M2D3: Generate gRNA plasmid

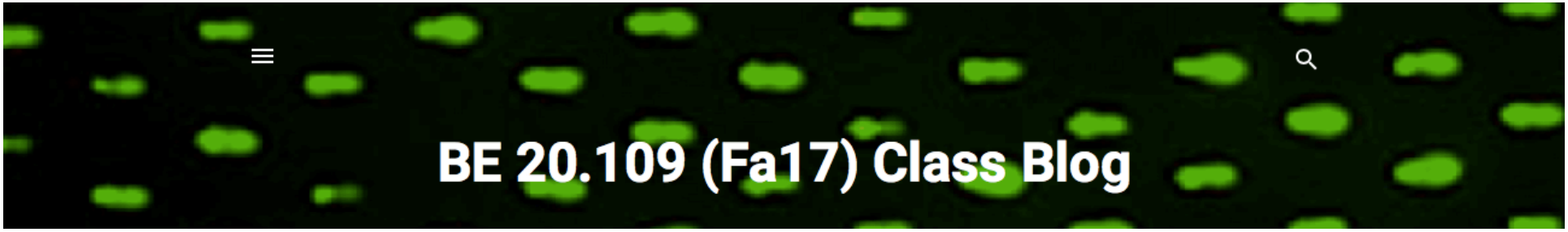
10/19/17

1. BE Communication workshop: Journal Club presentations
2. Pre-lab discussion
3. Set up reaction to generate gRNA plasmid

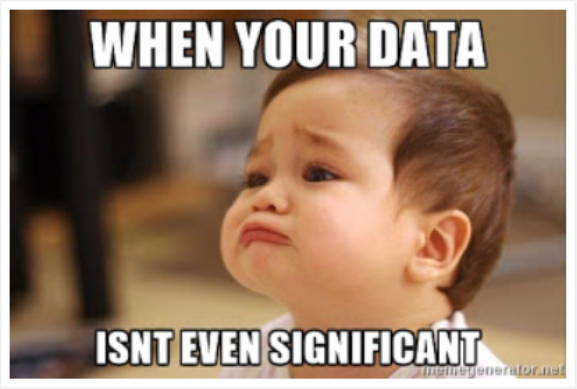
You can't change your journal paper after Friday @ 5pm. If you do change your paper before then email all instructors.

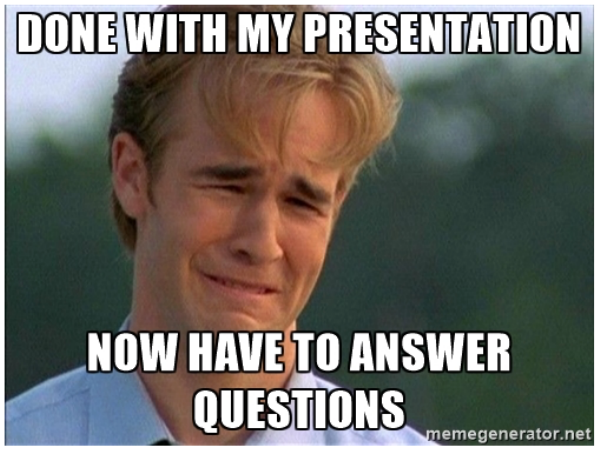
M2 major assignments

- Research Article (20%)
  - individual, submit on Stellar
  - word document
  - due 11/20 (no revision)
- Journal Club Presentation (15%)
  - individual, during lab section, video recorded
  - powerpoint slides due 1pm on Stellar Oct 24 or Oct 31
- Lab quizzes
  - M2D5 and M2D8
- Notebook (part of 10% Homework and Notebook)
  - one day will be graded by Eric announced M2D8
- Blog: http://be20109f17.blogspot.com/ (part of 5% Participation)
  - by 10/23 (Mod1 material)
  - by 11/21 (Mod2 material)

