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

10/8/19

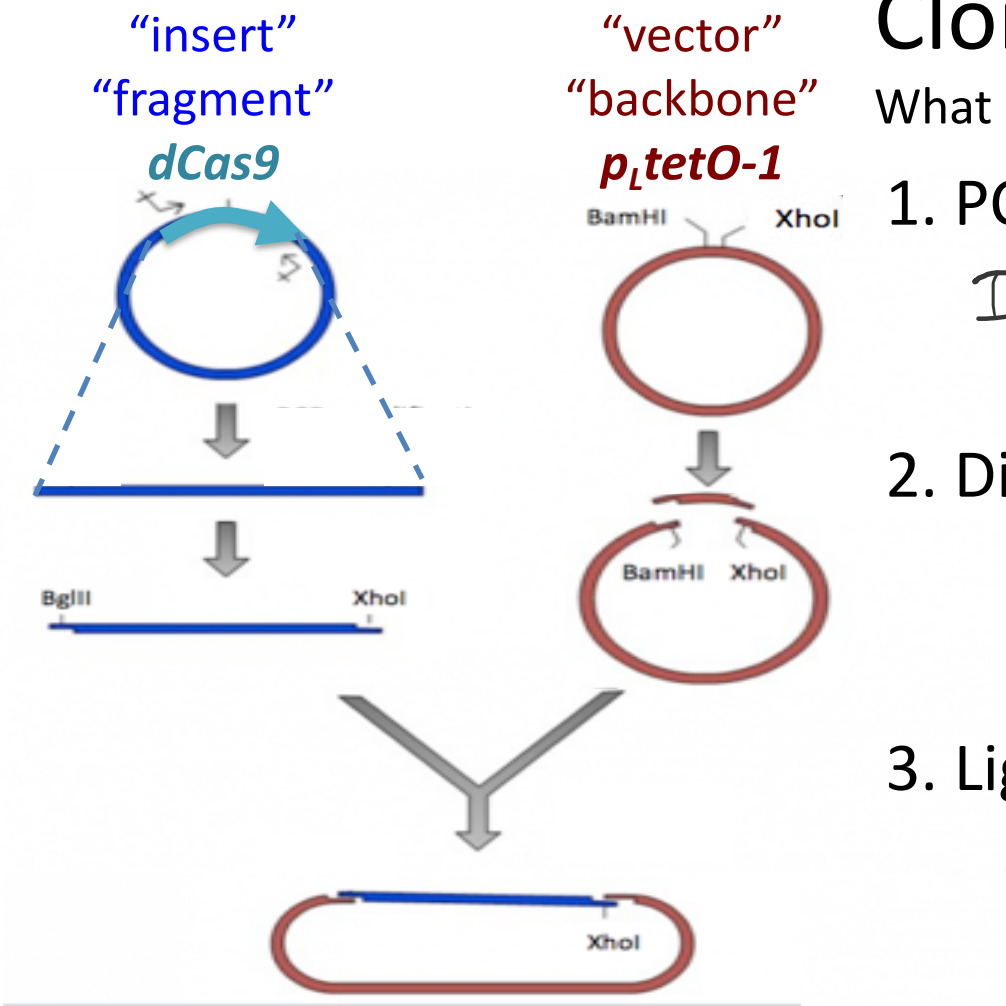
1. Design primers to dCas9
2. *In silico* PCR amplification, digest, and ligation
3. *Actual* (wet-lab) diagnostic digest of pdCas9

(Almost) done with Mod1!

- Data summary
  - draft due 10pm on Monday, October 14th
  - revision due 10pm on Saturday, October 26th
- Mini-presentation
  - due 10pm on Saturday, October 19th
- Blog post
  - due 10pm on ~~Thursday,~~ Tuesday October 15th

Cloning of pdCas9:

What enzyme is required?



