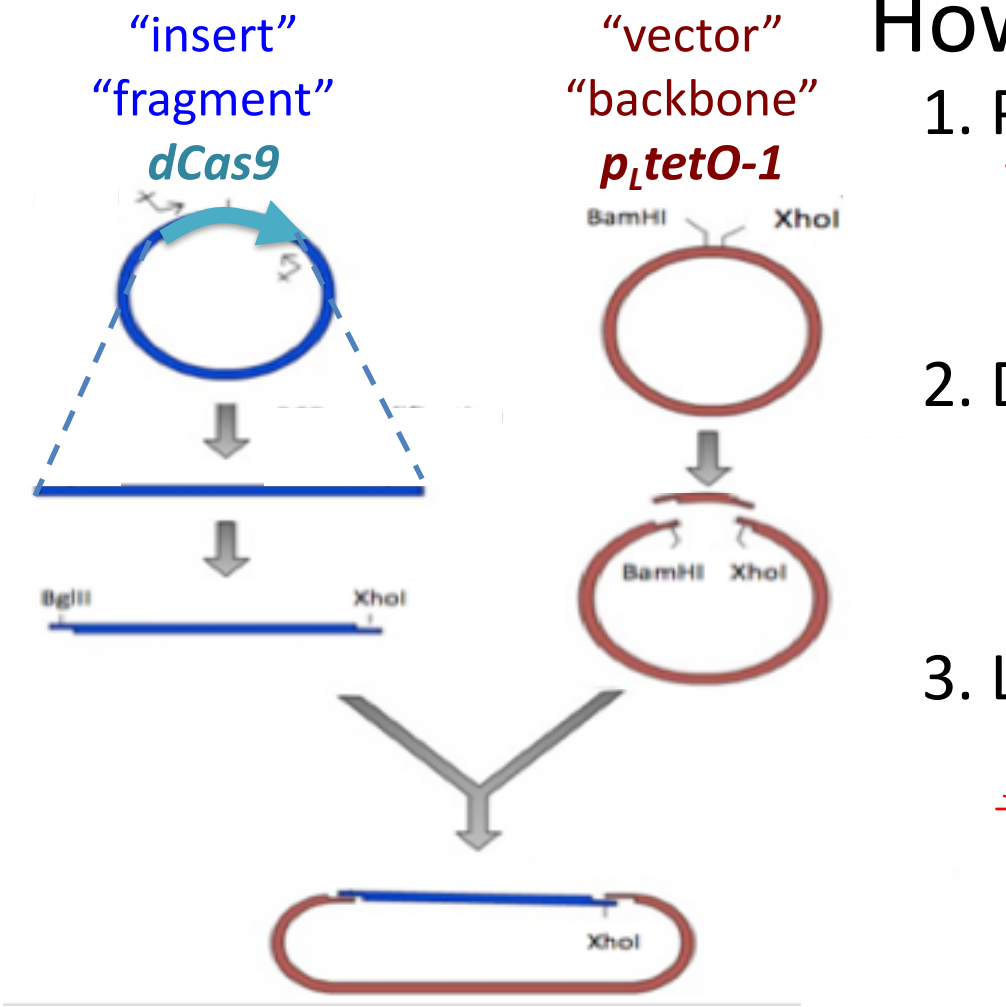
M2D1: Complete *in silico* cloning of dCas9 & actual confirmation digest

10/12/18

1. Design primers to dCas9
2. *In silico* PCR amplification, digest, and ligation
3. *Actual* (wet-lab) diagnostic digest of pdCas9

(Almost) done with Mod1!

