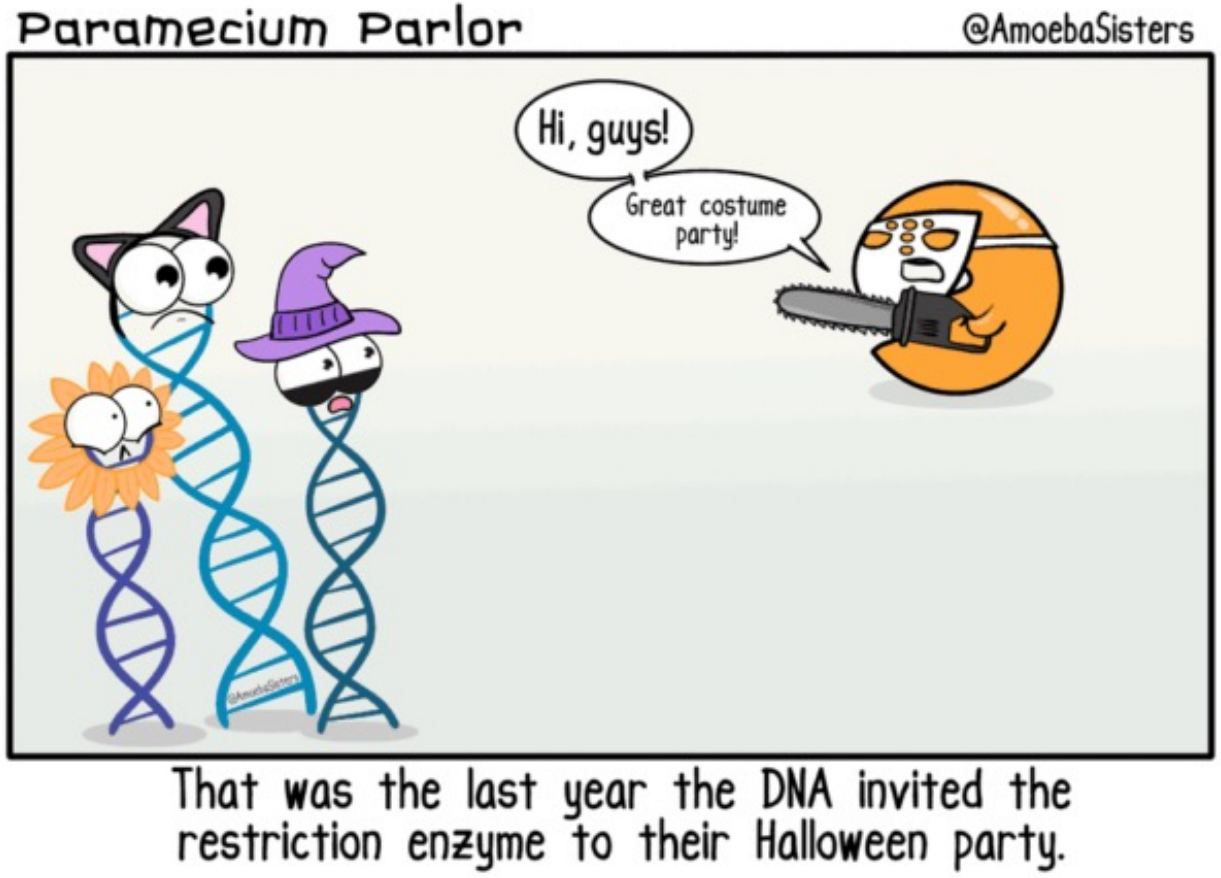
M2D2:
Complete in-silico cloning of protein expression plasmid

1. Prelab discussion
2. Complete DNA engineering exercise



Overview of M2: drug discovery

Determine putative PF3D7_20109-F21 binders via high throughput screening (SMM)

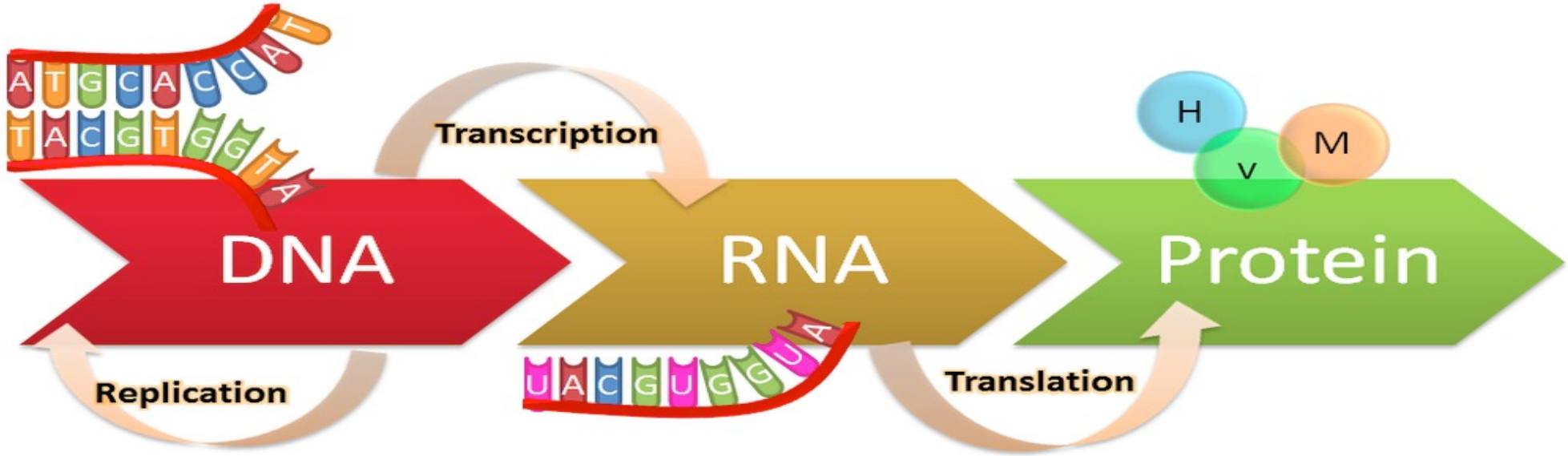
Create plasmid of PF3D7_20109-F21 to use in validation assays

