M2D3: Generate gRNA plasmid

10/20/17

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

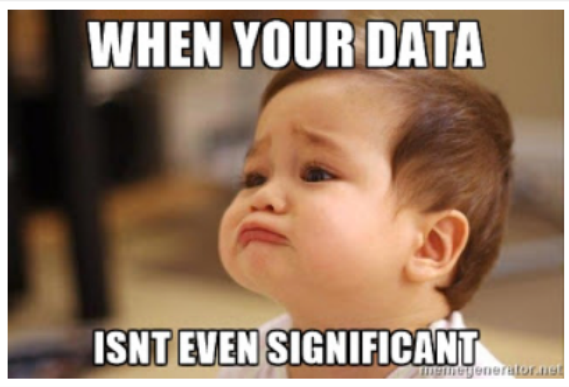
You can't change your journal paper after Saturday (10/21) @ 5pm. If you do change your paper before then email all instructors.

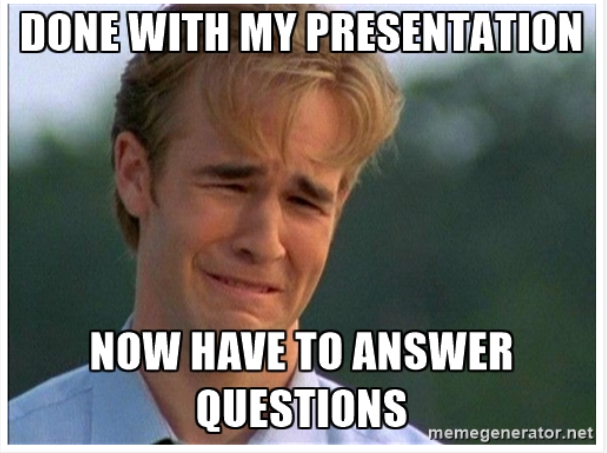
M2 major assignments

- 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 25 or Nov 1
- 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)

