Bootcamp: Molecular Biology Techniques and Interpretation

Bi8

Winter 2016

Today's outline

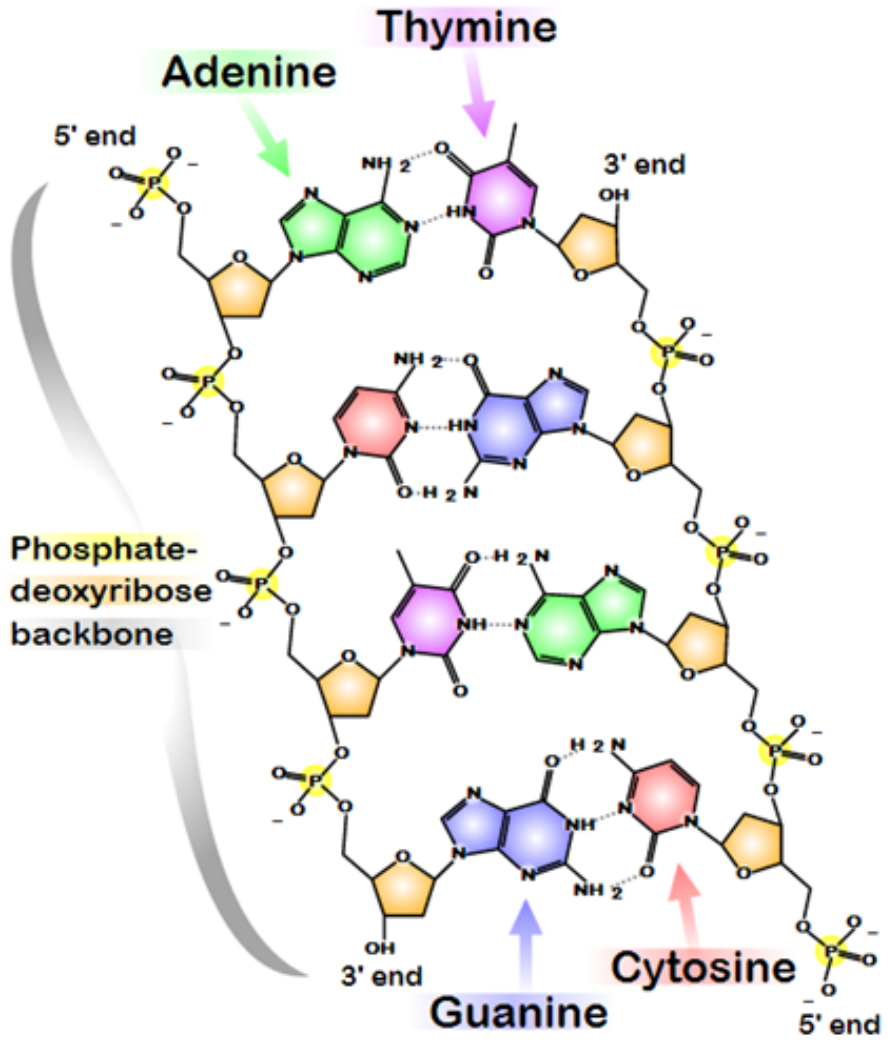
Detecting and quantifying nucleic acids and proteins:

- Basic nucleic acid properties
- Hybridization
- PCR and Designing Primers
- qPCR
- Sequencing
- Gel electrophoresis
- Blotting
- Designing Probes
- In situ hybridization (ISH)
- Fluorescent ISH (FISH)

Manipulating DNA:

- Cloning
- Enzyme toolkit
- cDNA Libraries
- Reporter genes
- Databases

Effect of salt, temperature and pH on nucleic acid duplex formation



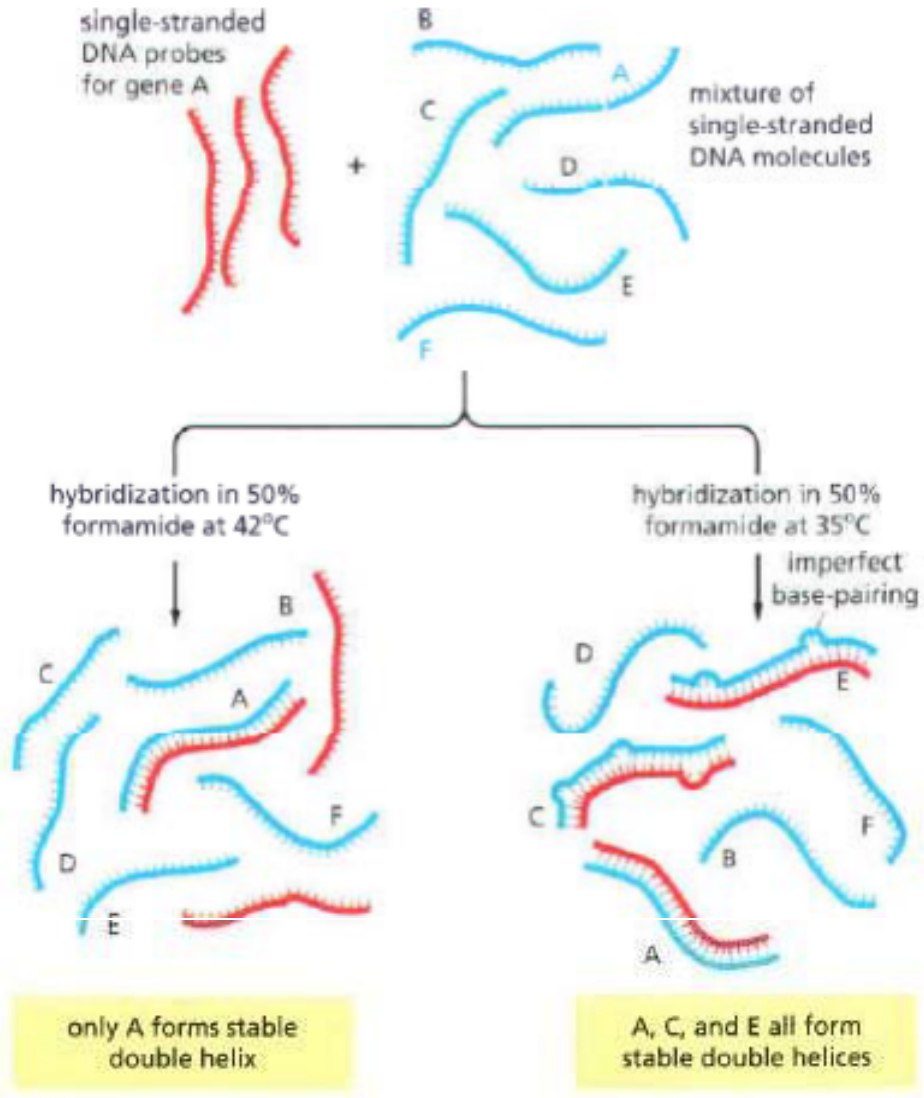
Equilibrium of interactions
hydrogen-bonding, pi-pi interactions vs. charge repulsion

