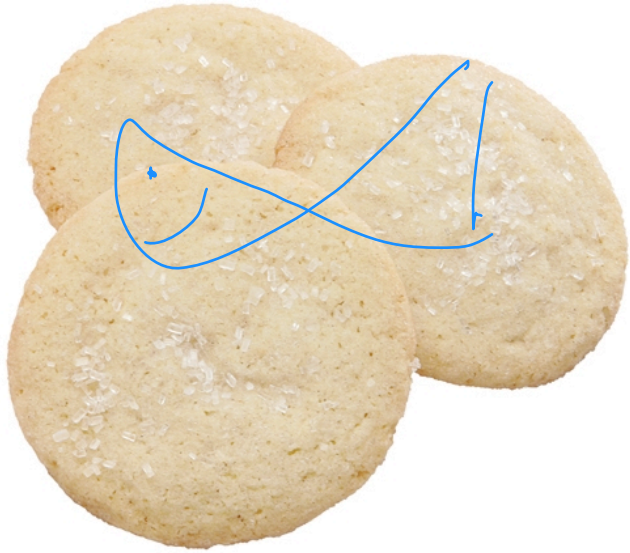
M1D1:
DNA engineering using PCR

9/16/15

Lab business

1. Lab treat…

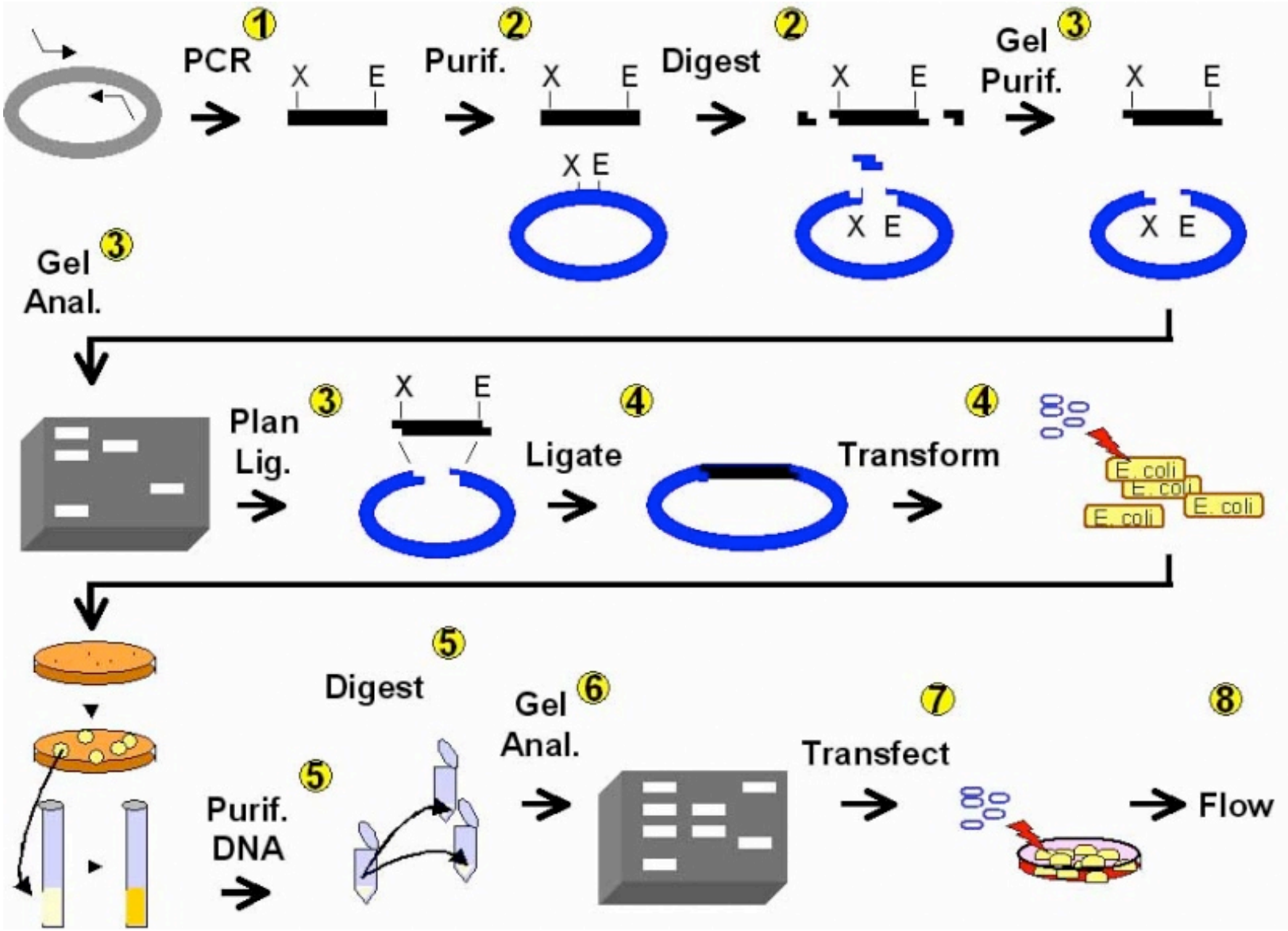


2. Prelab discussion
   - More course details
   - Primer design
   - PCR
3. First official notebook entry

