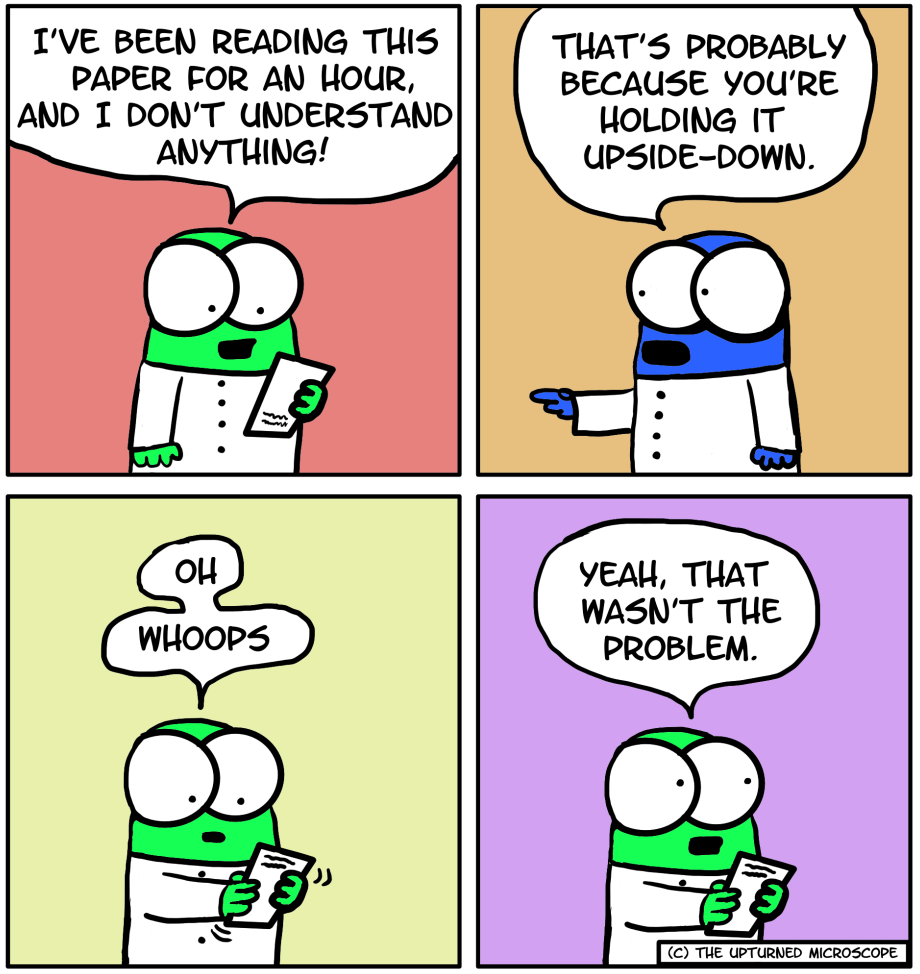
M2D1: Complete in-silico cloning of protein expression plasmid

1. Prelab discussion

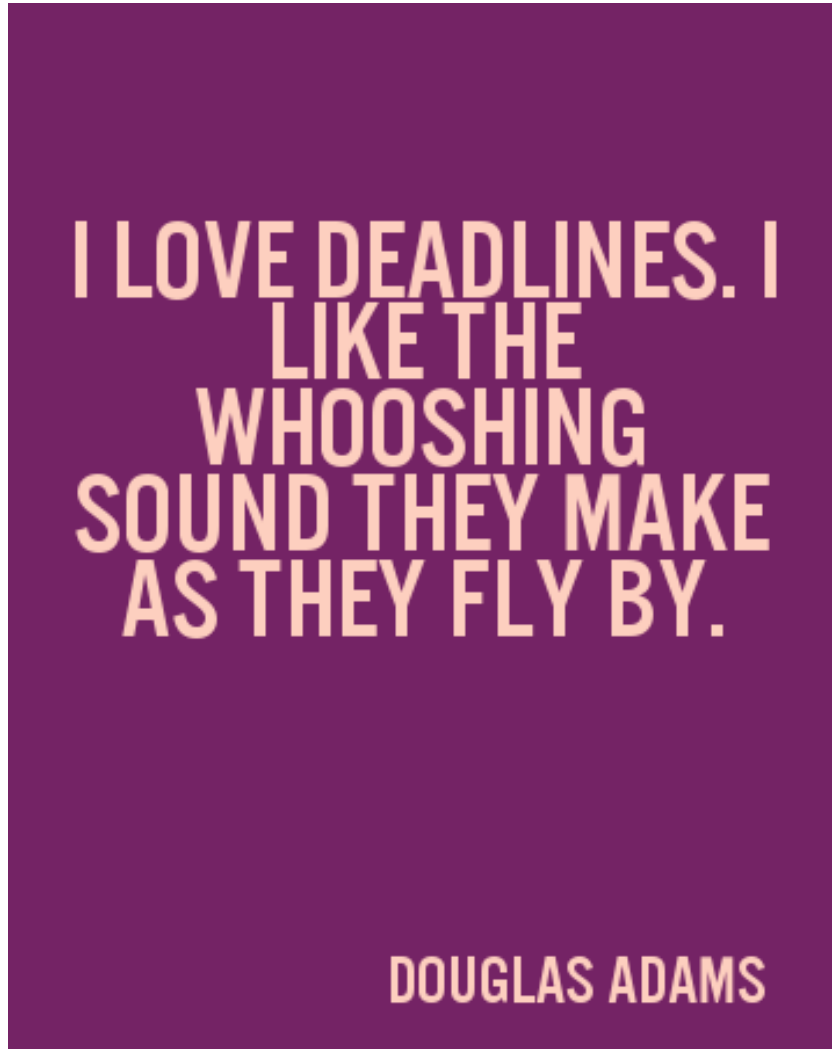
2. Complete *in silico* cloning exercise

3. Set up confirmation digest



Mod 2 Major Assignments

- Journal Article presentation (15%)
  - Individual
  - Presentations on 10/24 & 10/26
- Research article (20%)
  - Individual
  - due 11/20
- Laboratory quizzes (collectively 5%)
  - M2D4 and M2D7
- Notebook (collectively 5%)
  - Entry graded by Simone 24 hr after M2D7
- Blog (part of 5% Participation)
  - due 10/28 & 11/21 via Slack channel