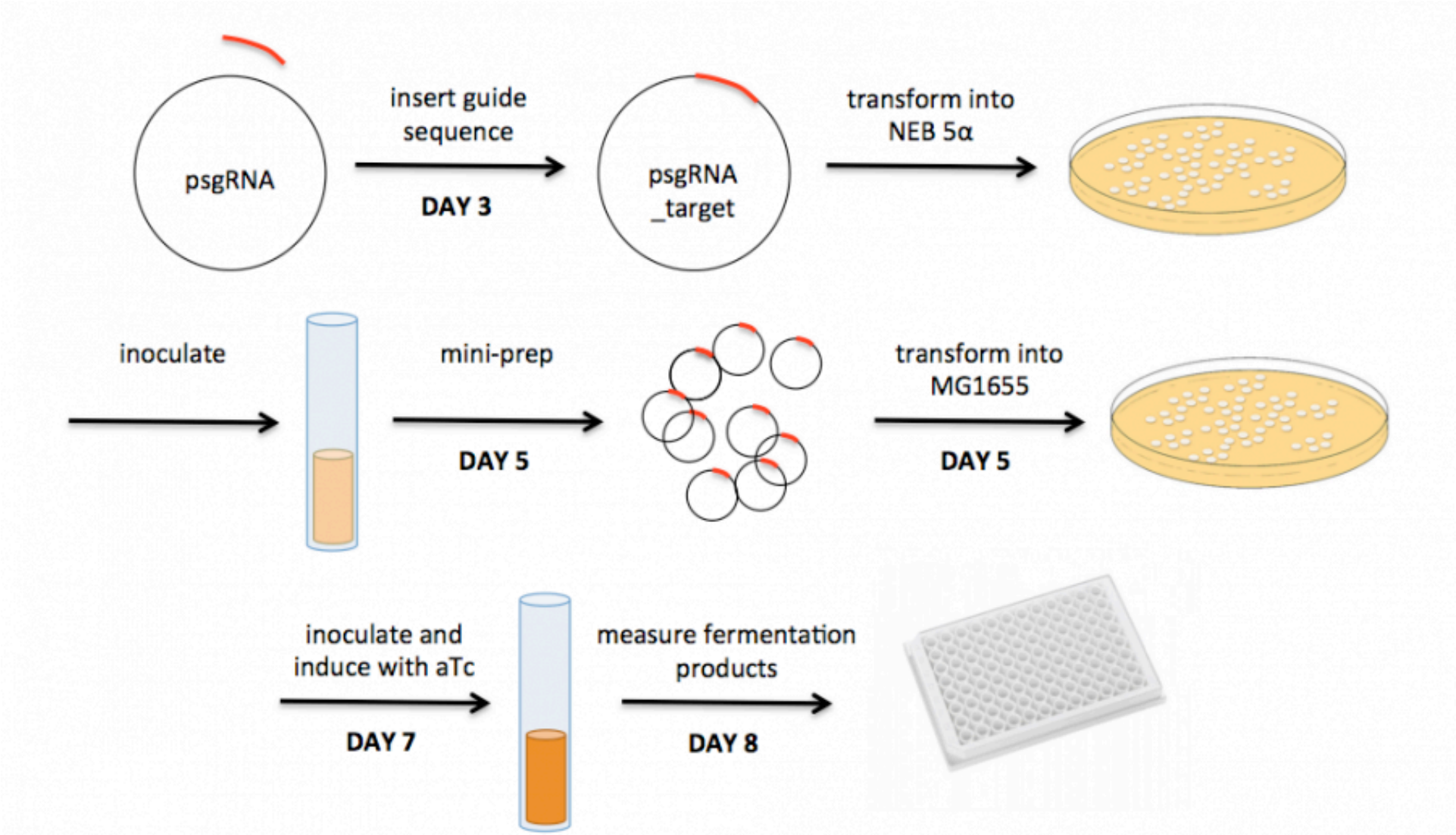


- You will receive an invitation to join the blog tomorrow
- Possible topics listed on the blog
- Details about use:
  - Do not publish MIT logo
  - Do not post photographs with names tagged
  - Do not write malicious comments
  - Do not plagiarize





