M2D1: Complete *in silico* cloning of dCas9

10/12/16

1. Pre-lab Discussion
2. Design primers to dCas9
3. *In silico* PCR amplification, digest, and ligation
4. REAL diagnostic digest of pdCas9

(Almost) done with Mod1!

- Mini-presentation
  - due 10pm on Saturday, October 14
  - You don't have to be exhaustive; tell a focused story
- Data summary
  - ✓ draft due 10pm on Wednesday, October 11
  - receive all comments by Monday, October 16th
  - revision due 10pm on Sunday, October 22nd
- Blog post
  - due 10pm on Monday, October 23

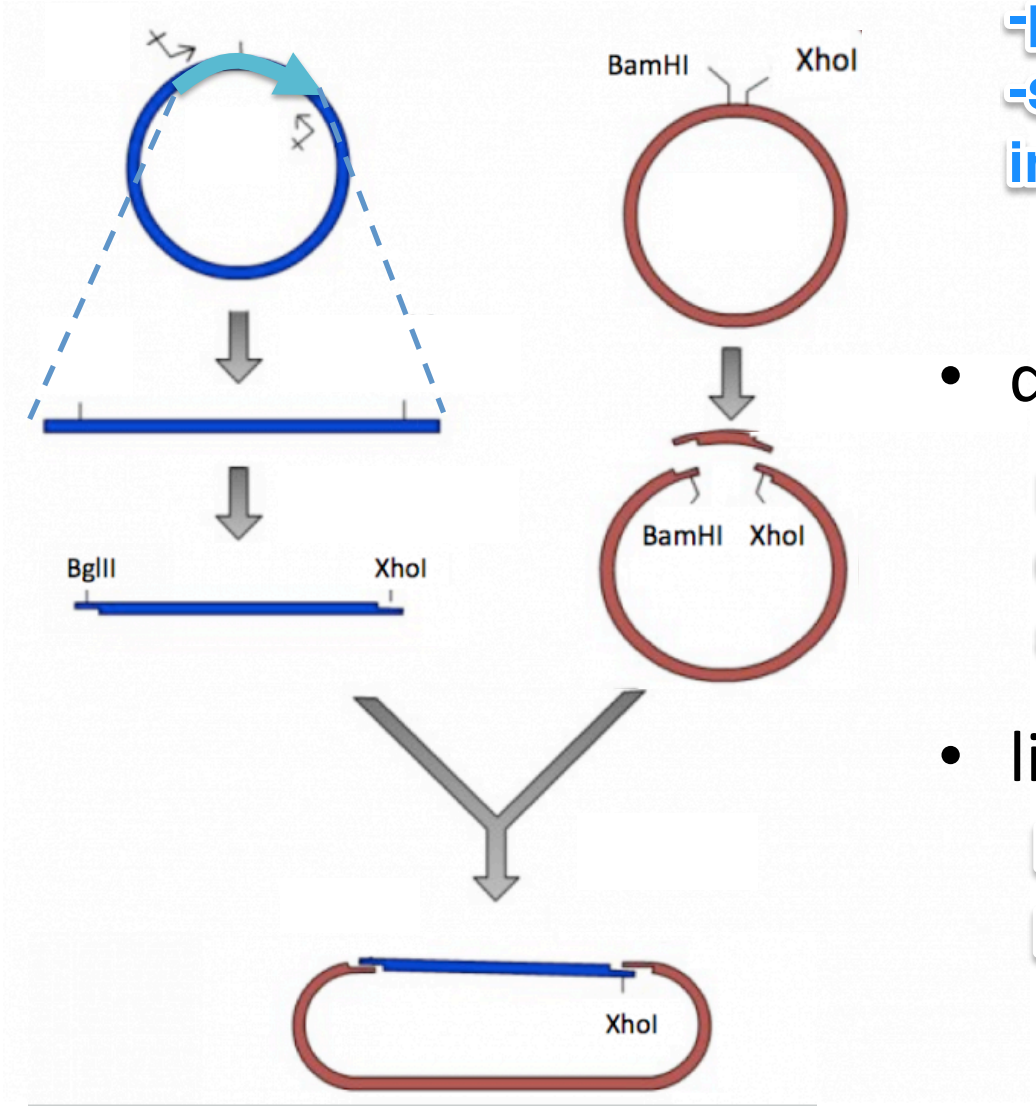
Sign up for journal club (M2D2 homework)

- Pick 1 of 20 papers, or suggest your own
- Present M2D4 (October 24) or M2D6 (October 31)
- Sign up by adding your name next to paper [LM/TR/Rainbow]
  - first come first serve!
  - 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				

"insert"
"fragment"
dCas9

"vector"
"backbone"
$p_L$tetO-1



How is DNA engineered?

