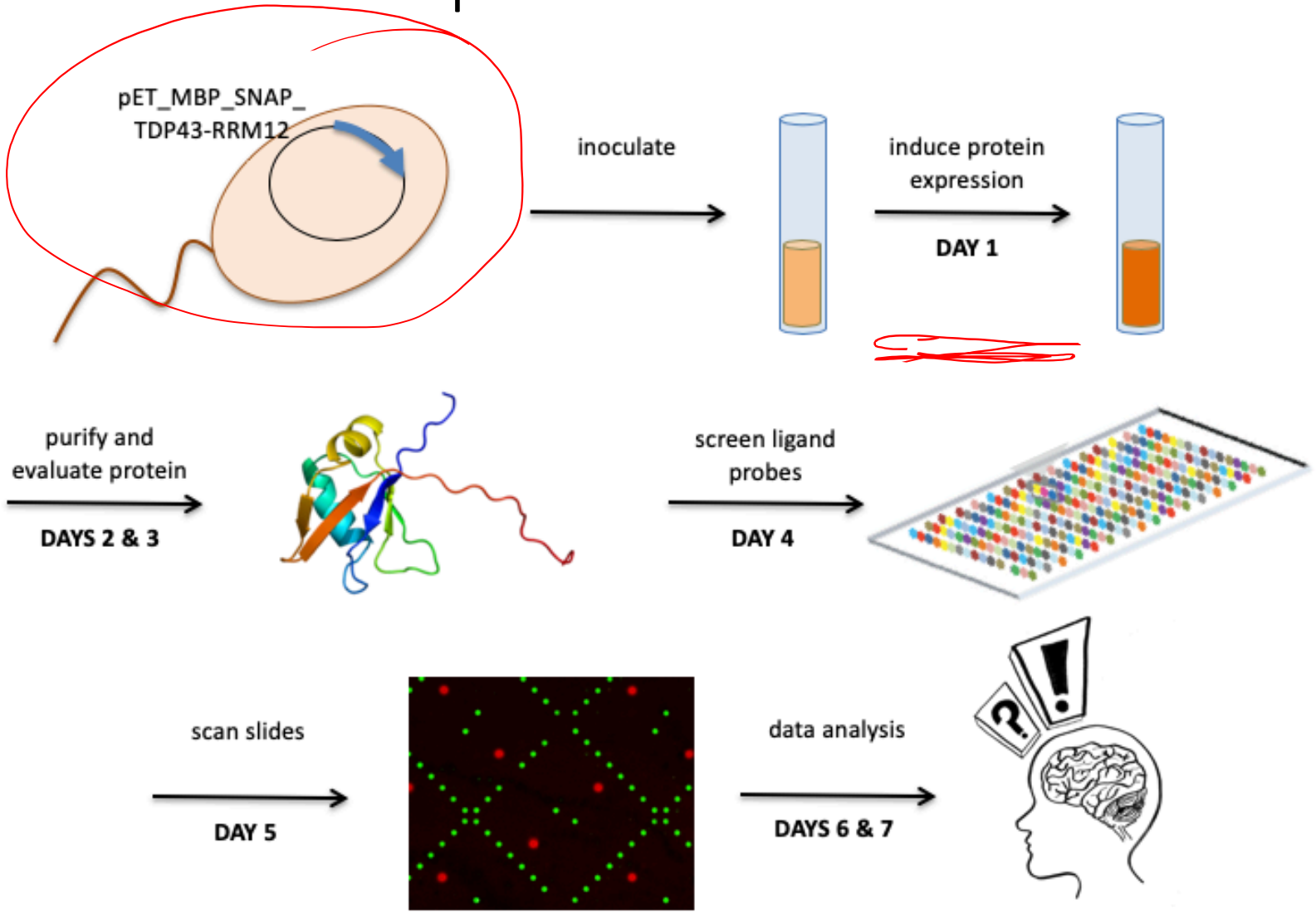
M1D1: Complete in silico cloning and induce TDP43 protein expression

1. Laboratory Orientation quiz
2. Prelab discussion
3. Build protein expression vector – *in silico*
4. Confirm protein expression vector – in lab

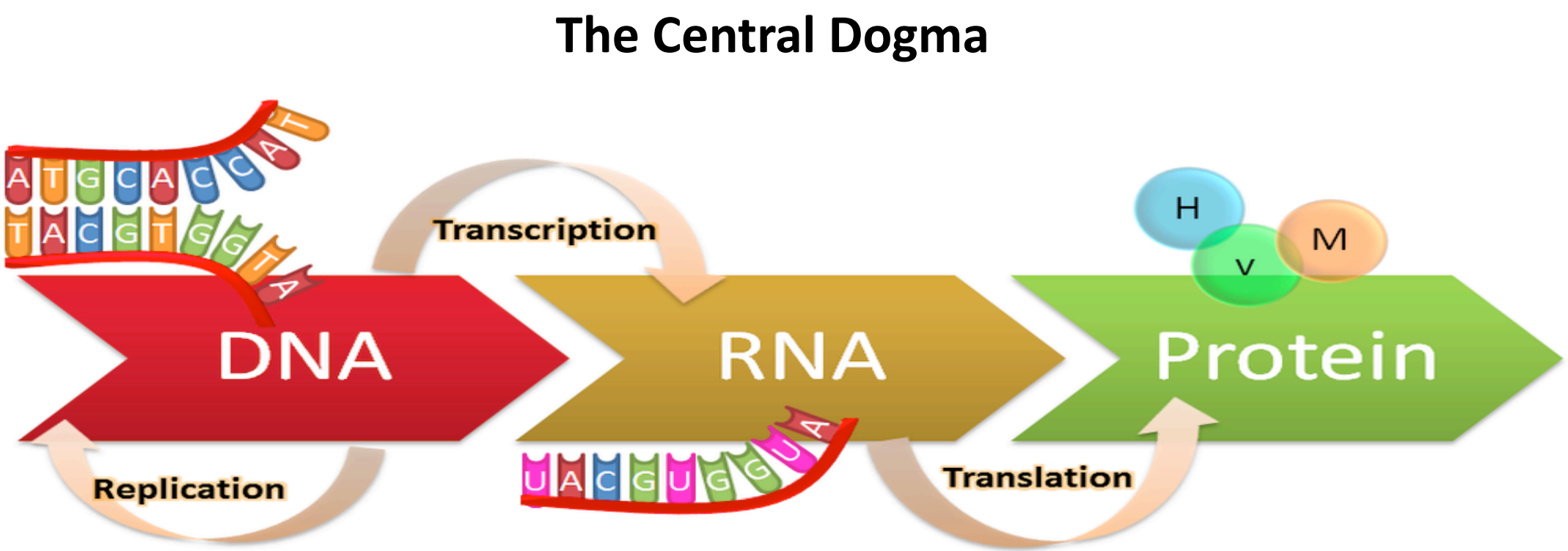
Important Dates for Mod 1!

