M2D1: Complete *in silico* cloning of dCas9

10/13/16

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

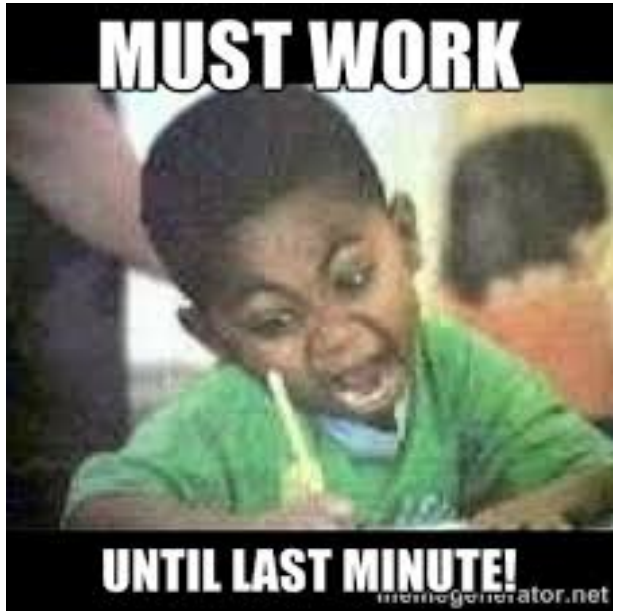
(Almost) done with M1!

- Mini-presentation
  - due 10pm on Saturday, October 15
  - You don't have to be exhaustive; tell a story
- Data summary
  - draft due 5pm on Wednesday, October 12
  - receive all comments by Wednesday, October 19th
  - revision due 5pm on Monday, October 24
- Blog post
  - due 5pm on Tuesday, October 25

Reflection assignments

- **Due dates**
  - Mod1:October 25 at 5 pm
  - Mod2: November 20 at 5 pm
  - Mod3:December 7 at 10 pm
  - Final blog: December 14 at 10 pm
- There are suggested blog topics but feel free to be creative.
- A few additional notes:
  - Do not publish MIT logo
  - Do not post photographs with names tagged
  - Do not write malicious comments
  - Do not plagiarize

“A few summers ago I had a job working in a marine biology lab. It turns out I don't like marine biology that much, but one thing that stuck with me about the experience was a phrase [my mentor used] ... ‘However long you think it's going to take, double it and add two.’ ... Human beings are amazing at overestimating their own competence. Whether it's how long an group meeting will take, or how many hours you will spend studying for an exam, or how many months it will take to finish your UROP project, or how much time you need to finish your Mod2 report, however long you think it's going to take: double it and add two.” -Former 20.109er via our class blog