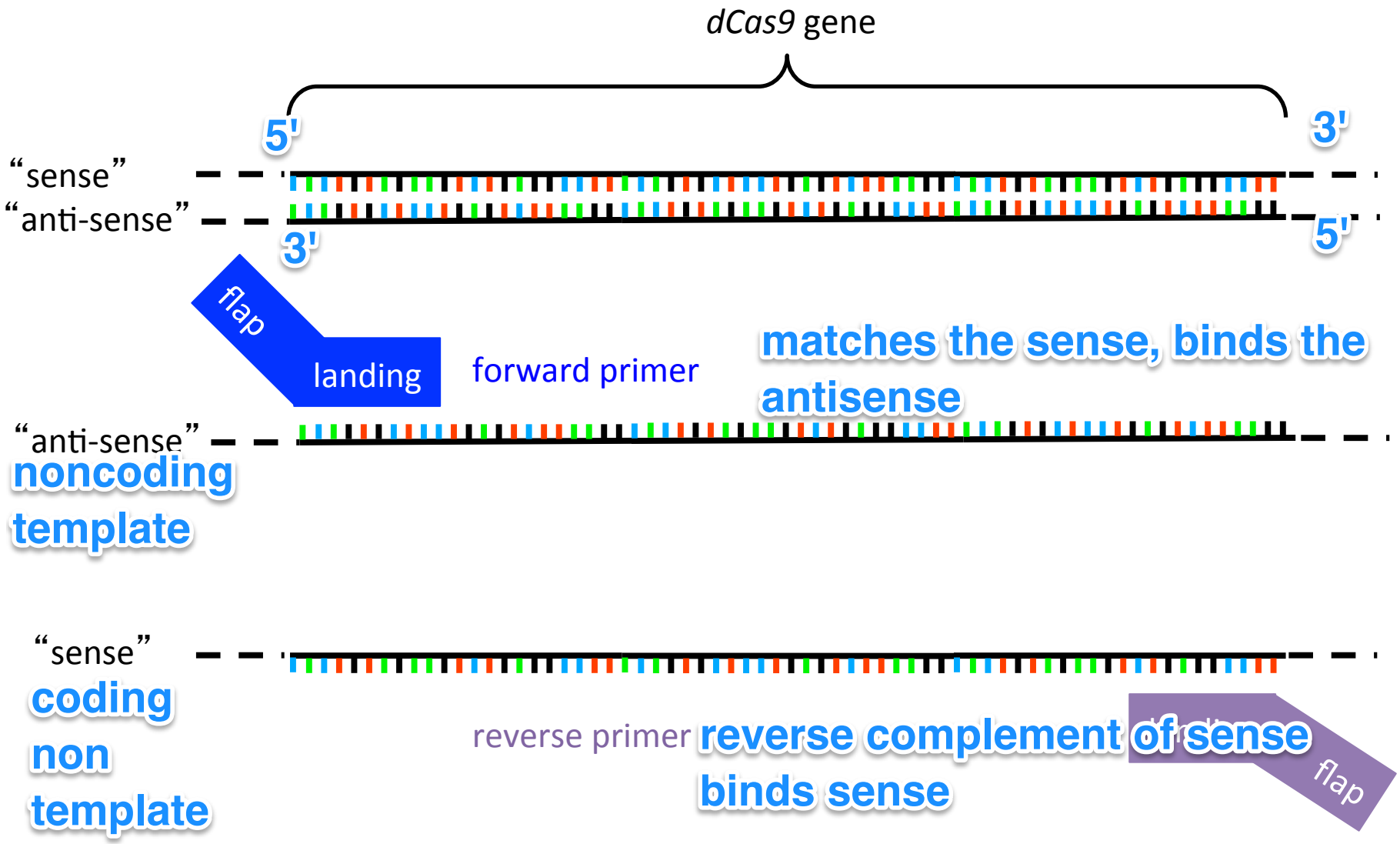
- PCR amplification of DNA:
  -polymerase chain reaction
  -specific primers to the gene of interest

- digestion:
  restriction enzymes
  endonucleases
  sticky ends (or blunt ends)

- ligation:
  ligase: seal backbone, phosphodiester bond

How do you design primers?

