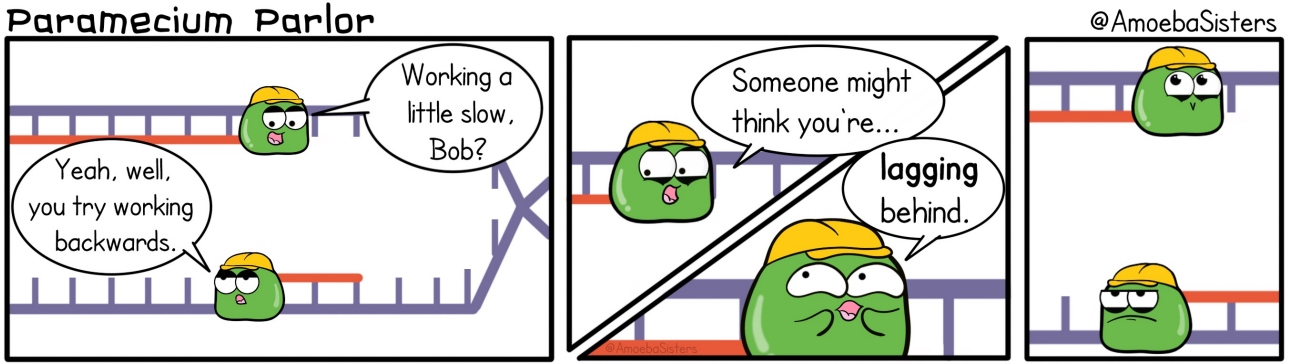
Finish this (start PCR) before prelab

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 20 uL volume)
   - 2 uL gRNA (forward primer)
   - 2 uL RevP (reverse primer)
   - 16 uL nuclease-free water
3. Take PCR tube and label with your team
   - 10.25 uL nuclease-free water
   - 1.25 uL primer mix (from #2) → OK to use P20
   - 1 uL pgRNA plasmid
   - 12.5 uL Q5 Hot Start 2x Master Mix

M2D3: Clone psgRNA expression plasmid

1. Set up reaction to generate gRNA_target plasmid
2. Pre-lab discussion
3. Continue generation of psgRNA
4. Transform pgRNA_target into bacteria



Mod2 Overview

Research goal: Increase the yield of commercially valuable byproducts in *E.coli* using CRIPSRi technology to target genes involved in mixed-acid fermentation pathway.

Last Lab:

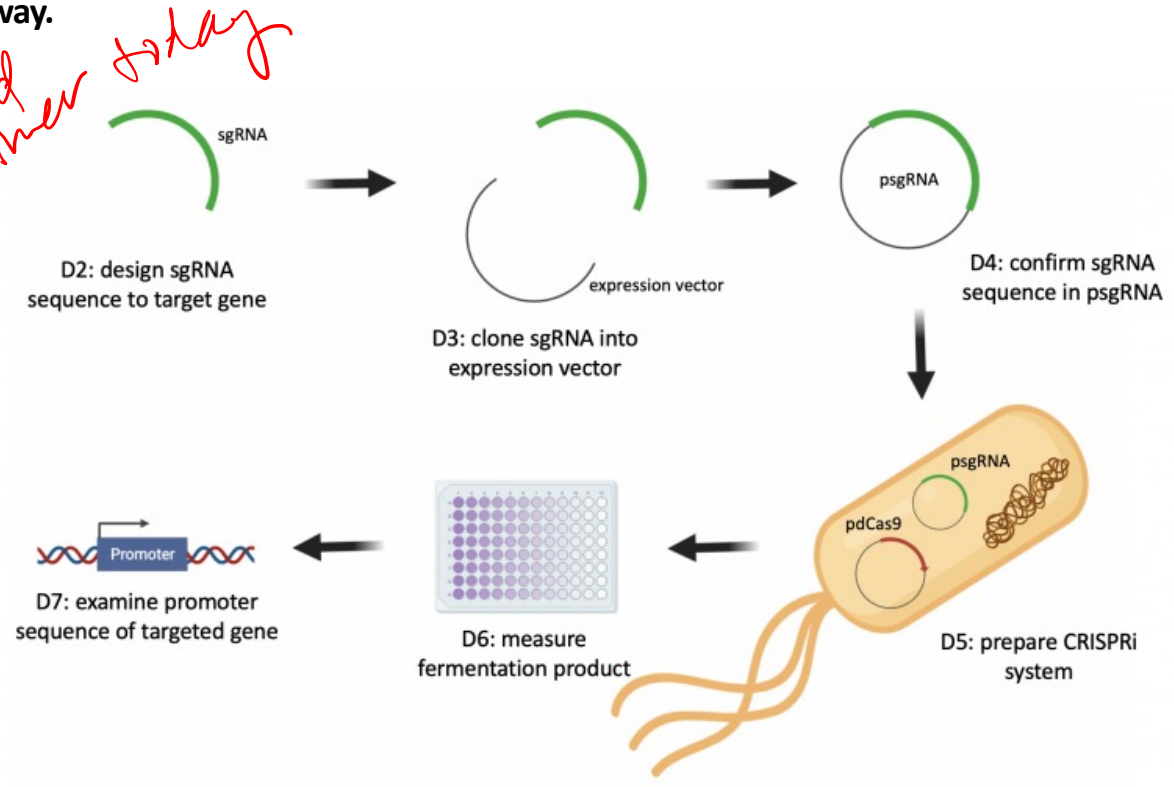
Chose metabolic gene of interest and designed sgRNA to target it

This Lab:

Clone sgRNA into vector to create plasmid that targets gene of interest

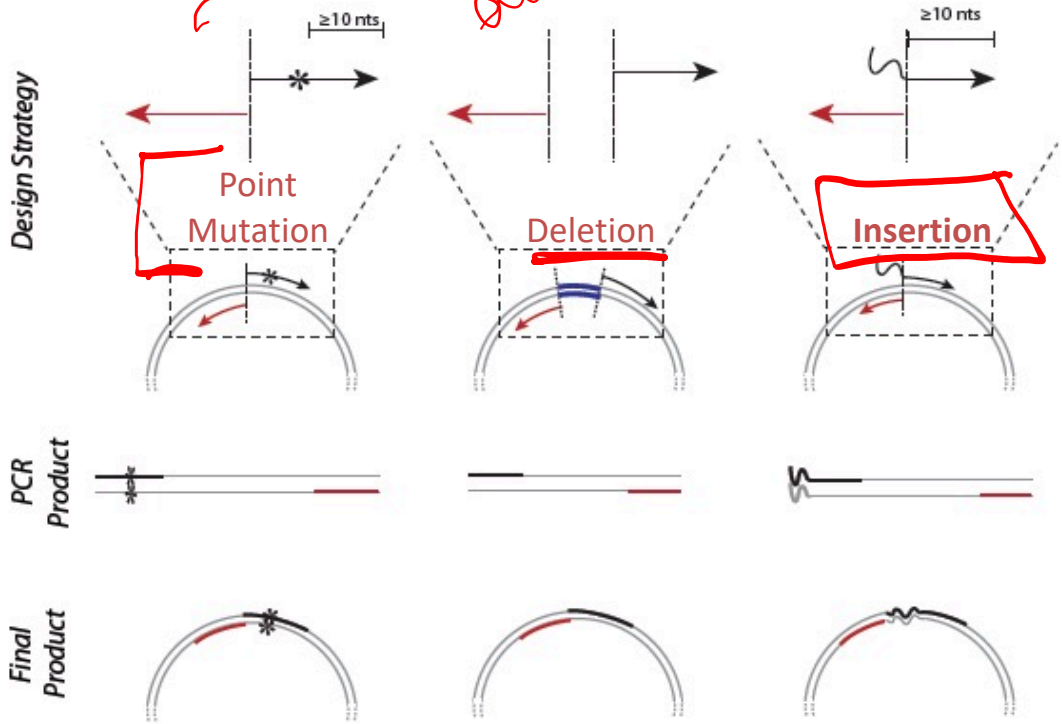
Next Lab:

Confirm correct sgRNA cloning and do preliminary CRISPRi system preparations

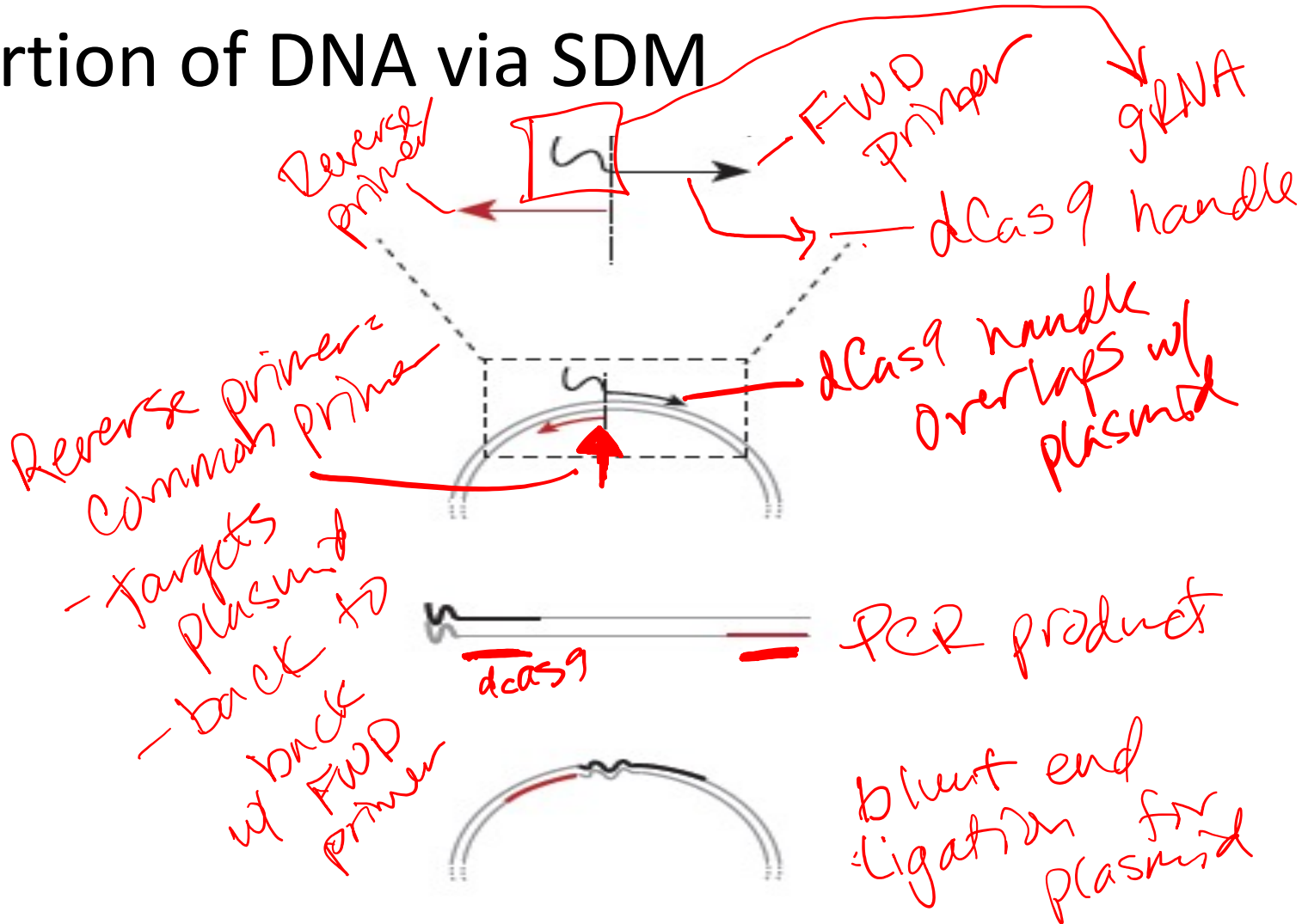


Use site-directed mutagenesis (SDM) to engineer plasmid DNA

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

