

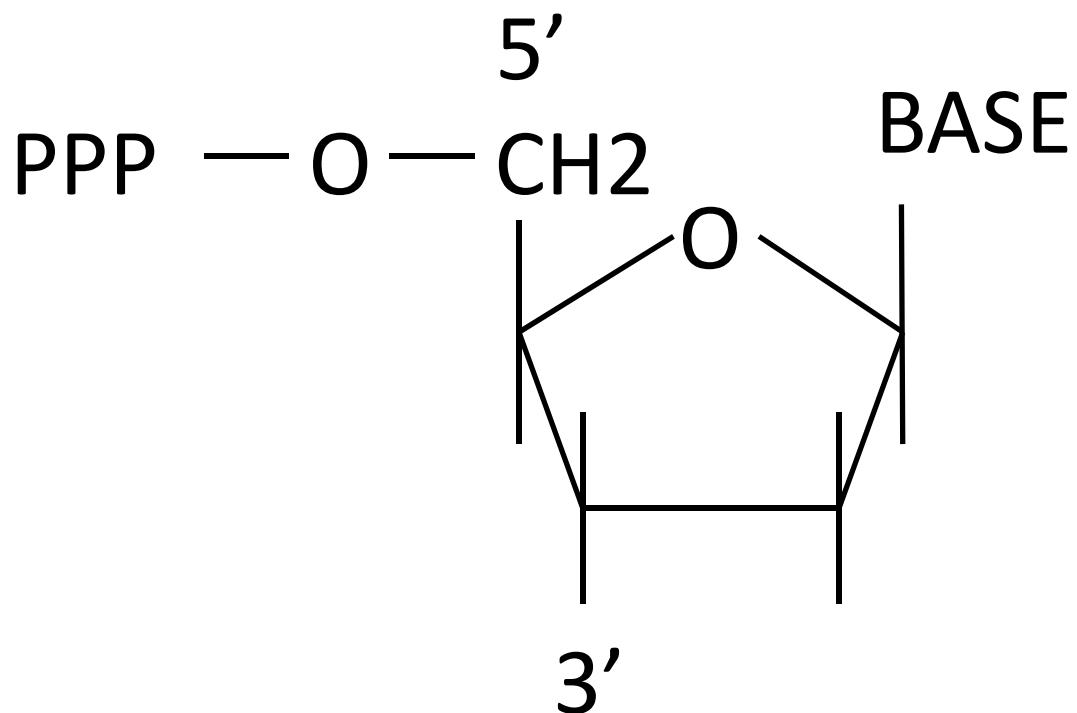
# Purified DNA Molecules Can Be Sequenced Rapidly

- DNA Sequencing
  - Important to determine the sequence of nucleotides of the cloned gene
  - Chain termination sequencing (Sanger method)
  - Computer automated sequencing
    - ddNTP's are each labeled with a different fluorescent dye
    - Samples are separated on a single-lane capillary gel that is scanned with a laser beam
    - Creates different color patterns for each nucleotide
    - Converted by computer to the sequence

# Sanger Method

- ✓ in-vitro DNA synthesis using 'terminators', use of dideoxynucleotides that do not permit chain elongation after their integration
- ✓ DNA synthesis using deoxy- and dideoxynucleotides that results in termination of synthesis at specific nucleotides
- ✓ Requires a primer, DNA polymerase, a template, a mixture of nucleotides, and detection system
- ✓ Incorporation of di-deoxynucleotides into growing strand terminates synthesis
  - ✓ Synthesized strand sizes are determined for each di-deoxynucleotide by using gel or capillary electrophoresis
    - ✓ Enzymatic methods

# Dideoxynucleotide



no hydroxyl group at 3' end  
prevents strand extension

# The principles

- Partial copies of DNA fragments made with DNA polymerase
- Collection of DNA fragments that terminate with A,C,G or T using ddNTP
  - Separate by gel electrophoresis
  - Read DNA sequence

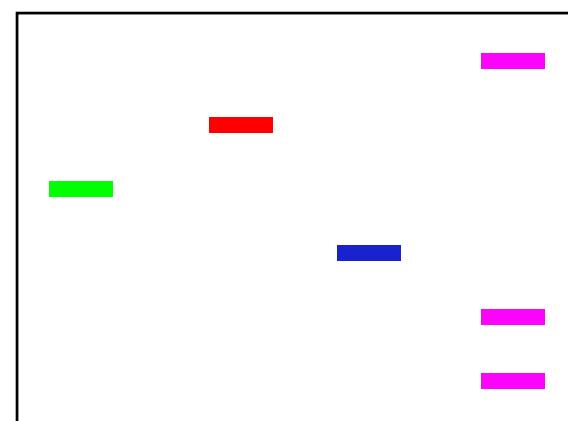
3' — CCGTAC — 5'  
primer 5' — 3'

