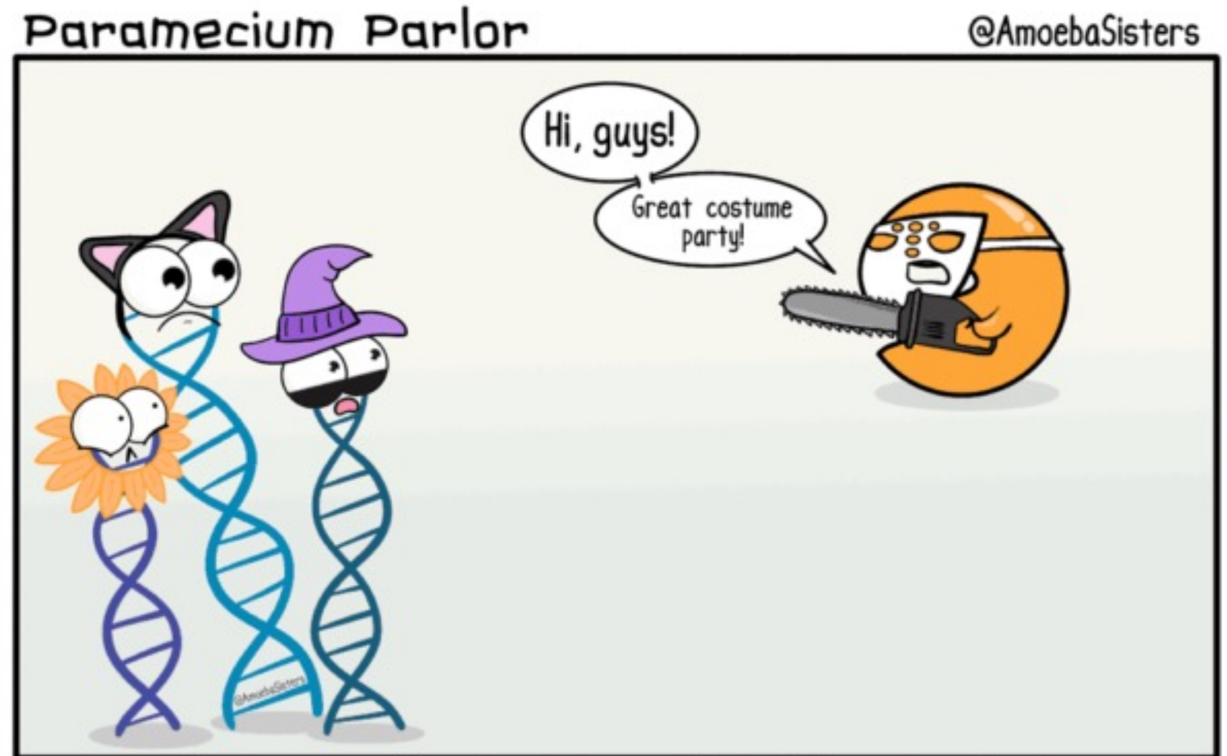
M2D1: Complete in-silico cloning of protein expression plasmid

1. Prelab discussion
2. Complete DNA engineering exercise



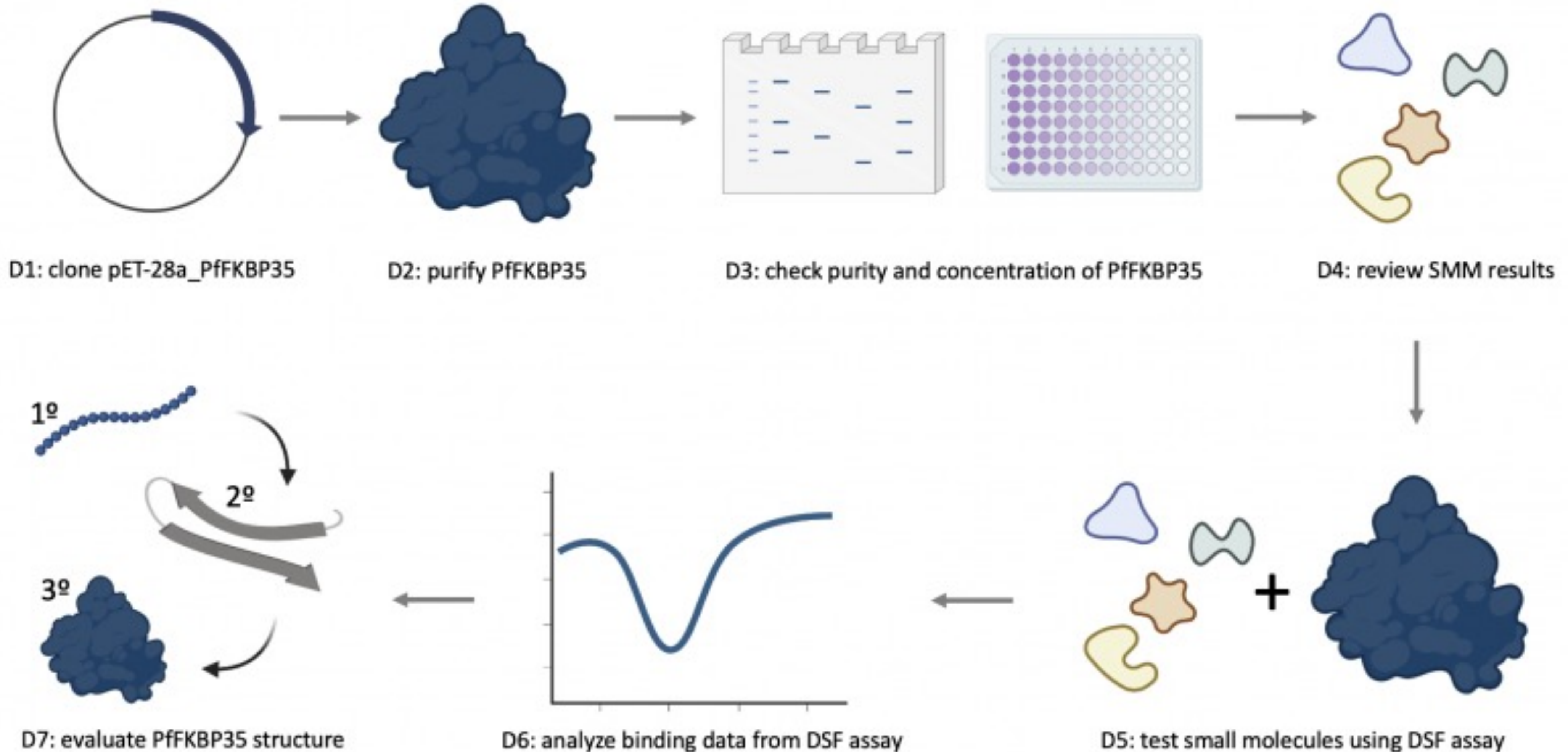
That was the last year the DNA invited the restriction enzyme to their Halloween party.