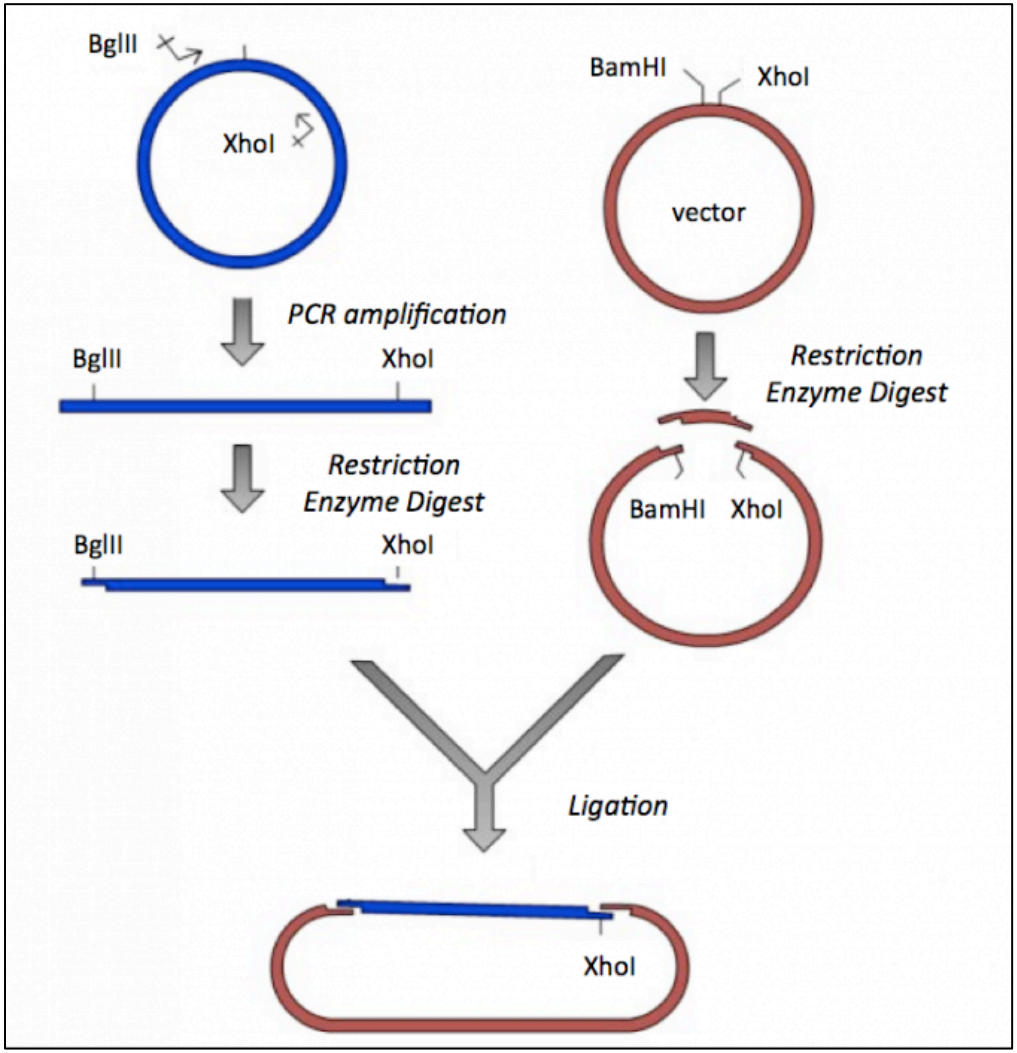


Sign up for journal club (M2D2 homework)

- Pick 1 of 20 papers, or suggest your own
- Present M2D4 (October 25) or M2D6 (November 1)
- 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				

How do we engineer DNA?



1. Amplify DNA

2. Digest: sticky ends, compatible ends

Mix together amplified DNA and expression vector

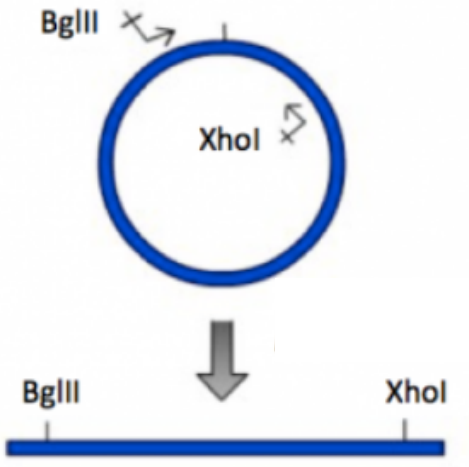
3. ligation: sealing backbone of DNA

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
  - break hydrogen bonds
- Anneal
  - T_m(primer) = depends on primer sequence
  - T_{anneal} ~ T_m(primer) – 5°C
- Extend
  - 72 °C (for Taq)
  - 1 min / 1000 bp