- 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 Joe 24 hr after M1D7
- Blog (part of 5% Participation)
  - due 3/16 via Blogspot

Overview of Mod1 Experiments



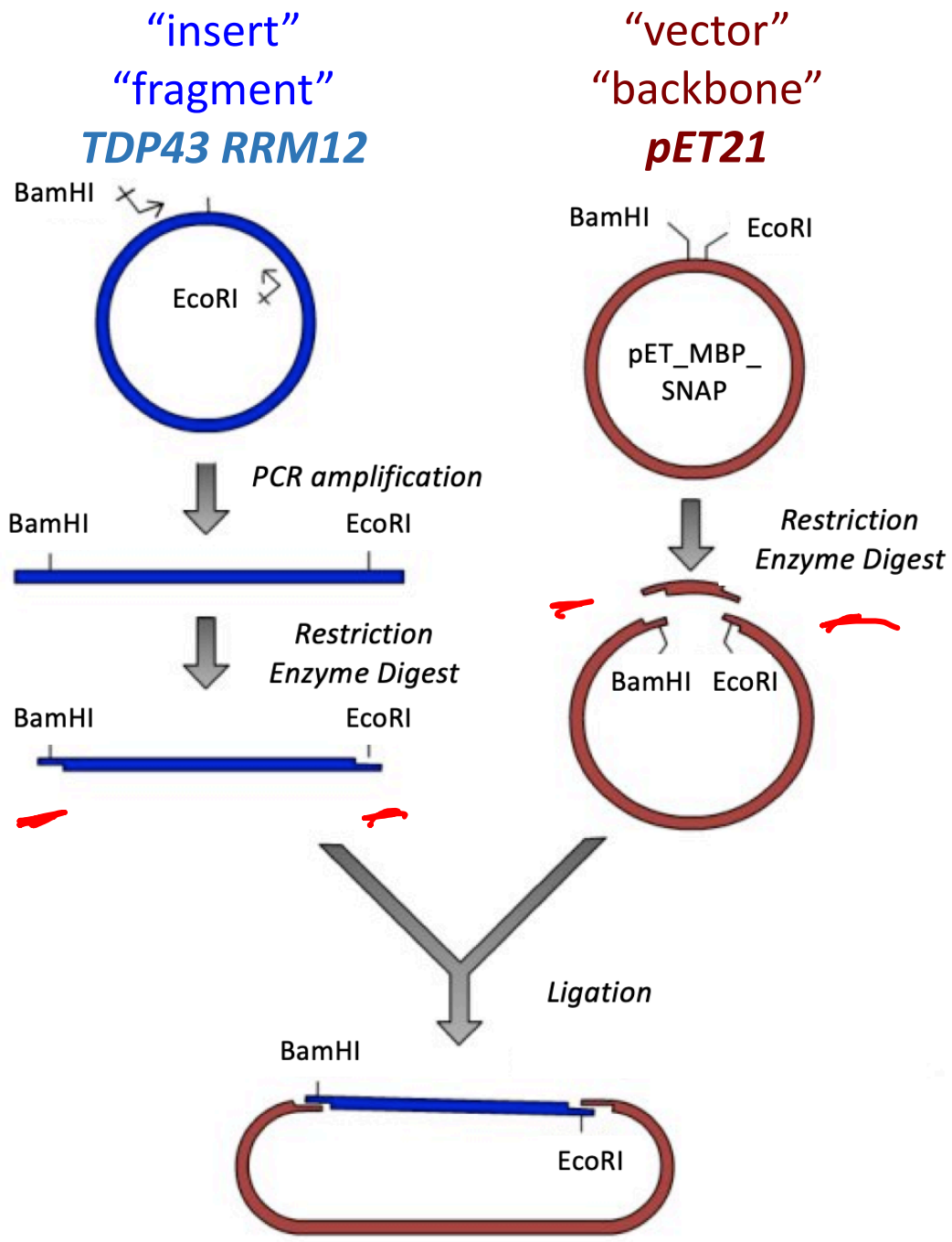
How are proteins made?