Homework

Choosing a story for your Journal Article presentation

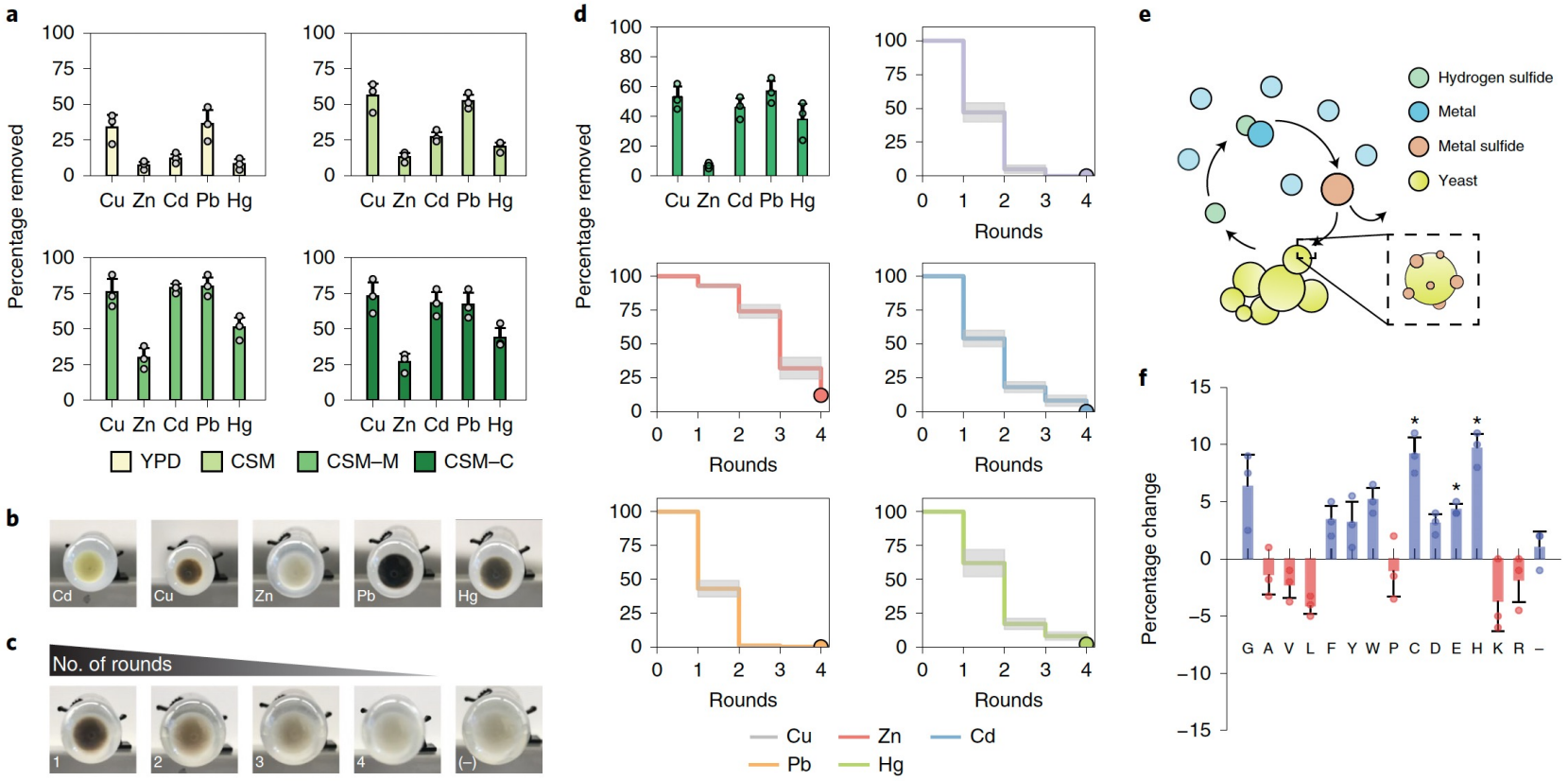
Map out a story from your Journal Article using figures

- In the Journal article presentation you will take your selected paper and present it in **10 minutes**
  - It's almost impossible to present an entire paper effectively in that time
- Most papers have a couple of **main storylines** that lead to an overall conclusion
  - Choose one of those as your focus and present the data that builds that part of the story

- Answer wiki questions to help you map out a story you can tell in 10 minutes

- ☐ What is the main conclusion of the paper?
- ☐ What four figures are the most important in supporting the main conclusion? Why?
- ☐ How do the figures work together to tell a story? How does this story lead you to the main conclusion of the paper?

What counts as a figure?

