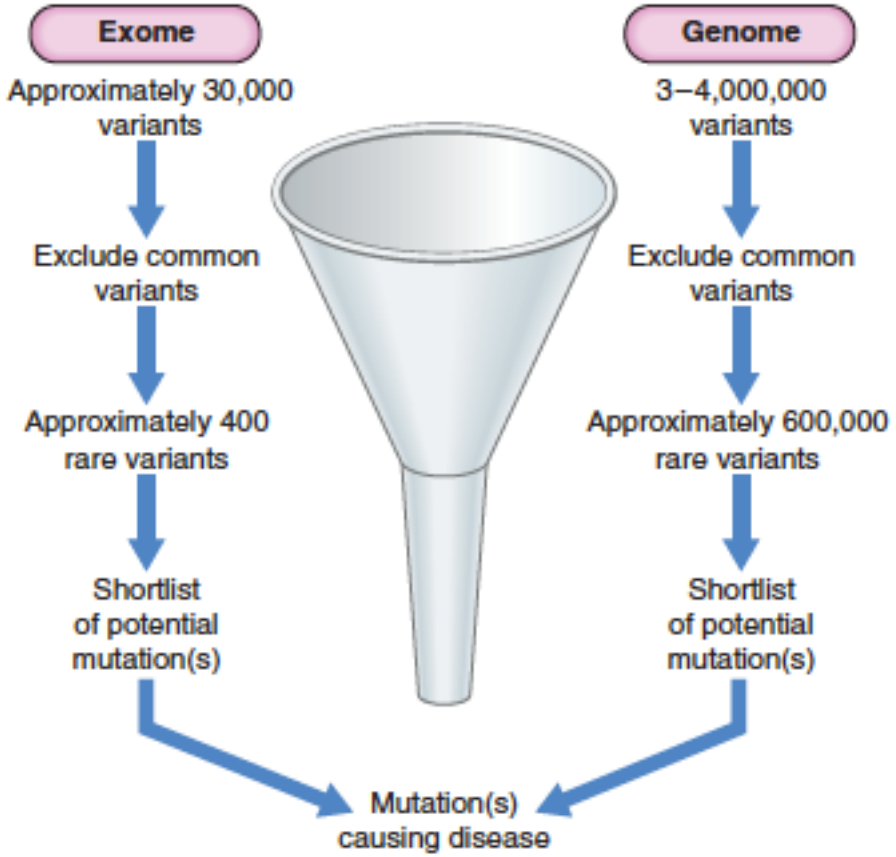



Pattern associated with good prognosis
Pattern associated with poor prognosis
Lower expression of this gene in tumor tissue compared to normal tissue
Higher expression of this gene in tumor tissue compared to normal tissue
Expression of this gene is the same in tumor and normal tissue




Pathways toward human disease gene identification

Filtering variants obtained by exome and genome sequencing to identify pathogenic mutation causing rare diseases.



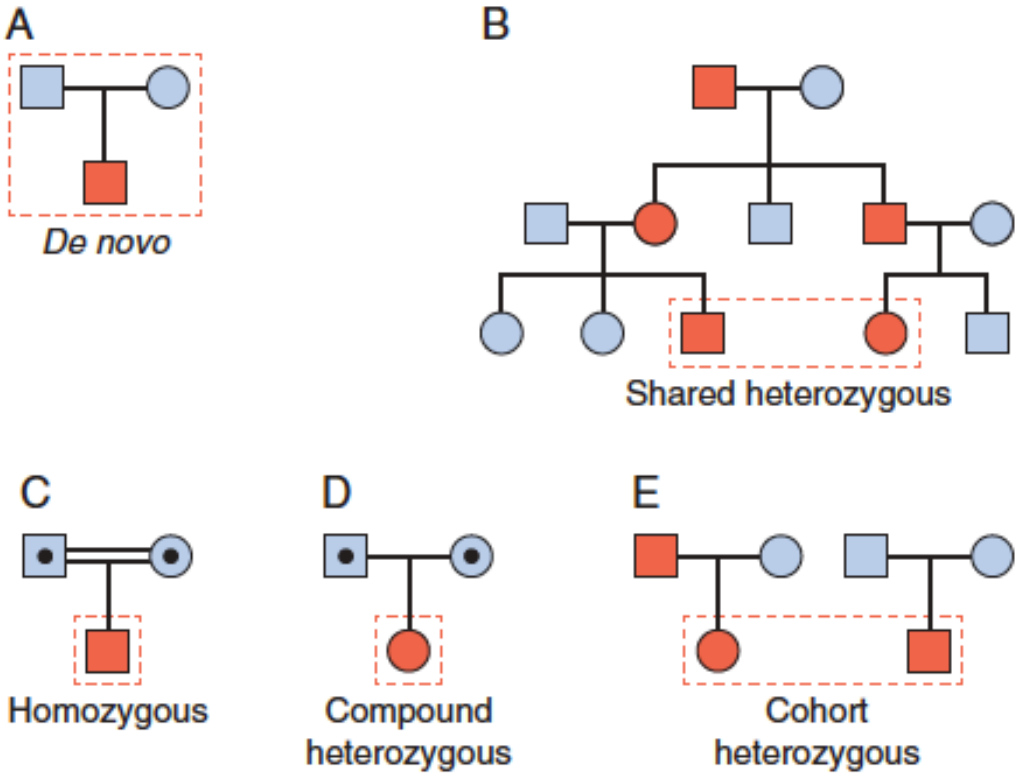



FIGURE 4.5 Strategies for disease gene identification by exome or genome sequencing. The red dashed boxes indicate individuals within pedigrees whose samples are analyzed by exome or genome sequencing. (**A**) Trio analysis of an affected patient and their unrelated, unaffected parents to detect heterozygous *de novo* mutations. (**B**) Linkage approach of sequencing the two most distantly related affected individuals in a dominant pedigree to identify shared heterozygous variants that include the pathogenic mutation. (**C**) Analysis of a proband from a consanguineous pedigree to identify homozygous variants in a gene within a homozygous region. (**D**) Analysis of a proband born to unaffected, unrelated parents to identify compound heterozygous mutations in a single gene. (**E**) Cohort analysis of unrelated, affected individuals who share a distinctive phenotype to identify heterozygous mutations in the same gene.

PCR
https://www.youtube.com/watch?v=oE-QpvoNnWE

GEL ELECTROPHORESIS
https://www.youtube.com/watch?v=vq759wKCCUQ
https://www.youtube.com/watch?v=8RBs0Ghg_48

RFLP
https://www.youtube.com/watch?v=MQnsCEhPFTU

MLPA
https://www.youtube.com/watch?v=gfLJxKuqleY

SANGER SEQUENCING
https://www.youtube.com/watch?v=FhlKYsc_9_A

NGS
https://www.youtube.com/watch?v=shoje_9IYWc