Finish this (start PCR) by 1:25pm

1. Make 100 uM gRNA stock (e.g. 20 nmol → 200 uL nuclease-free water)
2. Make primer mix (10 uM of each primer in 100 uL volume)
   - 10 uL gRNA (forward primer)
   - 10 uL RevP (reverse primer)
   - 80 uL nuclease-free water
3. Label PCR tube and label TRColor
   - 10.25 uL nuclease-free water
   - 1.25 uL primer mix (from #2)
   - 1 uL pgRNA plasmid
   - 12.5 uL Q5 Hot Start 2x Master Mix

M2D3: Generate gRNA plasmid

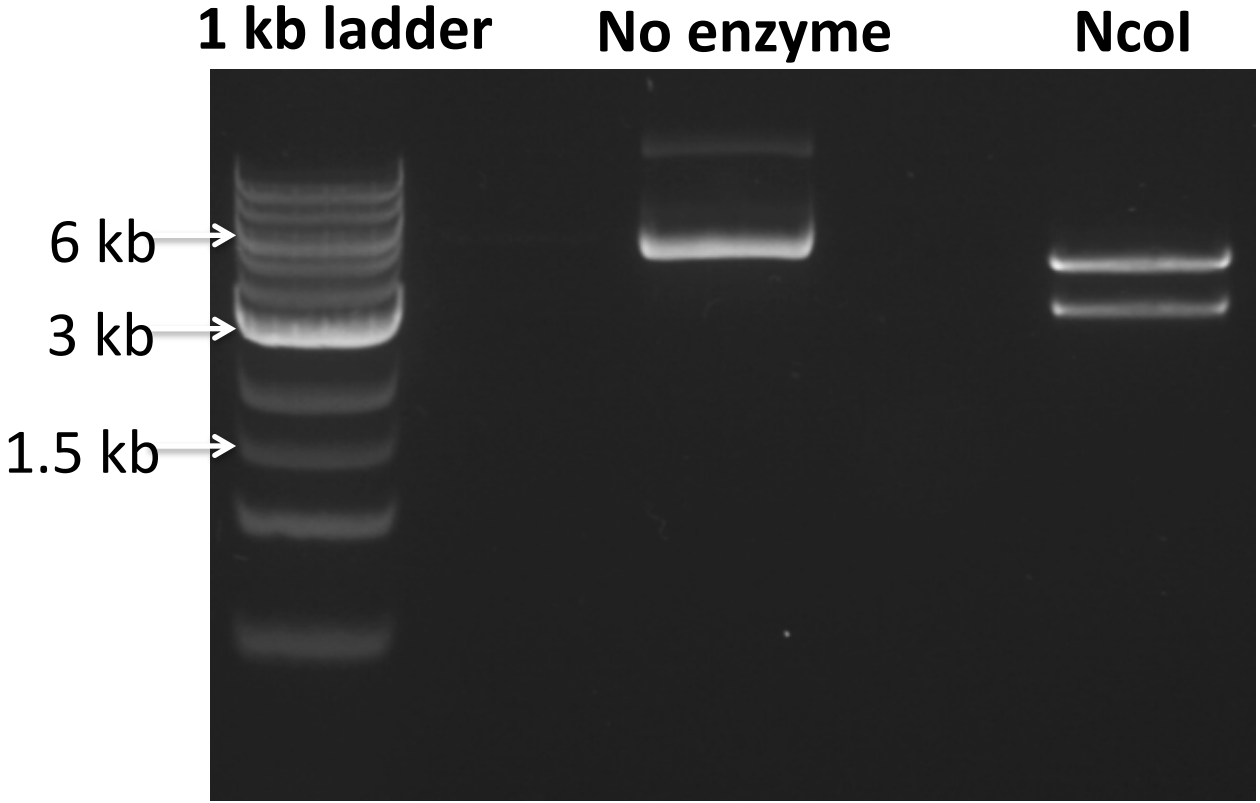
10/18/18

1. Start PCR
2. BE Communication workshop: Journal Club presentations, 1:30pm in 56-614
3. Pre-lab discussion part 1
4. Set up reaction to generate gRNA_target plasmid
5. Pre-lab discussion part 2 (if needed)
6. Transform pgRNA_target into bacteria

