Finish this (start PCR) by 2:00pm

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~~ 50 uL volume)
   - 5 uL gRNA (forward primer)
   - 5 uL RevP (reverse primer)
   - 40 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

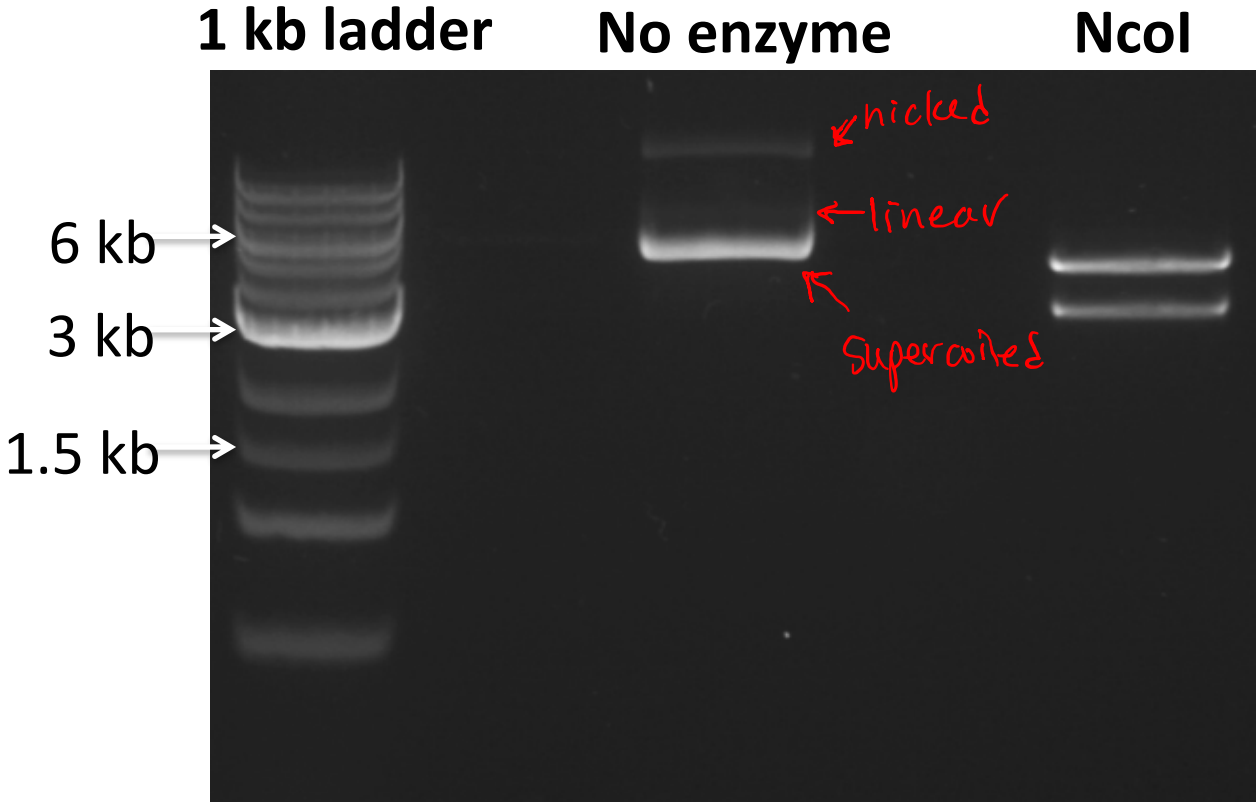
10/18/18

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

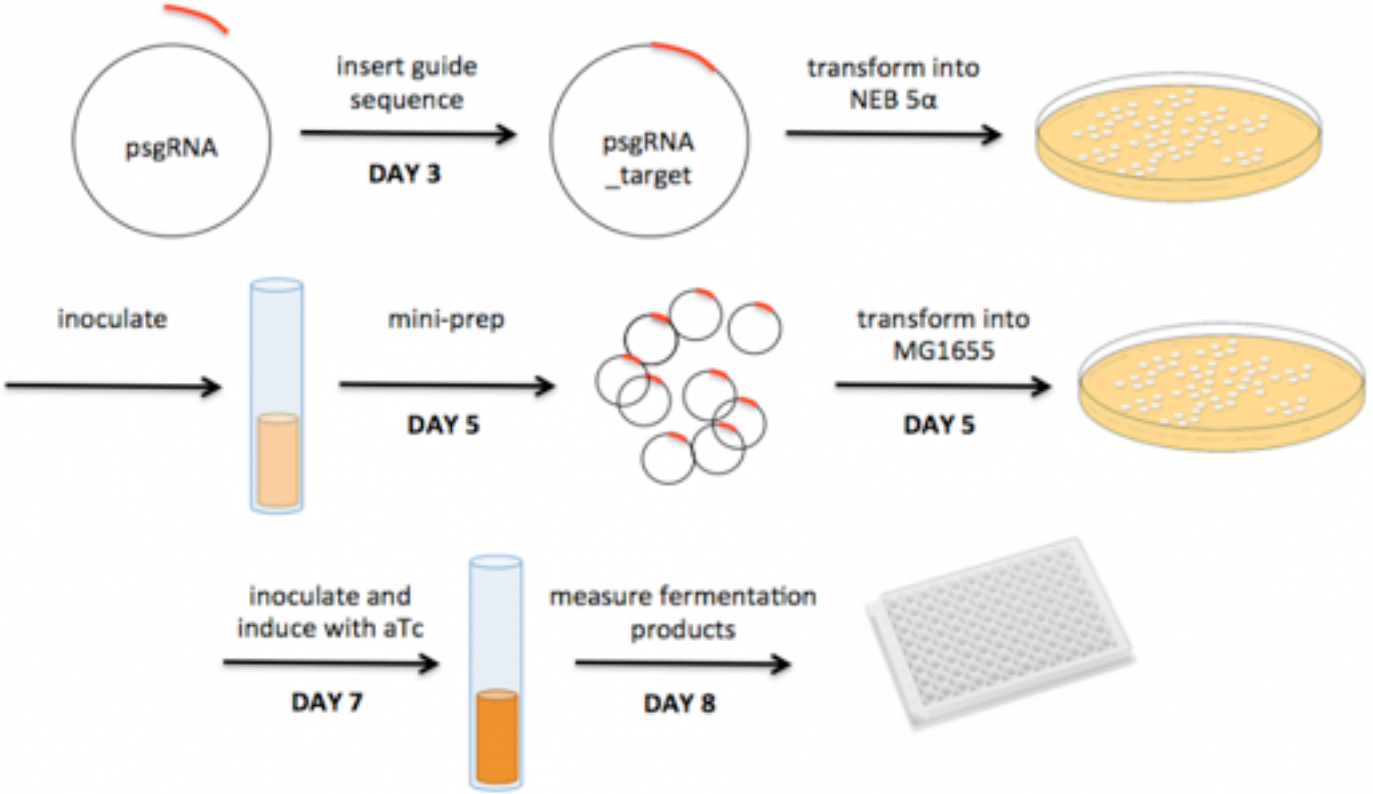
- Data summary revision due tomorrow (Saturday 10/20)