Mod 2 Major Assignments

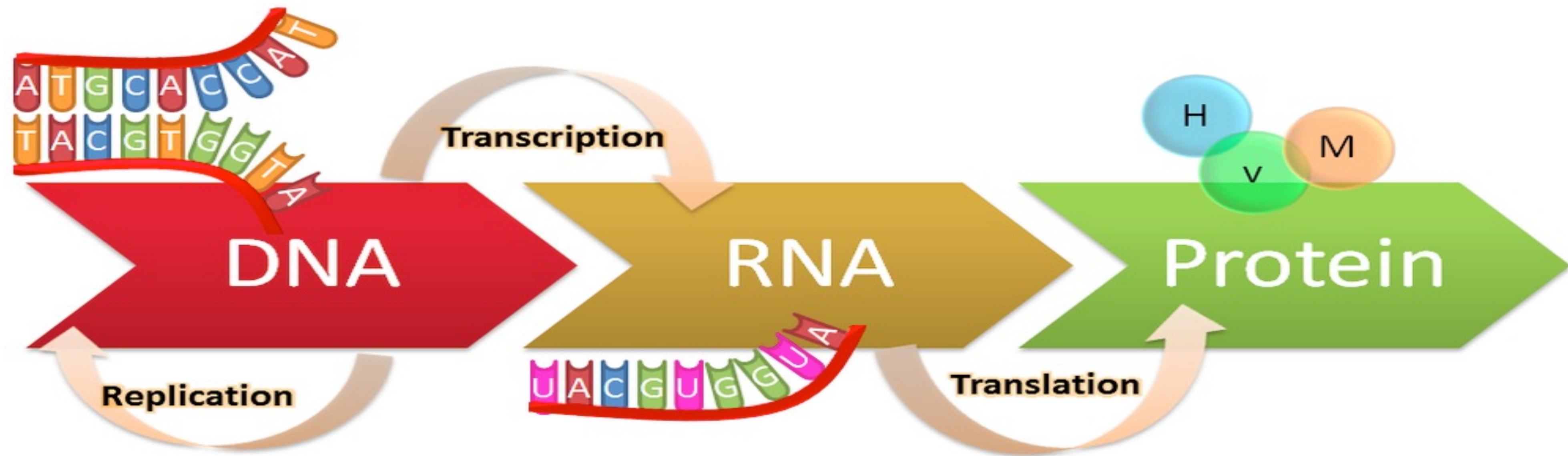
- Journal Article presentation (15%)
  - Individual
  - Presentations on 11/1 & 11/3
- Research article (20%)
  - Individual
  - due 11/21
- Laboratory quizzes (collectively 5%)
  - M2D4 and M2D7
- Notebook (collectively 5%)
  - one entry will be graded by Chyna 24 hr after M2D7
- Blog (part of 5% Participation)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