1. PCR amplification of DNA:

DNA polymerase

2. Digestion:

restriction enzymes = endonucleases

3. Ligation:

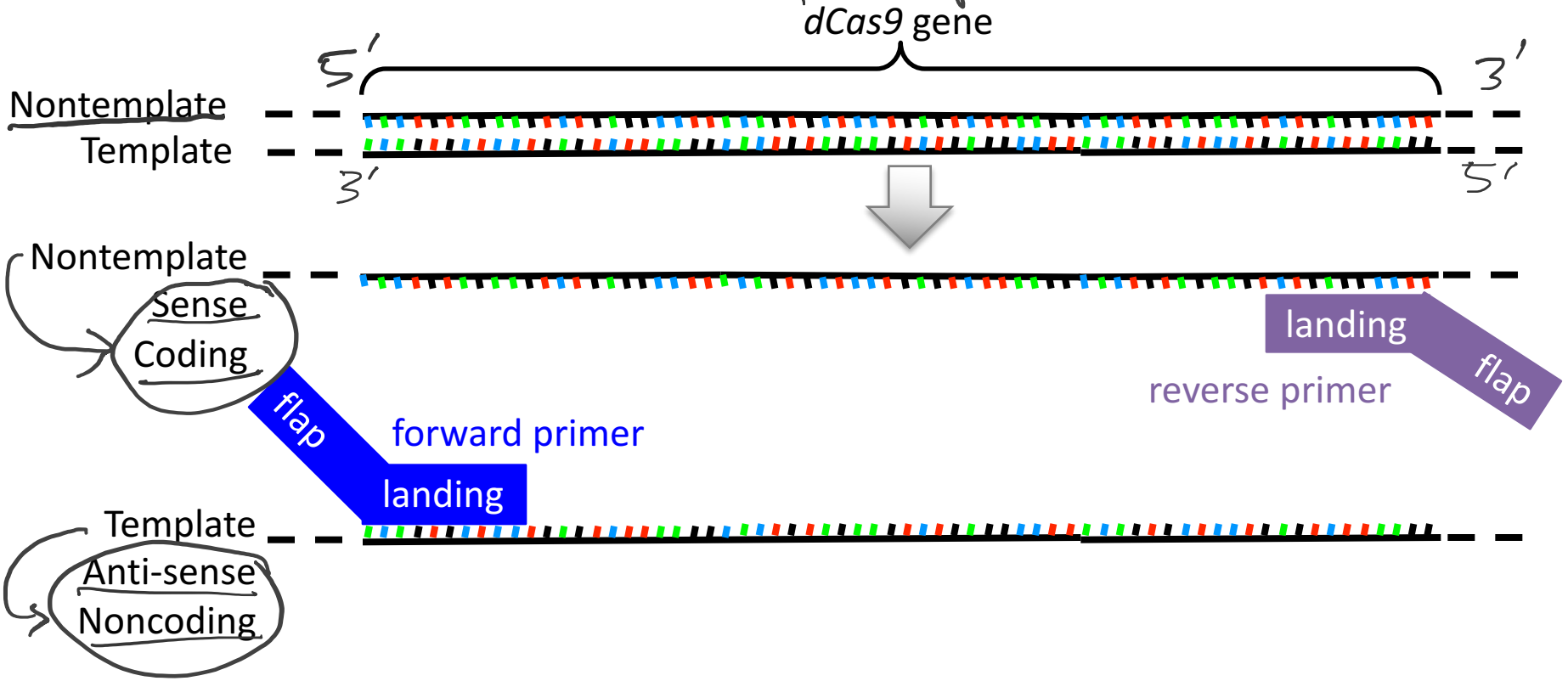
DNA ligase

PCR amplification of DNA: Defining terminology

forward primer: same seq. as nontemplate strand, binds template

reverse primer: Reverse complement of nontemplate, binds nontemplate

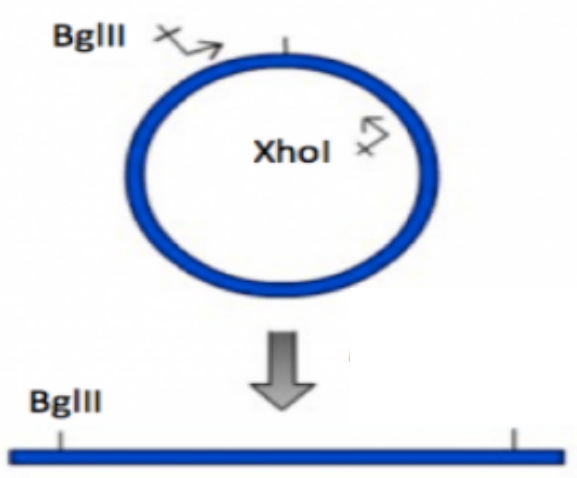
Report all DNA seq 5'→3'