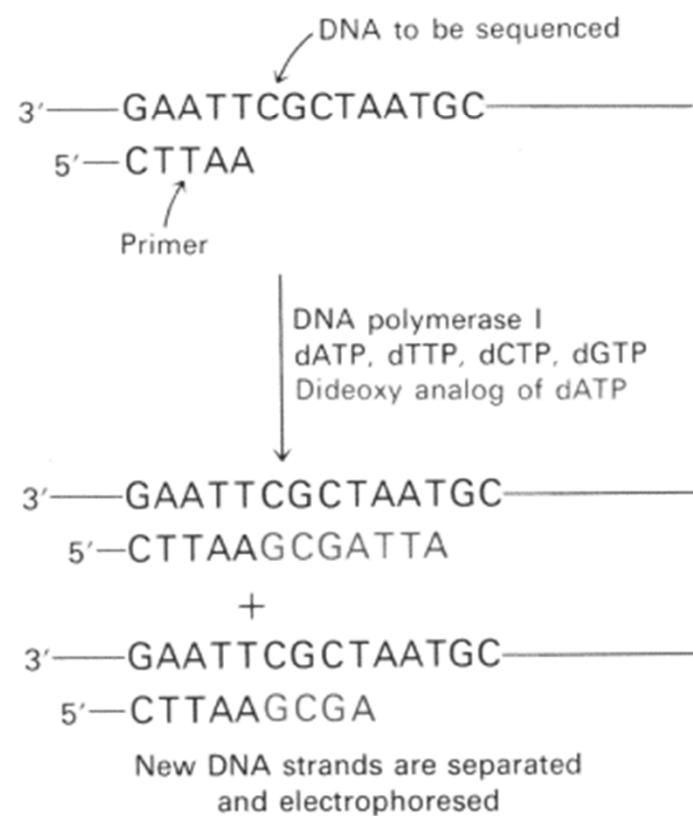
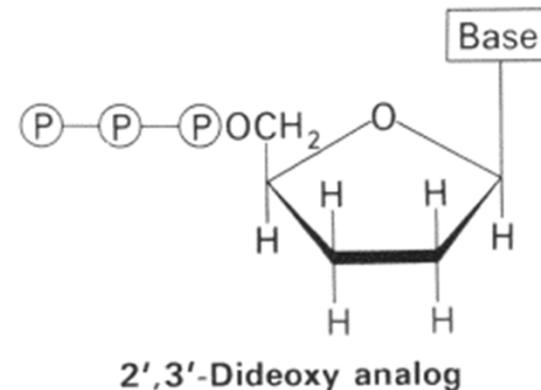
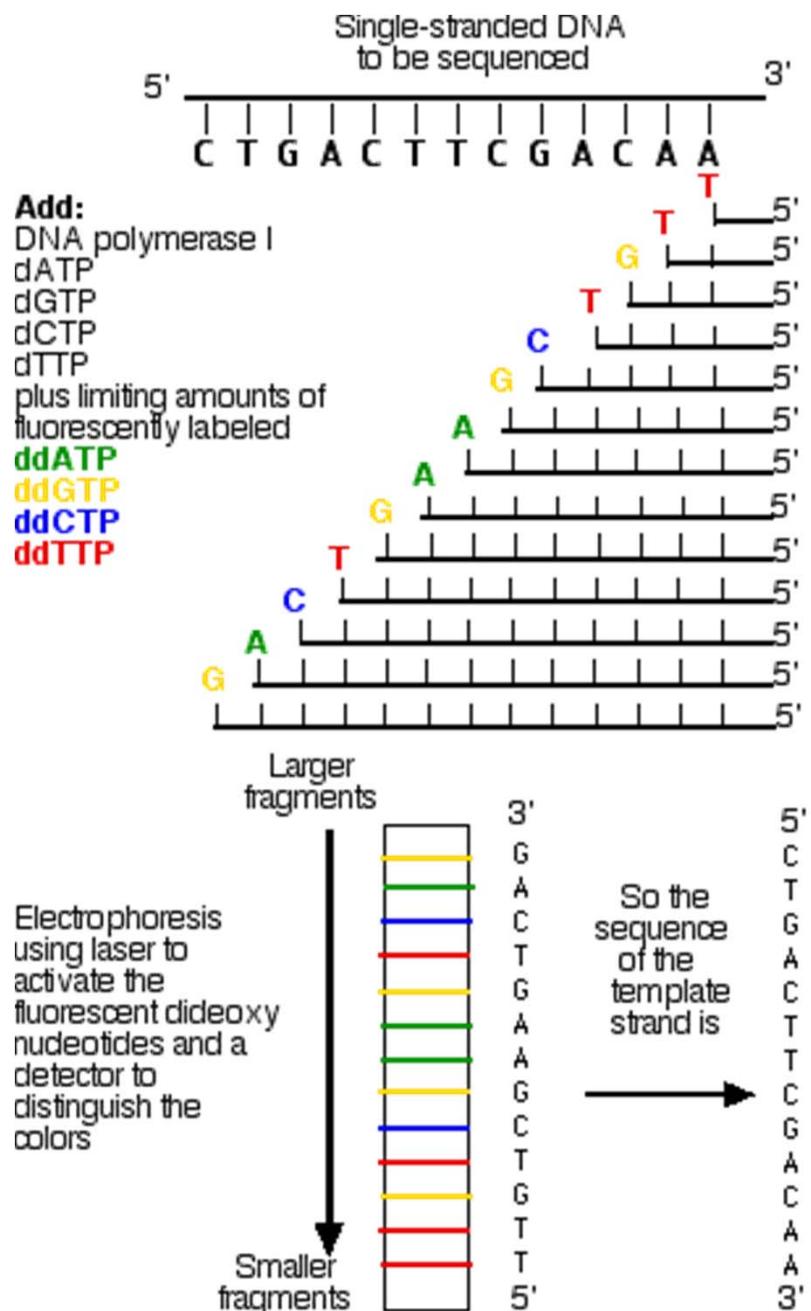
↓ dNTP

ddATP ddTTP ddCTP ddGTP

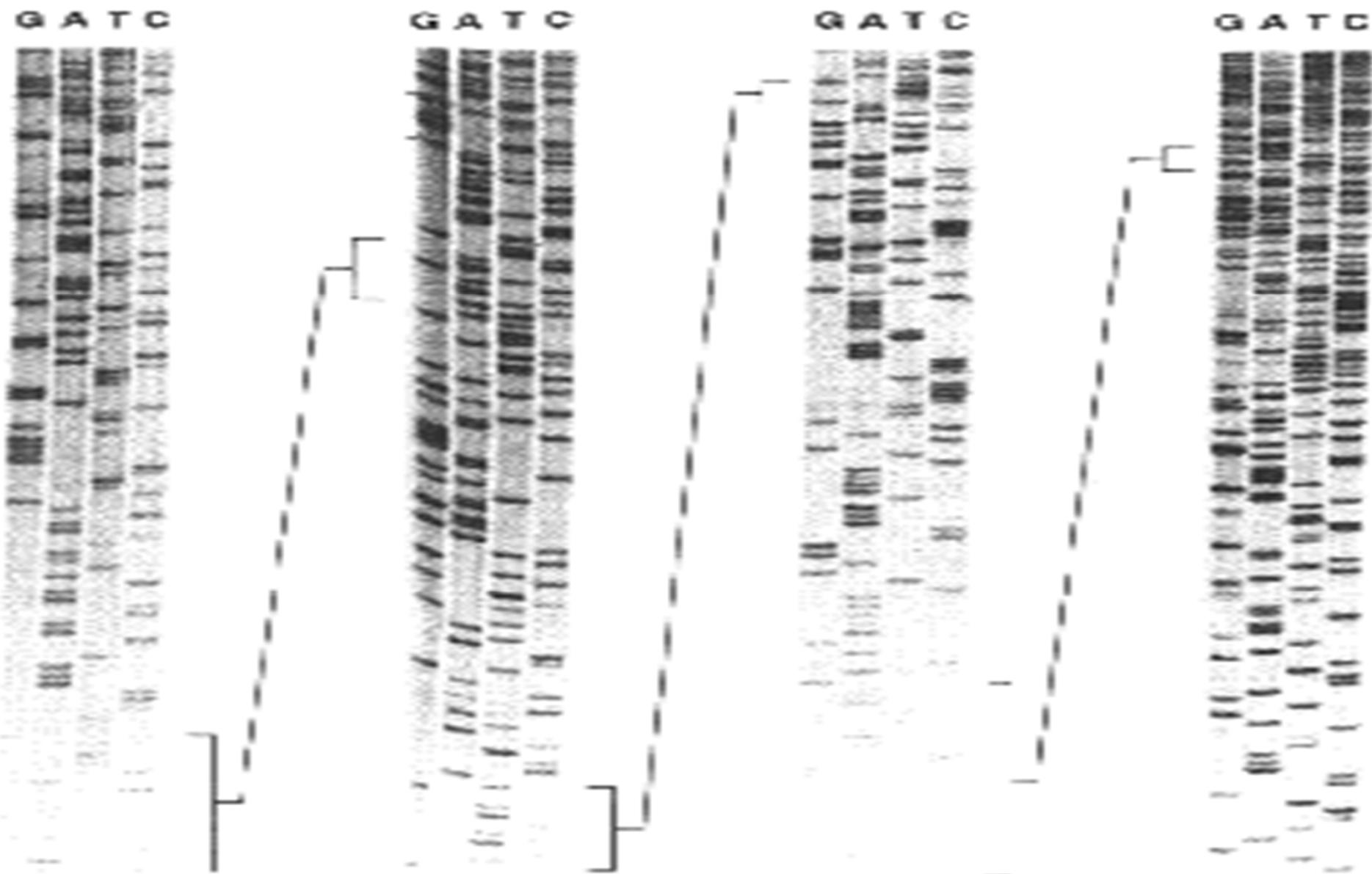
— GGCA — GGCAT — GGC — G  
— GGC — GGCATG

