Nucleic Acid Techniques

What are the techniques used to work with DNA?

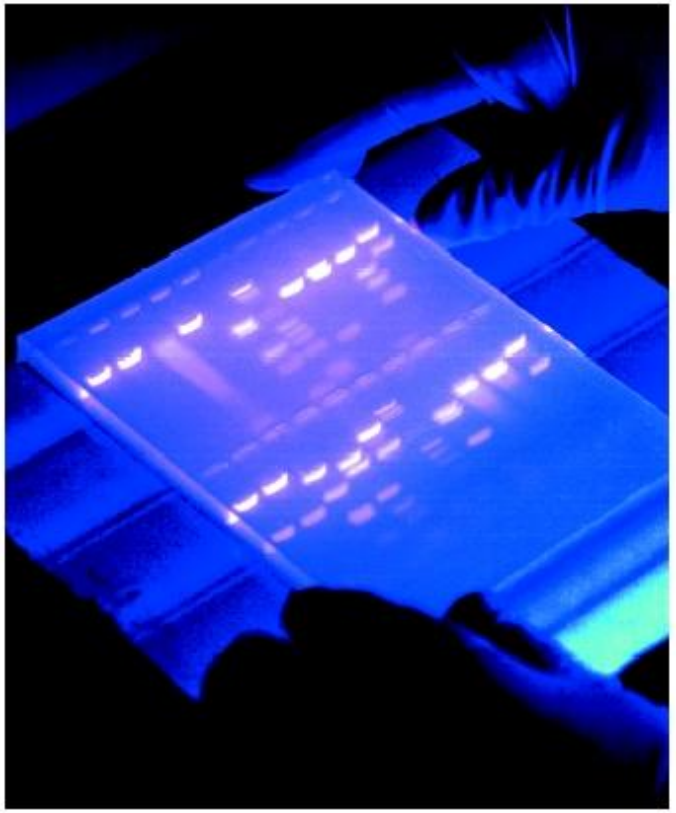
How is DNA cloned?

_____

_____

How is DNA produced in the lab?

_____



DNA Gel Electrophoresis

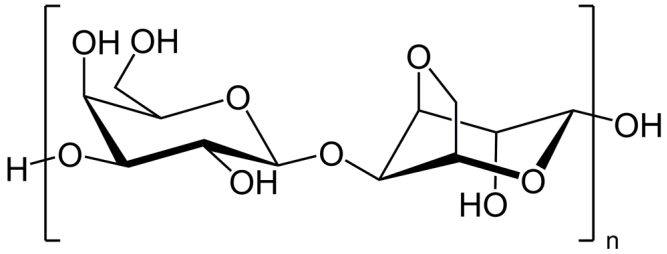
Electrophoresis - movement of charged particles _____

_____

Gel matrix:

- agarose - a polysaccharide _____

_____



In SDS-PAGE, SDS gives all proteins in a sample an overall "-" charge. Proteins move toward "+" ,anode, of electric field.

What is the charge of DNA?

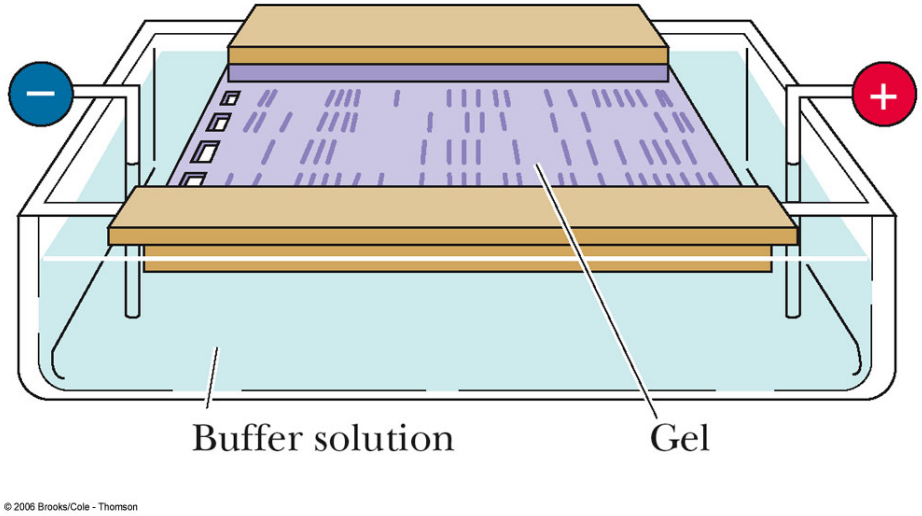
_____ in nucleotide

DNA in a sample will move _____

_____

DNA in sample is separated by size

- large size moves _____
- small size moves _____



DNA Gel Electrophoresis

How to visualize DNA after gel electrophoresis?