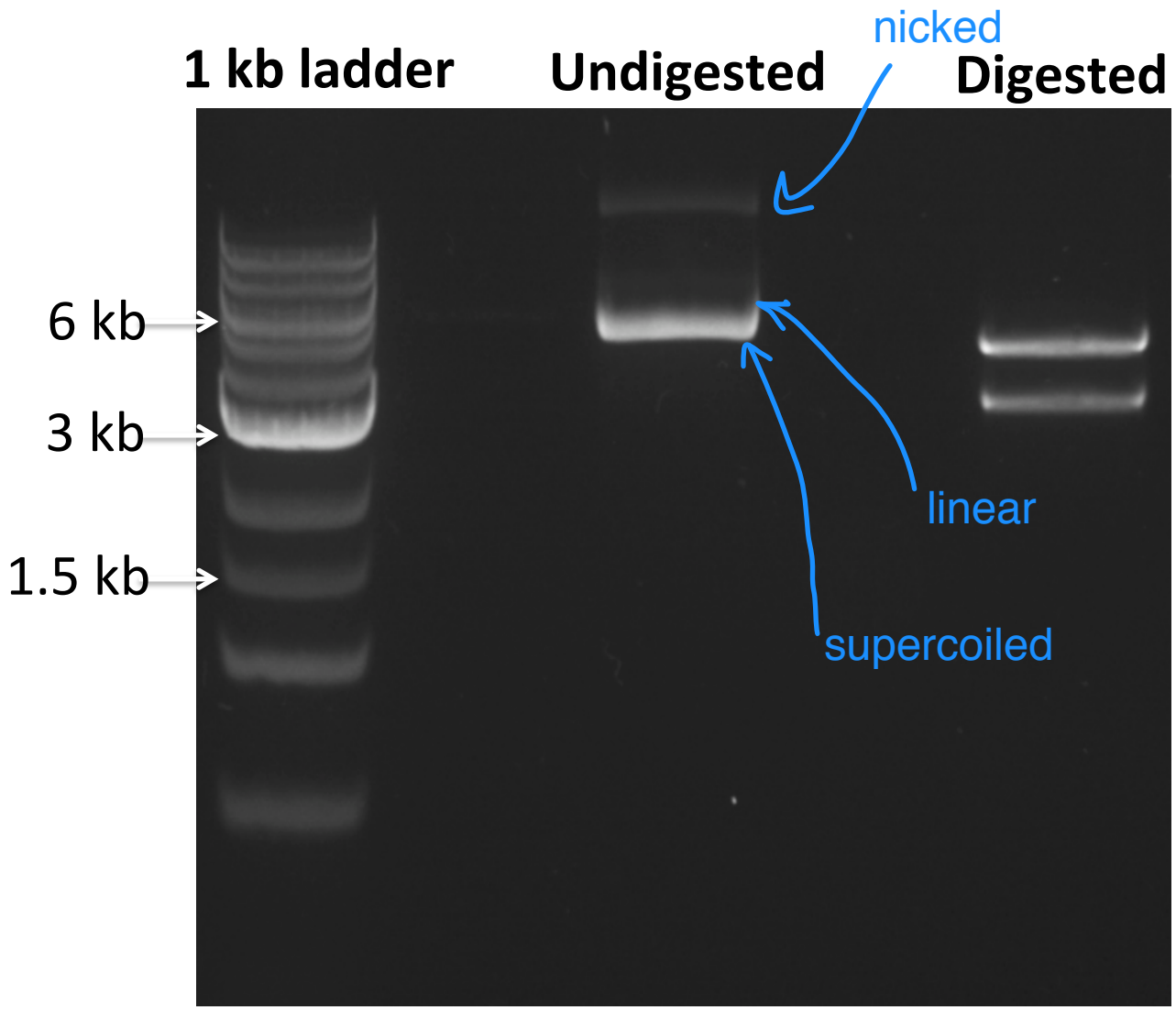
BE 20.109 (Fa17) Class Blog

- You will receive an invitation to join the blog soon
- 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  - Do not publish MIT logo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