Salt
More salt screens backbone charges decreasing strand repulsion

Temperature
Higher temperature causes separation of the DNA strands or 'melting'

pH
Alkaline and acidic pH can both denature DNA

Hybridization



Higher temperature requires higher degree of complementarity

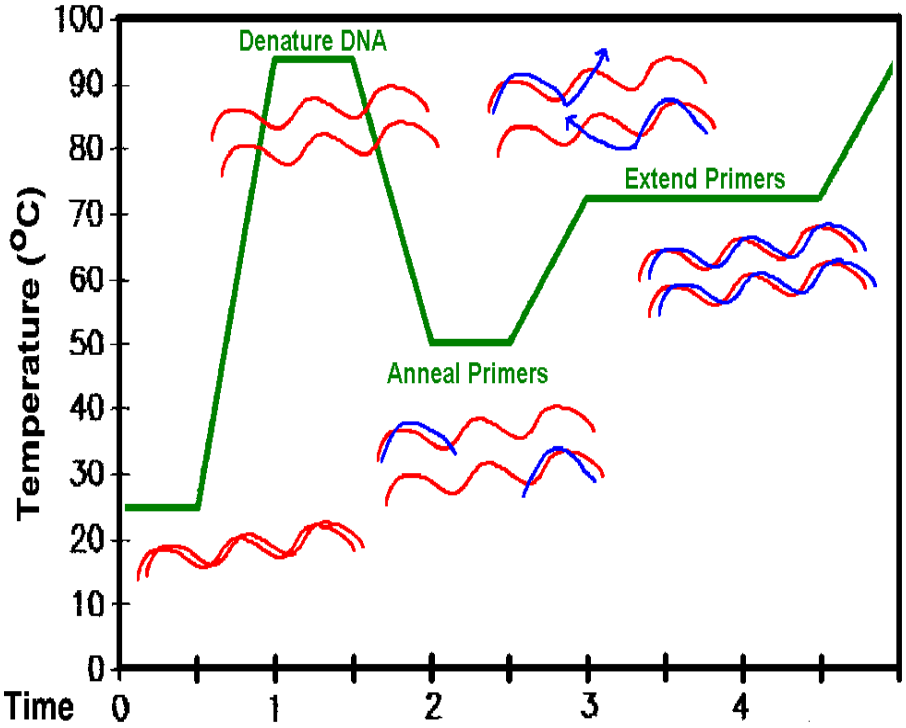
Alberts et al., Molecular Biology of the Cell

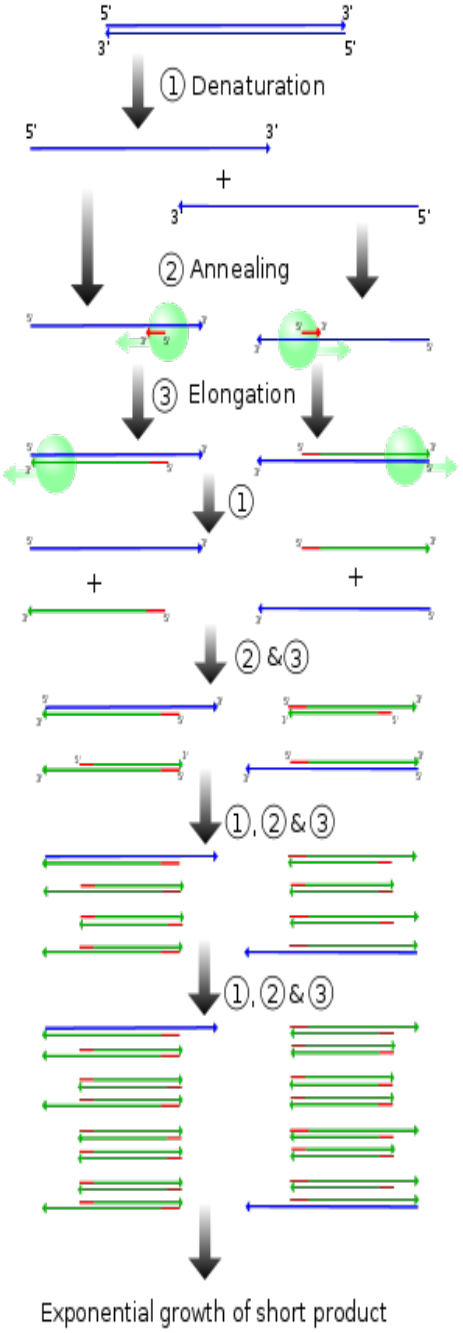
Polymerase Chain Reaction (PCR) amplifies DNA

Reaction mixture contains:

DNA Polymerase
2 Primers
dNTP (DNA nucleotides)
$MgCl_2$
Double stranded DNA Template