- Journal club starts on ~~Tuesday~~ Wednesday! (See evaluation rubric on wiki. Also, if coming to office hours related to it, email us ahead of time so we can be sure to read your paper)
- *If you do change your paper before then email all instructors.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

Confirmation digest results of pdCas9



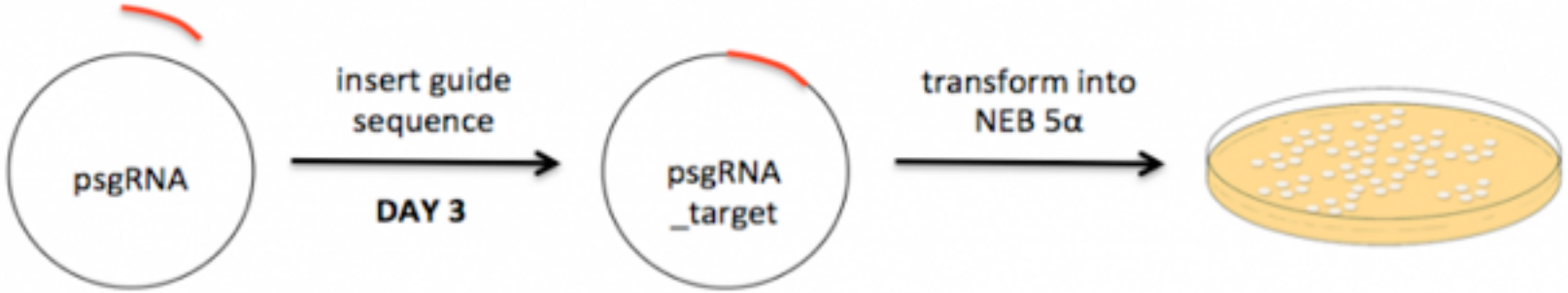
- Digest band locations depend on restriction enzymes used
- DNA can be supercoiled, linear, or nicked