- Mini-presentation
  - due 10pm on Saturday, October 13
  - You don't have to be exhaustive; tell a focused story
- Data summary
  - ✓ draft due 10pm on Wednesday, October 10
  - revision due 10pm on Saturday, October 20th
- Blog post
  - due 10pm on Thursday, October 11th

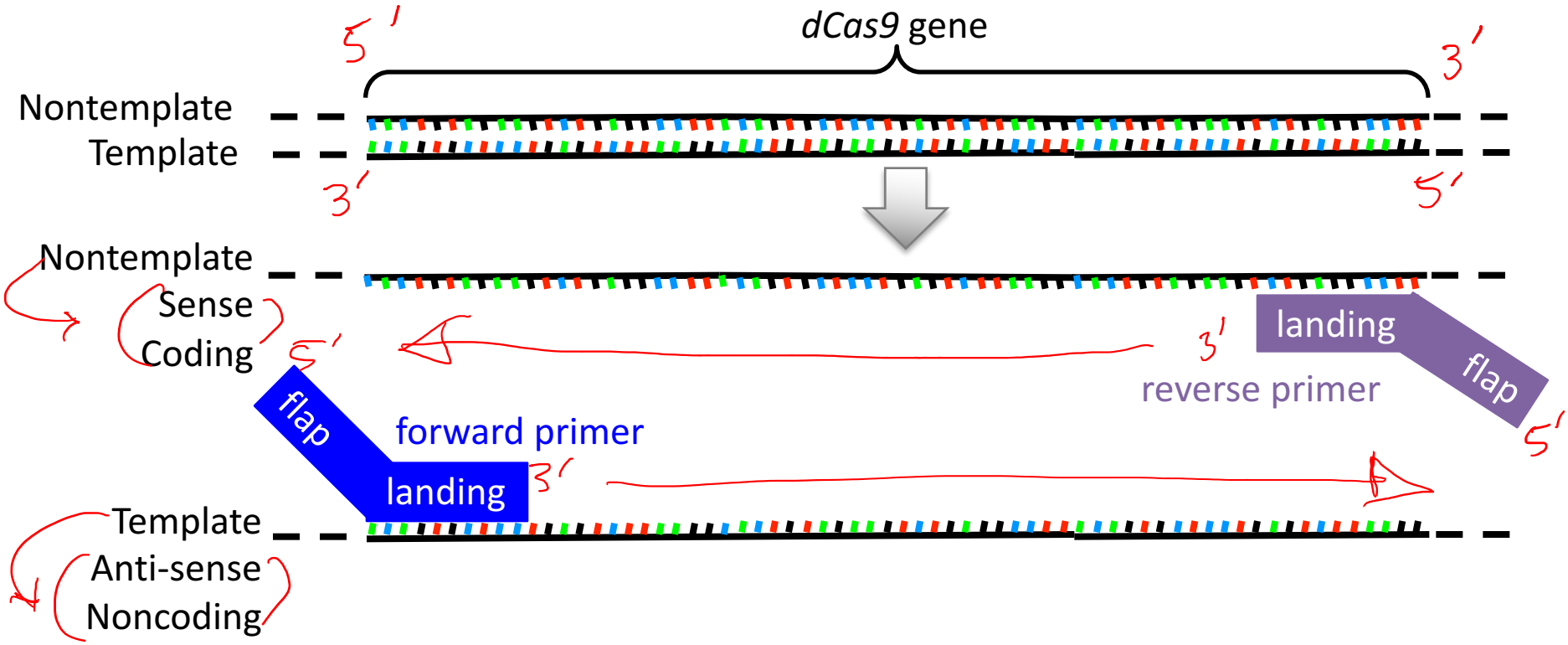


How is DNA engineered?

1. PCR amplification of DNA:
   DNA polymerase
   primers specific to gene seq.
   nucleotides (dNTPs)
2. Digestion:
   restriction enzymes
   ↳ endonucleases
   • sticky end or blunt
3. Ligation:
   DNA ligase
   ↳ seals phosphodiester bond in backbone