Express PF3D7_20109-F21 (from plasmid) in bacteria and purify protein

Assess purity and concentration of purified protein

Use purified protein to validate binding of small molecules identified in SMM

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

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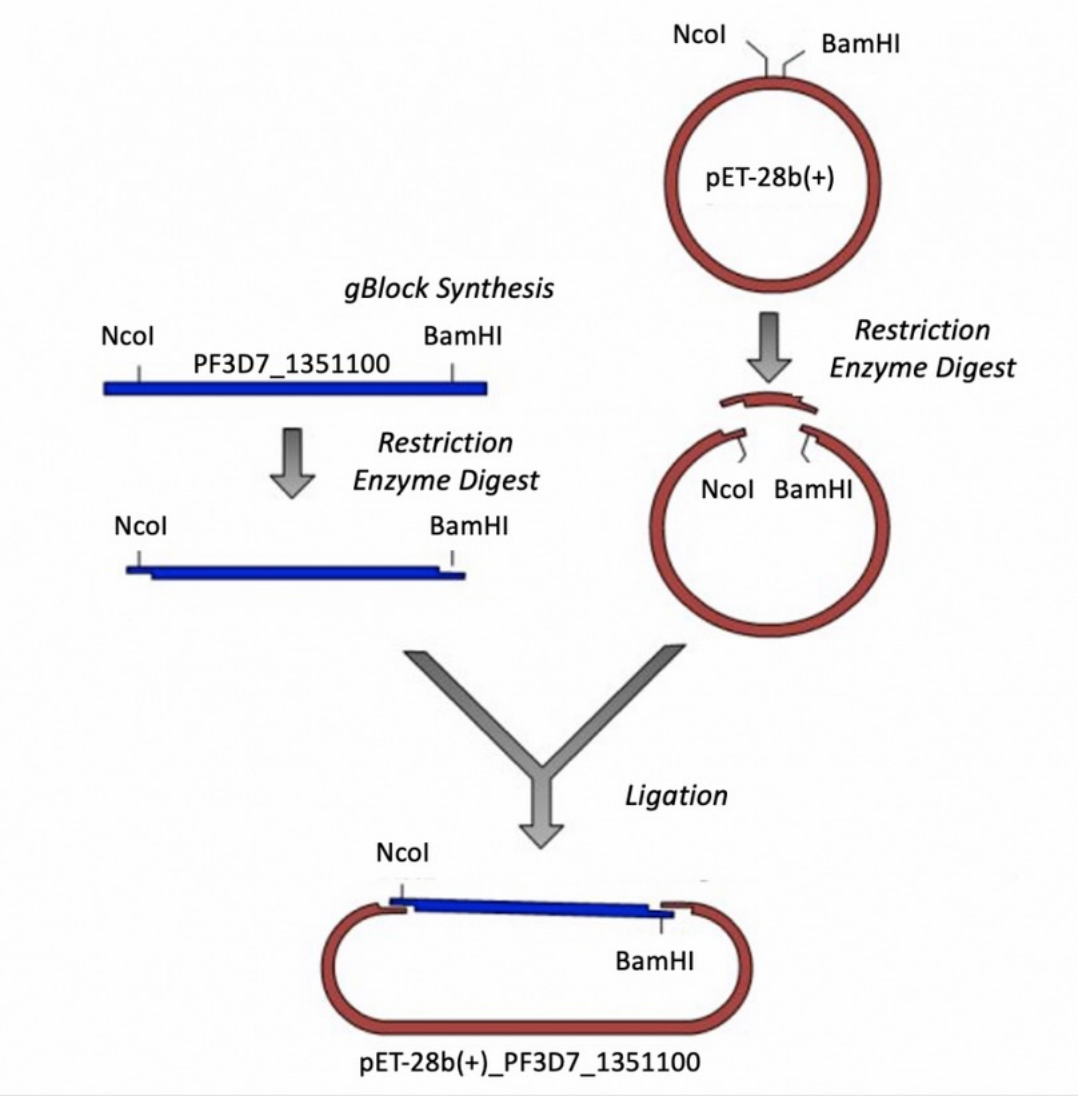
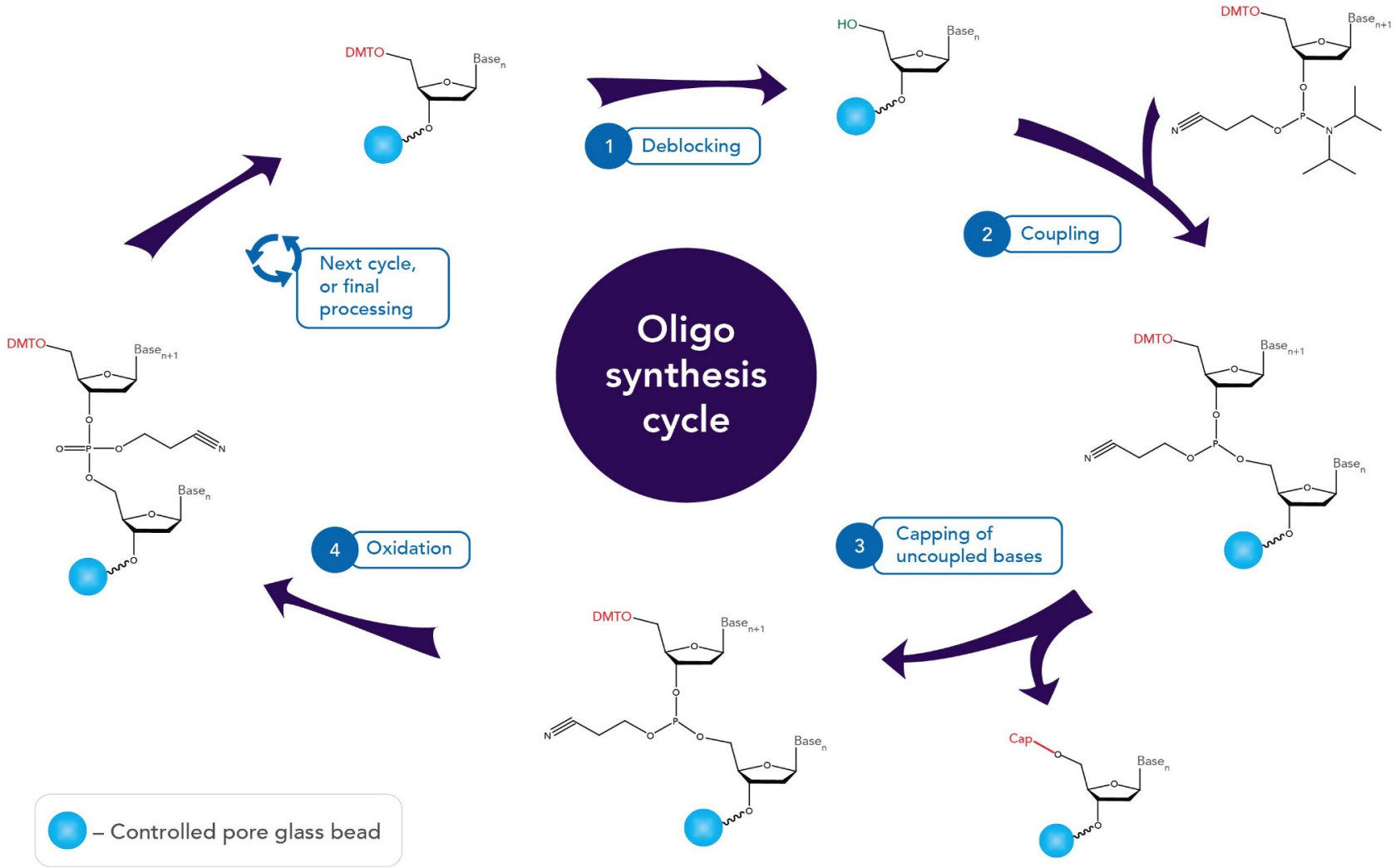
What if we want to make a specific protein?

