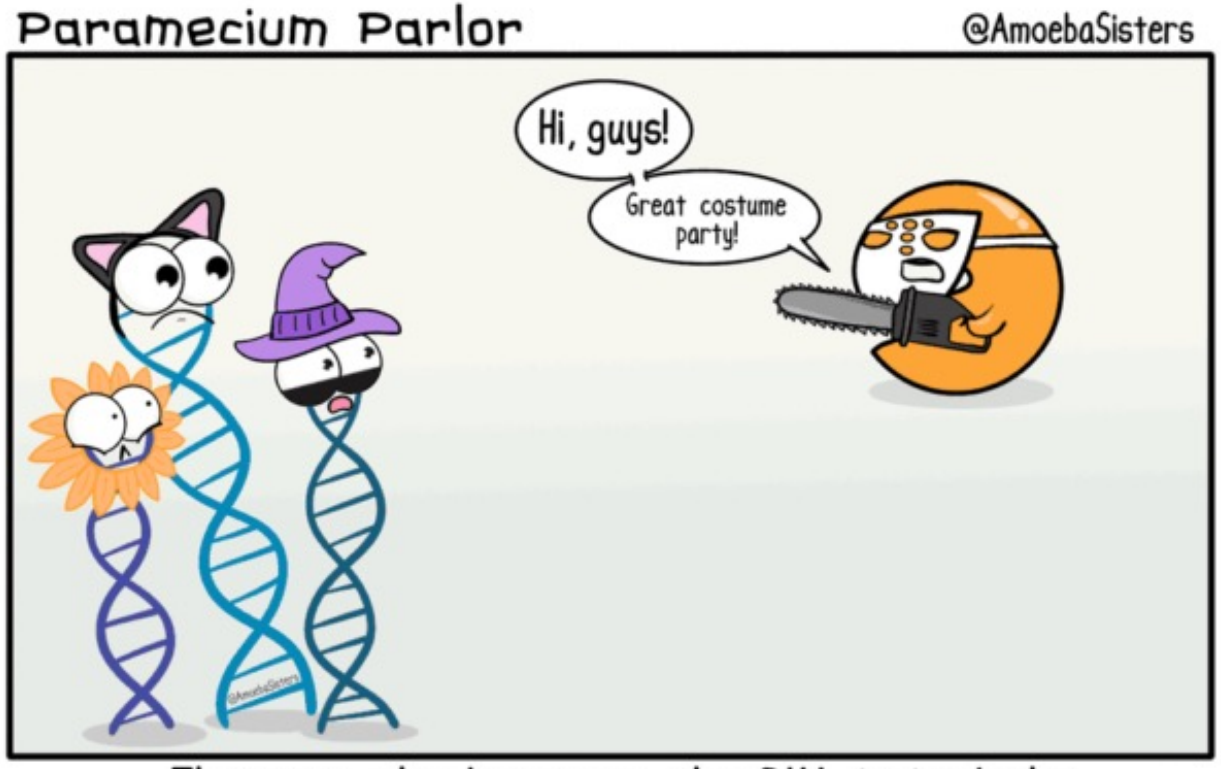
M2D1: Complete in-silico cloning of protein expression plasmid

