

# MIDI: DNA Engineering using PCR

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9/10/13

1. Pre-lab discussion
2. Lab practical quiz (~40 min)
3. Primer Design
4. PCR Set-up

Note: Start your lab notebook today!

*Announcements.*

- 1) CV/Resume wkshp - 9/11 - see handout 5-7pm
- 2) BE Networking Mixer - 9/19 Koch

Let's start with the wiki.

# A few instructions / observations about Evernote:

- You may bring and use your own laptop.
- You can have one notebook open on the web-hosted version and one on the desktop.
- Work together to copy & modify protocols from the wiki -- you may work off one notebook for this.
- You must write **your own** front/back matter (statement of purpose & interpretation/conclusion).

# From protocol to lab notebook

1. Begin by adding the correct amount of water to a 200 ul PCR tube. Add that amount +1 ul to a second PCR tube.
2. Next add the primers to each reaction. Be sure to change tips between additions.
3. Next add template to the first reaction tube.
4. Finally add PCR Master Mix to each tube, pipetting up and down to mix. Leave your tubes on ice until the entire class

**Statement of purpose:** Today we will design primers to [do xyz task]. Then we will prepare [xyz DNA] by PCR to use as [xyz component] for later cloning.

Design primers for GFP insert (M1D1 Part 1)

See attached Word document.

PCR to make GFP insert (M1D1 Part 2)

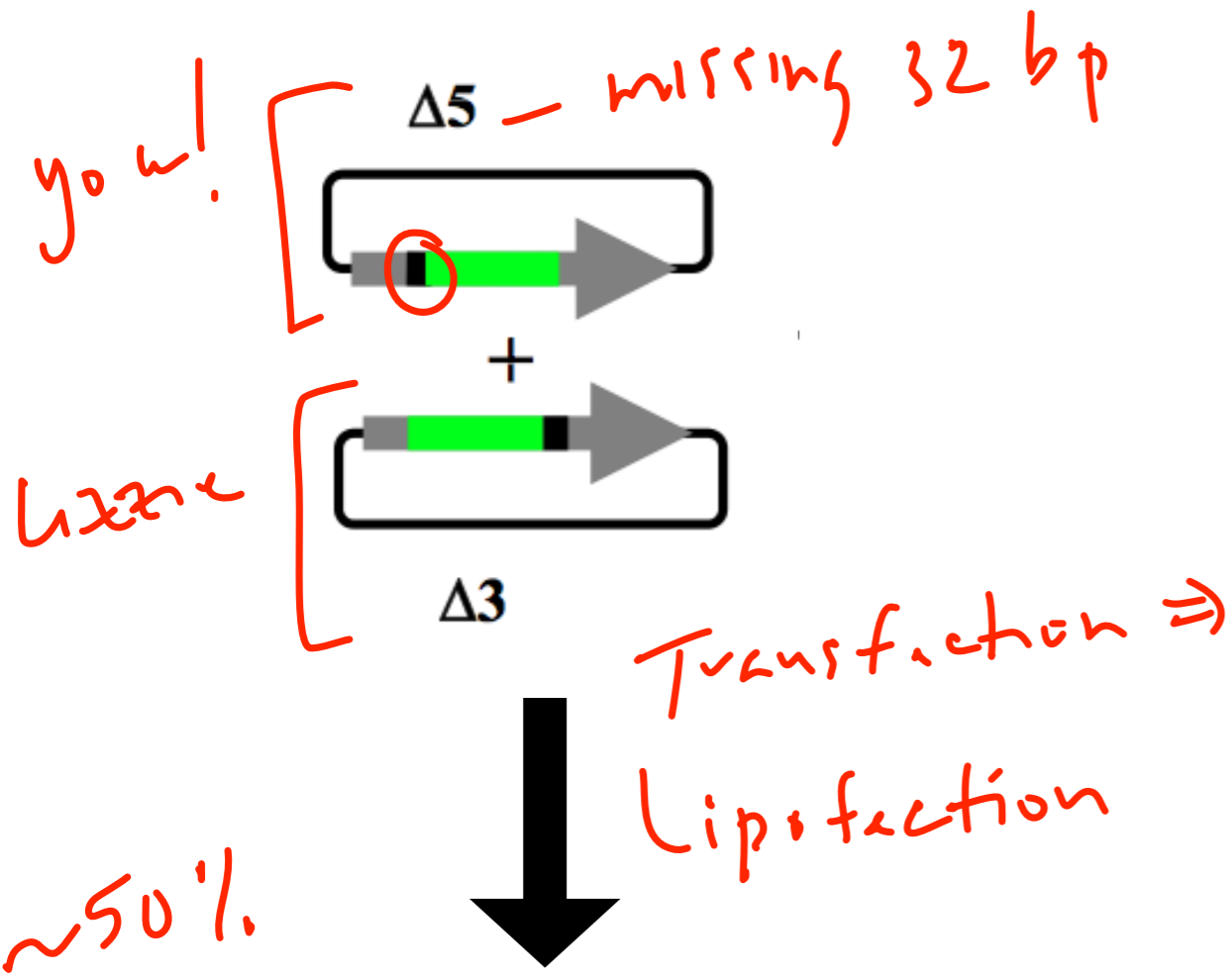
*Copy protocol and fill in exact volumes for #1.*