Mod 2 experimental overview



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

Today's goal: make psgRNA_target

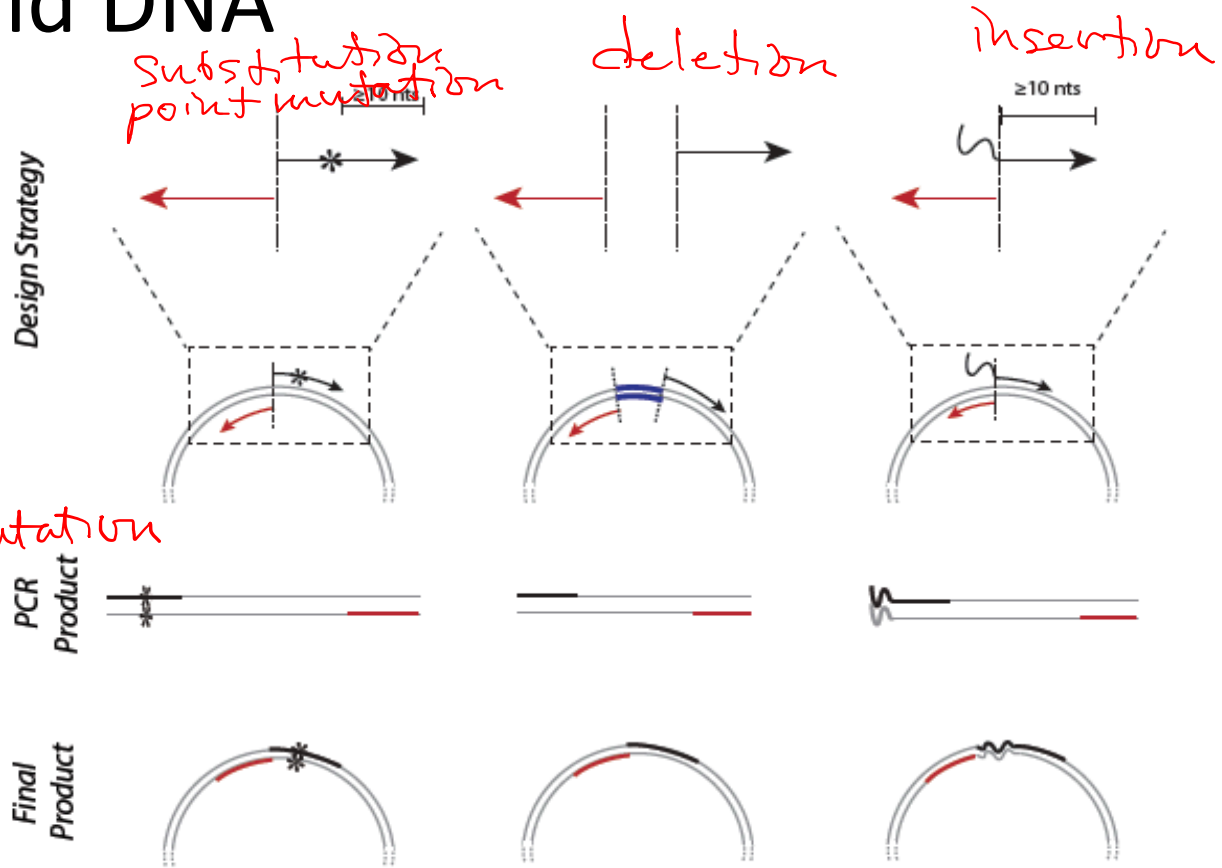


Insert gRNA sequence into expression vector to make pgRNA_target using site directed mutagenesis