1. Prelab discussion
2. Complete DNA engineering exercise
3. Set up confirmation digest of plasmid

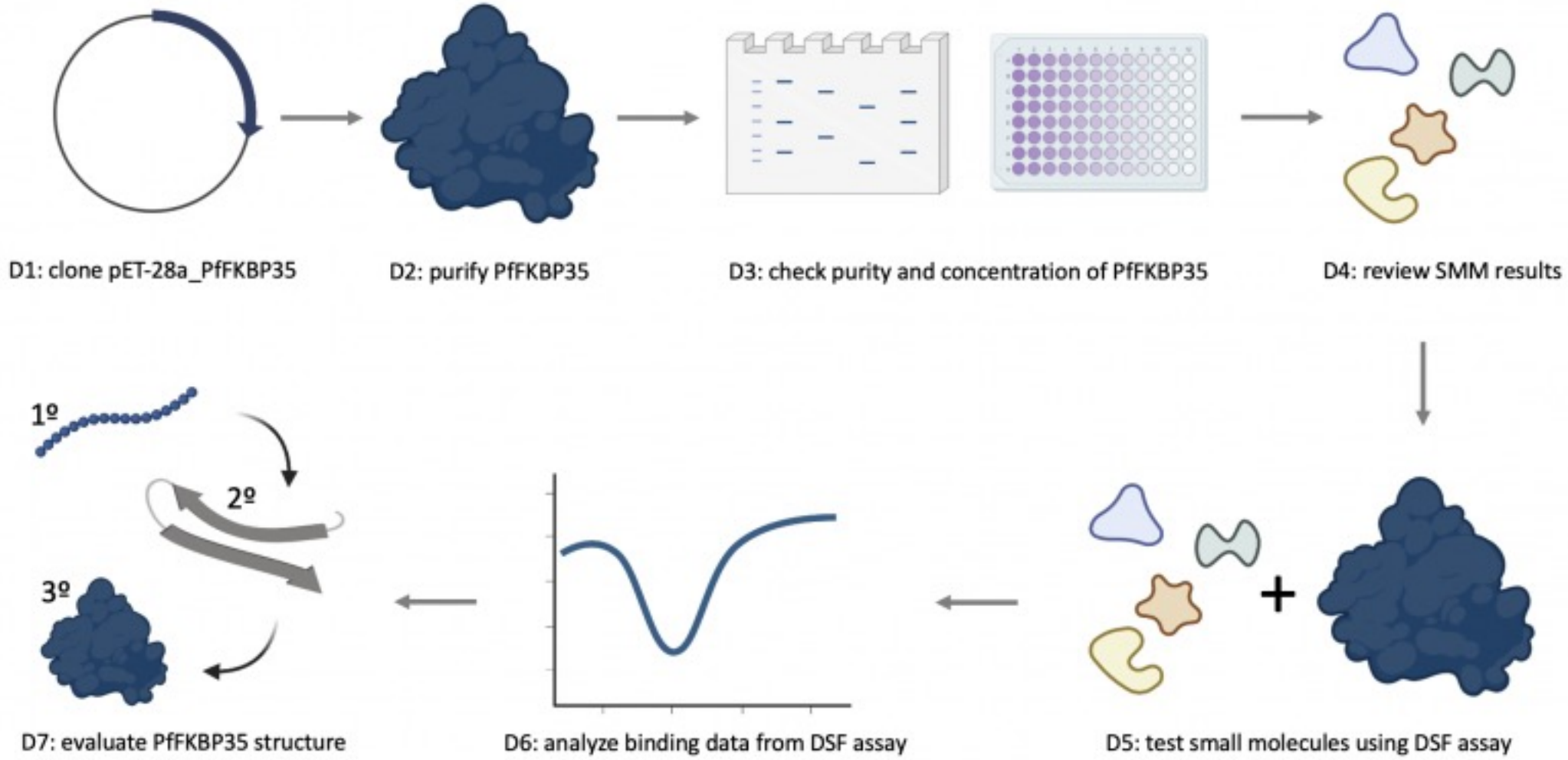


Mod 2 Major Assignments

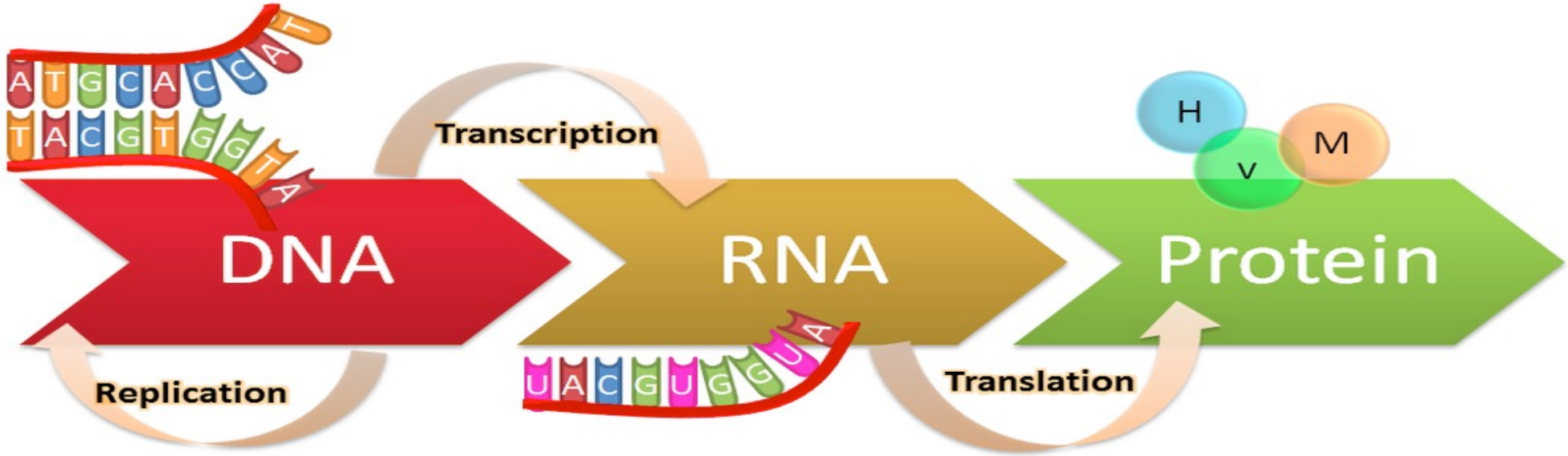
- Journal Article presentation (15%)
  - Individual
  - Presentations on 11/1 & 11/3
- Research article (20%)
  - Individual
  - due 11/21
- Laboratory quizzes (collectively 5%)
  - M2D4 and M2D7
- Notebook (collectively 5%)
  - one entry will be graded by Chyna 24 hr after M2D7
- Blog (part of 5% Participation)
  - due 11/5 & 11/22 via Slack channel

Overview of M2: drug discovery

Research goal: Test small molecules for binding to the *Plasmodium falciparum* FKBP35 protein using a functional assay.



How are proteins made?



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

What if we want to make a specific protein?

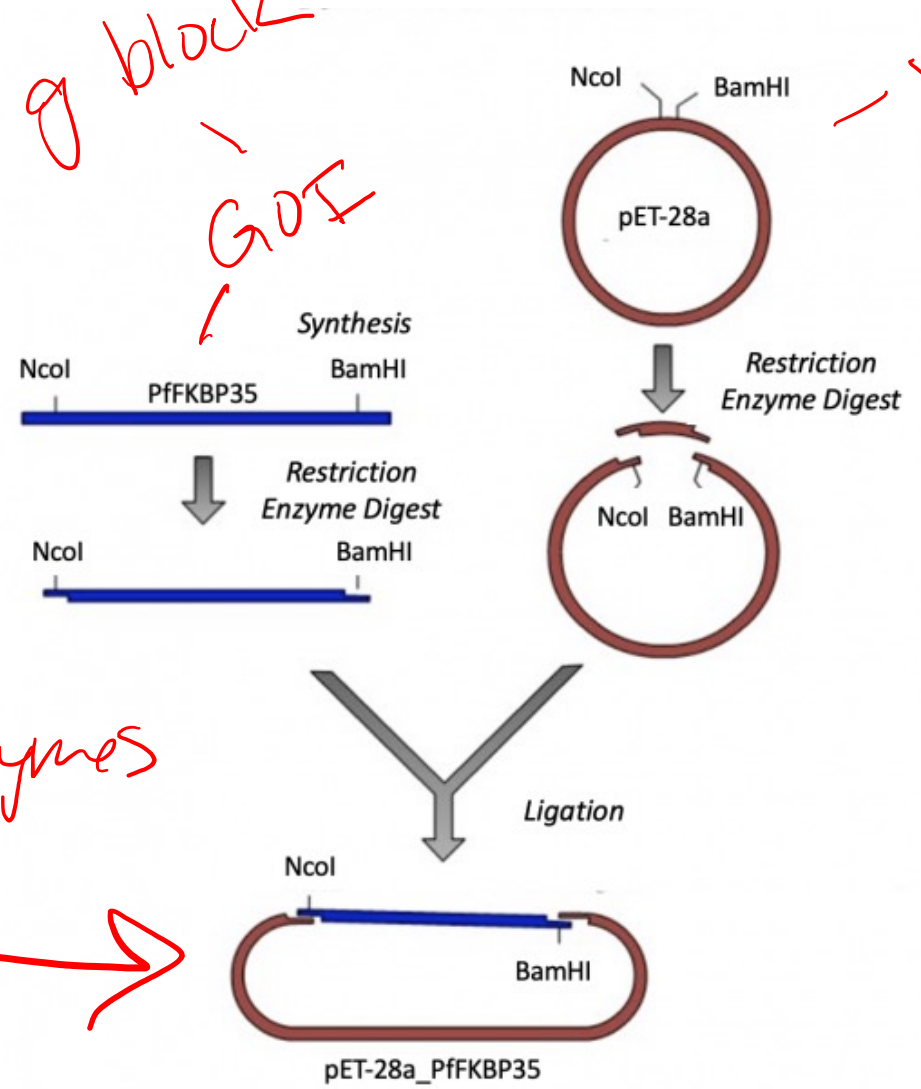
- Chemically synthesize protein by successively linking each amino acid
  - Complicated, **have to make each protein,** expensive