specific to our gene

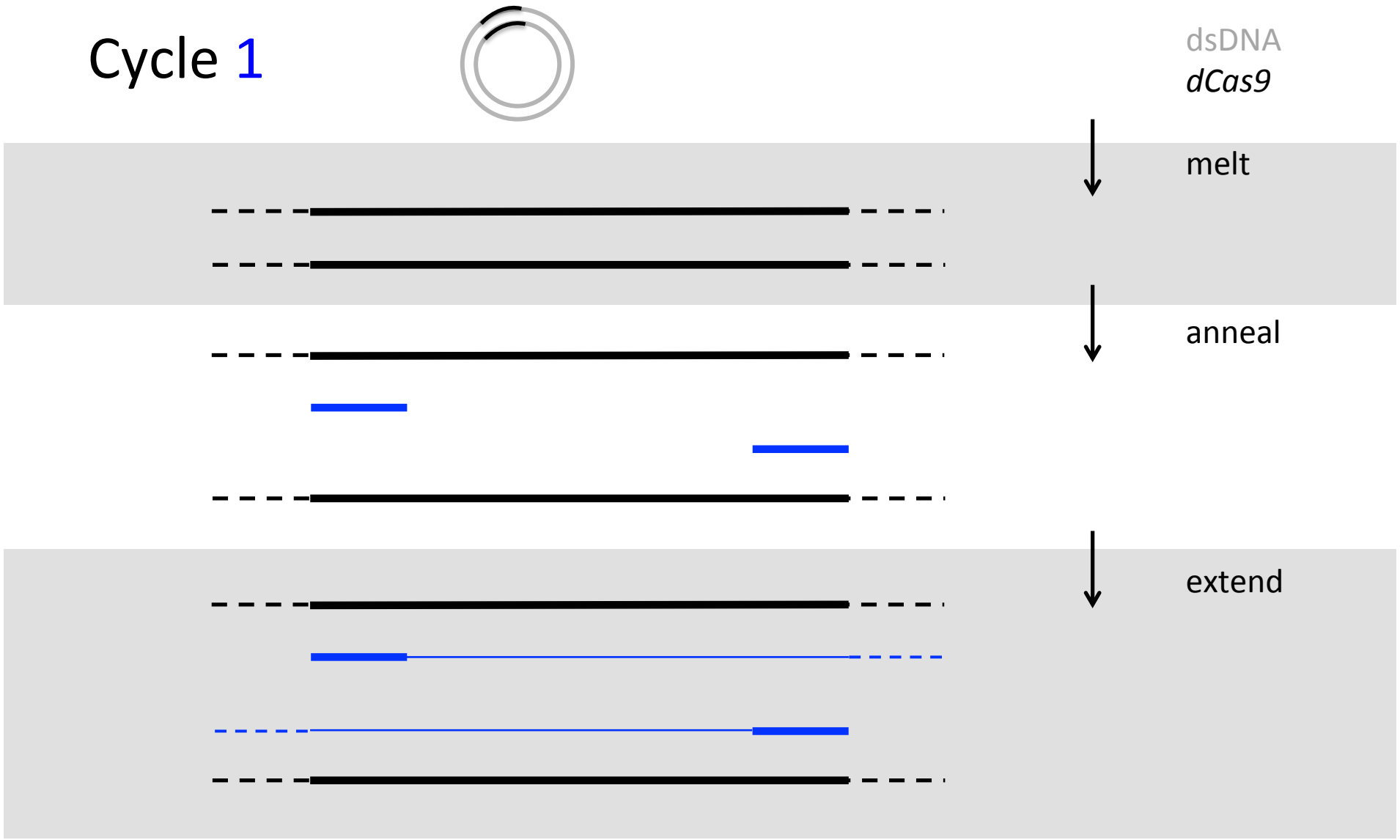


- Template DNA
  - dCas9 gene
- Primers
  - specific match to dCas9
  - option to add bases, *e.g.* endonuclease recognition sequence

