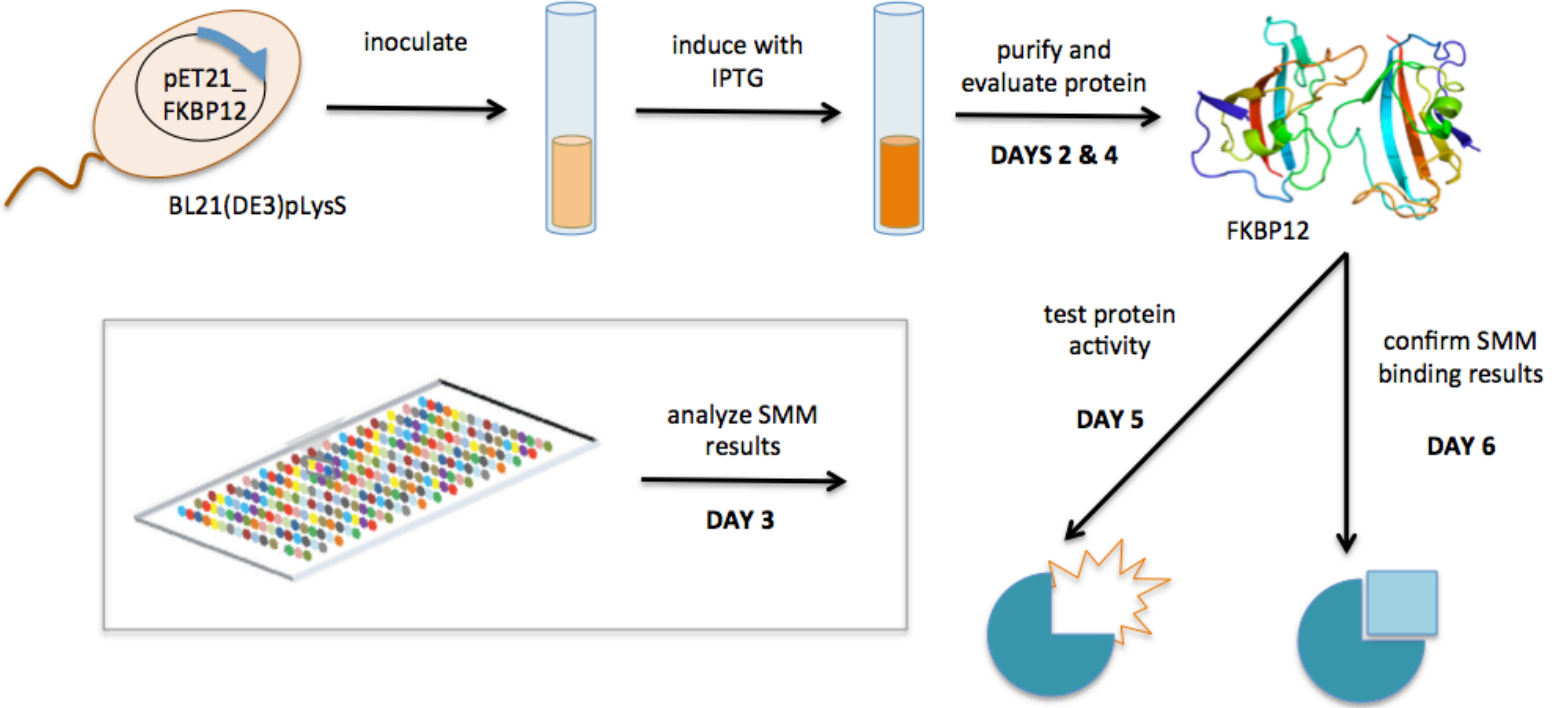
M1D1:
In silico cloning and confirmation digest of protein expression vector

1. Laboratory Orientation quiz
2. Prelab discussion
3. Build protein expression vector - virtually
4. Confirm protein expression vector - actually