Office hours

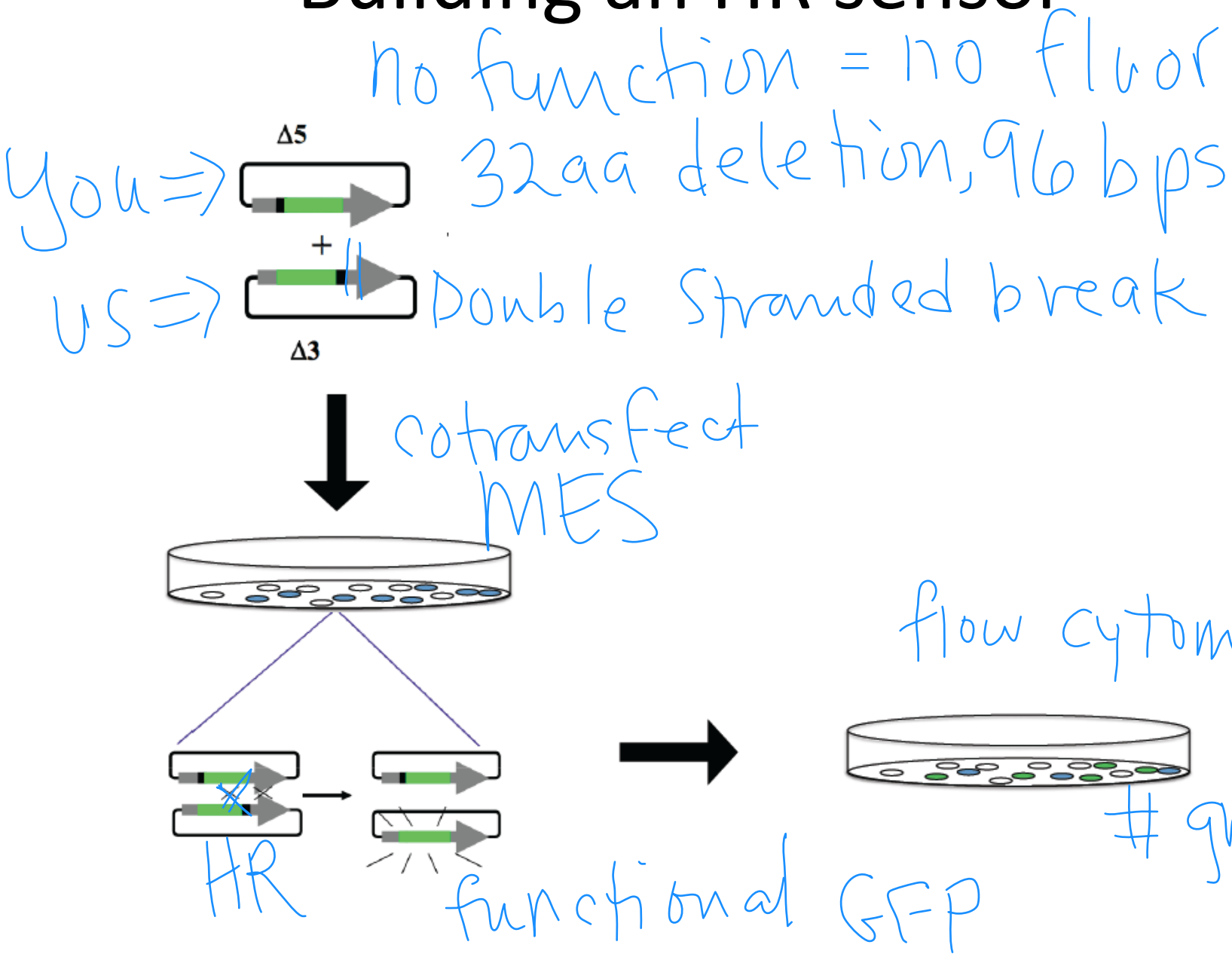
- Noreen in 16-317
  - Monday and Wednesday 1-3p
- Maxine in 16-239
  - Thursdays and Fridays 10-11a
- Leslie in 16-429b
  - Monday 12-2p and Tuesday 4-5p
- By appointment
  - Send an email with your availability