1. PCR amplification of DNA

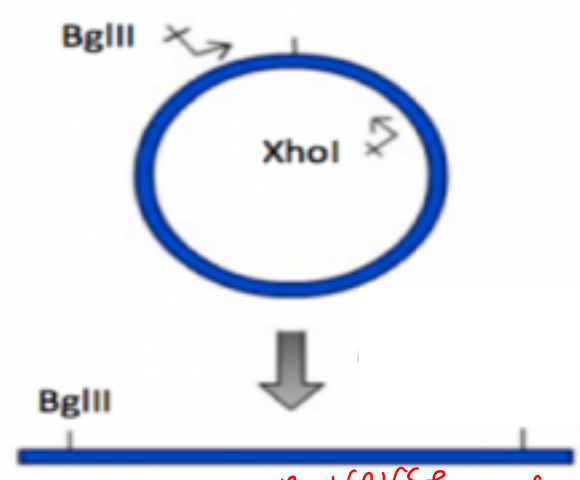
Defining terminology



1. PCR amplification of DNA

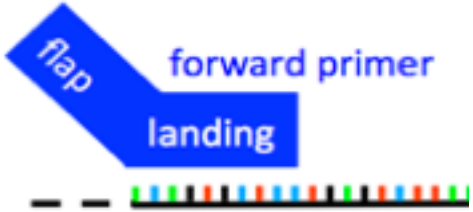
Designing primers

- **Landing sequence**: match to dCas9
- **Flap sequence**: contains endonuclease recognition sequence and junk DNA



1. PCR amplification of DNA

Primer design guidelines



- Length: 17-28 base pairs
  long enough to be specific
  short enough for easy annealing
- GC content: 40-60%
  - GC has 3 hydrogen bonds; AT has 2 hydrogen bonds
  - GC clamp at ends
- T_m(primer) < 65 °C] ideal for specific priming
- Avoid secondary structures
  - hairpins
  - complementation w/in primer sequence
- Avoid repetitive sequences
  - Max of 4 di-nucleotide repeats (ex. ATATAT)
  - Max of 4 bp in a run (ex. GATGGGG)