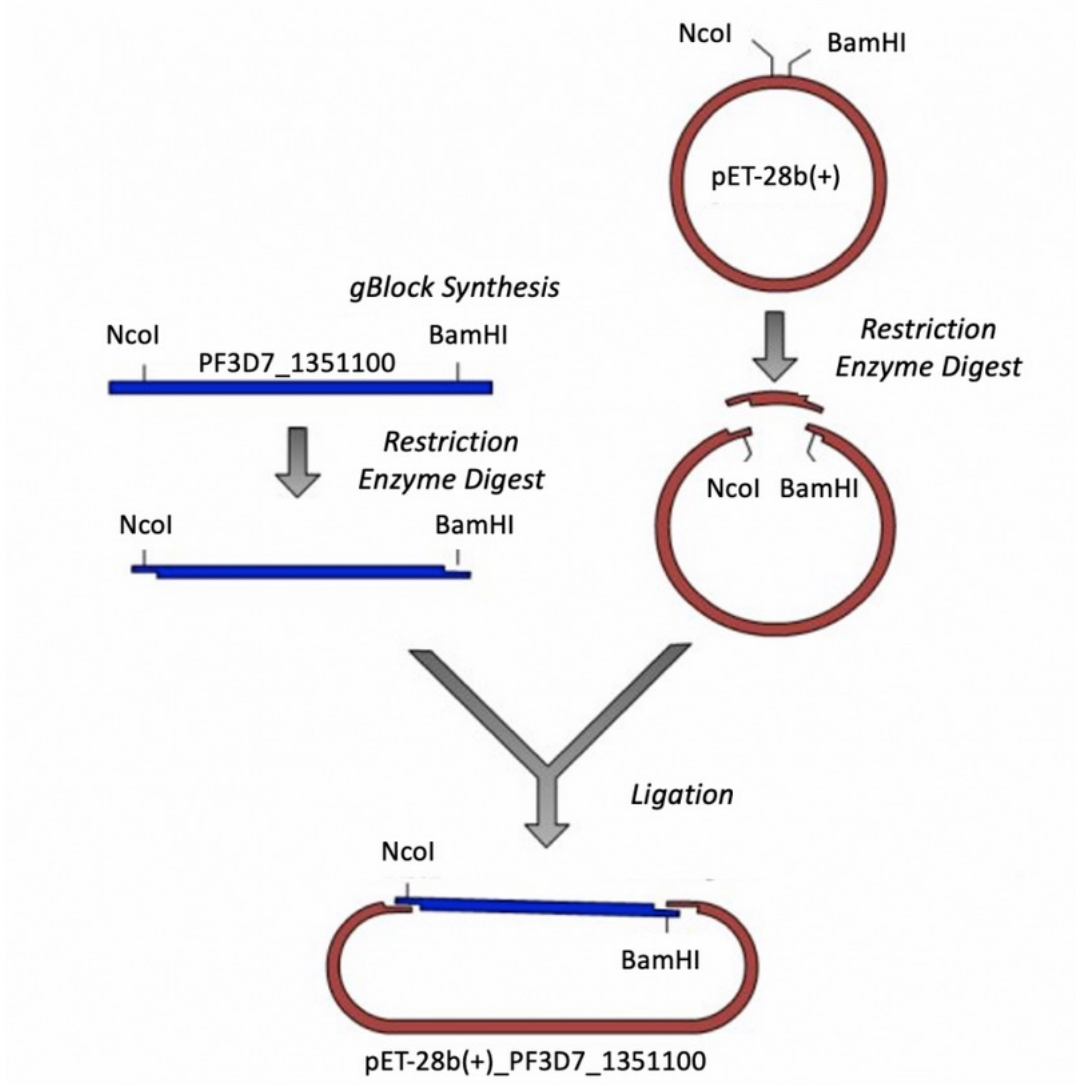
- Who are the players?
  - Insert
  - Vector
- What is the process?
  - Digestion
  - Ligation



How do we synthesize the insert?

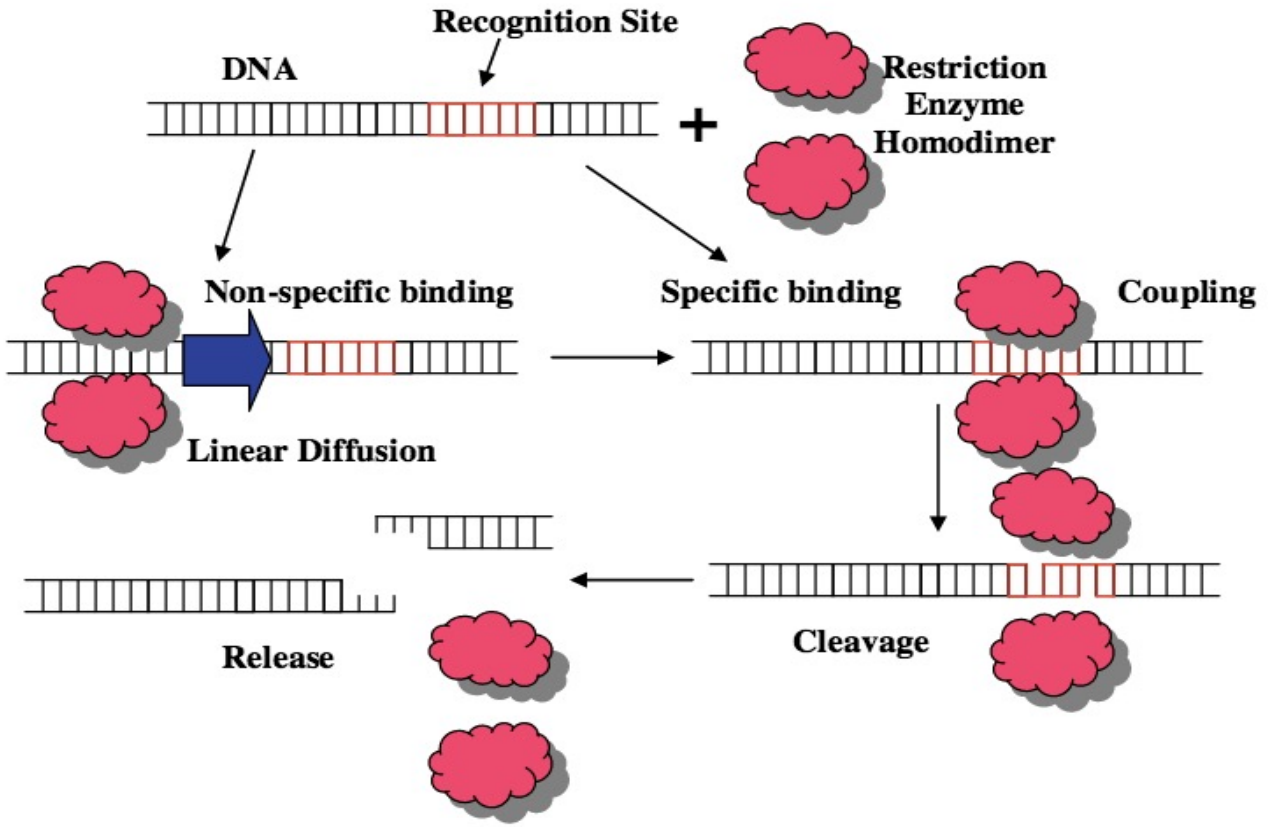
- Deblocking
- Coupling
- Capping
- Oxidization