Mod 1 overview

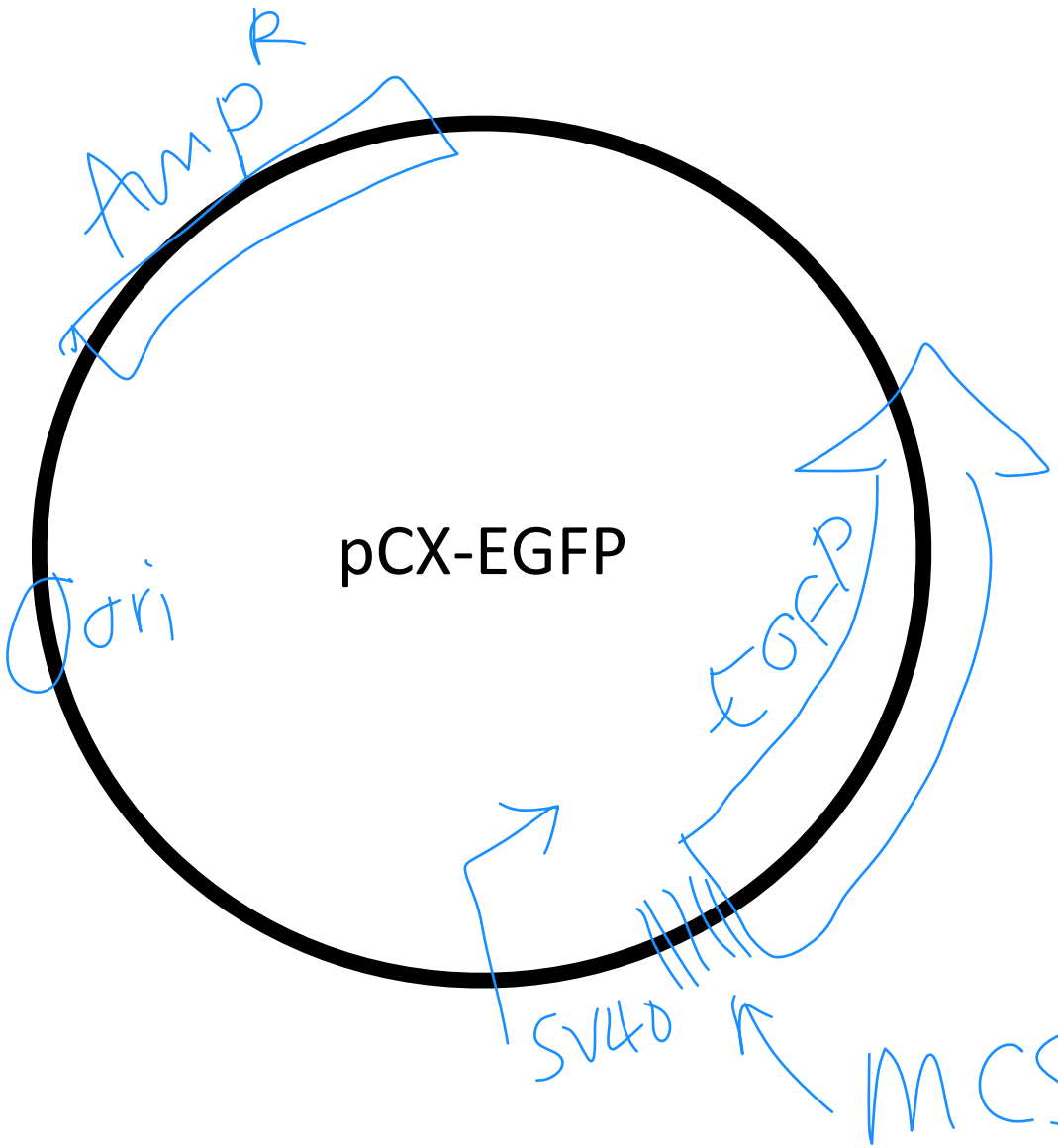


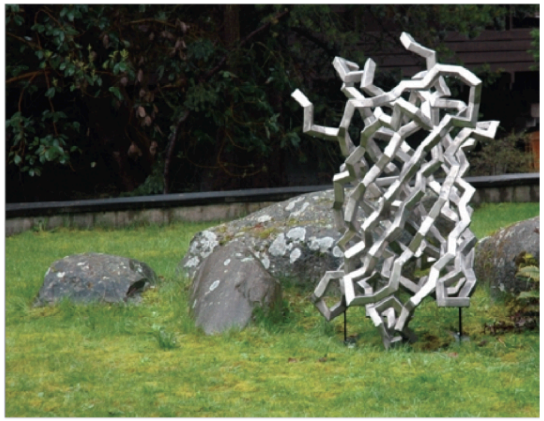
Building an HR sensor

no function = no fluor



