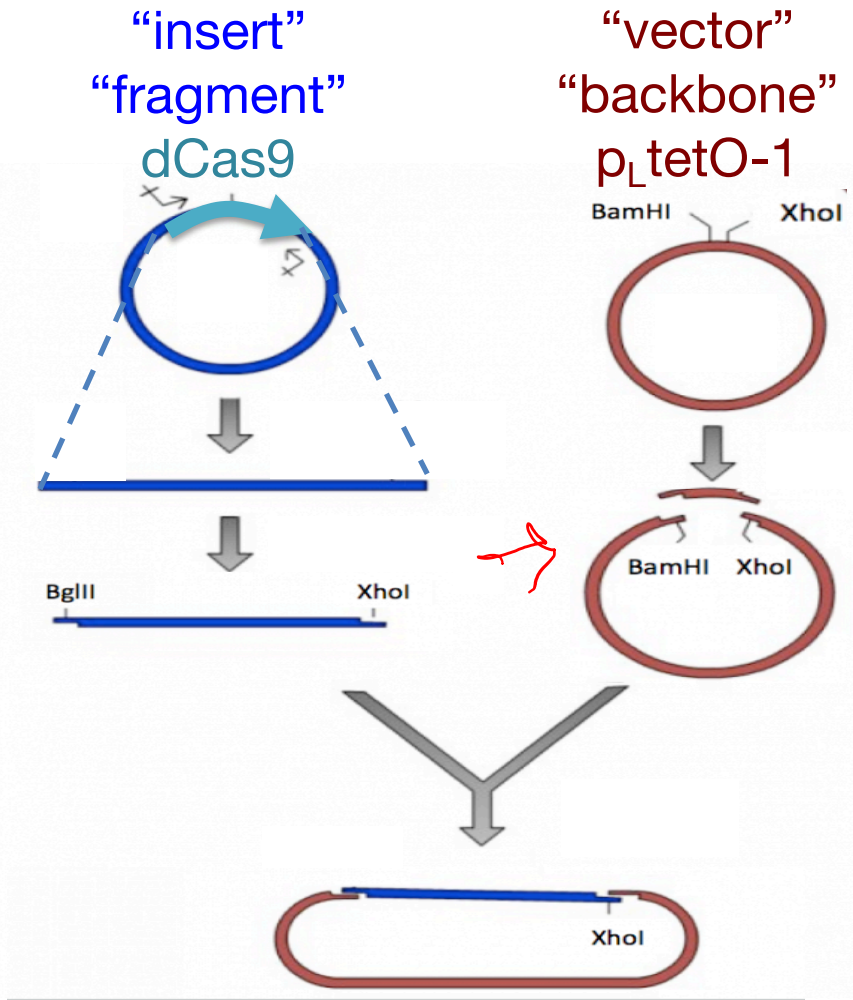
M2D1: Complete *in silico* cloning of dCas9 & confirmation digest

10/9/19

1. Design primers to dCas9
2. *In silico* PCR amplification, digest, and ligation
3. 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 Tuesday, October 15th
    - Reflect on Mod1 – prompts on Wiki
    - Meant to be fun