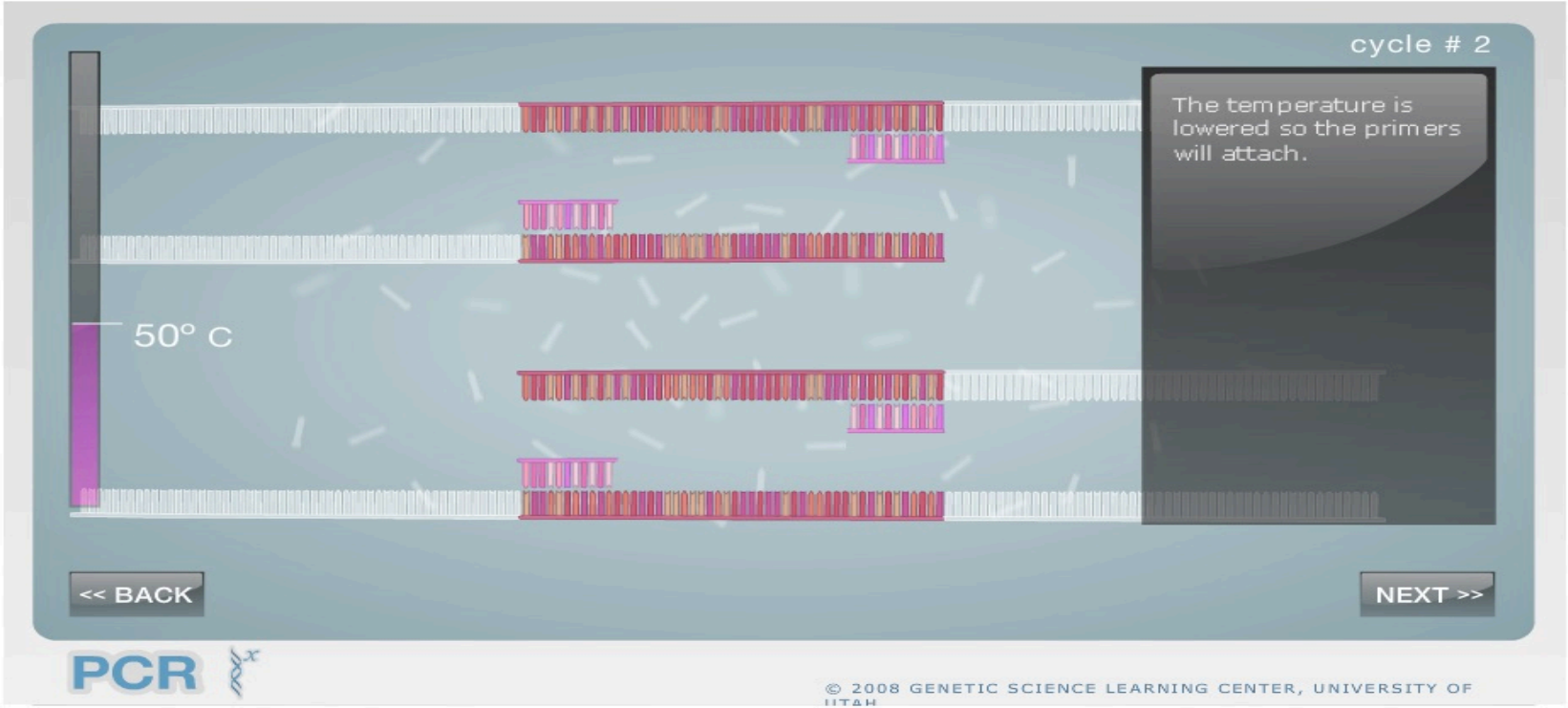
1. PCR amplification of DNA

Three major PCR steps—which temperature & why?

- Melt
  - 95 °C
  - hydrogen bonds break
- Anneal
  - T_m(primer) = 1/2 primer annealed to target
  - $T_{anneal} \sim T_m$(primer) – 5°C ↑ probability primer bound
- Extend
  - 72°C (for Taq)
  - 1000 bases/min

