M1D1:
DNA engineering using PCR

9/15/15

Lab business

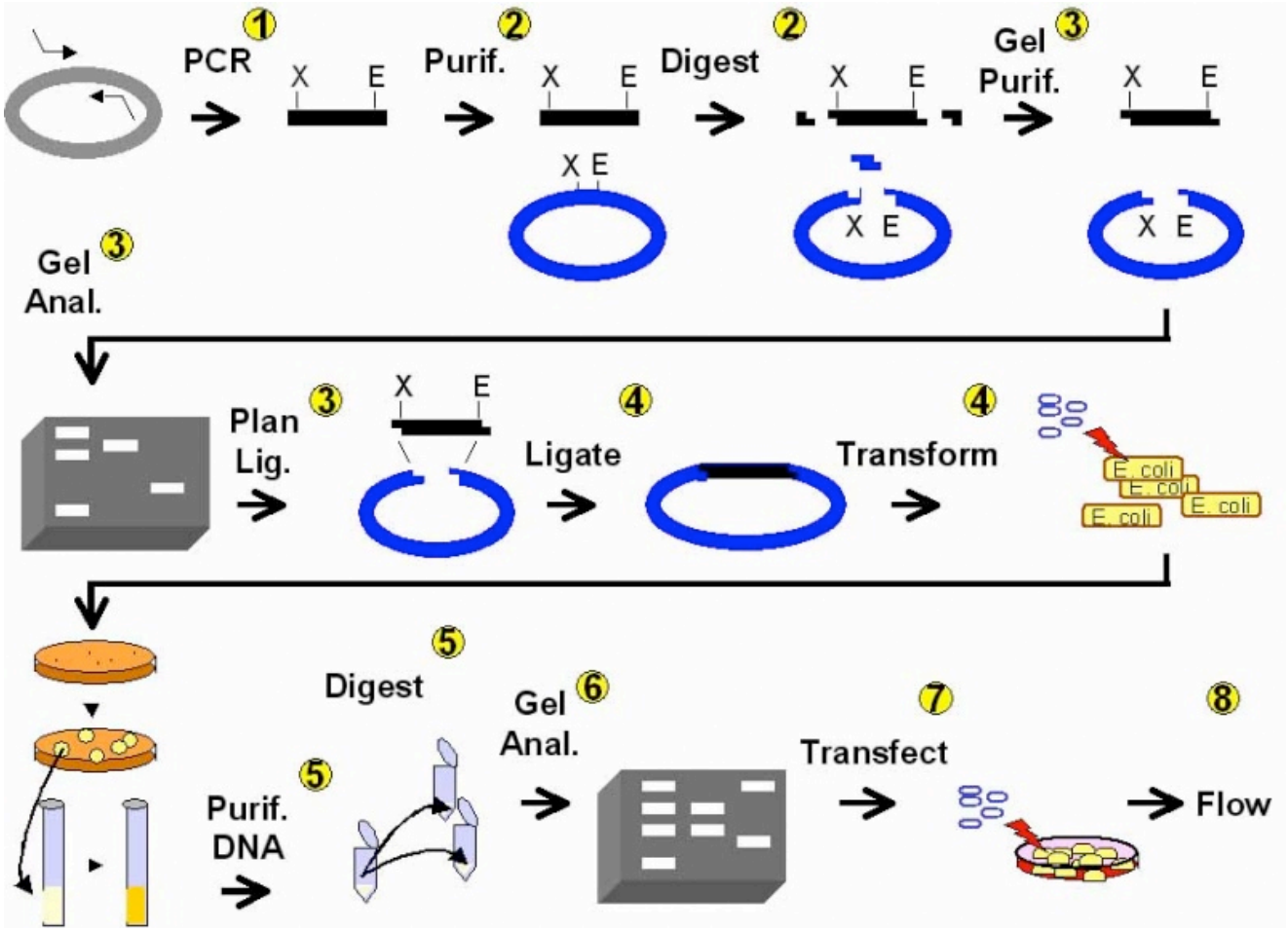
1. Lab treat…

2. Prelab discussion
   - More course details
   - Primer design
   - PCR
3. First official notebook entry

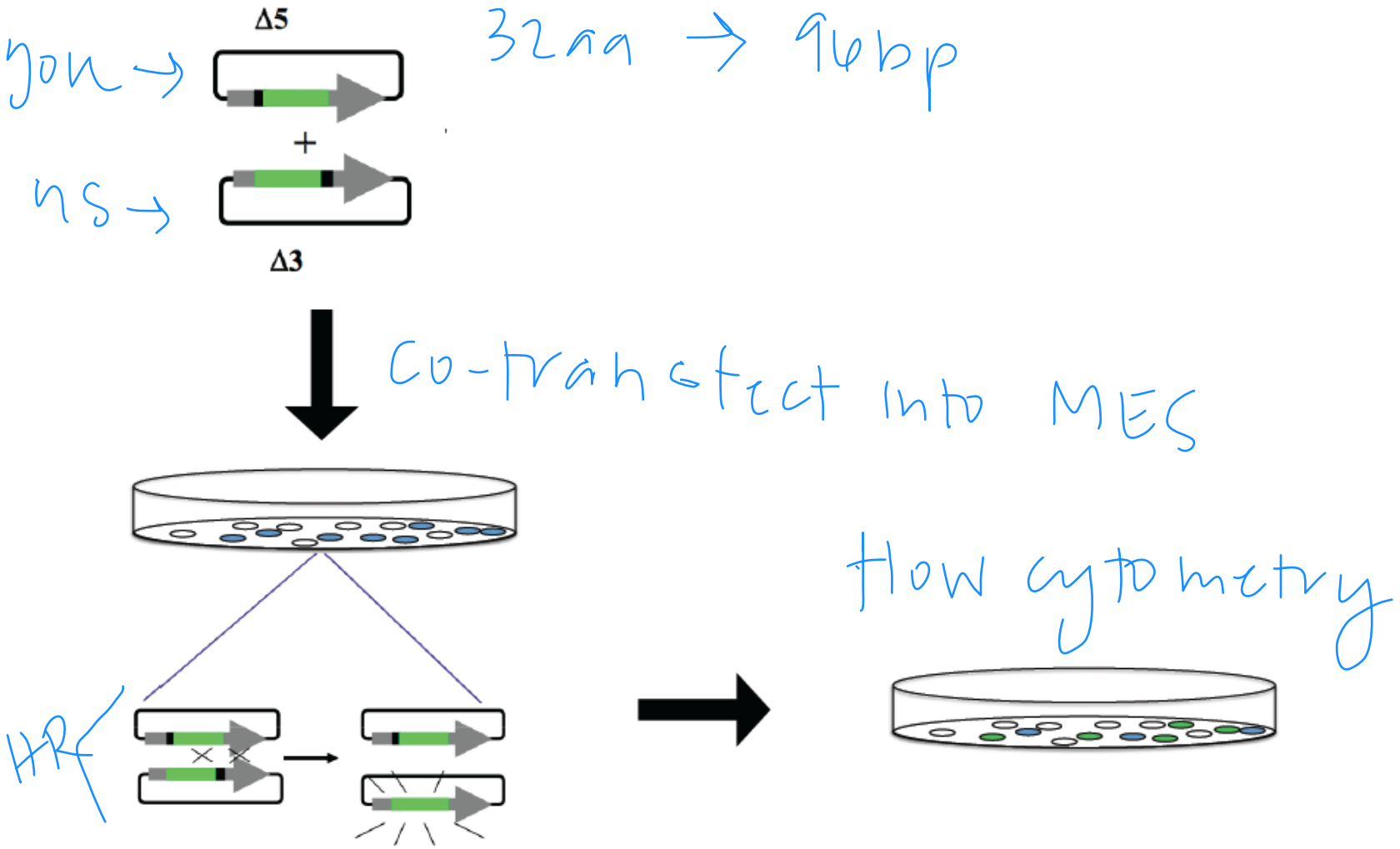
Office hours

- Noreen in 16-317
  - Monday and Wednesday 1-3p
- Maxine in 16-239
  - Thursdays and Fridays 10-11a
- Leslie in 16-429b
  - Monday 12-2p and Tuesday 4-5p
- By appointment
  - Send an email with your availability

