

M1D1:

Complete *in silico* cloning and confirmation digest of protein expression vector

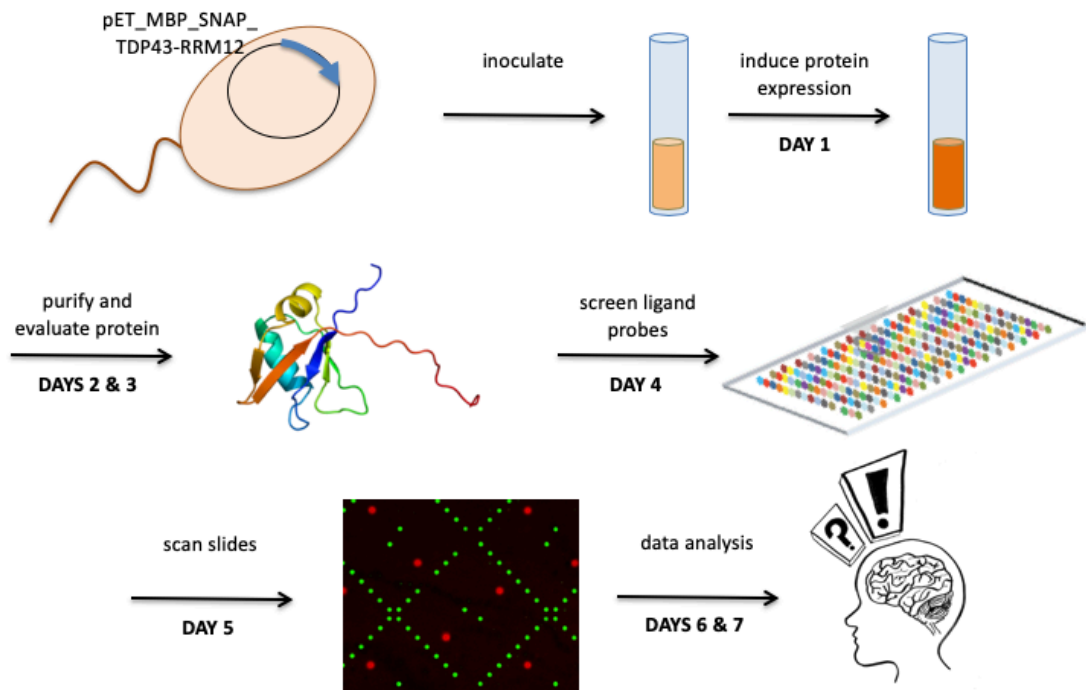
1. Laboratory Orientation quiz
2. Prelab discussion
3. Build protein expression vector - virtually
4. Confirm protein expression vector - actually

Mark your calendars!

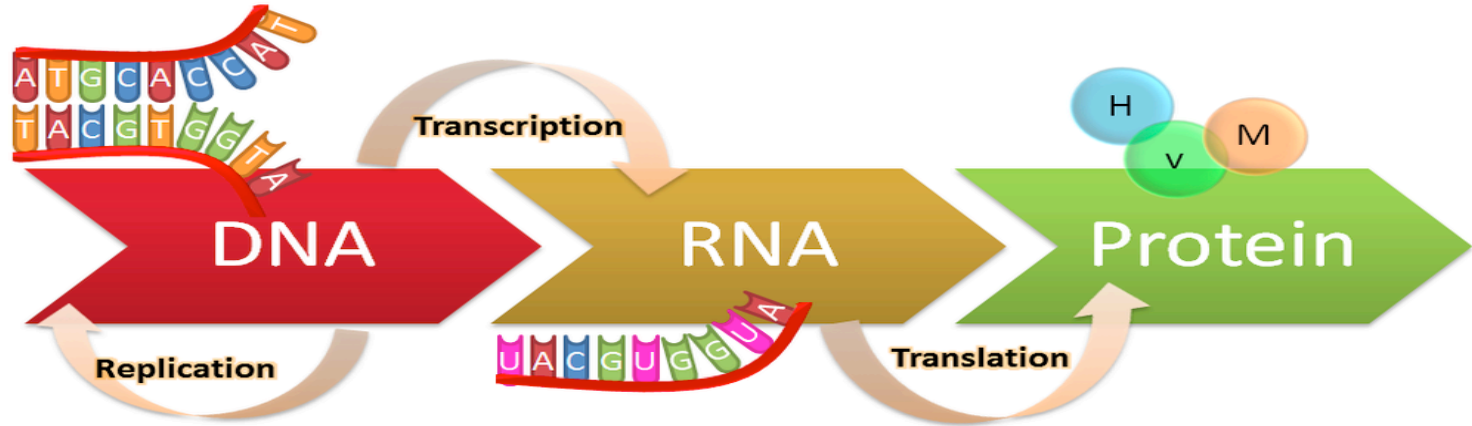
- **Data summary** (15%)
 - completed in teams and submitted via Stellar
 - draft due 3/8, final revision due 3/22
 - format in bullet points
- **Mini-presentation** (5%)
 - completed individually and submitted via Gmail
 - due 3/15
- **Laboratory quizzes**
 - scheduled for M1D4 and M1D7
- **Notebook** (part of 10% Homework and Notebook)
 - one entry will be graded by Kevin 24 hr after M1D7
- **Blog** (part of 5% Participation)
 - due 3/16 via Blogspot