NcoI
BamHI
pET-28b(+)
gBlock Synthesis
NcoI
BamHI
PF3D7_1351100
Restriction
Enzyme Digest
Restriction
Enzyme Digest
NcoI
BamHI
NcoI
BamHI
Ligation
NcoI
BamHI
pET-28b(+)_PF3D7_1351100

Digestion: restriction enzymes

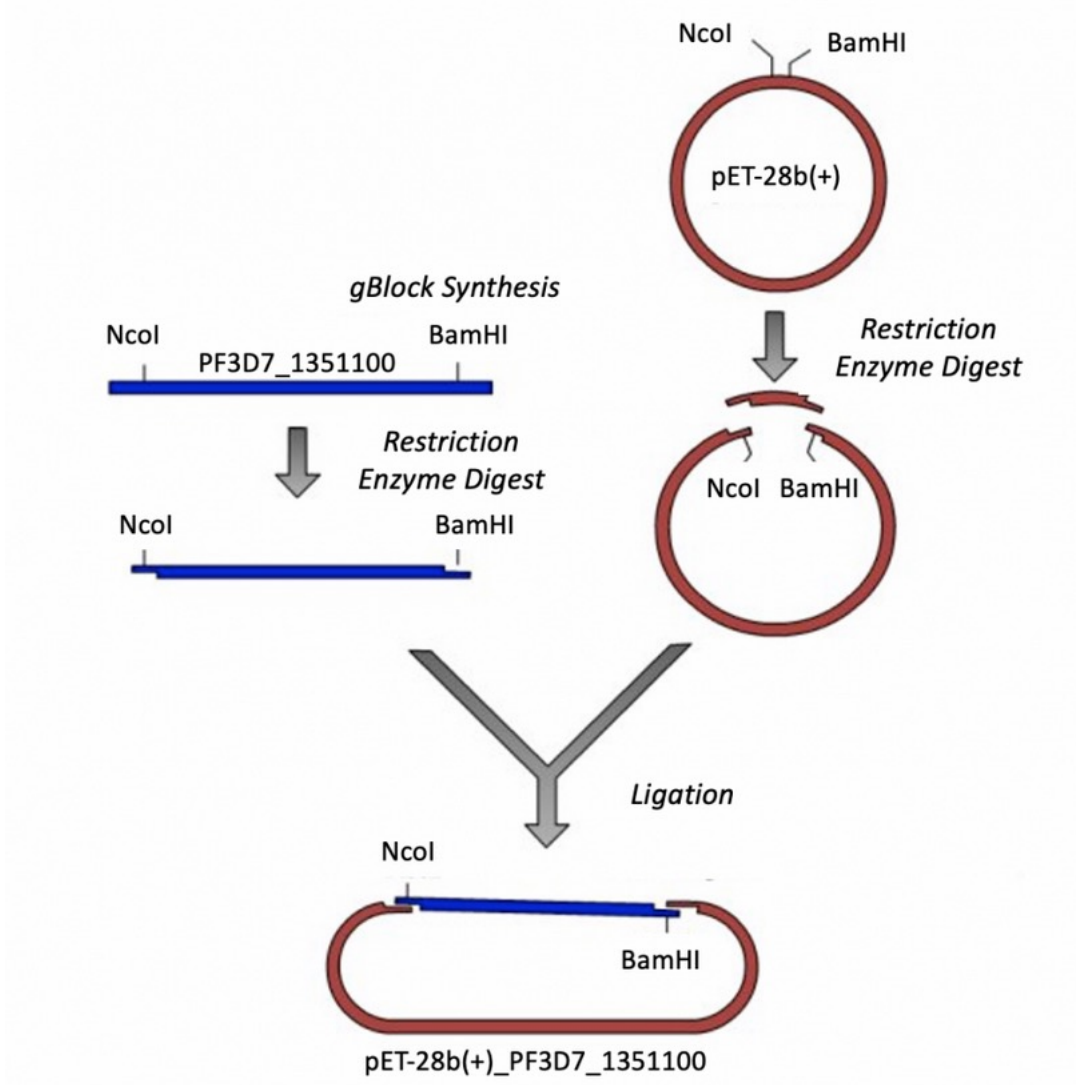
- Function as homodimers
- Each dimer contains active site that cleaves backbone at site of palindromic recognition sequence
- Results in cleavage of both strands