Announcements

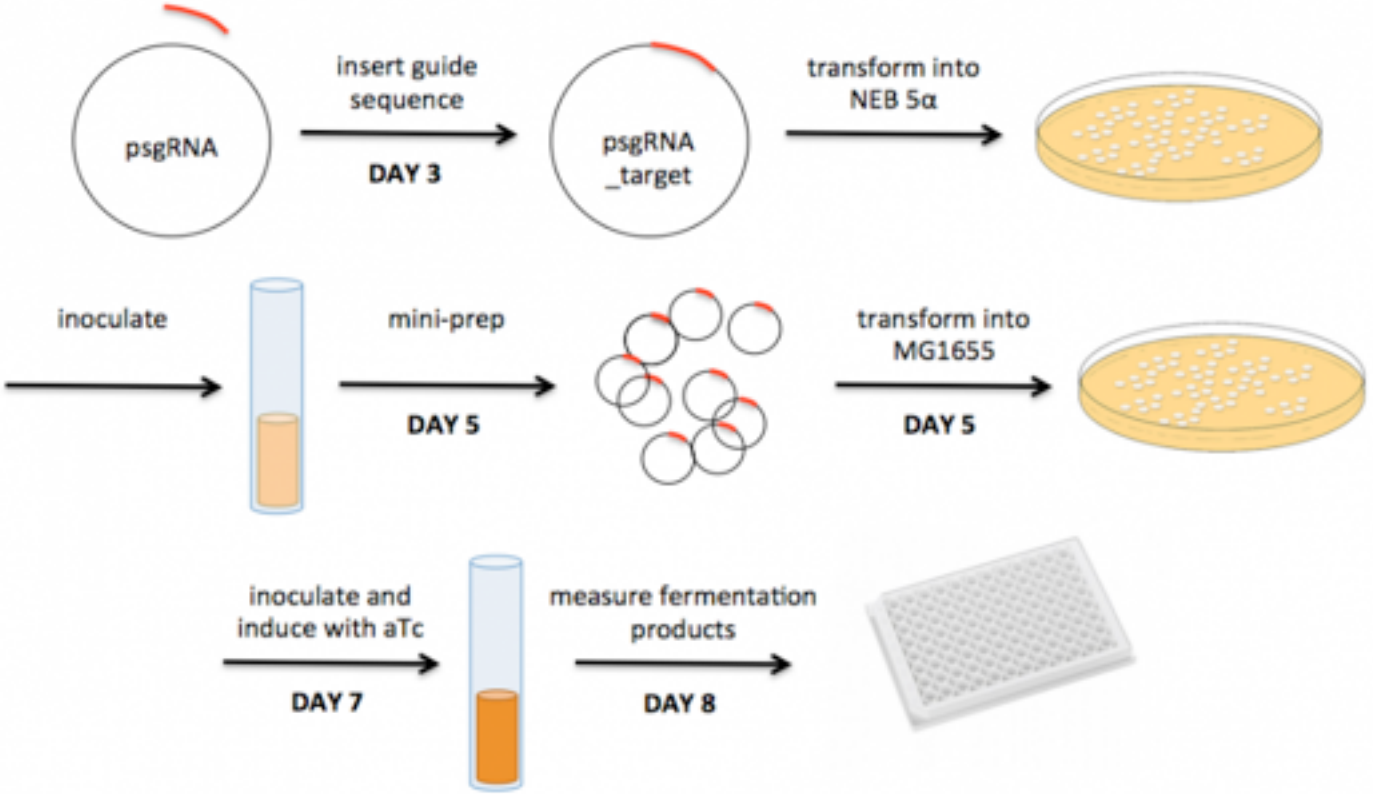
- Data summary revision due Saturday 10/20
- Journal club starts on Tuesday! (See evaluation rubric on wiki. Also, if coming to office hours related to it, email us ahead of time so we can be sure to read your paper)
- *If you do change your paper, please email all instructors. You can't change your journal paper after Saturday (10/20) @ 5pm.*