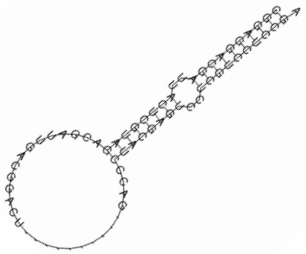
all primers 5' to 3'



Primer design guidelines

- Length
  - 17-28 base pairs
  - long enough to be specific
  - short enough so Tm no so high
- GC content
  - 40-60%
  - GC clamp at ends

    AT=2, GC=3; GC more stable
- T_m(primer)
  - < 65 °C
- Avoid Secondary structures

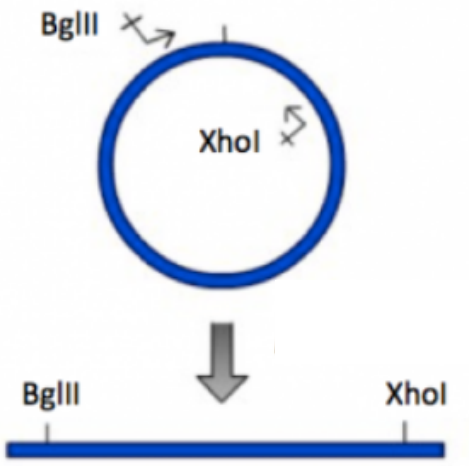


  - hairpins
  - complementation w/i primer sequence
- Avoid repetitive sequences
  - di-nucleotides > 4 (ex. ATATAT)
  - runs > 4 bp (ex. GATGGGGG)

Using PCR to generate *dCas9* flanked by restriction enzyme recognition sites

3 major steps in the PCR cycle: which temperature and why?

- Melt
  - 95 °C
  - dsDNA denatures
- Anneal
  - T_m(primer) = 1/2 primers annealed to target
  - T_{anneal} ~ T_m(primer) – 5°C
- Extend
  - 72°C (for Taq)
  - 1 min / 1000 bases