- Synthesize RNA encoding the protein
  - RNA degrades easily
  - Amplification: 1 RNA -> Many Proteins

- Create DNA encoding the protein → plasmid DNA
  - Highly stable, easily transformed into bacteria
  - Amplification Cascade: 1 DNA -> Many RNA -> Many Proteins

What if we want to make a specific protein?

- Who are the players?
  - Insert
  - Vector
- What is the process?
  - Digestion
  - Ligation



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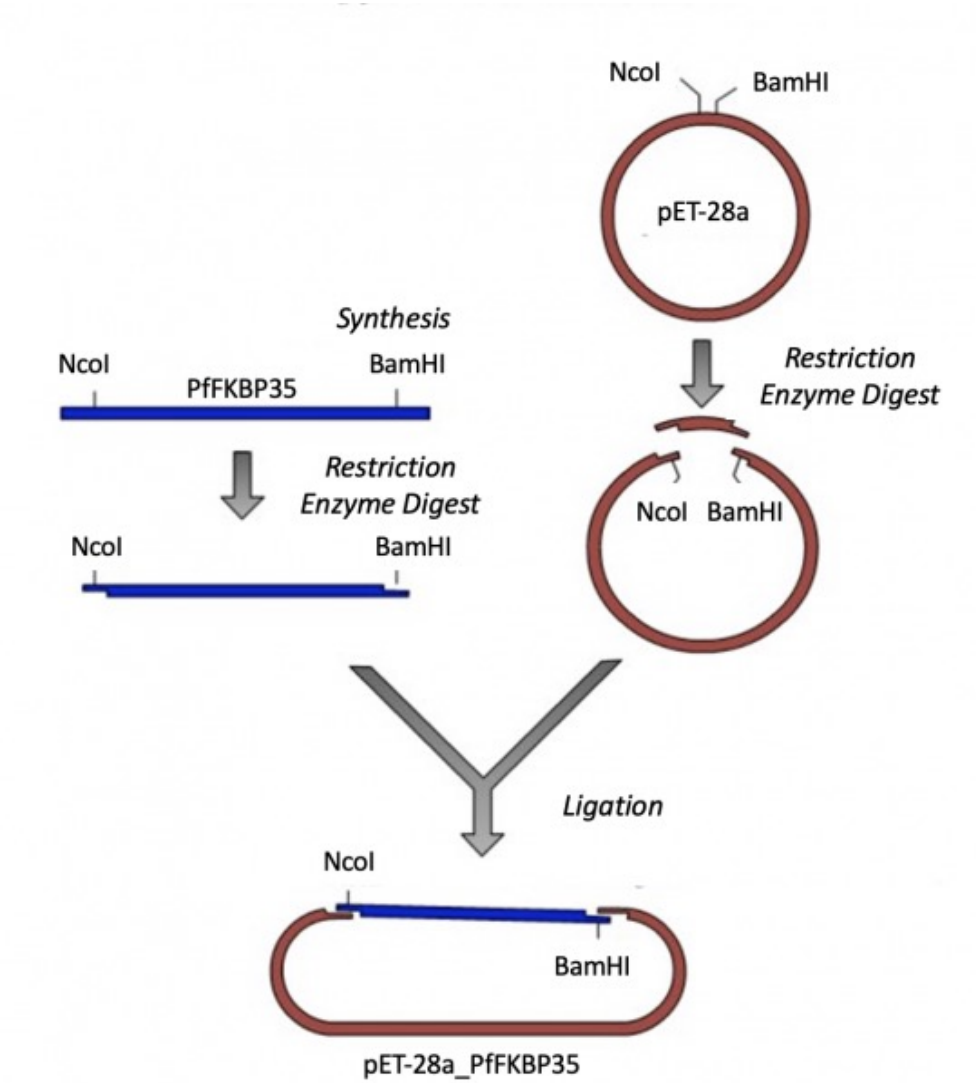
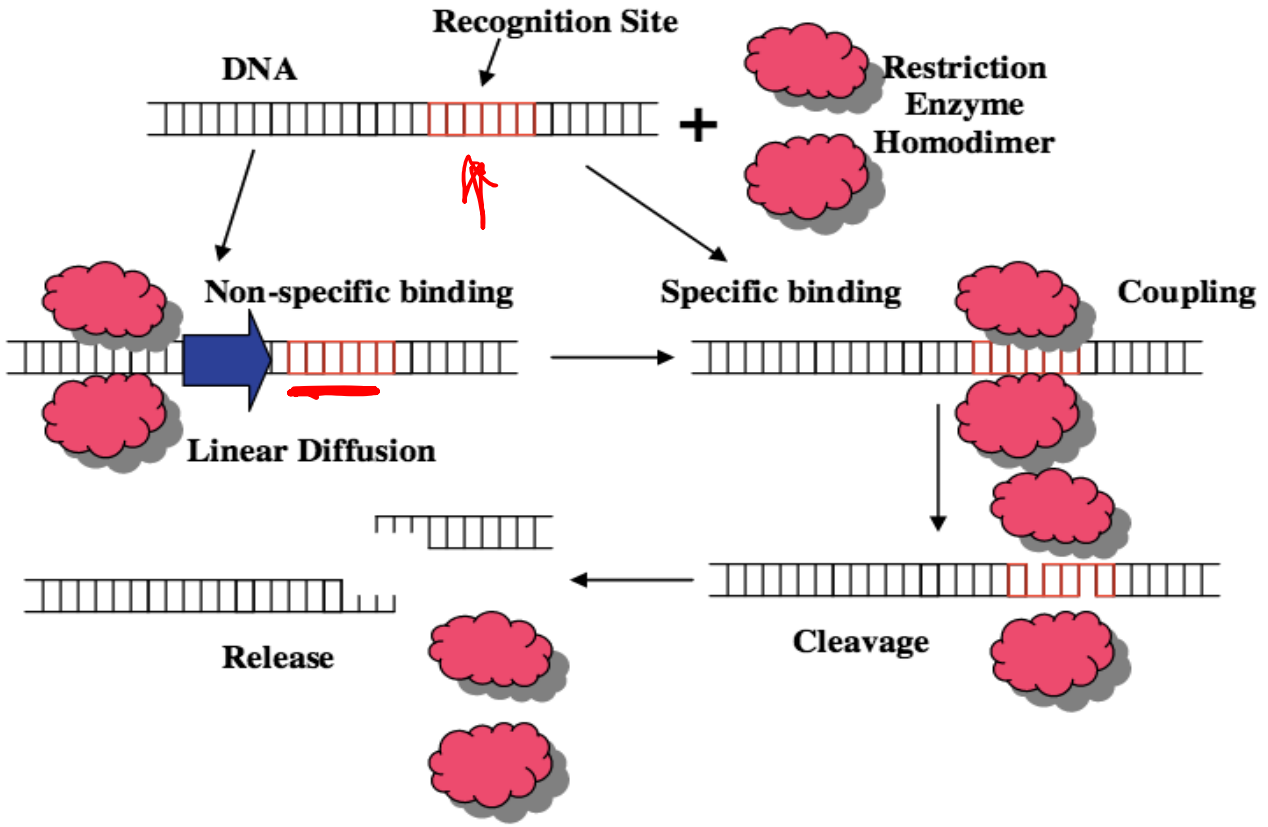