A T C G

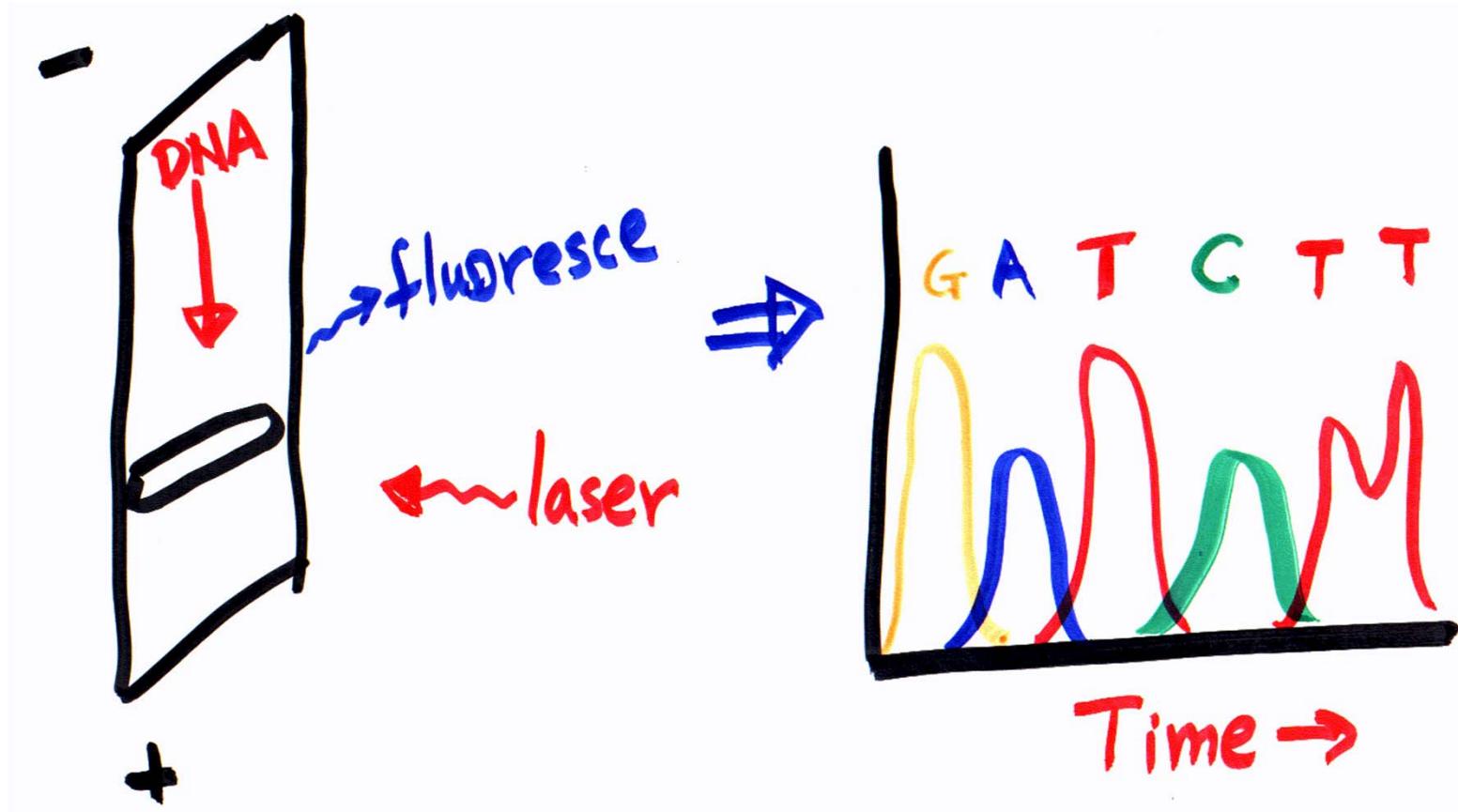




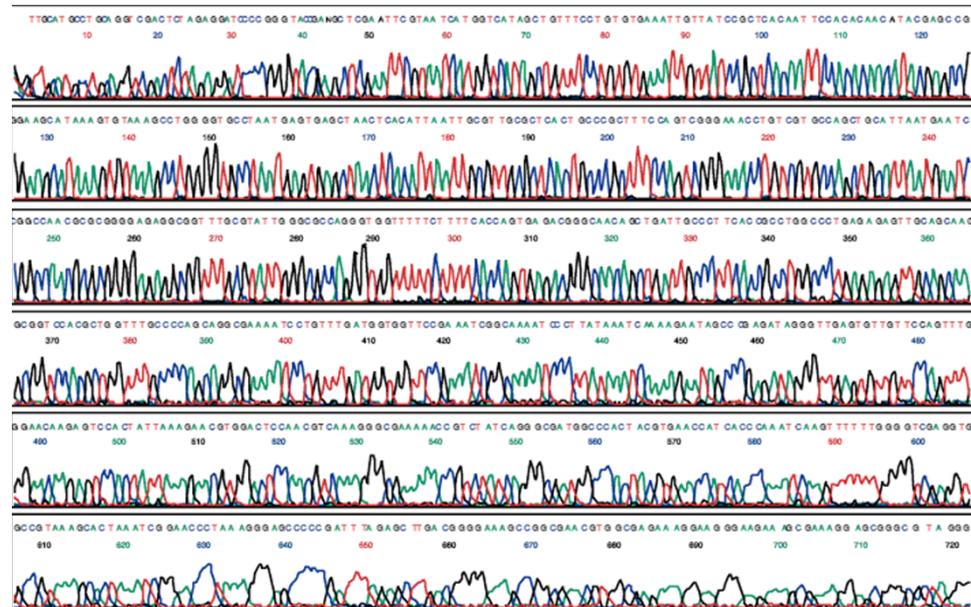
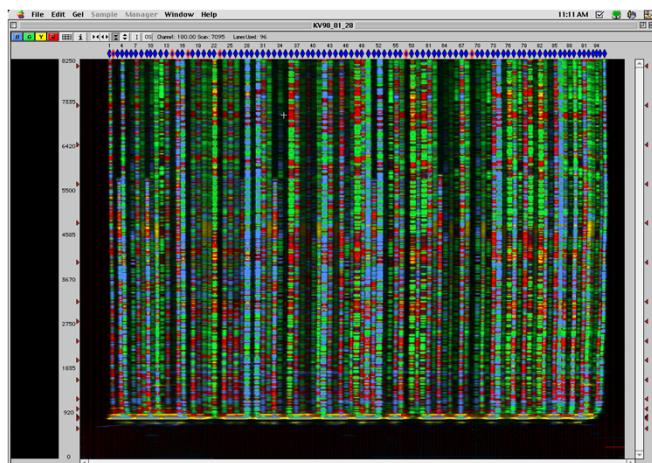
# Sanger Method Sequencing Gel