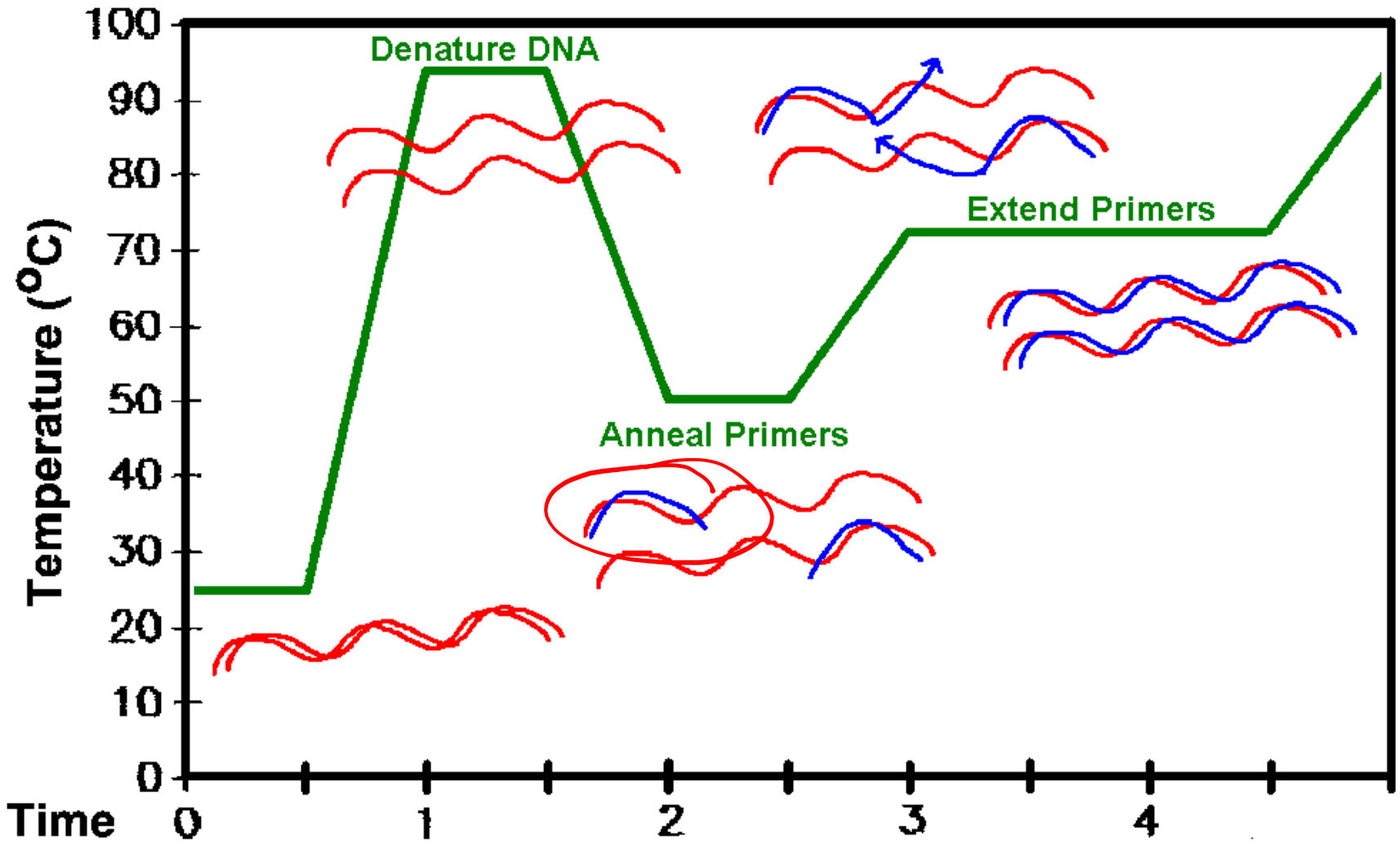
http://genius.com/Biology-genius-the-central-dogma-annotated

What if we want a specific protein?