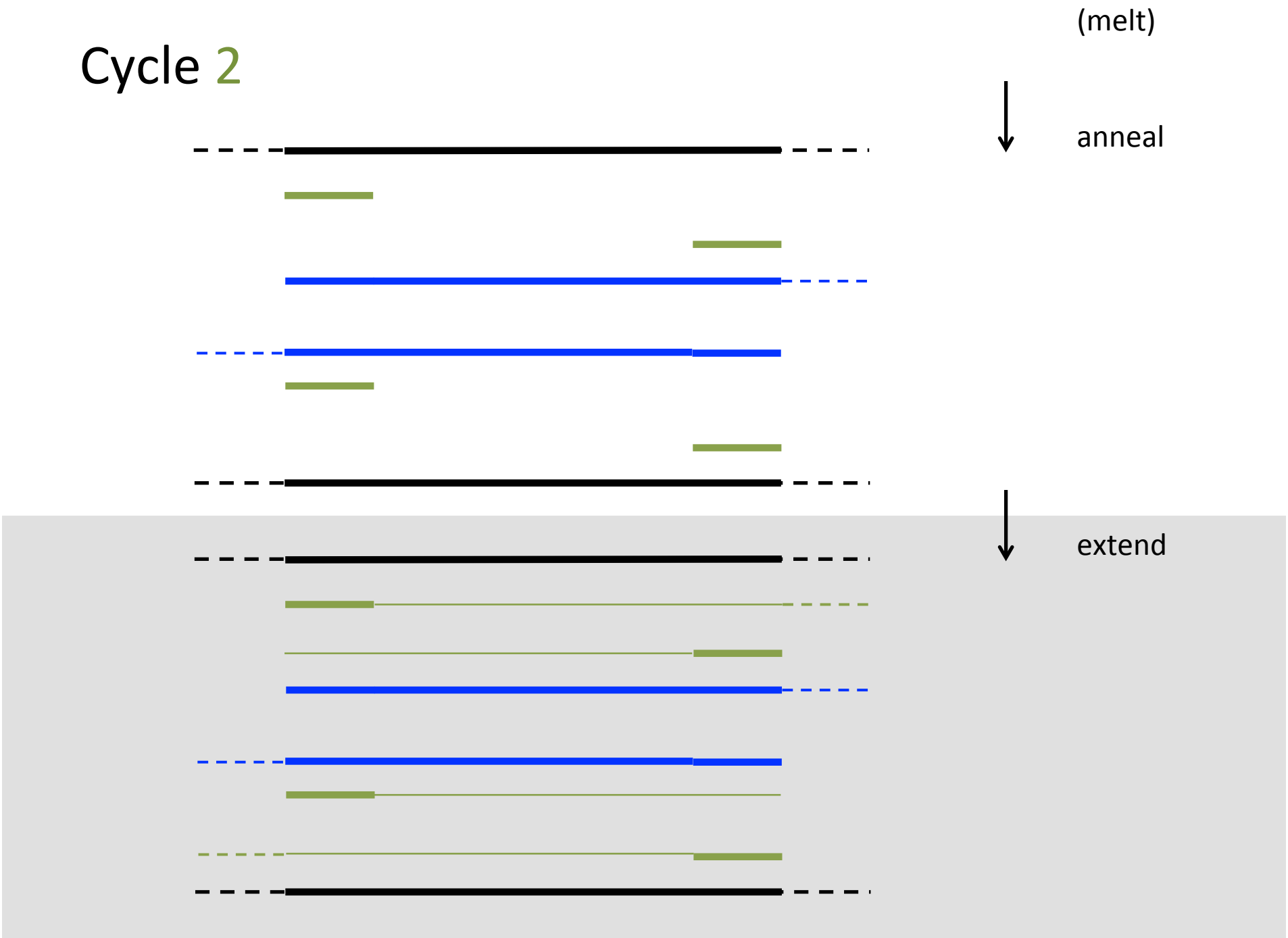
My favorite PCR animation..

learn.genetics.utah.edu