Digestion: restriction enzymes

- Function as homodimers
- Each dimer contains active site that cleaves backbone at site of palindromic recognition sequence
- Results in cleavage of both strands

→ sticky ends



Digest reagents and conditions

Reagents

→ REs

→ DNA template
- insert
- vector

→ Buffer/H_2O

Conditions

- Temperature:

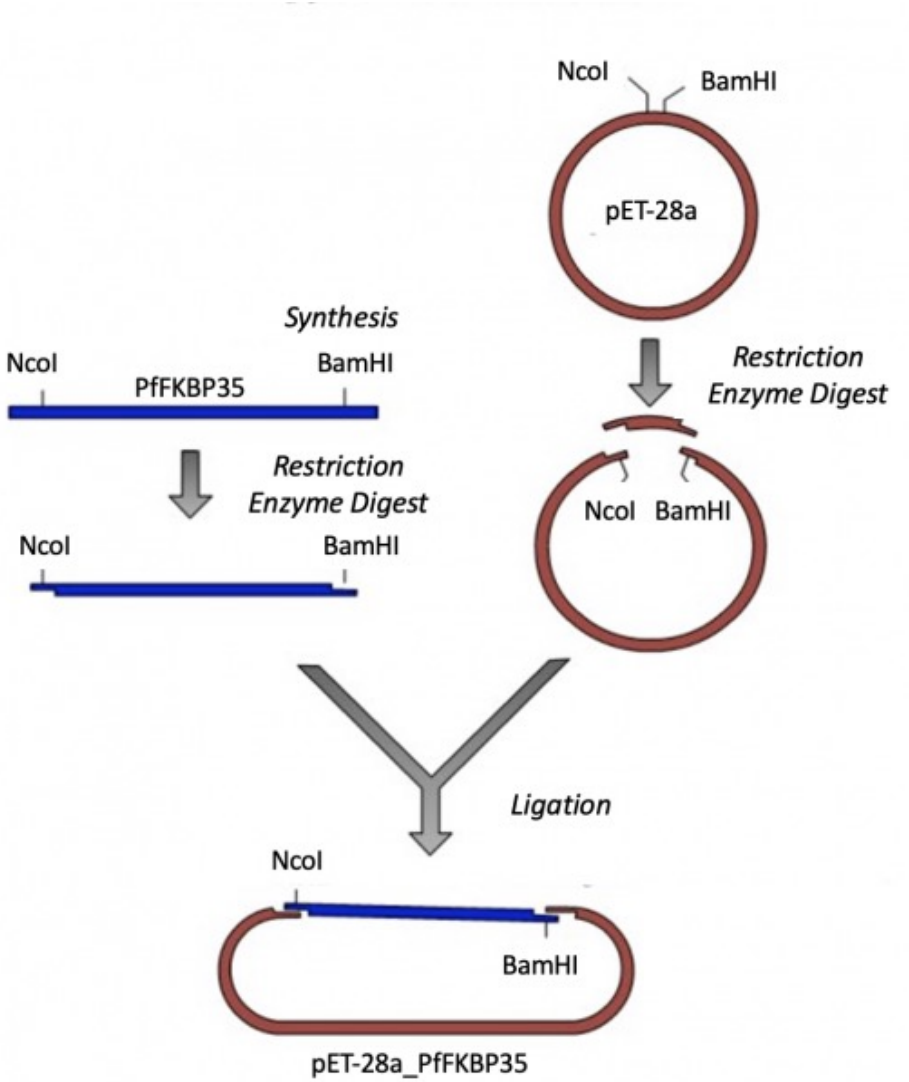
37°C

- Time:

1hr - O/N

→ Star activity

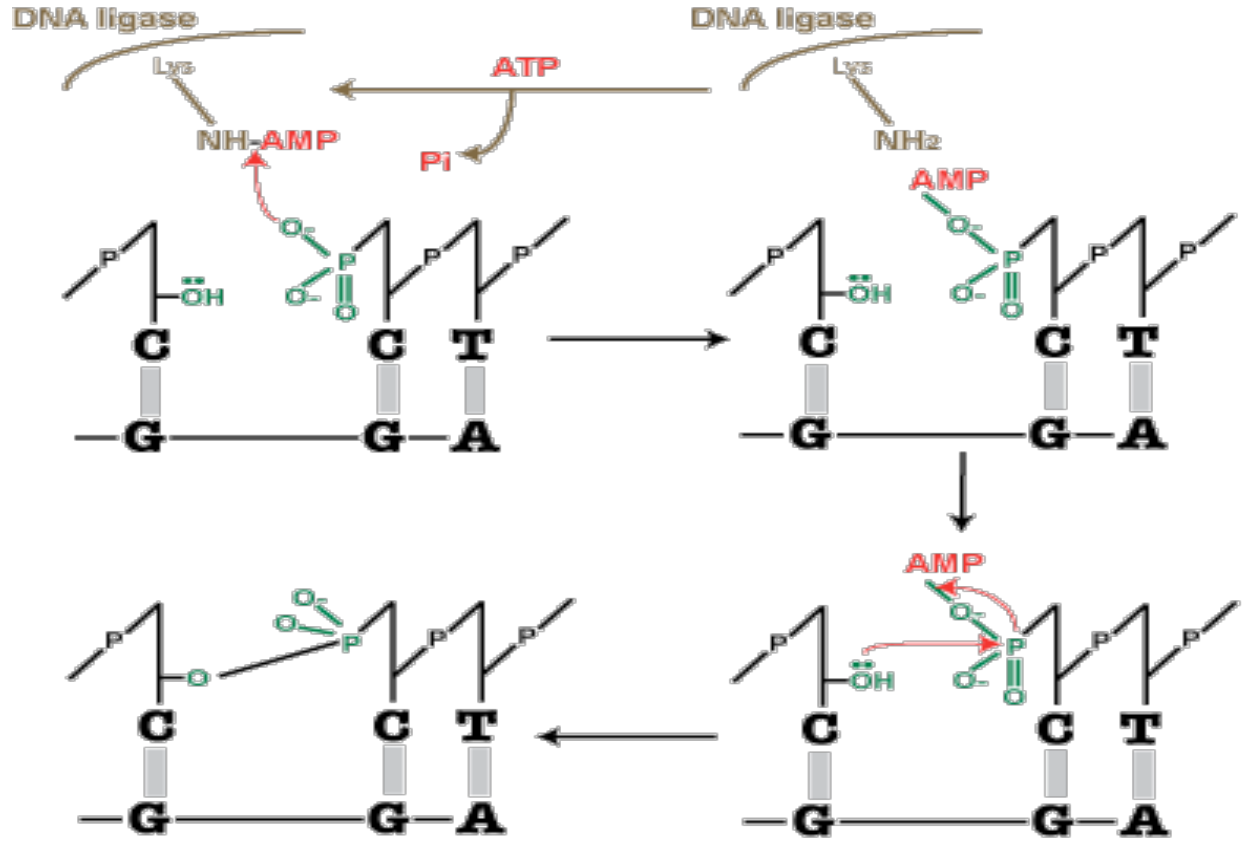
- Who are the players?
  - Insert
  - Vector
- What is the process?
  - Digestion
  - Ligation