*Optionally confirm (say, with checkboxes) key details such as adding Master Mix last, template only to experimental sample.*

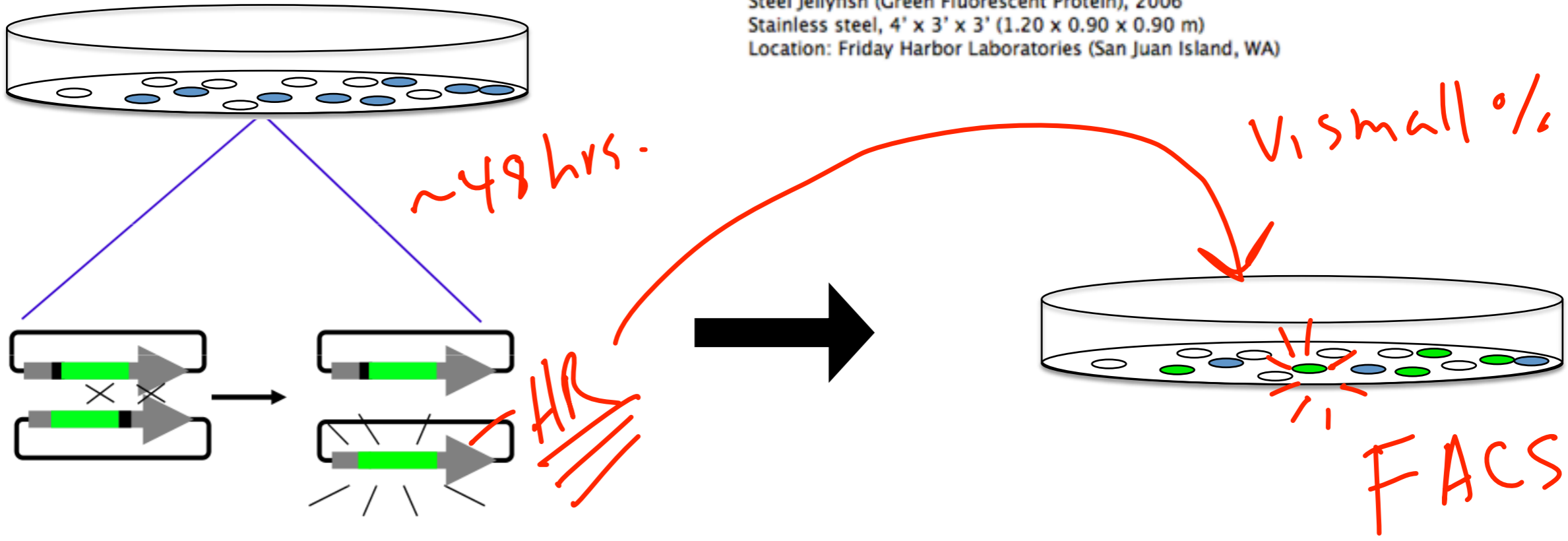
*Add unique notes: Rxn ready at 3 pm → on ice → thermal cycler started at 4.*