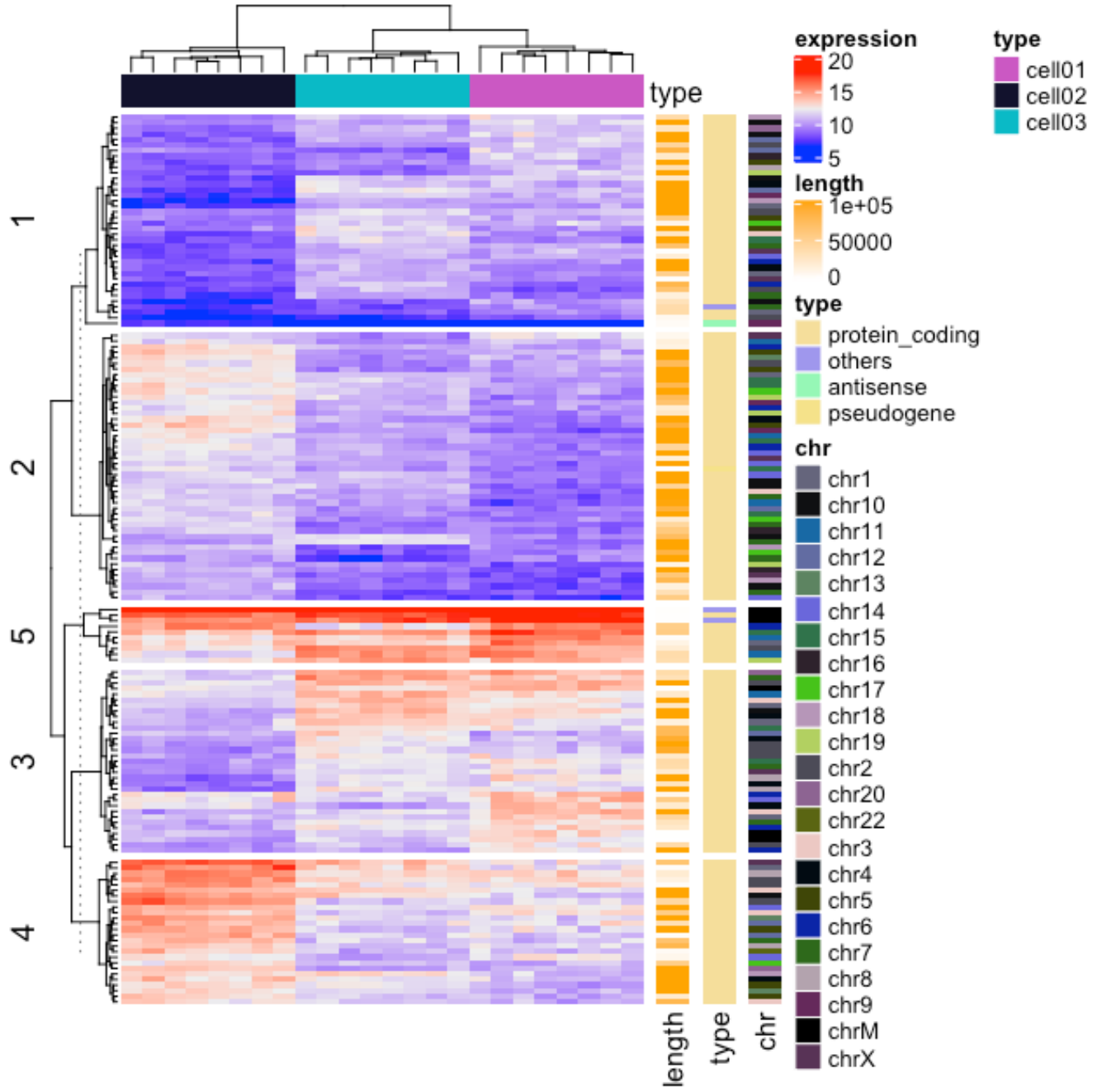


- Does it work to show this entire multipanel figure on a slide?
- Think about the story
  - What panel(s) are going to give the best **take-home message** to support the story
  - What panels will **present** well?
  - What experiments do you **understand** the best?

What if you have a complicated but necessary figure?

- If you can’t avoid it, give a **potential strategy** for how you will make it manageable in your presentation

- **Remember:** you can’t put data in a presentation that you don’t mention



Labwork

Clone a protein expression plasmid

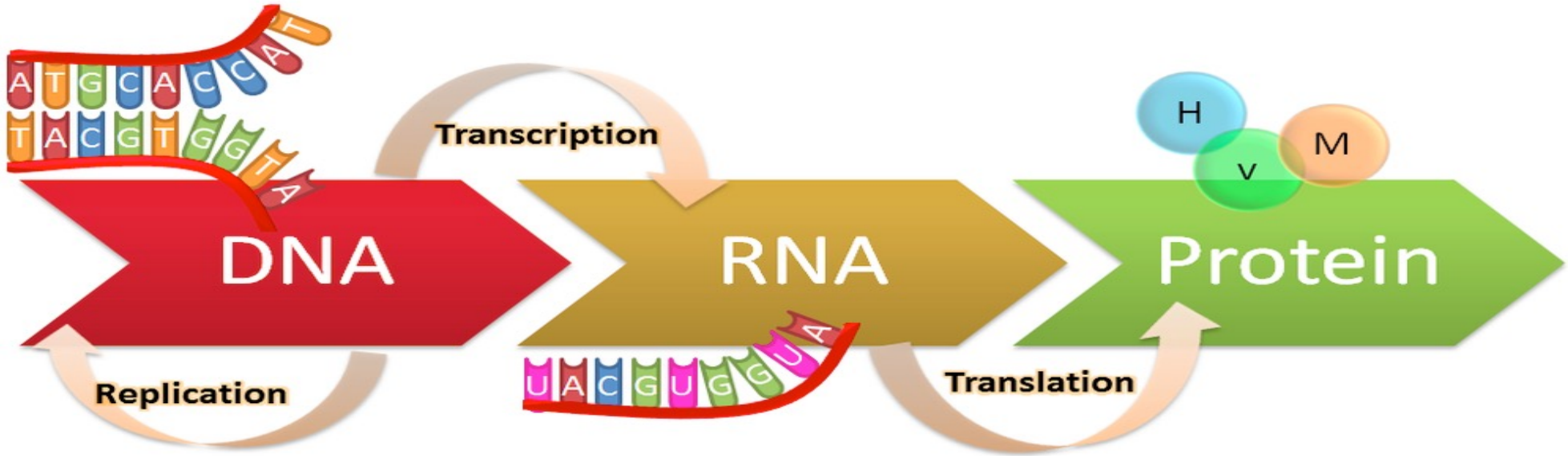
Mod 2 Overview: Drug discovery

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

- Malaria is a life-threatening infectious disease caused by parasites including the species *Plasmodium falciparum*
  - Drug resistance is a serious problem in treating malaria worldwide so **developing novel therapeutics is essential**

- This module will focus on characterizing a set of **small molecules** that could eventually become valuable therapeutics
  - These molecules are proposed to interact with a P. falciparum protein known as **PfFKBP35**
  - Start these experiments by making and purifying this protein for study

How are proteins made?



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

What if we want to make a specific protein?