PCR reactions are performed on a programmable thermocycling heat block



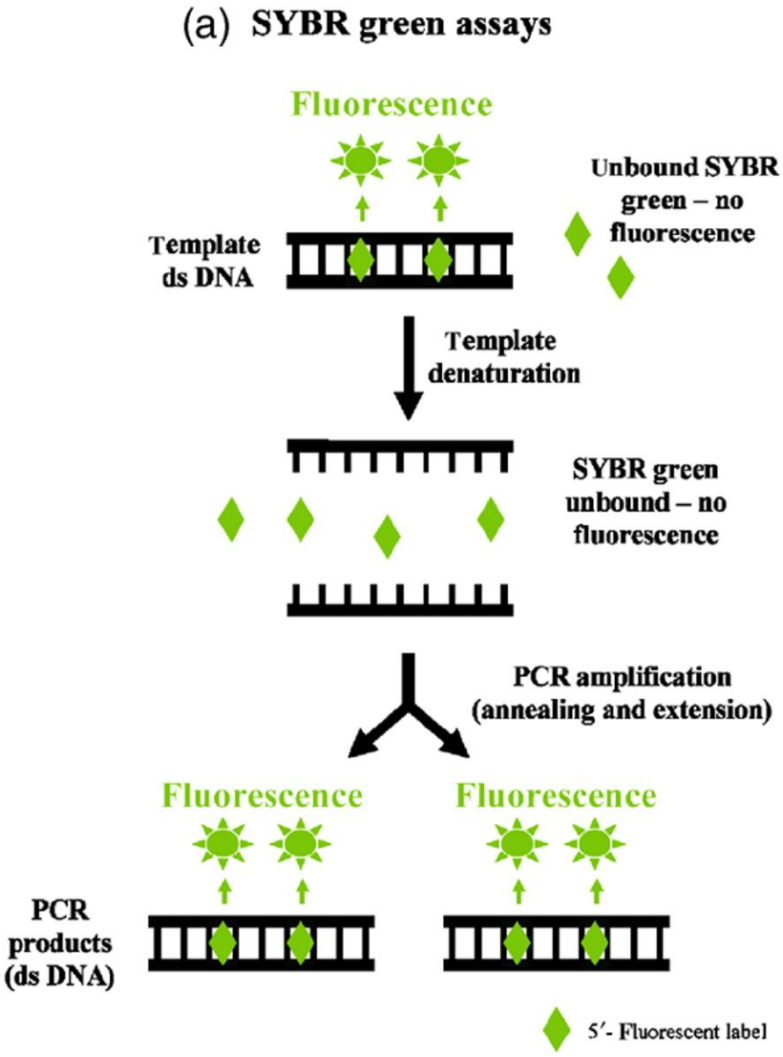


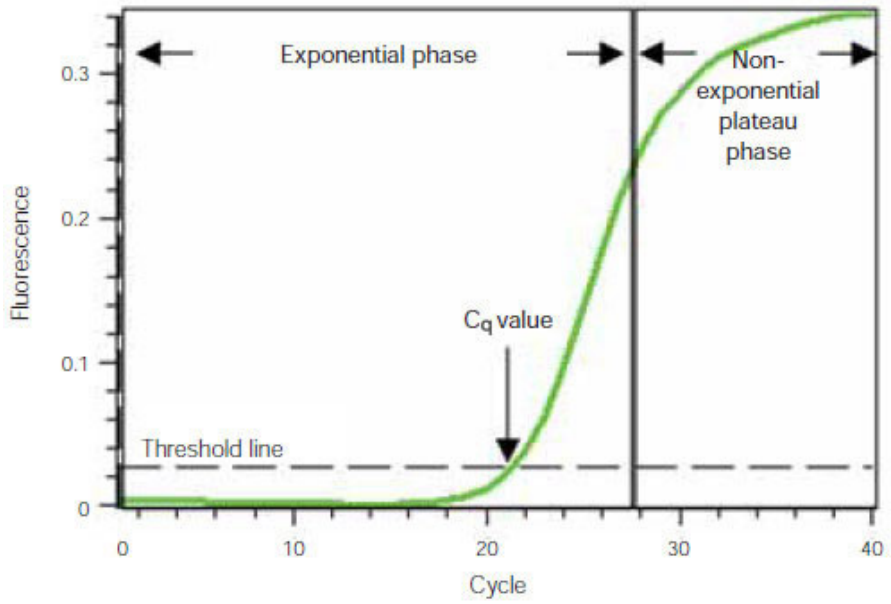
How to choose/design a PCR primer

PCR primers should be unique to the region you intend to amplify

- –Length (optimal length 18-22 bp)
- –Sequence complementarity to the ends of your target DNA
- –Melting temperature (Tm) should be similar between the two primers (~55-60°C)
- –Primers shouldn't form secondary structure

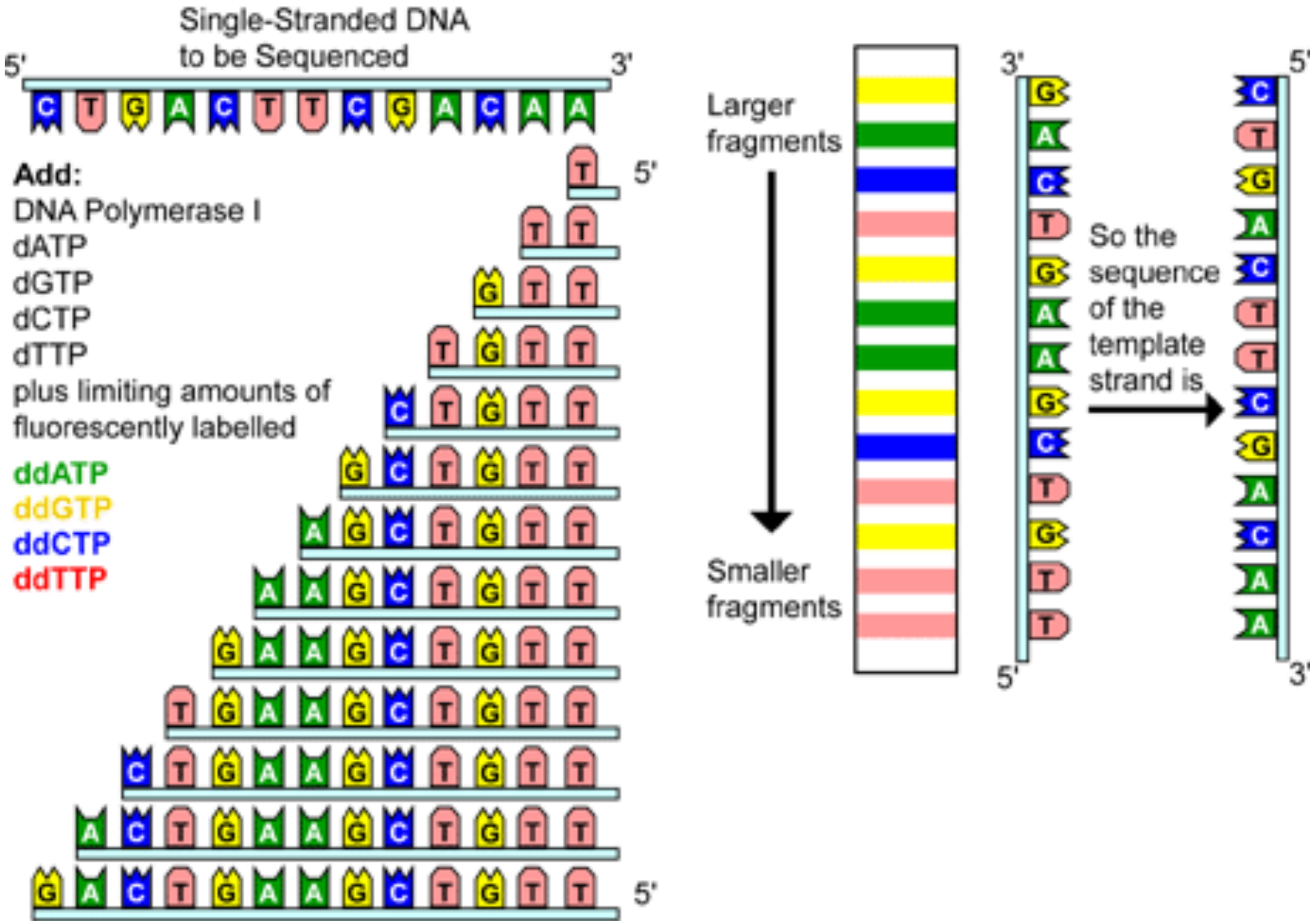
Quantitative PCR (qPCR) enables quantification of DNA/cDNA