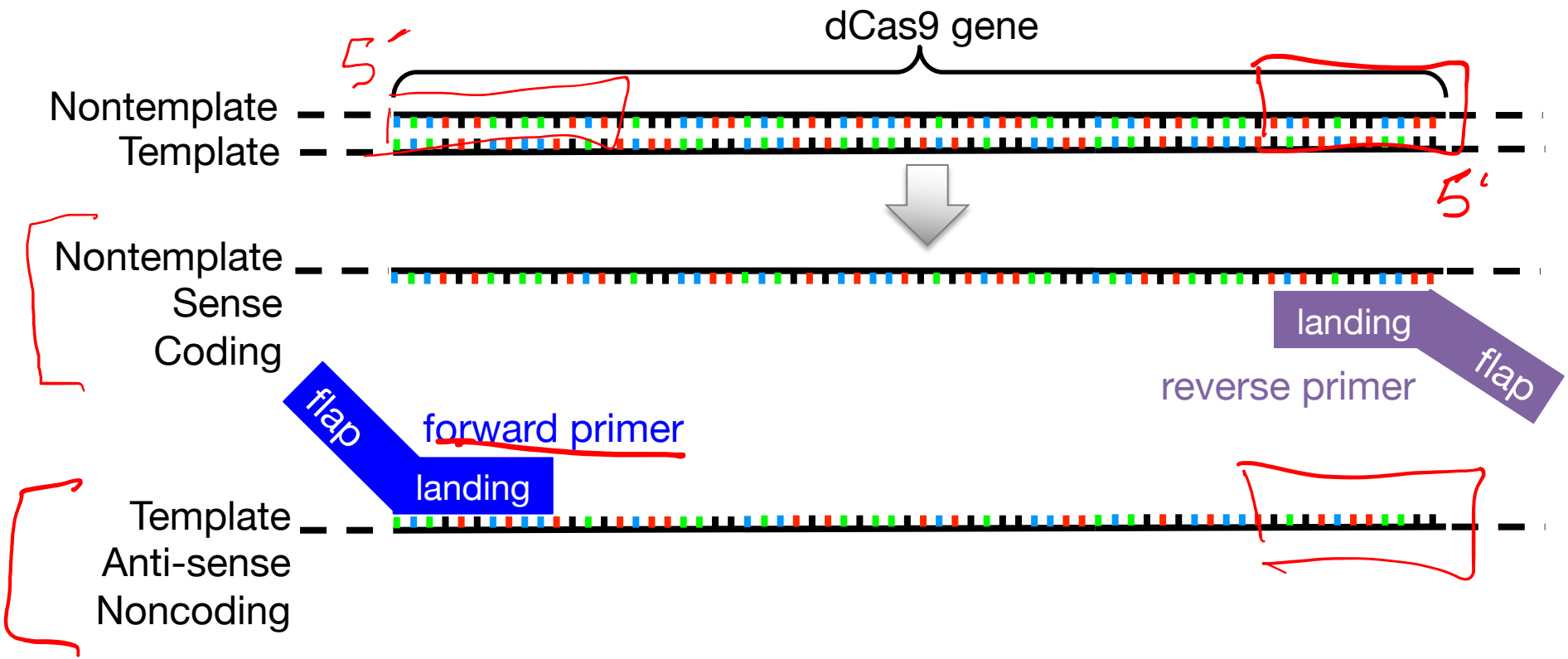


How is DNA engineered?

1. PCR amplification of DNA:
   Primers, Template | GOI
   Nucleotides, DNA polymerase
2. Digestion:
   Restriction Enzymes
   (Endonucleases)
3. Ligation:
   DNA Ligase

1. PCR amplification of DNA

Defining terminology

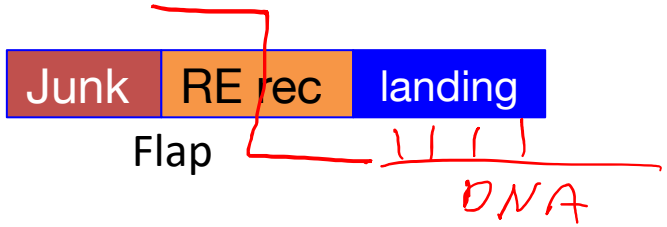


1. PCR amplification of DNA

Designing primers

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

Why junk DNA? to NT



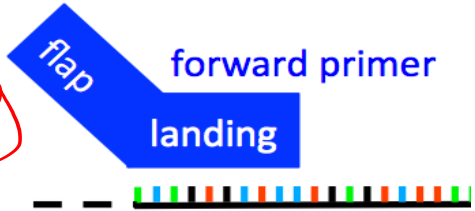
FWD Primer: Same sequence as nontemplate DNA
Binds template

REV Primer: Binds nontemplate DNA
Reverse complement sequence of nontemplate

1. PCR amplification of DNA

Primer design guidelines

specific vs good annealing



- 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 Melting Temp: T_m
- 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)

50% bound to DNA; 50% unbound

1. PCR amplification of DNA

Three major PCR steps—which temperature & why?