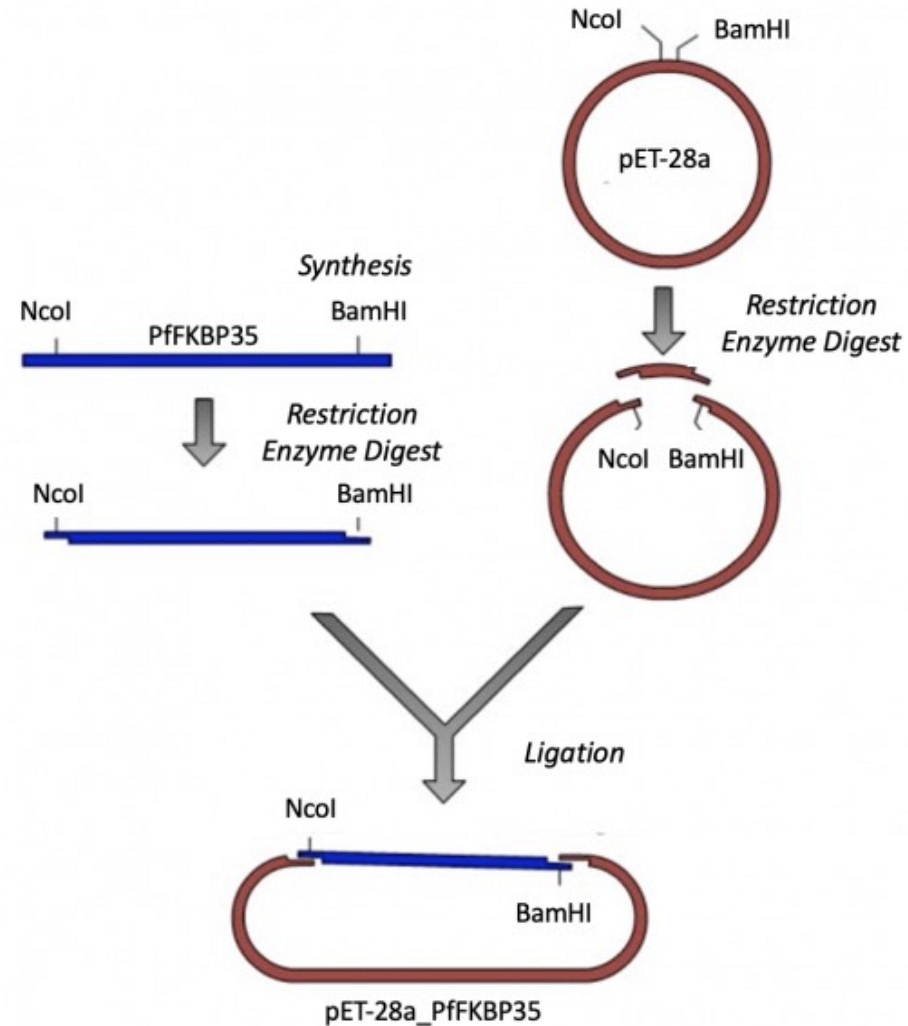
- Chemically synthesize protein by successively linking each amino acid
  - Complicated, **have to make each protein,** expensive

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

