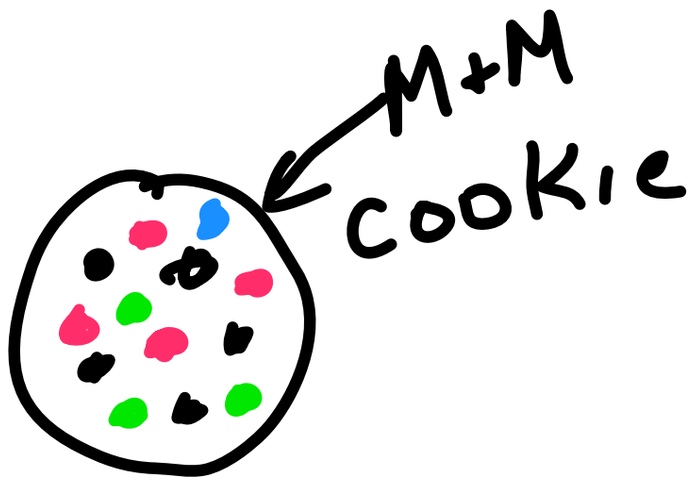
M1D1: Clean and Cut DNA

9/9/14

1. Pre-lab discussion

2. Lab practical = 1st lab treat



3. Primer Design

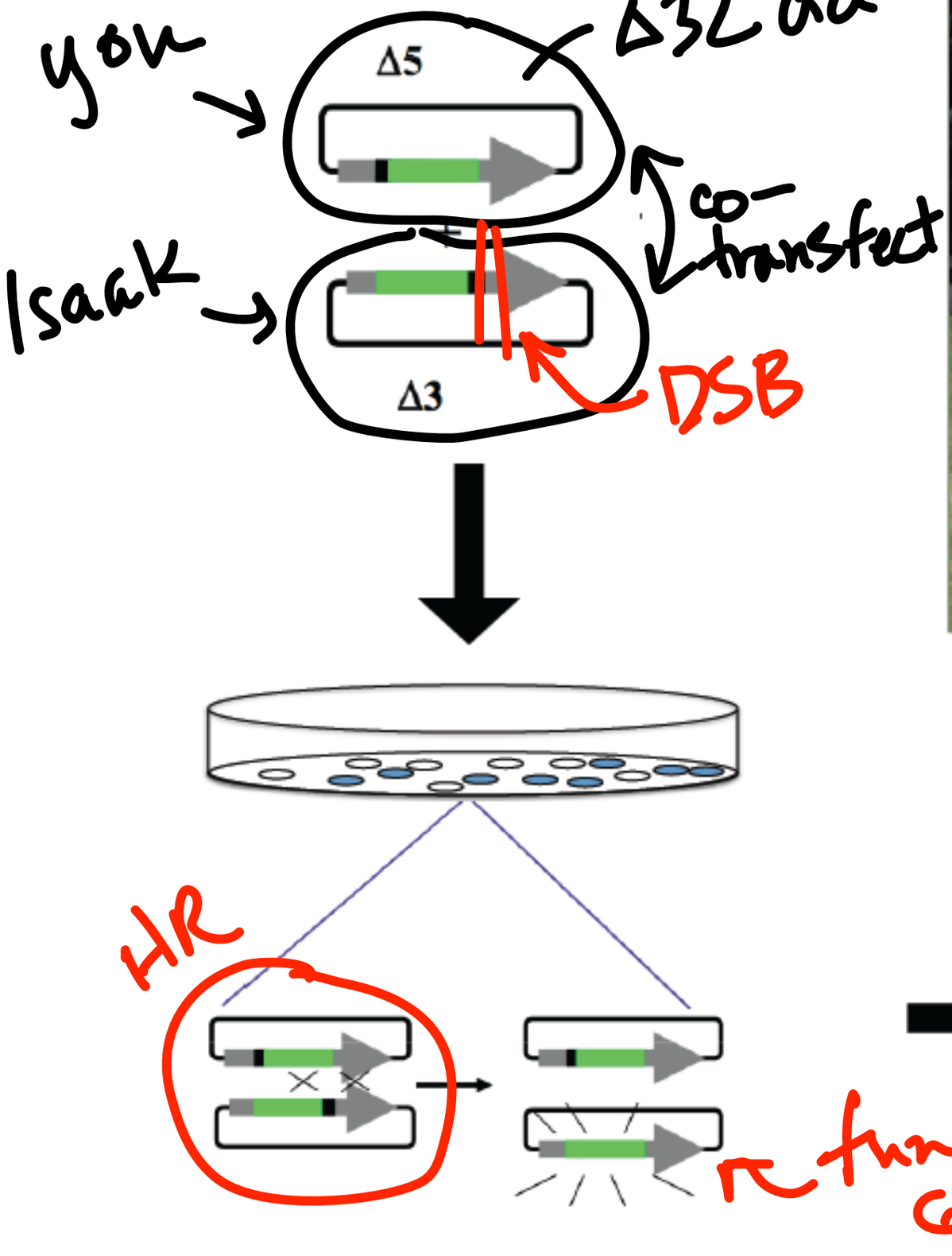