Slide courtesy of Agi

# Module I Overview:



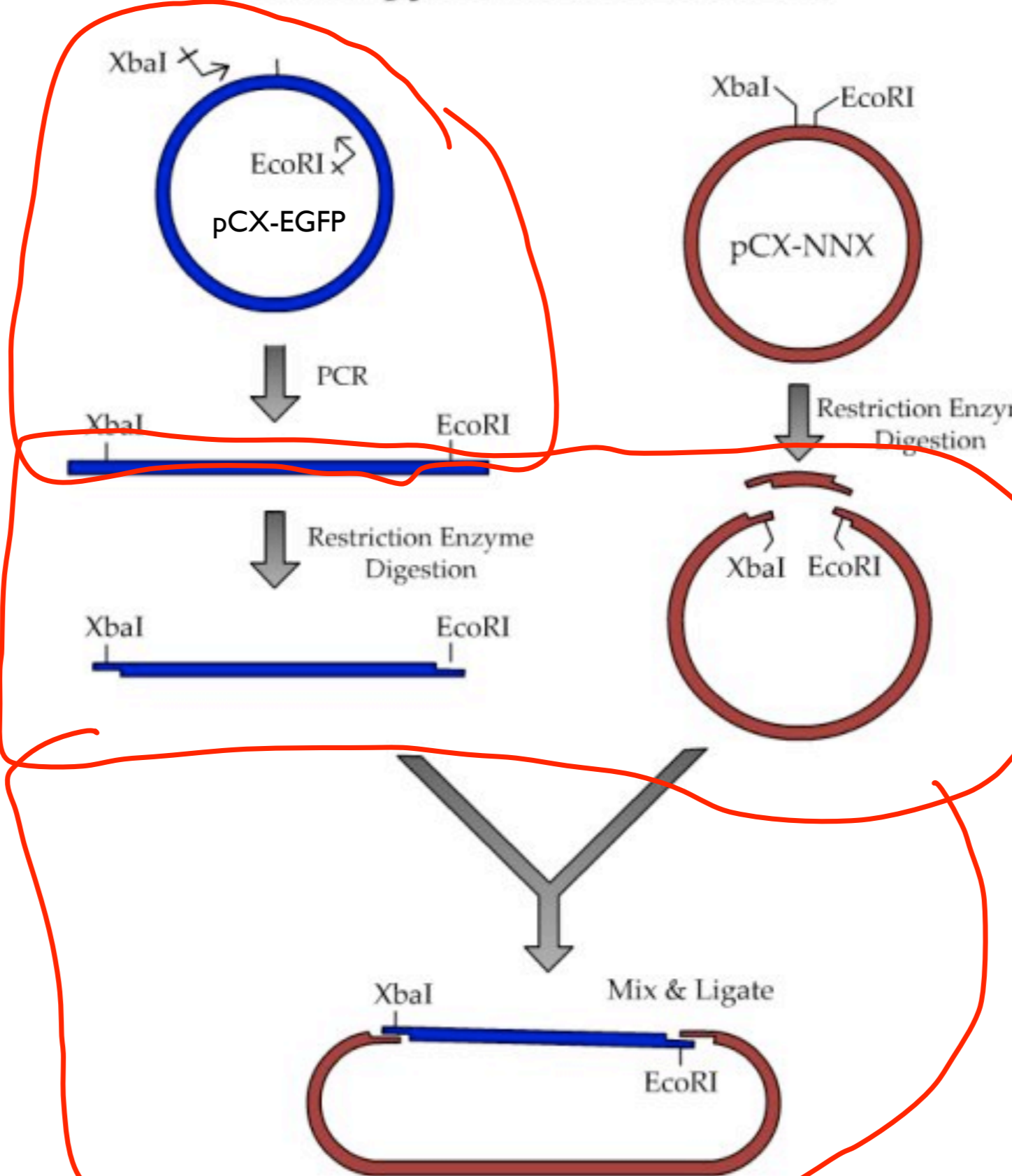
Julian Voss-Andrea  
 Steel Jellyfish (Green Fluorescent Protein), 2006  
 Stainless steel, 4' x 3' x 3' (1.20 x 0.90 x 0.90 m)  
 Location: Friday Harbor Laboratories (San Juan Island, WA)