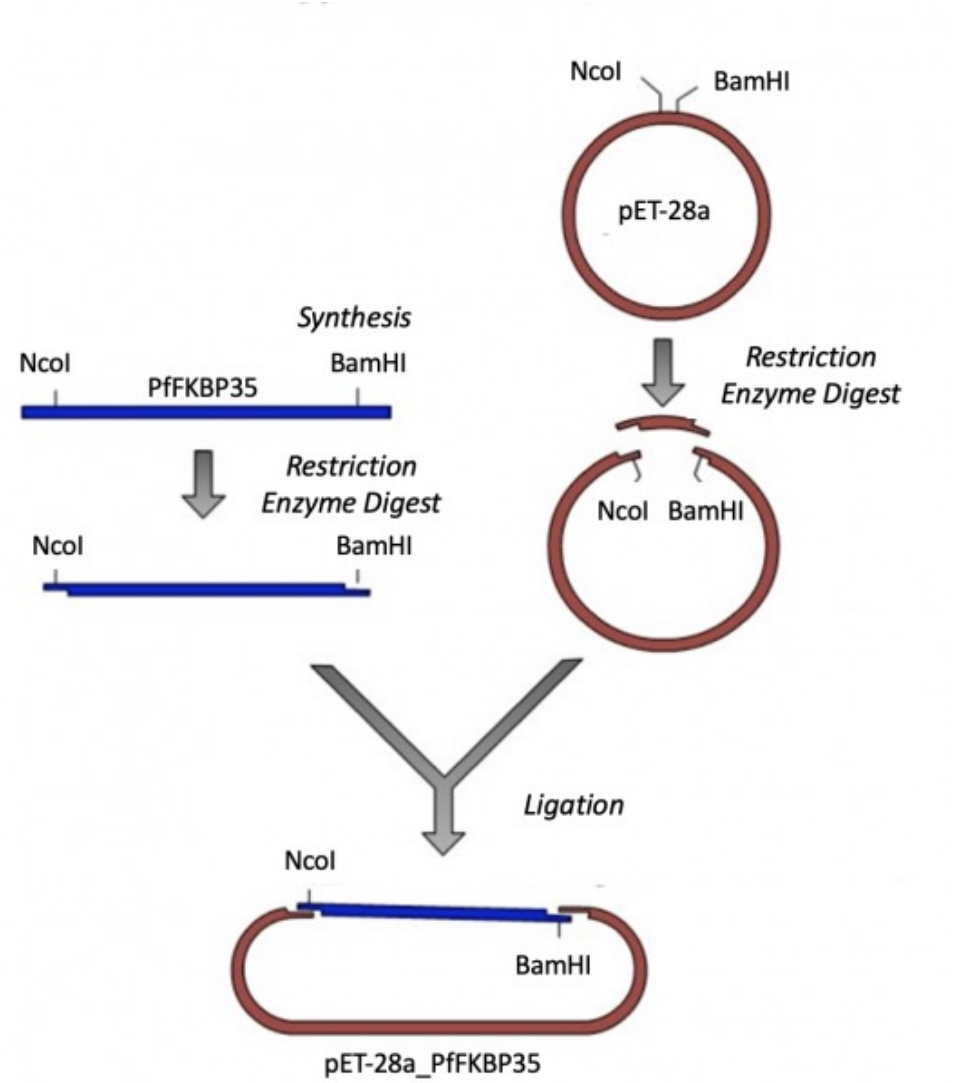
- Chemically synthesize protein by successively linking each amino acid
  - Complicated, **have to make each protein,** expensive

- 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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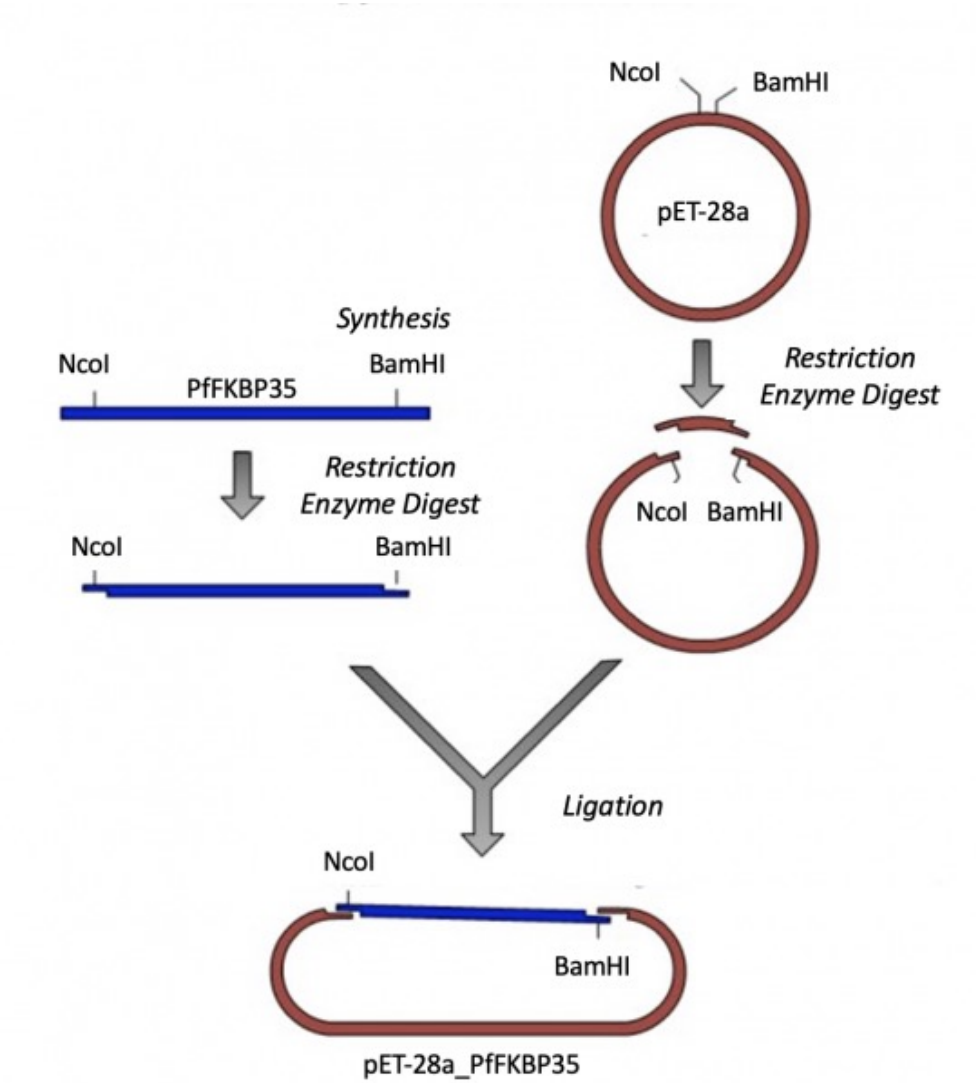
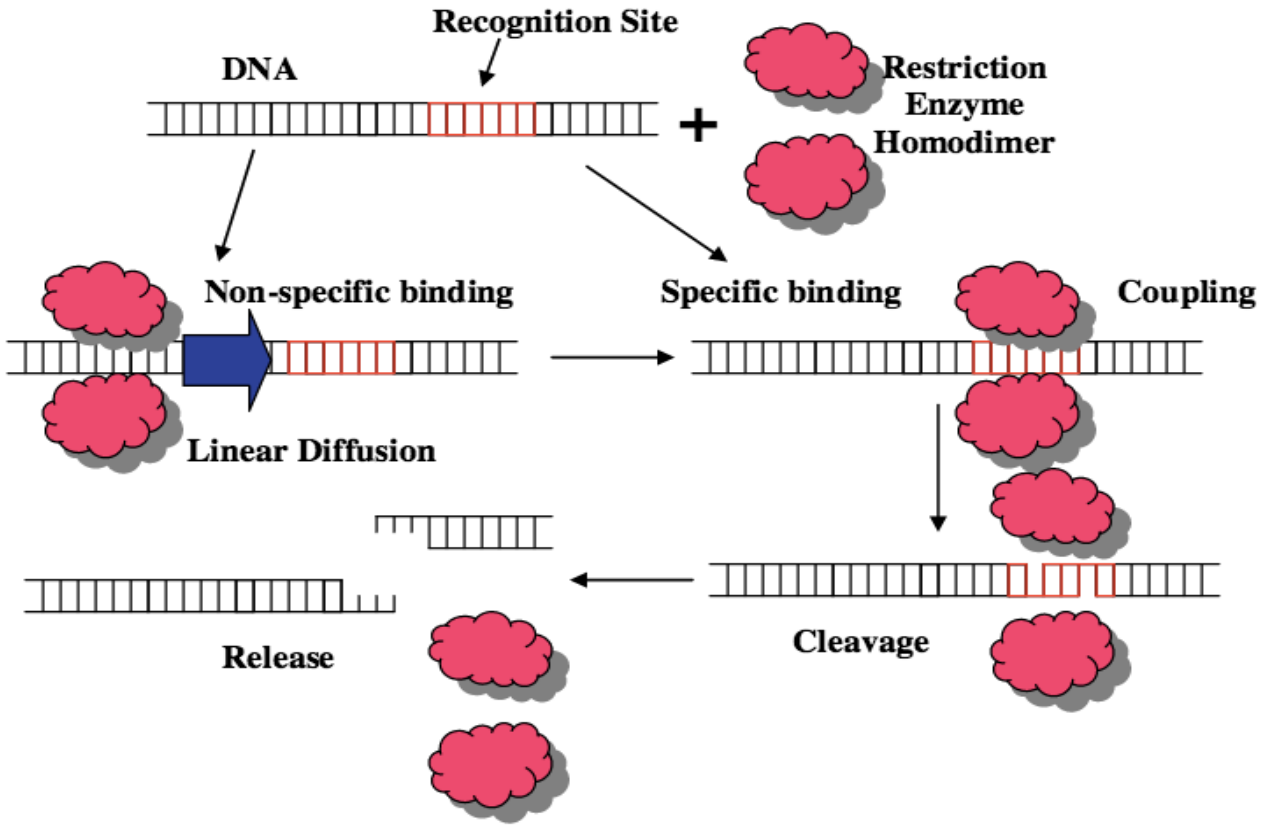