# Electrophoresis



# Sample Output

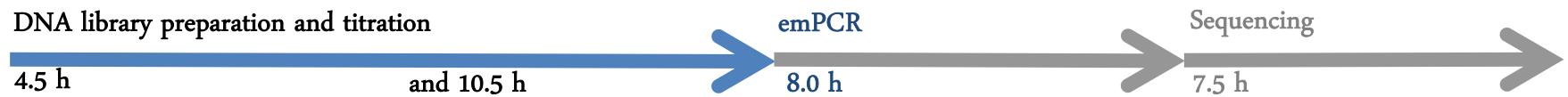
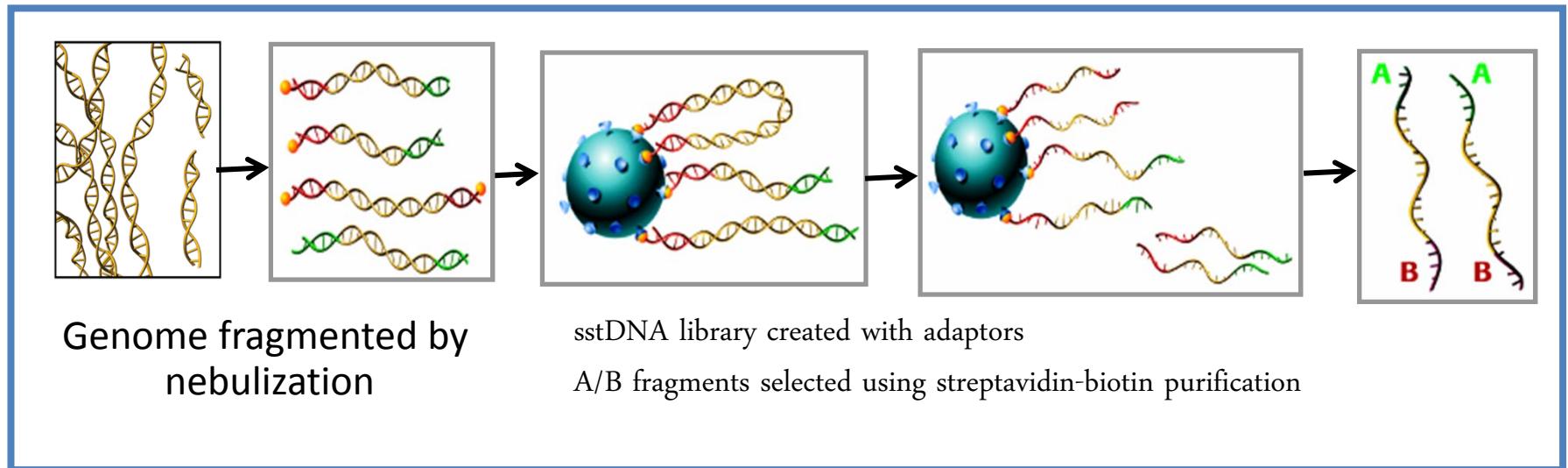