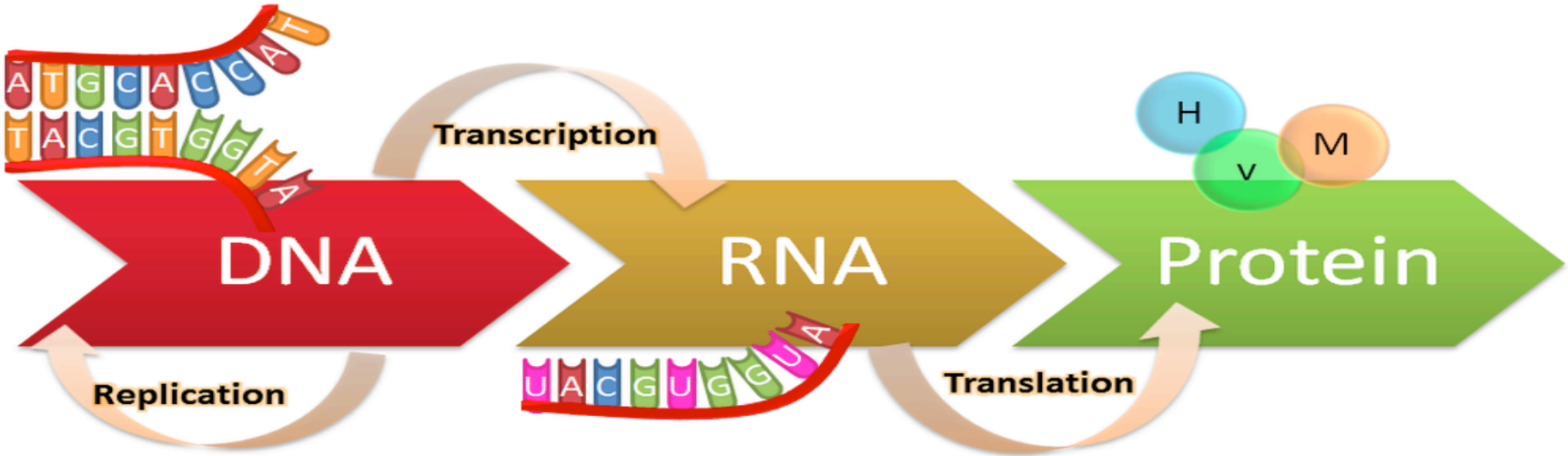
Mark your calendars!

- Data summary (15%)
  - completed in teams and submitted via Stellar
  - draft due 3/11, final revision due 3/25
  - format in bullet points
- Mini-presentation (5%)
  - completed individually and submitted via Gmail
  - due 3/16
- Laboratory quizzes
  - scheduled for M1D4 and M1D7
- Notebook (part of 10% Homework and Notebook)
  - one entry will be graded by ~~Casper~~ Michaela 24 hr after M1D7
- Blog (part of 5% Participation)
  - due 3/17 via Blogspot

Overview of Mod 1 experiments



How are proteins made?



http://genius.com/Biology-genius-the-central-dogma-annotated

What if we want a specific protein?