Mod 2 experimental overview



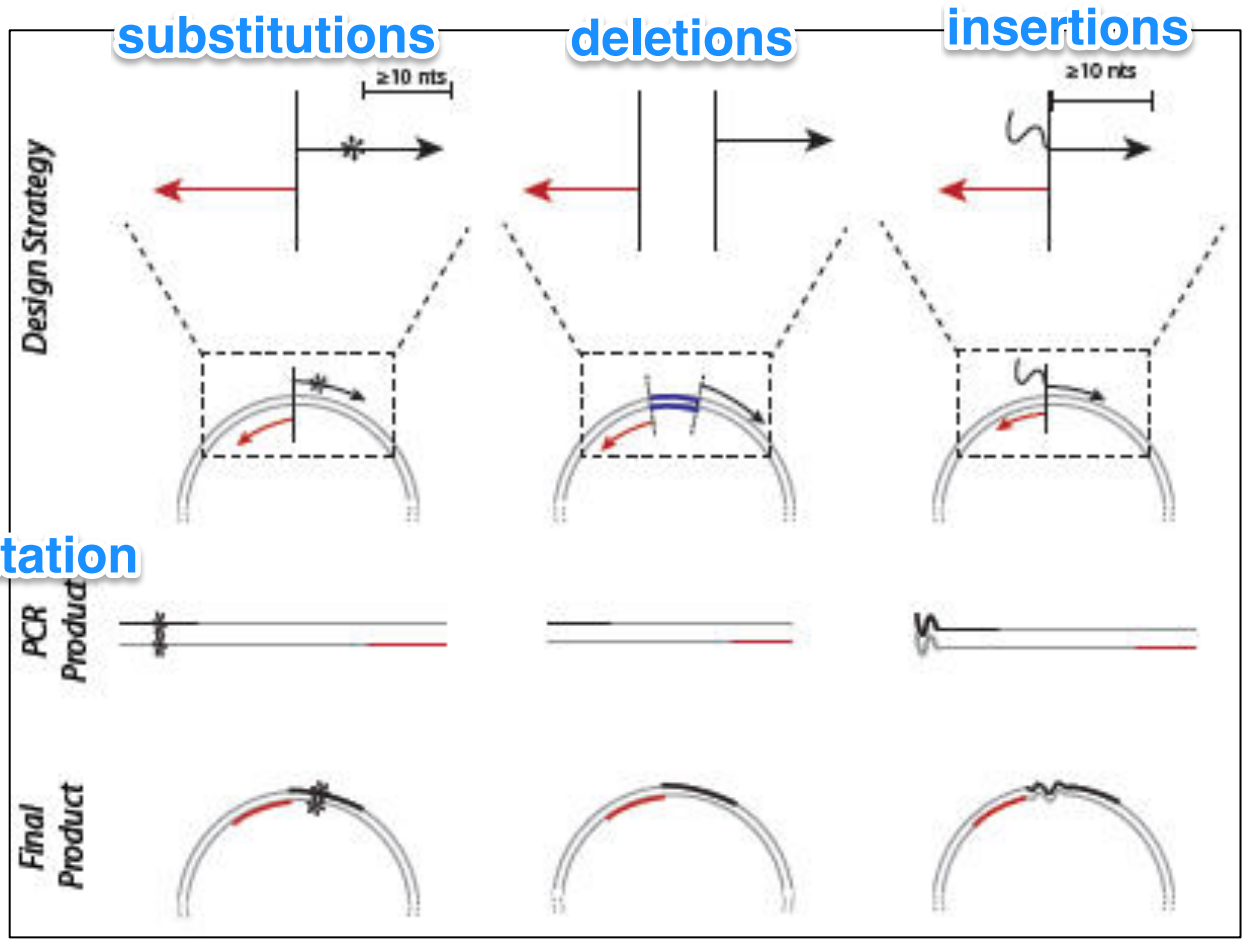
Use of Site-directed mutagenesis (SDM) to engineer plasmid DNA: NEB Q5 SDM kit