Overview of Mod 1 experiments

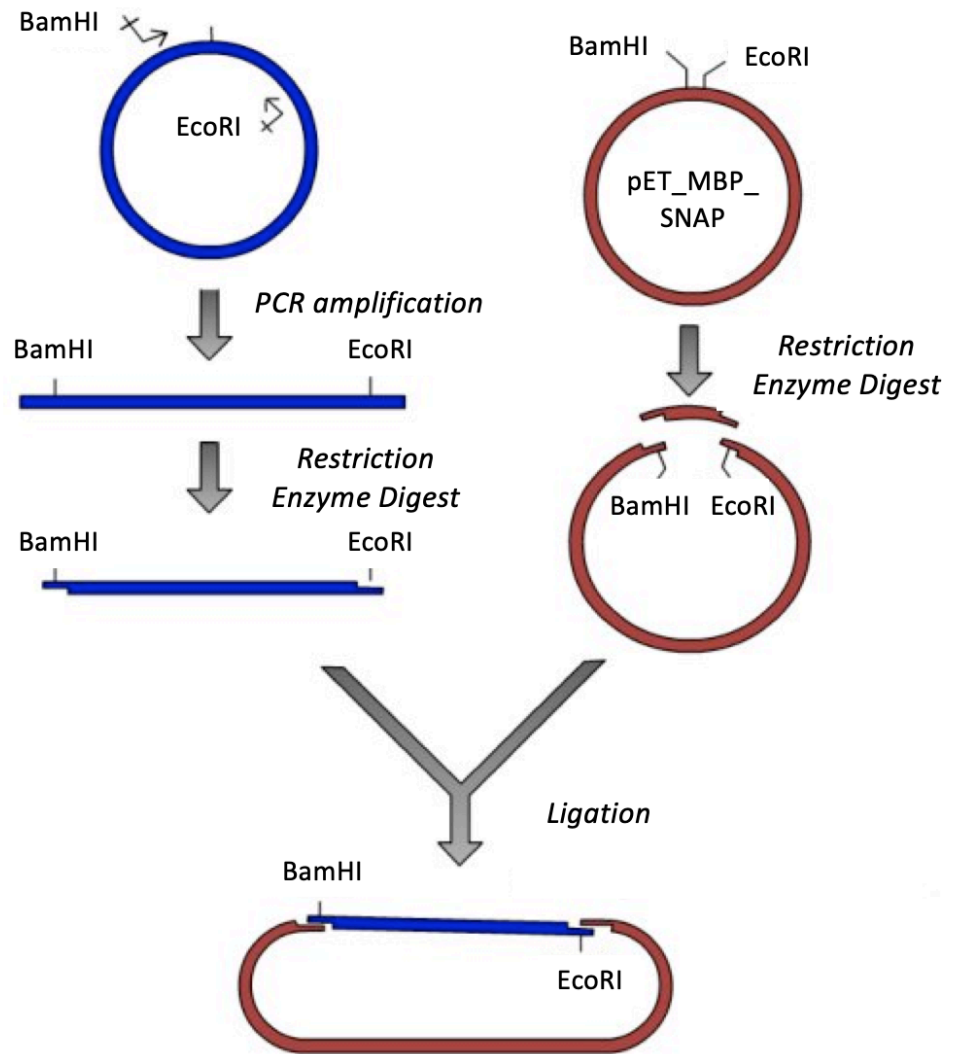


How are proteins made?