# Step I: Build the system!

Roadmap for Plasmid Construction

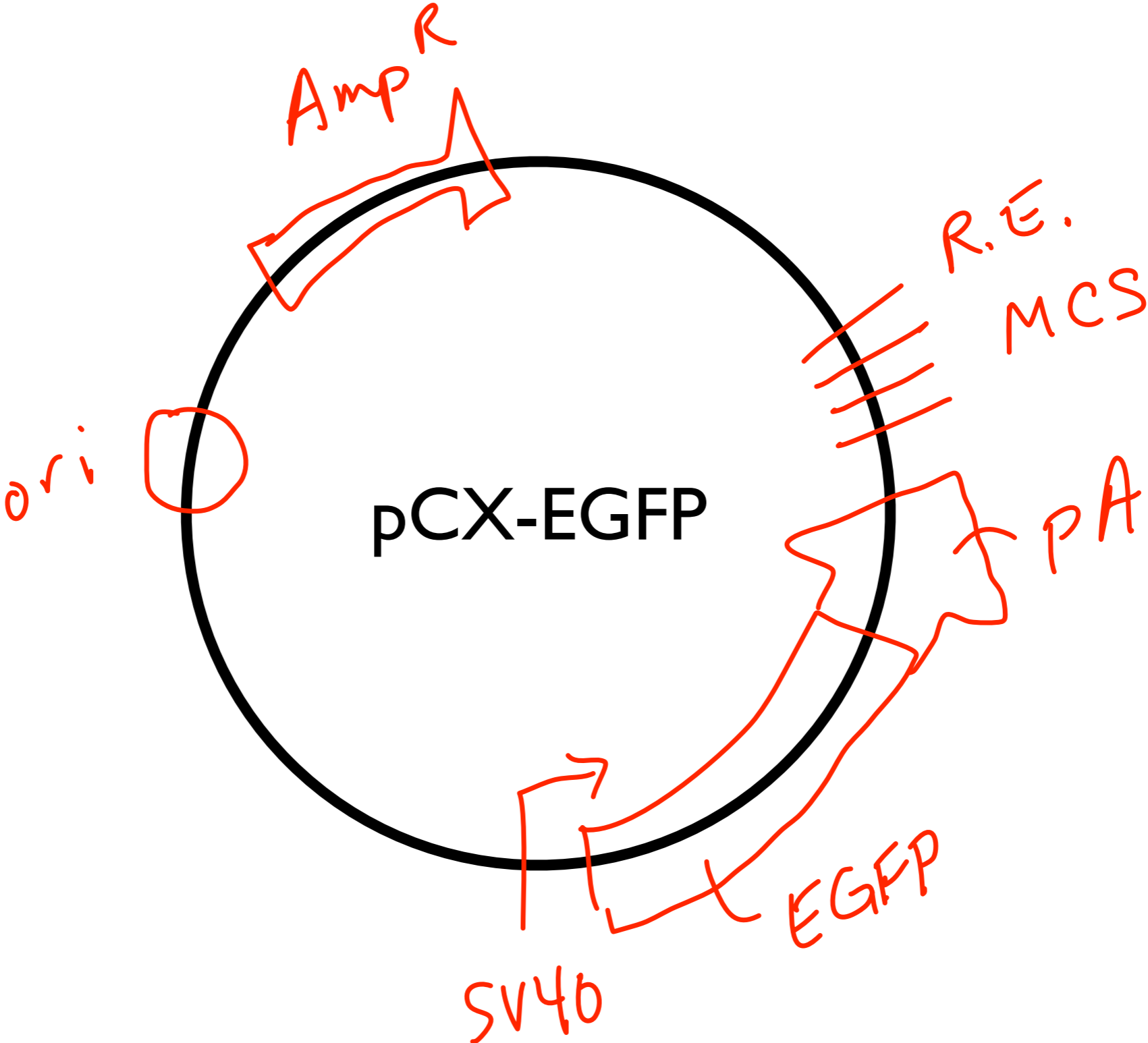