Digestion: restriction enzymes

- 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

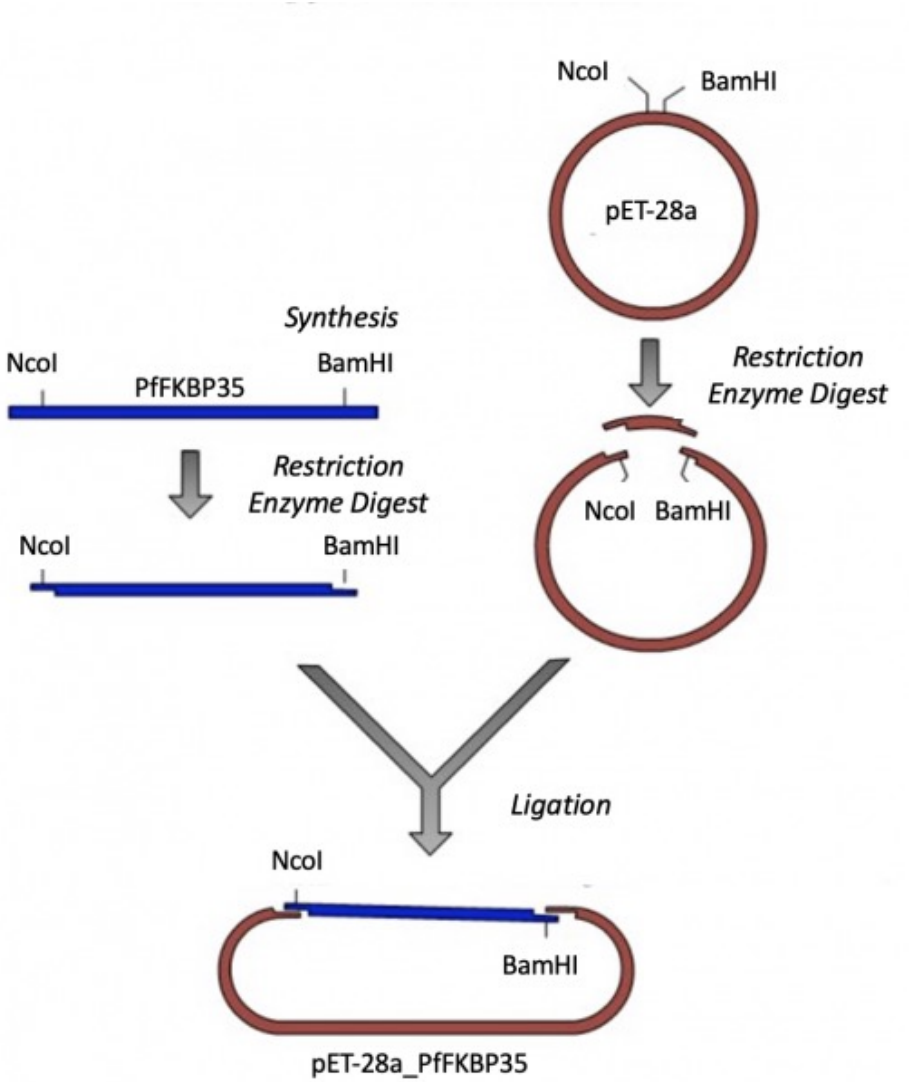
Reagents

Conditions

- Temperature:

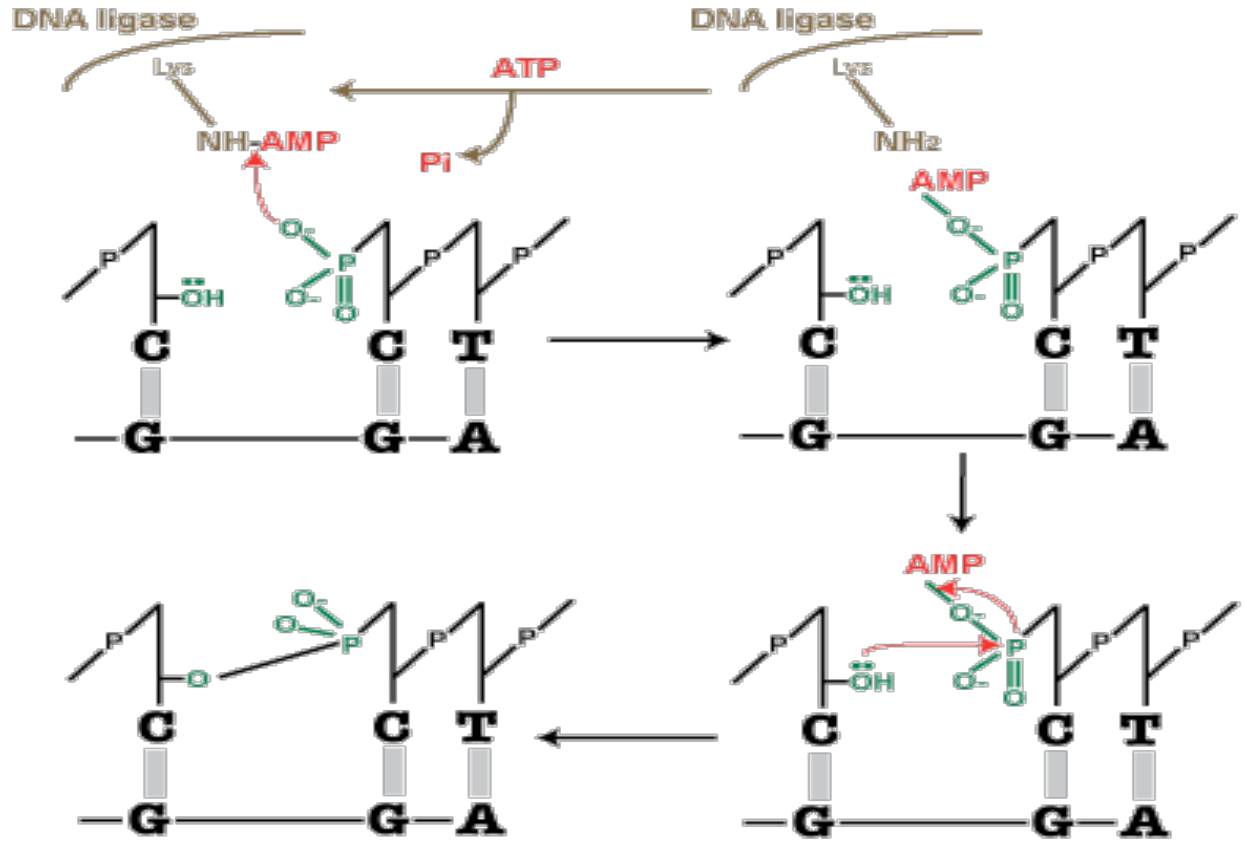
- Time:

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

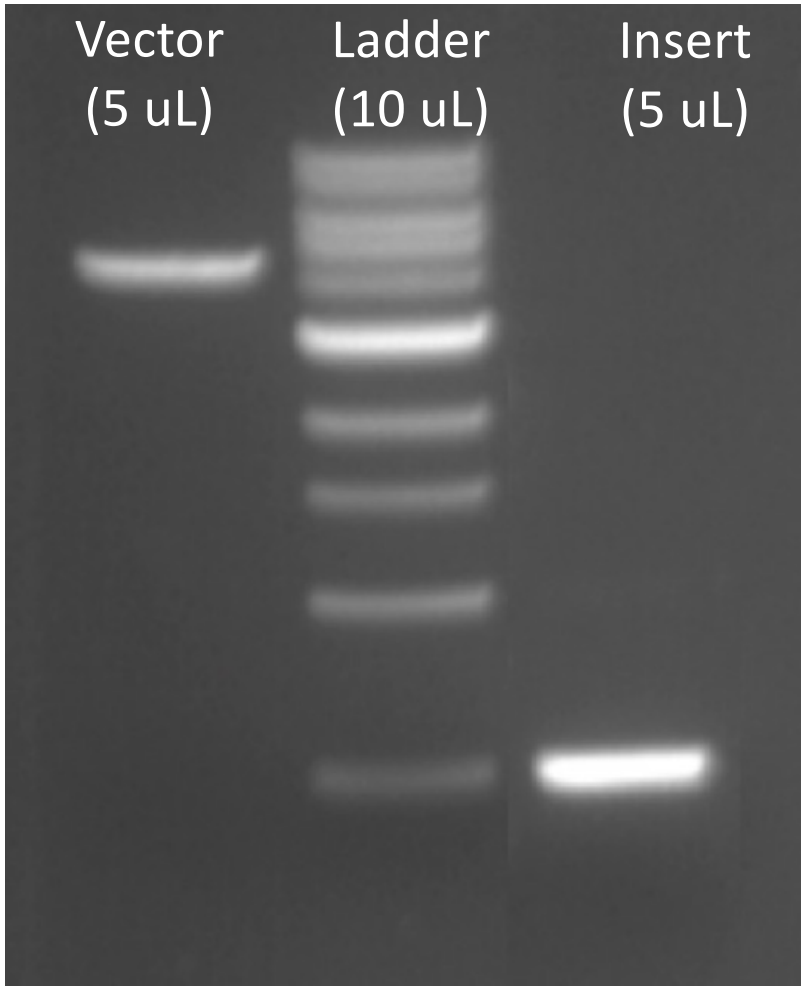


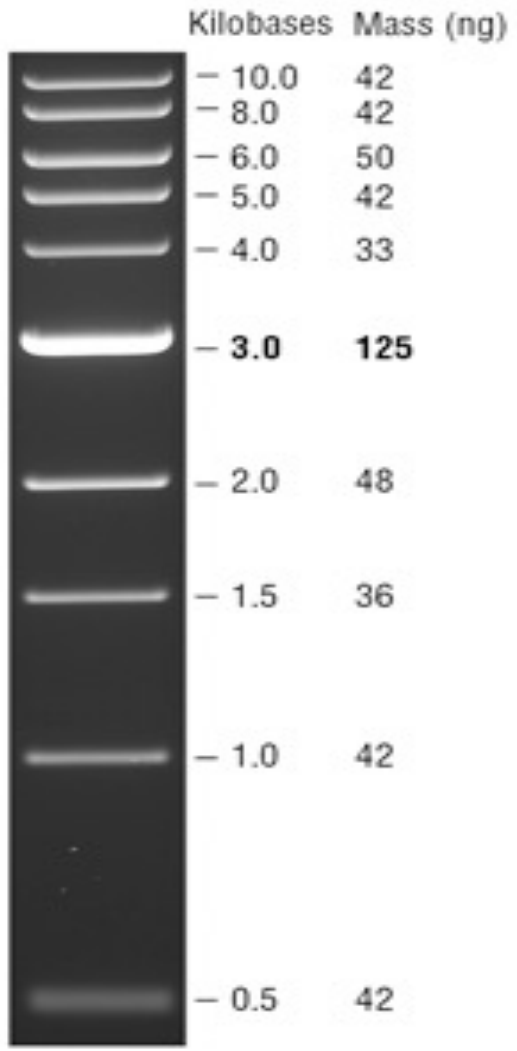
Ligation: T4 DNA ligase

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



Ligation conditions





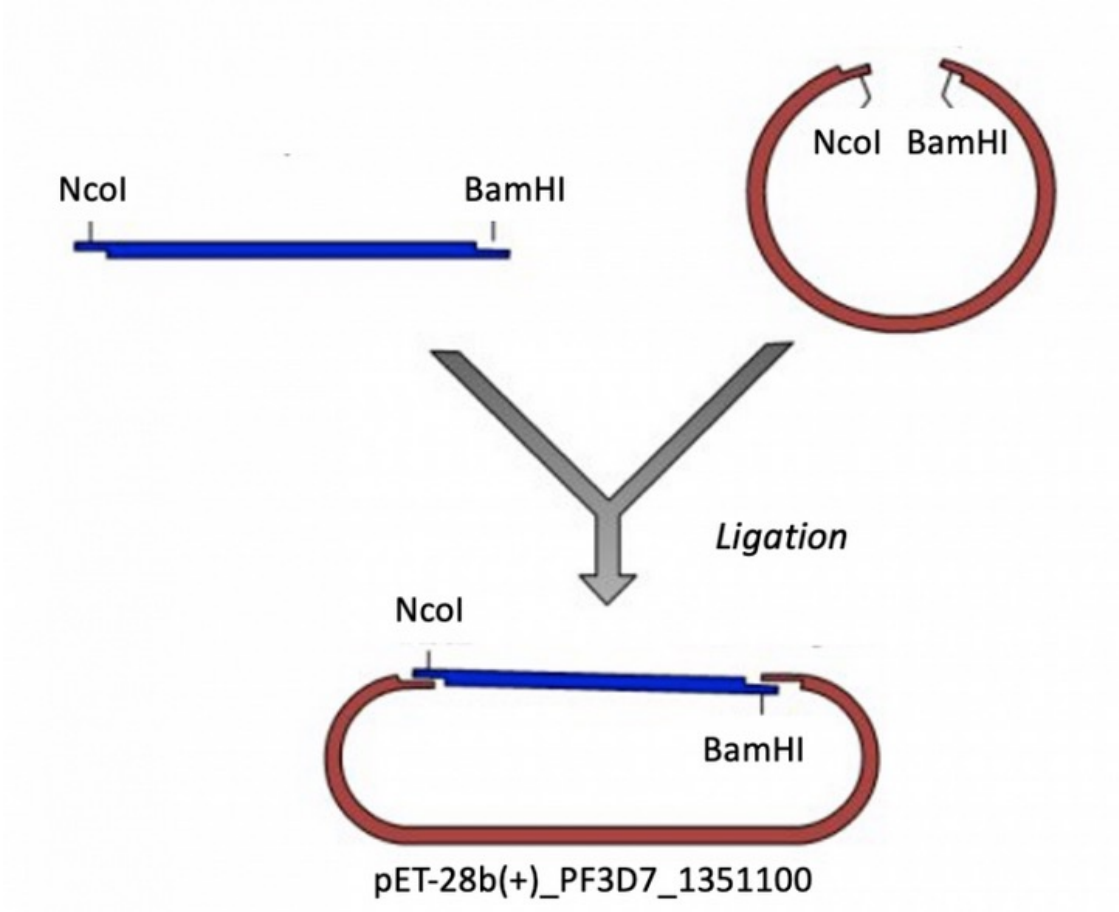
- Ideally, want 3:1 molar ratio of insert:backbone
- Calculate molar amounts from measured concentrations and known sizes of DNA molecules

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

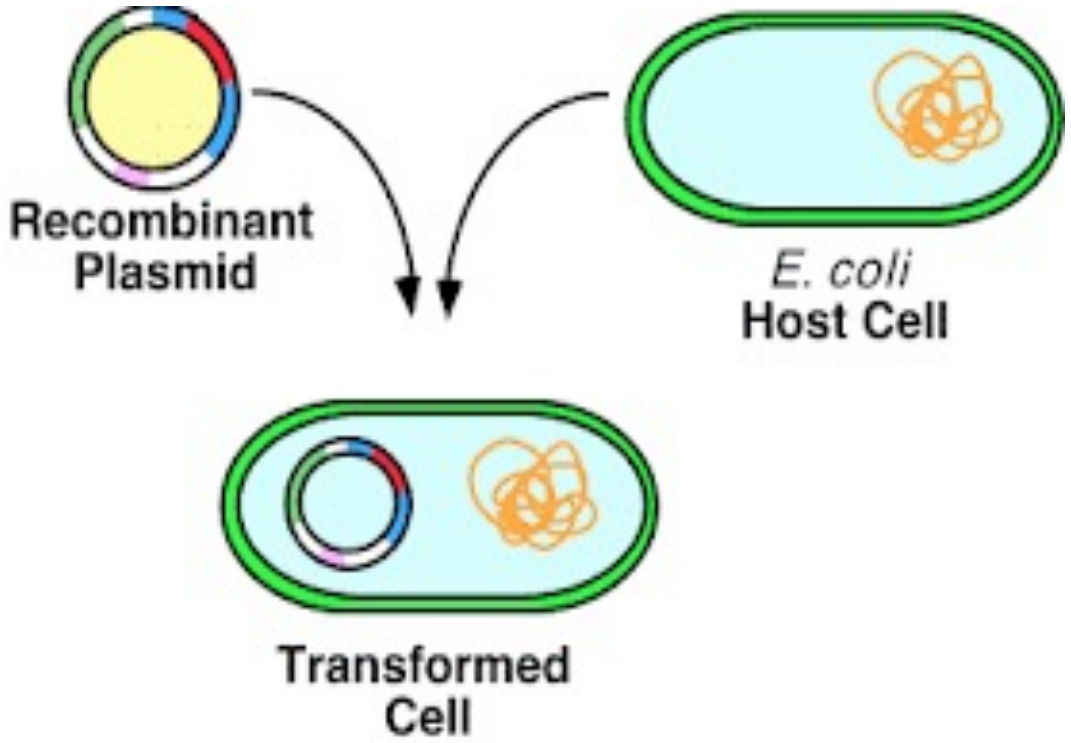
How do we confirm the cloning product?