1 lane

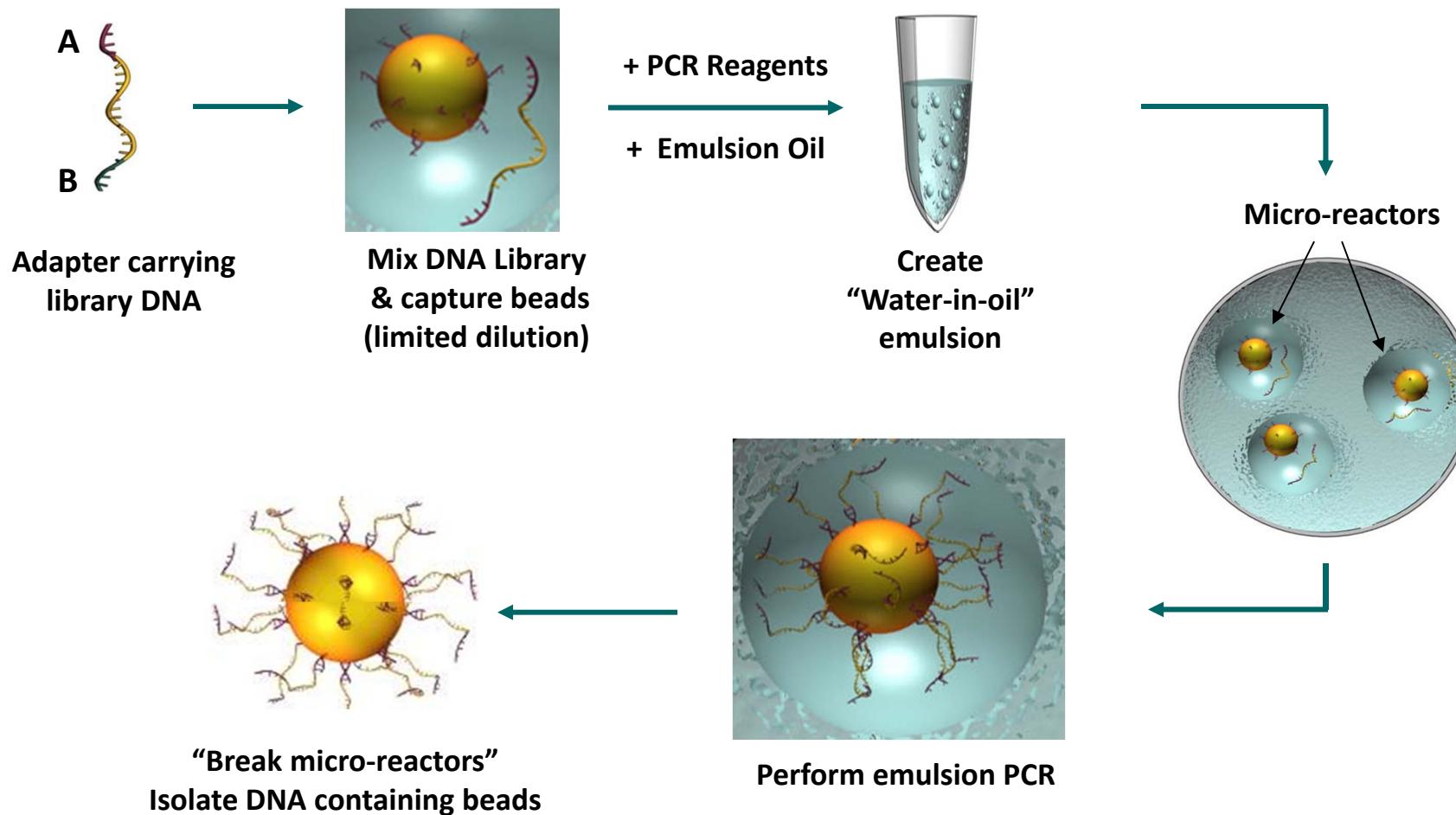
# 454 Pyrosequencing System

# Sequencing Workflow

## *Library Preparation*

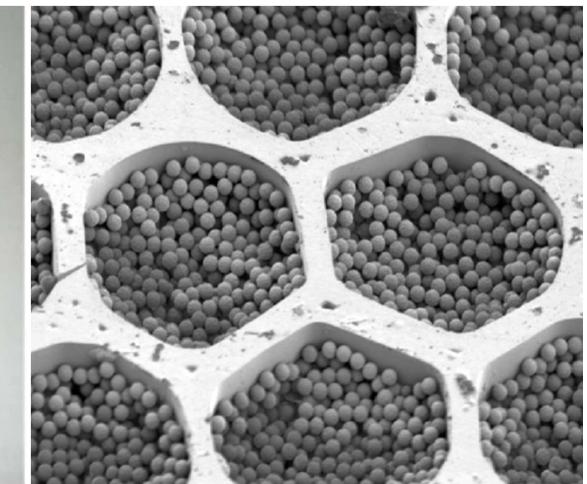
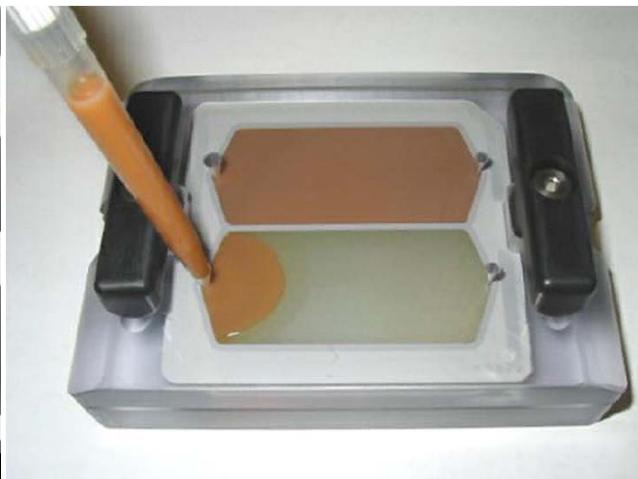
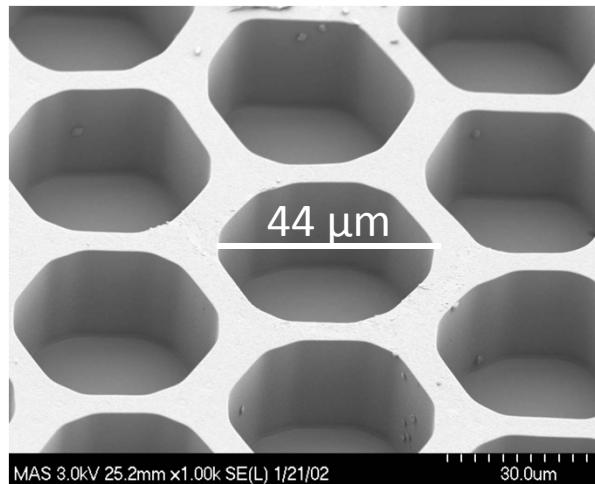
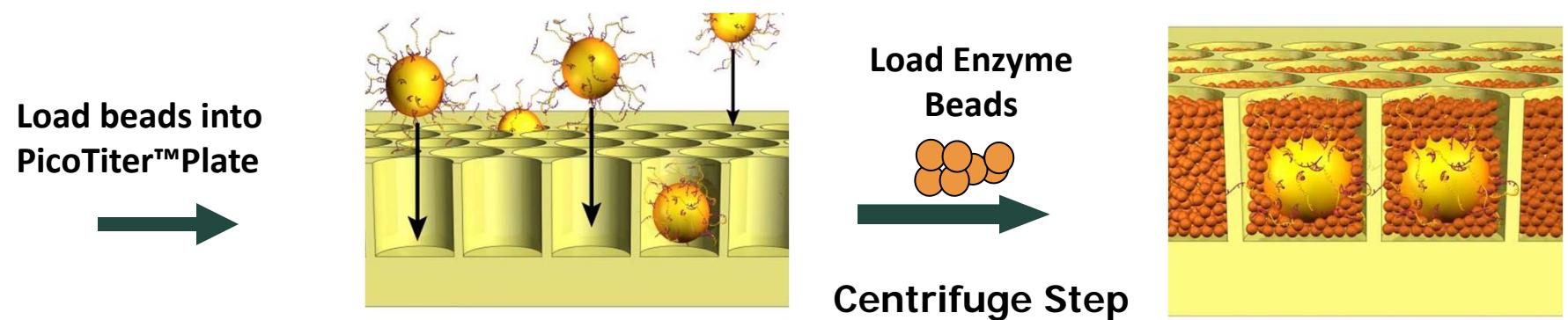