Confirmation digest results of pdCas9



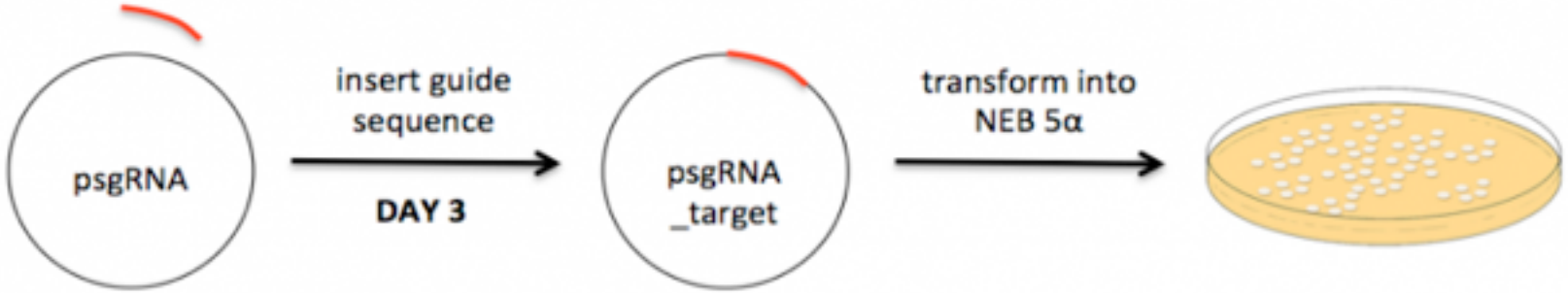
- Digest band locations depend on restriction enzymes used
- DNA can be supercoiled, linear, or nicked

Mod 2 experimental overview



http://engineerbiology.org/wiki/20.109(F18):Module_2

Today's goal: make psgRNA_target



Insert gRNA sequence into expression vector to make pgRNA_target using site directed mutagenesis

Note: sgRNA = gRNA

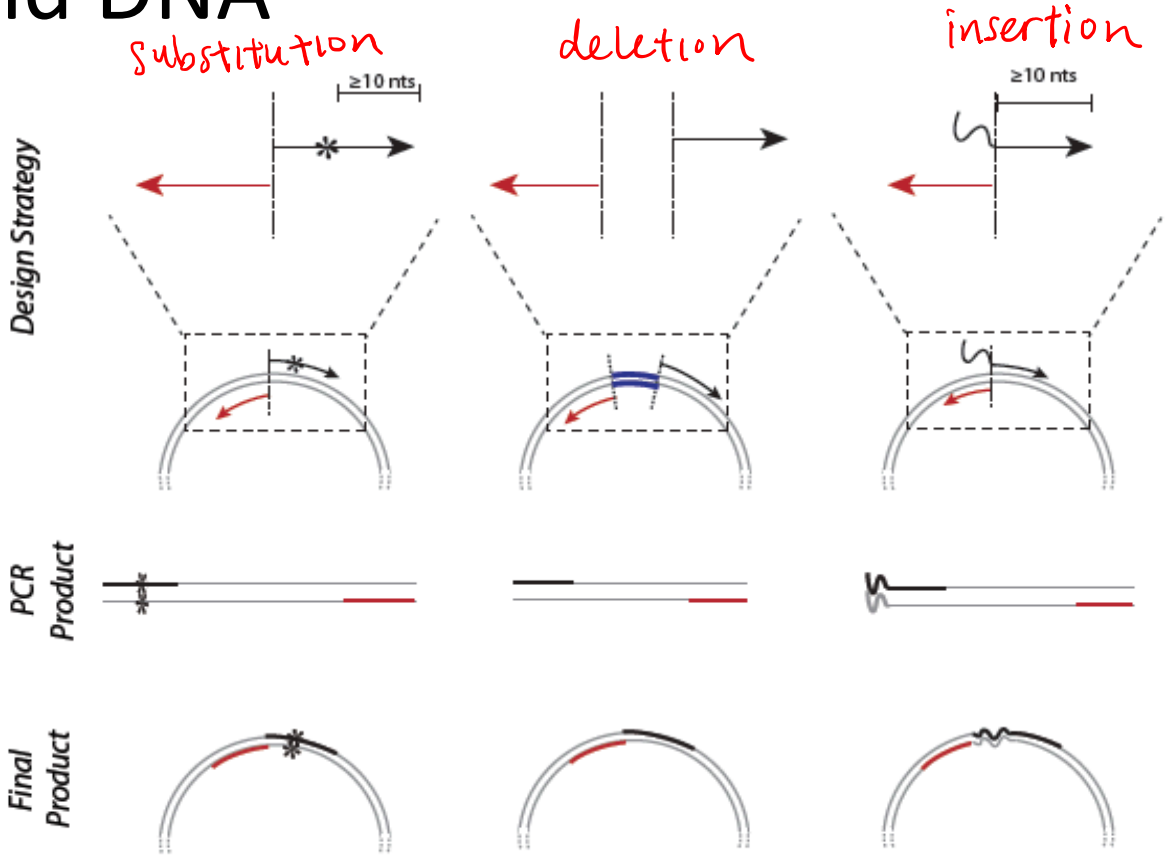
Use site-directed mutagenesis (SDM) to engineer plasmid DNA

