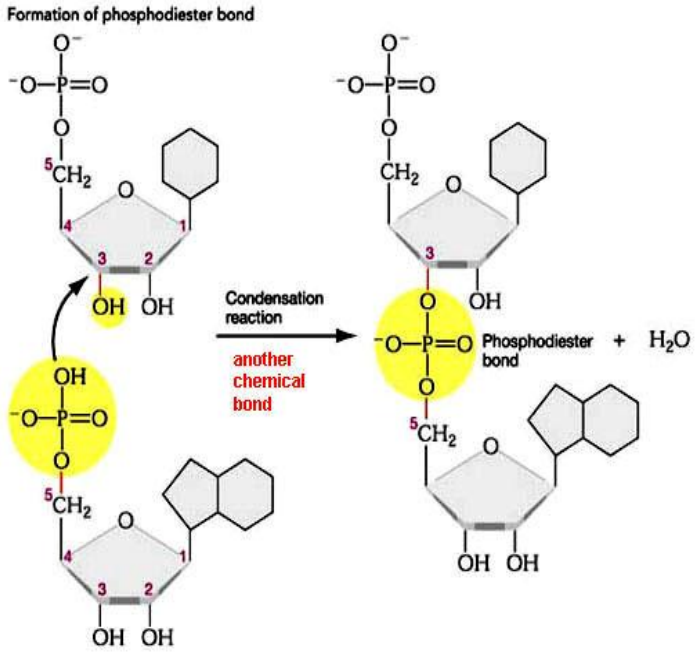


MCDB 140L Day 9

Outline

- Quiz
- Come in Tues, Thurs AND Sunday!
- Review of last time
- Today's techniques
- Lab Write-up
- Before you start

Come in Tues, Thurs, and Sunday

- Come in tomorrow morning and move your ligation reaction into the freezer
- Come in on Thursday before noon to move your transformation plates to the fridge
- Come in on Sunday afternoon to start overnight cultures of your recombinants