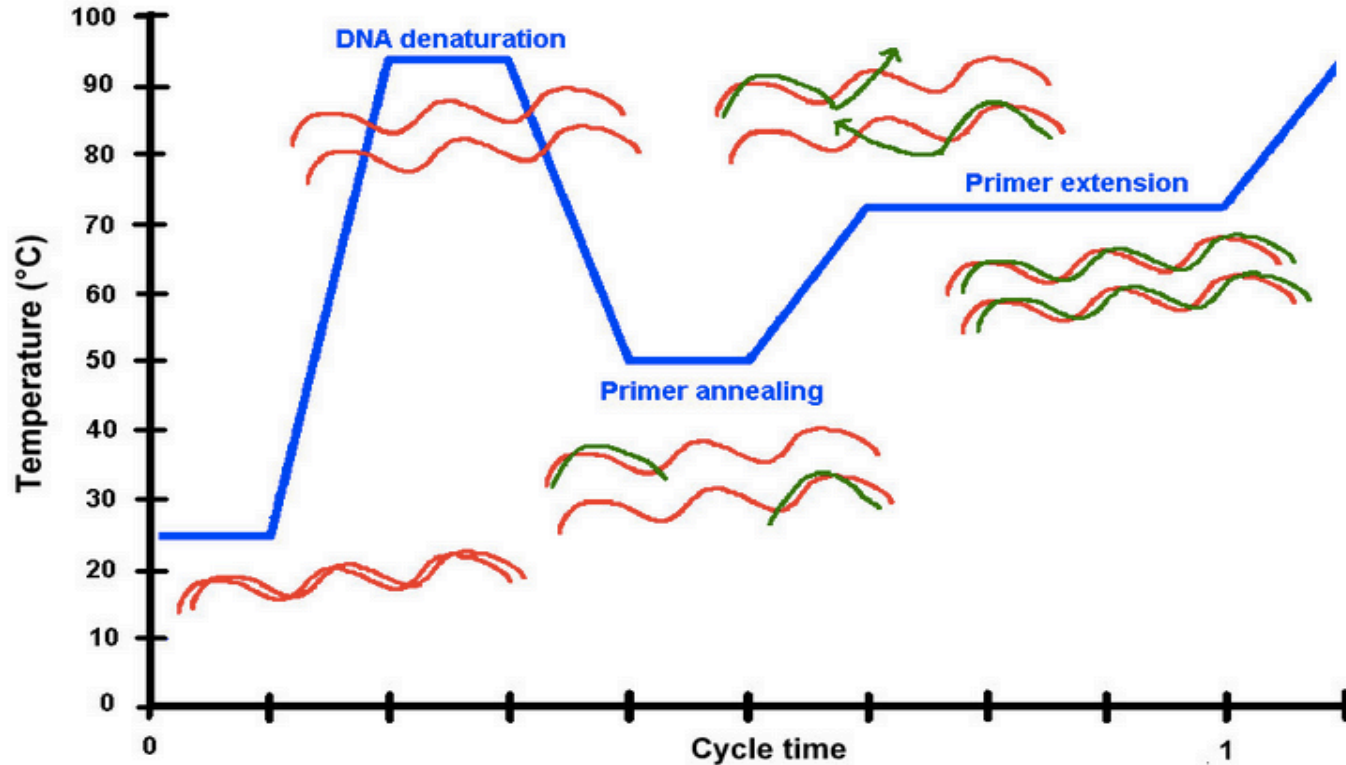


What if we want specific protein?