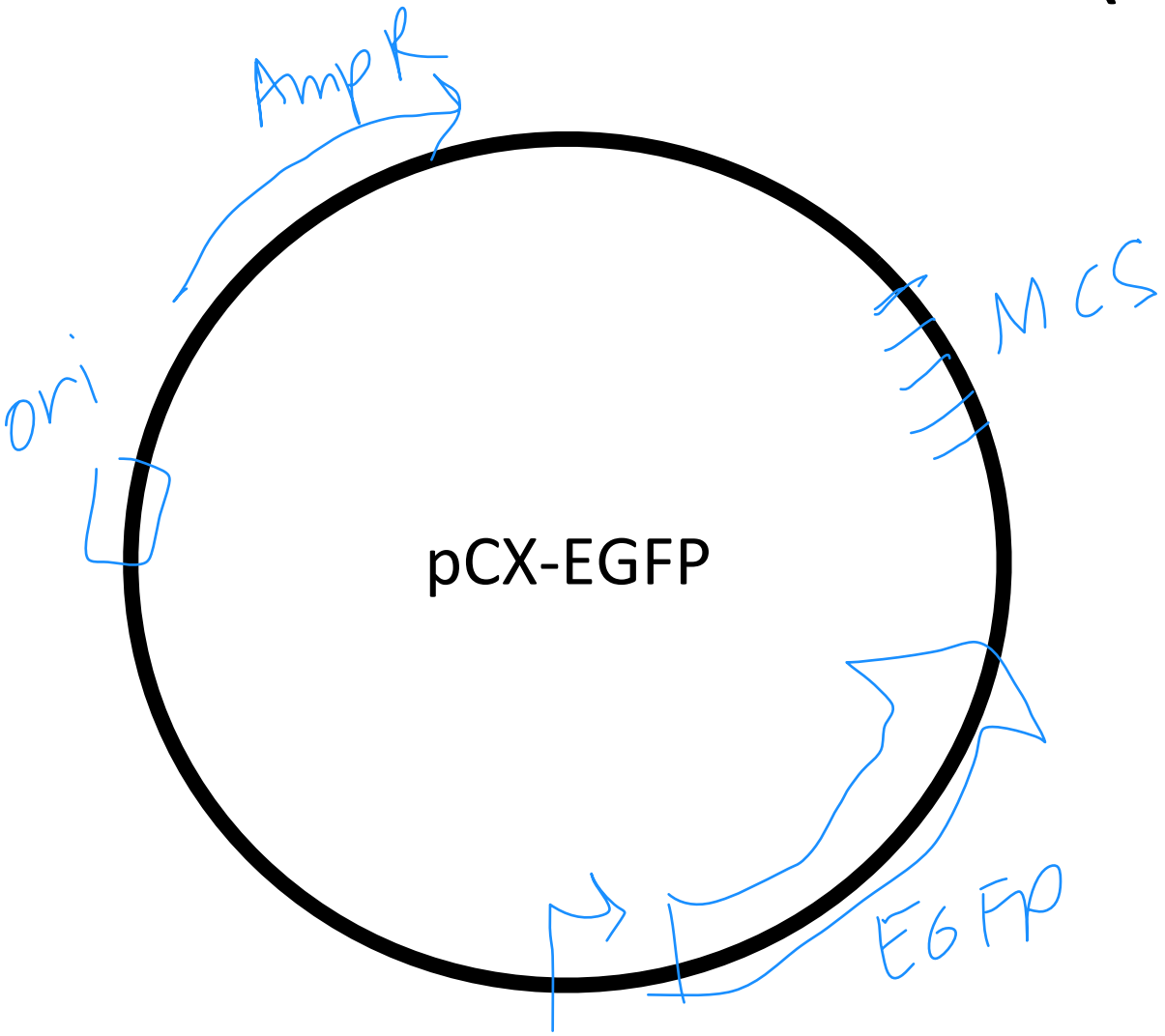
Mod 1 overview

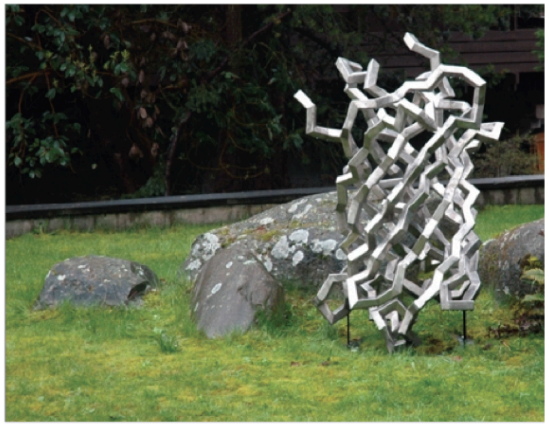


Building an HR sensor



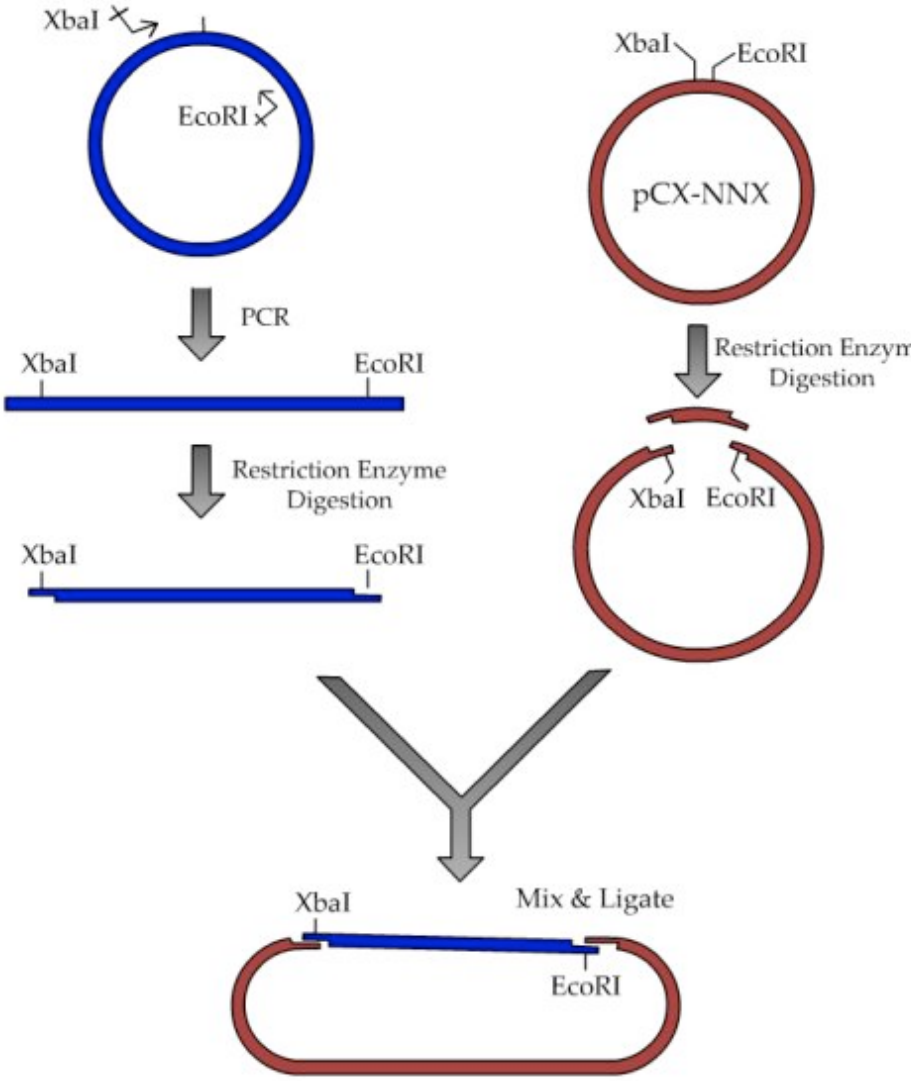
A closer look at (E)GFP





Julian Voss-Andreae
Steel Jellyfish (Green Fluorescent Protein), 2006
Stainless steel, 4' x 3' x 3' (1.20 x 0.90 x 0.90 m)
Location: Friday Harbor Laboratories (San Juan Island, WA)

How do we engineer DNA?