- Amplification

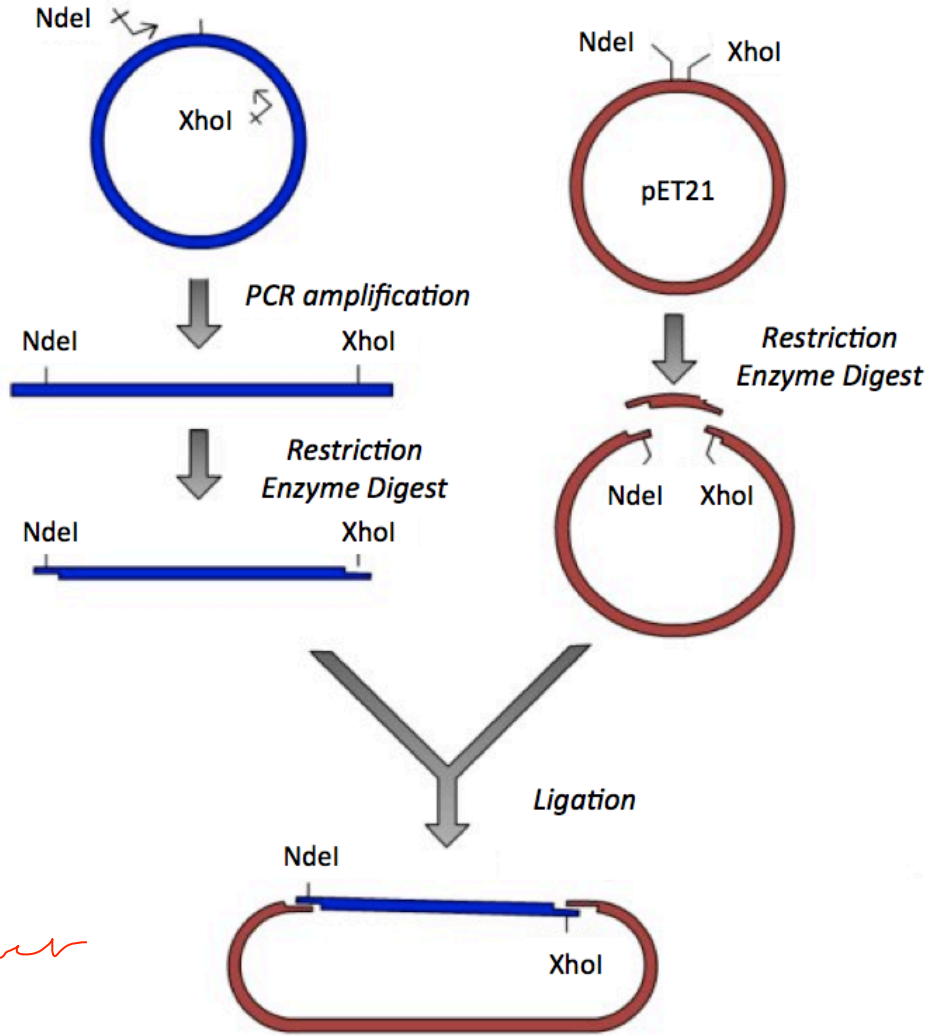
  gene of interest

- Digestion

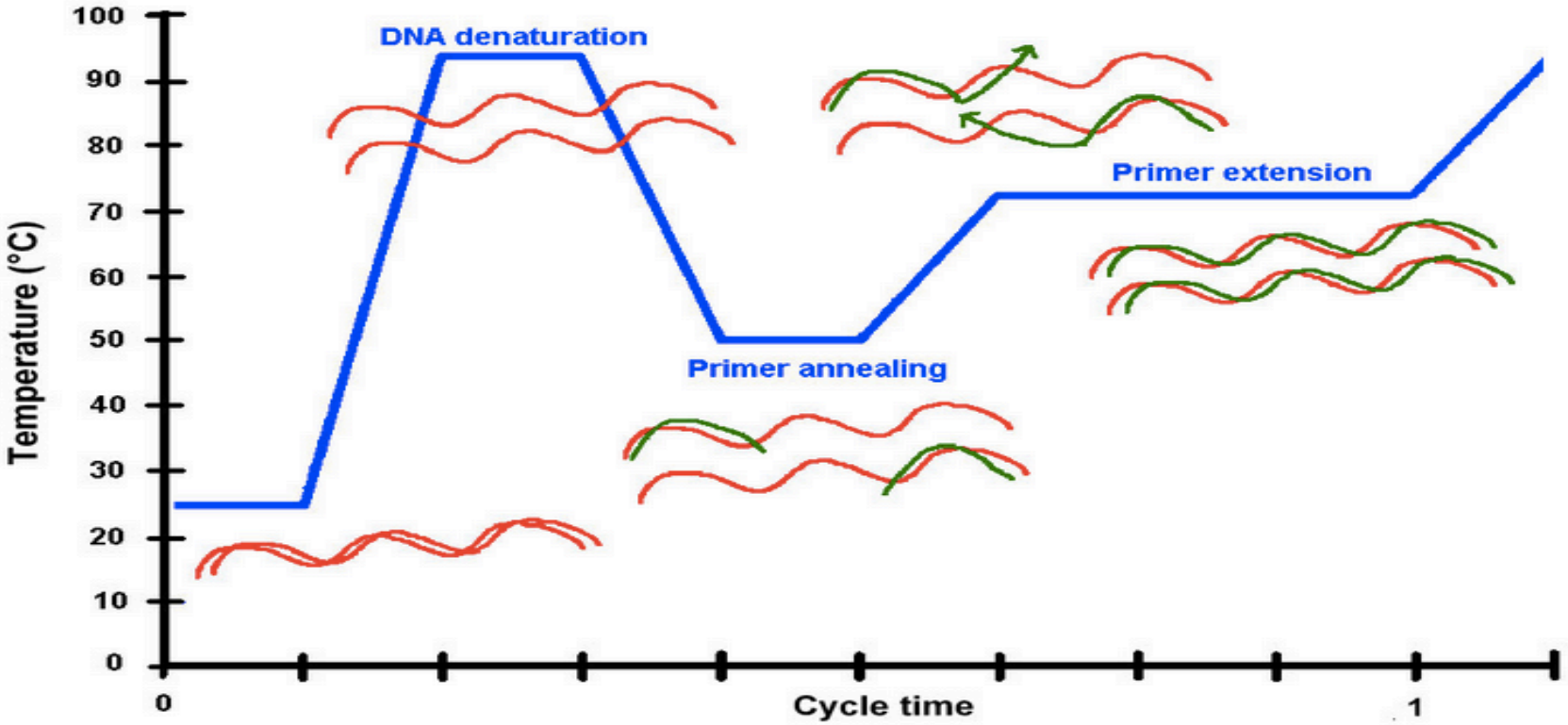
  generate sticky ends
  open the vector