- Create DNA encoding the protein
  - 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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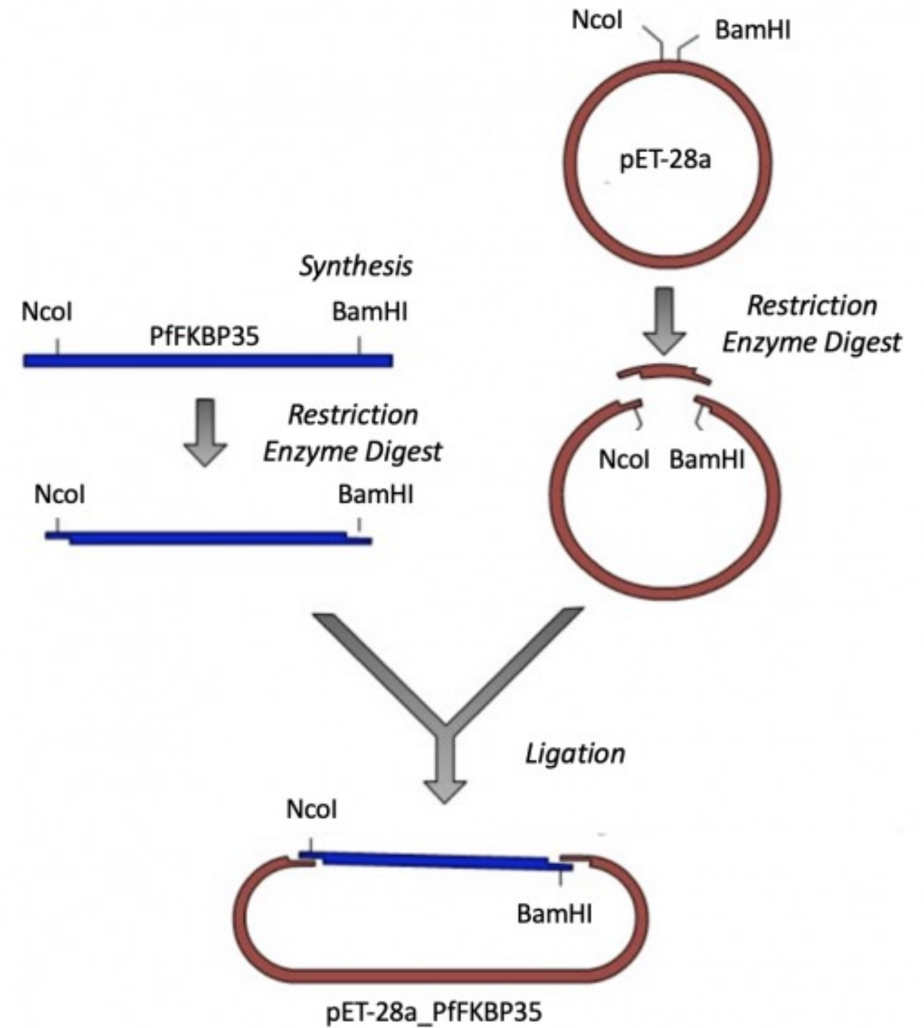
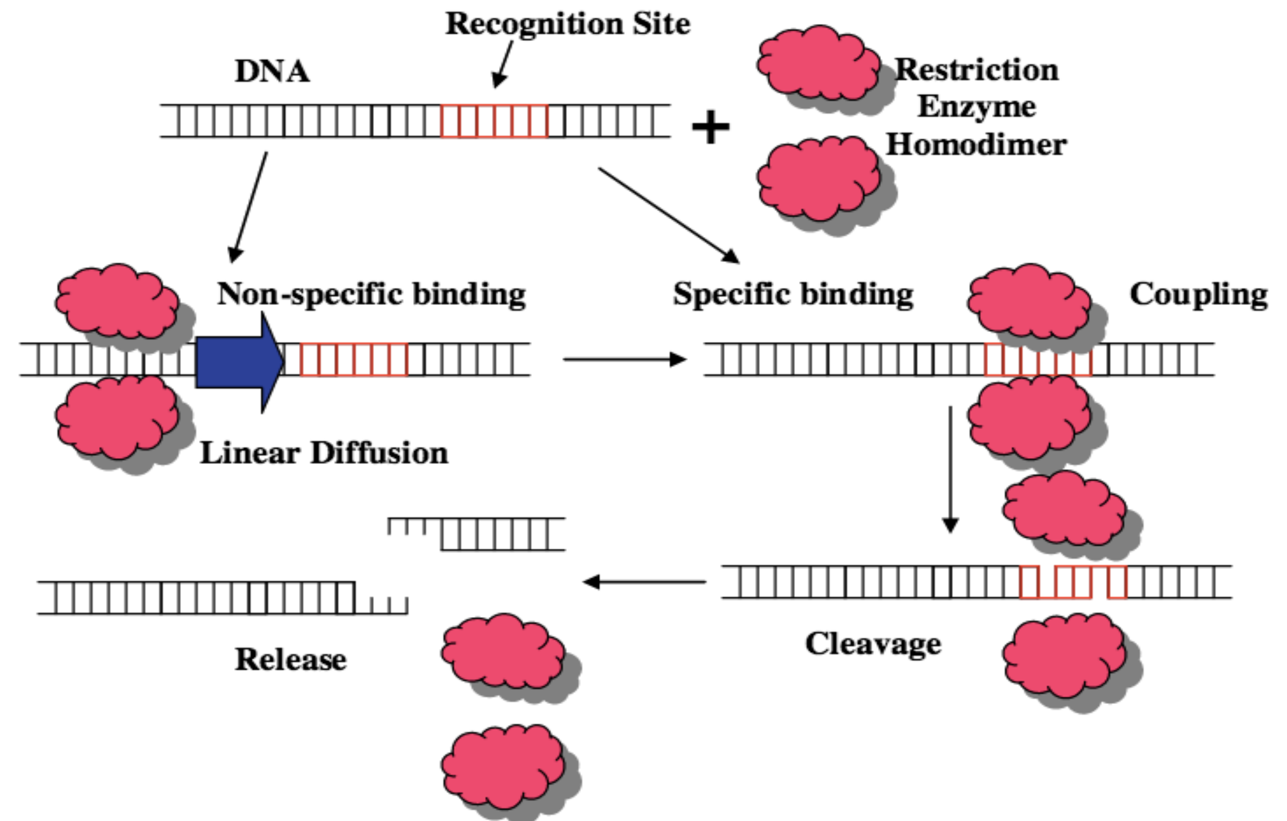