- Amplification
- Digestion
- Ligation

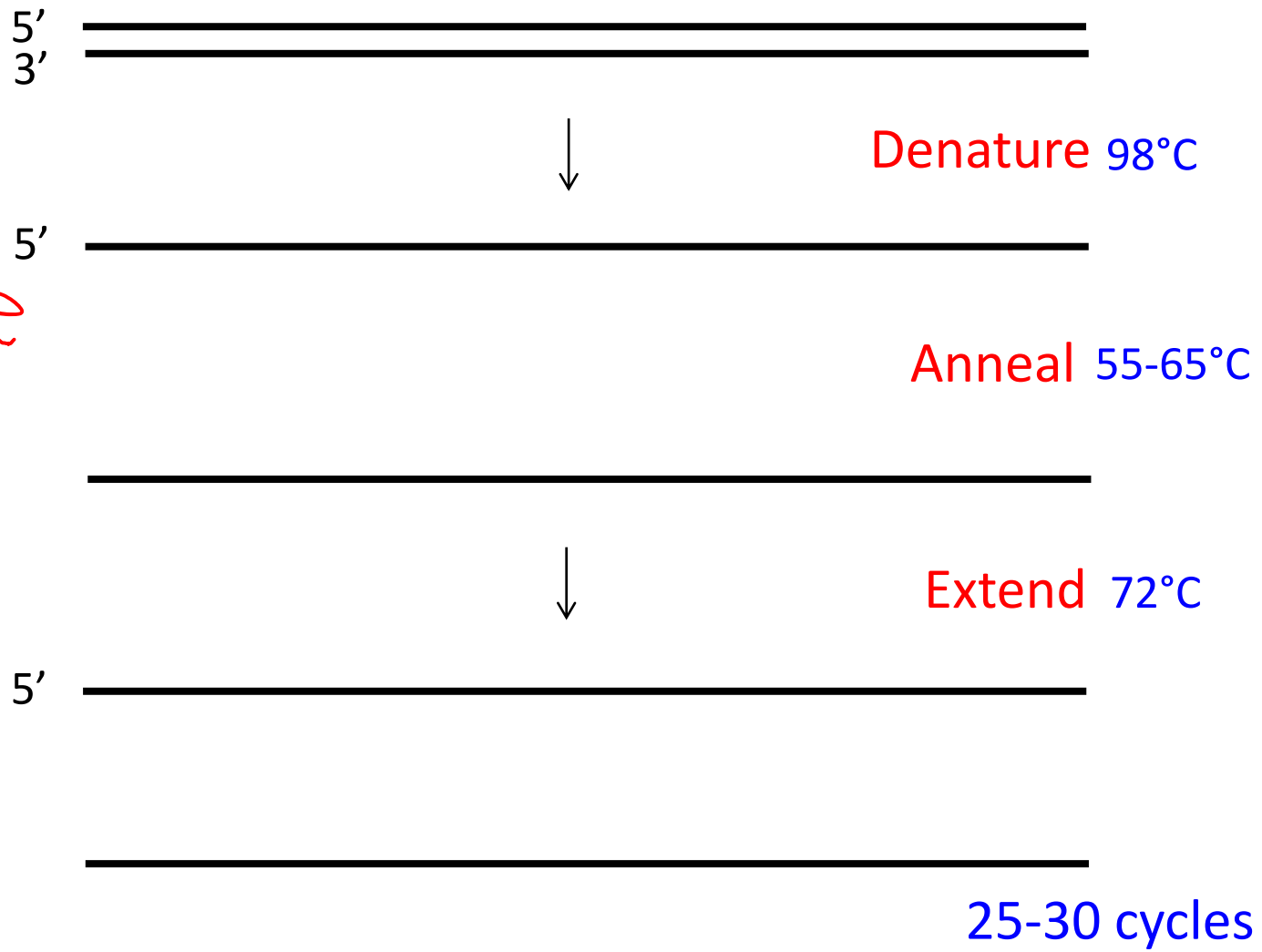


Amplification: PCR cycling



1) Amplification– PCR reagents and conditions

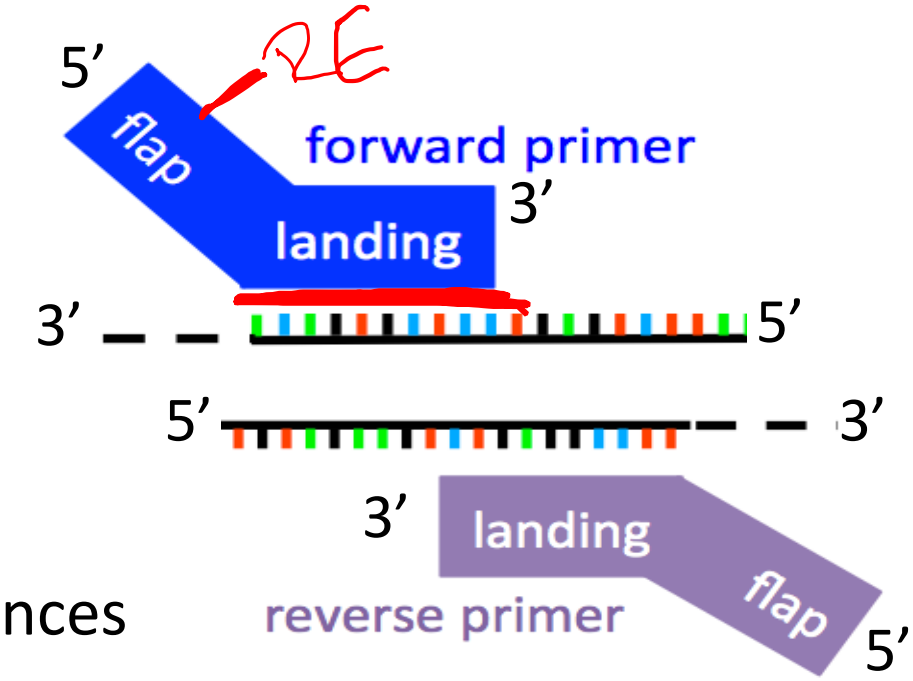
Reagents
Primers
DNA Template
dNTPs
DNA polymerase
Buffer (Mg^{2+})



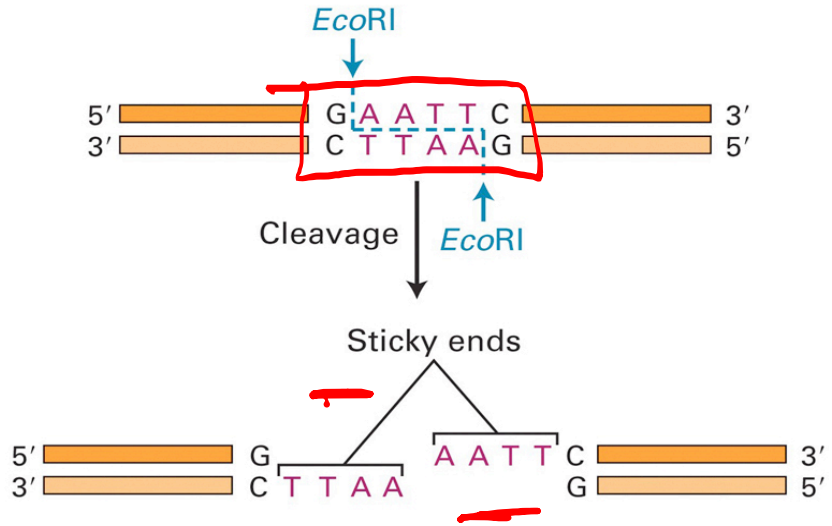
1) Amplification—Guidelines for primer design

- **Landing sequence**: match to TDP43 gene, RRM12 region
- **Flap sequence**: endonuclease recognition sequence, junk DNA

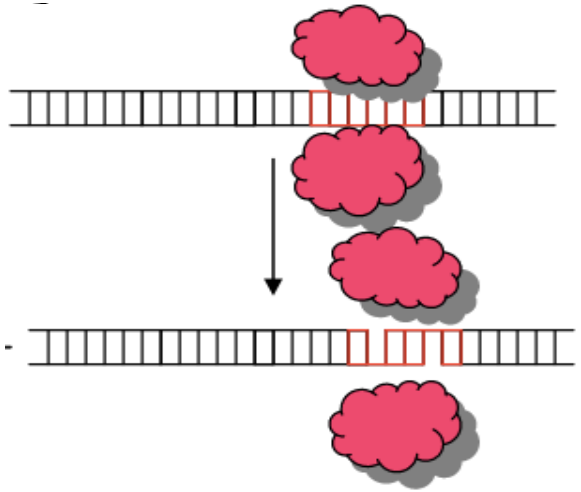
  - Length (landing sequence): 17-28 bp
  - GC content: 40-60 %
  - Melting temp of primer : < 65 °C
  - Avoid secondary structure and repeat sequences
    (e.g. hairpins, primer dimers, ATATAT)