- Ligation

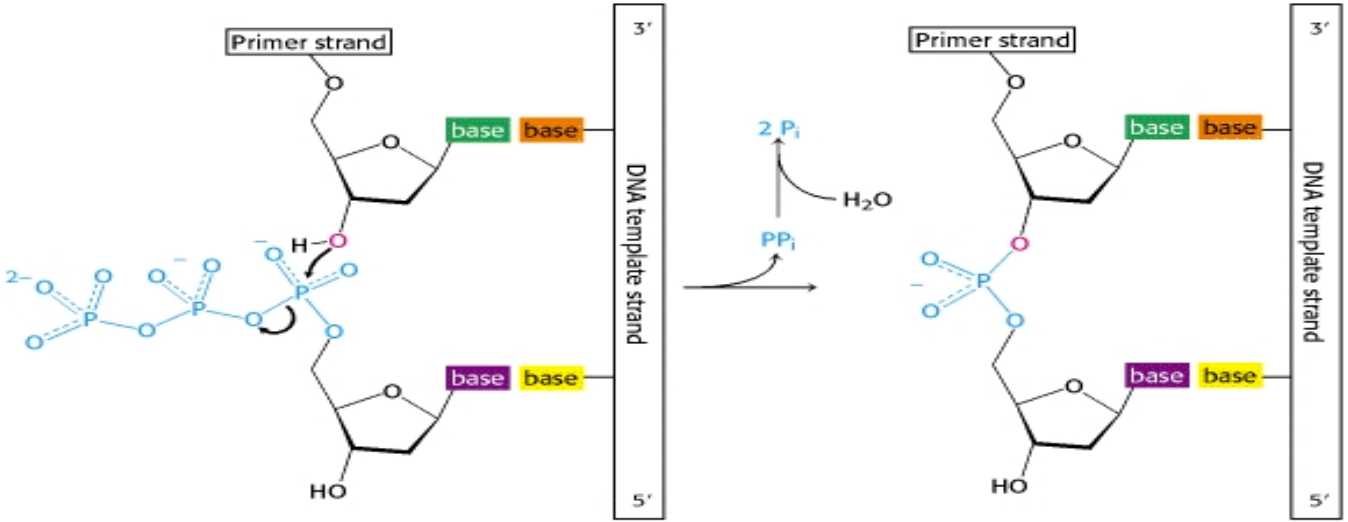
  glues everything together



Amplification: PCR cycling



Amplification: DNA polymerase

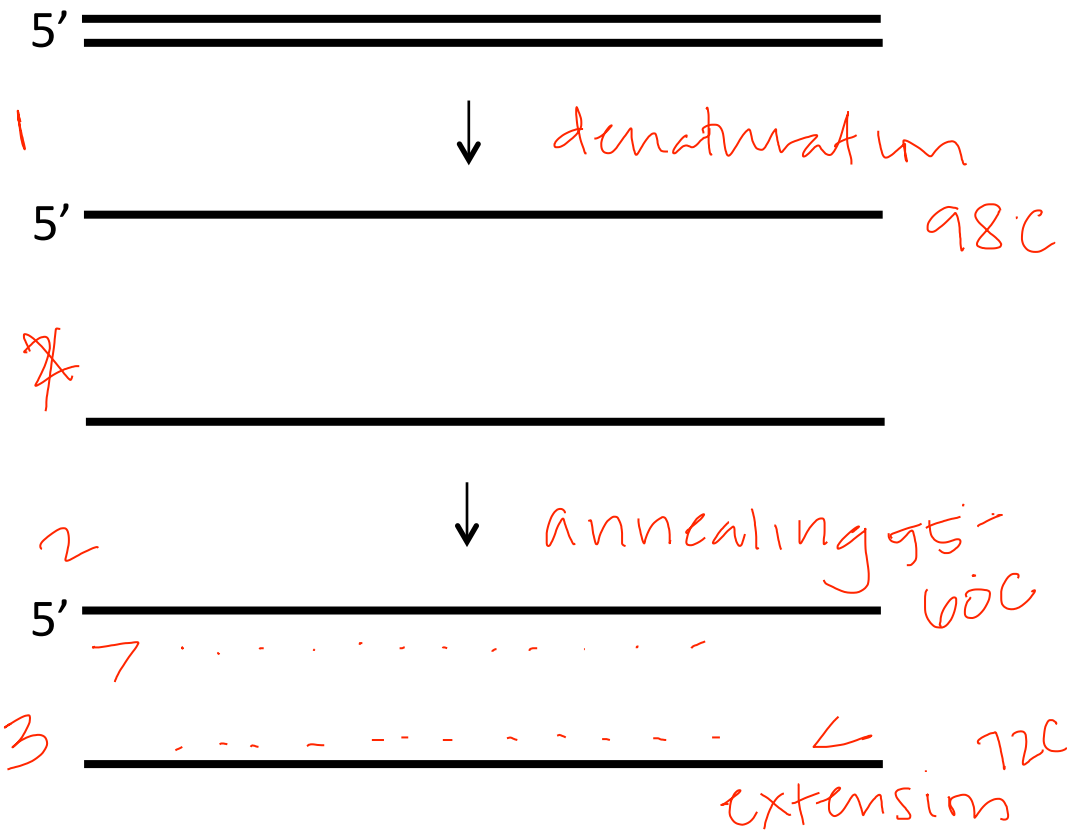


- Catalyzes formation of polynucleotide chains
- Requires a primer base-paired to template

http://www.ncbi.nlm.nih.gov/books/NBK22374/

PCR reagents and conditions

Reagents
dNTPs
polymerase
primers
template
buffer



A closer look at primer design

- Length: 17-28 bp

  Specificity of binding (to template)