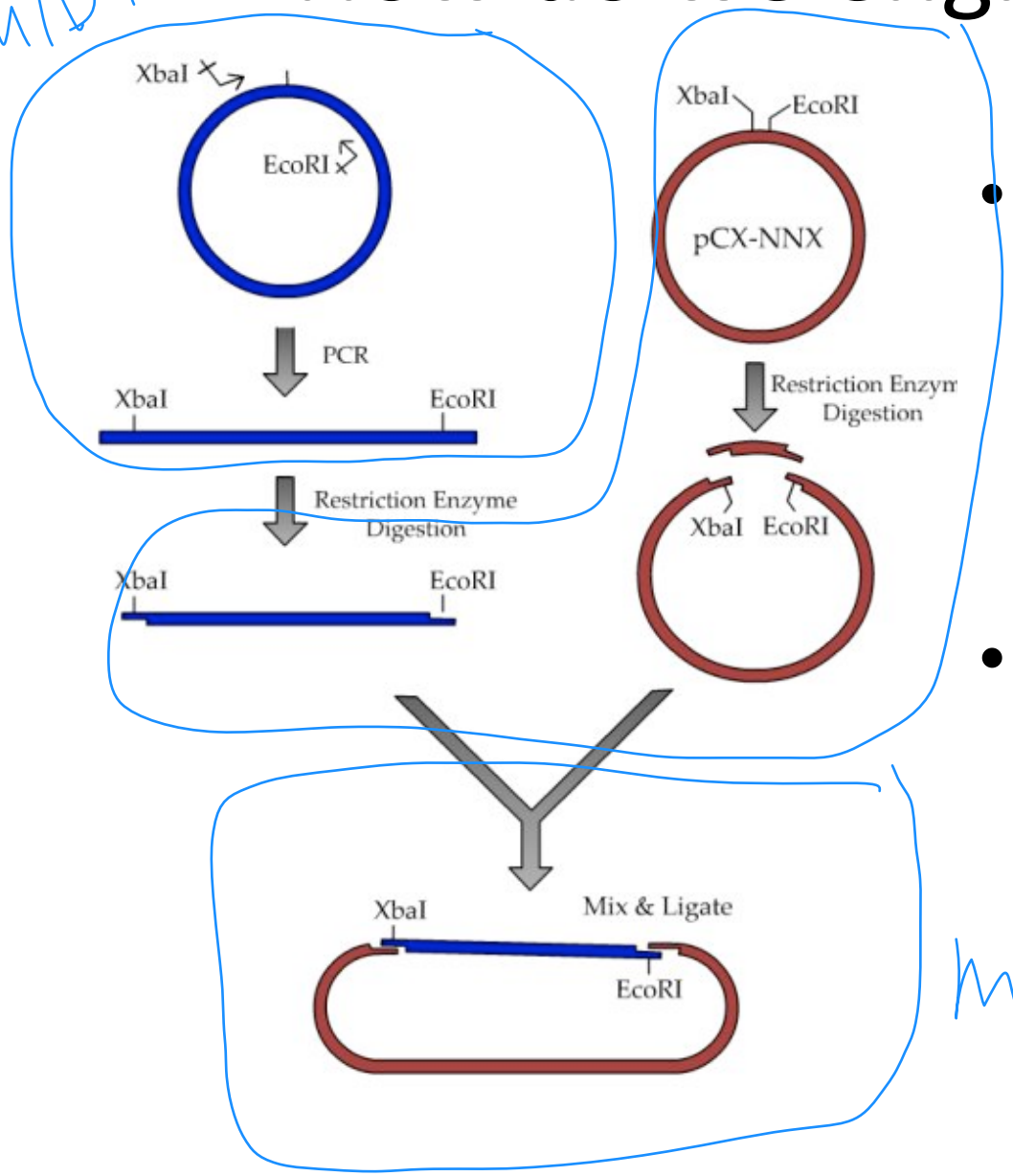
A closer look at (E)GFP





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?