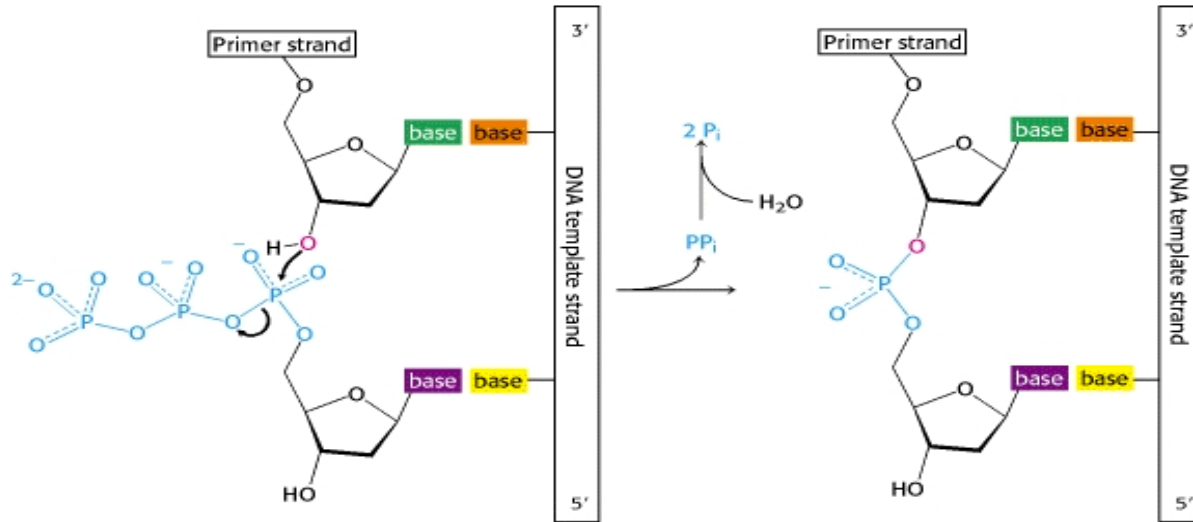
- Amplification
- Digestion
- Ligation



Amplification: PCR cycling



Amplification: DNA polymerase



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

PCR reagents and conditions

Reagents
polymerase DNA
primers
dNTPs
buffer
template

5' 

↓ ↑ T

5' 

flap seq.
landing seq.



↓

5' 



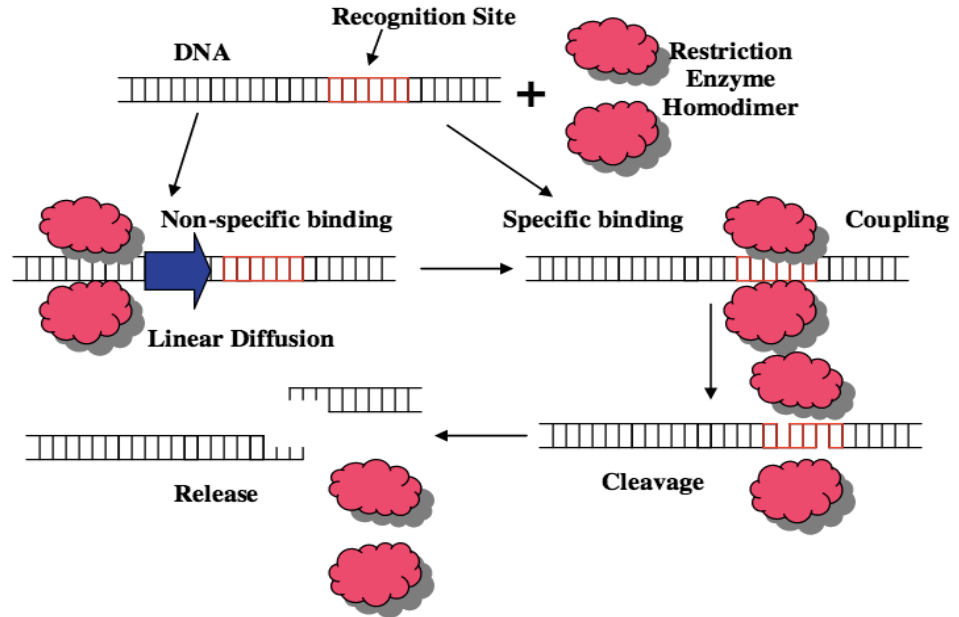
A closer look at primer design

- Length: 17-28 bp
- GC content: 40-60 %
- T_m : < 65 °C
- Avoid secondary structure and repeat sequences