Note: sgRNA = gRNA

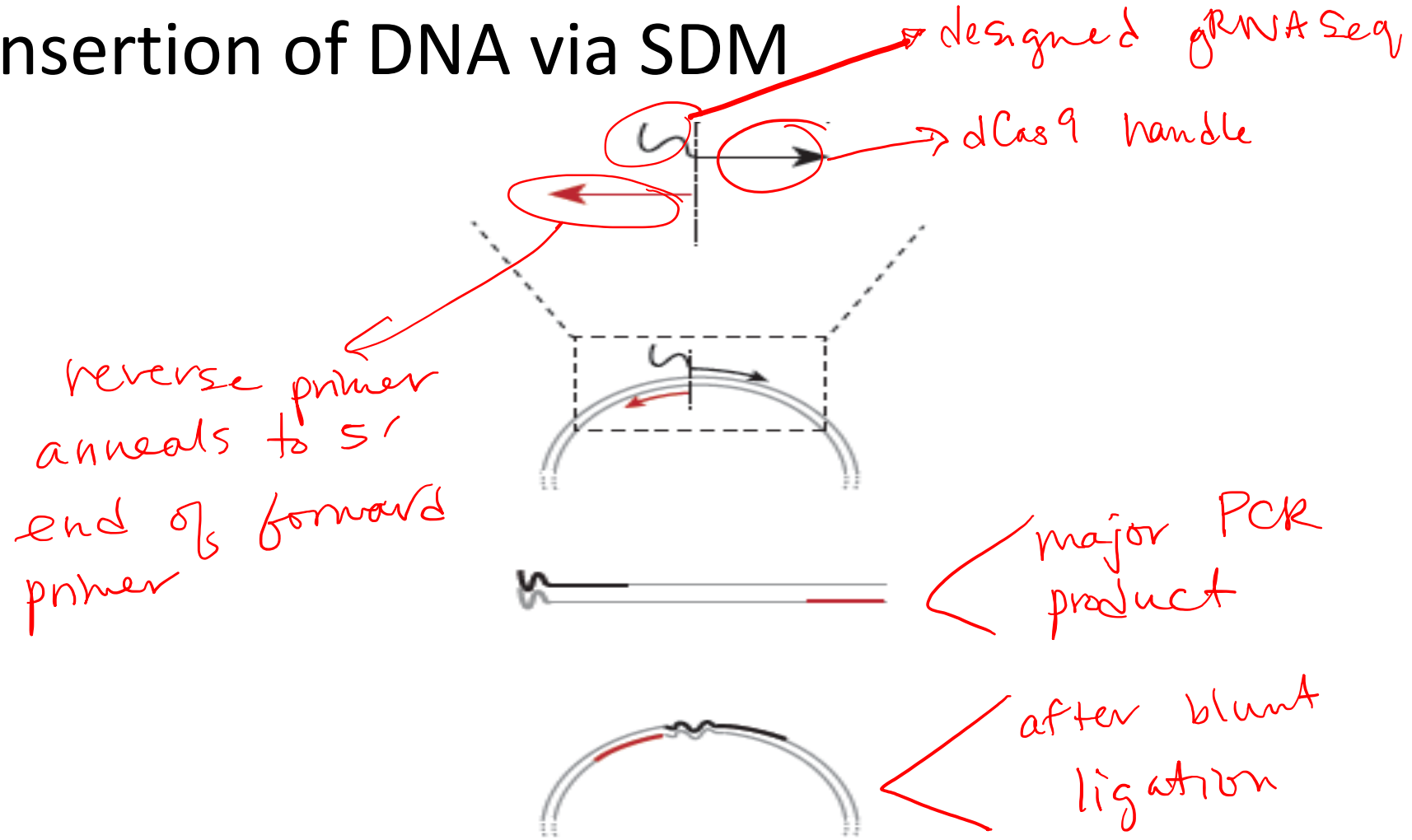
Use site-directed mutagenesis (SDM) to engineer plasmid DNA