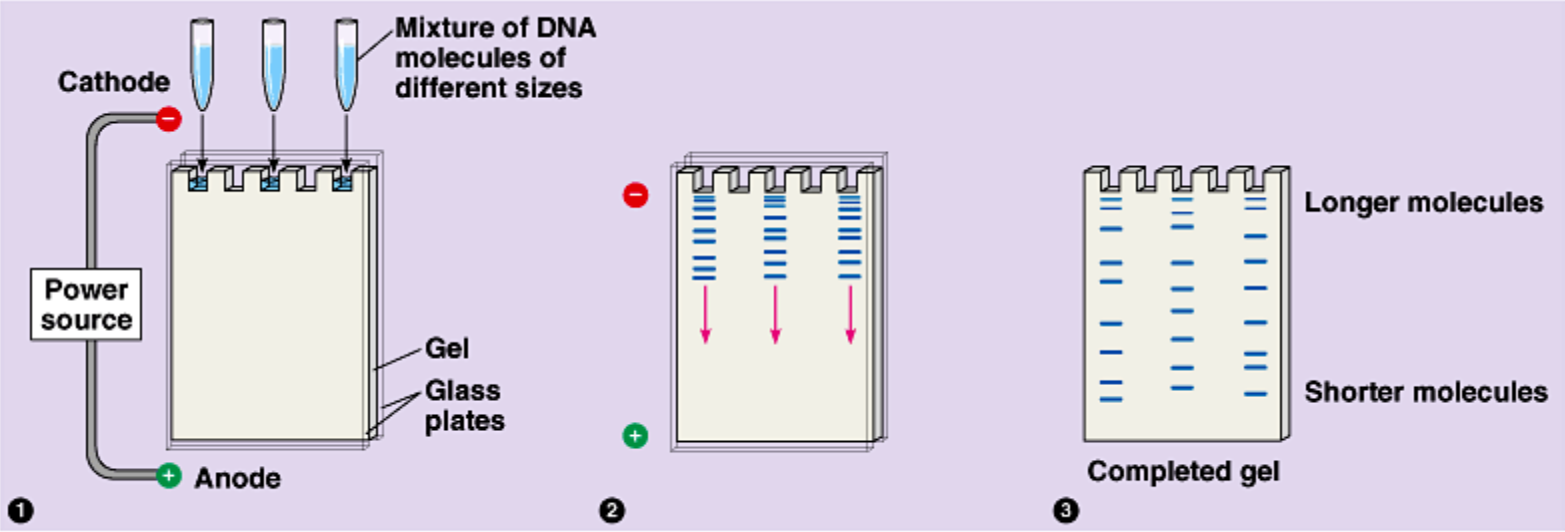
SYBR Green specifically binds double-stranded DNA by intercalating between base pairs and fluoresces only when bound to dsDNA.

How to sequence DNA



http://www.daviddarling.info/encyclopedia/D/DNA_sequencing.html

Gel Electrophoresis: separation of nucleic acids or proteins based on molecular weight



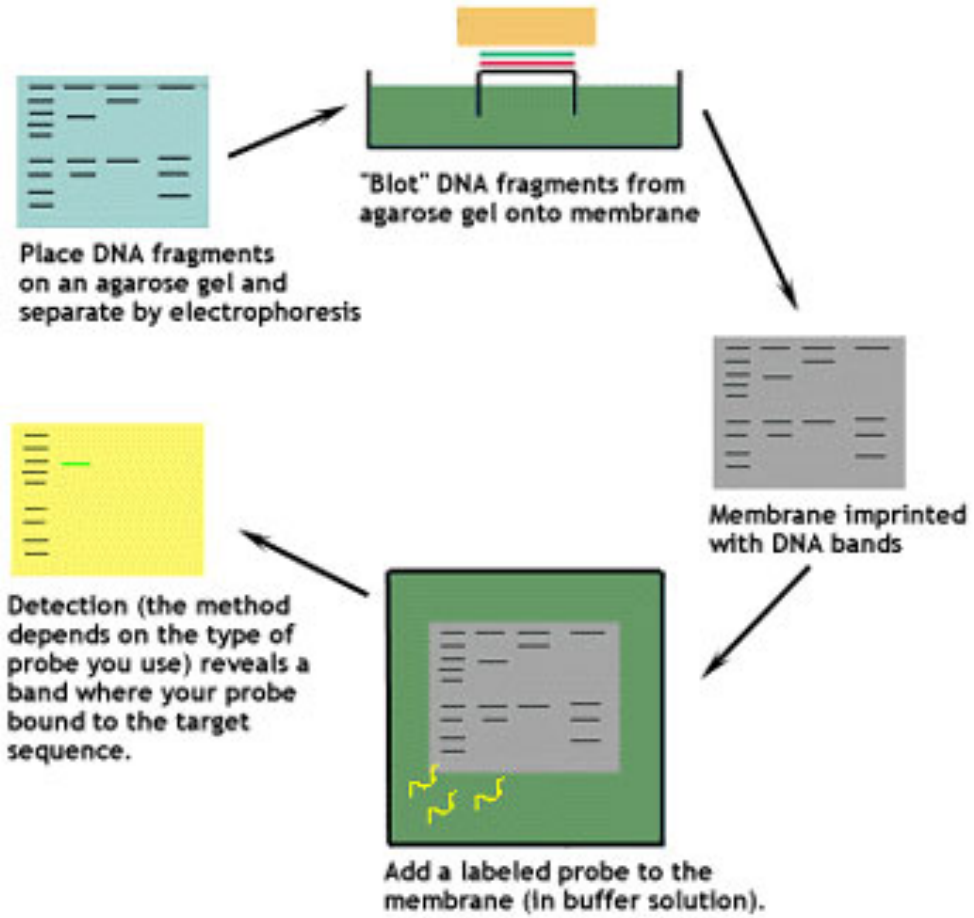
DNA Agarose Gel

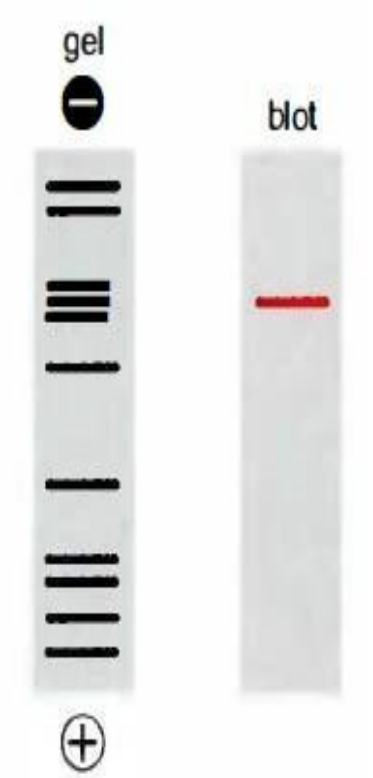
- DNA is negatively charged, so it will be pulled towards the anode

Protein SDS-PAGE

- Proteins must first be denatured and coated with SDS (to make it negatively charged) prior to being run a gel

