Finish this by 1:30pm

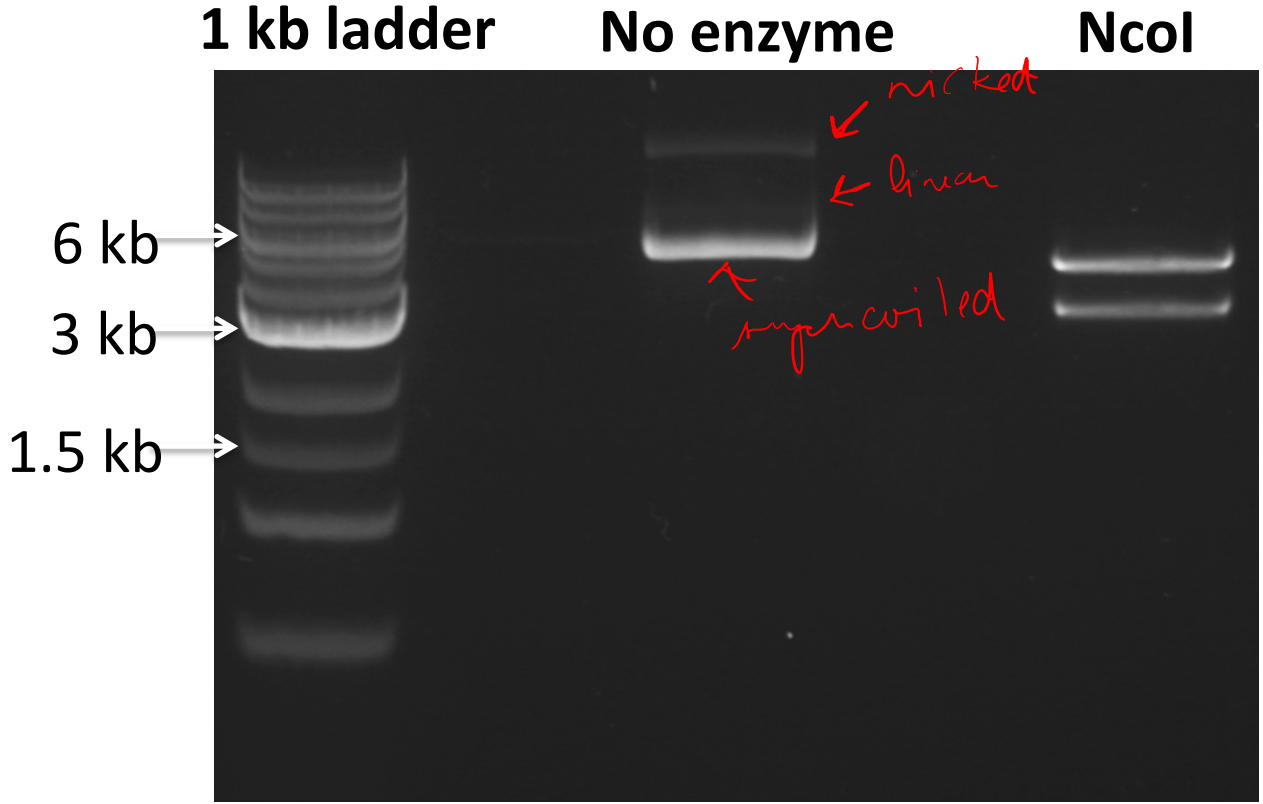
1. Make 100 uM gRNA stock (e.g. 20 nmol → 200 uL nuclease-free water)
2. Make primer mix, final concentration 10uM of each primer
   - 5 uL gRNA (forward primer prepped in #1, 100uM stocks in your ice bucket)
   - 5 uL RevP (reverse primer, on front bench)
   - 40 uL nuclease-free water
3. Label one PCR tube with Color marker (team color)
   - 10.25 uL nuclease-free water
   - 1.25 uL primer mix (prepped #2)
   - 1 uL pgRNA plasmid (25ng/ul)
   - 12.5 uL Q5 Hot Start 2x Master Mix