# Emulsion Based Clonal Amplification



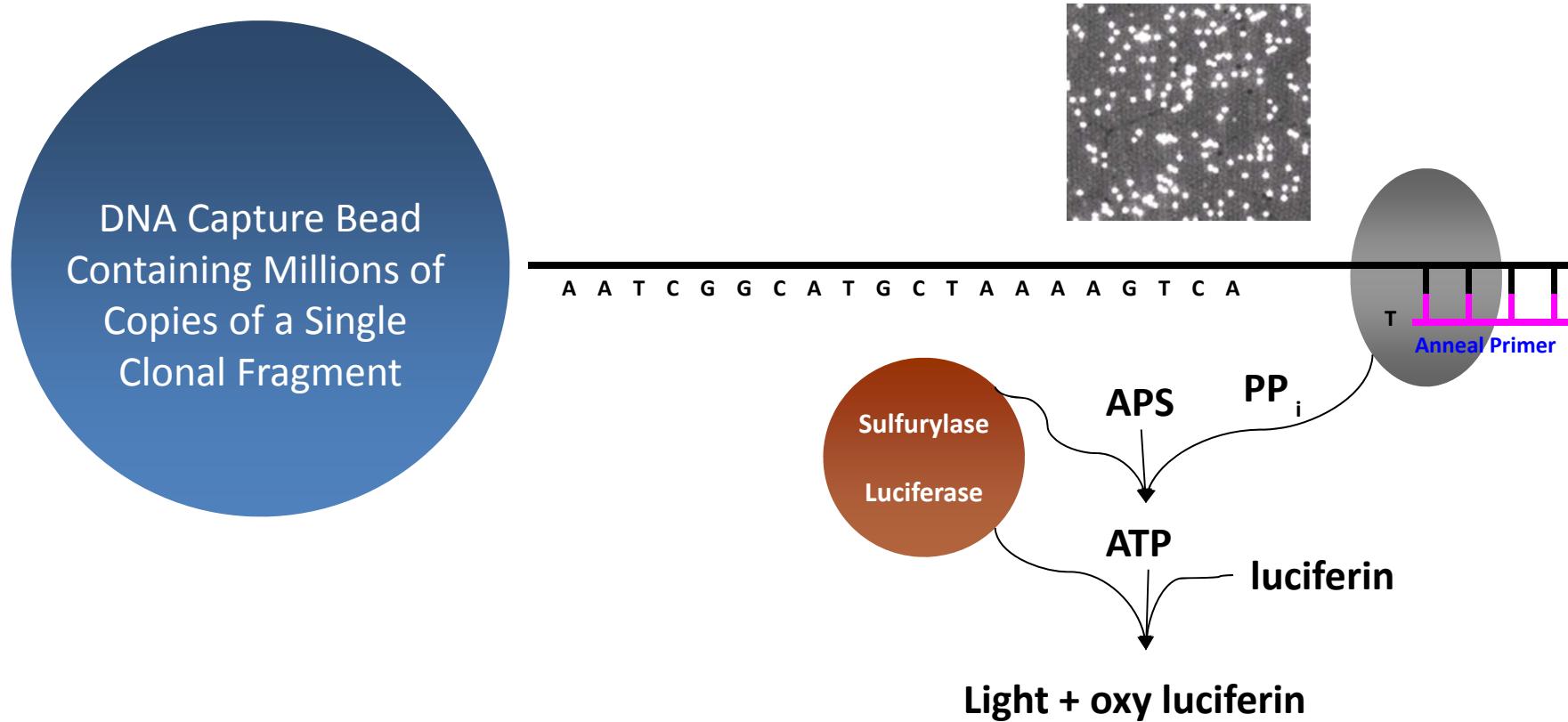
- Generation of millions of clonally amplified sequencing templates on each bead
- No cloning and colony picking

# Depositing DNA Beads into the PicoTiter™Plate



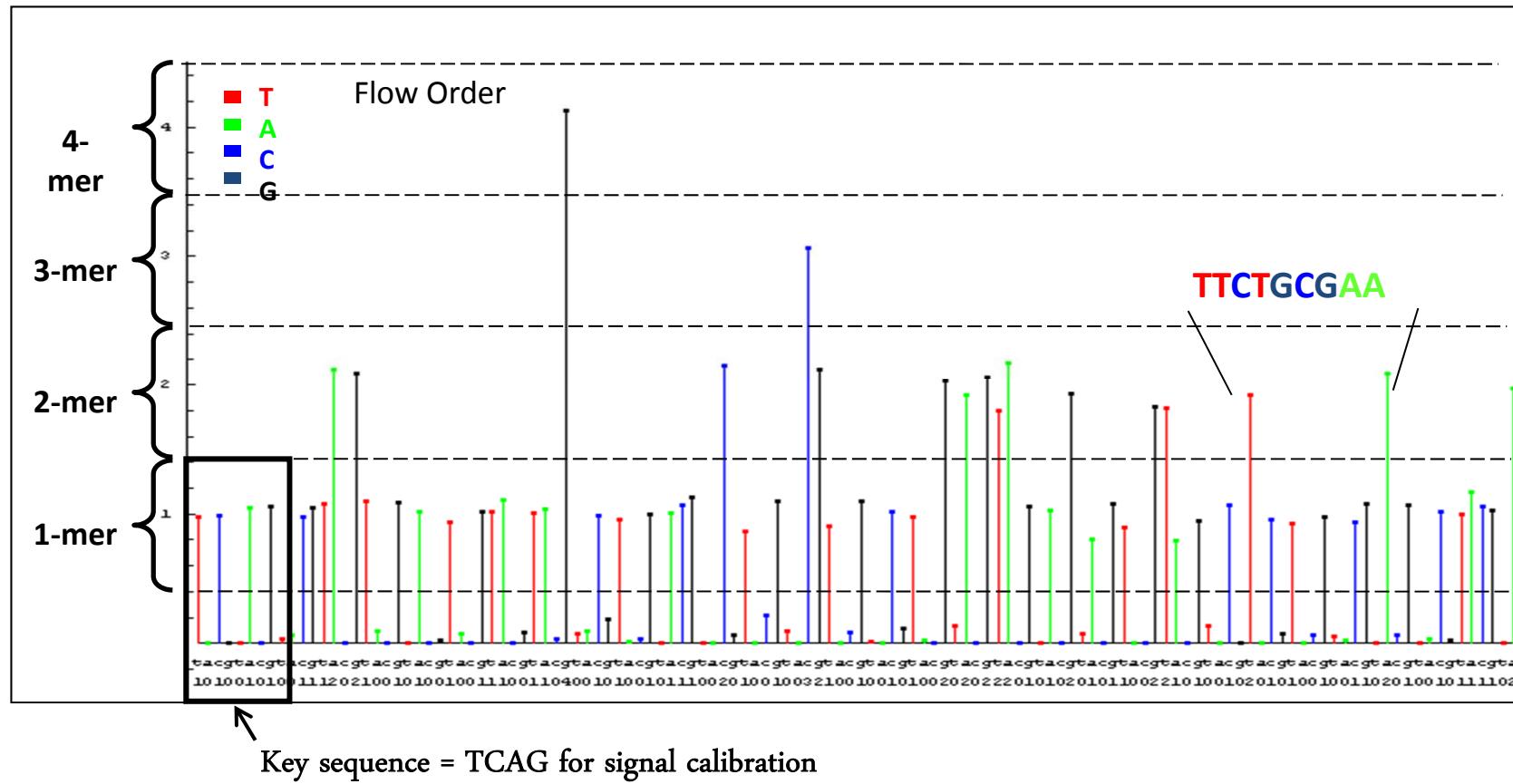
# Sequencing-By-Synthesis

- Simultaneous sequencing of the entire genome in hundreds of thousands of picoliter-size wells
- Pyrophosphate signal generation