- GC content: 40-60 %

  DNA binding efficiency

- Tm : < 65 °C

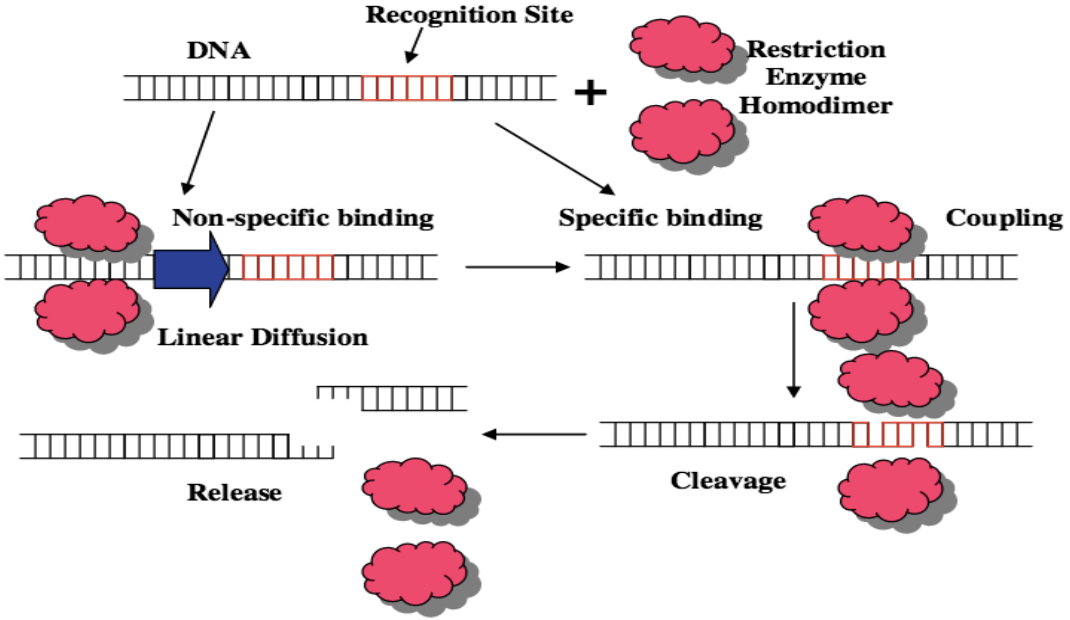
  DNA binding efficiency

- Avoid secondary structure and repeat sequences

ATATATAT

Digestion: restriction enzymes

- Function as homodimers
  - Each dimer cleaves backbone at site of palindromic recognition sequence