primer: primer binding

Digestion: restriction enzymes

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



Digest reagents and conditions

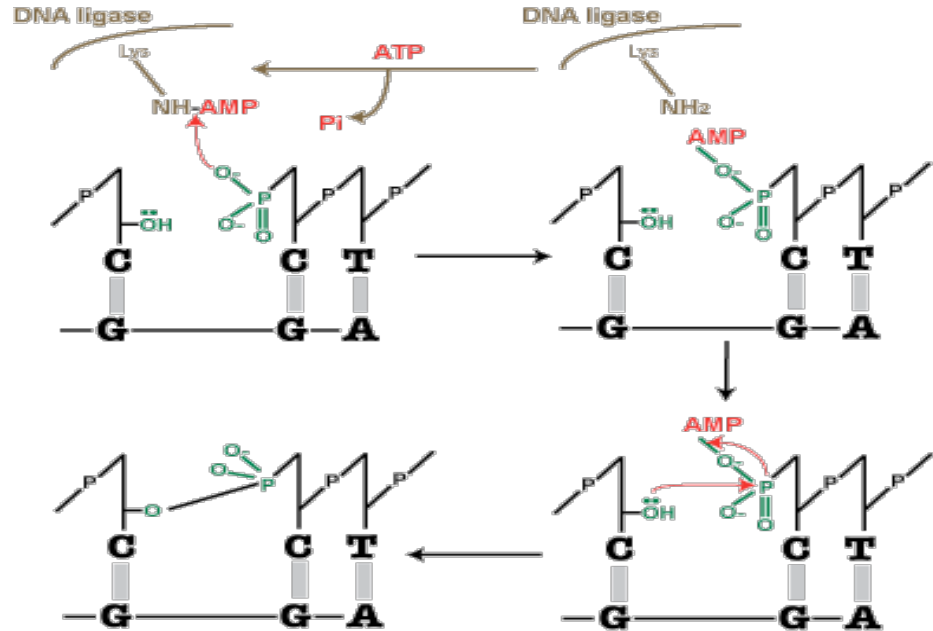
Reagents
enzyme (RE)
buffer (AMP)
DNA

- Temperature

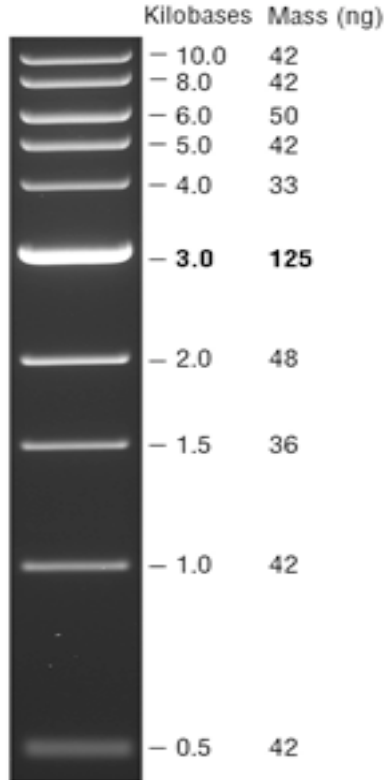
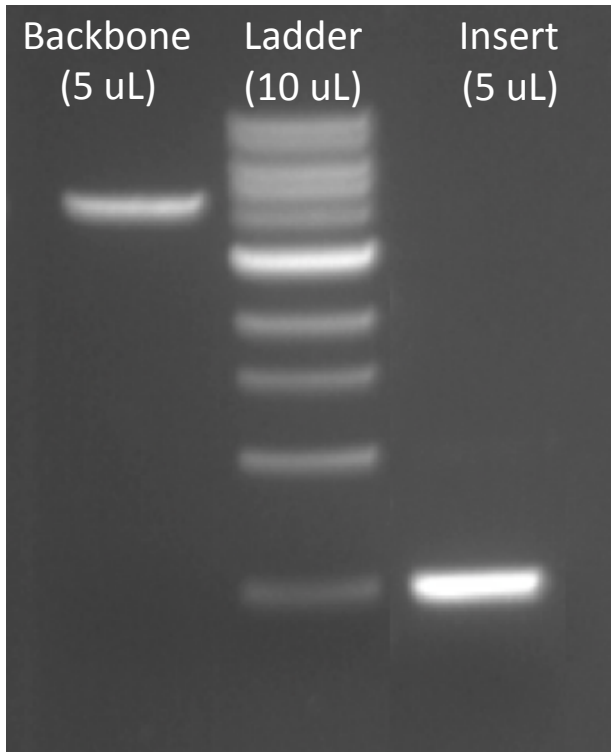
- Time