- Create specific, targeted changes in double-stranded plasmid DNA

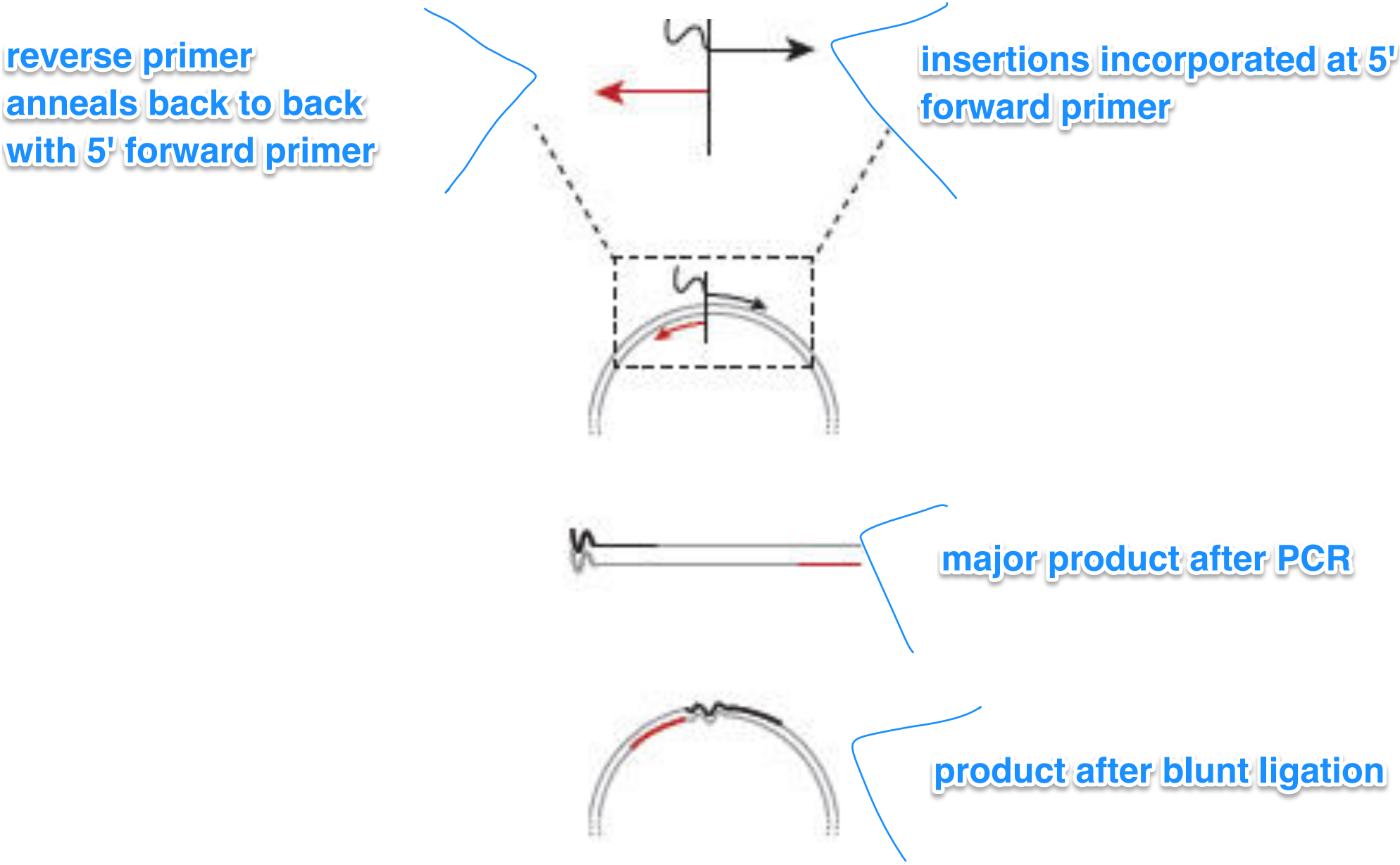
- Forward primer: **contains the desired mutation**

