Review: Plasmid Isolation (miniprep)

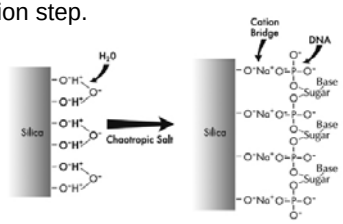
*adapted from Qiagen Miniprep Handbook

P1 : Resuspension buffer (contains RNase A)
- RNase will degrade RNA after cell lysis

P2: Alkaline (high pH, NaOH) lysis buffer w/ SDS
- NaOH lyses the cell, SDS solubilizes lipids and proteins as well as DNA.
- Do not incubate too long or shake/vortex vigorously→ Releases chromosomal DNA and irreversibly denatures/shears your plasmid **BAD**

N3: Neutralization buffer (Acetic acid, Guanidine hydrochloride)
- Neutralized and adjusted to high salt binding conditions. High "chaotropic" salt causes denatured proteins, chromosomal DNA, cellular debris and SDS to precipitate while smaller plasmid DNA renatures correctly and stays in solution. *Complete precipitation is important. This is the major separation step.

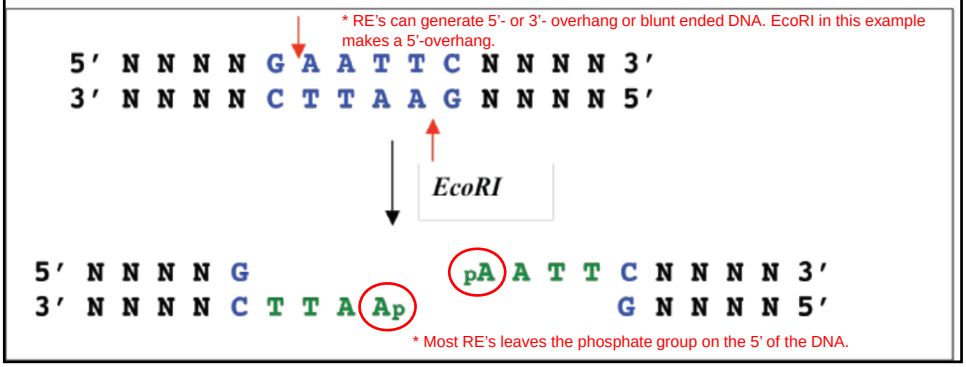
Spin column – silica membrane. DNA binds in high salt via salt bridge formation with silica and elutes in low salt at pH 7-8.5.



PB (IsoPrOH w/ GuHCl) - wash out protein
PE (70% Ethanol) - wash out salt
EB (Water or Tris/EDTA buffer) – Elute DNA; EDTA prevents DNA degradation.

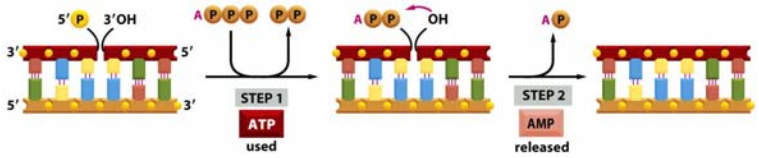
Restriction Enzymes

•RE's bind to DNA at a *specific* sequence and catalyze hydrolysis of phosphodiester bonds.
•Many different RE's have been isolated from various organisms.
•Restriction sites are often *palindromes*.



DNA ligase

- DNA ligase – join nicks or two pieces of DNA together



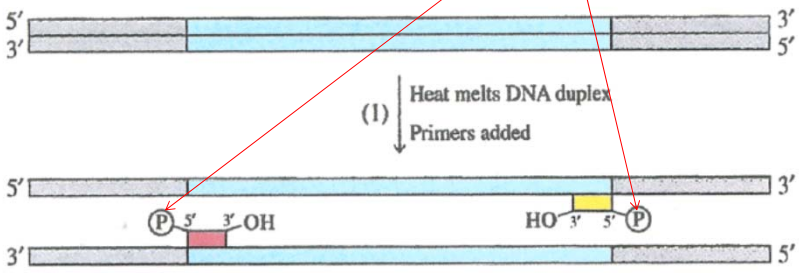
Problem: How to get large amount of a *specific* DNA sequence?