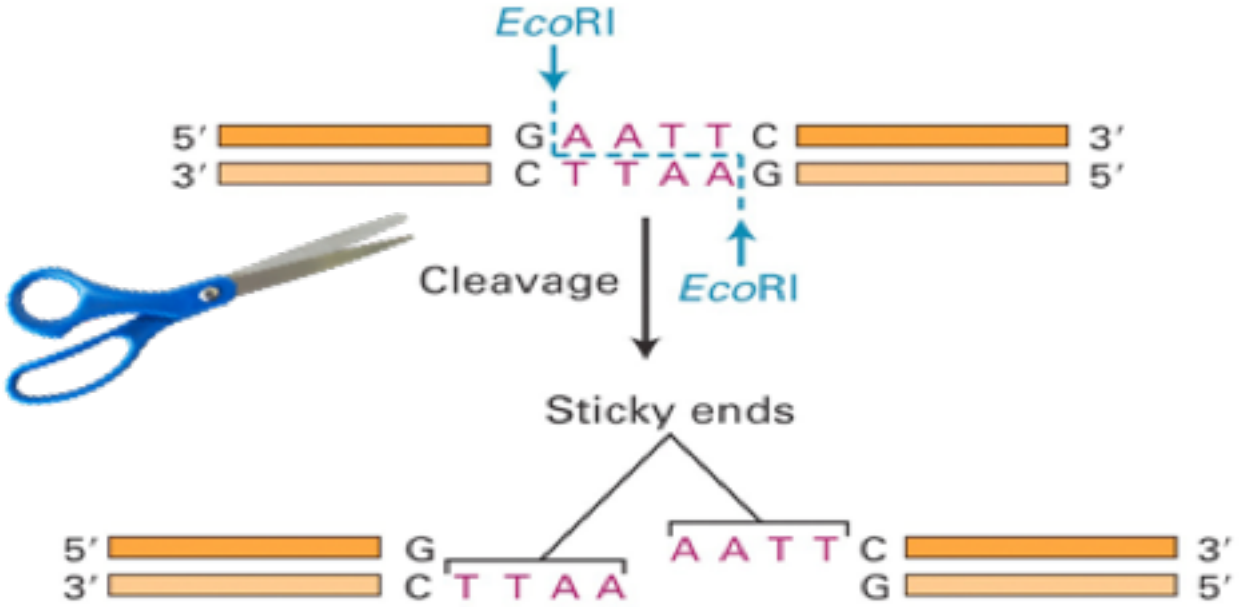
Leslie's favorite PCR animation

http://learn.genetics.utah.edu/content/labs/pcr/



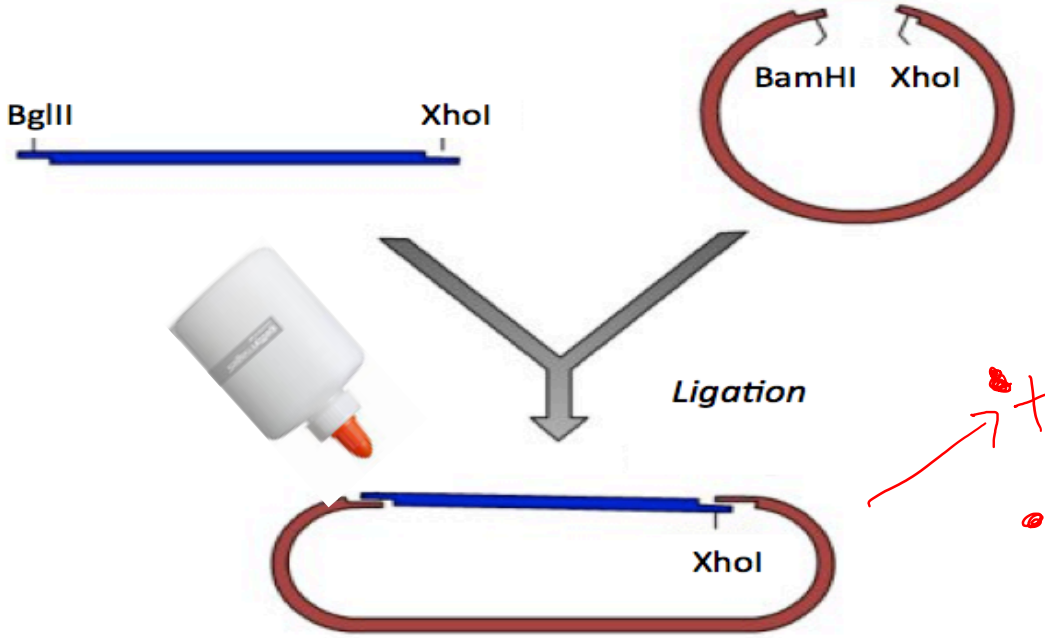
2. Digestion

Restriction endonucleases create sticky ends on dCas9 insert and plasmid backbone



3. Ligation

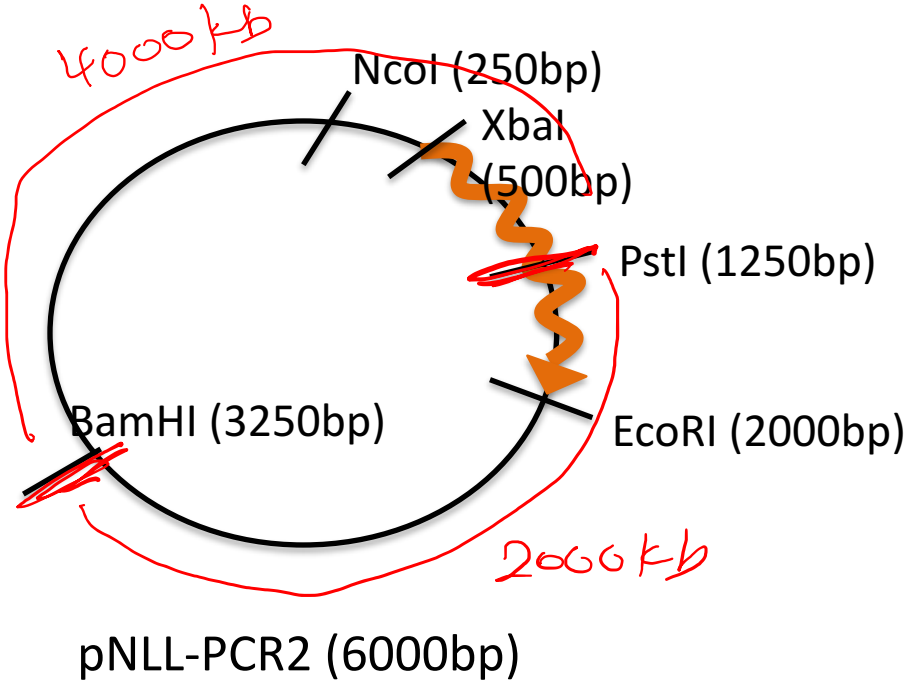
Insert dCas9 into expression vector (backbone) to create new plasmid (pdCas9)