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

  Contains desired mutation
- PCR product:

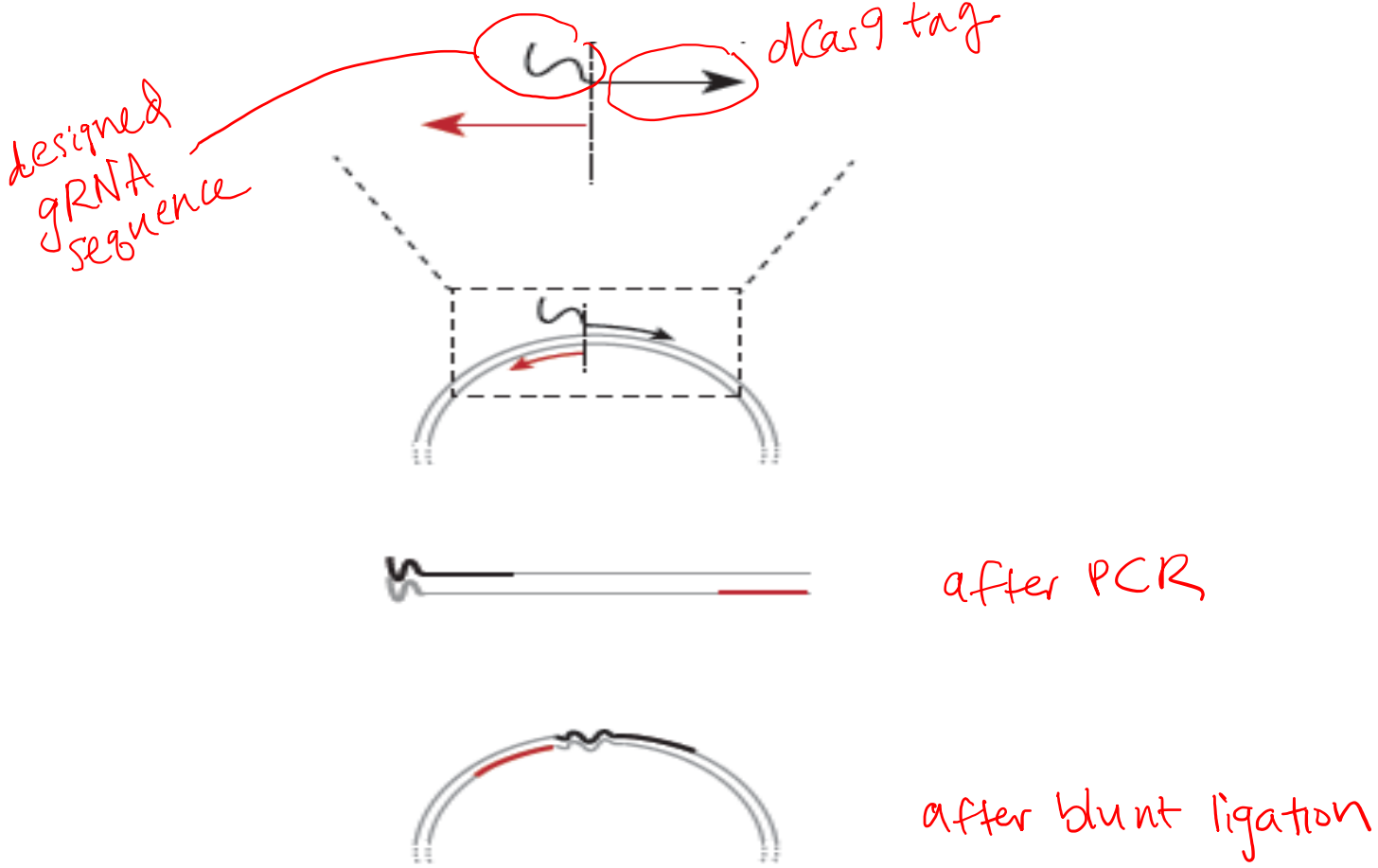
  linear
- Final product:

  circular plasmid



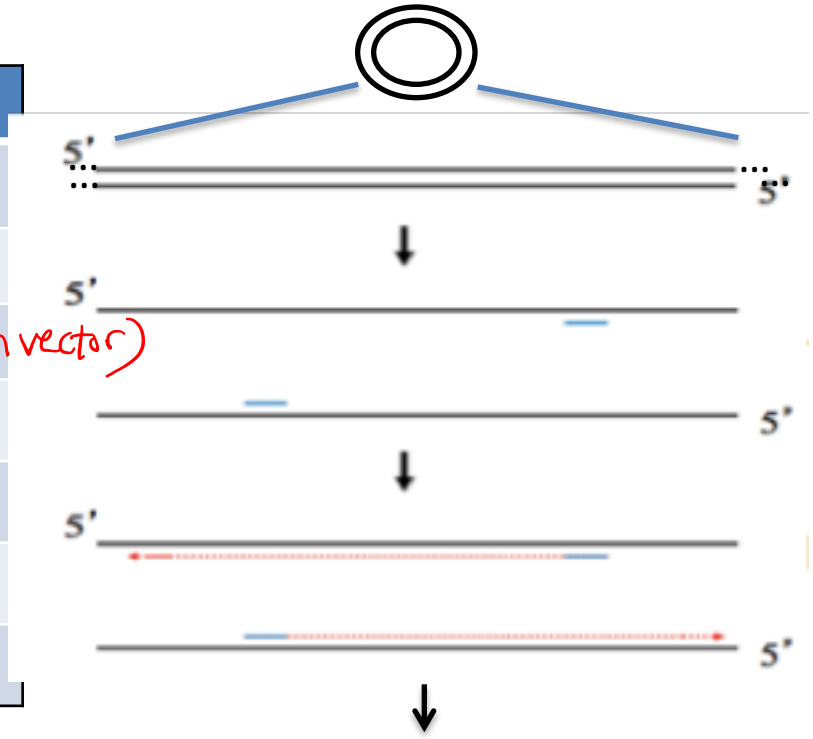
https://www.neb.com/products/e0554-q5-site-directed-mutagenesis-kit#Product%20Information