- *Polymerase Chain Reaction (PCR)*
  - PCR allows for amplification and isolation of a specific DNA segment from a small sample.
  - Can be used to append specific sequences (e.g., RE sites) at ends of amplified DNA & introduce mutations on specific sites (Site-directed mutagenesis)
  - Requires:
    - DNA sample
    - Thermostable polymerase
    - Oligonucleotide *primers* *Two required. Usually do not have 5' phosphate groups.
    - Deoxynucleoside triphosphates (dNTPs)

PCR

- We usually use primers without 5' phosphates (b/c cheaper).
- Usually, PCR is followed by RE digestion in which the 5'-P is exposed and subsequently used for ligation

STEP 1: Denaturation at ~95°C



STEP 2: Annealing or "hybridization" of primers at ~55°C

Primers are short (16-20 bp) segments of complimentary ssDNA

Primer 3'-TCGACGTAGAGTCCGG-5'

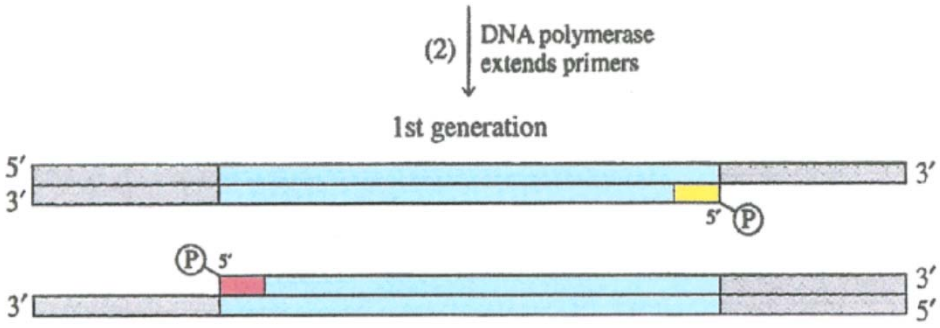
Template 5'-AGCTGCATCTCAGGCCNNNNNN…….

PCR

STEP 3: Elongation at ~70°C

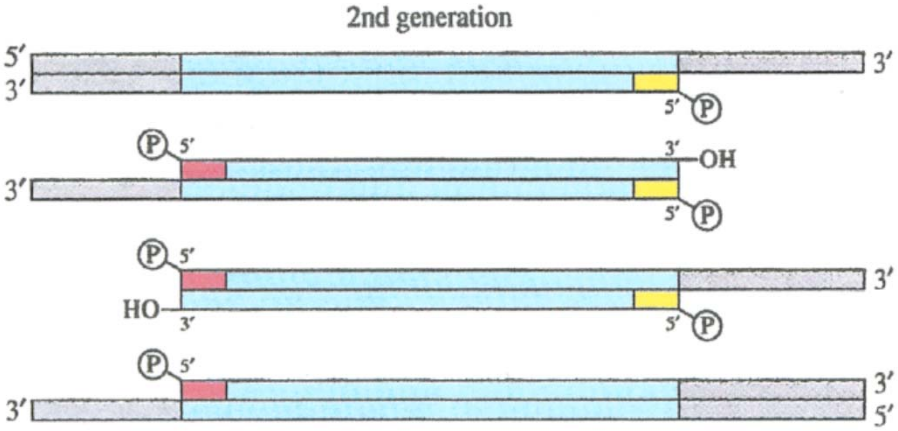
• Thermostable polymerase appends dNTPs to 3' end of primer using parent strand as template.

• Rates vary, but e.g., 1 min for 1 kbp.



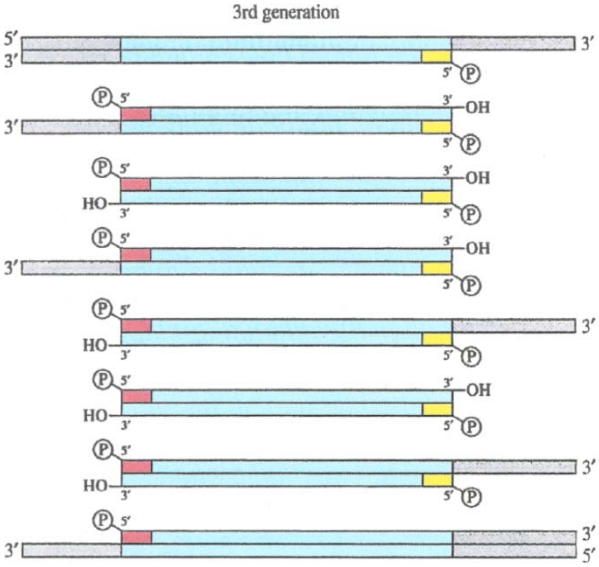
PCR

Repeat Denaturation, Annealing, Elongation…



PCR

Repeat Denaturation, Annealing, Elongation…



Problem: How to separate DNA fragments?

Gel Electrophoresis

- Polyacrylamide gel electrophoresis
  - 20bp - 2000bp
- Agarose gel electrophoresis
  - 300bp - 40,000bp

Electrophoresis - Principle

- (-) charged phosphate groups of DNA are attracted to a (+) electrode when a charge (potential) is applied.
  * What was the pH of the running buffer? Does it matter?
- DNA has evenly spaced charge (i.e., uniform charge density), thus it migrates according to **size**.