M2D3: Generate gRNA plasmid

10/17/19

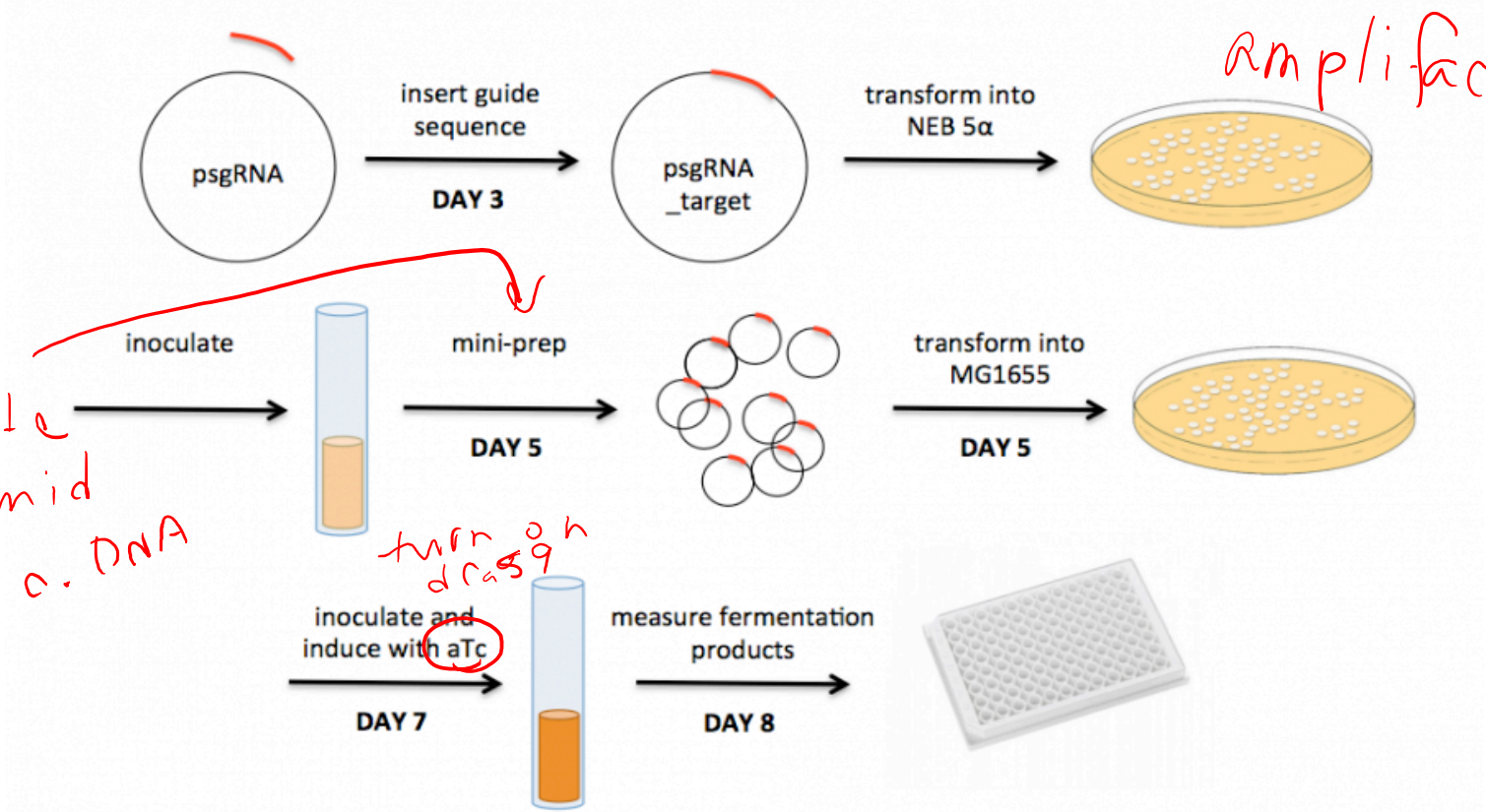
1. Start PCR
2. BE Communication workshop: Journal Club presentations, 1:30pm in 56-614
3. Pre-lab discussion part 1
4. Complete reaction to generate gRNA_target plasmid
5. Transform pgRNA_target into bacteria