2) Digestion—Create compatible ends on insert fragment and backbone

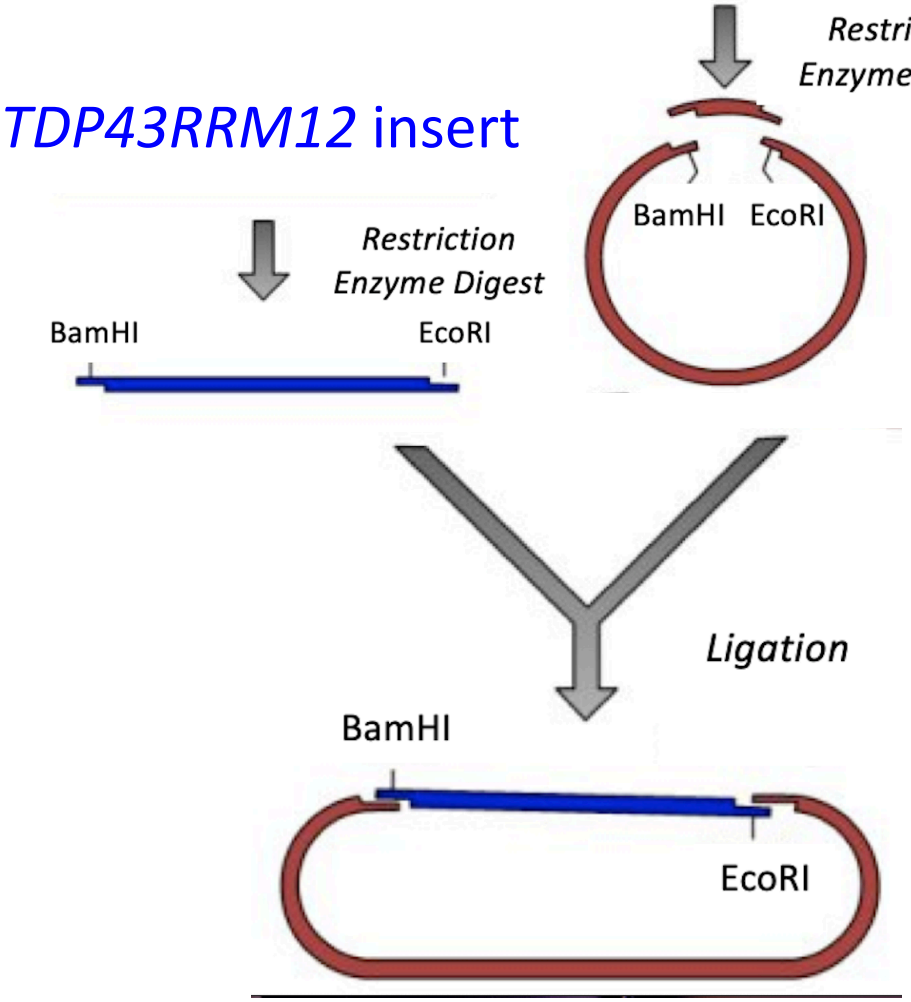


- many restriction enzymes function as homodimers
- binds palindromic sequences
- cleaves backbone