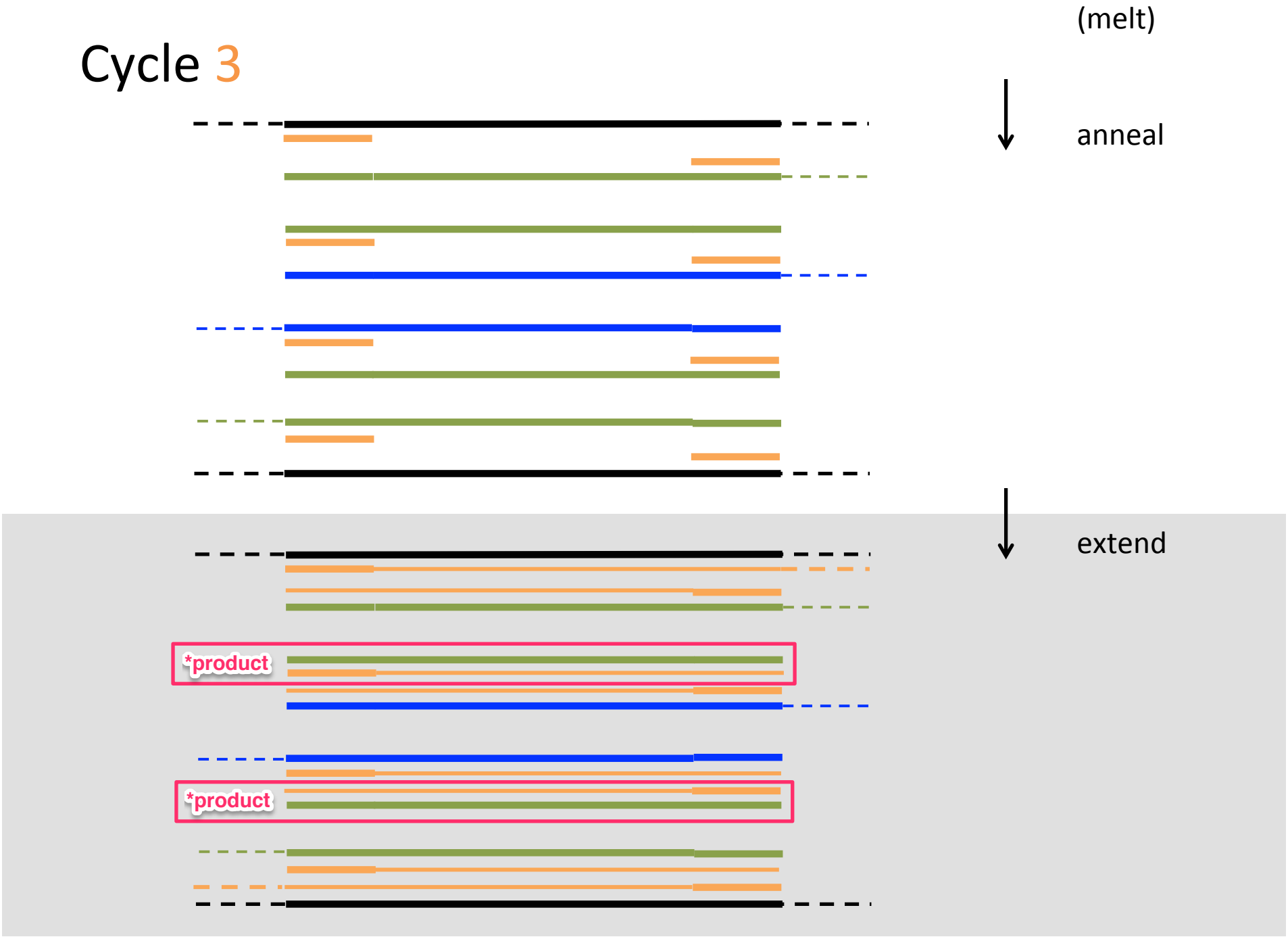
How many PCR cycles until only your amplicon?