- 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

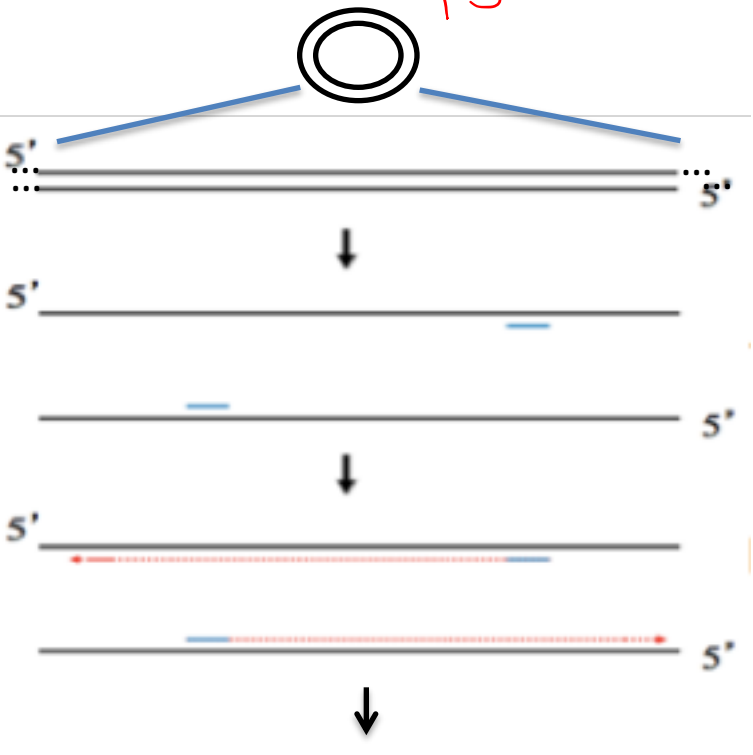
Insertion of DNA via SDM



SDM Part 1: PCR amplification of DNA

pgRNA plasmid

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



98 °C

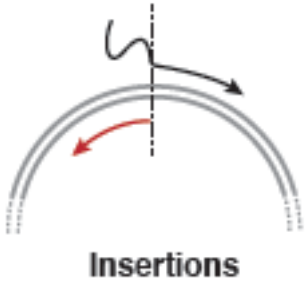
55 °C

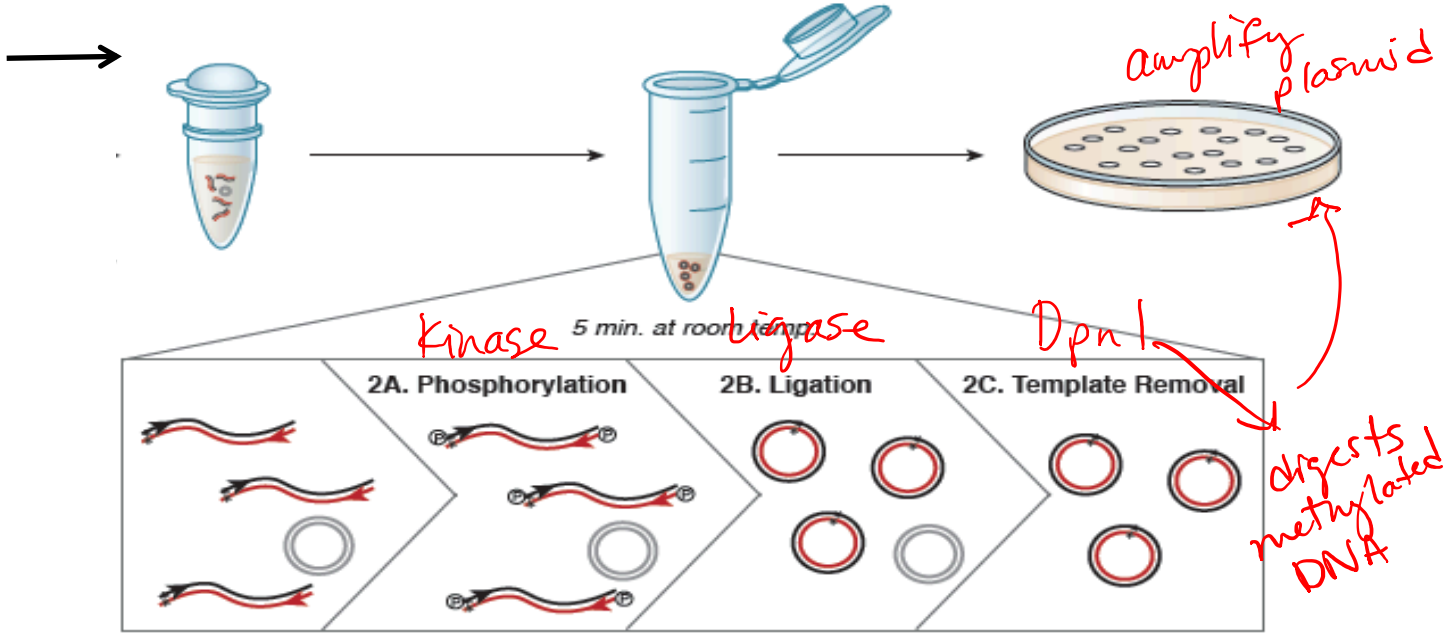
72 °C

Linear

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



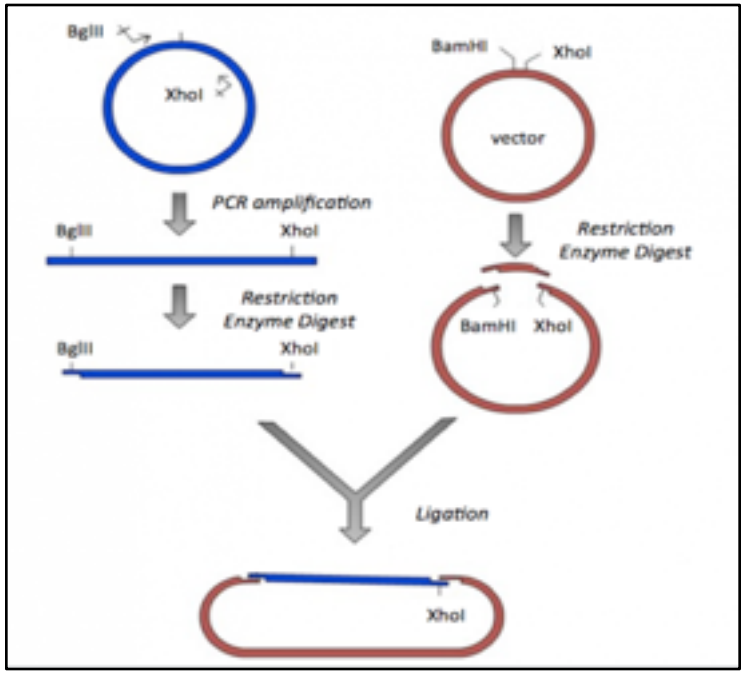