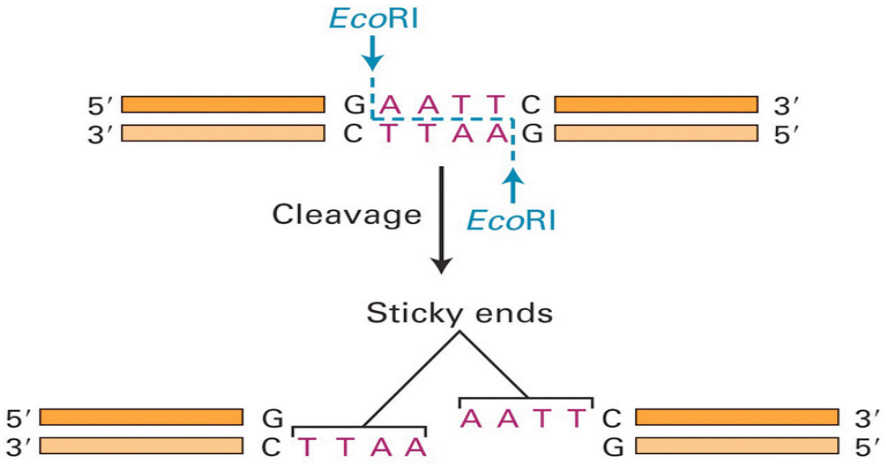
- Melt
  - 95 °C
  - Breaking Hydrogen bonds to Denature DNA
- Anneal
  - T_m(primer) = 50% bound/unbound
  - T_{anneal} ~ T_m(primer) – 5°C More primer on DNA

Δ for each primer

- Extend
  - 72 °C (for Taq) DNA Polymerase
  - 1000 bases/min

2. Digestion

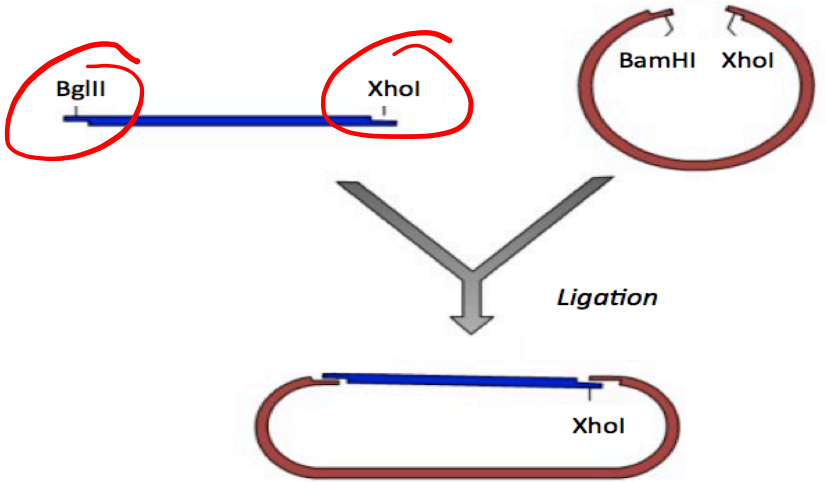
Restriction endonucleases create sticky ends on dCas9 insert and plasmid backbone