Digest reagents and conditions

Reagents

- Temperature:
- Time: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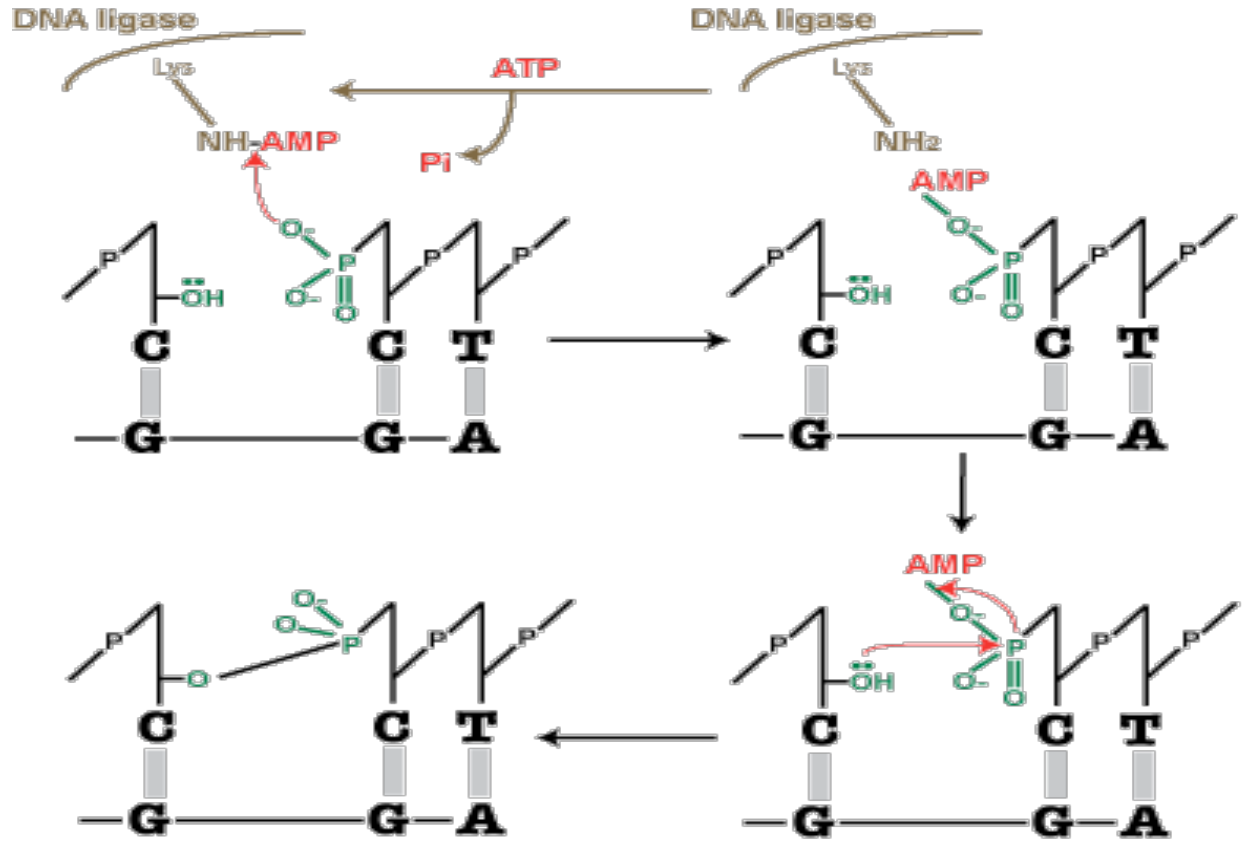
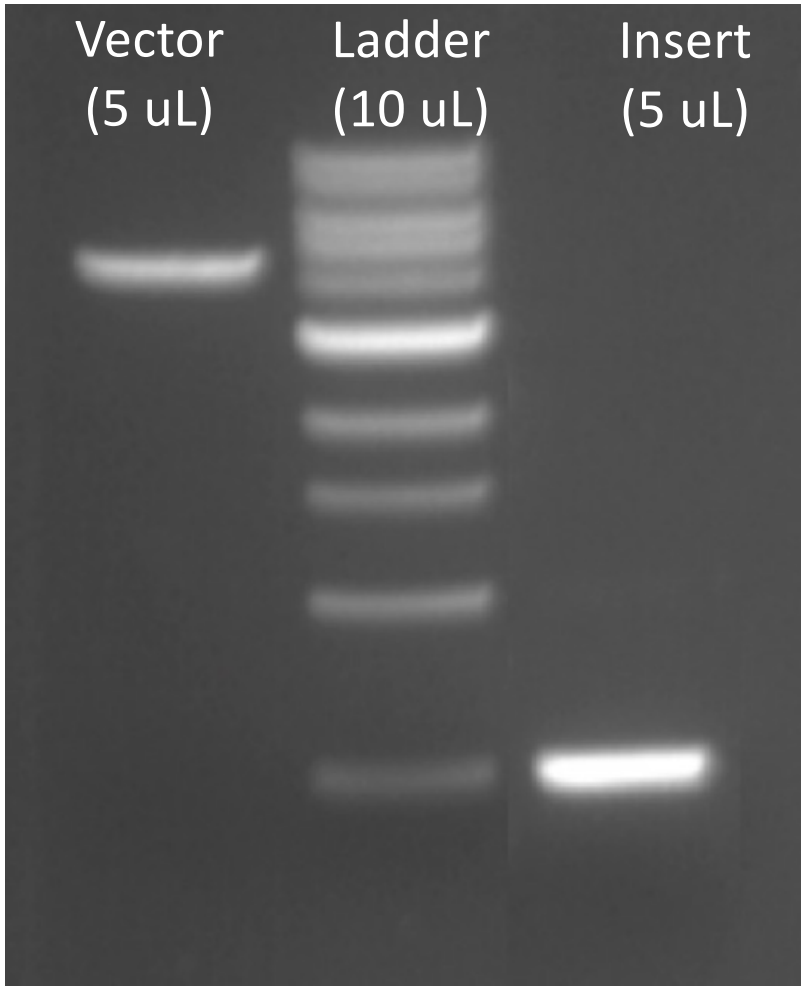
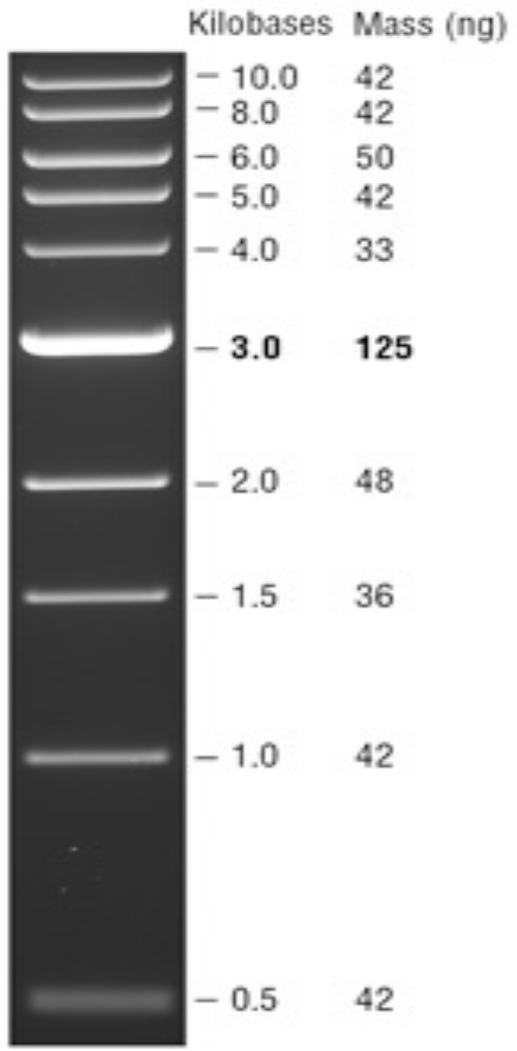
Ligation: T4 DNA ligase