https://www.neb.com/products/e0554-q5-site-directed-mutagenesis-kit#Product%20Information

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

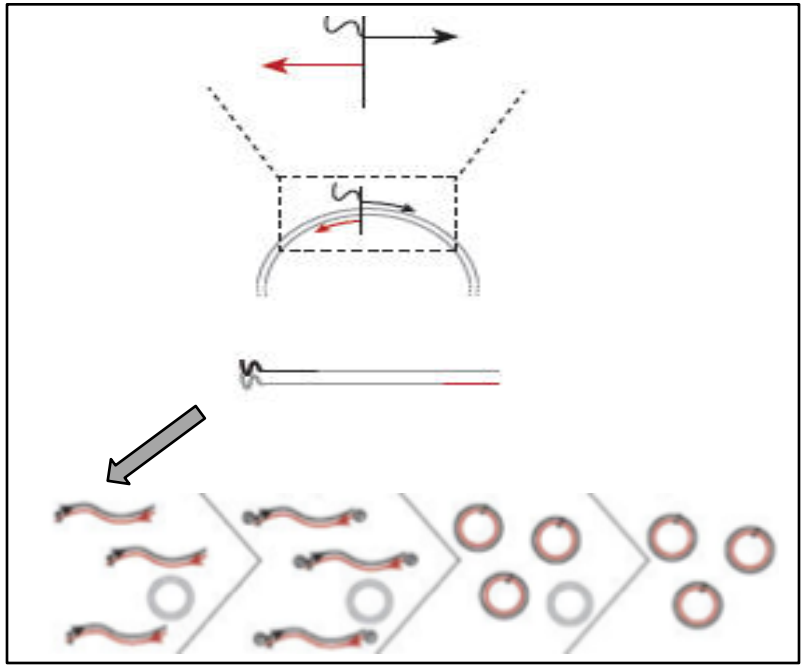
We have covered two ways to engineer DNA

"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)~~ Wednesday 10/24

Journal Club I

- Submit 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 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~~ FRIDAY (10/~~25~~ 26)—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