Digestion: restriction enzymes

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



Digest reagents and conditions

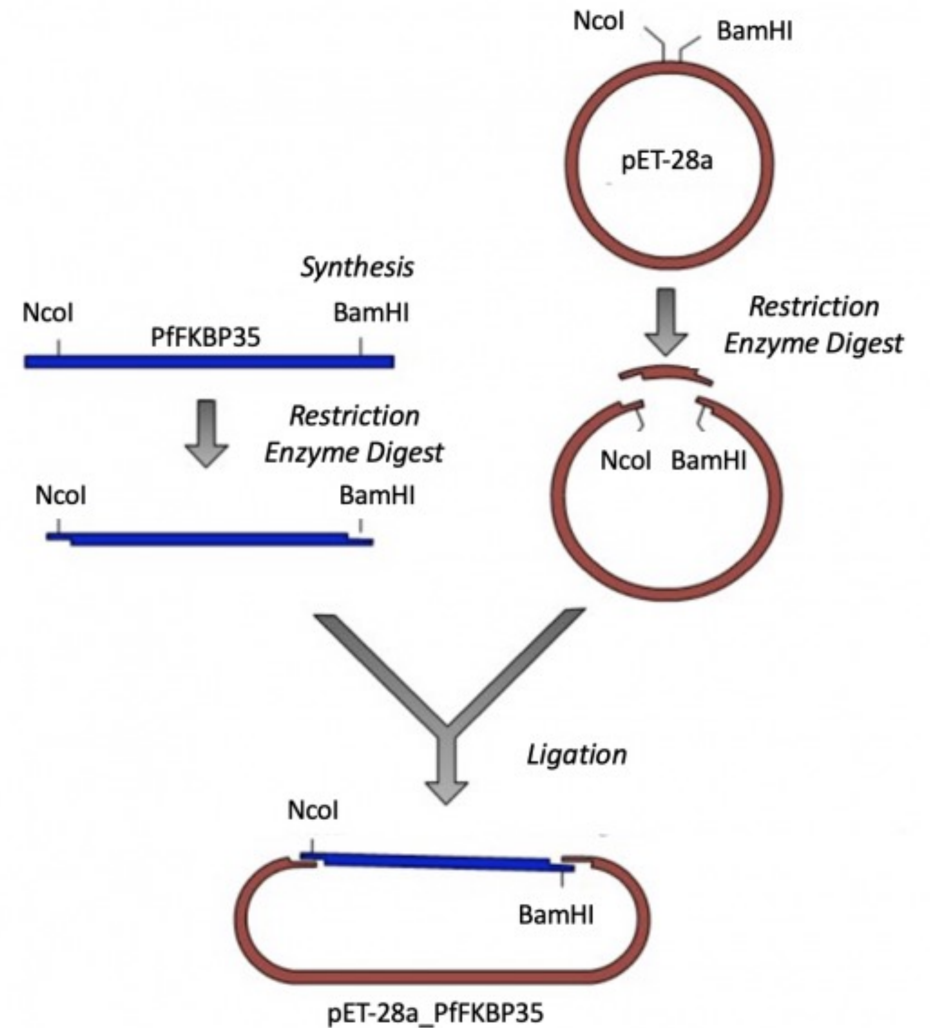
Reagents

Conditions

- Temperature:

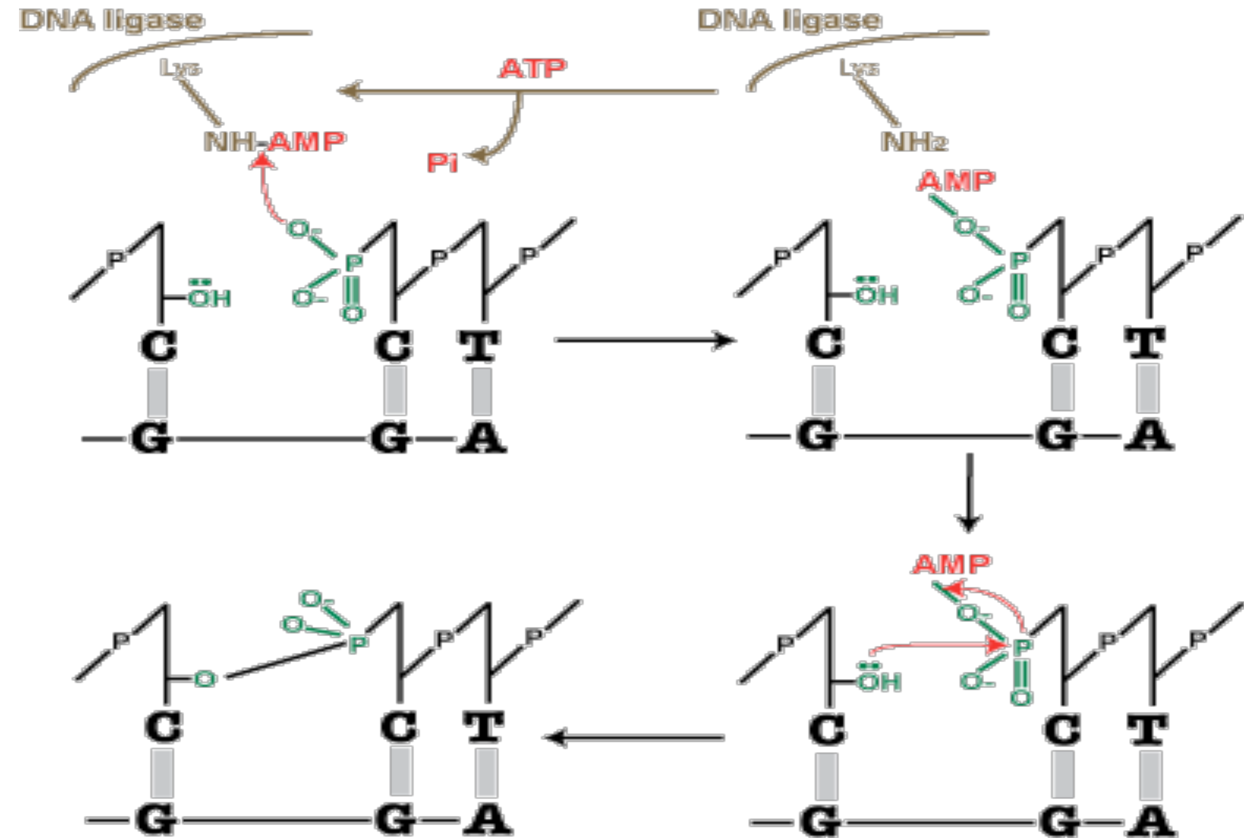
- Time:

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

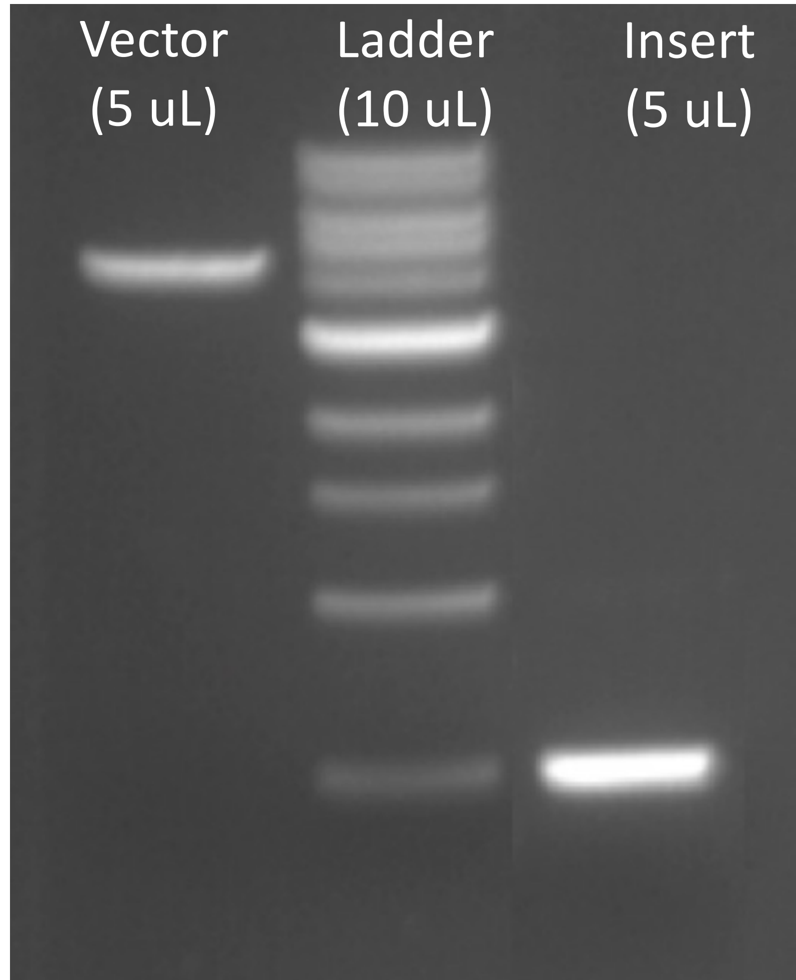


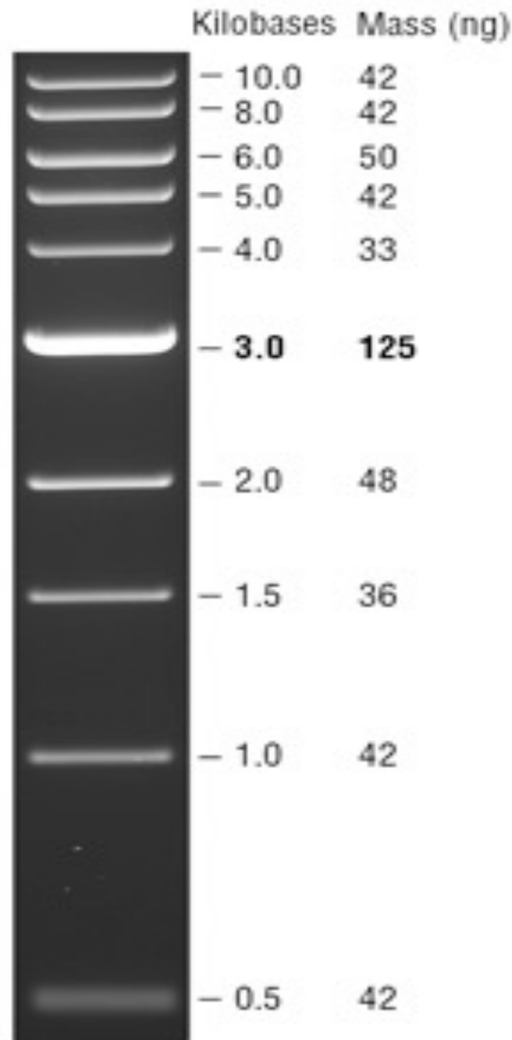
Ligation: T4 DNA ligase

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



Ligation conditions





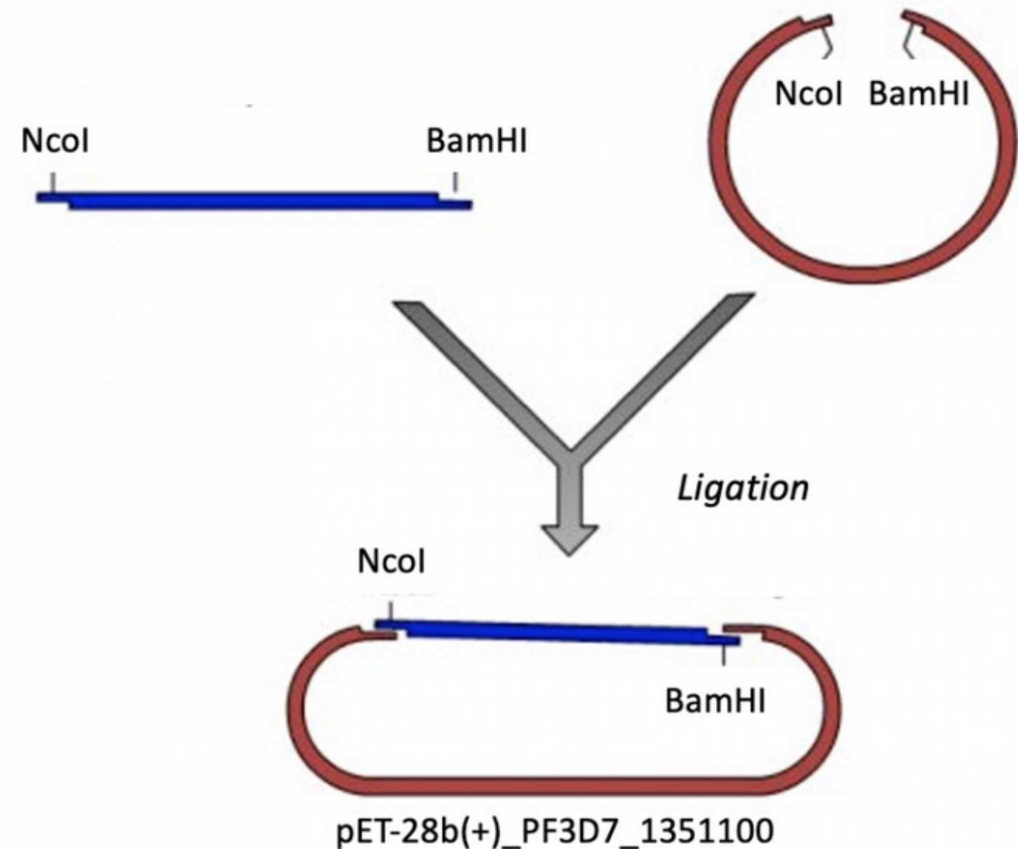
- Ideally, want 3:1 molar ratio of insert:backbone
- Calculate molar amounts from measured concentrations and known sizes of DNA molecules