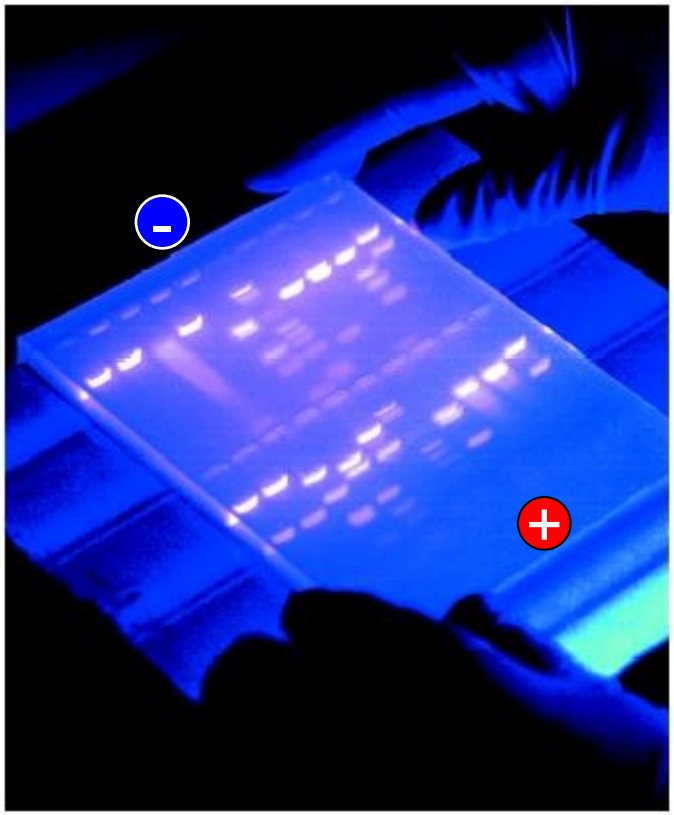
Ethidium Bromide (EtBr)

A small molecule that “stains” nucleic acids by _____

_____

Under ultraviolet light EtBr will _____

_____



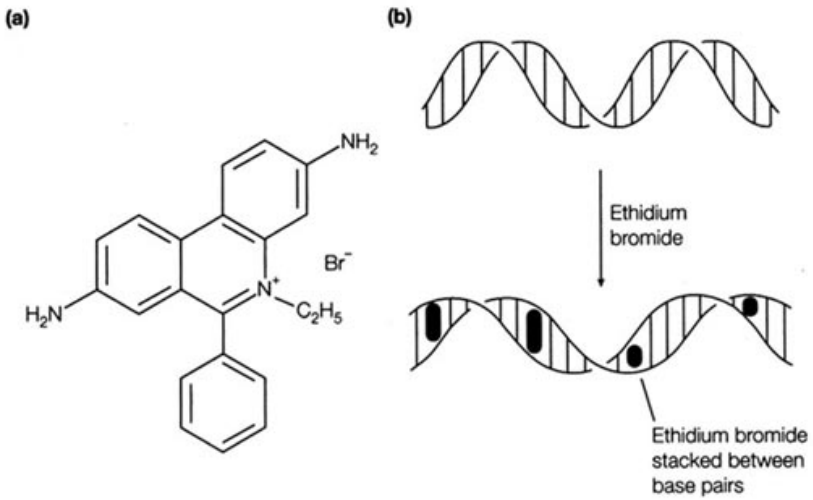


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

How to clone a gene?