Digest reagents and conditions

Reagents
enzyme
target (DNA)
buffer

- Temperature

37°C or 25°C

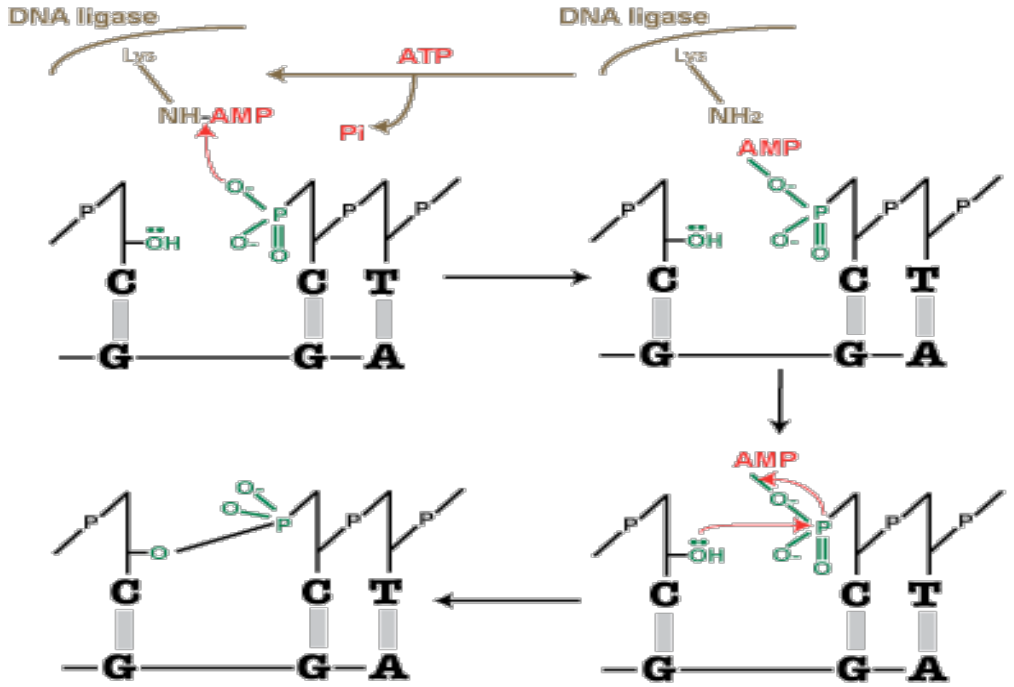
- Time

1 hr – ~~24~~ h

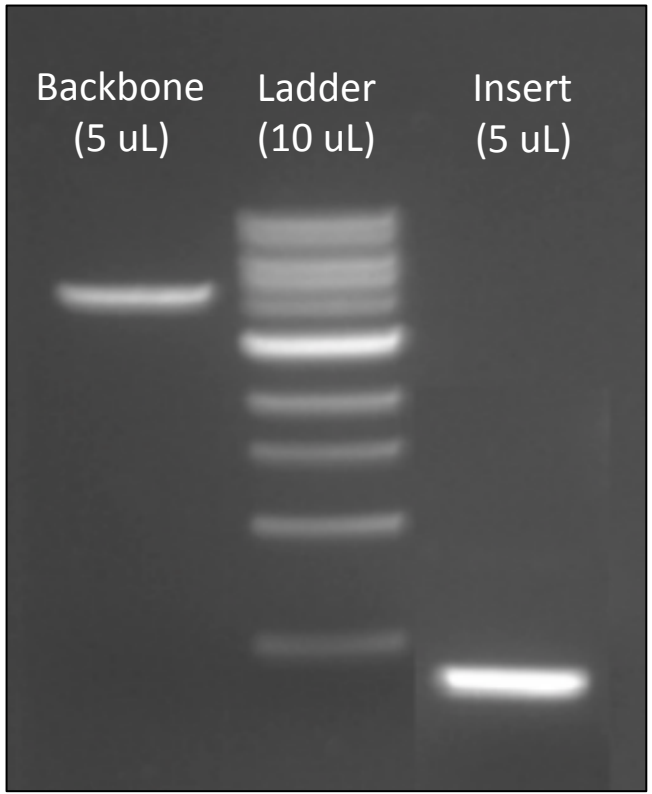
overnight.

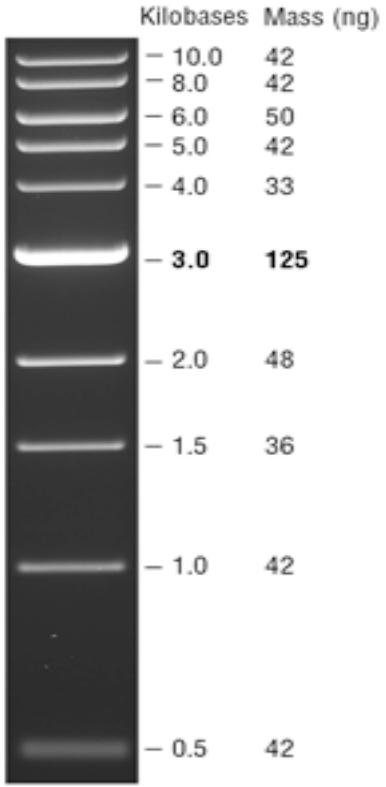
Ligation: T4 DNA ligase

- Forms covalent phosphodiester bond between 3’ OH acceptor and 5’ phosphate donor
- Requires ATP



Ligation conditions





- Ideally, want 4:1 molar ratio of insert:backbone
- Calculate molar amounts from concentrations and sizes of DNA molecules

Ligation calculations

1. Determine volume of backbone
   - Use backbone concentration = 40 ng/uL
   - Want 50 – 100 ng
2. Calculate moles of backbone
   - Vector = 5367 bp, MW bp = 660 g/mol
3. Calculate moles of insert
   - Insert = 351 bp, 4:1 ratio of insert:backbone
4. Calculate volume of insert
   - Use insert concentration = 20 ng/uL

How do we confirm the product?