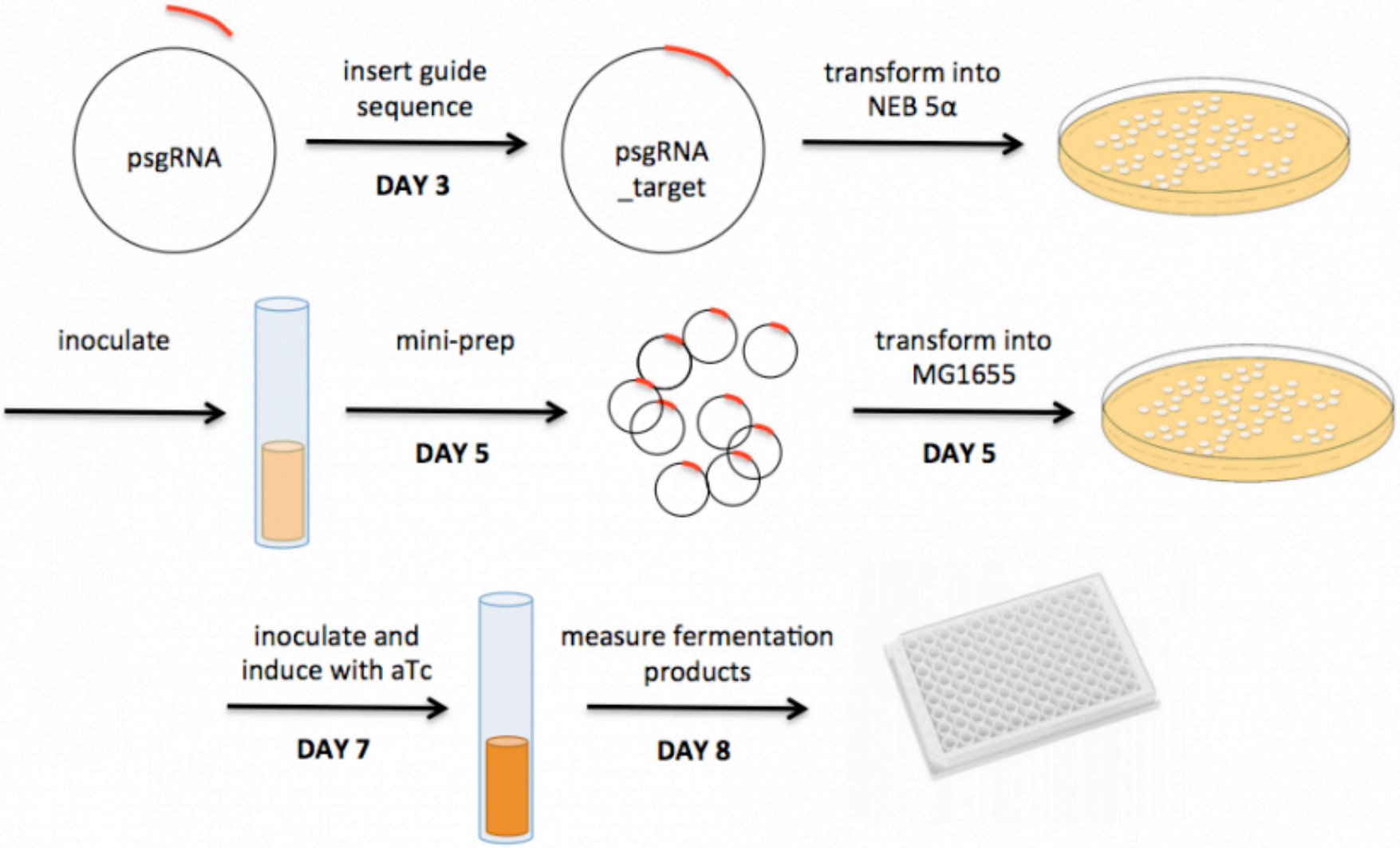


Confirmation digest results of pdCas9



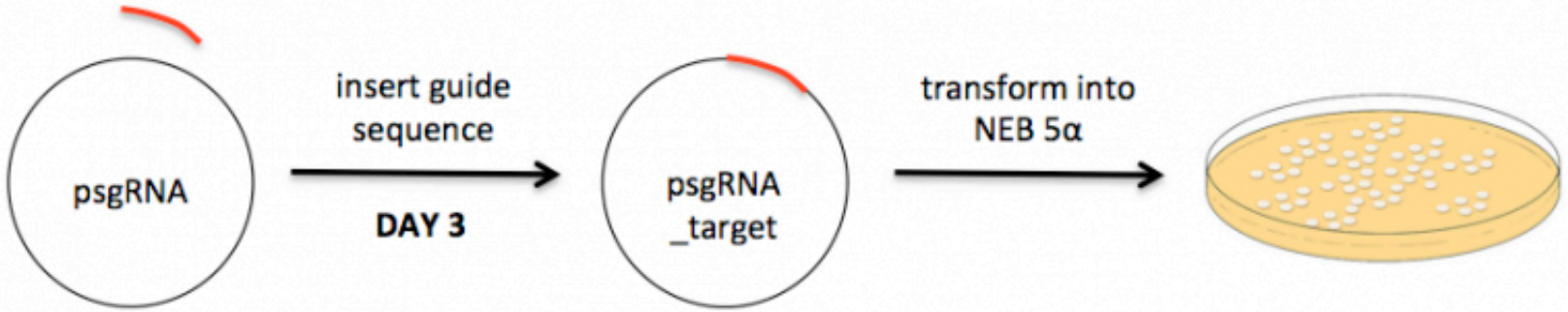
- Digest band locations depend on restriction enzymes used
- DNA can be supercoiled, linear, or nicked

Mod 2 experimental overview



http://engineerbiology.org/wiki/20.109(F17):Module_2

Today's goal: make psgRNA_target



Insert sgRNA sequence into expression vector to make psgRNA_target using site directed mutagenesis

Note: sgRNA = gRNA

Use site-directed mutagenesis (SDM) to engineer plasmid DNA

- NEB Q5 SDM kit
- Create specific, targeted changes in double-stranded plasmid DNA
- Forward primer:

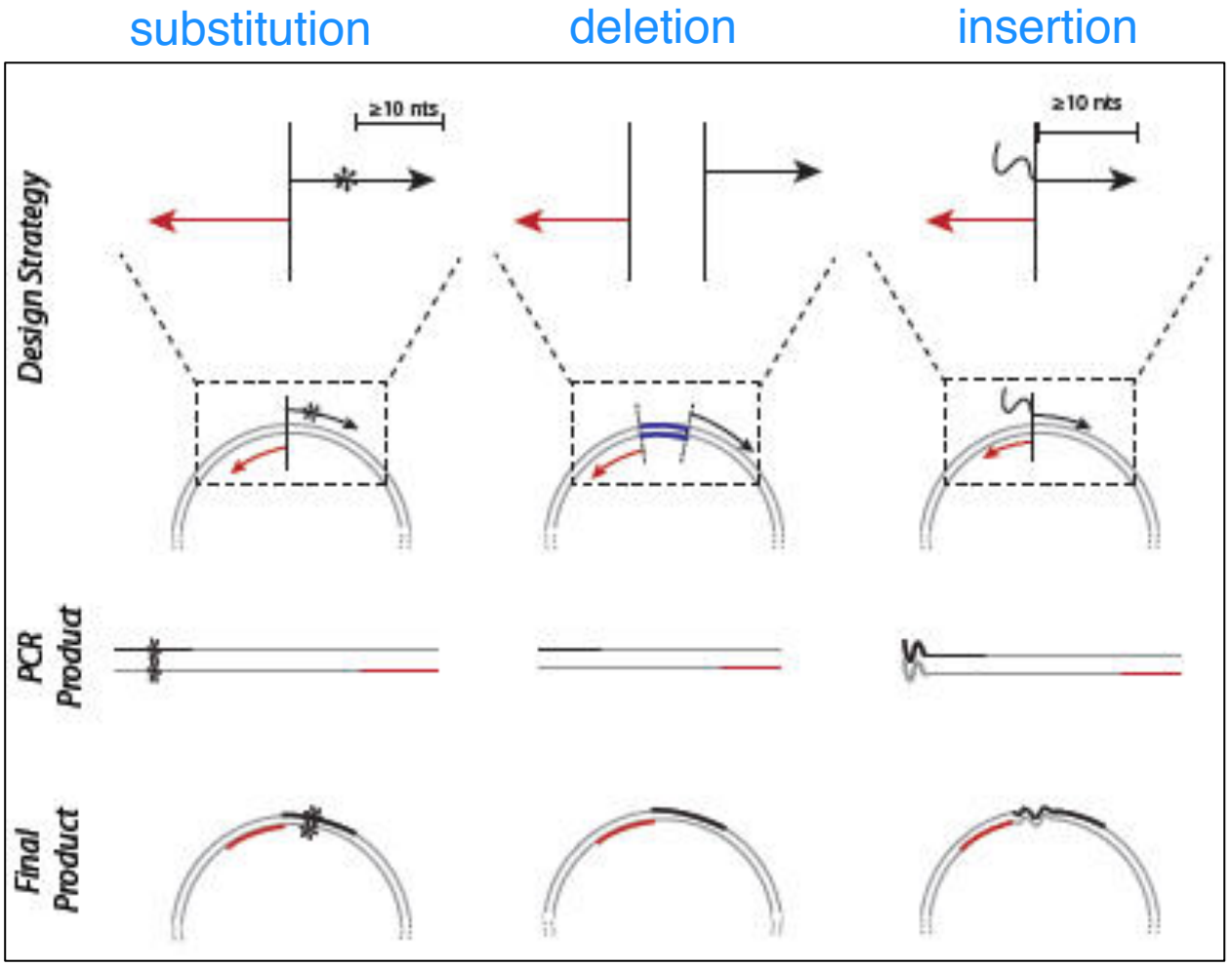
includes desired mutation

- PCR product:

linear

- Final product:

circular plasmid



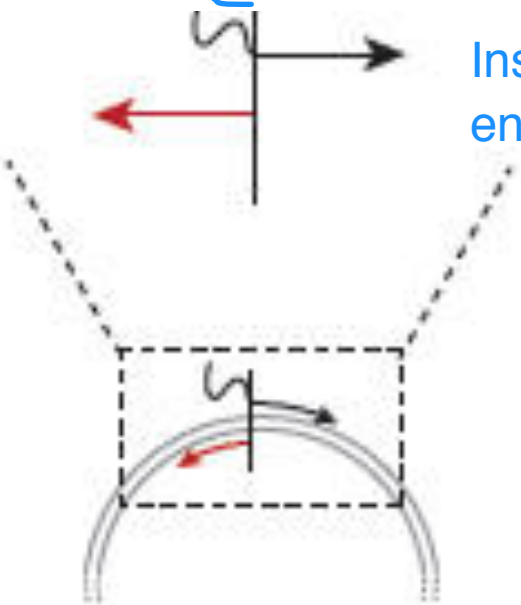
https://www.neb.com/products/e0554-q5-site-directed-mutagenesis-kit#Product%20Information