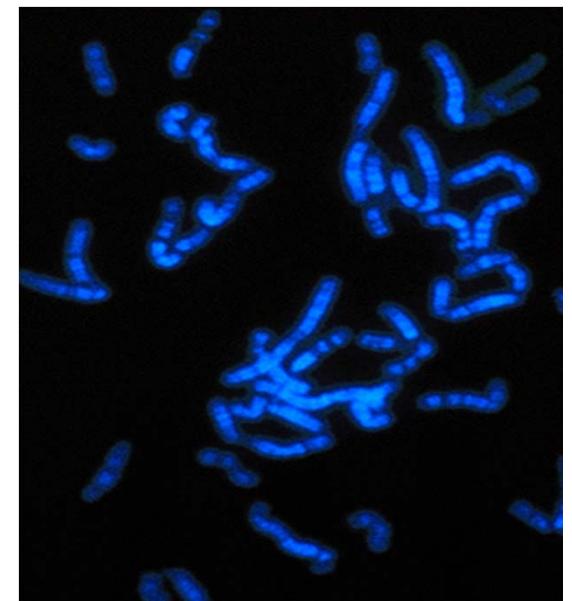
# GS FLX Data Analysis

## *Flowgram Generation*

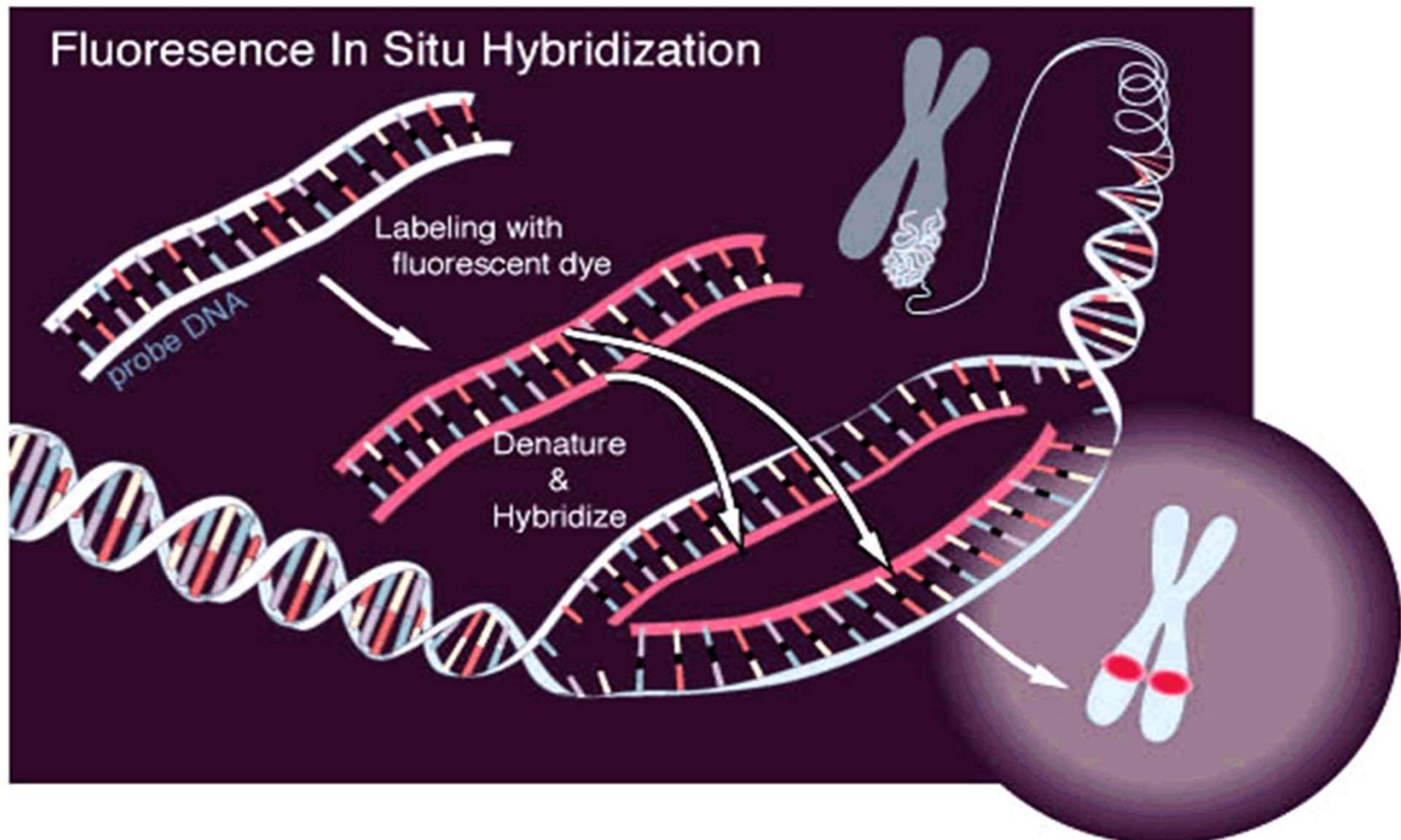


# Chromosome Location and Copy Number

- Identify the chromosome location of the cloned gene
- Determine if the gene is present as a single copy in the genome
- **Fluorescence *in situ* hybridization (FISH)**
- A process which vividly paints chromosomes or portions of chromosomes with fluorescent molecules
  - Chromosomes are isolated from cells and spread out on glass slide
  - cDNA probe for gene of interest is labeled with fluorescent nucleotides and incubated with slides
  - Probe will hybridize with complementary sequences on slide
  - Slide is washed and exposed to fluorescent light
  - Wherever probe bound, it is illuminated to indicate the presence of that gene