- PCR product: **linear**

- Final product: **circular plasmid**



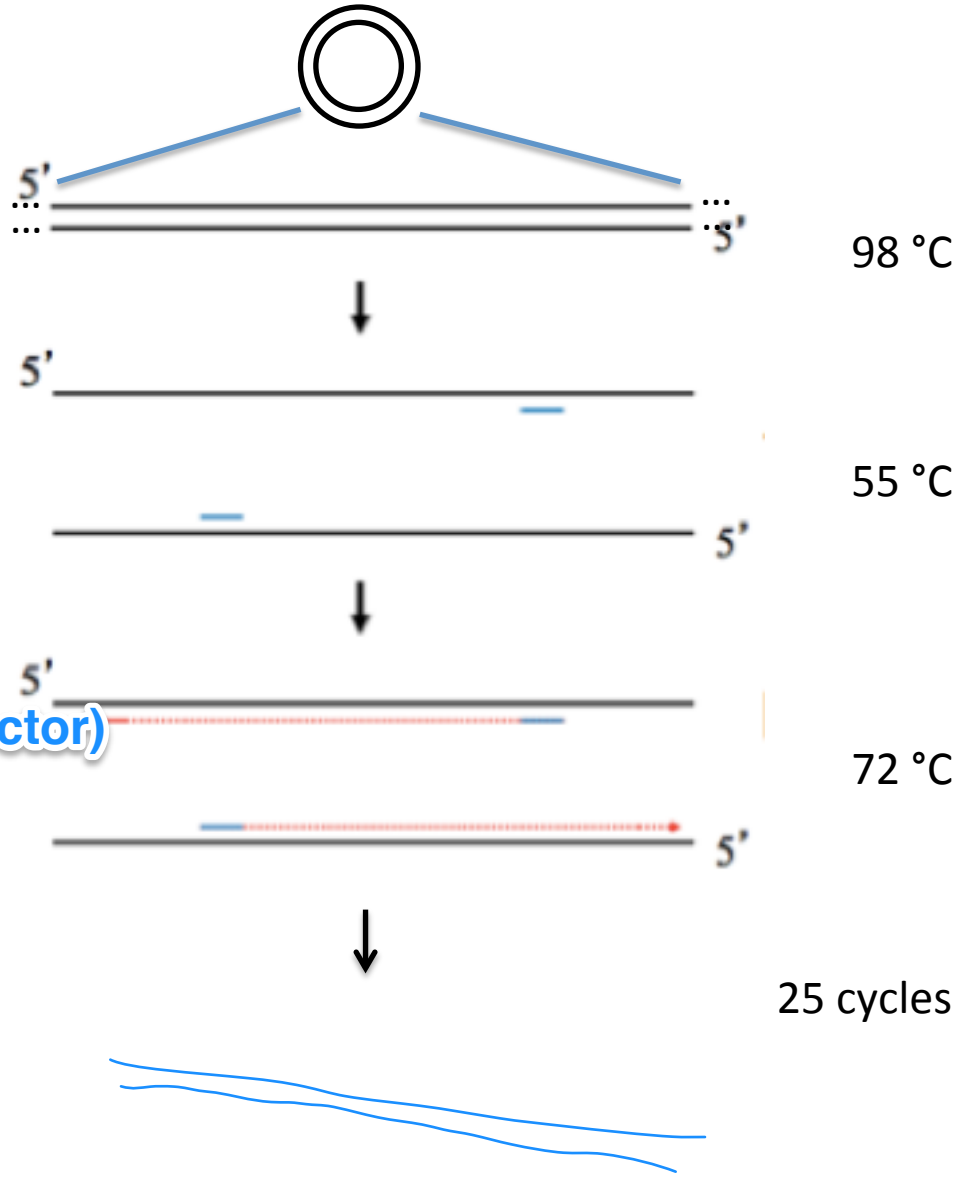