Confirmation digest results of pdCas9



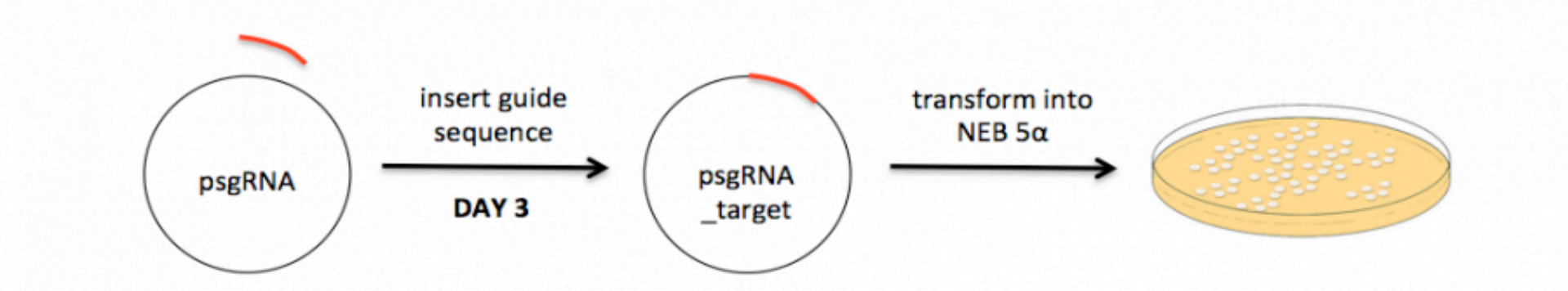
- Digest band locations depend on restriction enzymes used
- DNA can be supercoiled, linear, or nicked