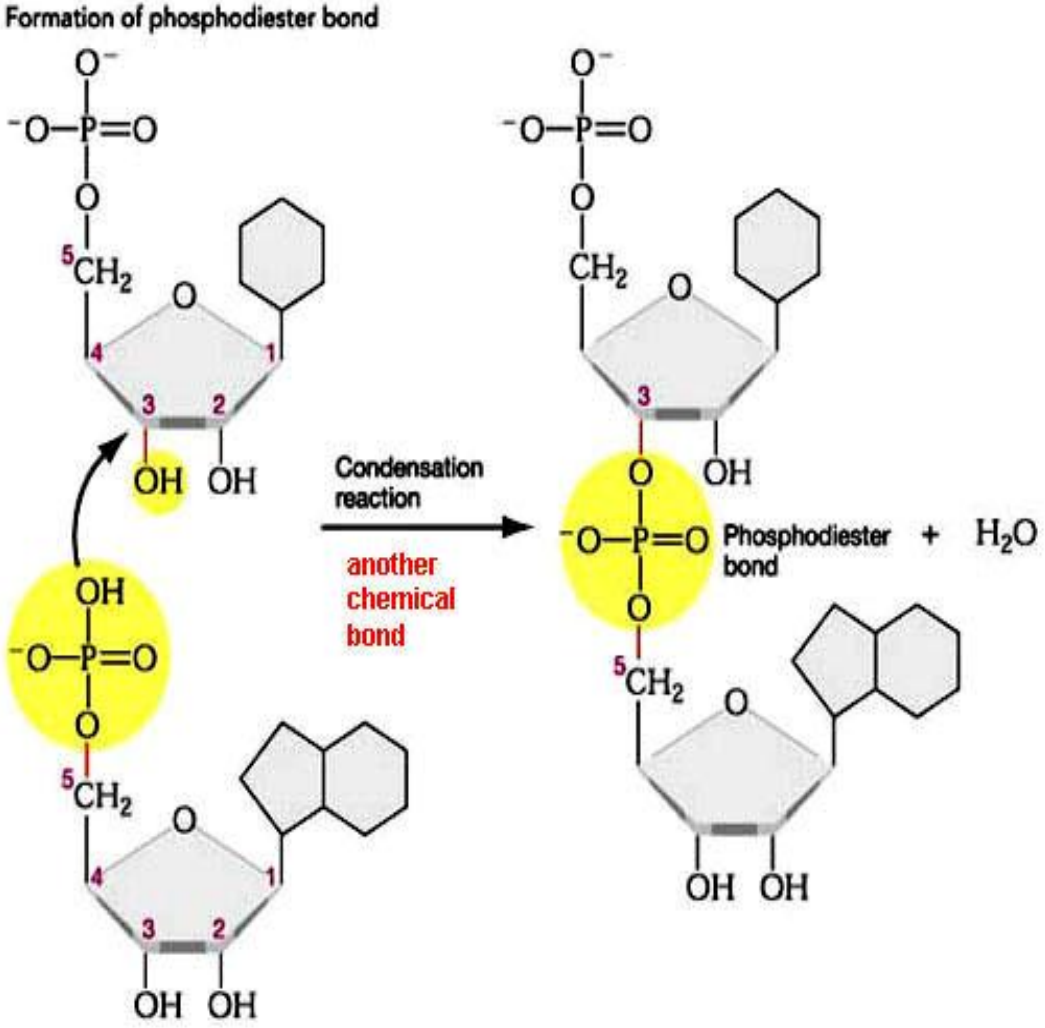
Review of last time

- What did we do?
  - Digested both plasmid and insert with EcoRI
- Why did we do it?
  - Puts compatible sticky ends on vector and insert (prepares them for ligation)

Today's techniques 1 of 1

- Dephosphorylate plasmid
  - Purpose: remove 5' terminal phosphates from vector so the re-circularization of plasmid can't occur in ligation reaction
    - Without 5' phosphate the DNA ligase can't form a new phosphodiester bond in DNA backbone of empty vector
    - Digested PCR product does have 5' phosphates so 2 of the 4 possible bonds will be formed and the remaining nicks can be repaired by the *E. coli* host following transformation

Dephosphorylate plasmid

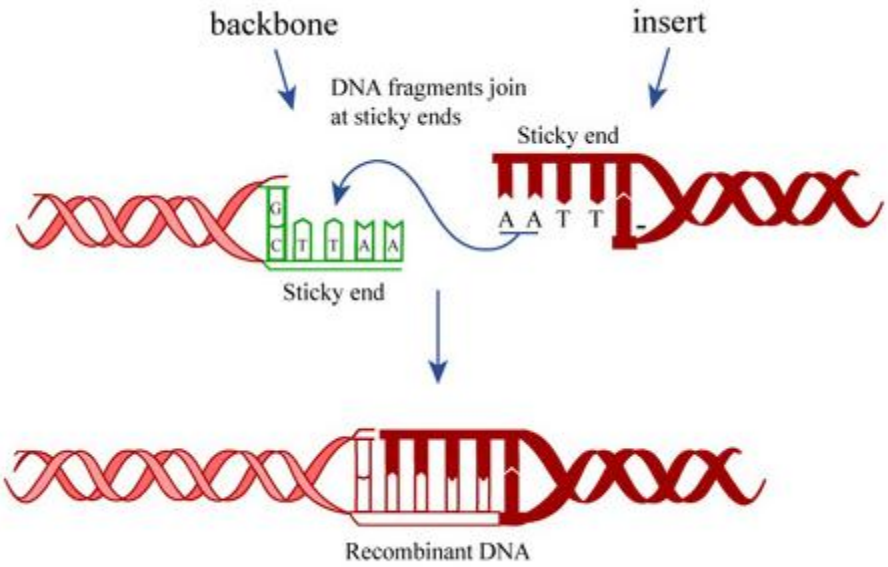


After phosphatase reaction…

- Check concentration of insert and vector
  - Purpose: ligations are more efficient when there is a 3-10-fold molar excess of insert:vector
    - Quantify based on EtBr fluorescence
    - Insert is ~600bp, vector is 3kb, therefore equal mass of each would be a 5 fold molar excess
    - We want a 3-fold molar excess, **don't setup ligation until I have looked at your gel!** Don't just blindly follow manual, figure out how much volume of each you'd need and adjust accordingly

After quantifying insert and vector…

- Ligate digested PCR product into digested and dephosphorylated vector
  - Purpose: to clone our PCR product
    - Ligase will catalyze formation of phosphodiester bonds (when 5’ phosphates are available)



Before you start

- Before you start:
  - Get ice and wear gloves!
- Step B2, run **5µl** of PCR product and vector with **1µl** 6X sample buffer

- Come in **tomorrow** **morning** and move your ligation reaction into the freezer