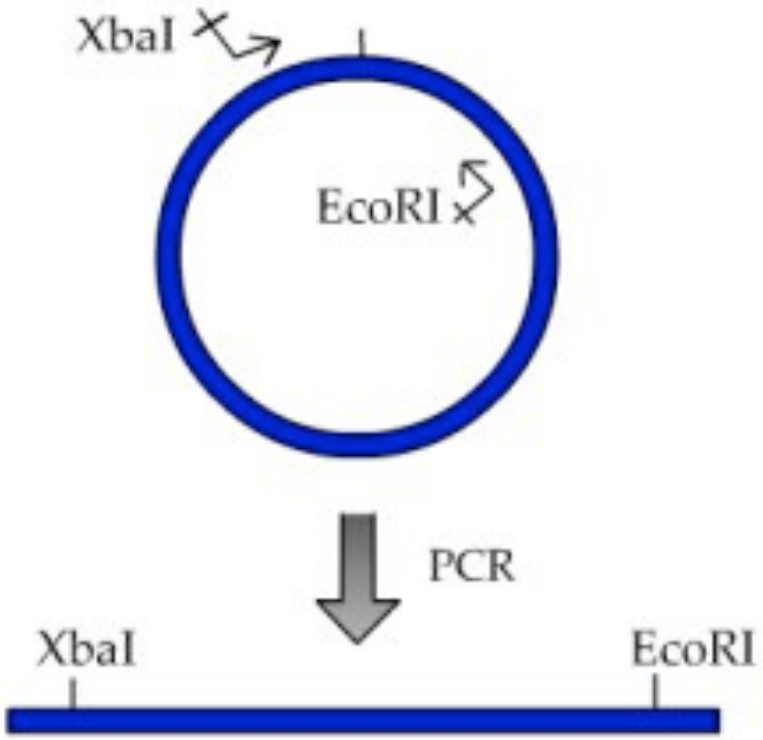
MID1

MID2

- PCR amplification
  - deletion
  - insertions
  - mutations *
- Restriction enzymes
  - cut/paste