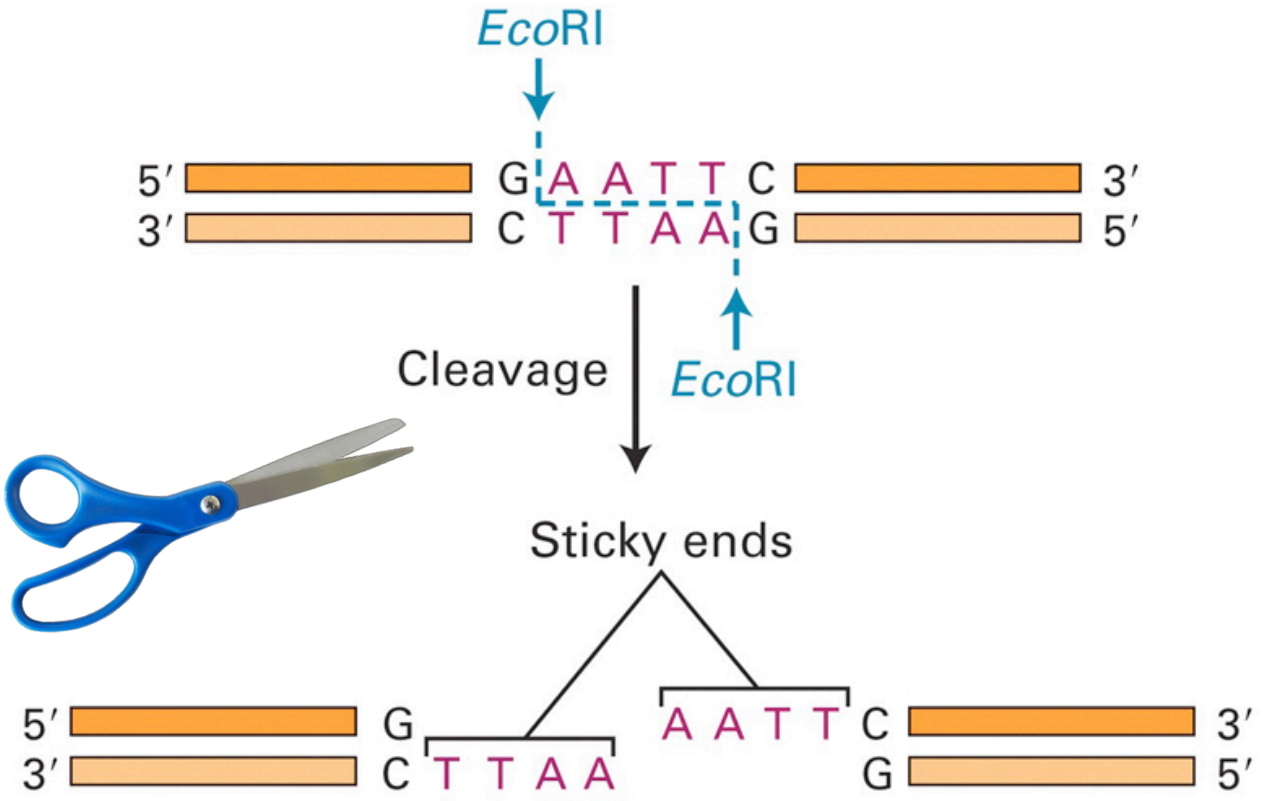


- Template DNA
  - dCas9 gene
- Primers
  - specific match to dCas9
  - option to add bases, *e.g.* endonuclease recognition sequence

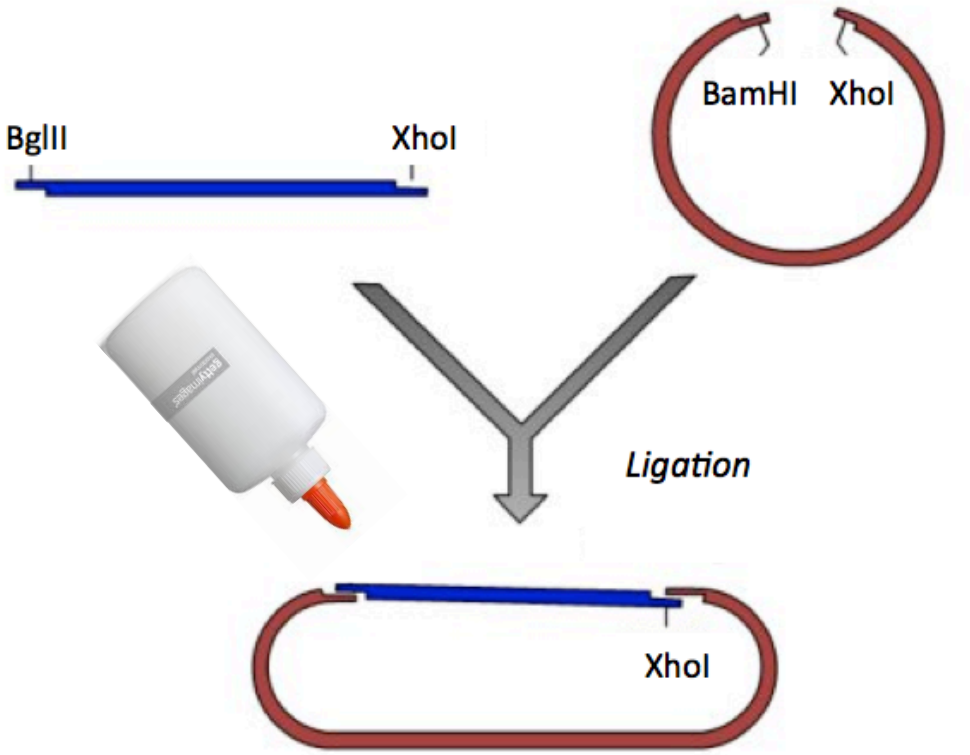
My favorite PCR animation..