Cycle 2

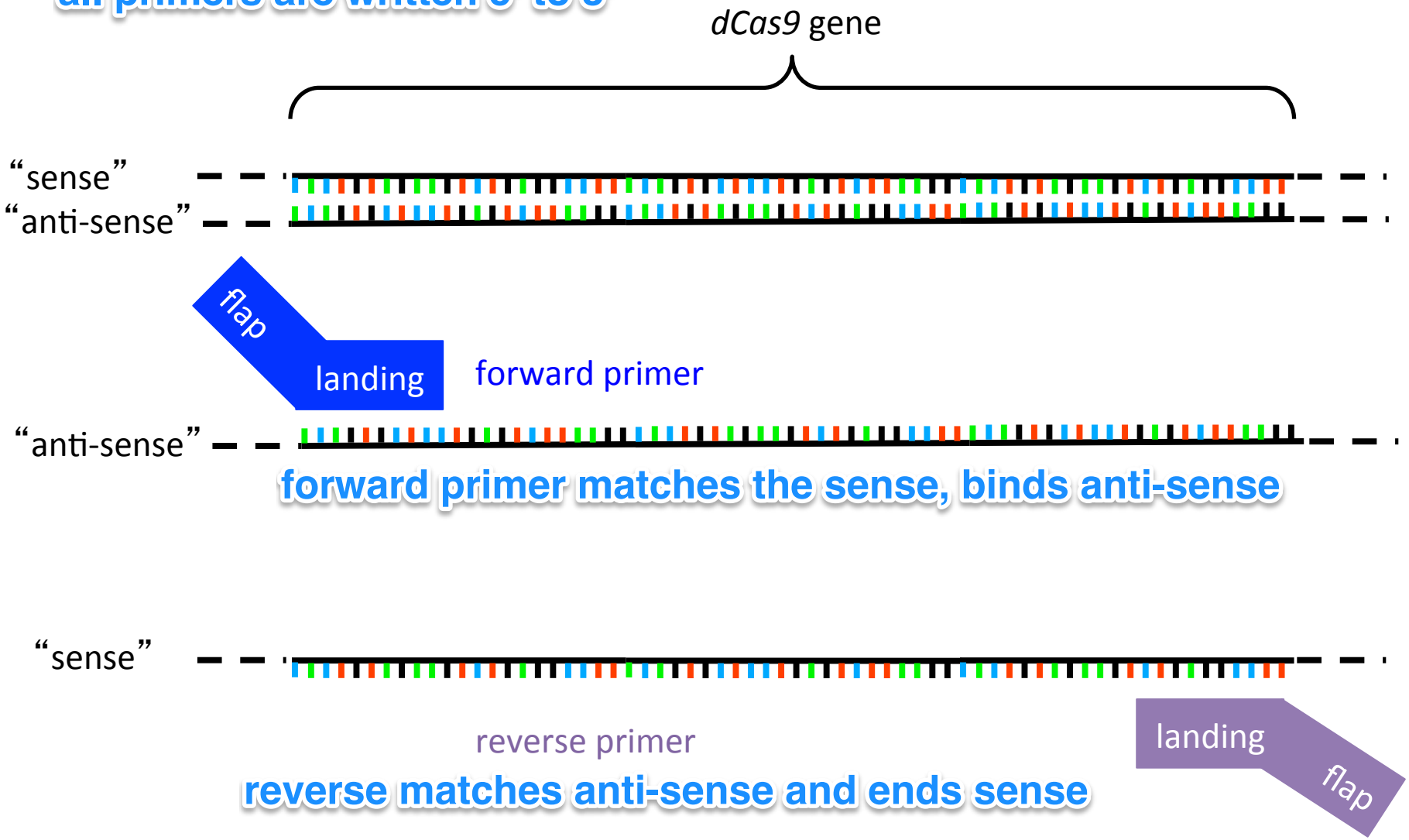


Cycle 3



How do you design primers?

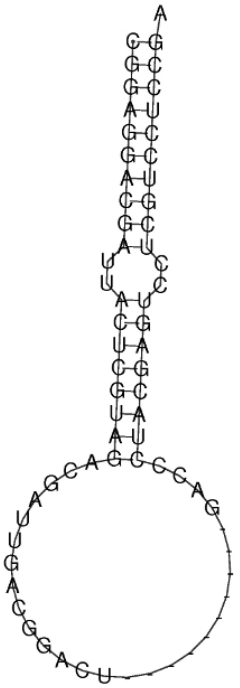
all primers are written 5' to 3'



Primer design guidelines

- Length
  - 17-28 base pairs
  - **adequate specificity and easy annealing**
- GC content
  - 40-60%
  - GC clamp at ends
- T_m(primer)
  - < 65 °C

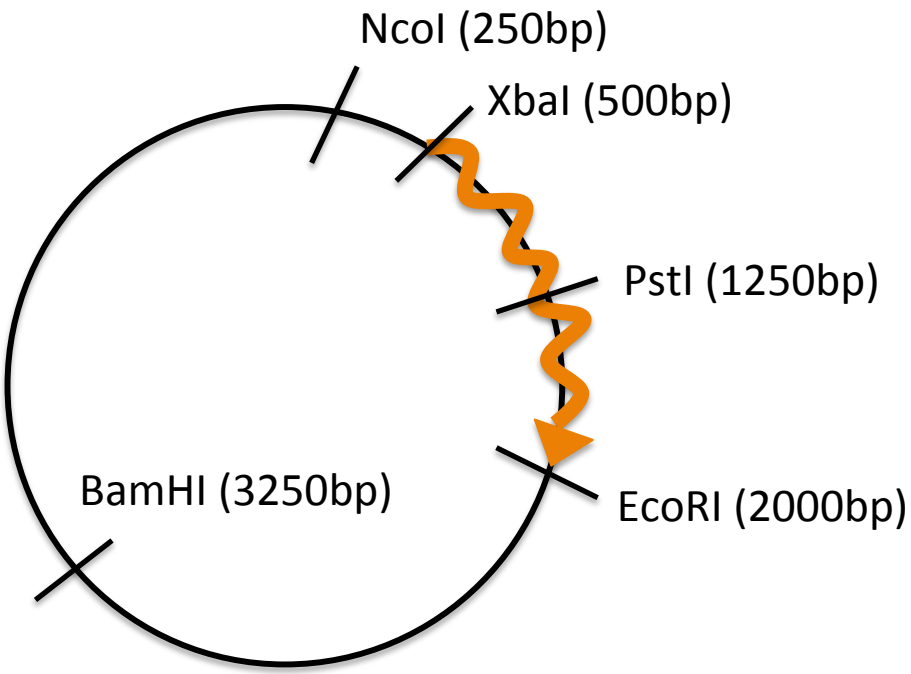
image from Primer Prospector Overview Tutorial