MID3-4

Using PCR to generate Δ5 EGFP



- Melt

94°C break H bond

- Anneal : TmP = melting temp of primer, 50% of primer bound to template

55°C

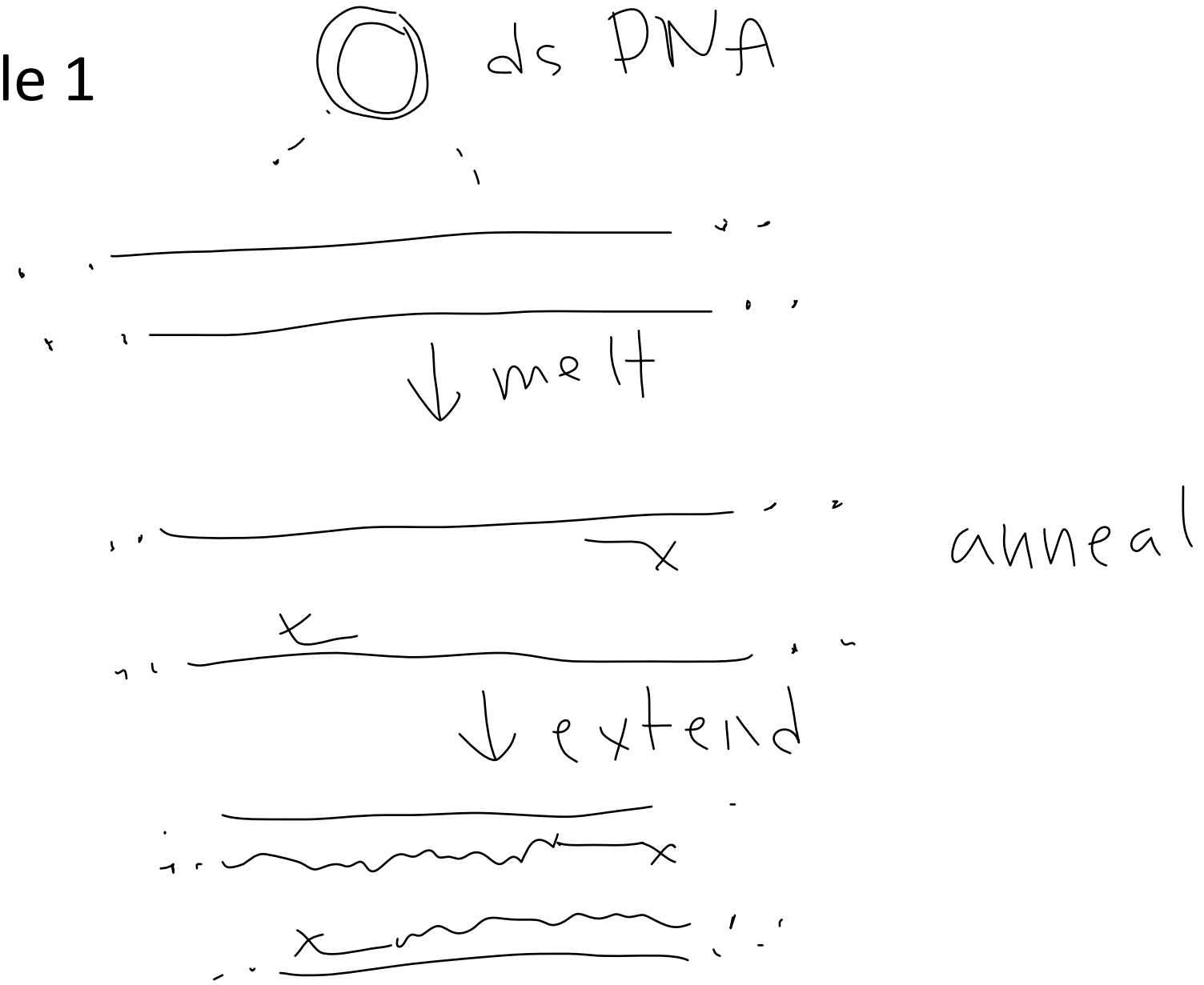
Anneal temp = TmP − 5°C

- Extend

72°C 1 min/1000b

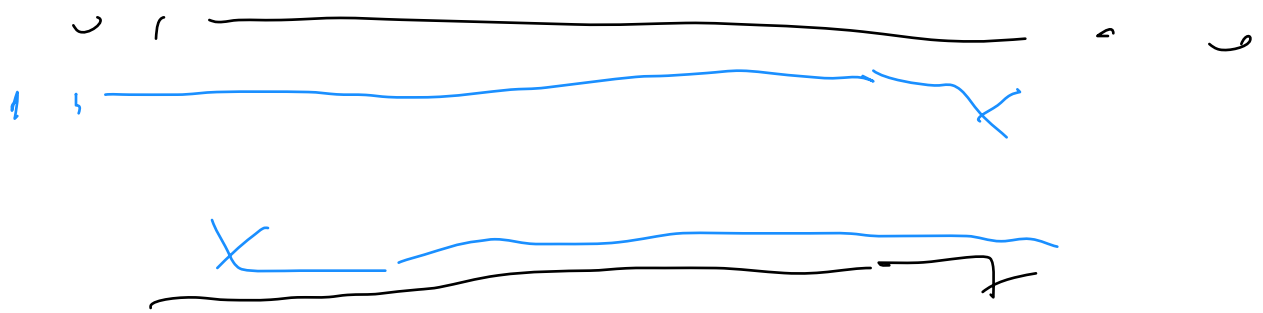
How many cycles until your amplicon?