- Transformation

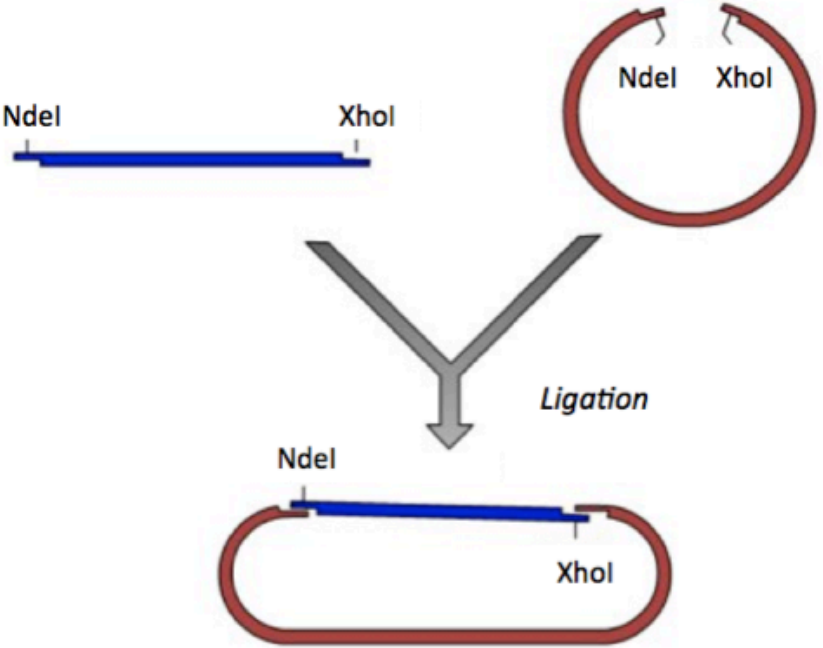
  Amplify cloning product

- Purification

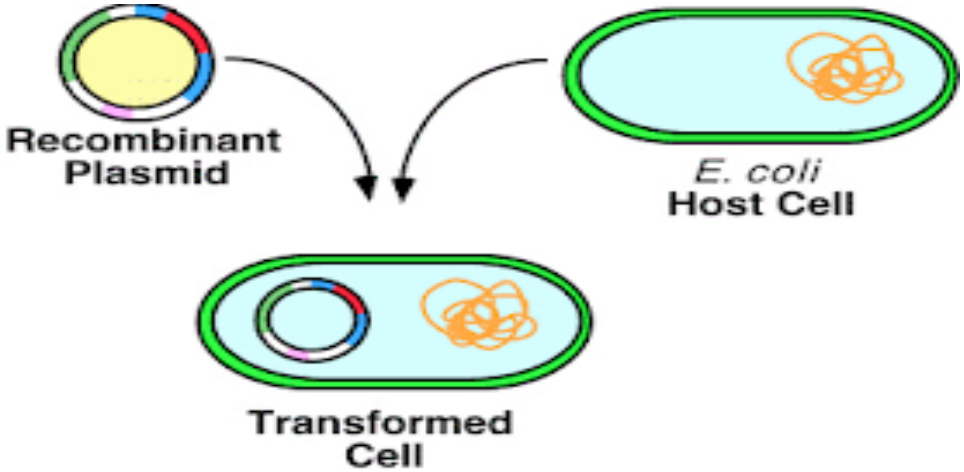
  retrieve from bacteria

- Digestion

  check

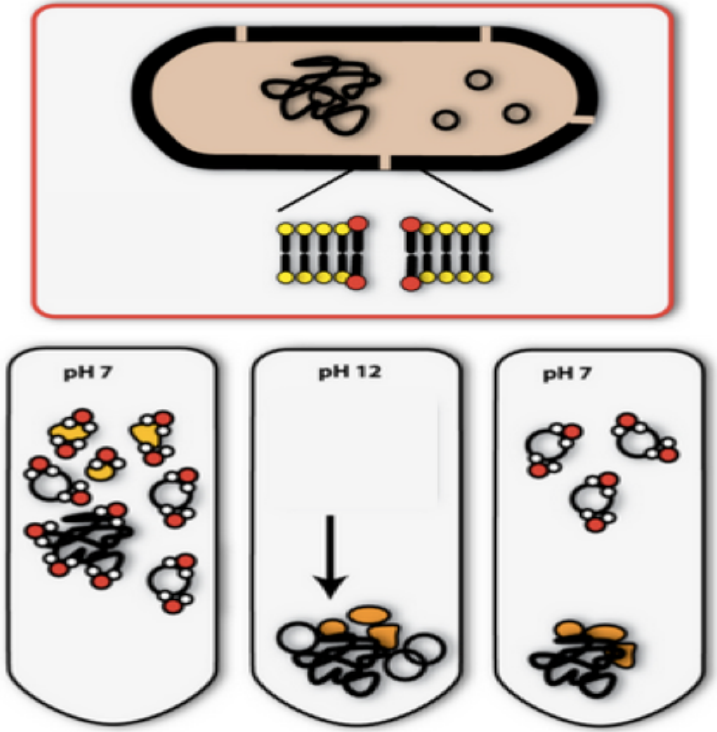


Transformation



1. Incubation
2. Heat shock
   - DNA taken in by competent cells
3. Recovery
4. Selection

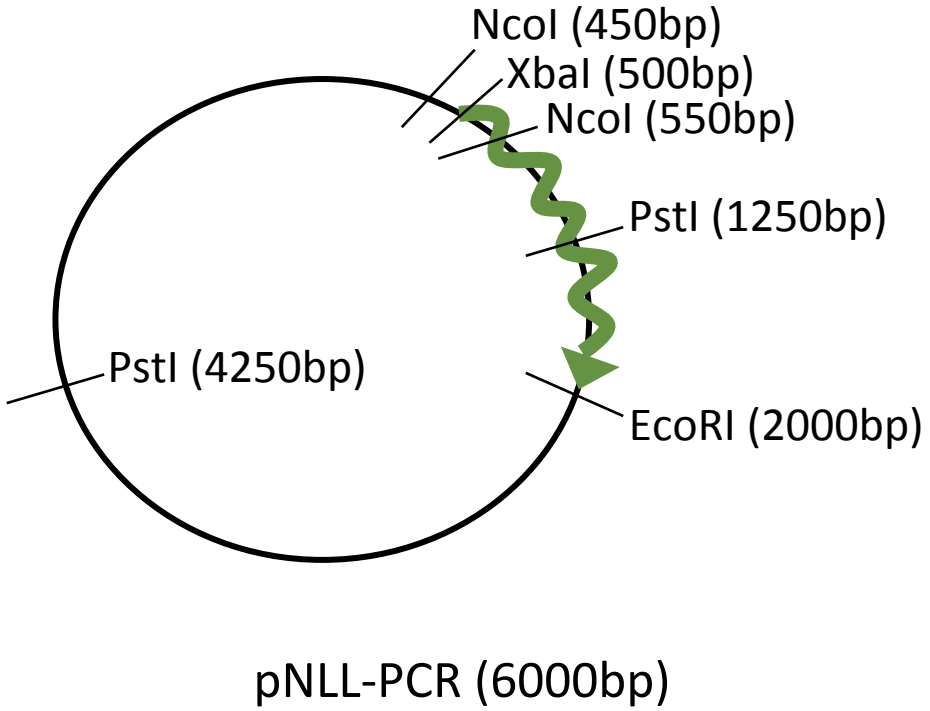
Purification



1. Resuspend cells
2. Lysis
3. Neutralization
   - Separates chromosomal DNA from plasmid DNA
4. Wash
5. Resuspend or elute DNA

Digestion, again

- Confirmation digests
- Ideally, will cut once in insert and once in vector
  - XbaI and EcoRI?
  - PstI?
  - NcoI?



What should go in your notebook?

Laboratory notebook entry component:	Points: Complete	Partial	Incomplete
Date of experiment (include Module#/Day#) and Title for experiment	1	0.5	0
Hypothesis or goal / purpose	1	0.5	0
Protocols (link to appropriate wiki sections)	1	0.5	0
Notes on protocol changes / clarifications	1	0.5	0
Observations	2	1	0
*Visual details			
*Qualitative information			
*Raw data			
Data analysis	3	1.5	0
*Calculations			
*Graphs and Tables			
Summary and interpretation of data	3	1.5	0
*What did you learn?			
*How does this information fit into the larger scope of the project?			
Information is clear	2	1	0
All days represented	1	0.5	0
OVERALL /15			

How should you format your notebook?

Insert H B I U S A x_2 x^2 More

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THURSDAY, 2/8

Hypothesis or goal:
What are you testing and what do you expect of your results?

Protocols: [include link to wiki]
Part 2: Construct pRSETb_FKBP12 in silico

- Include all work / notes / images / sequences generated.
- Be sure to note any interesting observations or protocol changes!

Part 3: Confirmation digest

- Include completed table with volumes.
- Include calculations.
- Be sure to note any interesting observations or protocol changes!

Summary and interpretations:
What, if any, conclusions can be made and what does this prepare you to do next?

How should you organize your notebook?

- Entitle your project “20.109(S19)_YourName”
  - Make each module a new folder
  - Make each day a new entry within module folder
- Share the project with Noreen and Michaela
  - Right-click and choose ‘settings’
  - Add collaborators by email

For today…

- Virtual cloning exercise to build pET21_FKBP12 expression plasmid
- Confirmation digest of pET21_FKBP12

For M1D2…

- Prepare a template for Benchling entries
- Complete in class exercises