pET21 vector

TDP43RRM12 insert



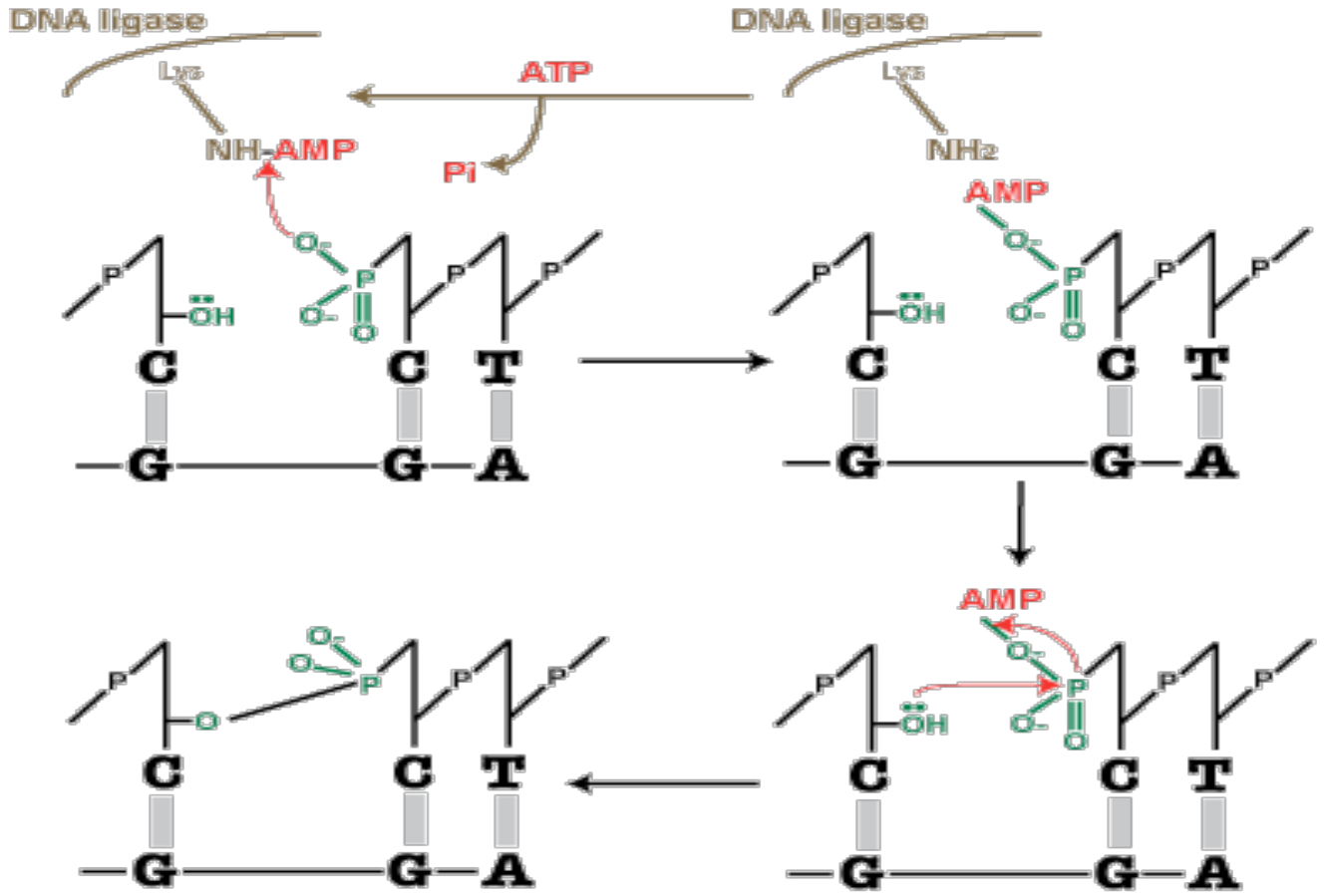
2) Digestion– Reagents and conditions

Reagents
RES
Buffer (ATP)
DNA

- Temperature
- Time

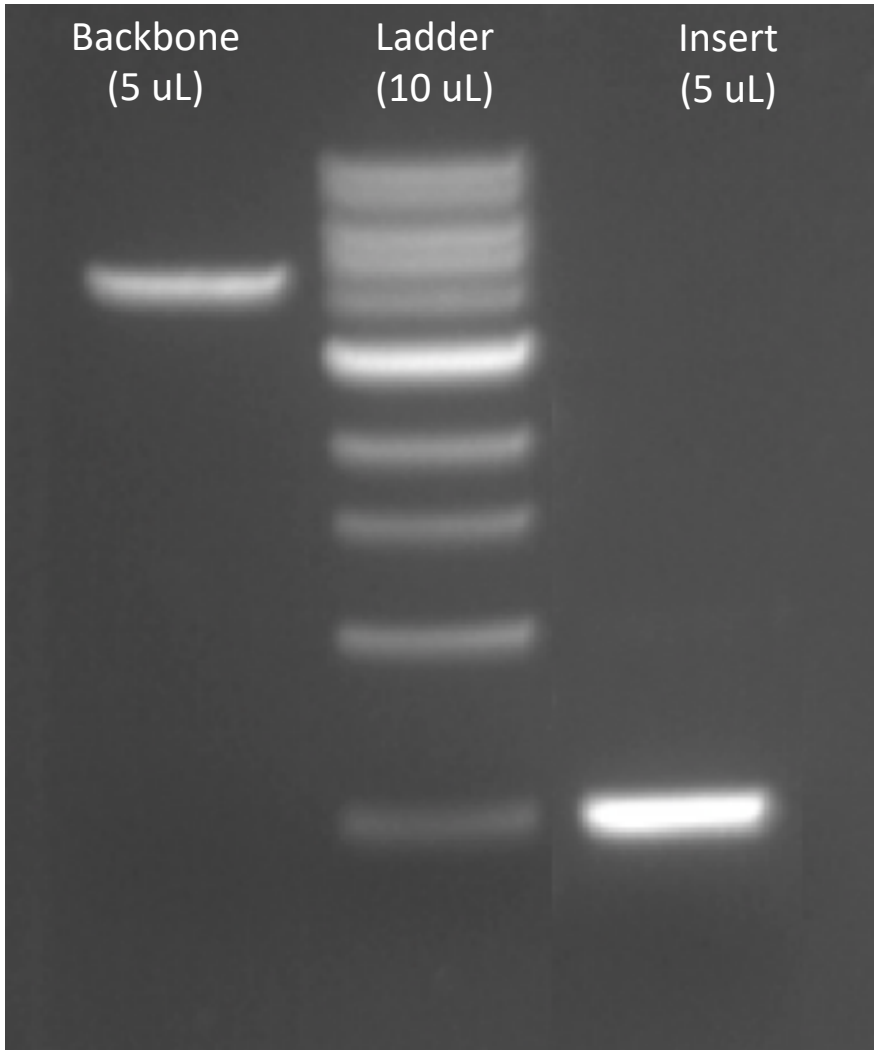
3) Ligation: T4 DNA ligase

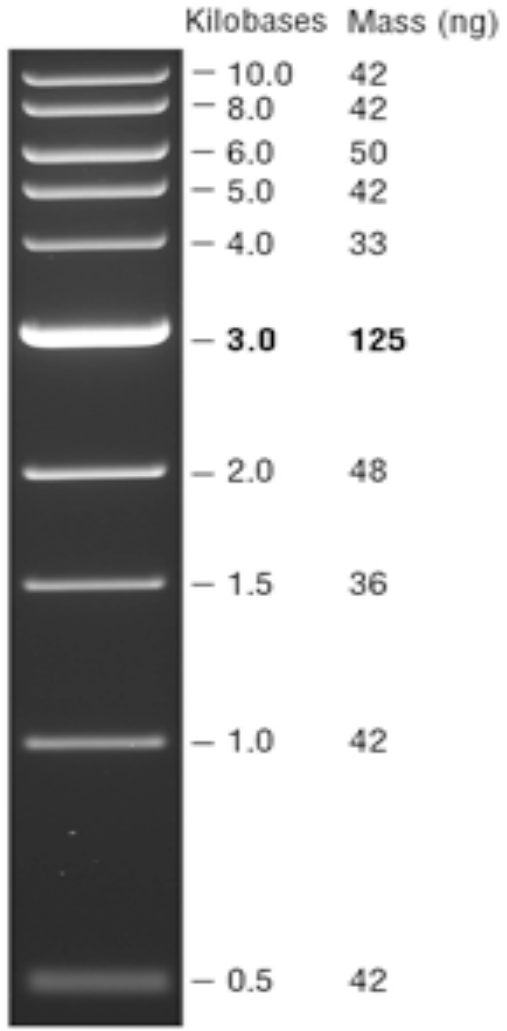
- Forms covalent phosphodiester bond between 3’ OH acceptor and 5’ phosphate donor
- Requires ATP



bitesizebio.com

3) 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

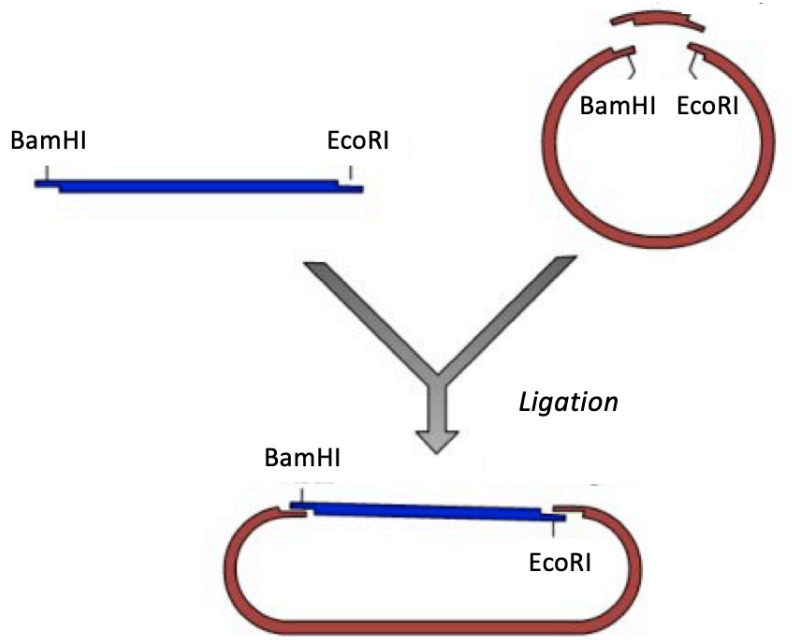
4. Calculate volume of insert
   - Use insert concentration = 20 ng/uL

How do we confirm successful ligations/ DNA plasmid production?