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

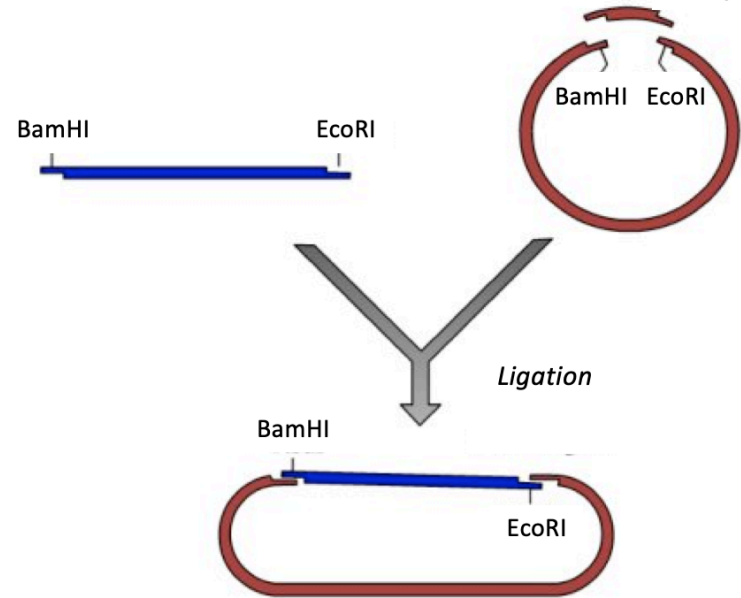
1. Determine volume of backbone
 - Use backbone concentration = **50** ng/uL
 - Want 50 – 100 ng
2. Calculate moles of backbone
 - Vector = **6837** bp, MW bp = 660 g/mol
3. Calculate moles of insert
 - Insert = **527** bp, **3**:1 ratio of insert:backbone
4. Calculate volume of insert
 - Use insert concentration = 20 ng/uL

How do we confirm the product?