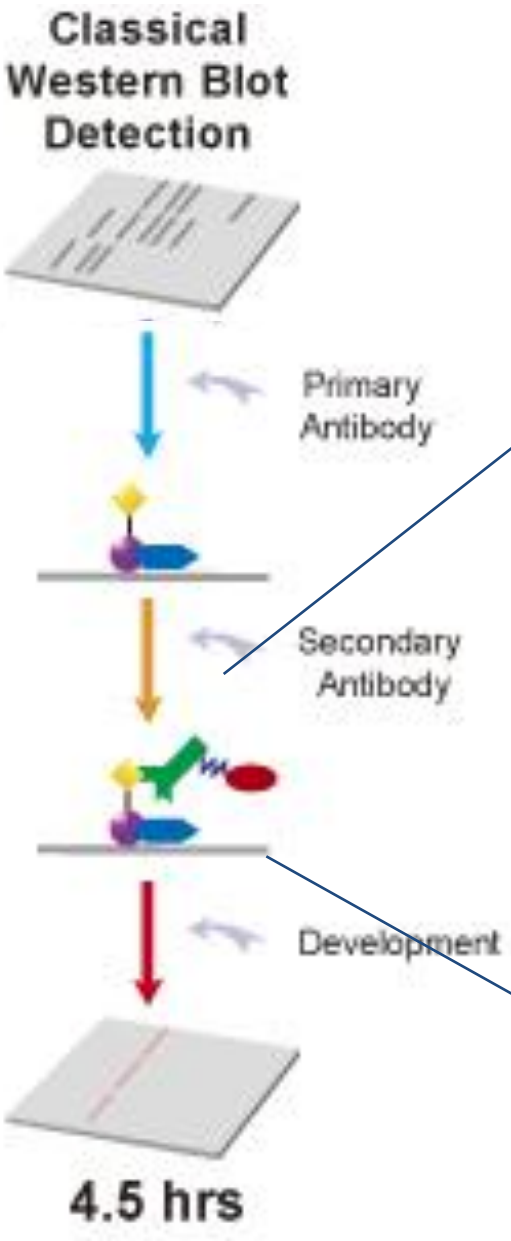
Blotting: Detecting presence of specific DNA/RNA sequences using probes