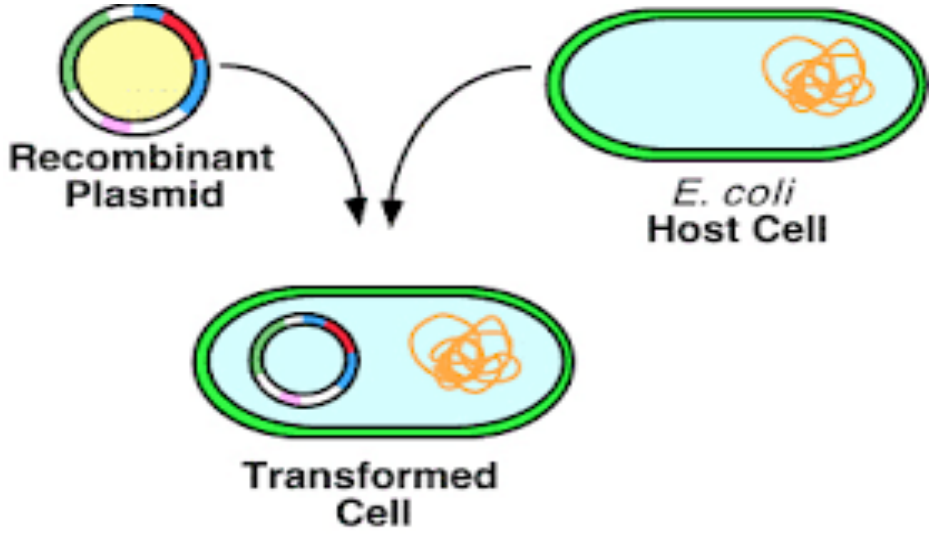
Method 1: Sequencing

Method 2: Diagnostic Digest

- Amplify plasmid
  - Transform into bacteria
- Purification
  - Separate plasmid from chromosomal DNA
- Digestion
  - Confirm the plasmid contains expected fragments