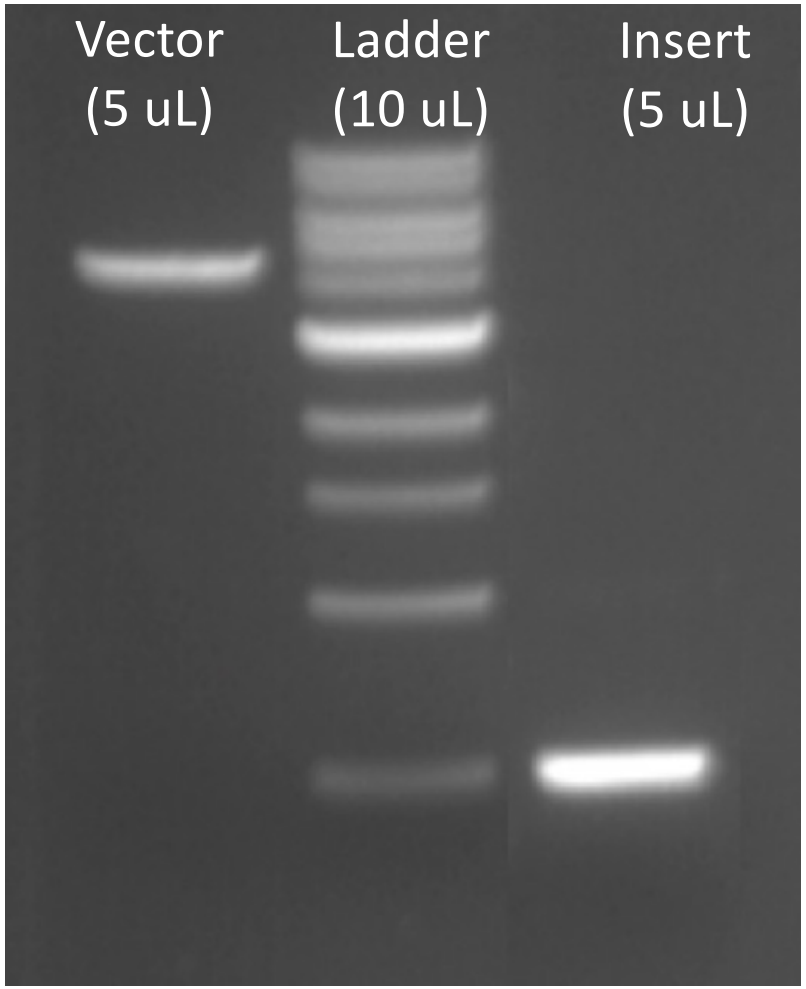
→ Bacteriophage

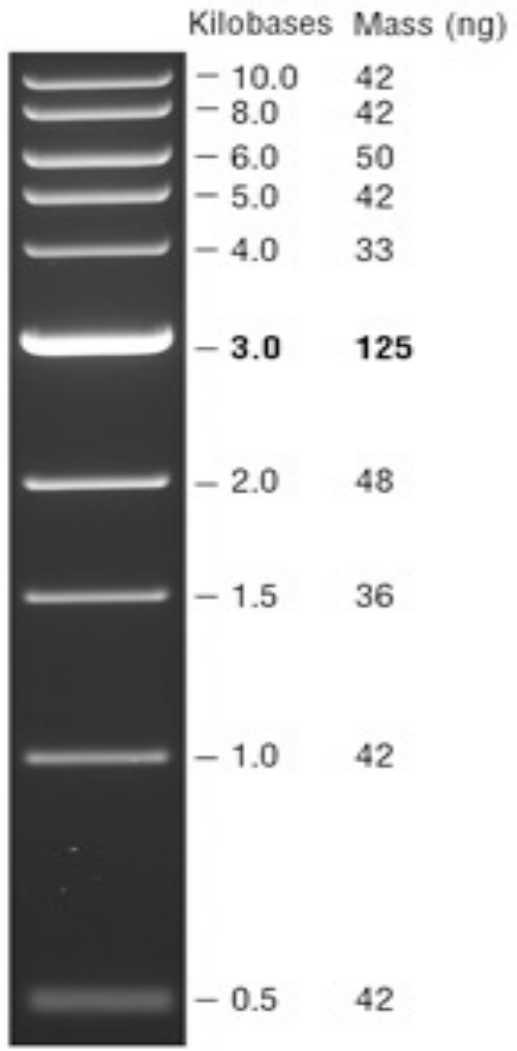
Ligation: T4 DNA ligase

- Functions as a carrier for AMP leaving group
- Forms covalent phosphodiester bond between 3’ OH acceptor and 5’ phosphate donor
- Requires ATP



Ligation conditions





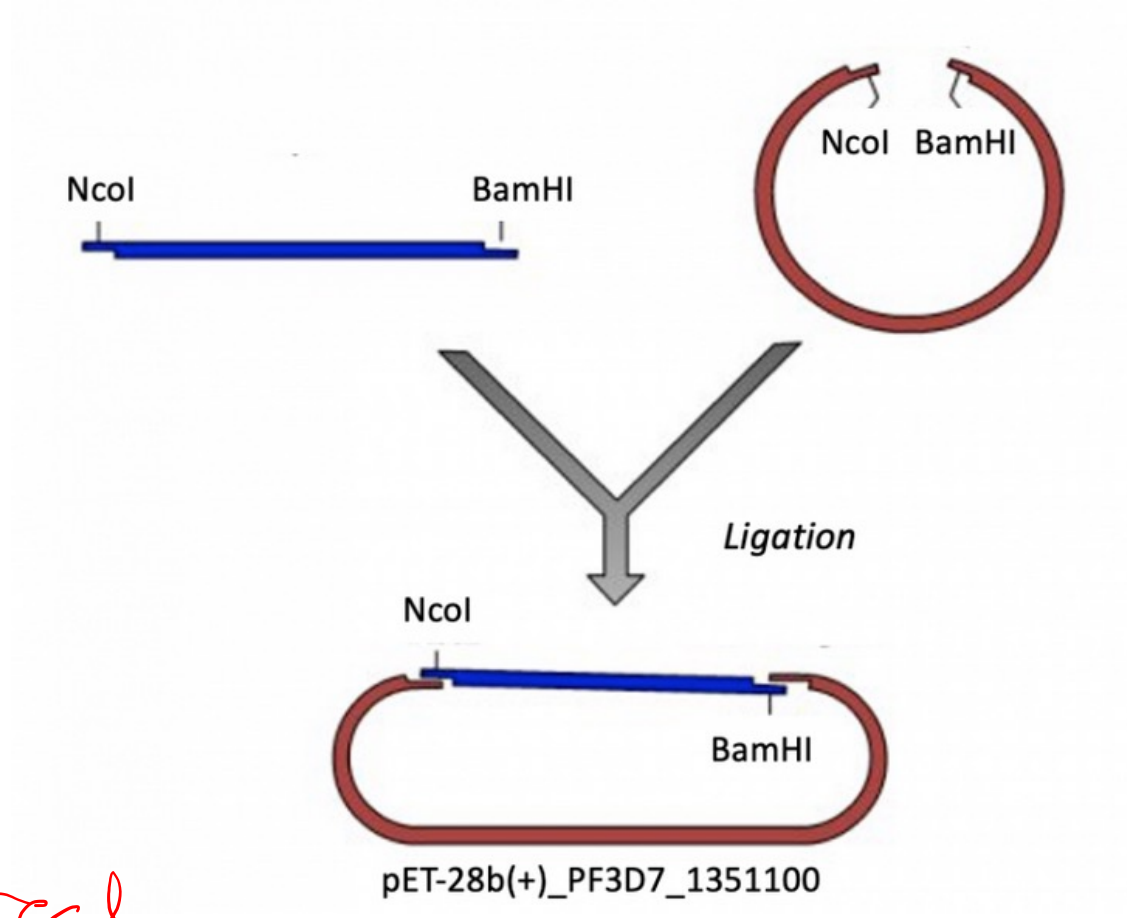
- Ideally, want 3:1 molar ratio of insert:backbone
- Calculate molar amounts from measured concentrations and known sizes of DNA molecules