Mod 2 experimental overview



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

Today's goal: make psgRNA_target

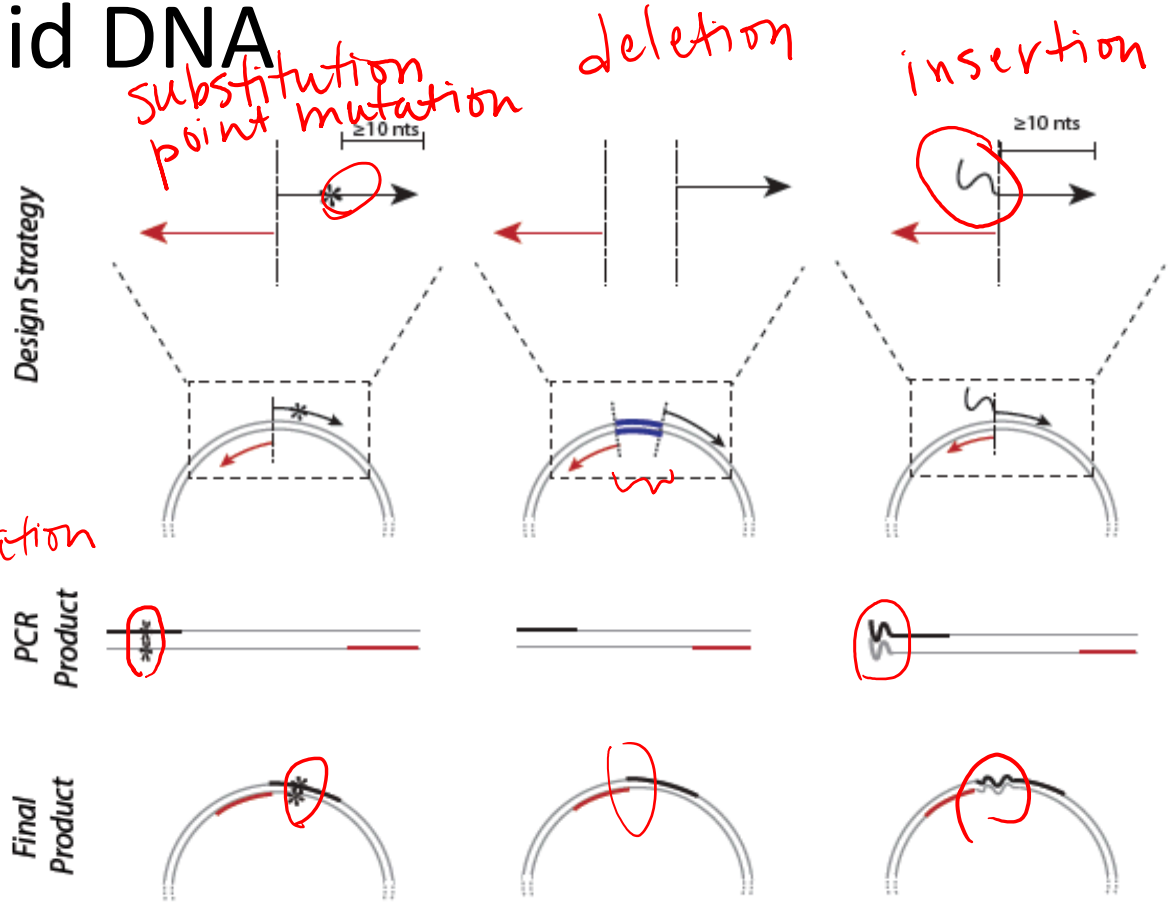


Insert gRNA sequence into expression vector to make pgRNA_target using site directed mutagenesis

Note: sgRNA = gRNA

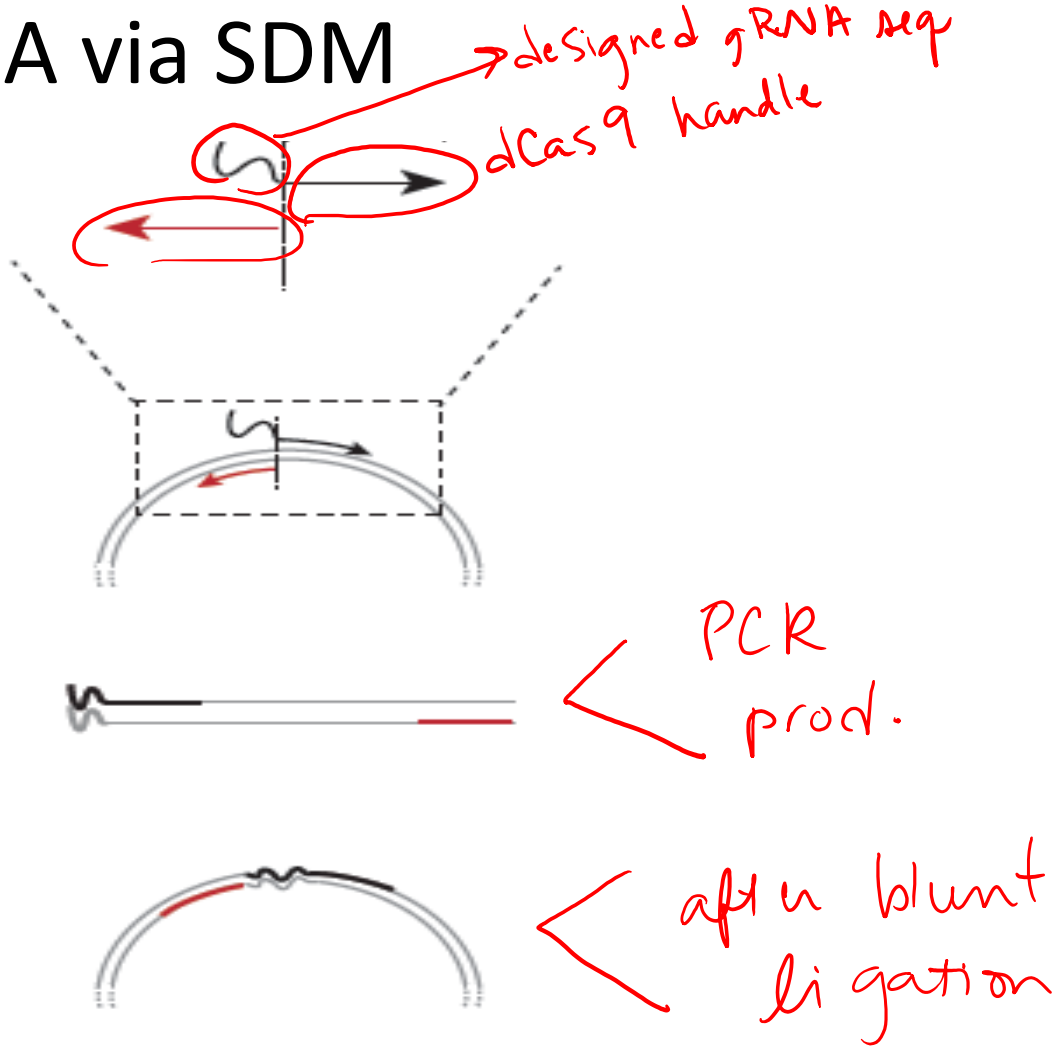
Use site-directed mutagenesis (SDM) to engineer plasmid DNA