learn.genetics.utah.edu

Digestion: dCas9 insert and plasmid backbone

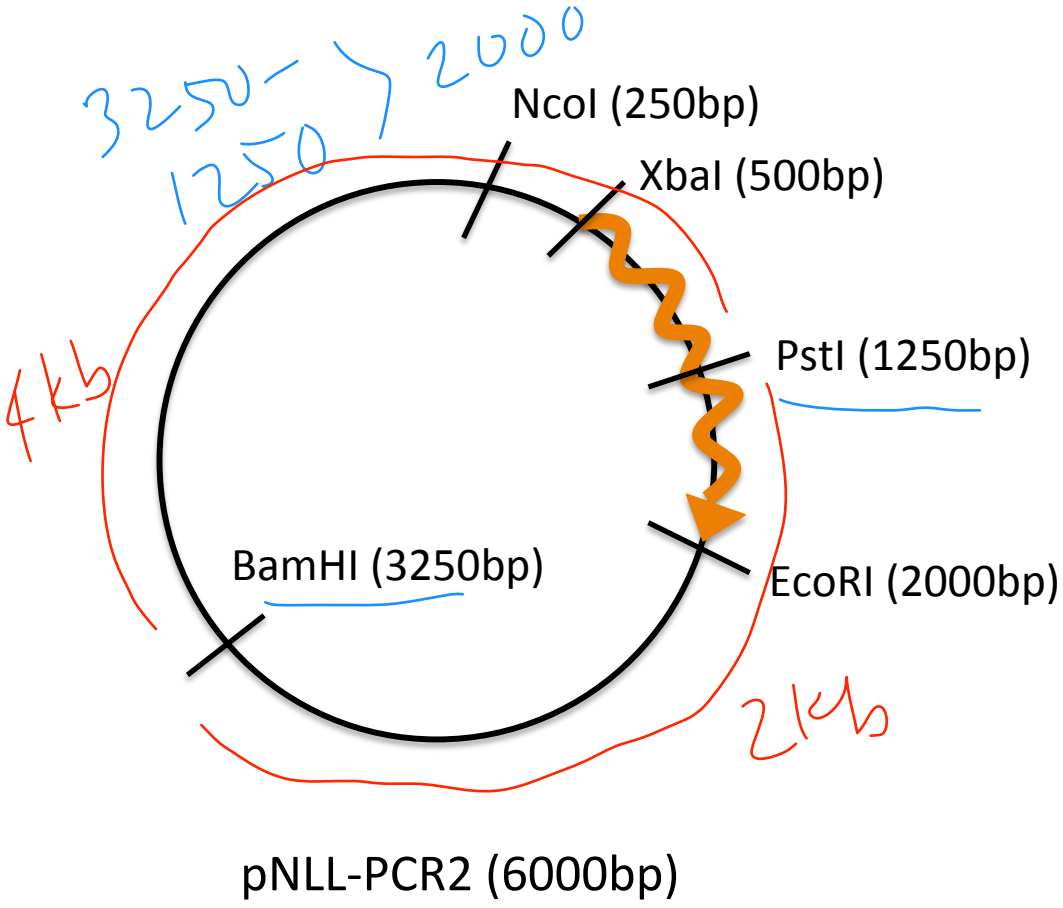


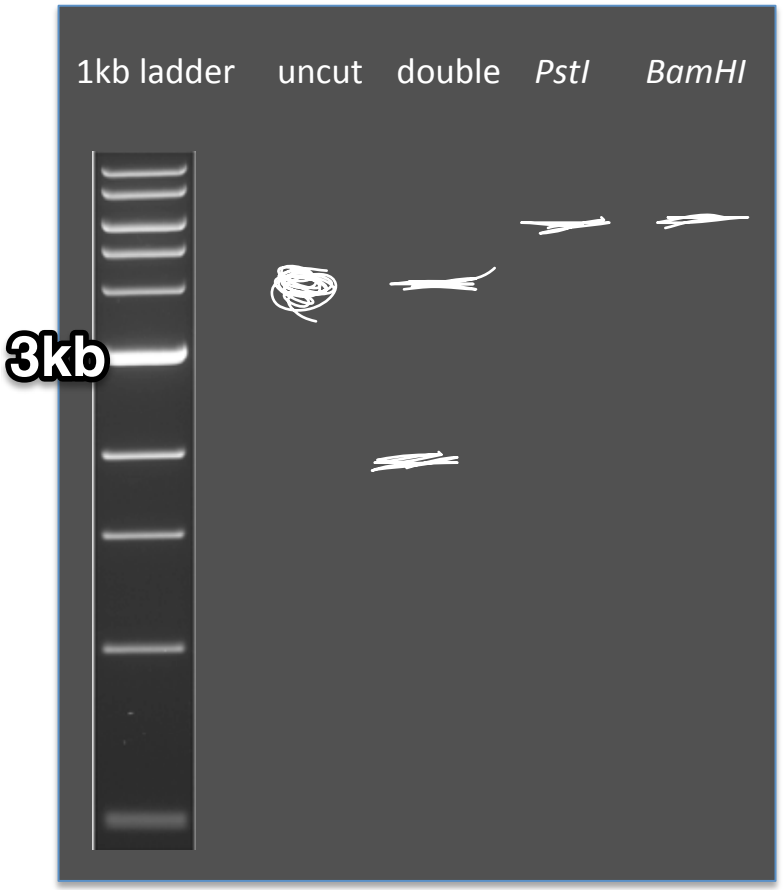
Ligation: dCas9 insert and plasmid backbone after digestion



Confirmation digest considerations

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





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 ** at 2:30pm we will all work through this calculation together
2. Set up confirmation digests of pdCas9 for agarose gel electrophoresis
   - choose restriction enzymes for diagnostic digest
   - calculate volumes of digest components
   - “set-up” digest and leave overnight at 37°C