Steps:

1. _____ with gene of interest
   many methods for this
2. cut DNA with _____
3. _____ plasmid vector
4. express gene on plasmid vector in _____

Restriction endonuclease - an enzyme that hydrolyzes (breaks, “cuts”) the _____

cut DNA at _____

cut sites are **palindromic** - _____

Named after organism from which they were isolated

Restriction endonuclease

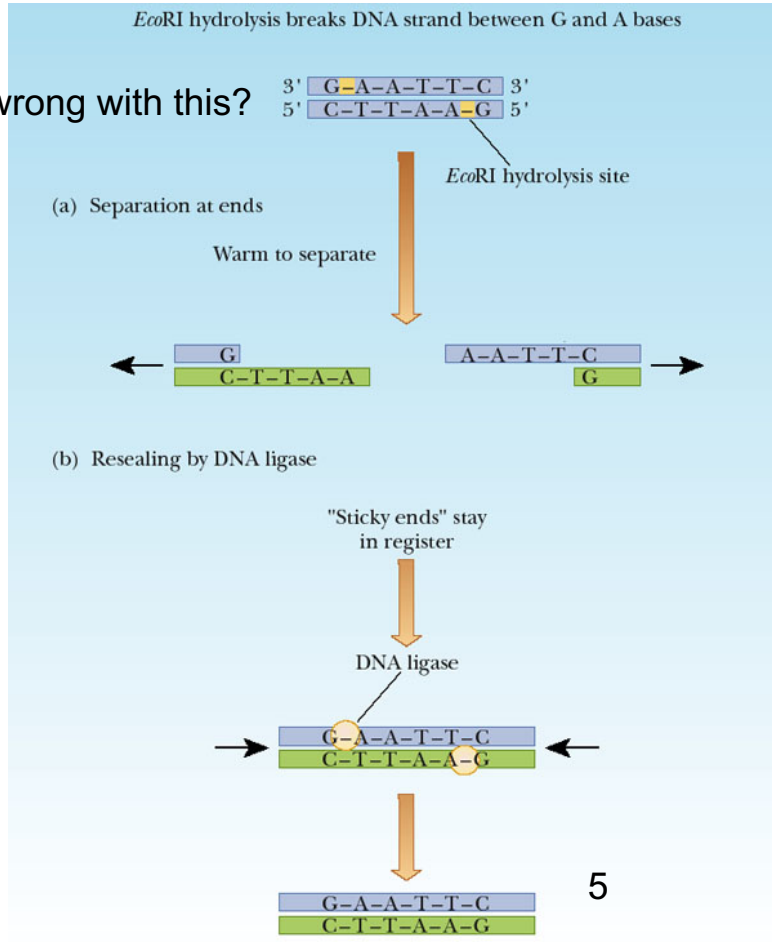
*Eco*RI - isolated from bacteria *Escherichia coli* (*E. coli*)

- recognizes sequence

5′A T G C T G **G A A T T C** G A G A C G 3′

3′T A G G A C **C T T A A G** C T C T G C 5′

What's wrong with this?



- Cutting DNA with restriction enzyme

_____

- _____

DNA ligase - enzyme that _____

_____

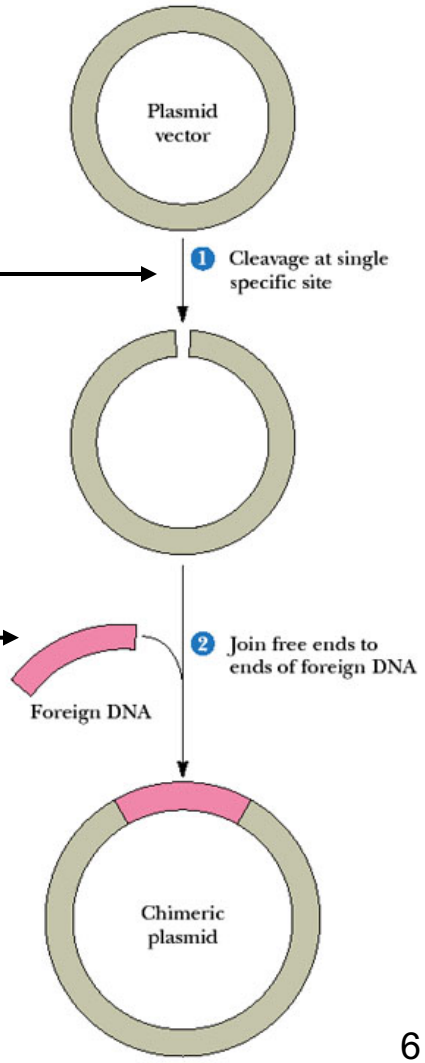
Plasmid Vectors

Plasmid - a small, circular DNA molecule _____
- contains site for _____
- _____

Cut plasmid with restriction enzyme to produce _____

Cut gene with restriction enzyme to produce compatible “sticky ends”