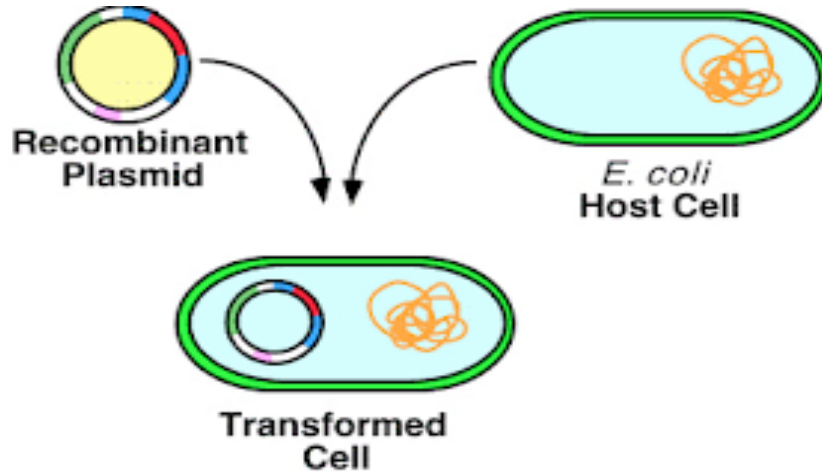
- Transformation

- Purification

- Digestion

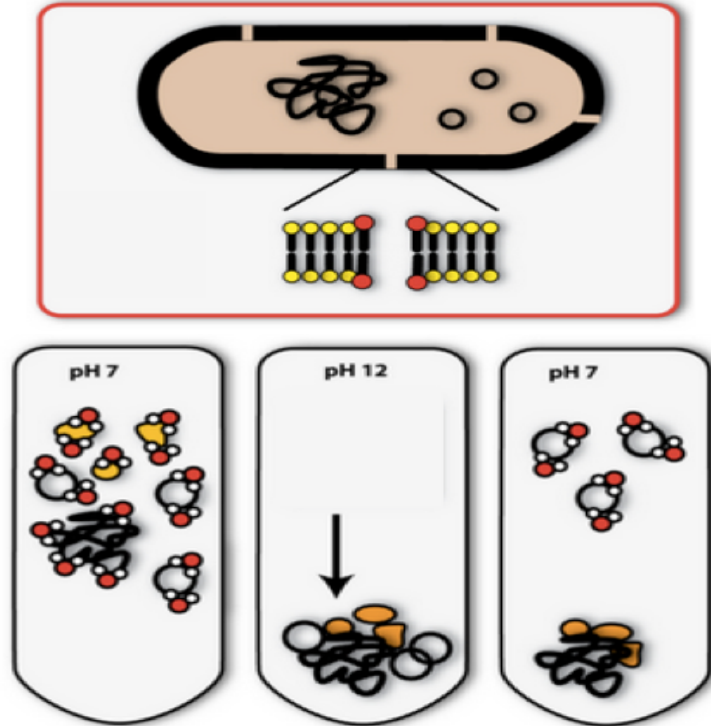


Transformation



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

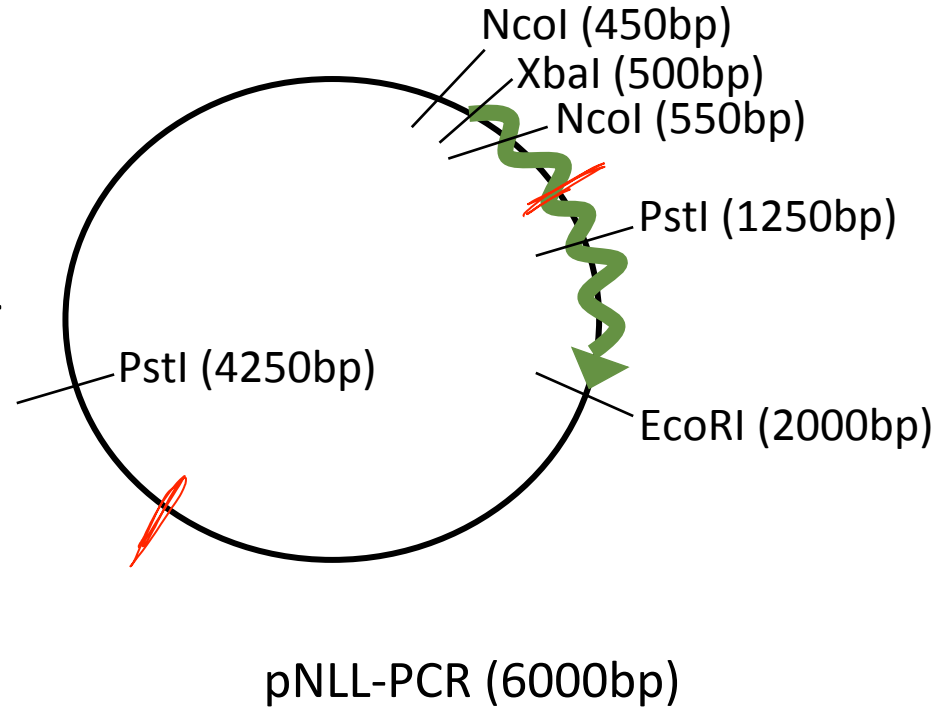
Purification



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

Digestion, again

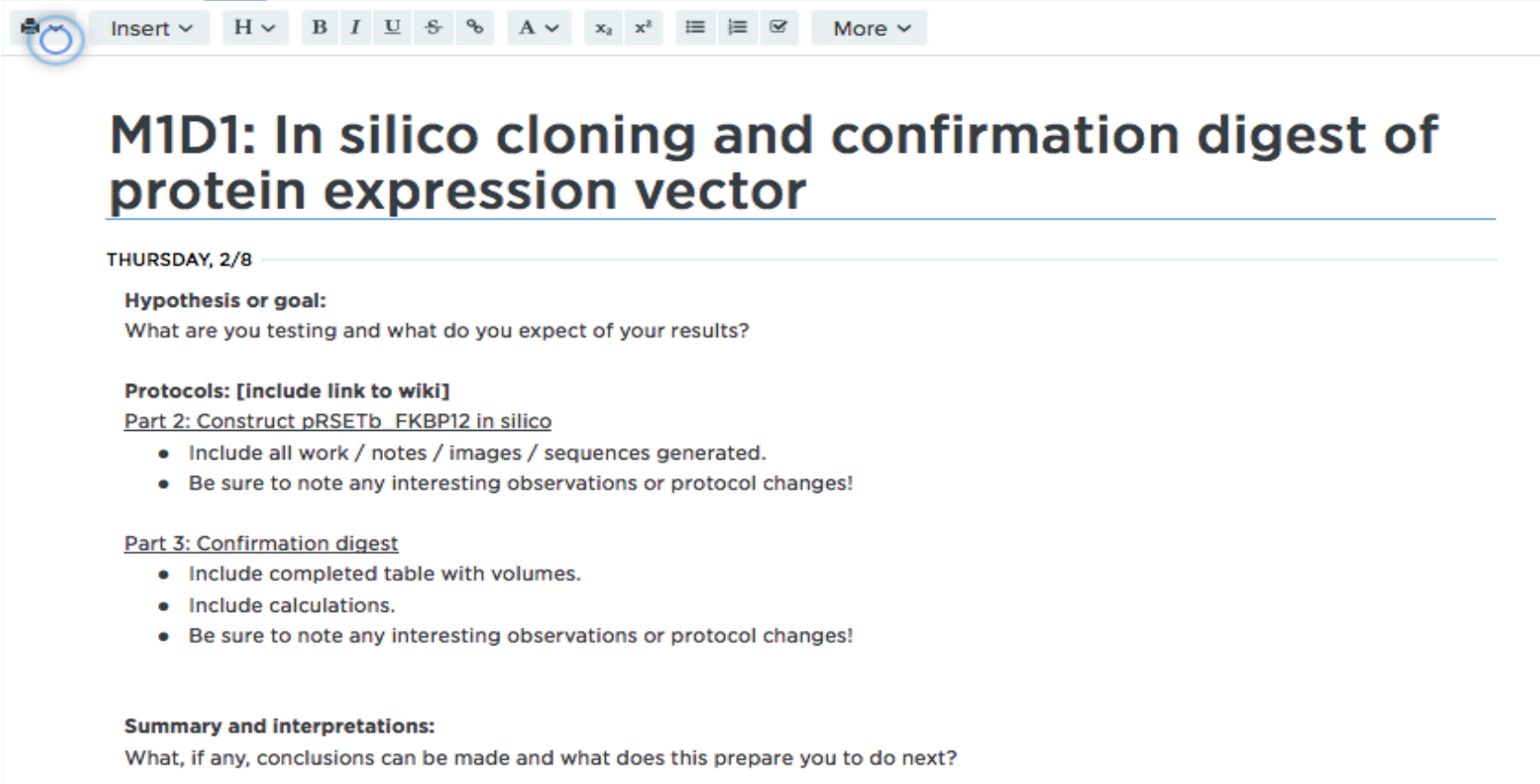
- Confirmation digests
- Ideally, will cut once in insert and once in vector
 - XbaI and EcoRI?
 - PstI?
 - NcoI?



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?



The image shows a screenshot of a digital notebook interface. At the top, there is a toolbar with various editing options like 'Insert', 'H', 'B', 'I', 'U', 'S', 'A', 'x₂', 'x²', and 'More'. Below the toolbar, the notebook content is displayed. It starts with a title 'M1D1: In silico cloning and confirmation digest of protein expression vector' followed by a horizontal line. Below the line is the date 'THURSDAY, 2/8'. The main content is organized into sections: 'Hypothesis or goal:', 'Protocols: [include link to wiki]', 'Part 2: Construct pRSETb FKBP12 in silico', 'Part 3: Confirmation digest', and 'Summary and interpretations:'. Each section contains specific instructions or bullet points.

Insert ▾ H ▾ B I U S A ▾ x₂ x² ☰ ☷ ☒ More ▾

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

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(S20)_YourName”
 - Make each module a new folder
 - Make each day a new entry within module folder
- Share the project with Noreen and Kevin
 - Right-click and choose ‘settings’
 - Add collaborators by email

For today...

- Virtual cloning exercise to build pET_MBP_SNAP_TDP43-RRM12 expression plasmid
- Confirmation digest of pET_MBP_SNAP_TDP43-RRM12

For M1D2...

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