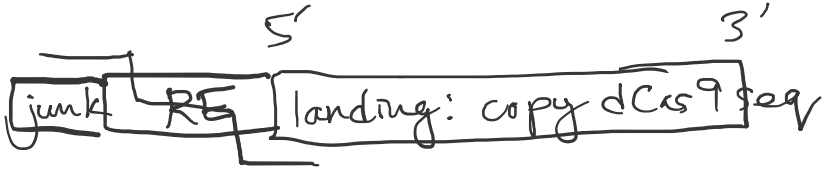
PCR amplification of DNA:

Designing primers

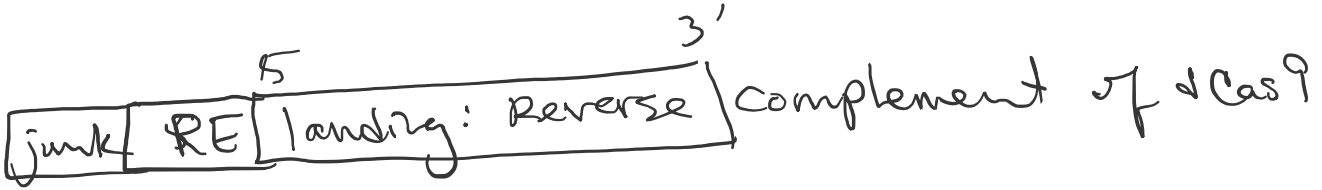
- **Landing sequence**: match to dCas9
- **Flap sequence**: contains endonuclease recognition sequence and junk DNA



forward primer:

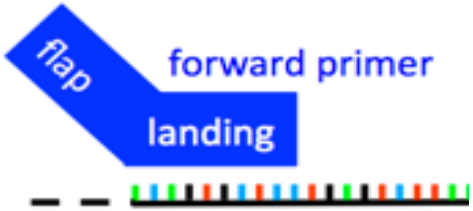


reverse primer:



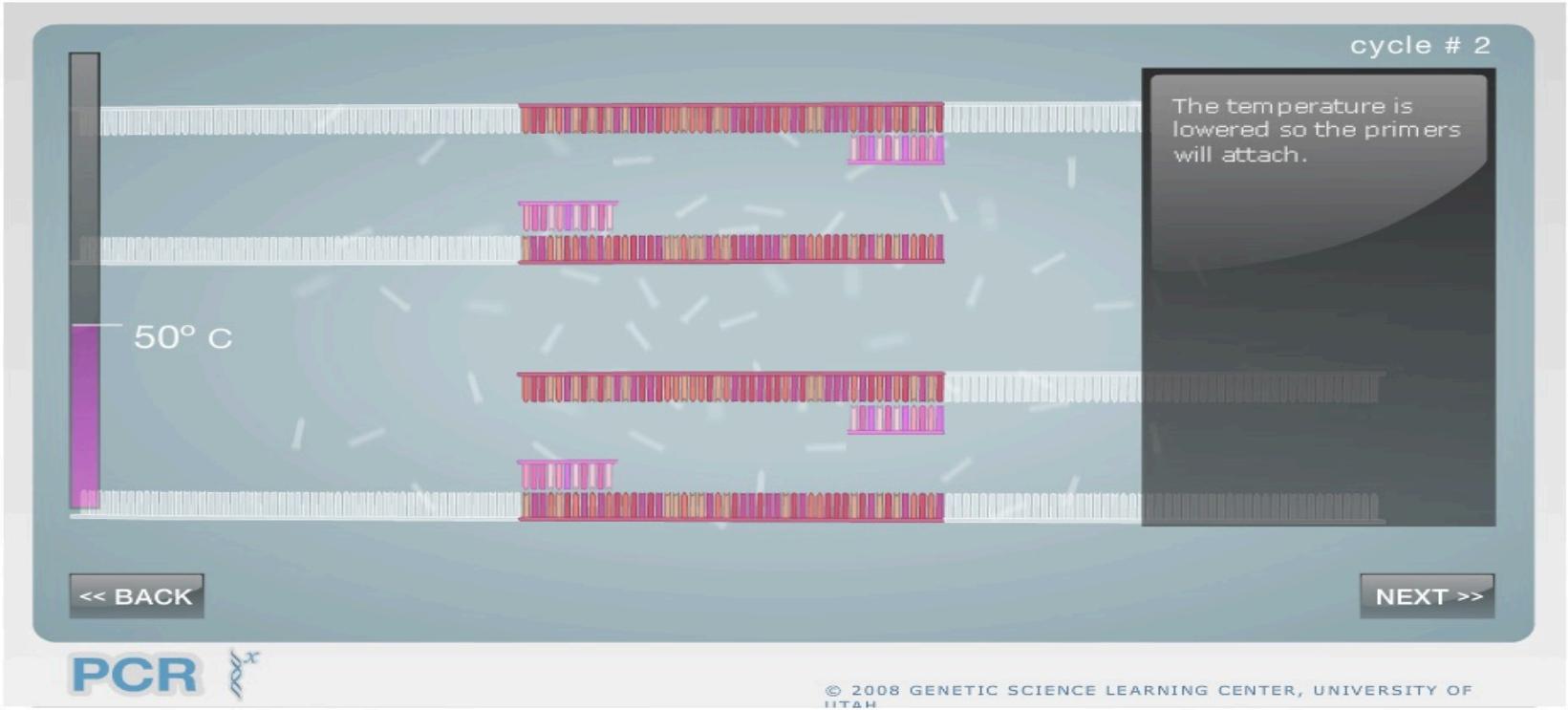
1. PCR amplification of DNA

Primer design guidelines



- Length: 17-28 base pairs
- GC content: 40-60%
  - GC has 3 hydrogen bonds; AT has 2 hydrogen bonds
  - GC clamp at ends
- T_m(primer) < 65 °C — 50% bound DNA, 50% unbound
- 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)

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



Three major PCR steps—which temperature & why?

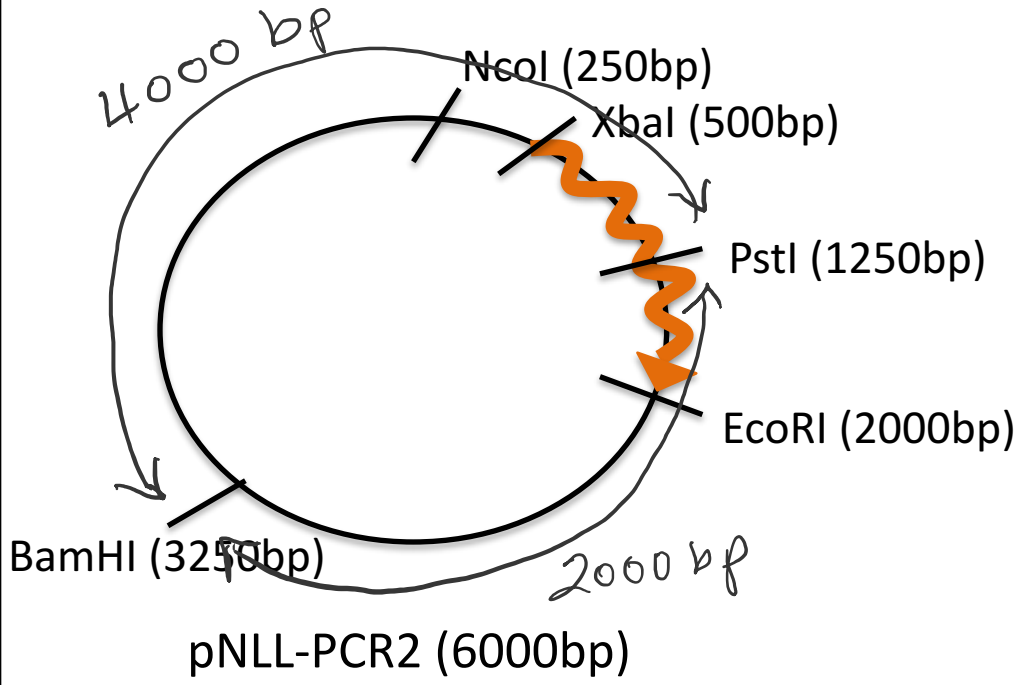
- Melt
  - 95 °C
  - disrupt hydrogen bonds between bases
    DNA denatures
- Anneal
  - T_m(primer) = 50% bound DNA
  - $T_{anneal} \sim T_m(primer) - 5°C$ lower temp = more primer bound to DOI
    must be adjusted for each primer
- Extend
  - 72 °C (for Taq) → DNA polymerase
  - 1000 bases/min