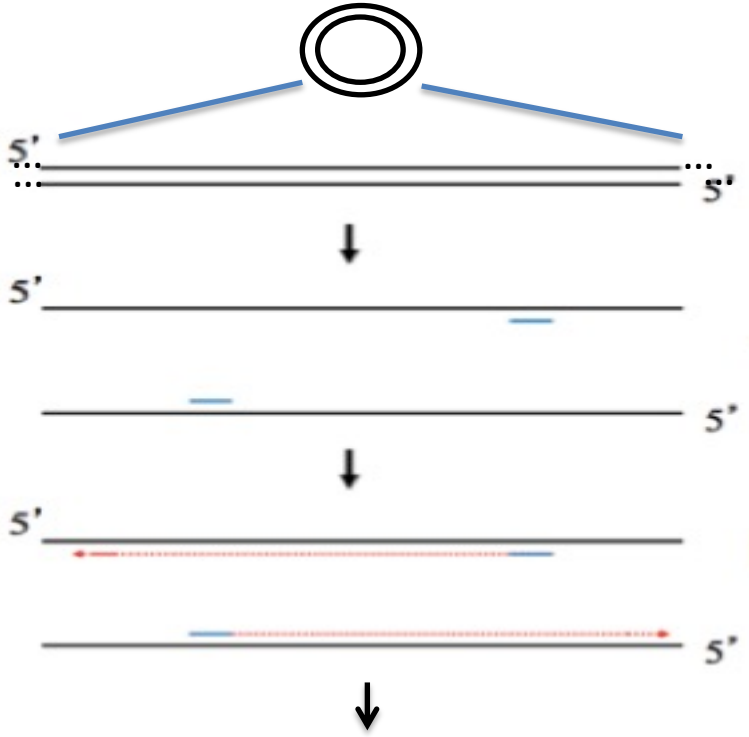


Insertion of DNA via SDM



SDM Part 1: PCR amplification of DNA

Ingredients
F primer
R primer
template → pSGRNA
dNTPs
Q5 taq → Polymerase – High fidelity
Buffer (pH, cofactors like Mg^{2+})
H_2O