Pro tips for ligation calculations

1. Determine volume of vector
   - Use backbone concentration = 50 ng/uL
   - Want 50 – 100 ng
2. Calculate moles of vector
   - Vector = (you will discover this in the exercise) bp, MW bp = 660 g/mol
3. Calculate moles of insert
   - Insert = (you will discover this in the exercise) bp, **3**:1 ratio of insert:vector
4. Calculate volume of insert
   - Use insert concentration = 25 ng/uL

How do we confirm the cloning product?

- Transformation
  - into bacteria
    E. Coli
  - amplify plasmid
- Purification
  - out of bacteria
  - mini prep / maxi prep
- Digestion
  - use RE - cut new plasmid - specific pcal pattern

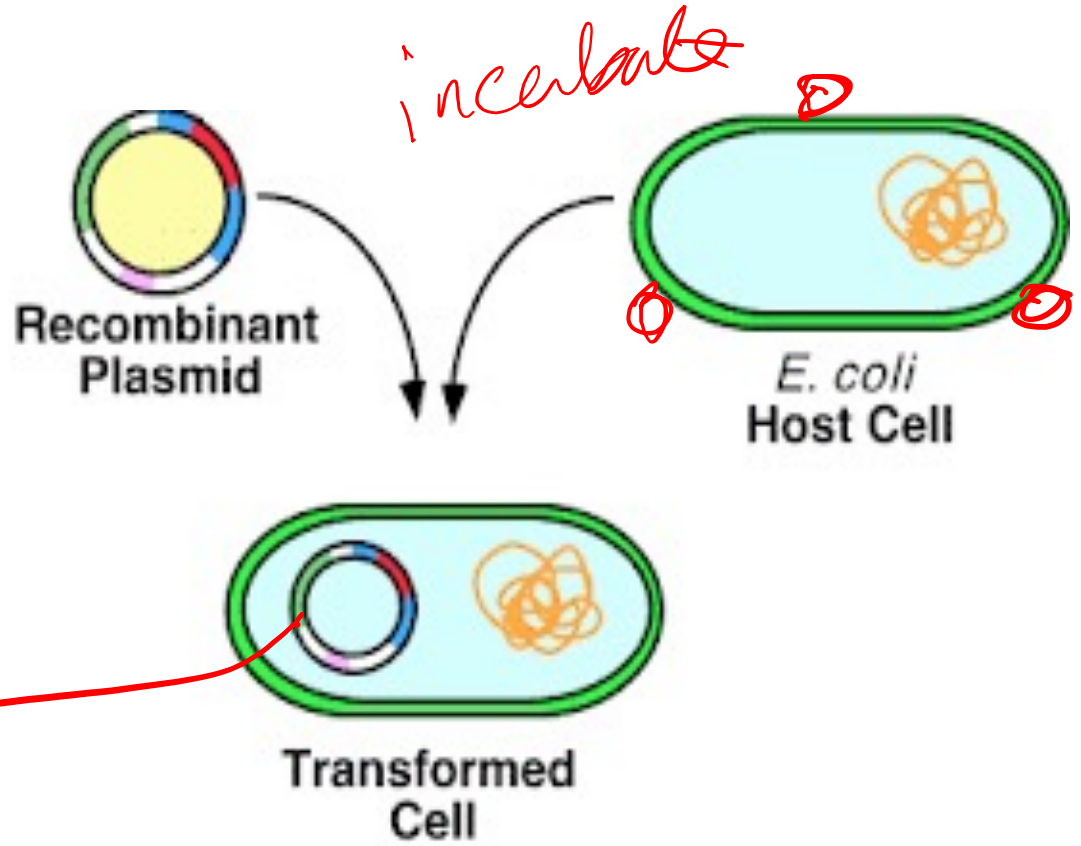


Transform plasmid into bacteria for amplification

1. Incubation