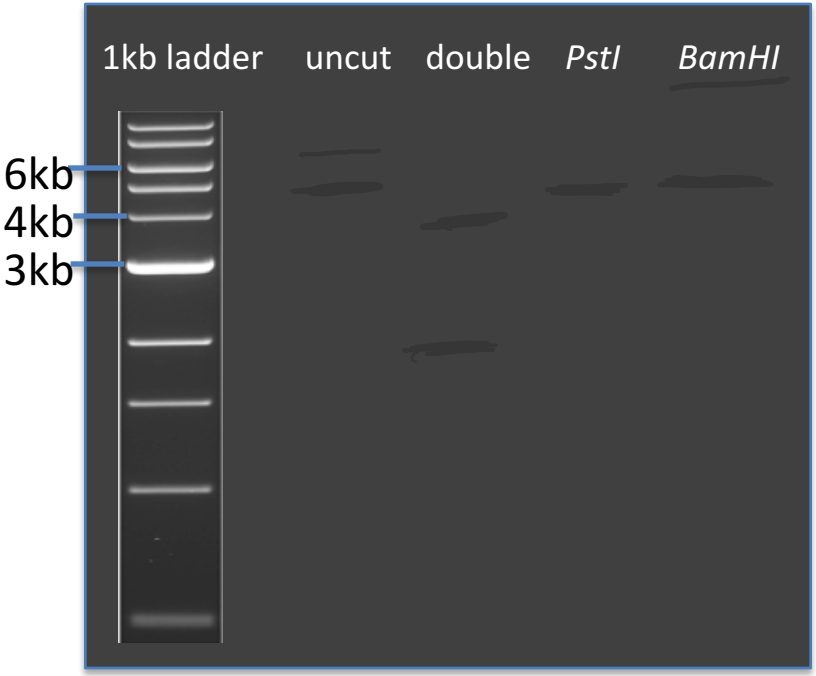
Next steps in cloning pdCas9…

1) PCR amplified dCas9
2) digest w/ RE PCR amplicon (dCas9) + vector
3) ligation Reaction
4) Incubate ligation products w/ competent bacteria
5) mini prep DNA from bacteria
6) diagnostic digest (confirmation digest)

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 22nd) or M2D6 (October 29th)
- Pick 1 of 20 papers, or suggest your own
- Reserve paper by adding name next to it **[LMM/TR/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				
5				
6				

M2D2 homework—Make a Journal Club 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).
- The information should be clear and large enough to read.
- Keep text to a minimum. (NO captions on slide!)
- The title should state the take-home message of the data that are shown.

Today in lab

1. Reproduce *in silico* (in Snap Gene) 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 3:15pm)
   - Choose restriction enzymes for diagnostic digest
   - Calculate volumes of digest components
   - Set-up digest and leave overnight at 37°C