MIDI



MIDI2  
MIDI3

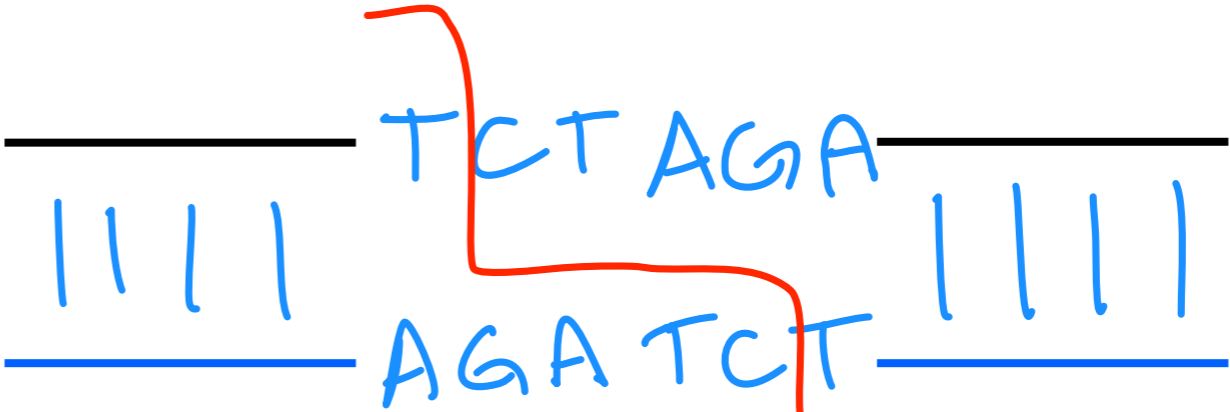
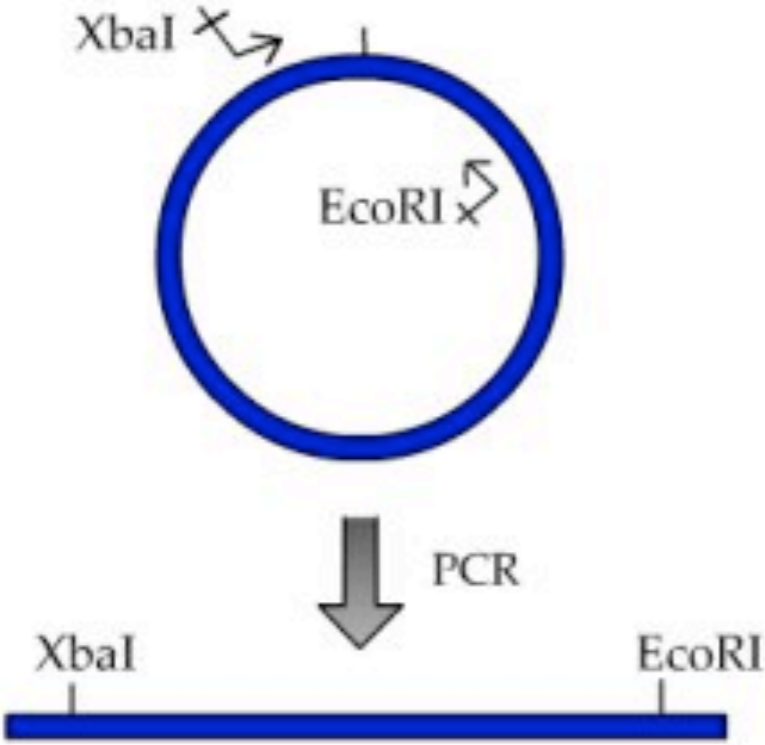
MIDI4