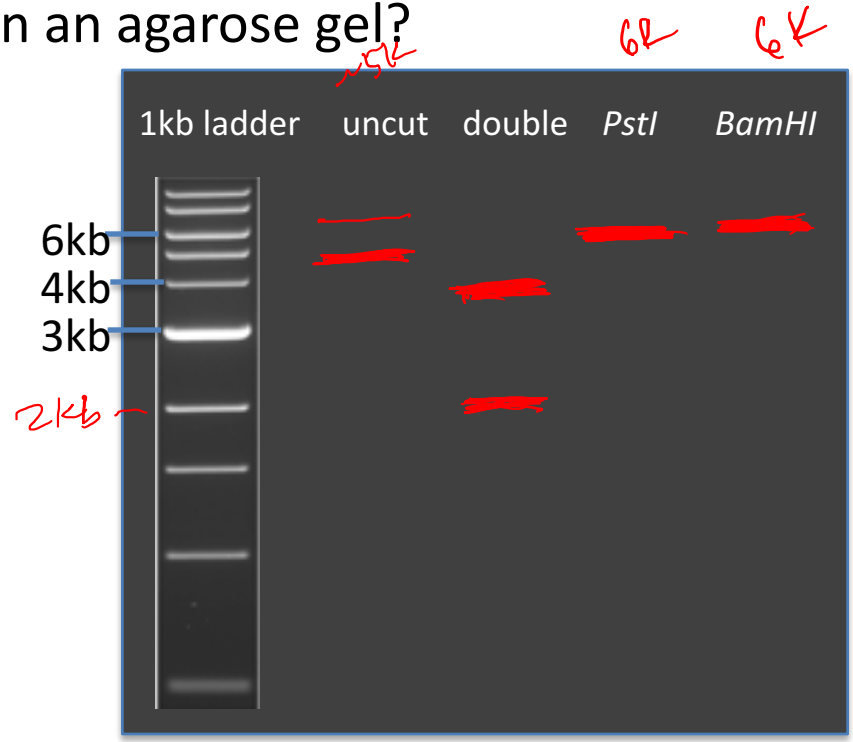


- transform into bacteria
- grow in ciamid culture + purify
- conf. digest seq

Confirmation digest considerations

- Do you have access to the enzymes?
- Are the two enzymes compatible?
- Are fragments easily distinguished on an agarose gel?





M2D2 homework—Sign up for Journal Club

- Sign up on wiki for which day you will present:
  M2D4 (October 24th) or M2D6 (October 31th)
- Pick 1 of 20 papers, or suggest your own
- Reserve paper by adding name next to it **[LMM/WF/Red]**
  - First come, first served!
  - Only one T/R and one W/F student per article
  - Don't pick a paper randomly

Slot	Day 4 (T/R)	Day 6 (T/R)	Day 4 (W/F)	Day 6 (W/F)
1				
2				
3				
4				

M2D2 homework—Make a presentation slide

To help you prepare for the Journal Club presentation, you will craft 1-2 slides using this article by Ji. et al. to present the data from Figure 2.

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

Today in lab

1. Reproduce *in silico* (in Benchling) the cloning of pdCas9
   - Design primers that would amplify the gene dCas9
   - Depict PCR amplification product
   - Digestion of dCAS9 PCR product and vector by restriction enzymes
   - Ligation of insert and vector
2. Set up confirmation digests of pdCas9 for agarose gel electrophoresis (start at 3pm)
   - Choose restriction enzymes for diagnostic digest
   - Calculate volumes of digest components
   - Set-up digest and leave overnight at 37°C