Add cut plasmid, cut gene, and DNA ligase

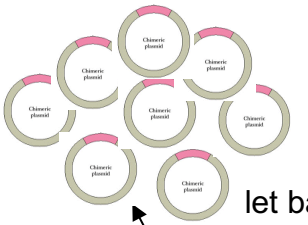


Plasmid Vectors

Plasmid can be put into bacteria to _____

_____

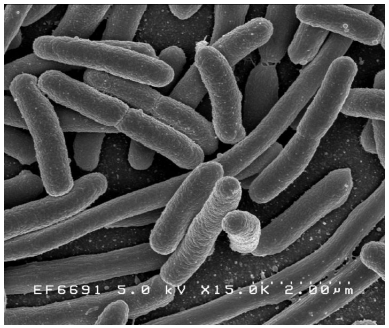
Gene in plasmid can also be expressed as protein to obtain large amounts of _____ _____ or structure identification.



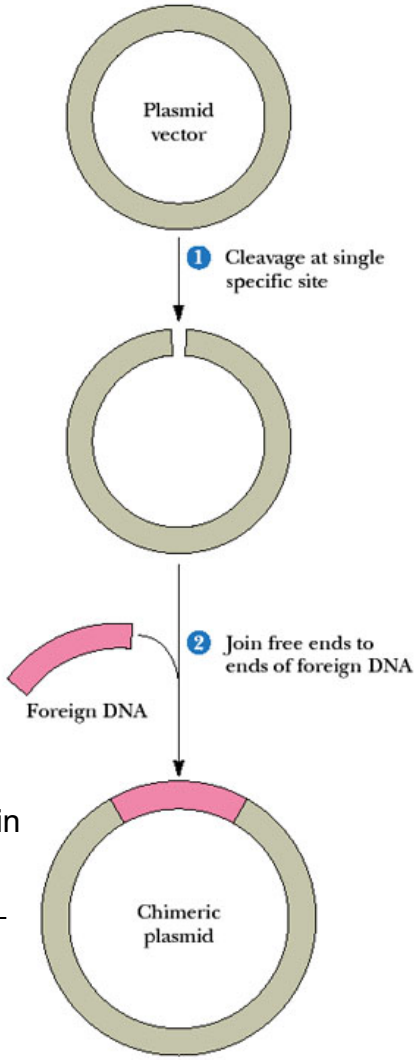
let bacteria make more plasmid, purify

protein

let bacteria make protein, purify



put plasmid in bacteria



Polymerase Chain Reaction (PCR)

Method for amplification of _____

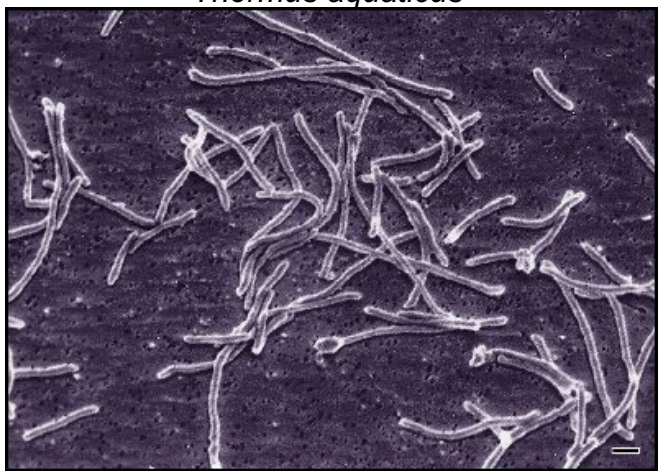
- _____
- obtain millions to hundreds of millions of DNA molecules