Southern = DNA **Northern = RNA**

Western blotting: Detecting the presence of specific proteins



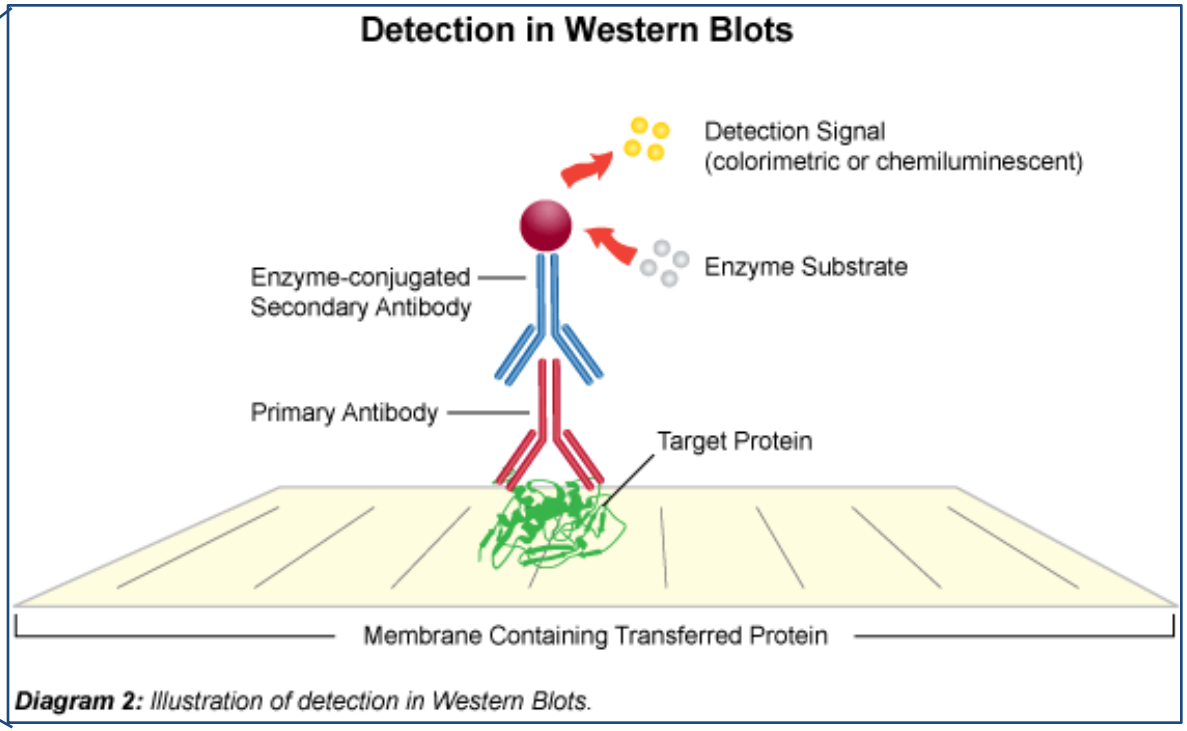


Diagram 2: *Illustration of detection in Western Blots.*

In situ hybridization detects nucleic acid localization in cells

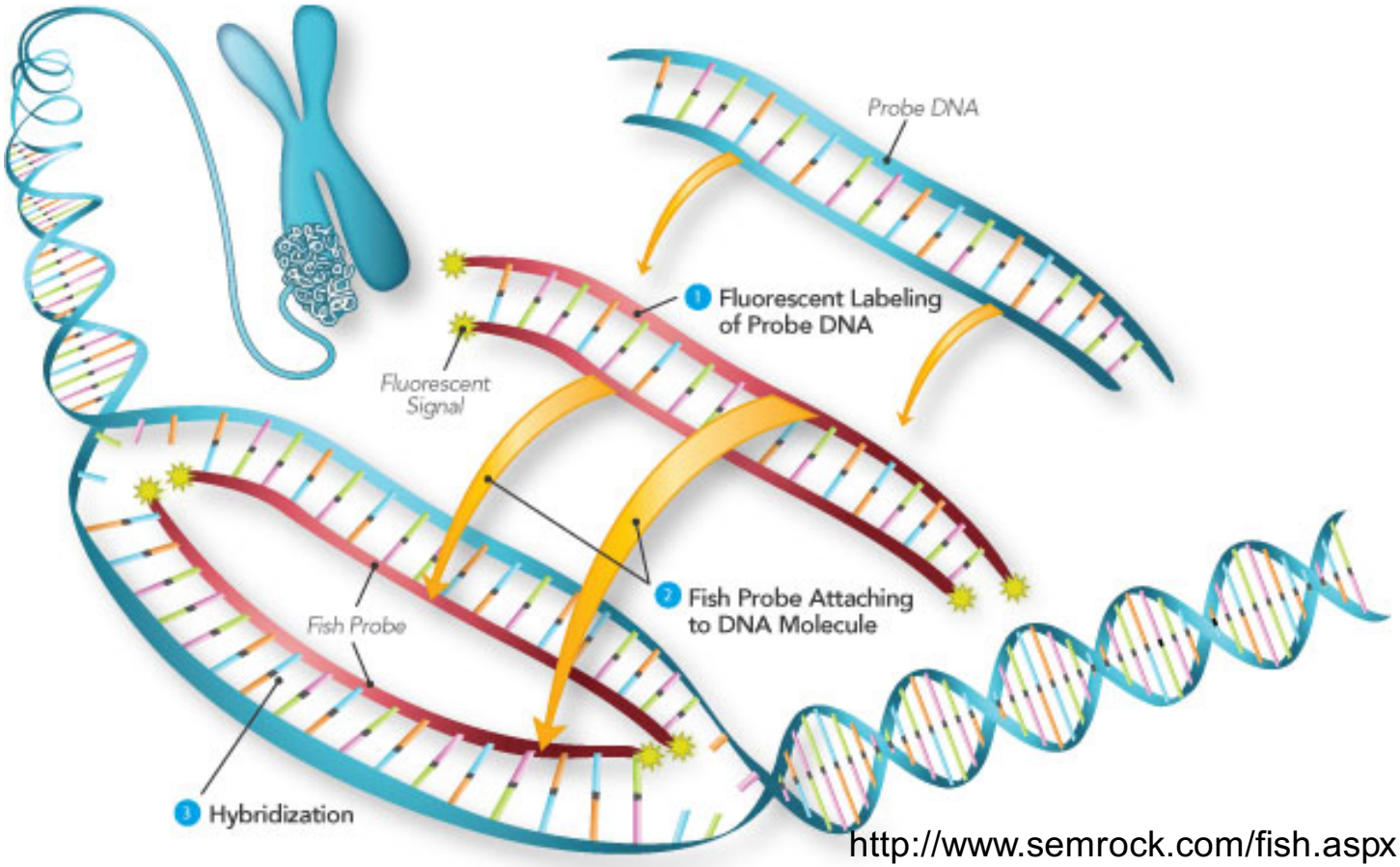
Can detect DNA or RNA

2 key features of probe design

- **Length**
- **Complementarity**

- **How specific do you want your probe to be?** ***Do you want to pick up highly similar sequences? Or more dissimilar sequences?***

Fluorescence *in situ* hybridization (FISH)



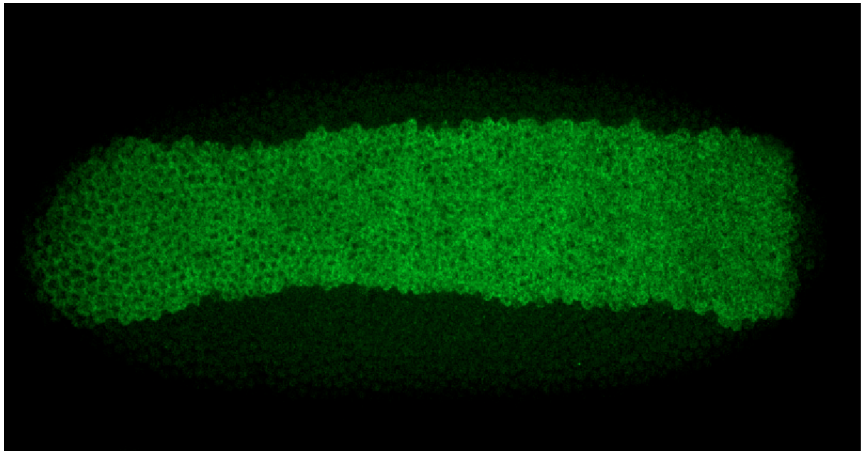
http://www.semrock.com/fish.aspx

Single or double stranded probes may be used.
Definitely use single stranded probes when targeting RNA.
Fluorescent probes are the most commonly used now, but older methods have different modes of detection (this method is known just as ISH)

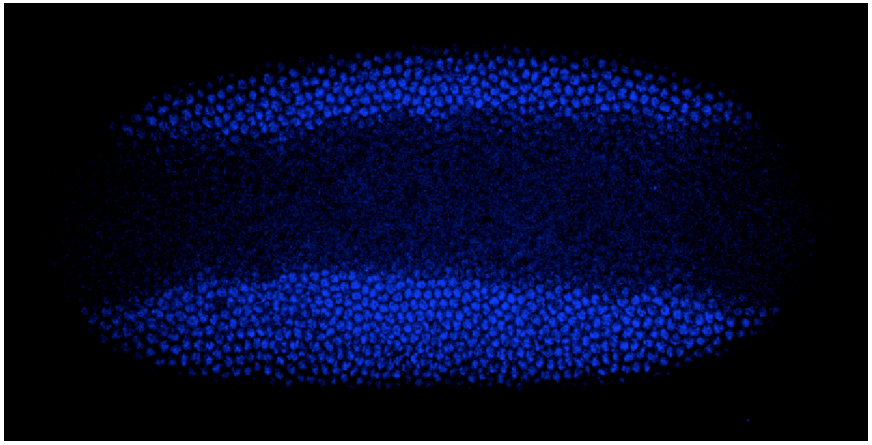
Fluorescence *in situ* hybridization (FISH)

Profiling expression of 2 different RNAs in *Drosophila* embryo

sna



sog



Cloning

- **What is Cloning?**
  - Process of modifying & introducing foreign DNA into a host cell
  - Recombinant DNA = modified DNA
  - Vector = circular piece of DNA which transports a gene into a host cell
  - Host cell containing inserted gene = clone

- **Why do Cloning?**
  - Molecular analysis of genes and their function depends on isolation and amplification of specific segments of DNA:
    - Sequencing
    - Expression of genes, proteins that affect biology pathways
    - Cell manipulation

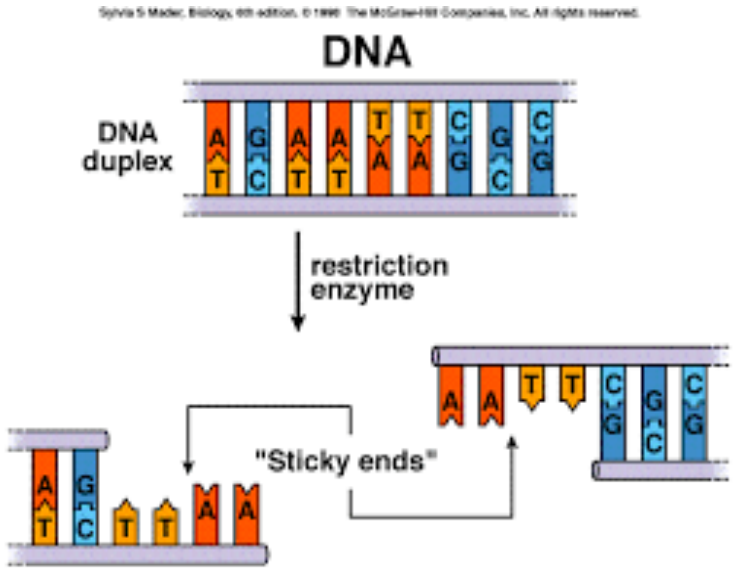
Enzyme toolkit for manipulating DNA

- DNA polymerase: copies a DNA strand from a complementary template

- Restriction enzymes: cut DNA at specific sequence sites
  - Multiple enzymes, each w/ unique recognition sequences