2 REs for DNA insert Directionality

3. Ligation

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



Major steps of cloning

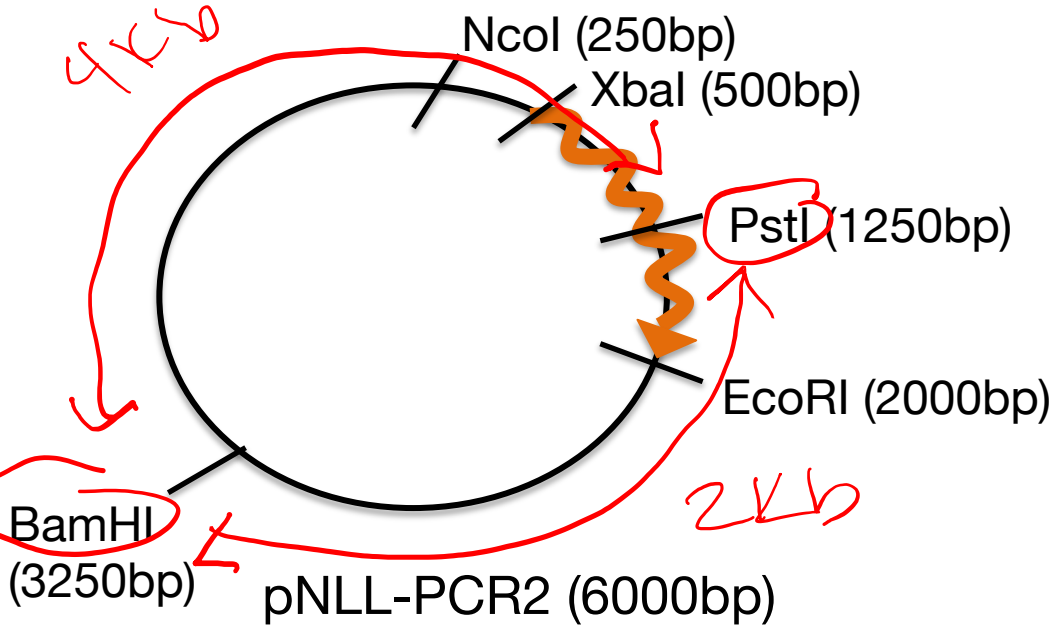
1) PCR amplicon
2) Digest amplicon & backbone w/ RE s
3) Ligation
4) Transform ligation products in competent bacteria
5) Mini Prep DNA from bacteria
6) Diagnostic / confirmation digest

COMPETANT

Confirmation digest considerations

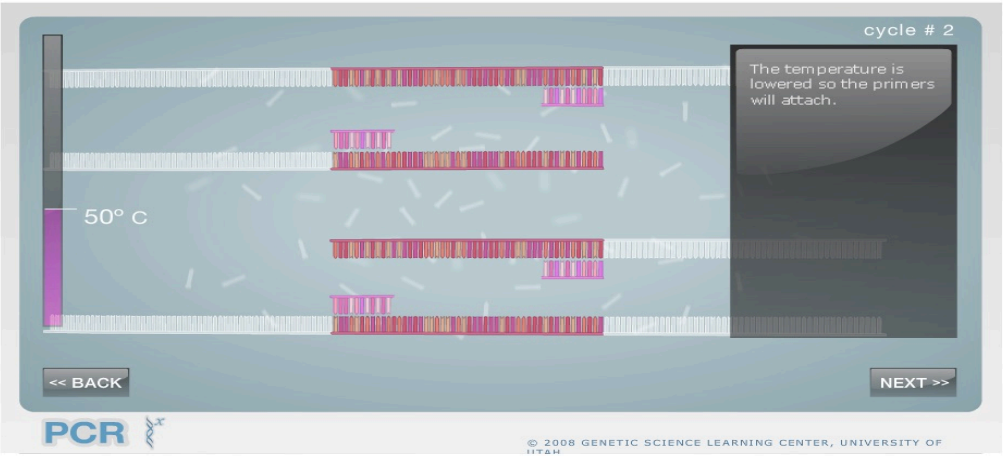
- Do you have access to the enzymes?
- Are the two enzymes compatible?
- Are fragments easily distinguished on an agarose gel?

WEB Double Digest Finder



Leslie's favorite PCR animation

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



- Feel free to watch on your own!
- Also goes through the 3 cycles to product concept that Noreen discussed in lectu

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
- 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 presentation slide

To help you prepare for the Journal Club presentation, you will craft 1-2 slides using the 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.

Regular ppt

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 3pm)
   - Choose restriction enzymes for diagnostic digest
   - Calculate volumes of digest components
   - Set-up digest and leave overnight at 37°C