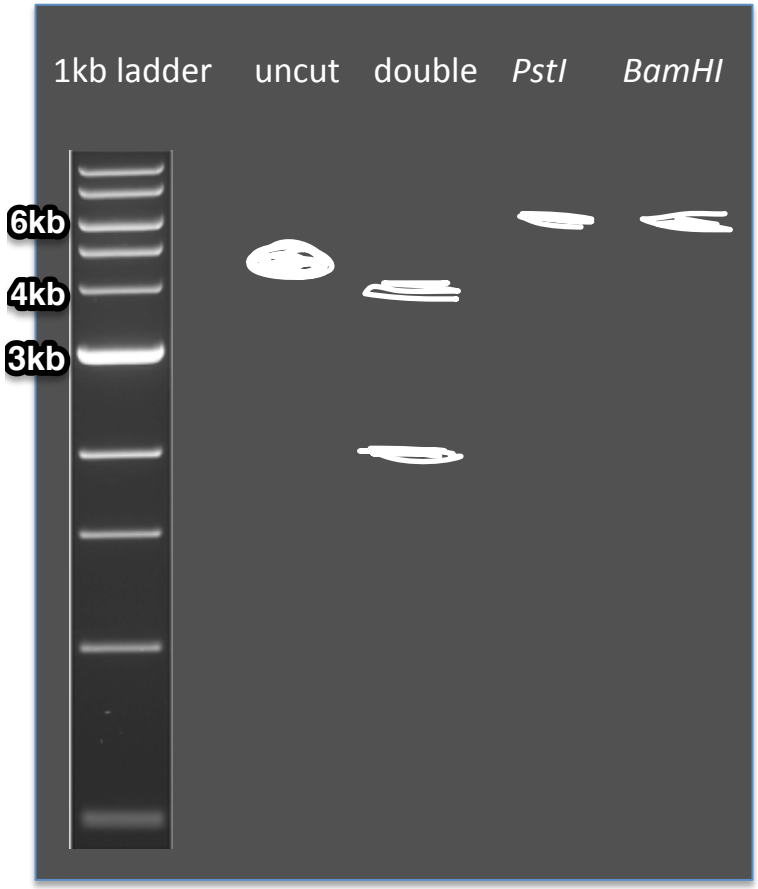
- Secondary structure
  - hairpins
  - complementation
- Repetitive sequence
  - di-nucleotides < 4
  - runs < 4 bp

Confirmation digest considerations

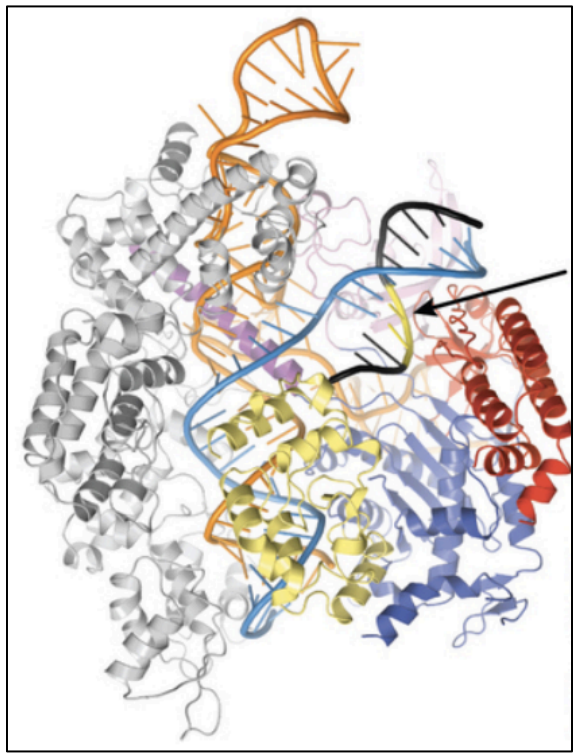
- Do you have access to the enzymes?
- Are the two enzymes compatible?
- Are fragments easily distinguished on an agarose gel?



pNLL-PCR2 (6000bp)



Today in lab



- Reproduce *in silico* (in Benchling) the cloning of pdCas9 *
  - PCR amplification of dCas9 insert
  - digestion of vector by restriction enzymes
  - ligation of insert and vector
- Set up confirmation digests for agarose gel electrophoresis
  - pdCas9 cut by restriction enzymes

* Cas9–sgRNA–DNA complex from *Nature* **513**, 569–573 (2014)