DNA polymerase must be able to _____ after being heated to 95˚C (203˚F)

Utilizes DNA polymerases from bacteria that _____

- *Thermus aquaticus*
- *Taq* DNA polymerase
- first found in the hot springs and geysers of Yellowstone Nat'l Park (50 - 80˚C, 122 - 176˚F)
- subsequently found near deep-sea thermal vents, over 100˚C (212˚C)
- purified DNA polymerase termed "Taq polymerase"

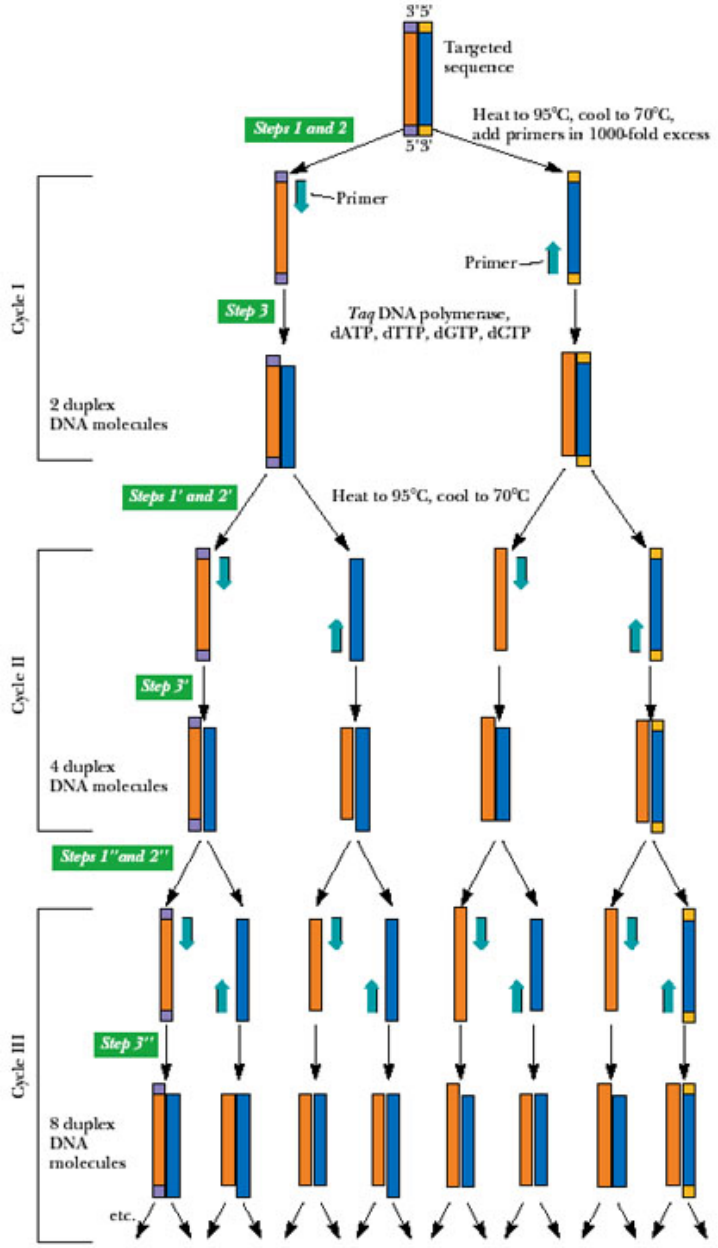
Thermus aquaticus



Polymerase Chain Reaction (PCR)

Kary Mullis
- "inventor" of PCR
- came up with idea to use DNA polymerase from -*Thermus aquaticus,* 1983
- awarded Nobel Prize in Chemistry, 1993

Polymerase Chain Reaction (PCR)



Requires:

DNA polymerase (*Taq*)

primer - short (15 - 30 bases) _____ used as base for *Taq* polymerase to synthesize _____

template - _____

Nucleotides - dATP, dTTP, dCTP, dGTP

cycle:

1. heat to 95˚C, _____
2. cool to 55˚C, _____ _____
3. heat to 70˚C, _____ _____ _____

http://www.youtube.com/watch?v=2KoLnIwoZKU

thermal cycler