# Where to we get the EGFP sequence?

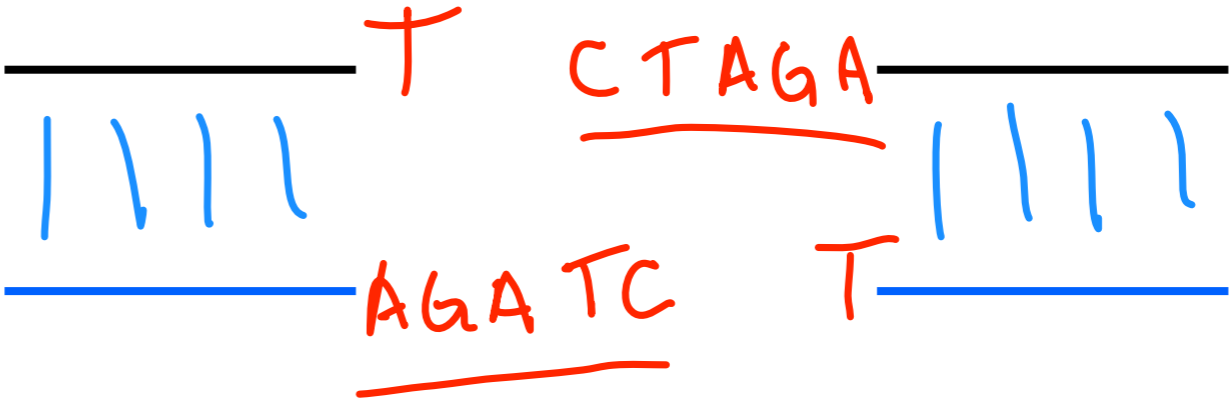


# Restriction enzyme sites are the glue for our project.

XbaI



37°C ~ 60min

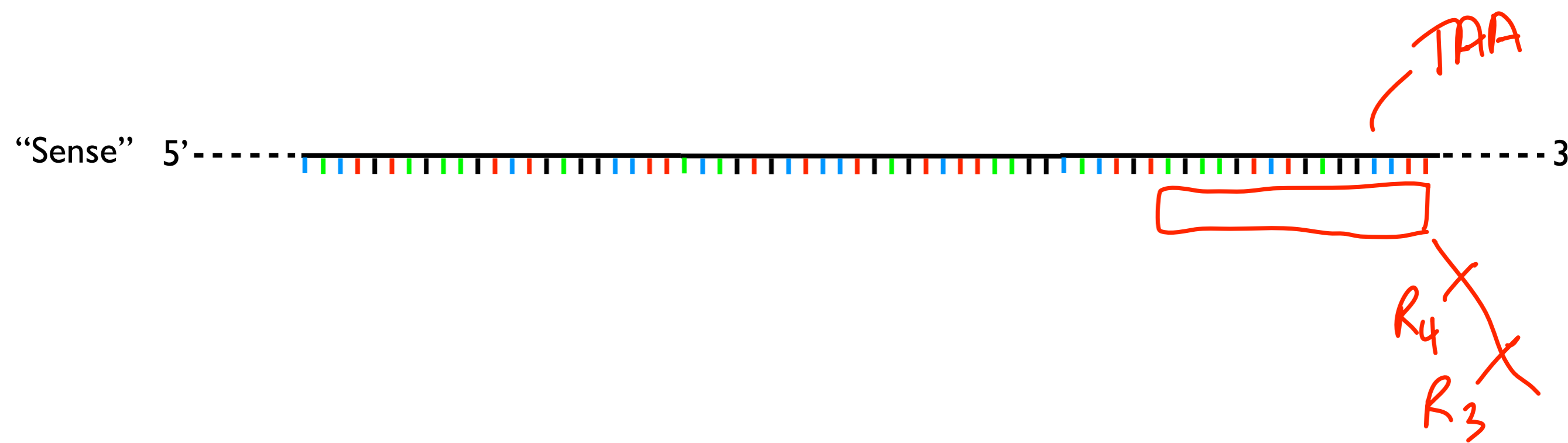
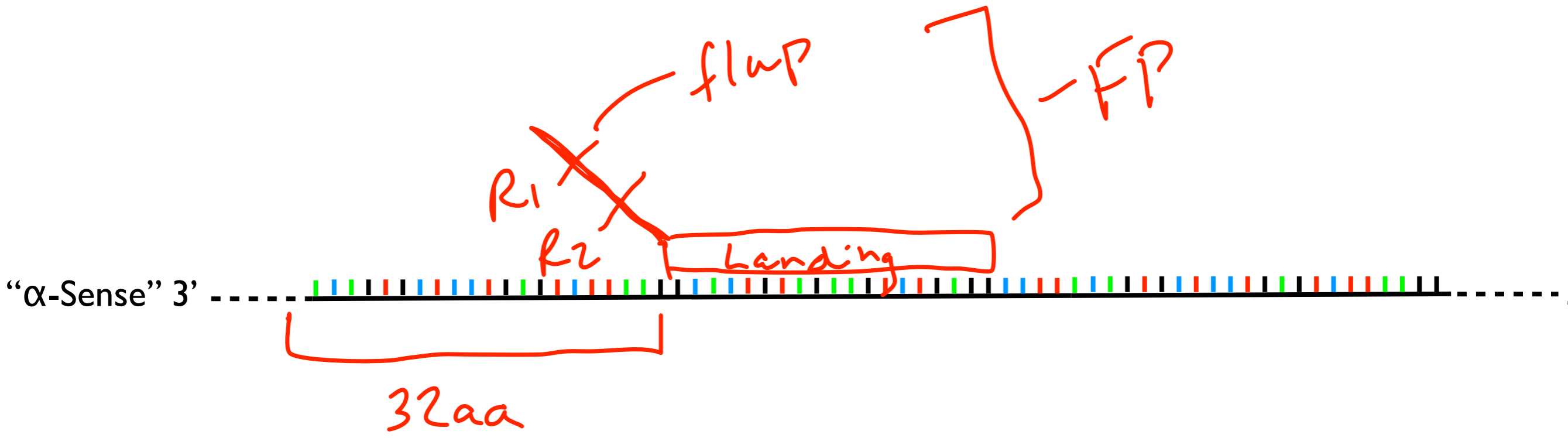
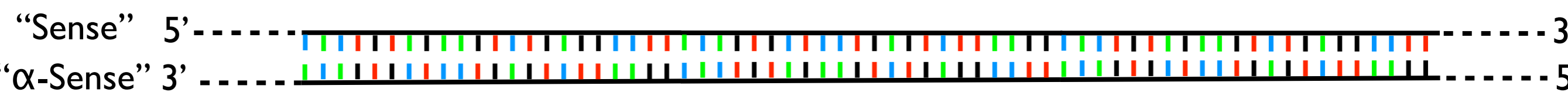


- cut DNA
- endonuclease
- palindrome
- sticky ends

Today you will engineer these sites into your PCR primers.