Insertion of DNA via SDM



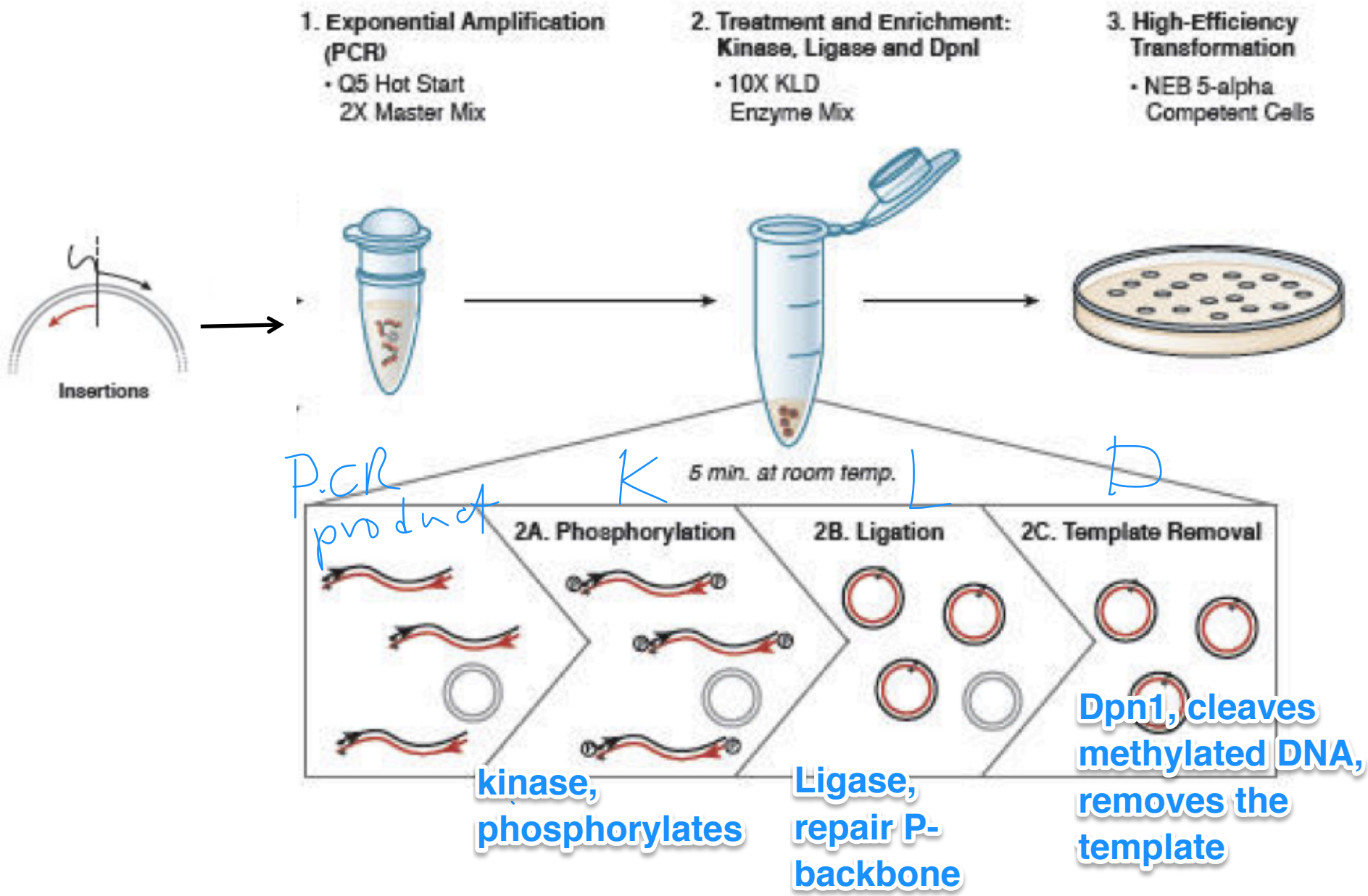
SDM ingredients and Thermocycling conditions