Insertion of DNA via SDM

gRNA sequence

Reverse primer anneals back to back with the 5' end of forward primer

Insertion incorporated at 5' end of forward primer

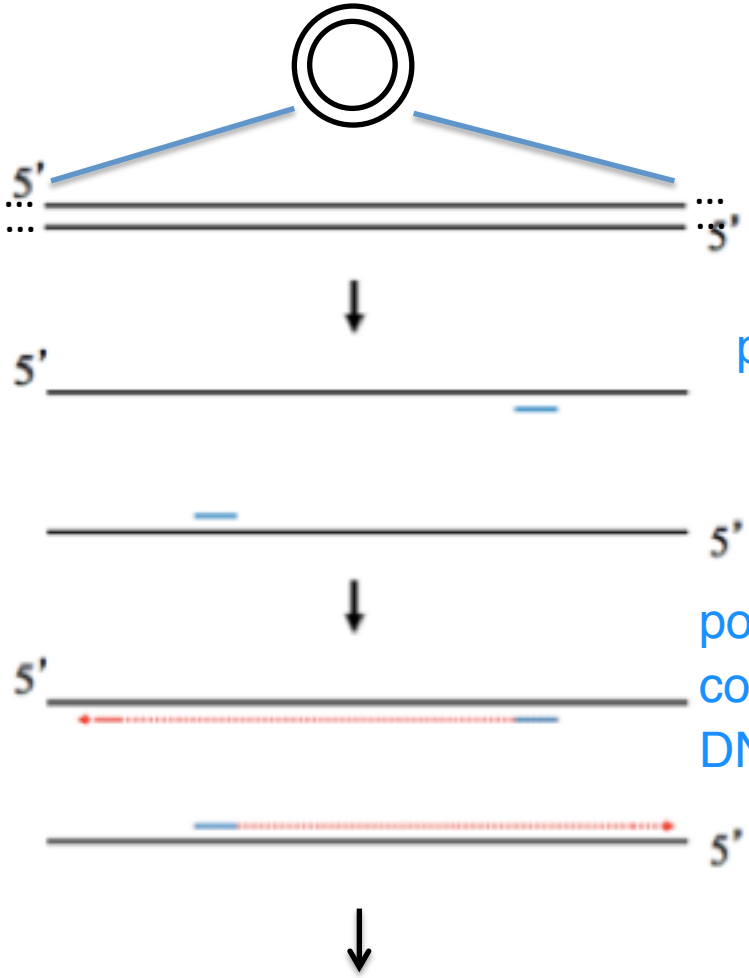


Major product after PCR

Product after blunt ligation

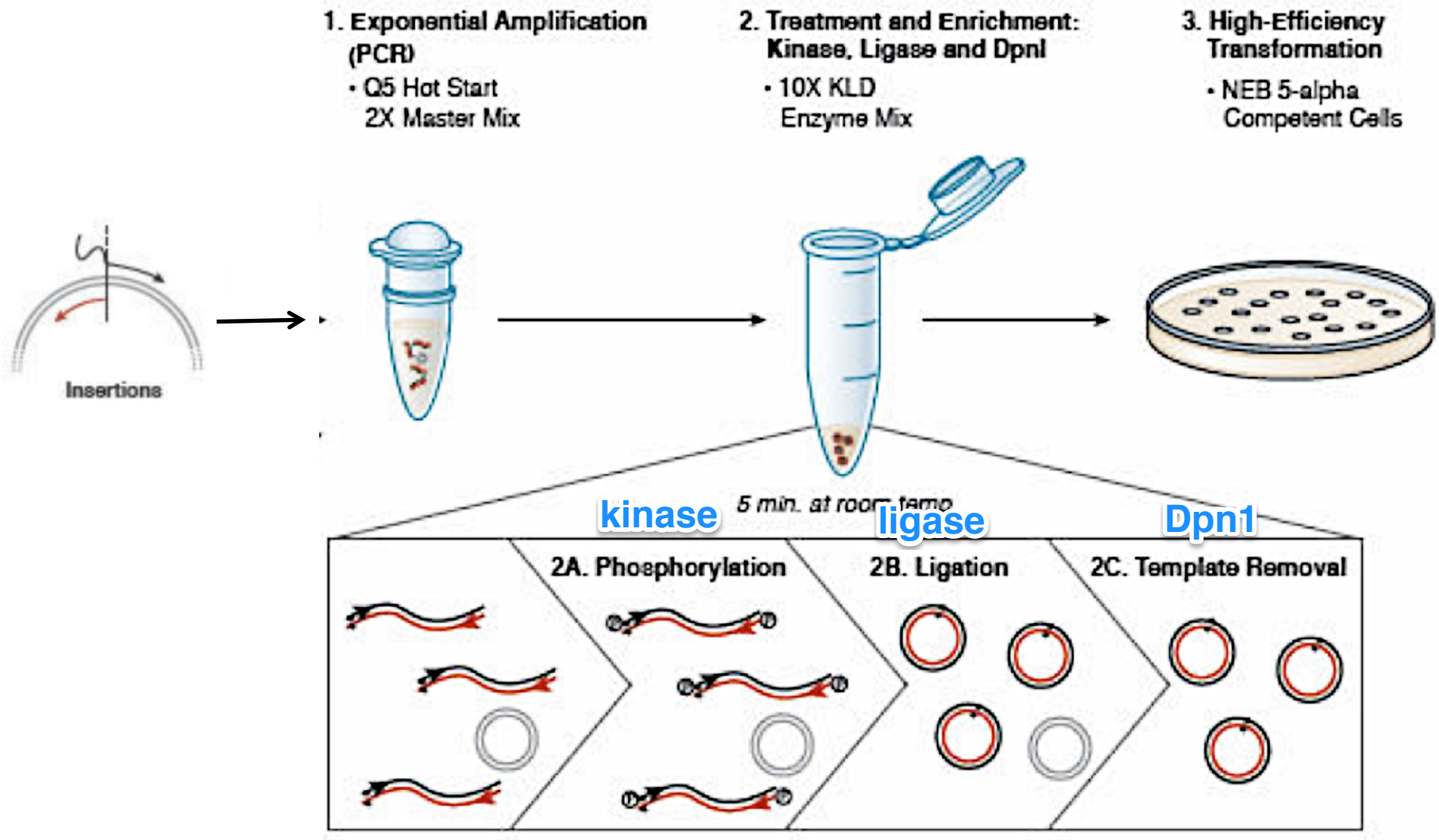
SDM Part 1: PCR amplification of DNA

Ingredients
Forward primer
Reverse primer
template (gRNA expression vector)
dNTPs
Polymerase
Buffer (pH, cofactors like Mg^{2+})
H_2O



25 cycles

SDM Part 2: Recover circular plasmid product using Kinase-Ligase-Dpn1 (KLD) enzyme mix