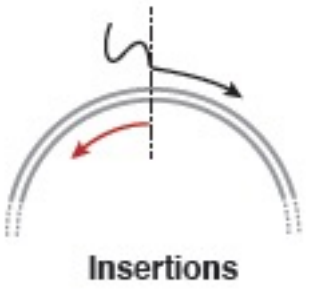
98 °C

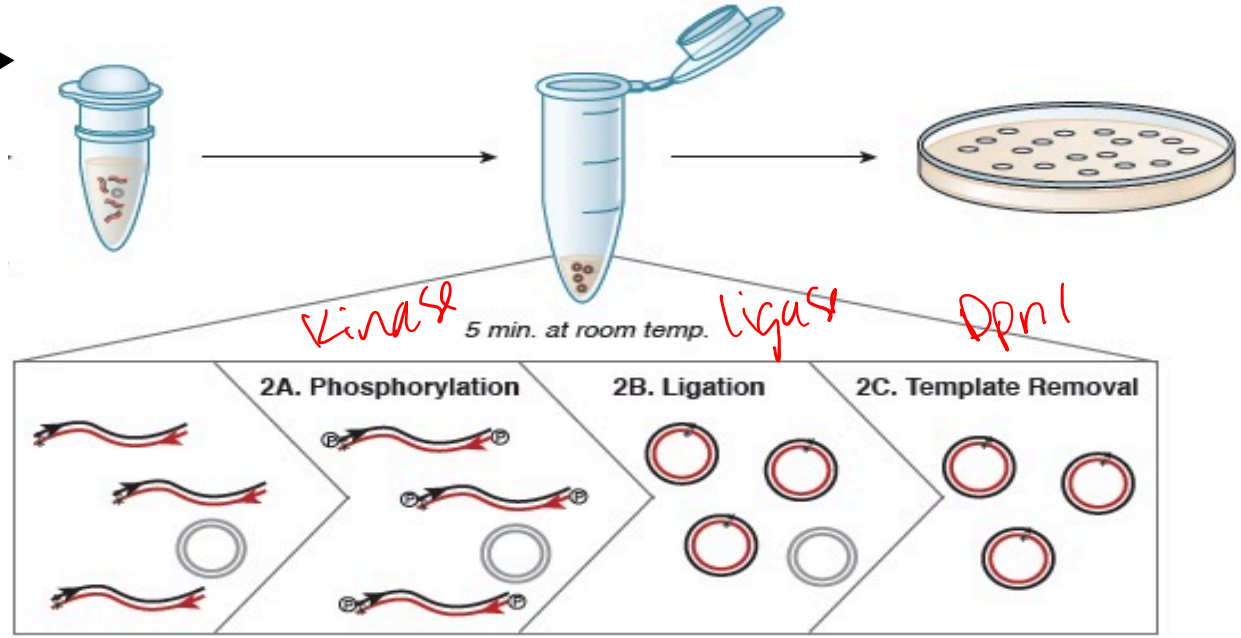
55 °C

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



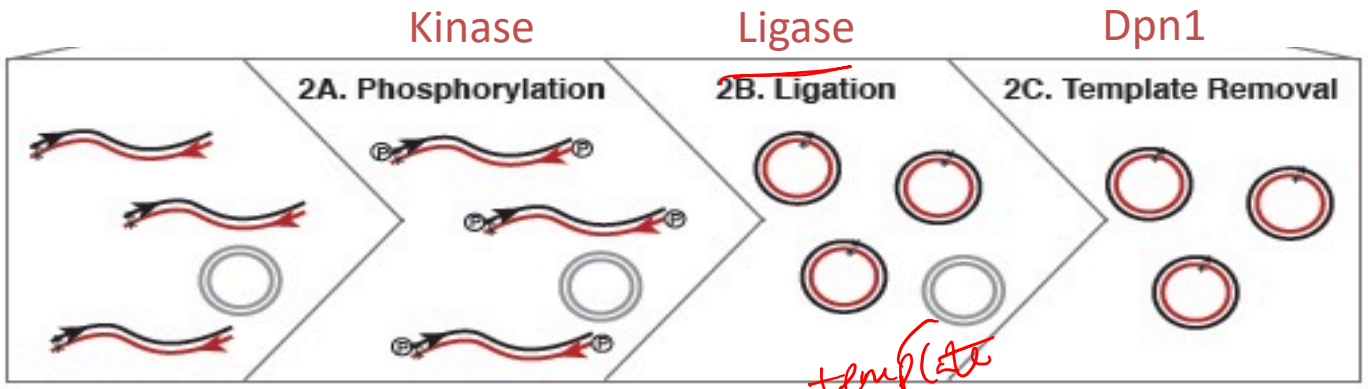


After PCR:
1) linear product w/ insert
2) template pSgRNA – no insert

Kinase
Ligase
DpnI

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

KLD reaction



template

K: phosphorylates PCR product ends - promotes ligation

L: ~~recirc~~ repair plasmid backbone - now circular

D: removes methylated DNA (template)
↳ cleave
↳ produced in bacteria

For today

1. Continue production of sgRNA plasmid
2. Work on journal club in down time

For M2D4…

1. Create a journal club slide and script for 1 figure from your paper
   - Use the homework checklist on the wiki for guidance

For homework, make a Journal Club presentation slide

Craft 1 slide using your JC article to present the data from a figure.