- NEB Q5 SDM kit
- Create specific, targeted changes in double-stranded plasmid DNA
- Forward primer: contain desired mutation
- PCR product: linear
- Final product: circular



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

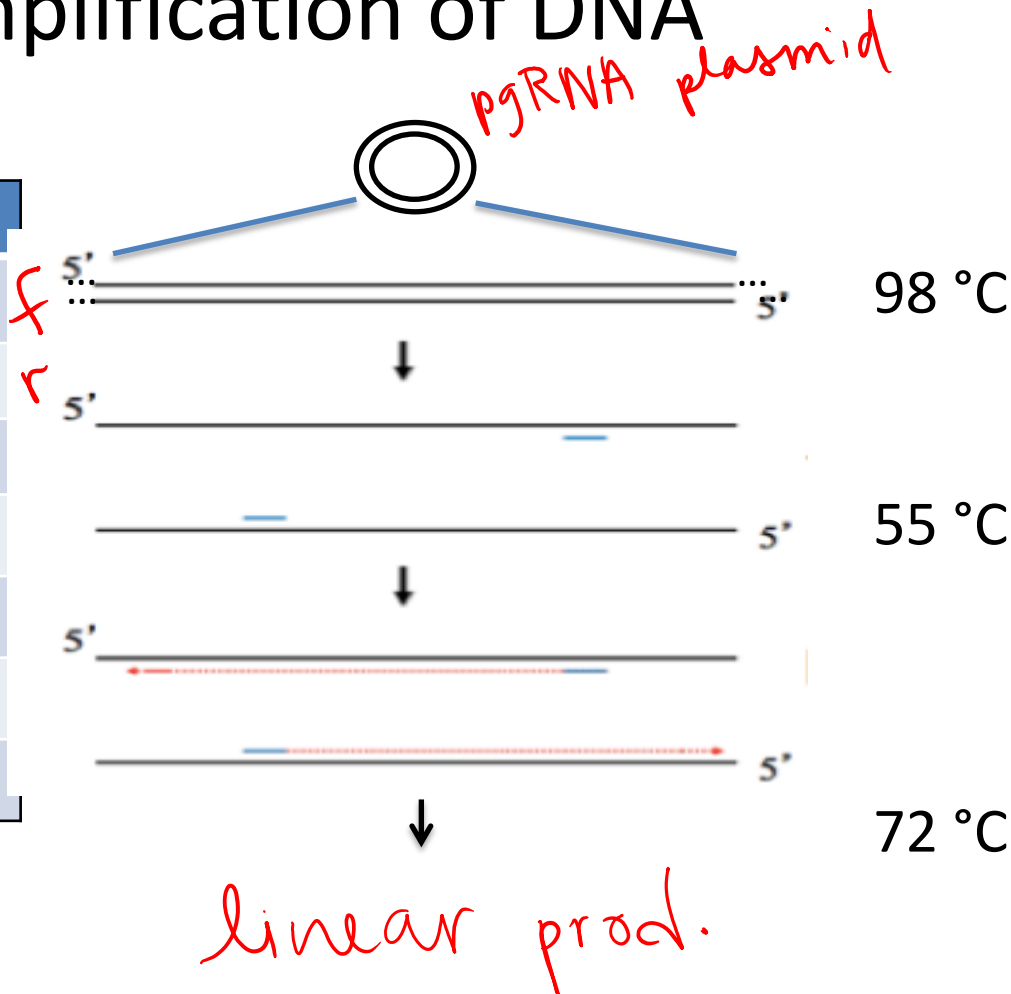
Insertion of DNA via SDM



SDM Part 1: PCR amplification of DNA

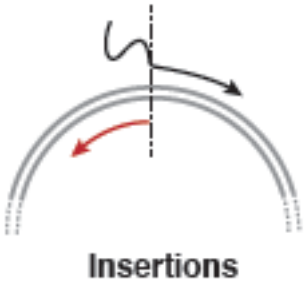
Ingredients
template
dNTPs
Polymerase
Buffer (pH, cofactors like Mg^{2+})
H_2O