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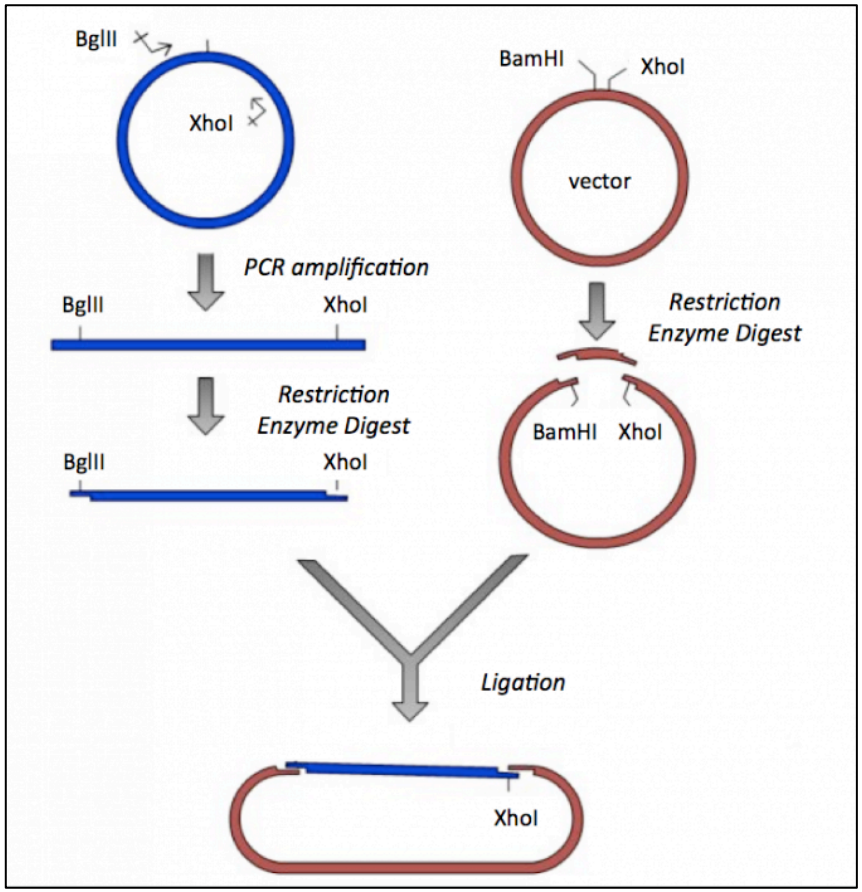
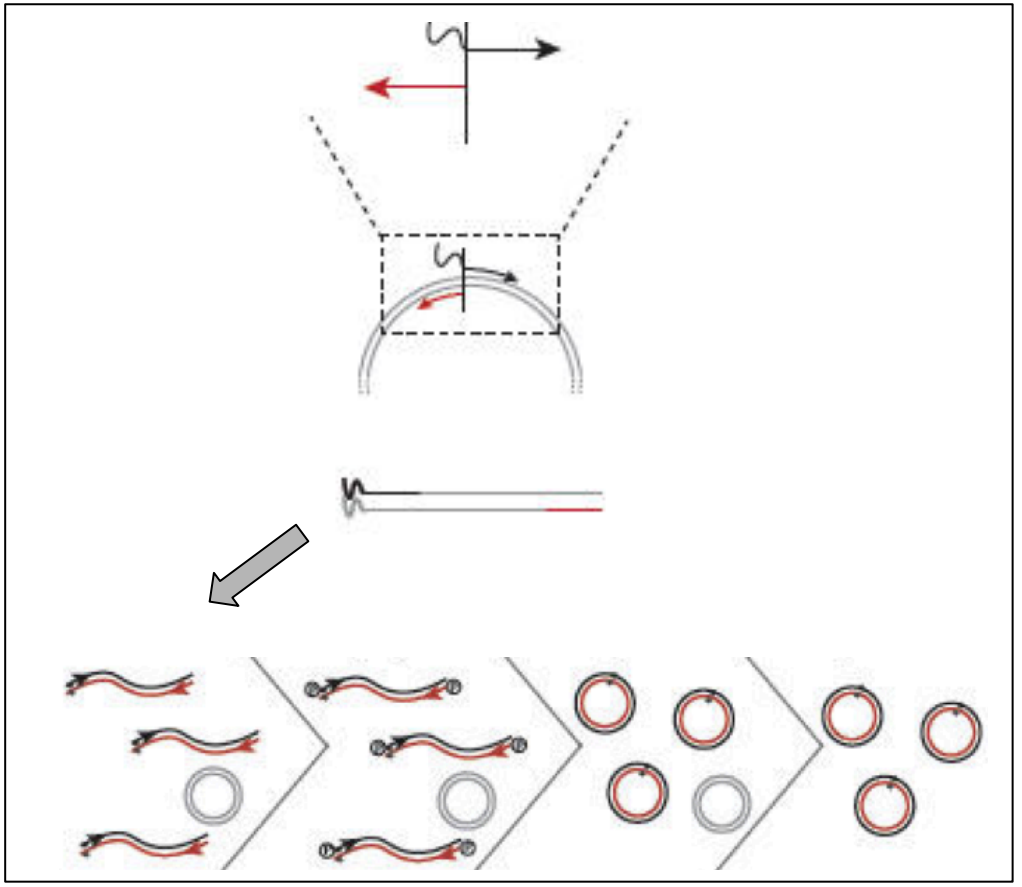
We have covered two ways to engineer DNA

"Traditional" plasmid cloning by restriction enzyme digest



pdCas9

Site directed mutagenesis



pgRNA_target

Wednesday (10/25)

Journal Club I

- Submit presentation slides to Stellar by 1pm Oct. 25th
- 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 are allowed to **pull figures directly from paper** to put on slides (title slide = citation)
- You will present from a mac
- 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

Friday (10/27)

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?

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 Data Summary revisions and Journal Club presentations in down time