# Primer design basics:



# Primer design basics:

primer = 10 bp

$$4^{10} \sim 10^6$$

- Length 17-28

- 50-60% G/C content

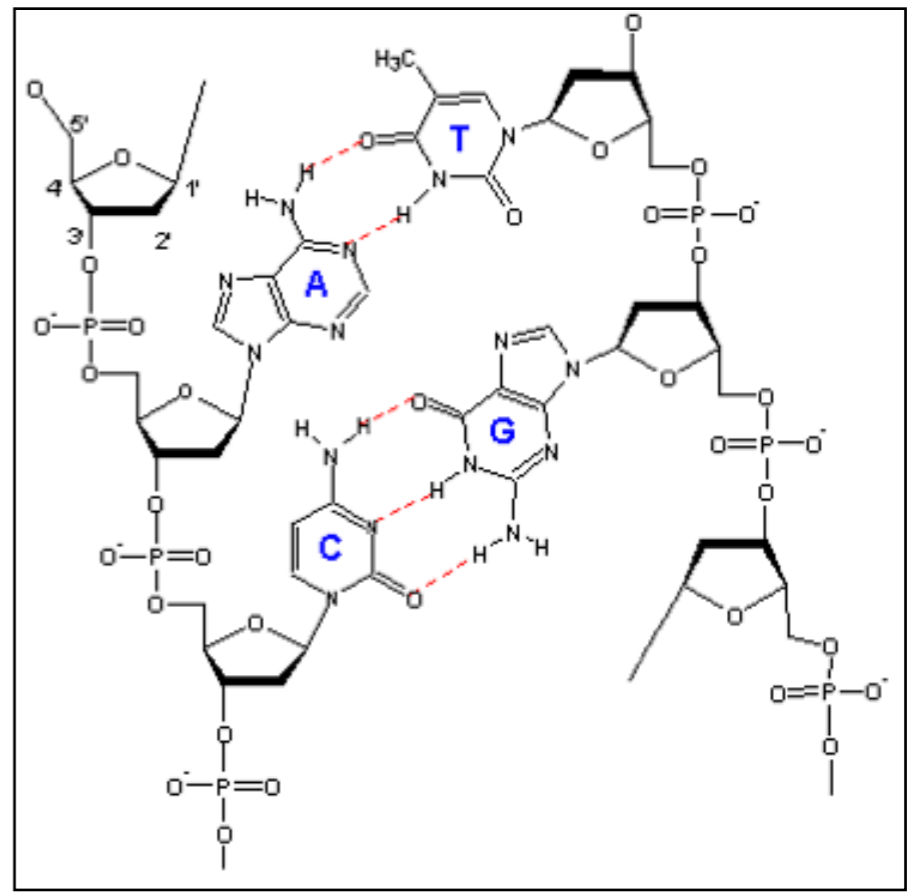
2° structure AT-rich

MT

- avoid hairpin  - watch the ends

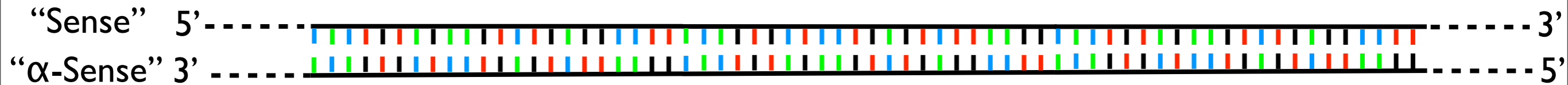
- primer dimers  $5'$              
 $3'$

AAAAAAGCGT



Thermodynamics of DNA Duplex, New Mexico State University

# PCR

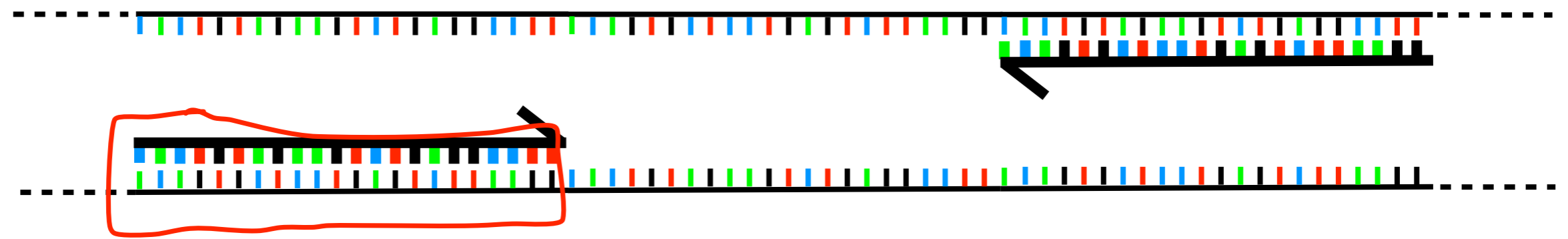


↓ Melting (94-98°C)

Denaturing =  
break H-bonds

↓ Annealing ( $T_{m,p} - 5^\circ\text{C}$ )

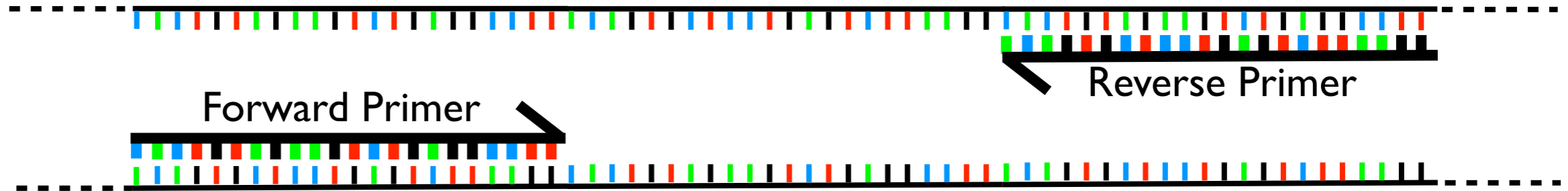
$T_{m,p}$  = primer melting temp



Landing  $T_m \sim 60^\circ\text{C}$



$T_a < T_{m,p}$   
Why?