Insertion of DNA via SDM



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



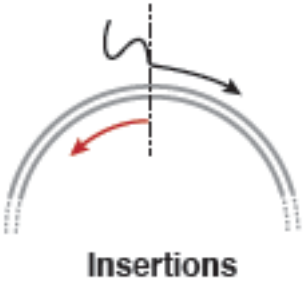
98 °C

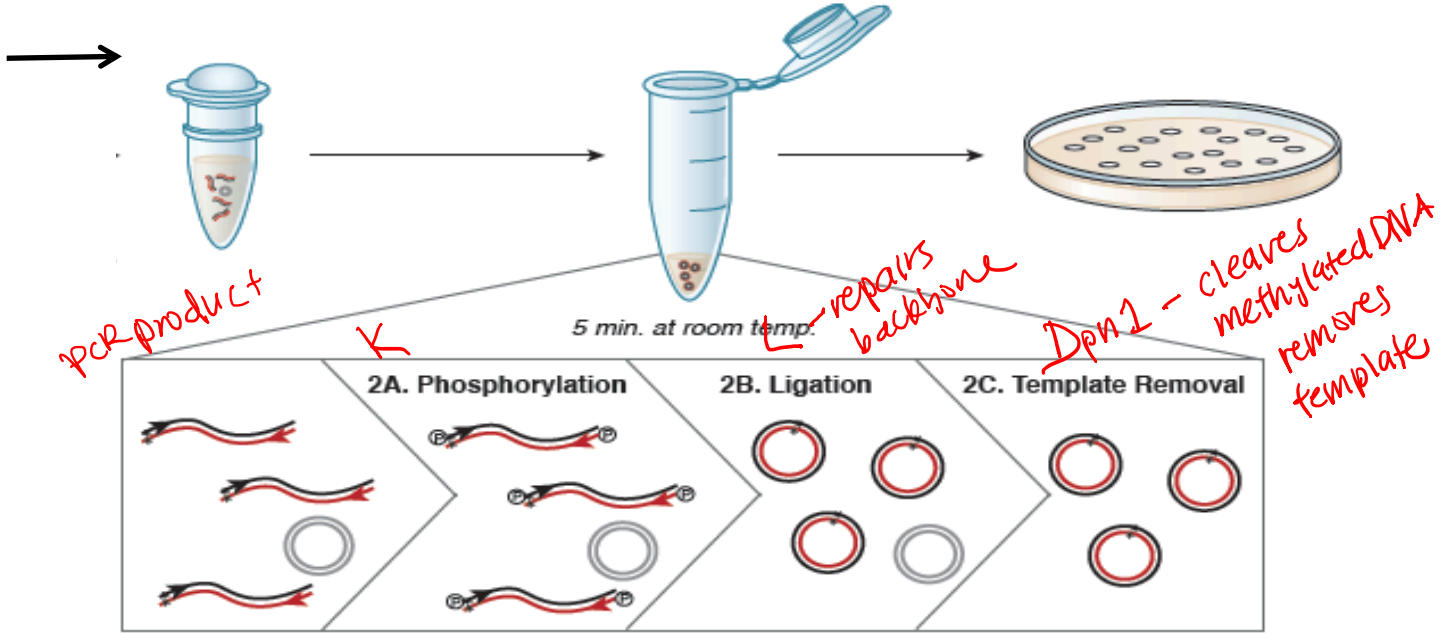
55 °C

72 °C

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

1. Exponential Amplificaion (PCR)
   - Q5 Hot Start 2x Master Mix
2. Treatment & Enrichment: Kinase, Ligase and DpnI
   - 10x KLD enzyme mix
3. High-efficiency transformation
   - NEB 5-alpha competent cells