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



Ligation conditions





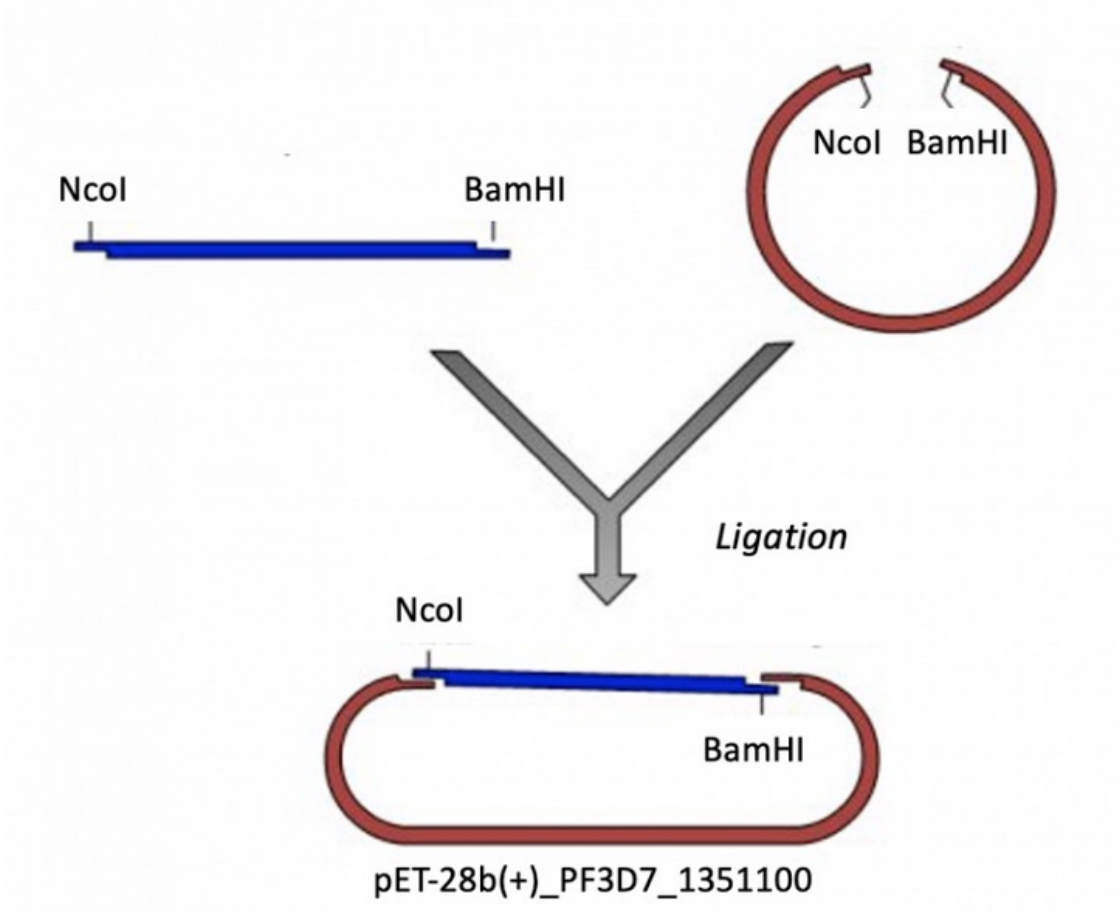
- Ideally, want 3:1 molar ratio of insert:backbone
- Calculate molar amounts from measured concentrations and known sizes of DNA molecules

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 discovery 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

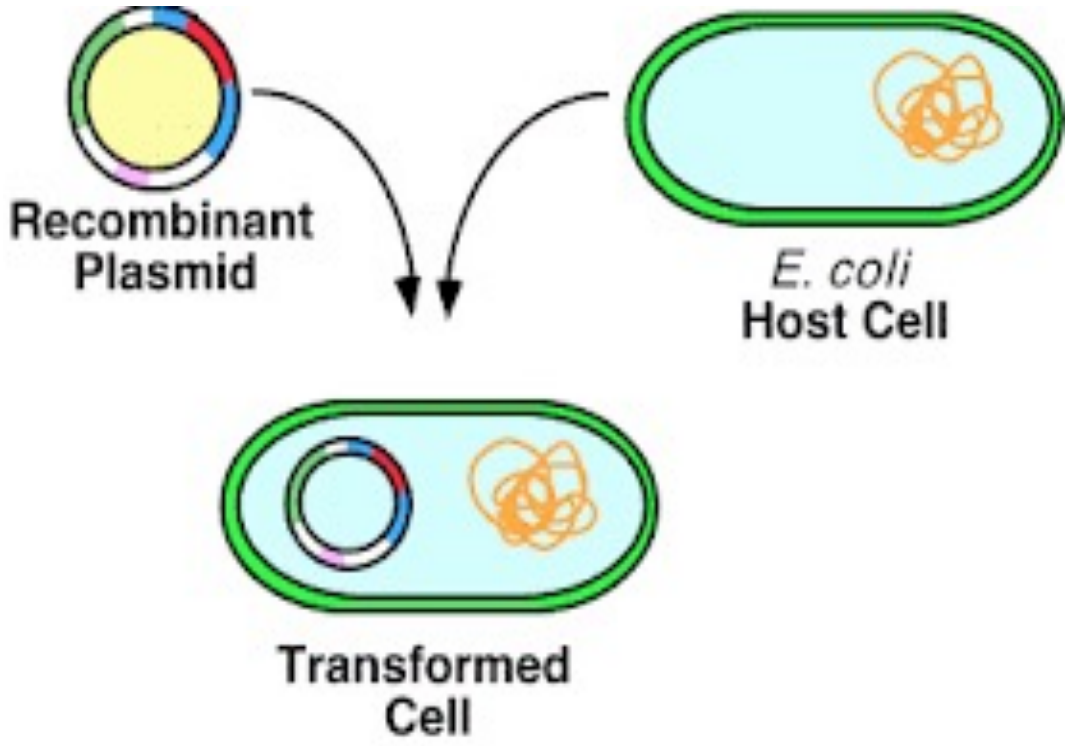
How do we confirm the cloning product?