- Transformation
- Purification
- Digestion



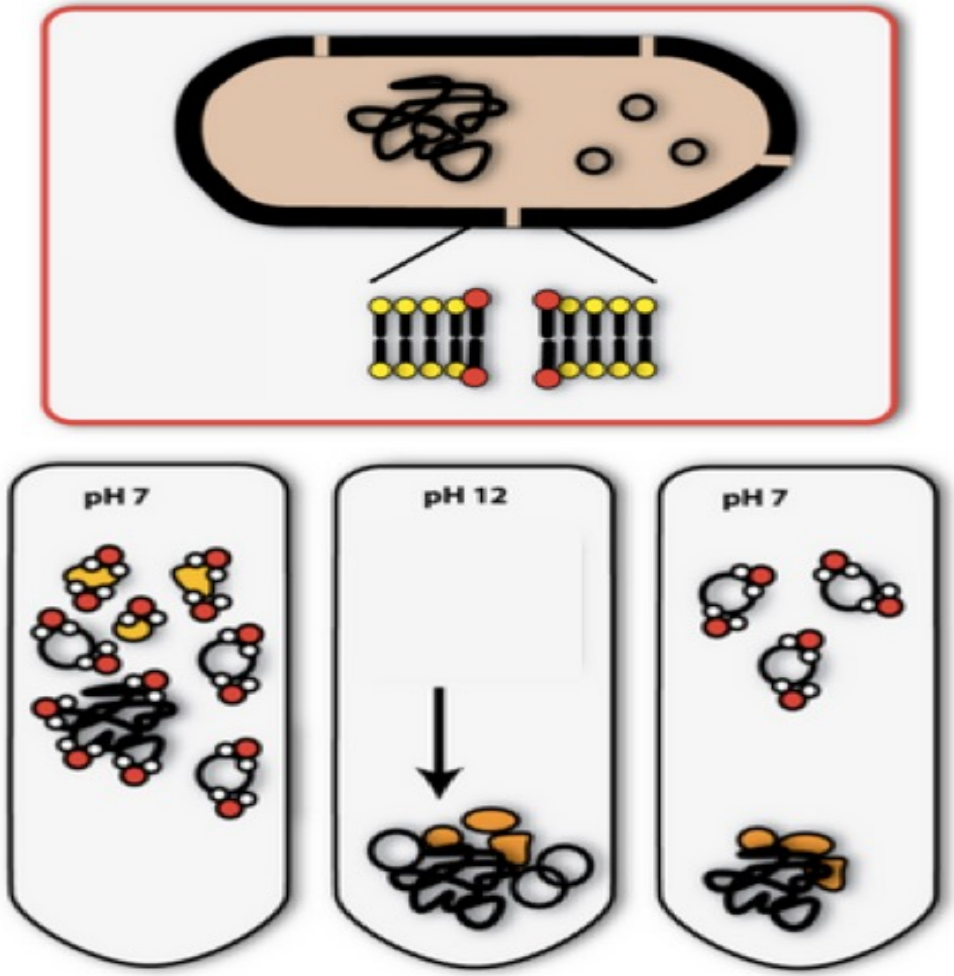
Transform plasmid into bacteria for amplification

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



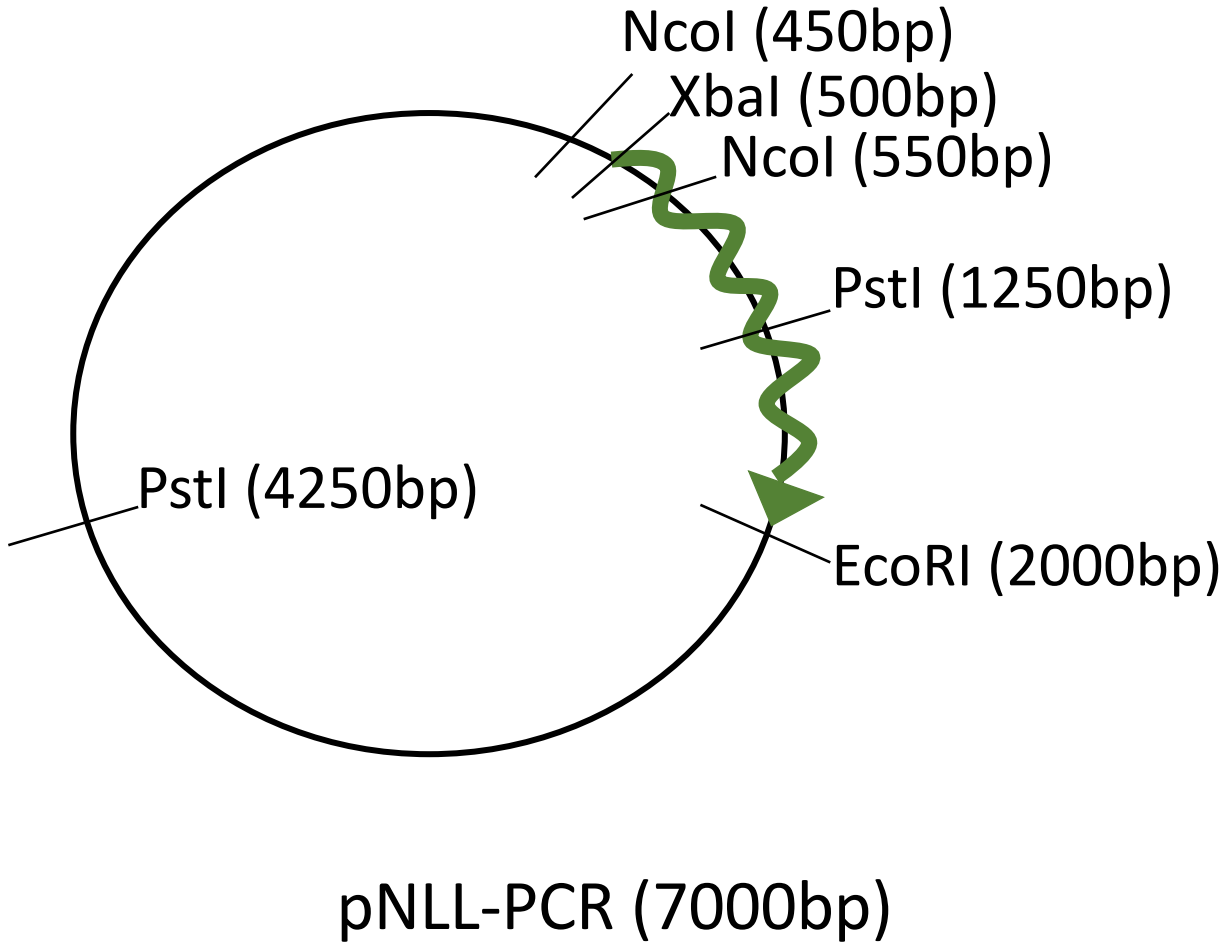
Purify amplified plasmid for confirmation

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 at the latest