- Your slide should show the data and highlight the key finding(s).
- The information should be clear and large enough to read.
- Keep text to a minimum. (NO figure captions on slide!)
- The title should state the take-home message of the data that are shown.

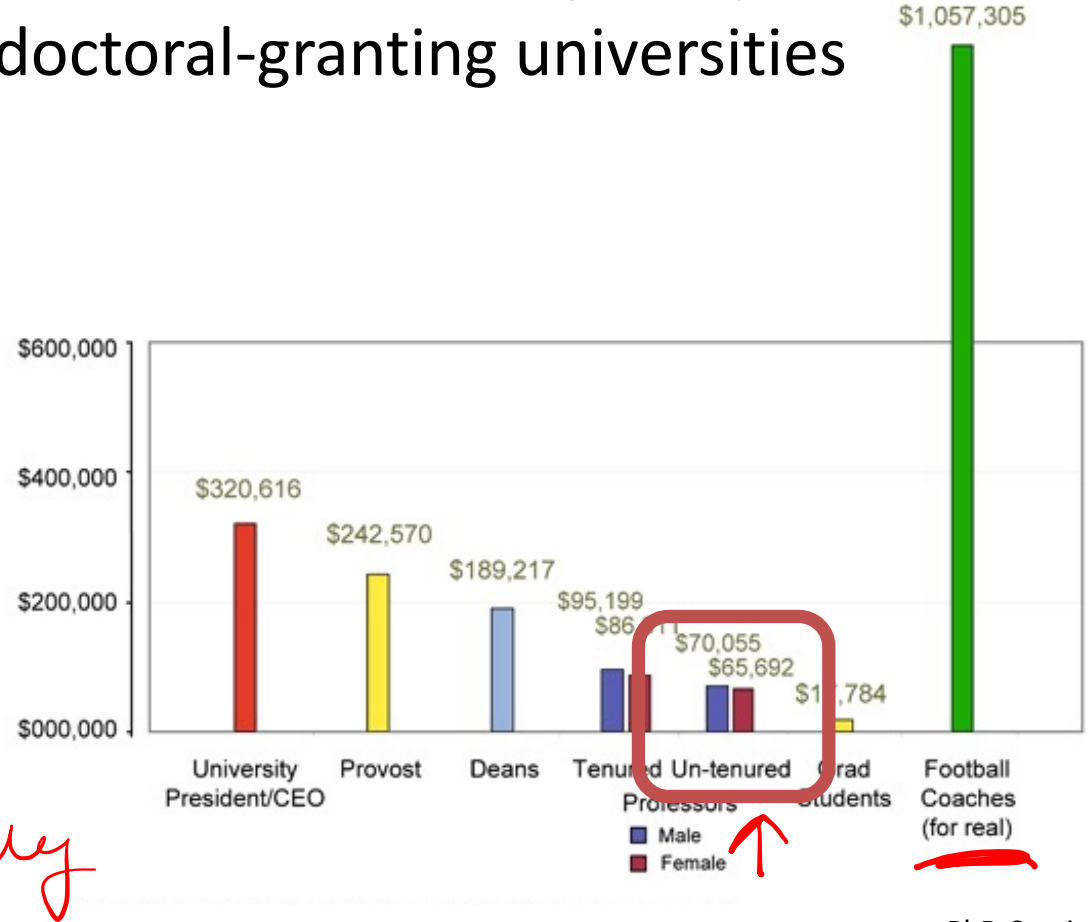
NO A, B, C.

no more than 1/2 of slide is text

EXAMPLE SLIDE: Football coaches are the highest paid academic employees at doctoral-granting universities

- Data represent expression of Y using method A
- Possibly something about the control(s), if applicable
- Perhaps an important note about the data that is not already stated in the title
- Transition to next slide…

in text or verbally



PhD Comics