SDM ingredients
F primer
R primer
polymerase
dNTPs
template (gRNA expression vector)
buffer (pH, cofactors Mg++)

H2O

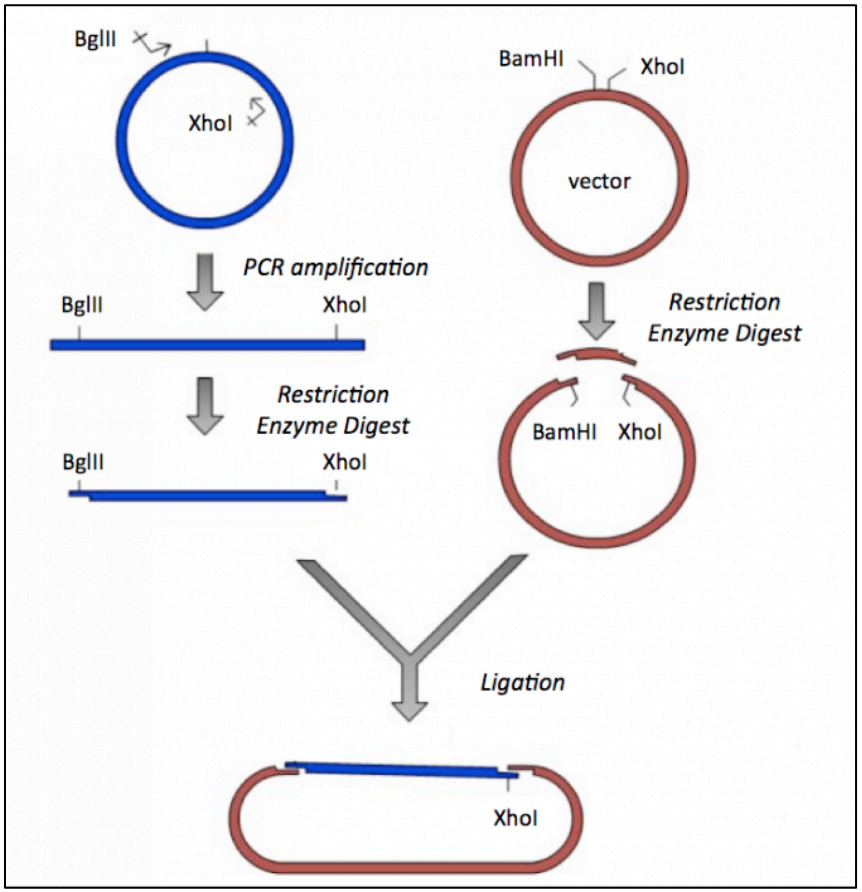


Additional steps necessary to recover circular plasmid product

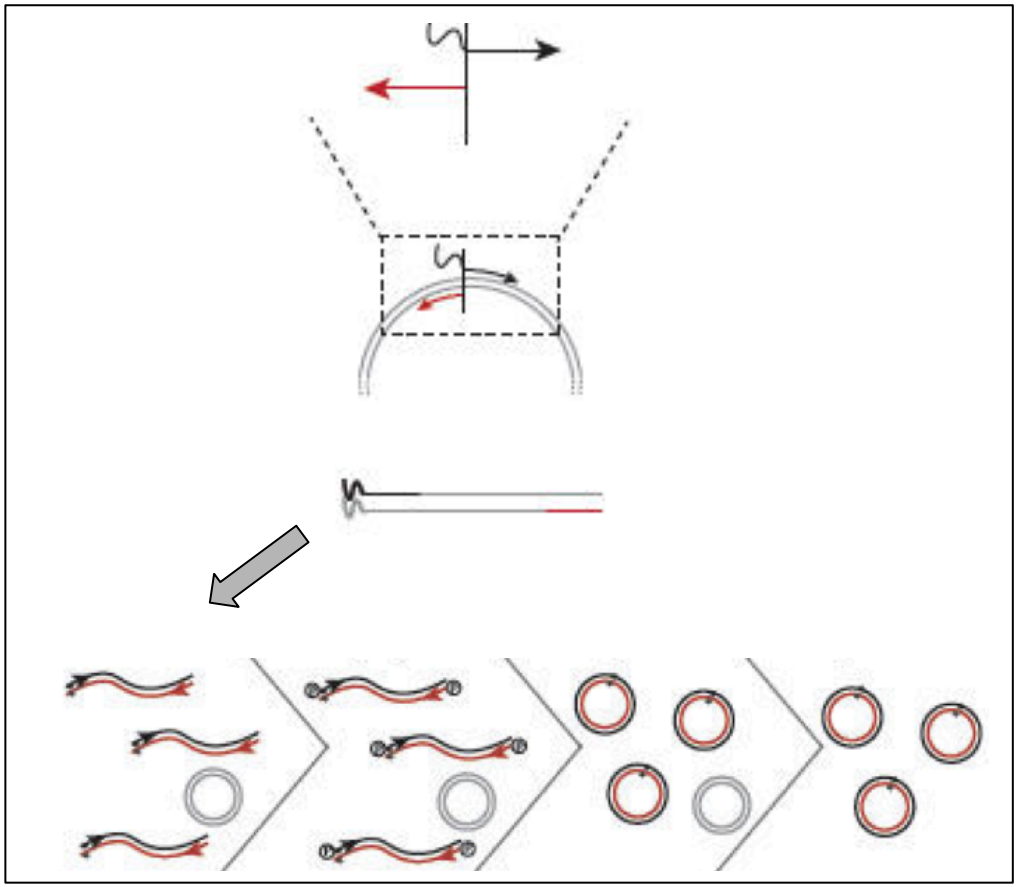


We have covered two ways to engineer DNA:

"Traditional" plasmid cloning by restriction enzyme digest



Site directed mutagenesis



Tuesday

Journal Club I

- Submit your presentation slides to Stellar by 1pm Oct. 24th
- Presentations should be 10min, PLEASE practice your talk out loud at least once
- Tell us a narrative from the paper, you don't have to (and probably can't) present all the data
- You will present from a mac (my computer)
- Q&A will start with student questions, asking questions counts toward your participation grade
- There will be SNACKS
- Please reach out to the instructors and discuss your paper in advance if you feel it will organize your thoughts/presentation

Thursday

M2D5 HW: Intro, Schematic, Discussion

- Draft Introduction
  - Draft the entire first “Big Picture” paragraph
  - overview/ topic sentence (first sentence) of each additional paragraph
  - references in text and brief summary of each reference
- Schematic of Mod2 experimental ***approach*** (not overview)
  - Create image (do not take and reference published schematics)
  - Include a figure title and caption
- Draft Discussion for confirmation agarose gel figure
  - Draft a paragraph