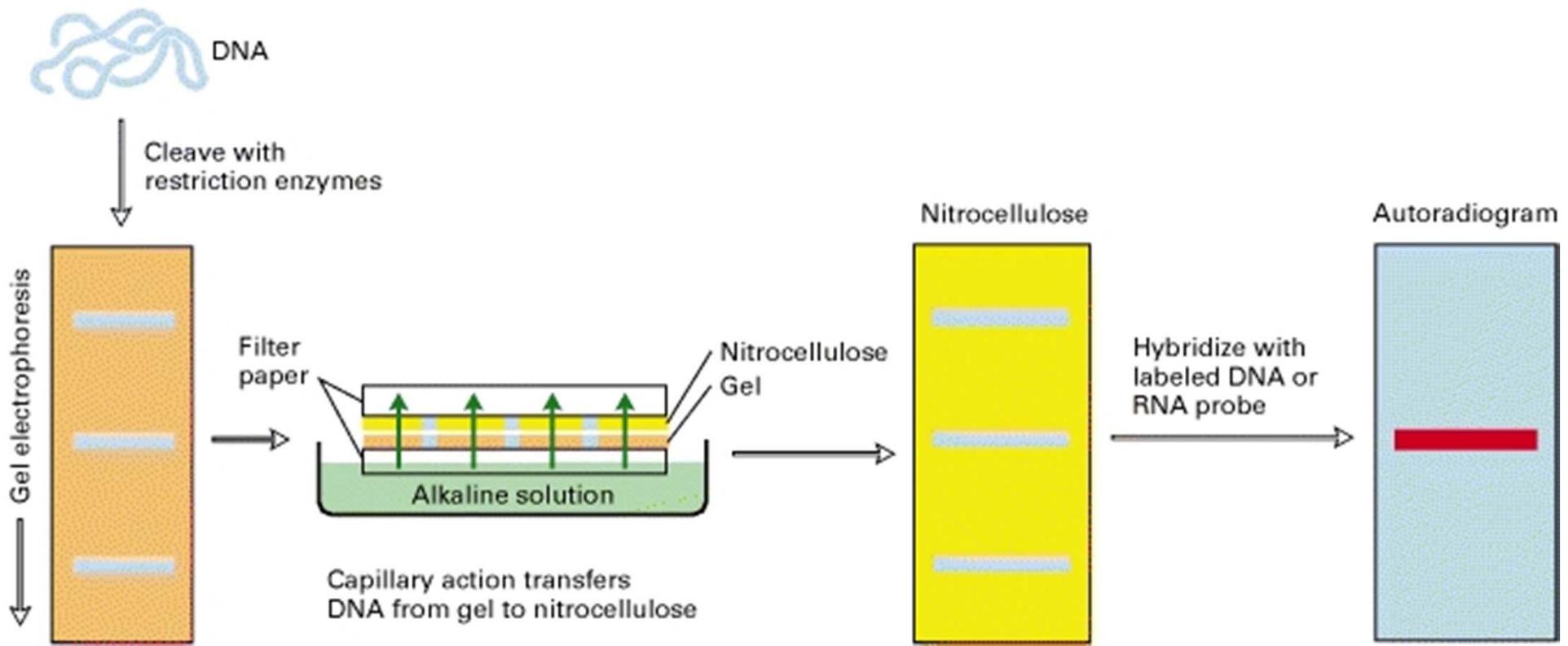
# FISH Procedure



# Chromosome Location and Copy Number

## Southern Blotting

- Digest chromosomal DNA into small fragments with restriction enzymes
- Fragments are separated by agarose gel electrophoresis
- Gel is treated with alkaline solution to denature the DNA
- Fragments are transferred onto a nylon or nitrocellulose filter (called blotting)
- Filter (blot) is incubated with a probe and exposed to film by autoradiography
- Number of bands on film represents gene copy number



**The Southern blot technique for detecting the presence of specific DNA sequences following gel electrophoresis of a complex mixture of restriction fragments.**

# Studying Gene Expression

- Techniques involve analyzing mRNA produced by a tissue
- **Northern blot analysis**
  - Basic method is similar to Southern blotting
  - RNA is isolated from a tissue of interest, separated by gel electrophoresis, blotted onto a membrane, and hybridized to a probe
- **Reverse transcription PCR**
  - Reverse transcription of mRNA is performed – converted into double-stranded cDNA
  - cDNA is then amplified with a set of primers specific for the gene of interest
  - Products electrophoresed on agarose gel

# Studying Gene Expression

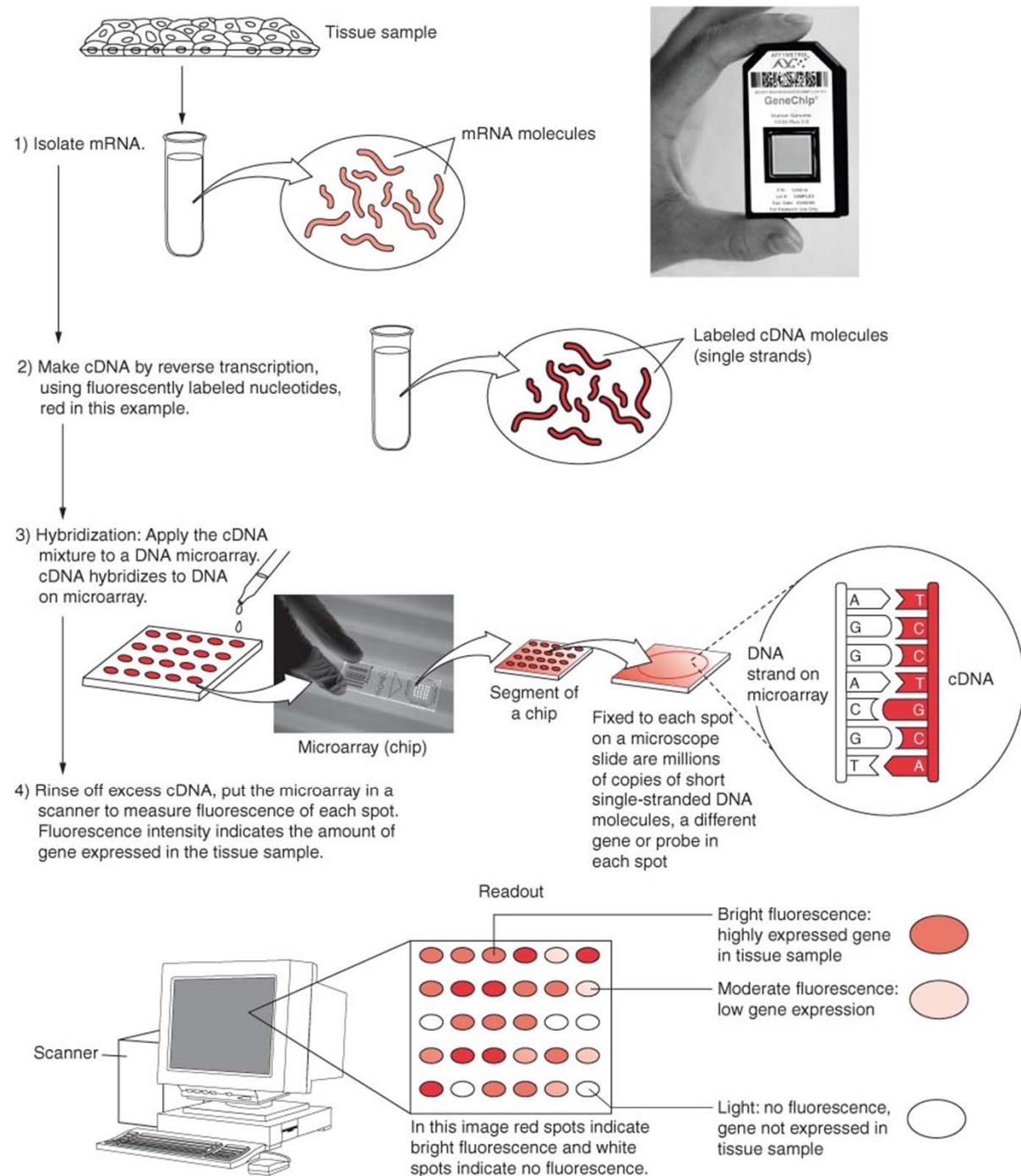
- *In situ* hybridization
  - Used to determine the cell type that is expressing the mRNA
  - Tissue of interest is preserved in a fixative solution and embedded in a wax-like substance
  - Tissue can be sliced into very thin sections attached to microscope slides
  - Slides are incubated with a probe to the gene of interest
  - Probe hybridizes with mRNA in cells
  - Probe is detected

# **Studying Gene Expression**

## **– Gene microarrays**

- DNA microarray analysis
- Single-stranded DNA molecules are attached onto a slide using a robotic arrayer fitted with tiny pins
- Can have over 10,000 spots of DNA
- Extract mRNA from tissue of interest, tag it with fluorescent dye, and incubate overnight with the slide
- mRNA will hybridize to spots on the microarray that have complimentary DNA sequences
- Slide is scanned with a laser that causes the spots to fluoresce

# Gene Microarrays



# *in situ* hybridization vs microarray

- *in situ* hybridization measures the expression and preserve the spatial information for a single gene.
- microarray extracts all mRNAs out of tissues and measure thousands of genes at the same time.