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

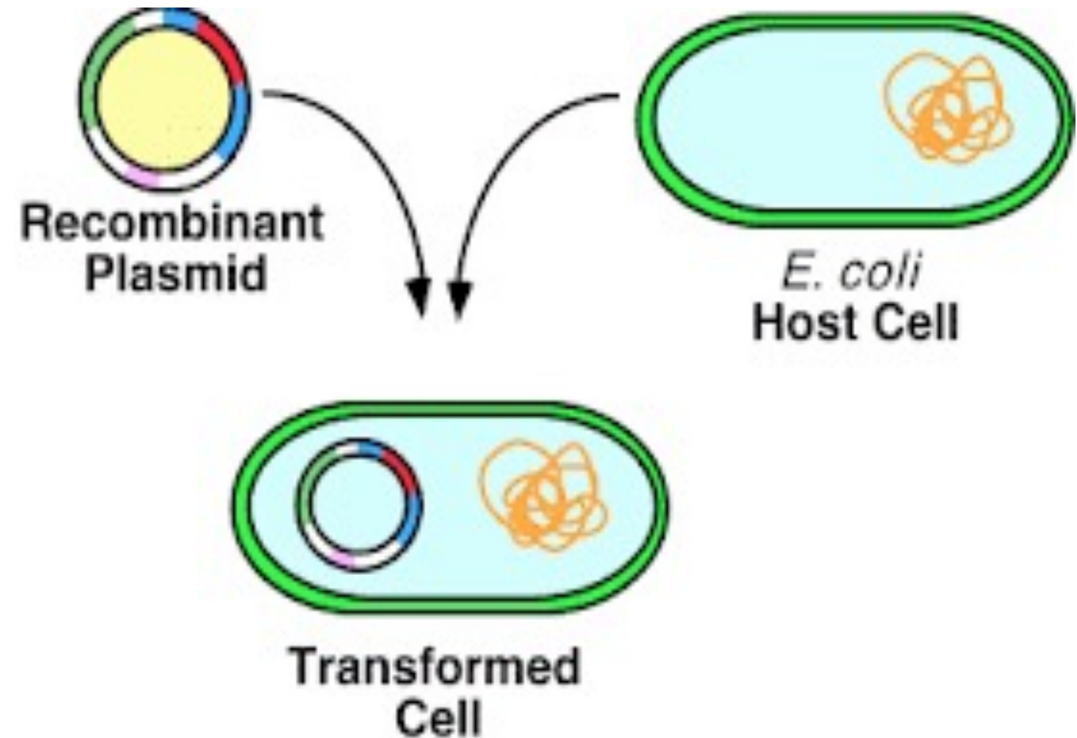
How do we confirm the cloning product?

- Transformation
- Purification
- Digestion



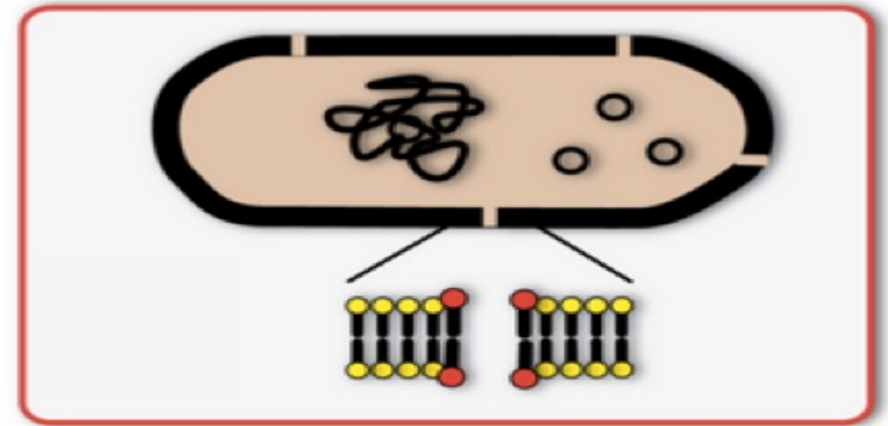
Transform plasmid into bacteria for amplification