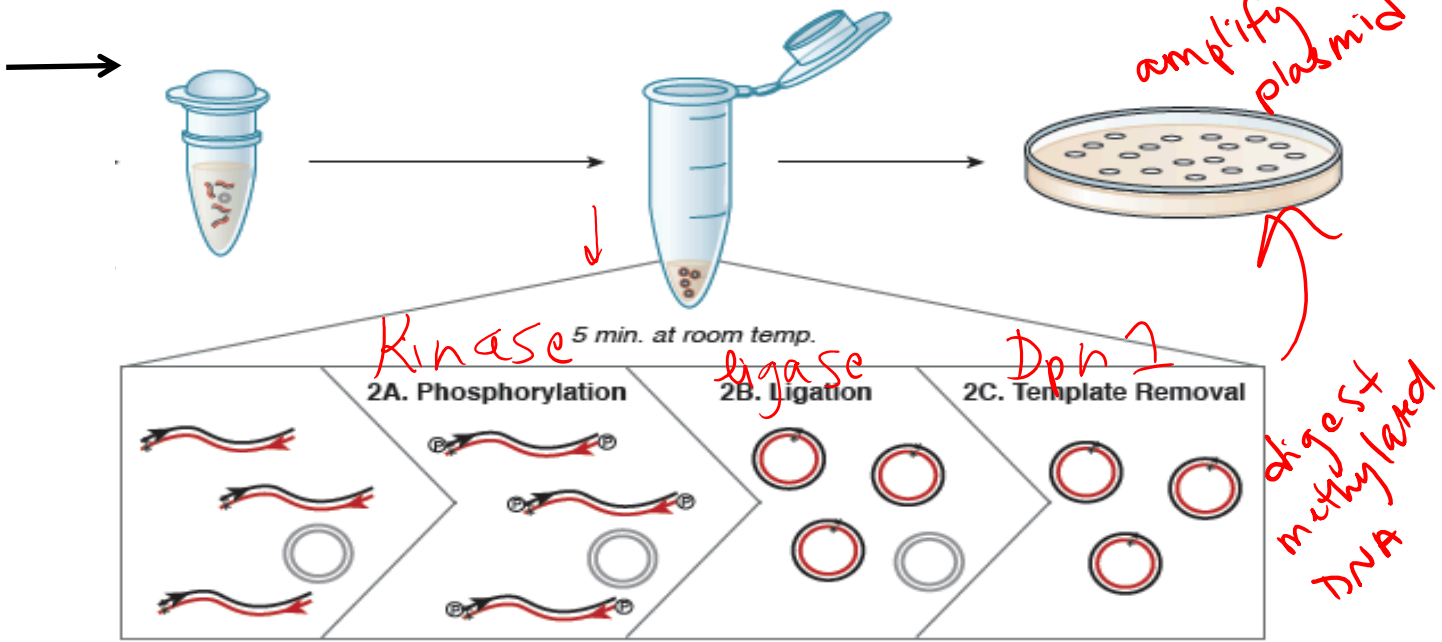
Q5 Hot start pfu



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





amplify plasmid

Kinase

Ligase

Dpn I

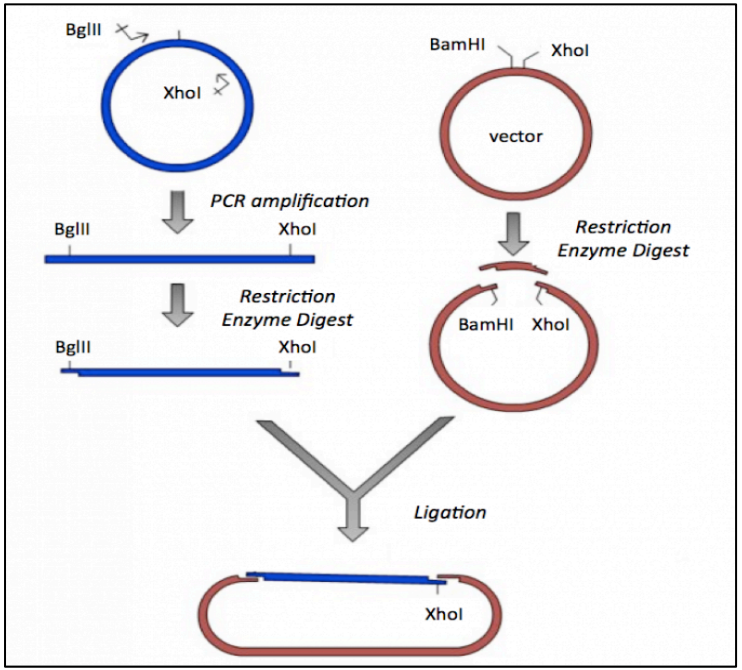
digest methylated DNA

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

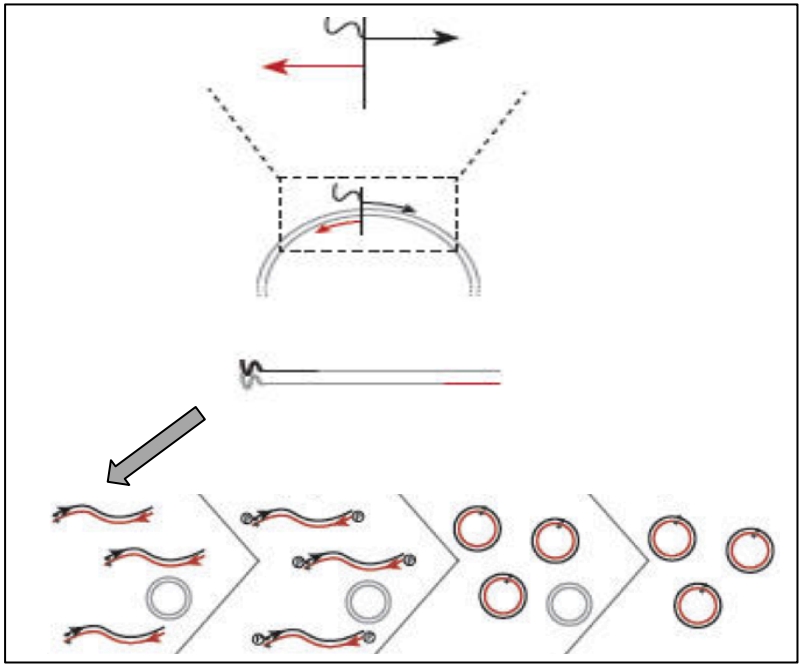
We have covered two ways to engineer DNA

"Traditional" plasmid cloning by restriction enzyme digest



pdCas9

Site directed mutagenesis



pgRNA_target

Today in lab…

1. Set up your gRNA insertion/amplification reaction using reagents at front bench
2. After the PCR cycling is complete:
   - complete KLD reaction
   - Transform into NEB5alpha bacteria
   - Spread bacteria onto ampicillin LB agar plate and leave at 37C
3. Work on Journal Club and Mini presentation in your downtime
   - *You can not longer change your journal club article or presentation date, email the instructors if there is a problem*

Mod2 assignments

- Research Article (20%)
  - Individual, submit on Stellar
  - Word document
  - Due 11/11 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
  - Journal Club I is Tuesday Oct. 22
- Lab quizzes (5%)
  - M2D5 (next Thursday!) and M2D8
- Notebook (part of 10% Homework and Notebook)
- Blog (part of 5% Participation)
  - By 11/1 (Journal club reflections)
  - By 11/12 (Mod2 material/research article)

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Tuesday (10/22)

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)
- FYI you will present from a lab computer (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 (today) and discuss your paper in advance if you feel it will organize your thoughts/presentation

Thursday (10/24)—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
    - *Same topics must be address as data summary background motivation*
  - References in text and brief summary of each reference at the end
- Schematic of Mod2 experimental ***approach*** (not overview)
  - Create image (do not take and reference published schematics or wiki images)
  - 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?