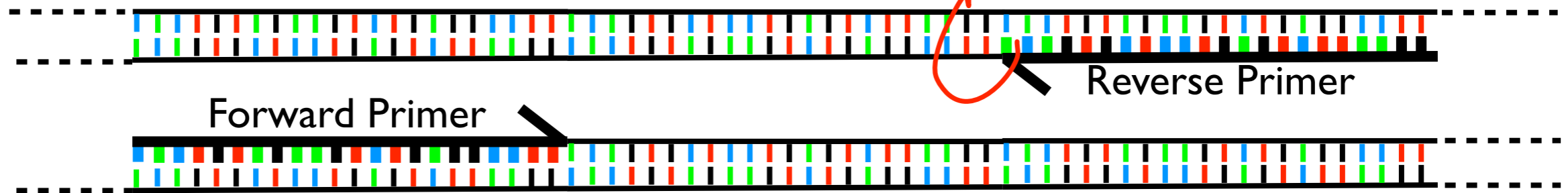


75% of primer is bound

Extension  $\sim 72^\circ\text{C}$

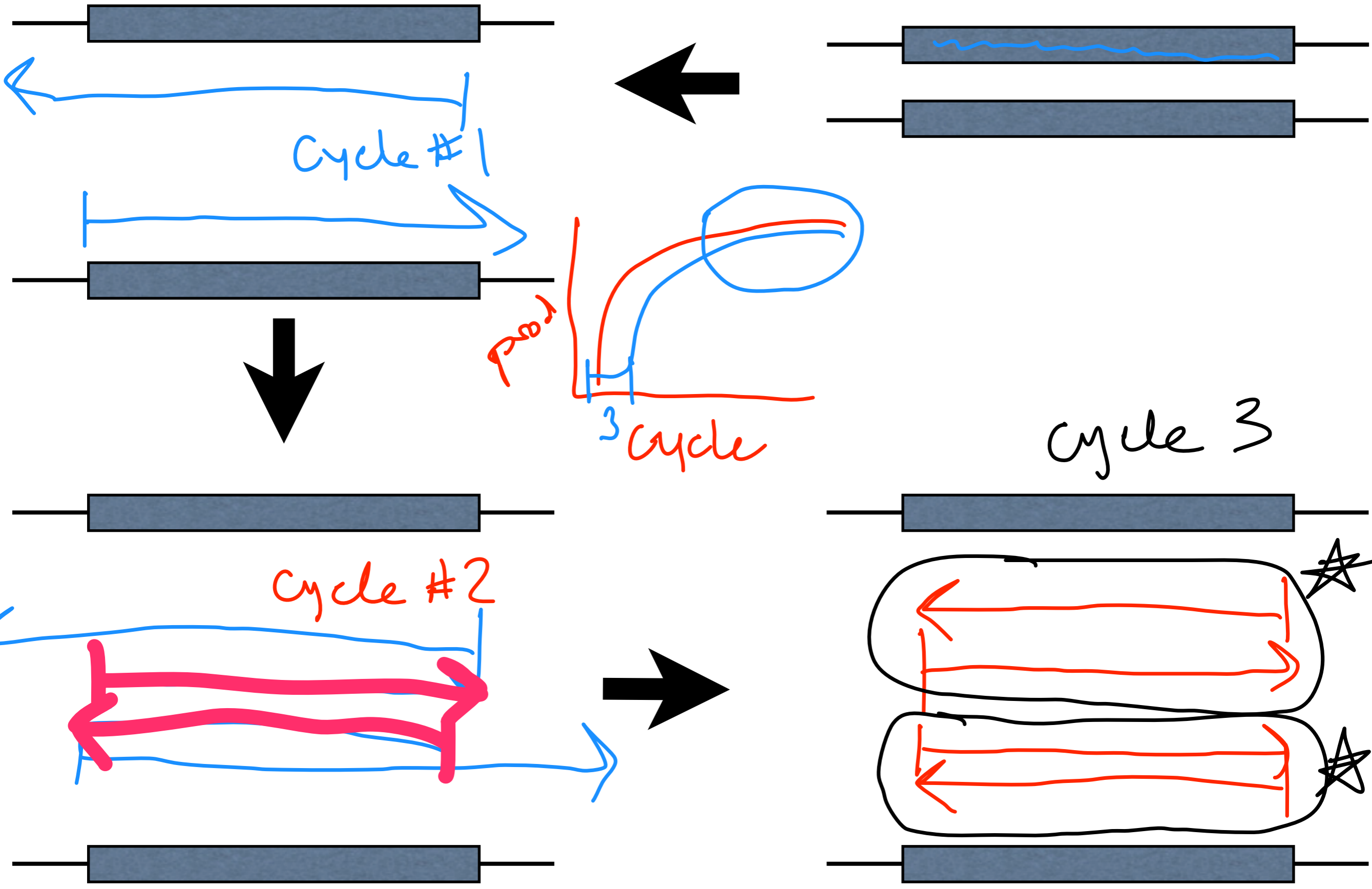


Extension temp is:



Extension time  
 $\sim 1 \text{ min/Kbp}$

# What happens in the 'real' PCR world?



# Your PCR:

Component	Purpose
primers (2)	define target
template	PCX-EGFP "pattern"
Master Mix	dNTPS
	polymerase
	buffers $\rightarrow$ $Mg^{2+}$

# Today in the lab:

- Lab practical (~40 min)
- Design PCR primers -- upload your final primer designs to your notebook!
- Set-up PCR reactions

# Next time in the lab:

- Cut and clean up your DNA
- Visit from Leslie

— Think about efficiency vs. accuracy

— Do not recalibrate pH meter