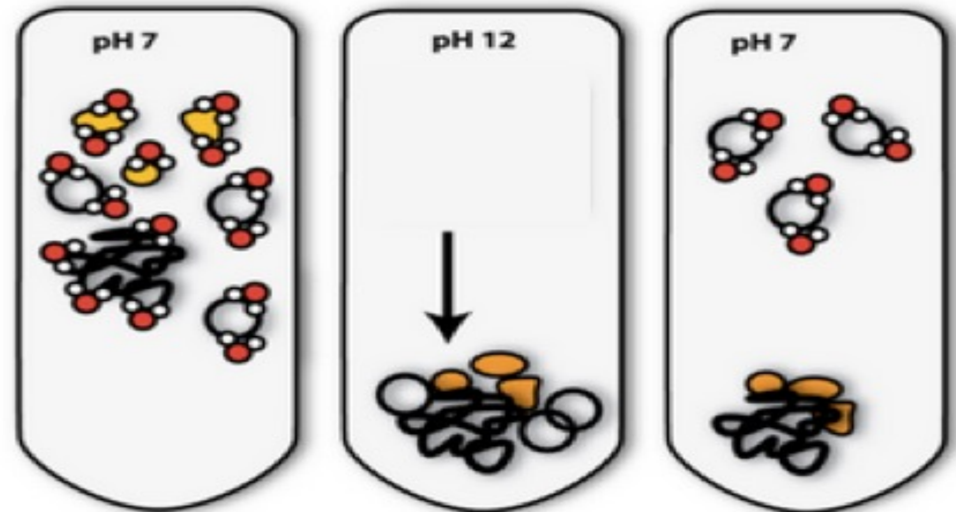
1. Incubation
2. Heat shock
3. Recovery
4. Selection



Purify amplified plasmid for confirmation

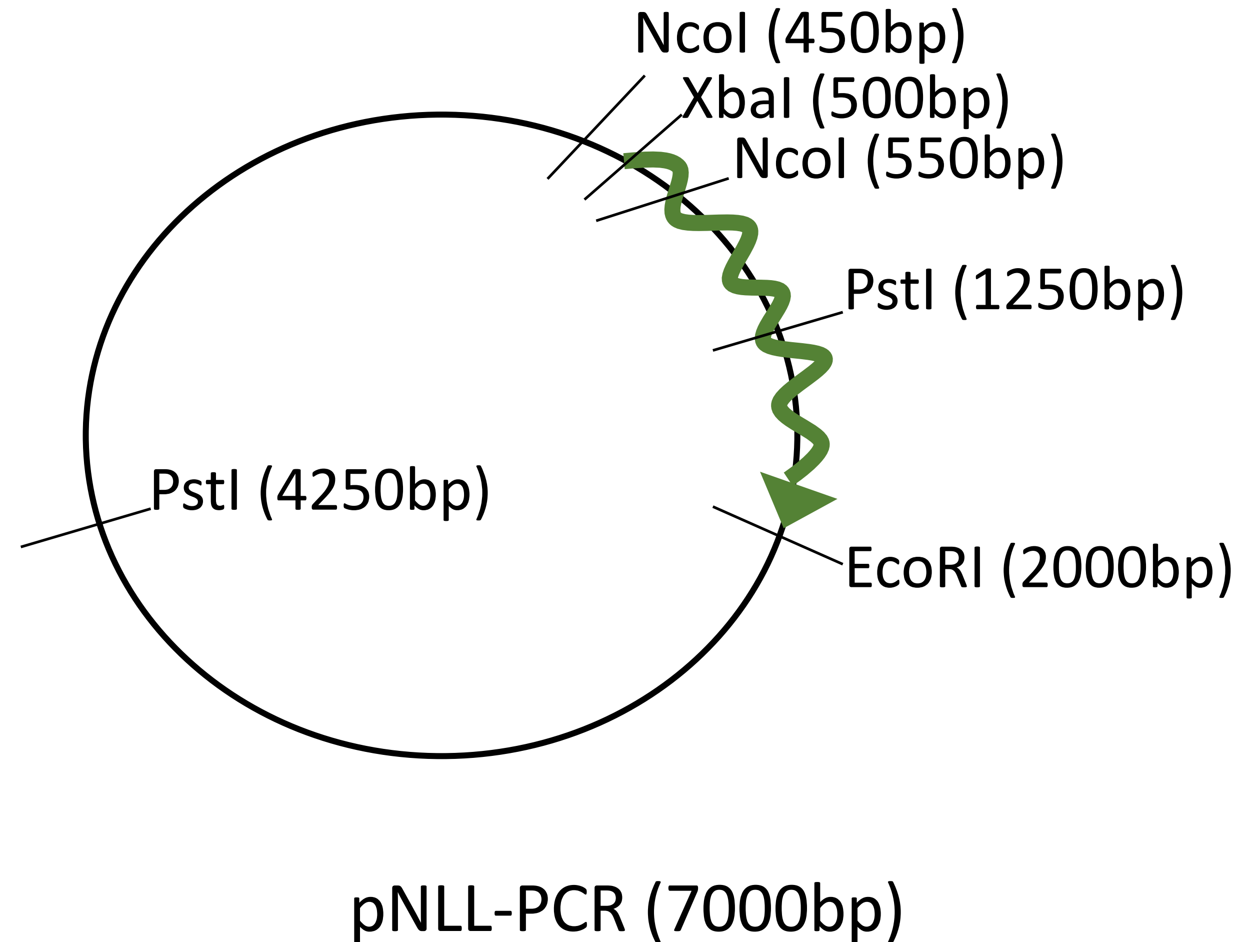
1. Resuspend cells
2. Lysis
3. Neutralization
4. Wash
5. Resuspend or elute DNA





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

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