- Ligases: joins DNA sequences together

- "Destruction" Nucleases
  - S1 and DNaseI: degrades DNA
  - RNaseA: degrades RNA

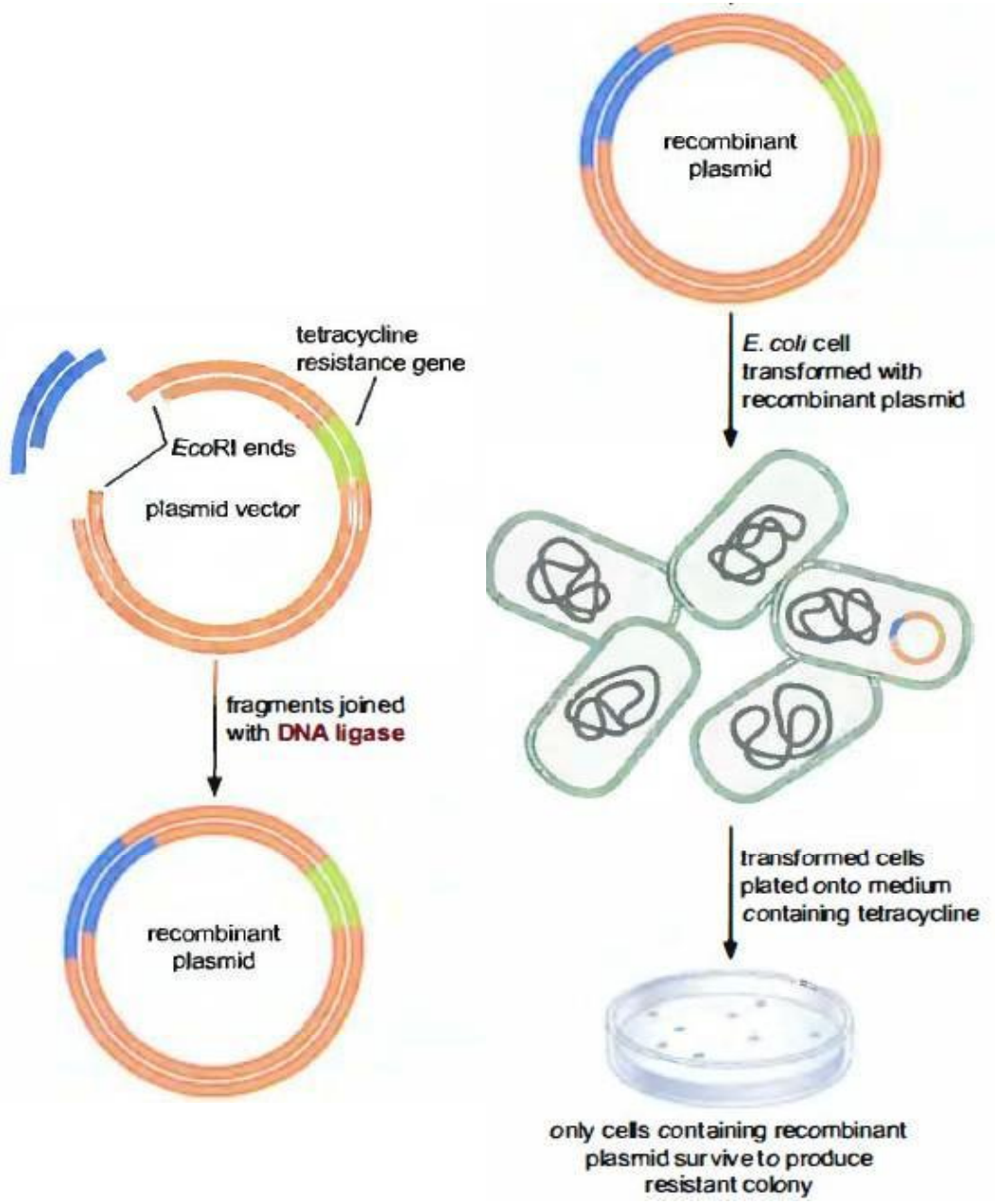


Cloning Procedure

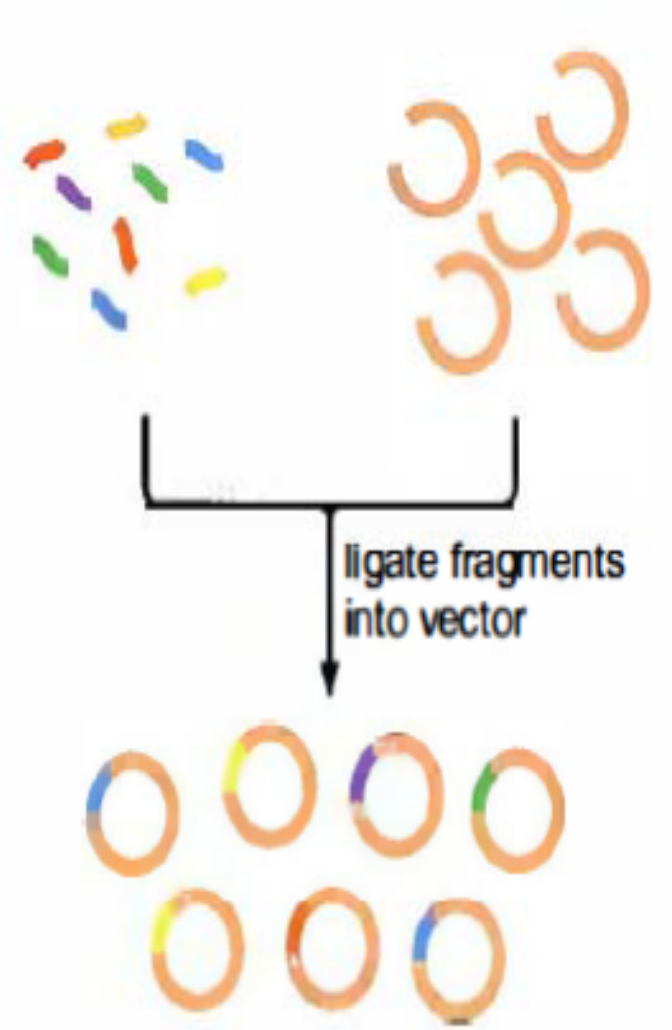
- Insert cleaved DNA fragments into a **plasmid vector**

- Allows replication within host (*E. coli)*
  - manipulate DNA
  - store it
  - generate a library of DNA molecules

- Vectors contain:
  - Origin of replication
  - Selectable marker (e.g. antibiotic resistance)
  - Restriction enzyme sites



Creating Libraries



A **DNA library** is a population of identical *vectors* or linear DNA that each carry a unique insert