Diagnostic Digest: Transformation to amplify DNA plasmid



1. Incubation of bacteria and DNA plasmid

2. Heat shock (or electroporation)

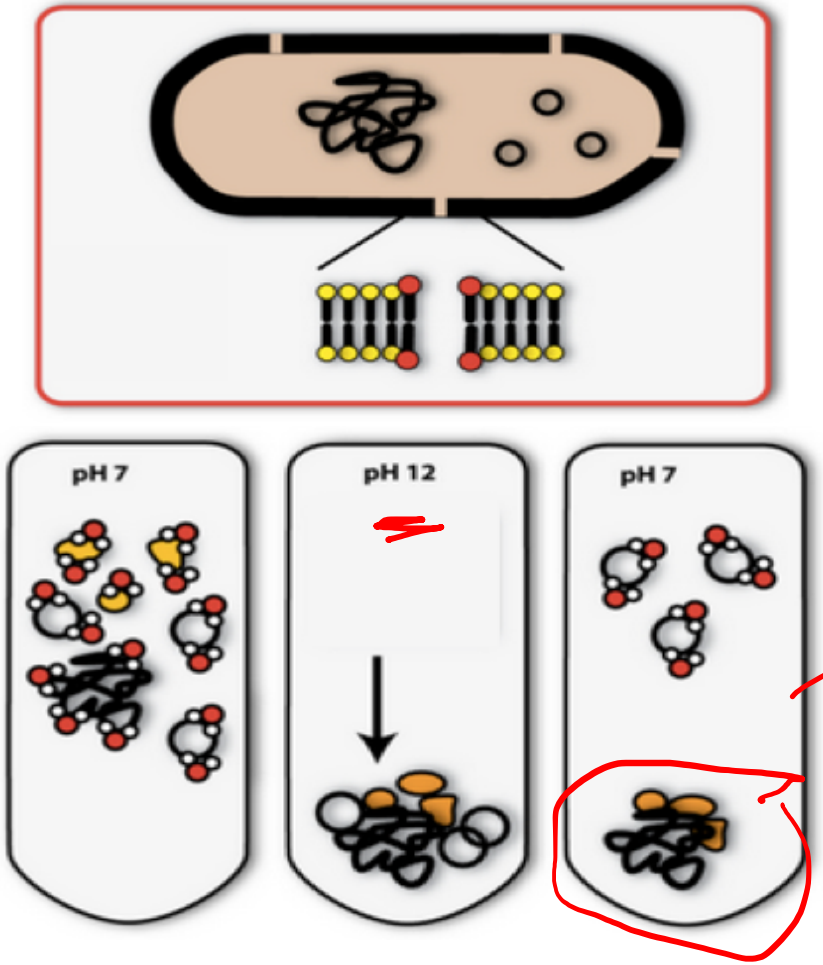
   -- DNA taken in by competent cells

3. Recovery at 37C

4. Selection for bacteria that have taken up the plasmid

Diagnostic digest: DNA Purification

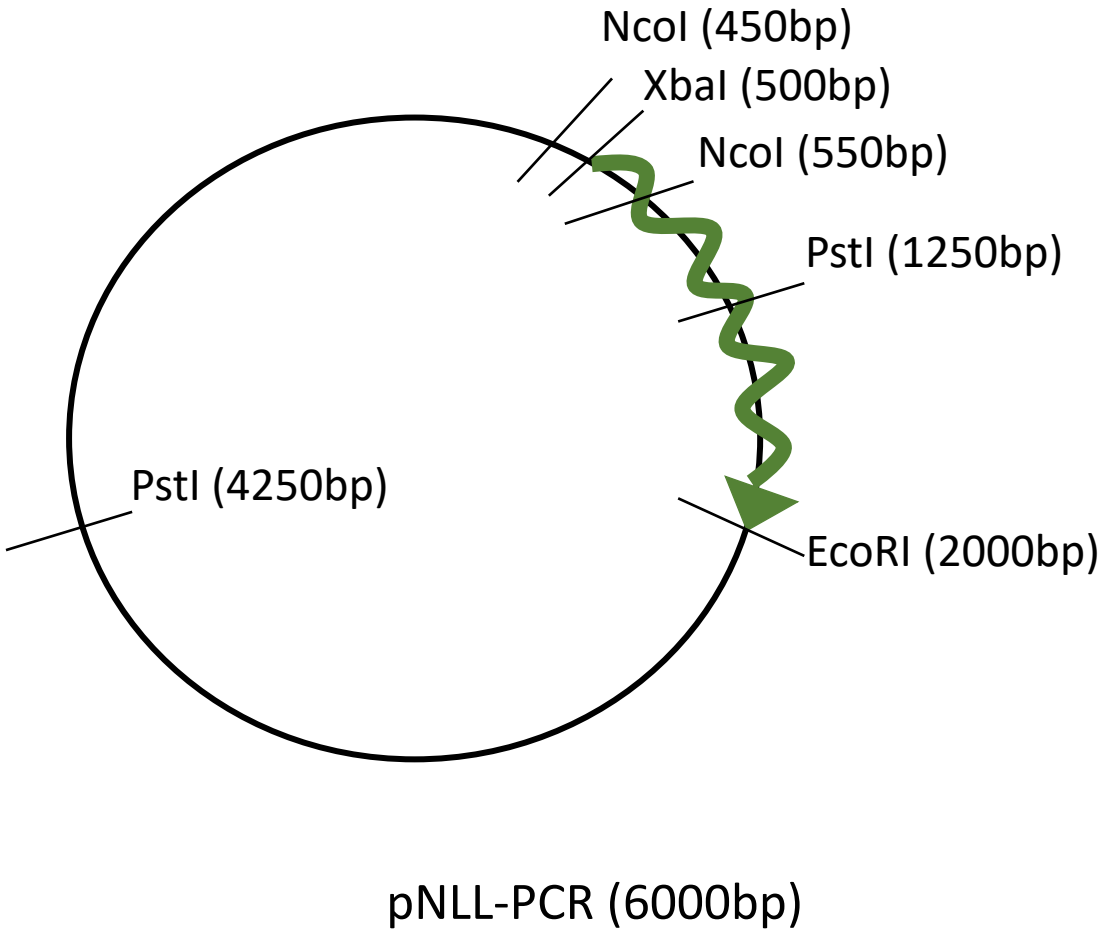
AKA: the mini-prep



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

Diagnostic Digest: Digestion (again)

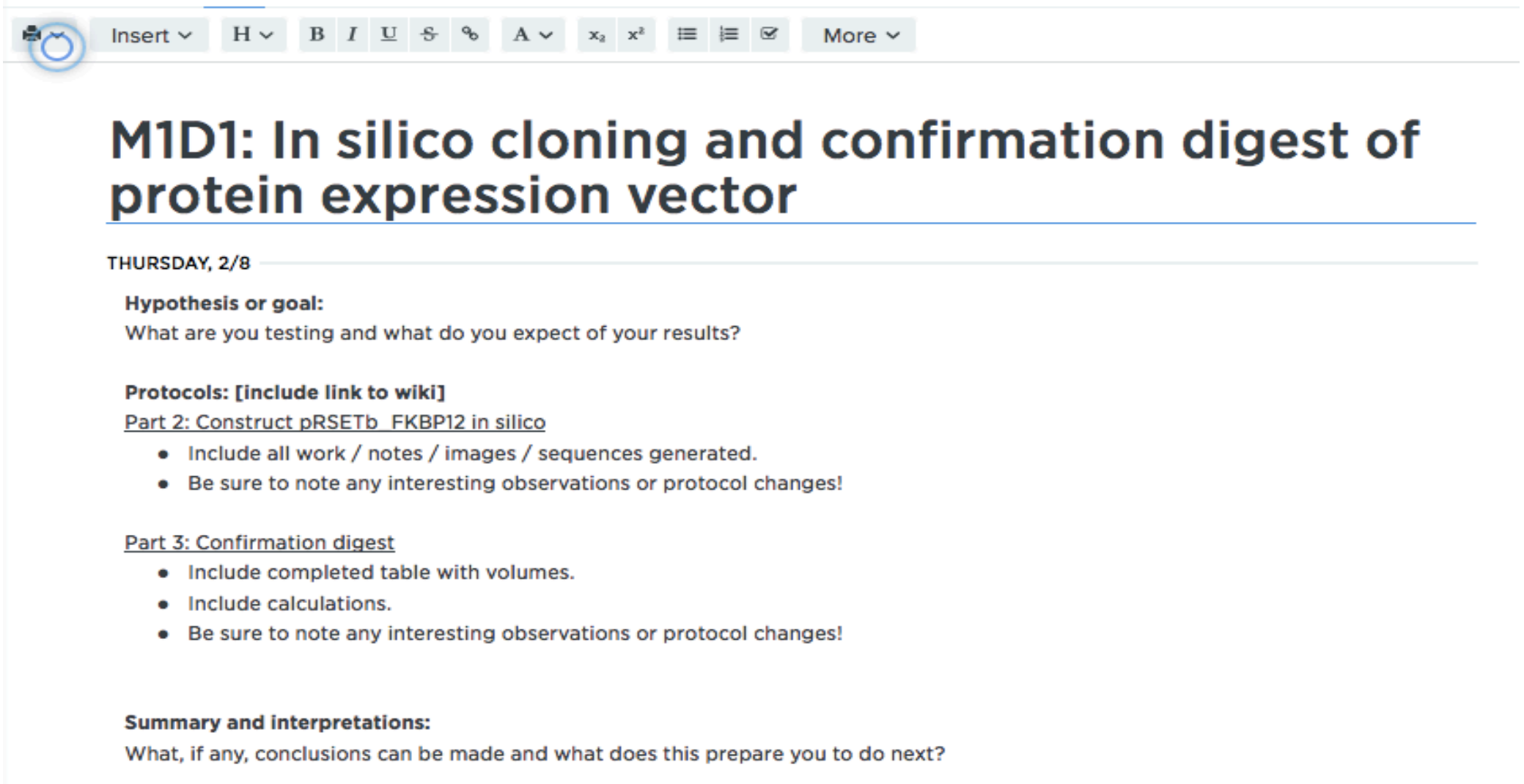
- Confirmation digests
- Ideally, will cut once in insert and once in vector
  - XbaI and EcoRI?
  - PstI?
  - NcoI?



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?



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:

Becky (rcmeyer@mit.edu) and **Joe (jkreitz@mit.edu)**

  - 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 (ligation calculation, etc)