Reporting and interpreting your data

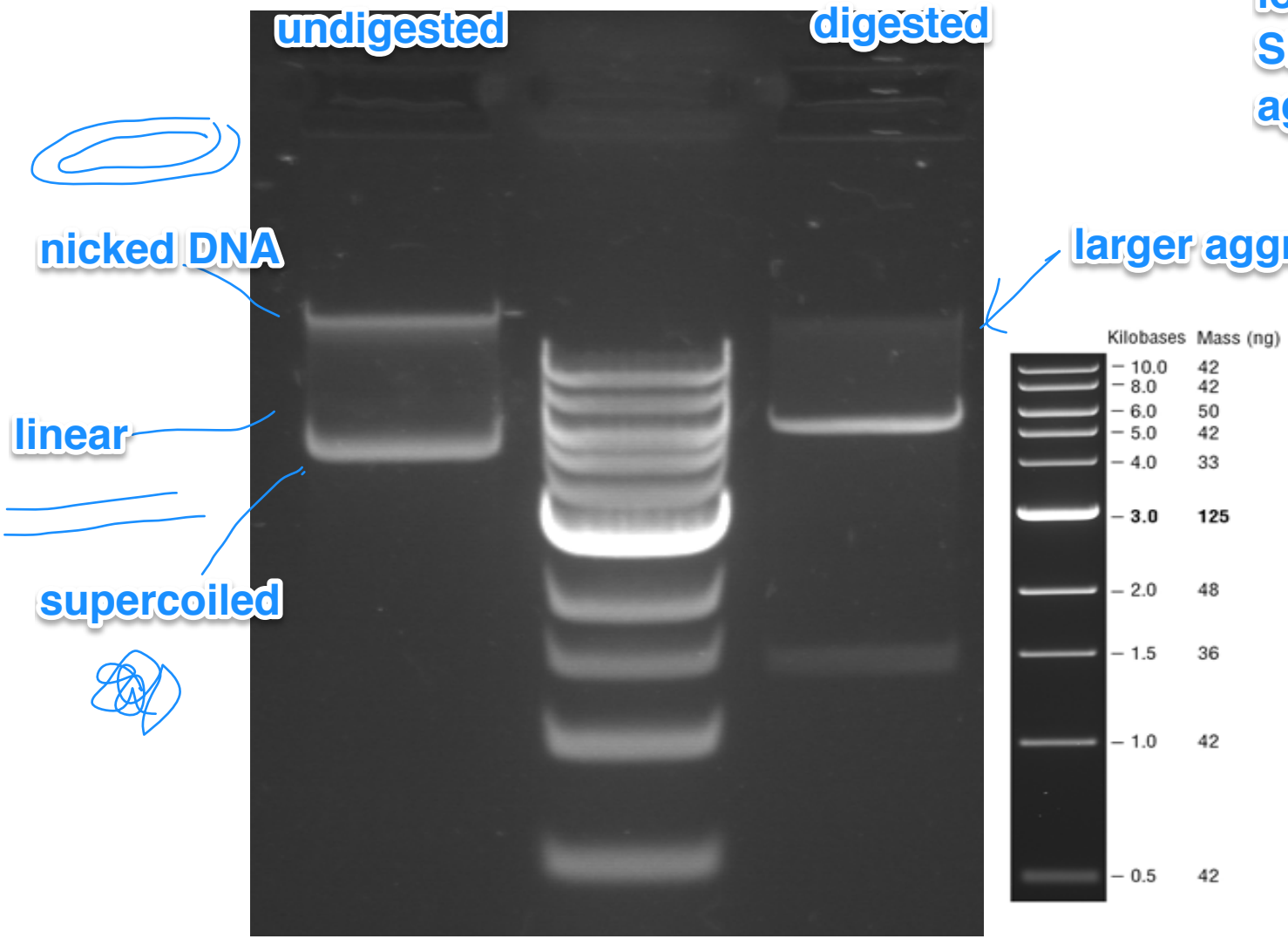
RESULTS

1. What was the overall goal of these data?
   - State concisely as an introductory sentence.
2. If applicable, what was the result of your control?
   - Was it expected?
3. What was your result?
   - Was it expected?
4. What does this motivate you to do next?
   - Specifically, what experiment follows?

DISCUSSION

1. What evidence do you have that your result is correct or incorrect?
   - How do your controls support your data?
2. In sum, what do your data suggest or indicate?
   - Do your data support your hypothesis? Why?
3. What does this motivate you to do next?
   - Specifically, what is the next research question?

Nicked, supercoiled, & linear DNA



loading dye did not have SDS, larger DNA aggregates present

Today in lab…

1. Find your gRNA oligo at the front bench and reconstitute in H_2O
2. Set up your gRNA insertion/amplification reaction using reagents at front bench
3. Work on your Journal Club presentations