Genomic library

- Constructed by ligating fragments of genomic DNA into vectors; each plasmid contains a part of the entire genome

cDNA library

- Constructed by ligating only coding regions into vectors

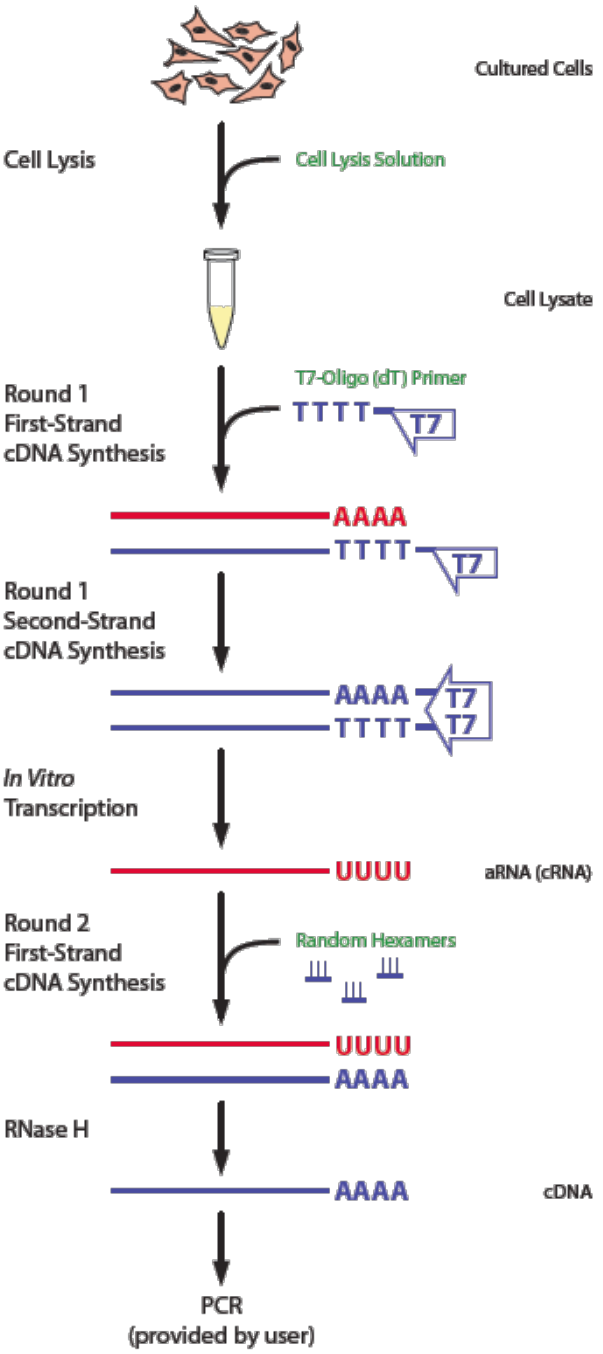
Molecular Biology of the Gene 5th ed

How to make a cDNA library

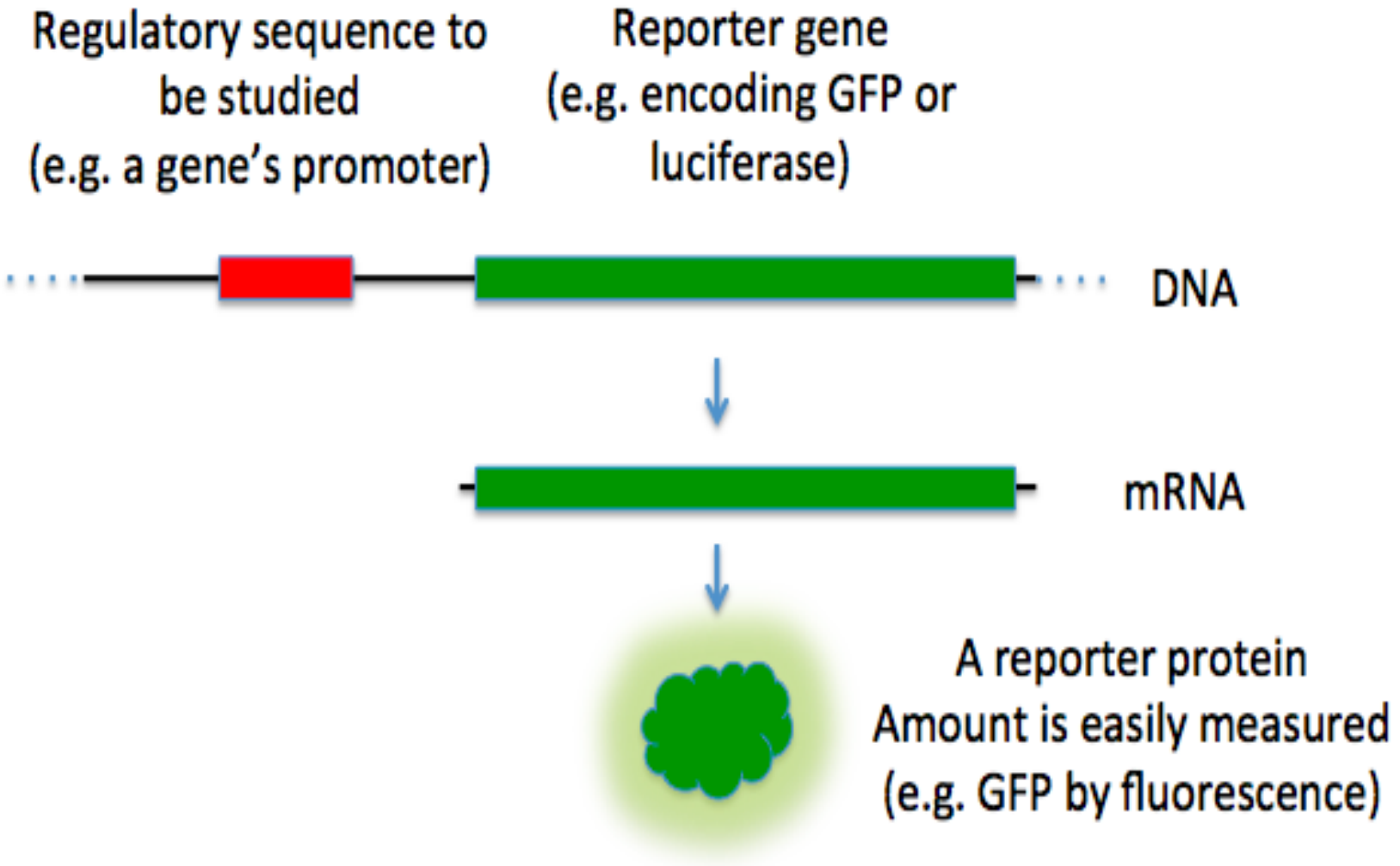
RNA → cDNA

reverse transcriptase

- mRNA isolated from an organism/tissue/cell directly encodes a gene and can be converted to DNA using **reverse transcriptase**
- The product is called **complementary DNA (cDNA)**.



Reporter genes



Reporter genes can be cloned into a plasmid and transfected into cells, forcing them to express the protein.

Databases

UCSC Genome Browser
http://genome.ucsc.edu/

PUBMED
(via Caltech)

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?otool=caitlib