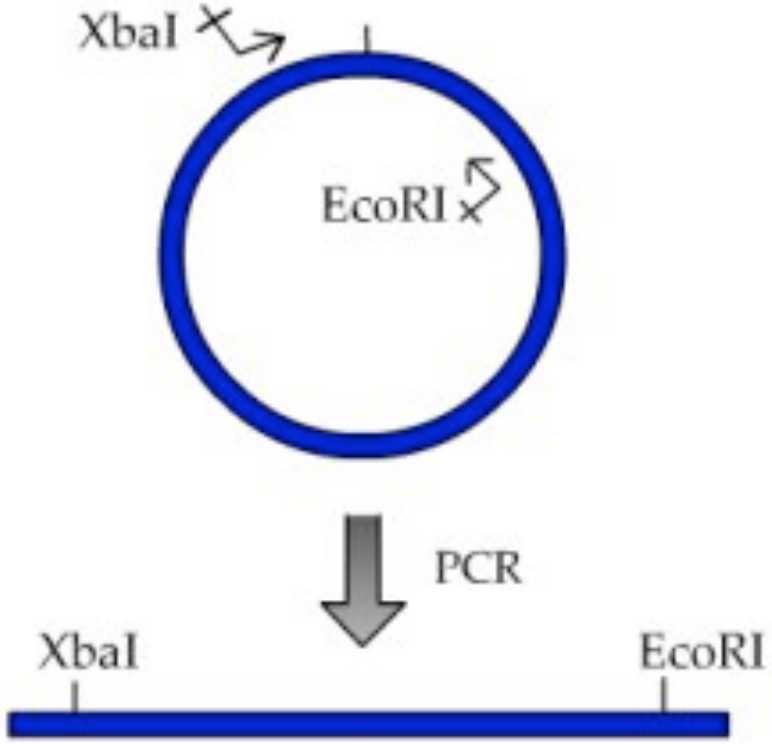
- PCR amplification

deletions in genes
insertion
mutations

- Restriction enzymes

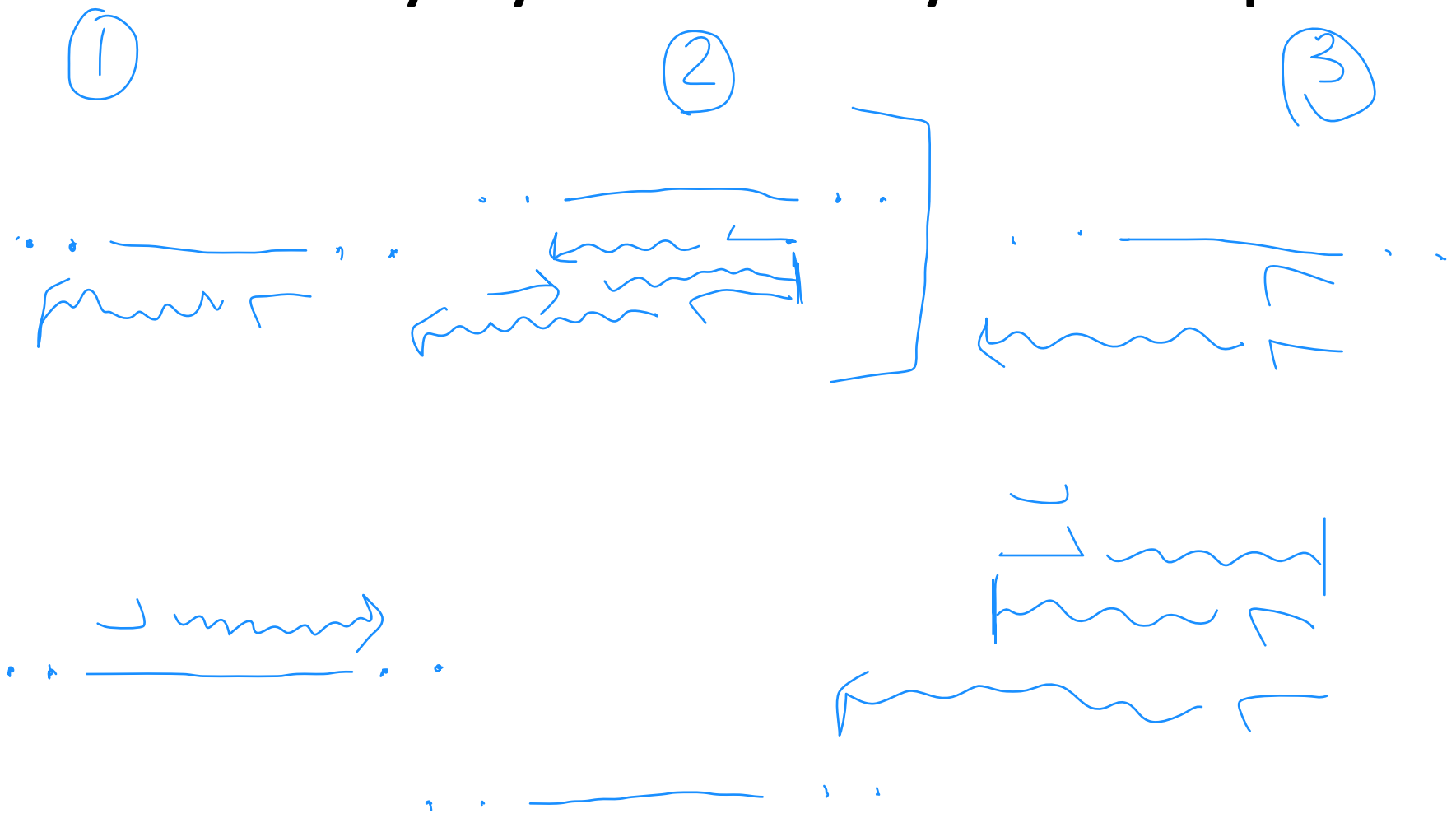
construction

Using PCR to generate Δ5 EGFP

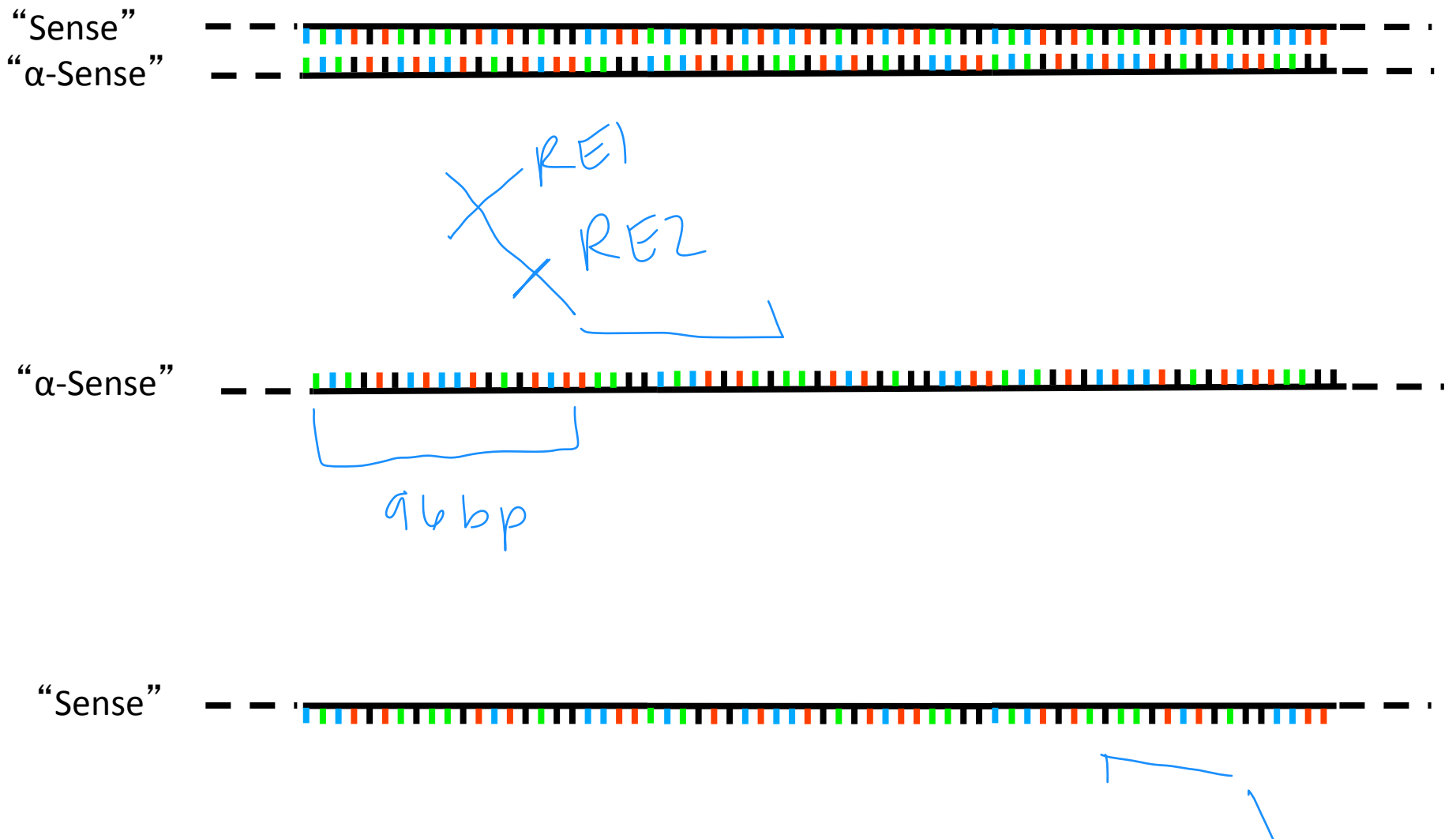


- Melt
- Anneal
- Extend

How many cycles until your amplicon?



Primer design overview



Primer design guidelines

1. Length 17-28bp
2. GC content 50-60% < Secondary melting
3. Secondary structure avoid hairpins 
4. Complementation primers dimers 
5. Repetitive sequence AAAA

PCR setup

Component	Purpose
template	EGFP
primers	Specificity
Master Mix	polymerase (Taq)
	dNTPs
	buffers, Mg^{2+}

What should be in your notebook?

- Copy/paste protocol from wiki
  - Add observations, measurements, analysis
- Work together to copy/modify protocols from wiki
- Front/back matter must be completed individually
- See the wiki for further details
  - Assignments tab

Today

- Design your PCR primers
  - Include primer sequence in your notebook!
- Prepare PCR
  - Include amounts in your notebook!
- Networking event
  - Thursday, September 24 at 5:30-7p
  - First floor lobby of the Koch