2. Heat shock

   42°C
3. Recovery
4. Selection

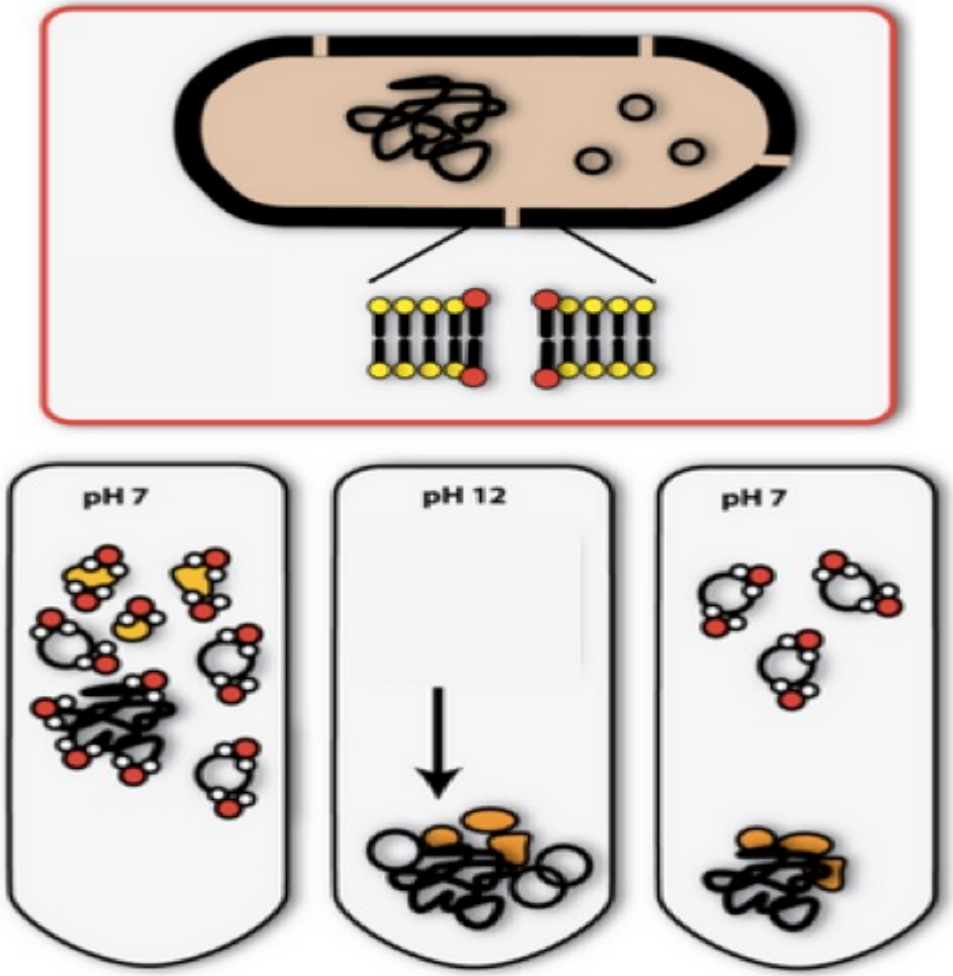
Antibiotic selection



Mini prep

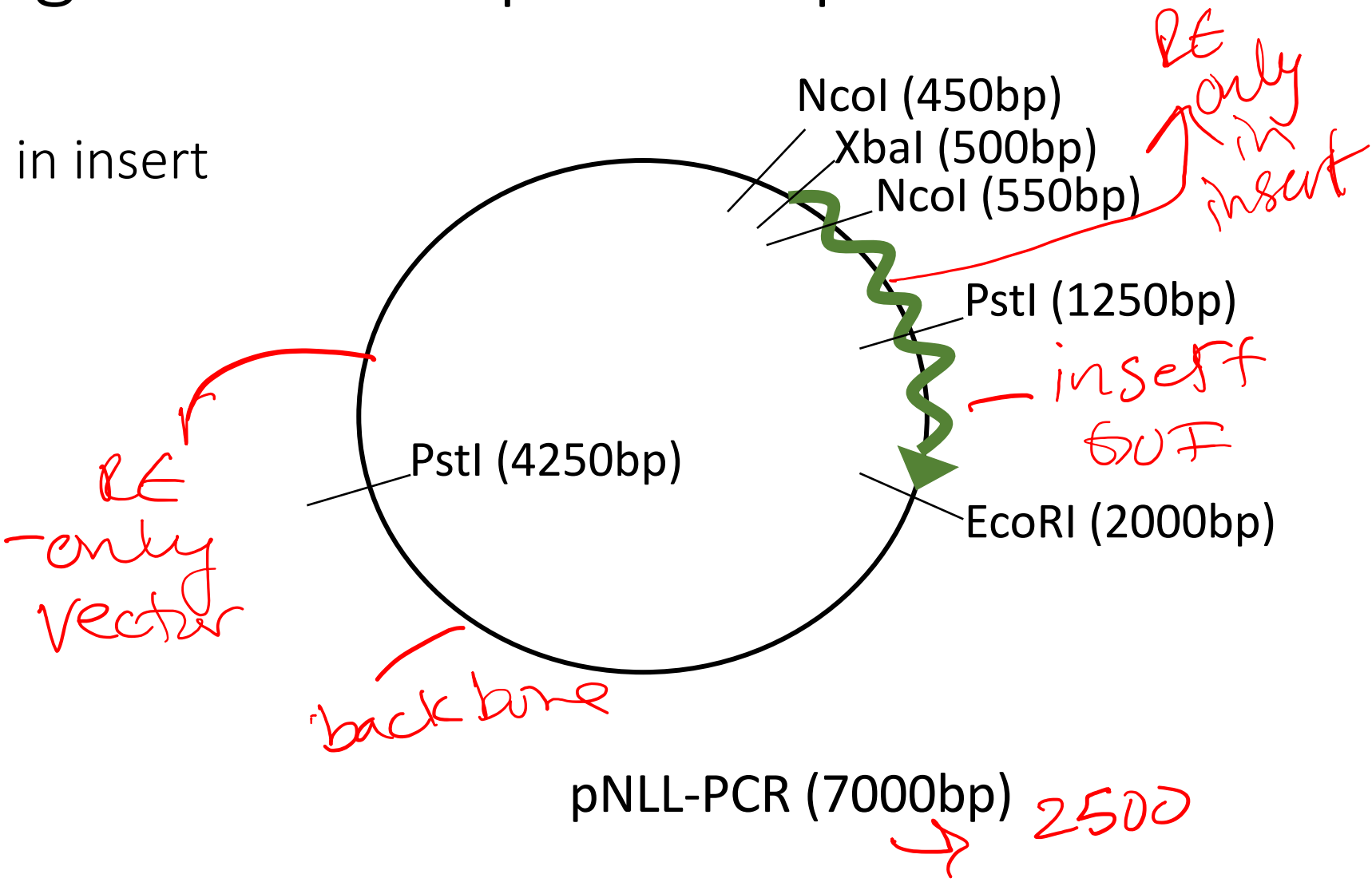
Purify amplified plasmid for confirmation

1. Resuspend cells
2. Lysis
   – DNA denaturization
3. Neutralization N3
4. Wash
5. Resuspend or elute DNA



Confirmation digest follows plasmid purification

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



For today…

- In silico cloning of your plasmid
- Set up restriction enzyme digest
  - Begin by 4:30pm

only one article / section

For M2D2…

- Sign up for your article for the Journal Article presentation on the wiki

- Read your journal article, chose the figures you want to be the focus of your story, and answer the questions on the wiki