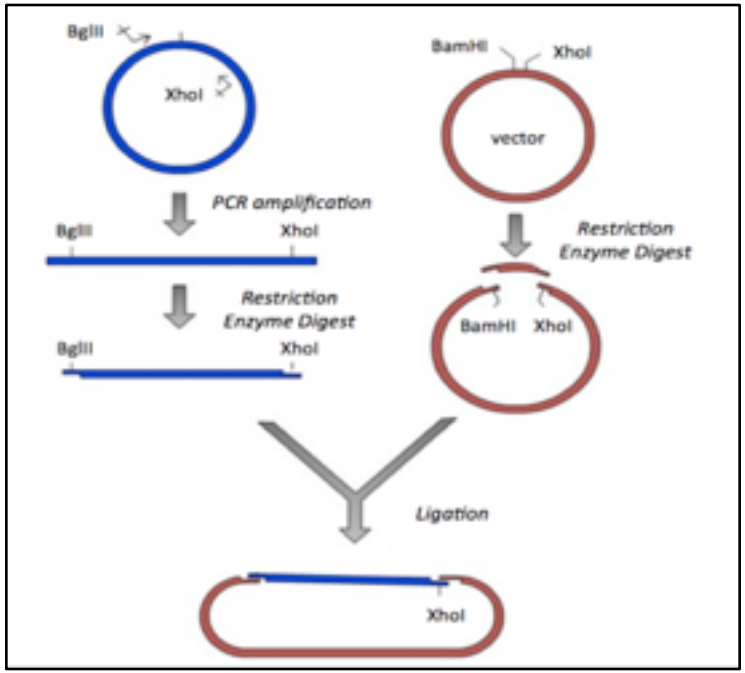


https://www.neb.com/products/e0554-q5-site-directed-mutagenesis-kit#Product%20Information

Phosphorylation video:
https://www.neb.com/tools-and-resources/video-library/the-mechanism-of-dna-phosphorylation

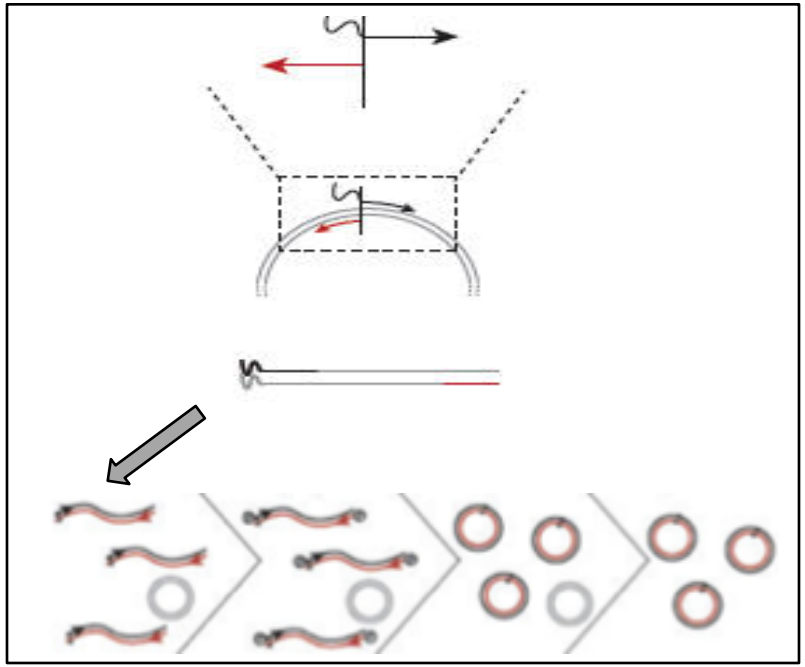
We have covered two ways to engineer DNA

"Traditional" plasmid cloning by restriction enzyme digest



pdCas9

Site directed mutagenesis



pgRNA_target

M2 major assignments

- Research Article (20%)
  - Individual, submit on Stellar
  - Word document
  - Due 11/12 by 10pm (no revision)
- Journal Club Presentation (15%)
  - Individual, during lab section, video recorded
  - Powerpoint slides due 1pm on Stellar, on the day of presentation
- Lab quizzes
  - M2D5 and M2D8
- Notebook (part of 10% Homework and Notebook)
- Blog (part of 5% Participation)
  - By 11/2 (Journal club reflections)
  - By 11/13 (Mod2 material/research article)

Tuesday (10/23)

Journal Club I

- Submit presentation slides to Stellar by 1pm Oct. 23rd
- 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

Thursday (10/25)—start homework early

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. Set up your gRNA insertion/amplification reaction using reagents at front bench
2. Work on Data Summary revisions and Journal Club presentations in down time