- Transformation
- Purification
- Digestion



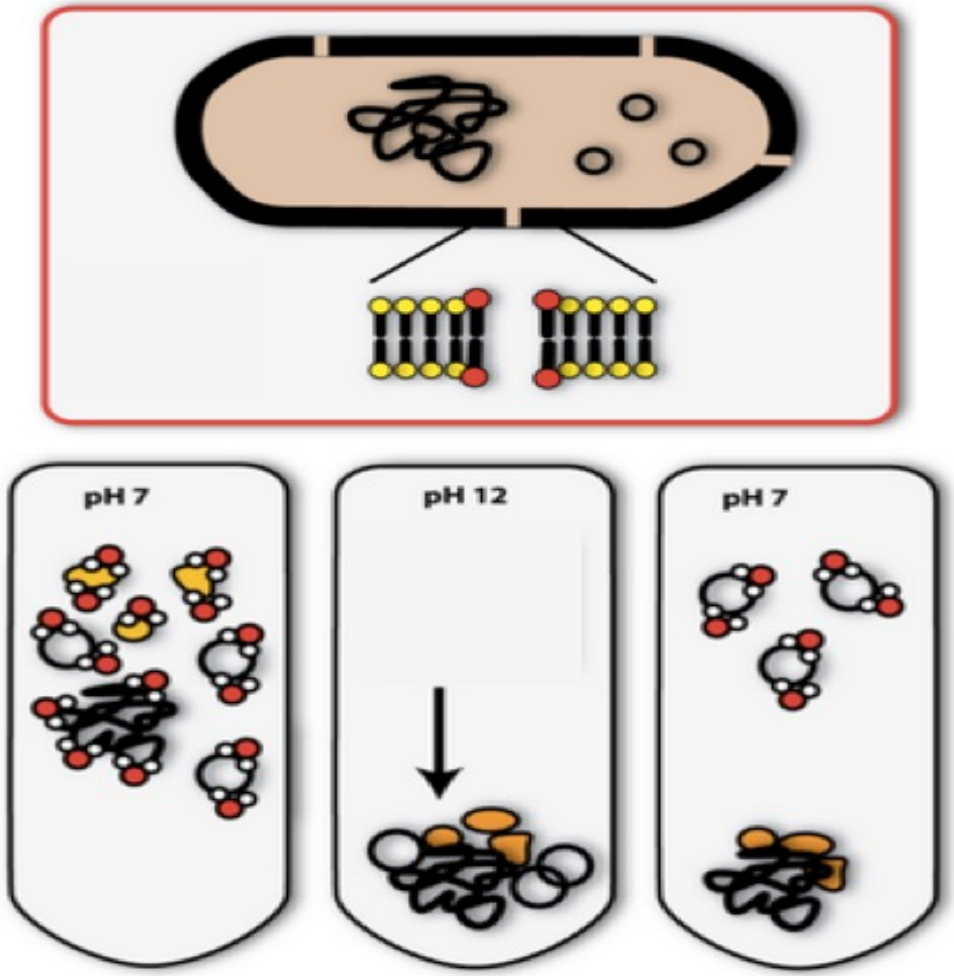
Transformation

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



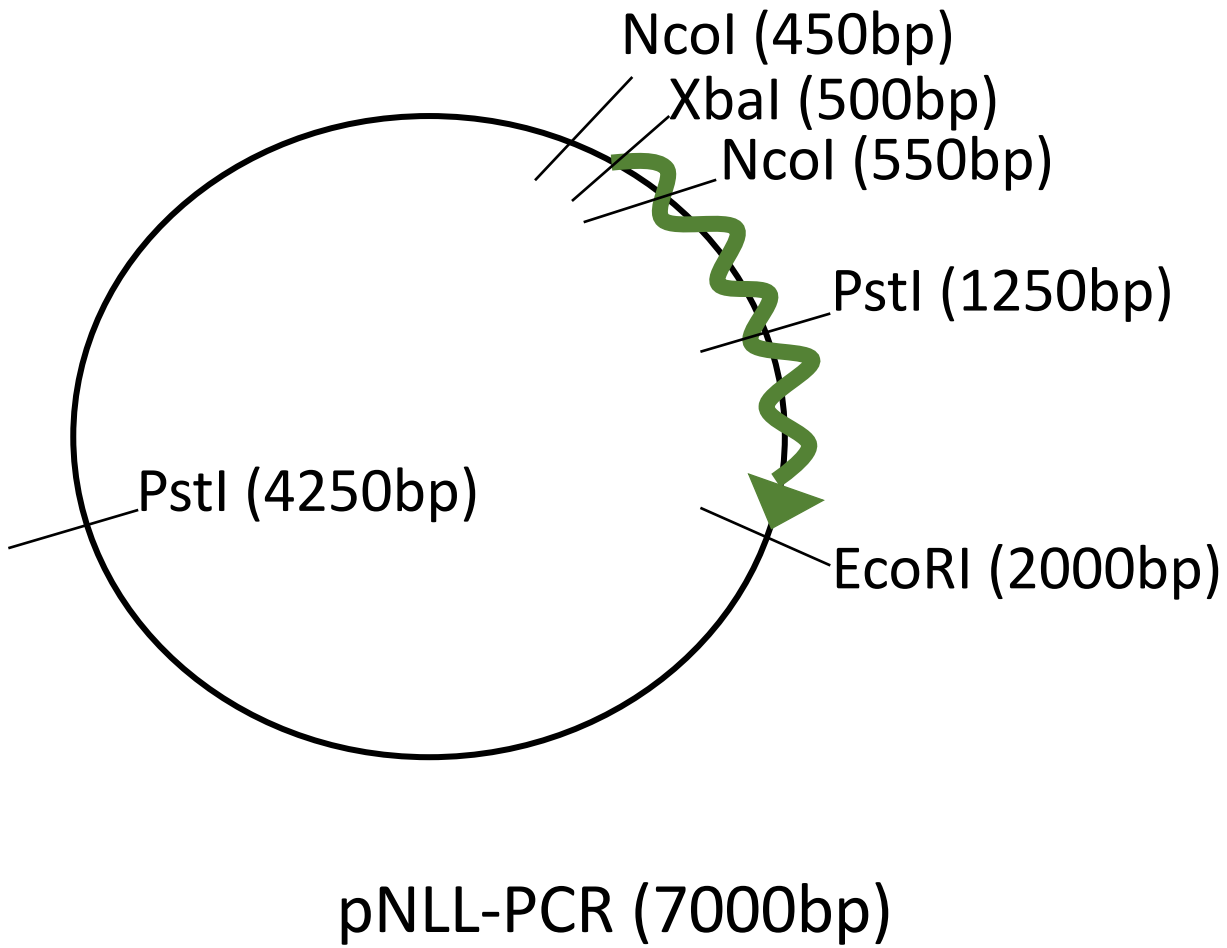
Purification

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

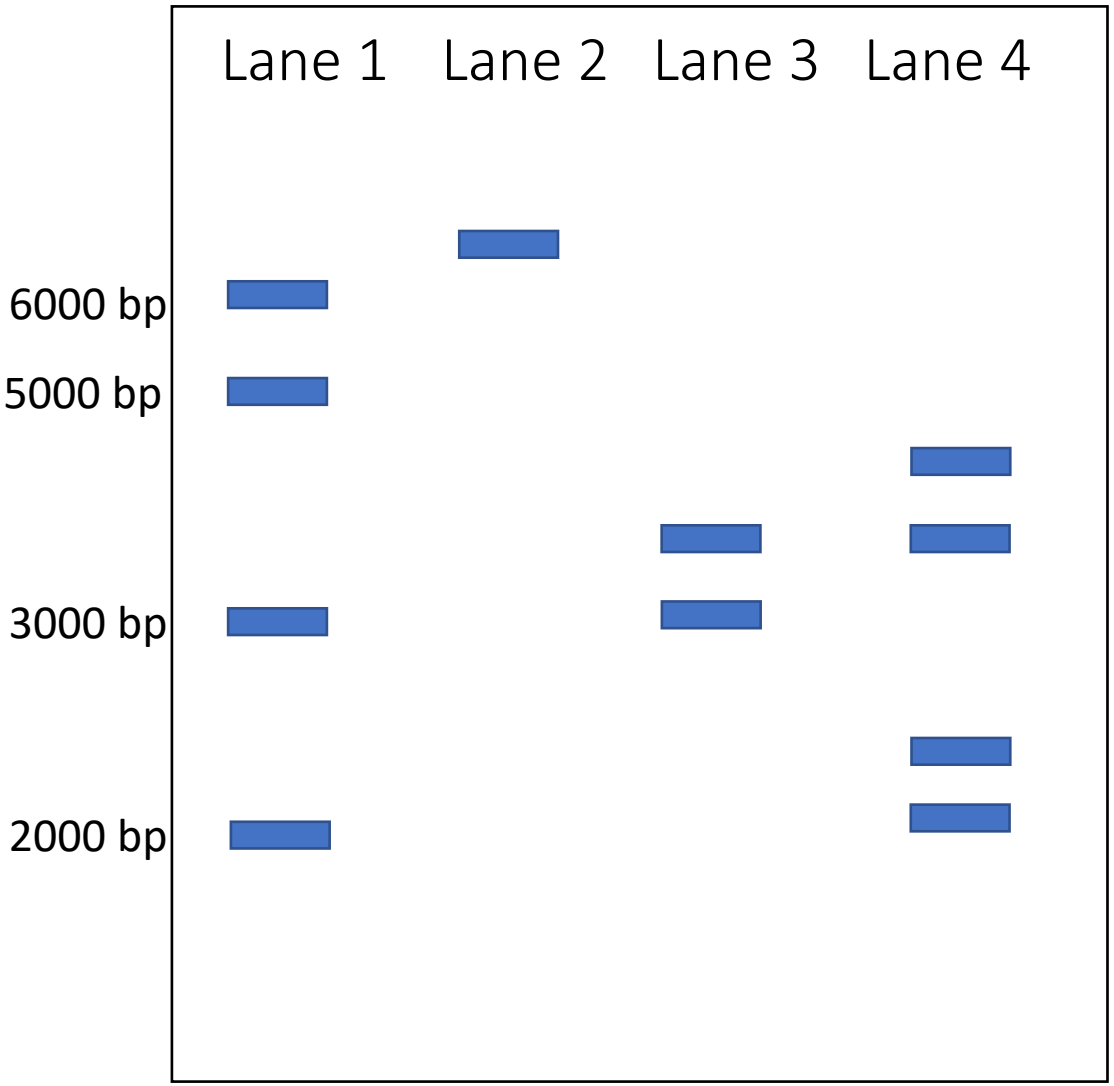


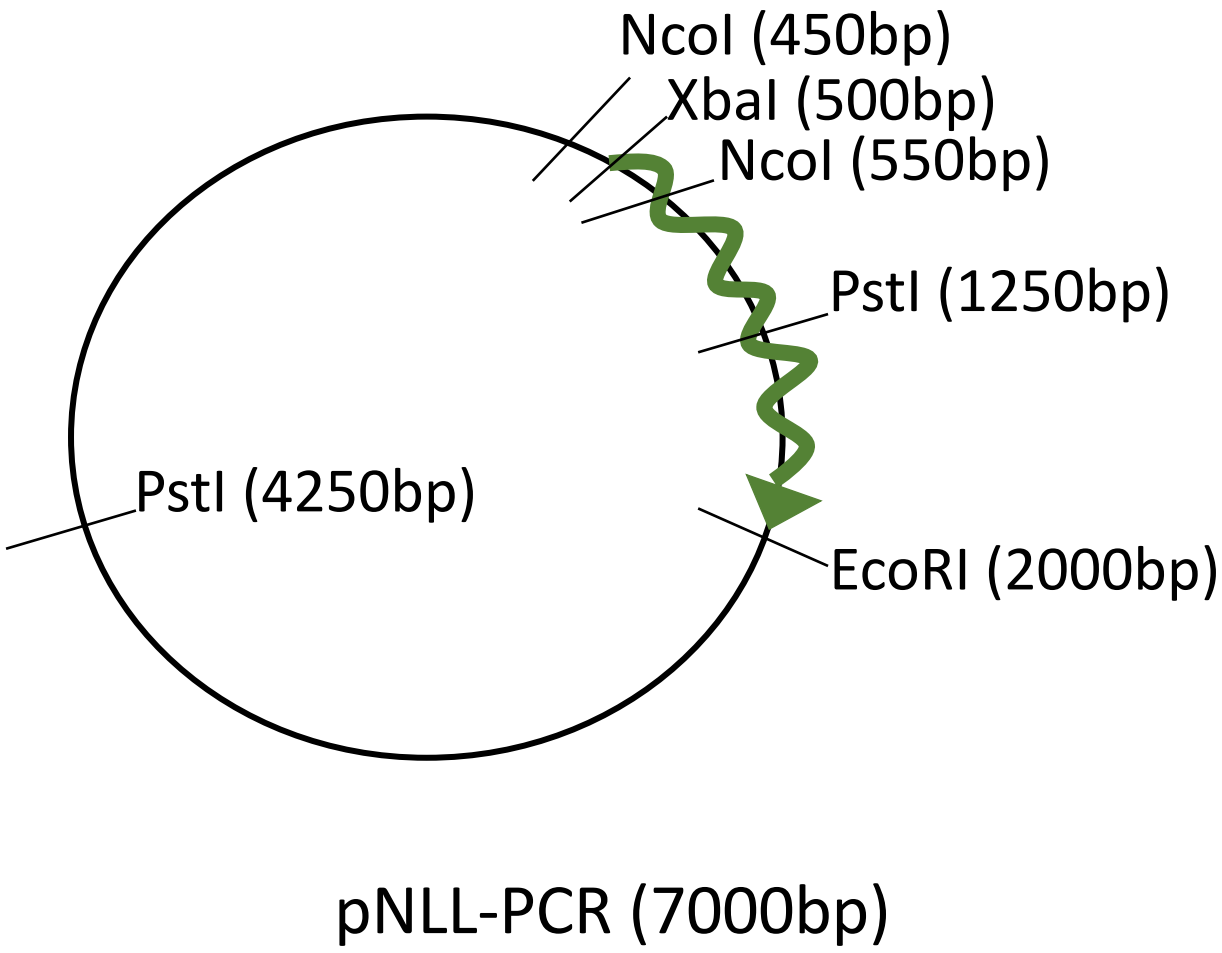
Digestion, again

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



PstI Digest: Ideal = 3000 bp and 4000 bp fragments





For today…

- In silico cloning of your plasmid
- Set up restriction enzyme digest

For M2D2…

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

- Email Noreen to reserve your JC presentation date