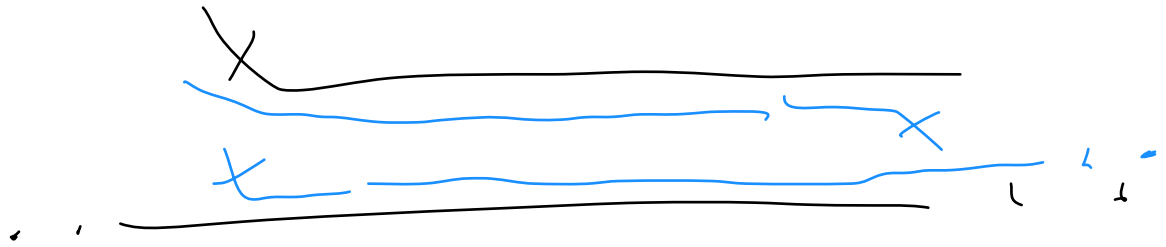
Cycle 1



melt not shown, blue = extend

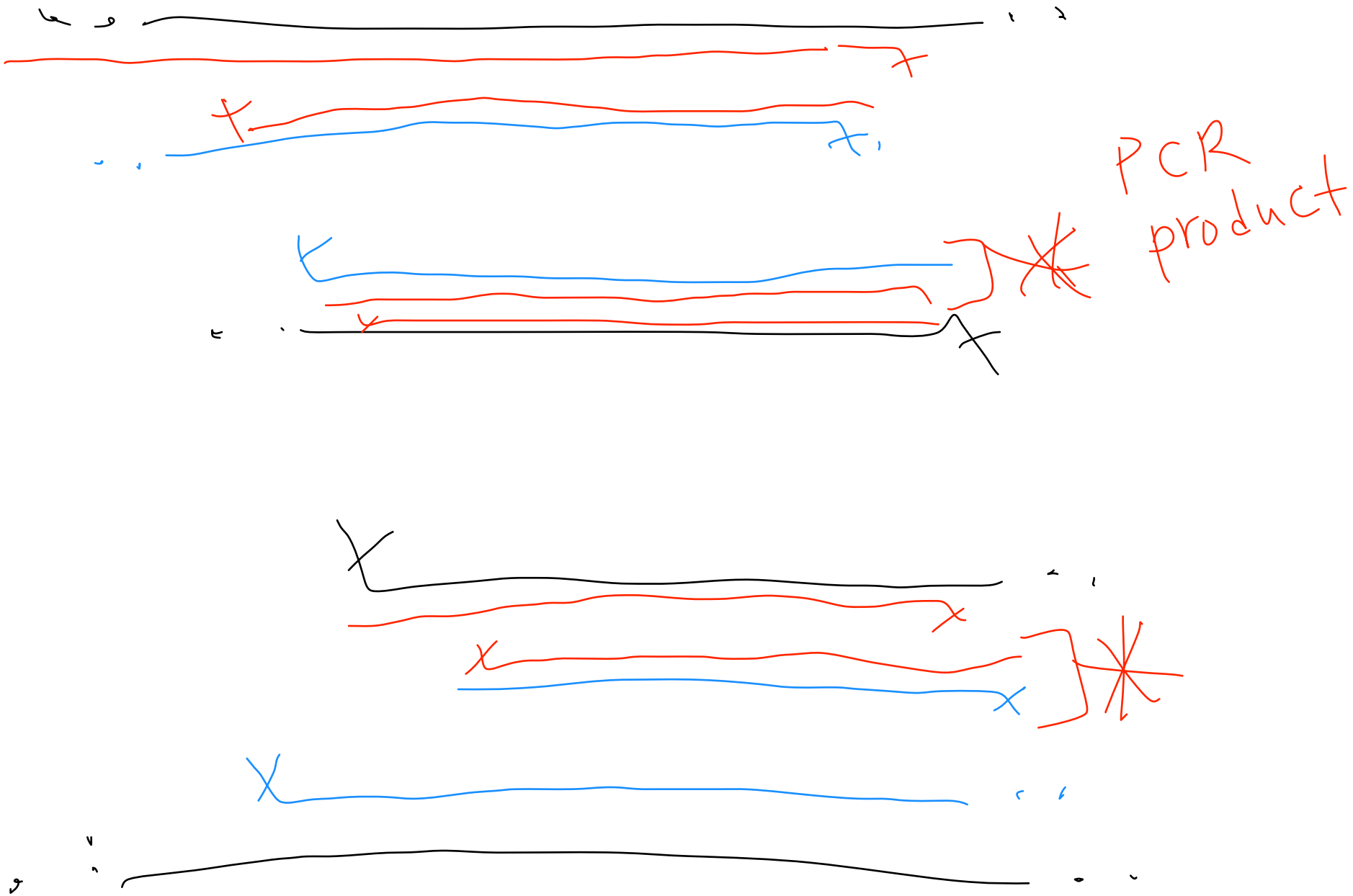
Cycle 2



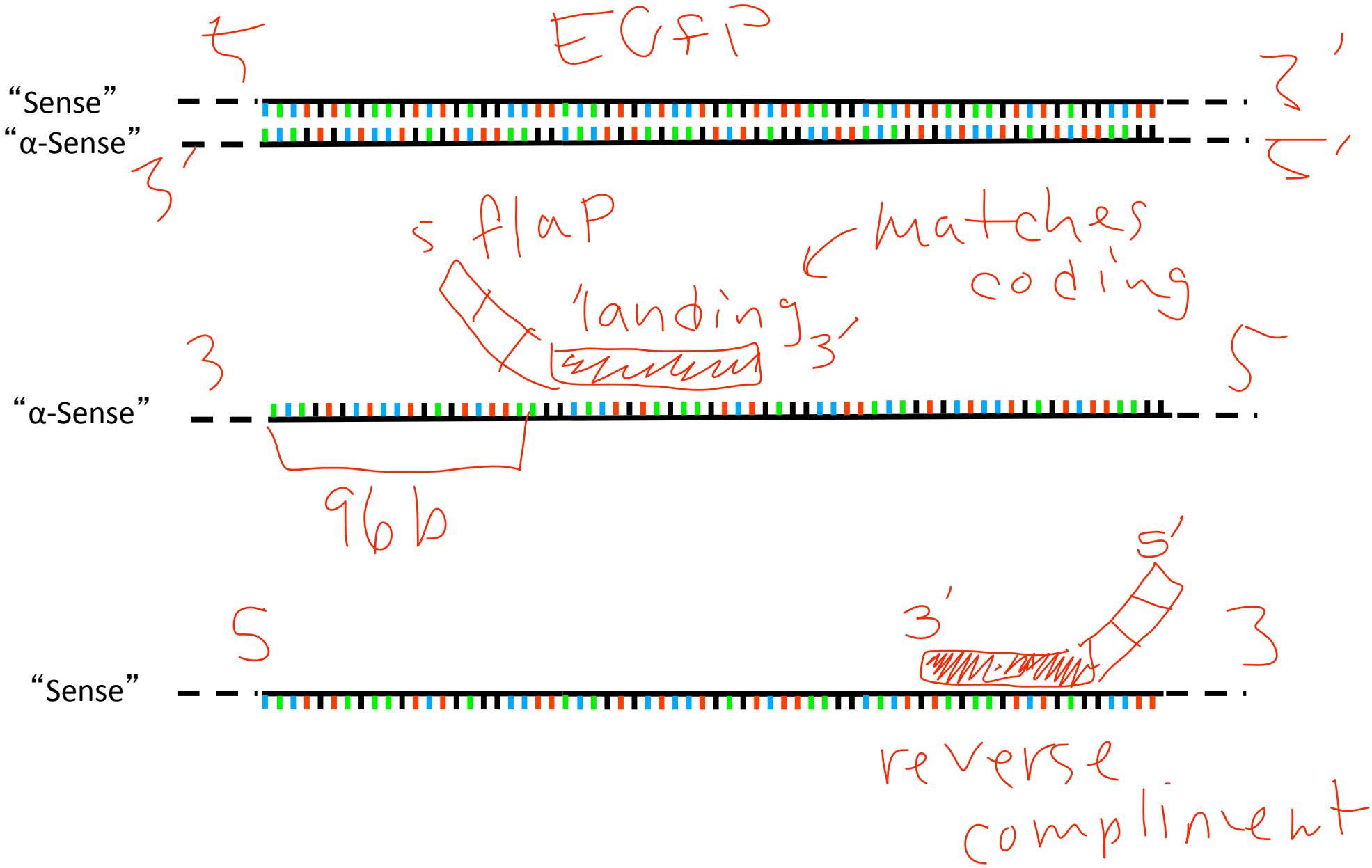


black=1 blue=2 red=3

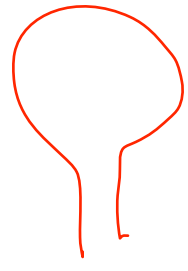
Cycle 3



Primer design overview



Primer design guidelines

1. Length 17-28 bp
2. GC content 50-60%
3. Secondary structure avoid hairpins 
4. Complementation primer dimers
5. Repetitive sequence non specific binding 2ndary structure

PCR setup

Component	Purpose
template	EGFP
primers (2)	amp specific seq.
Master Mix	Taq (polymerase)
	dNTP
	buffer, Mg^{++}

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
  - Link under Assignments tab

diff font color

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