4. Set-up PCR reactions

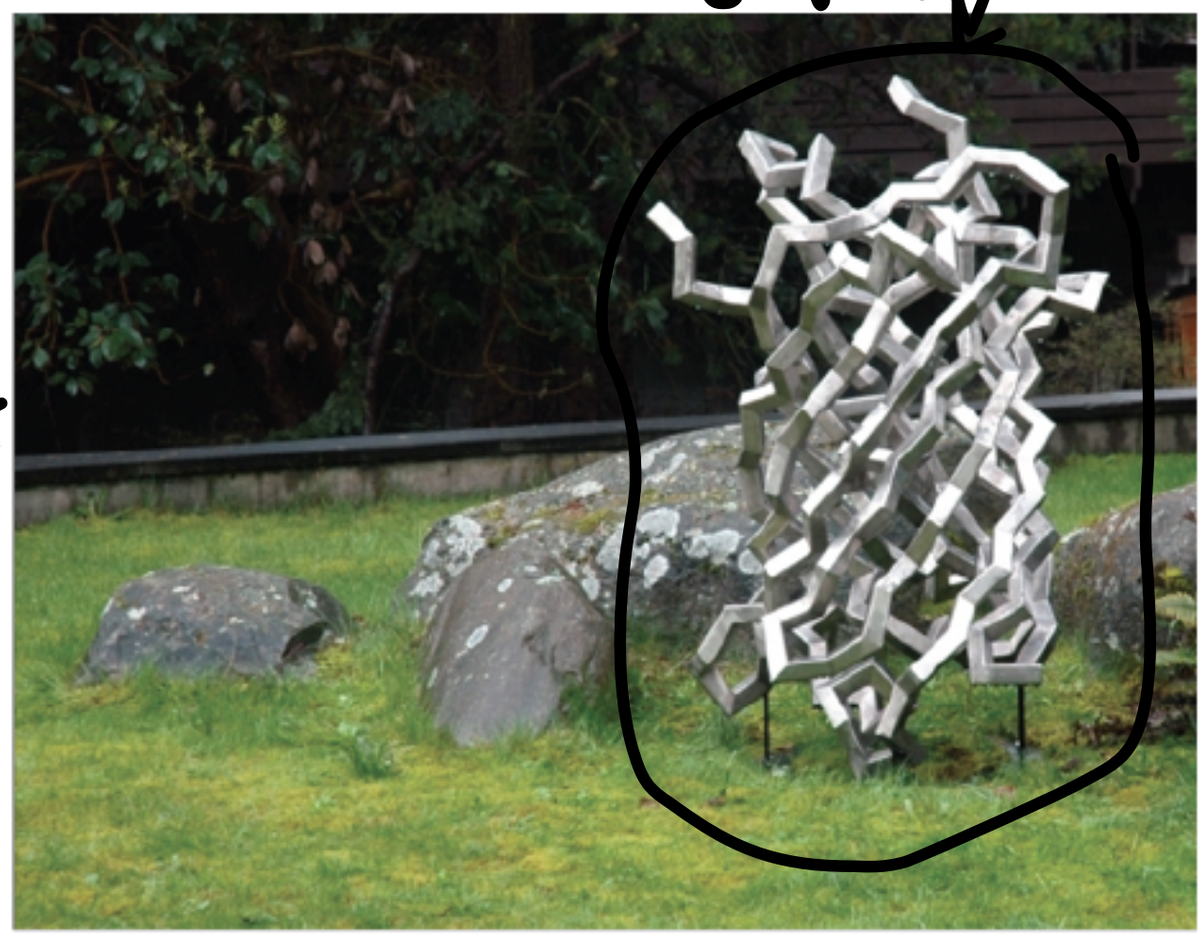
Start your lab notebooks today!

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).

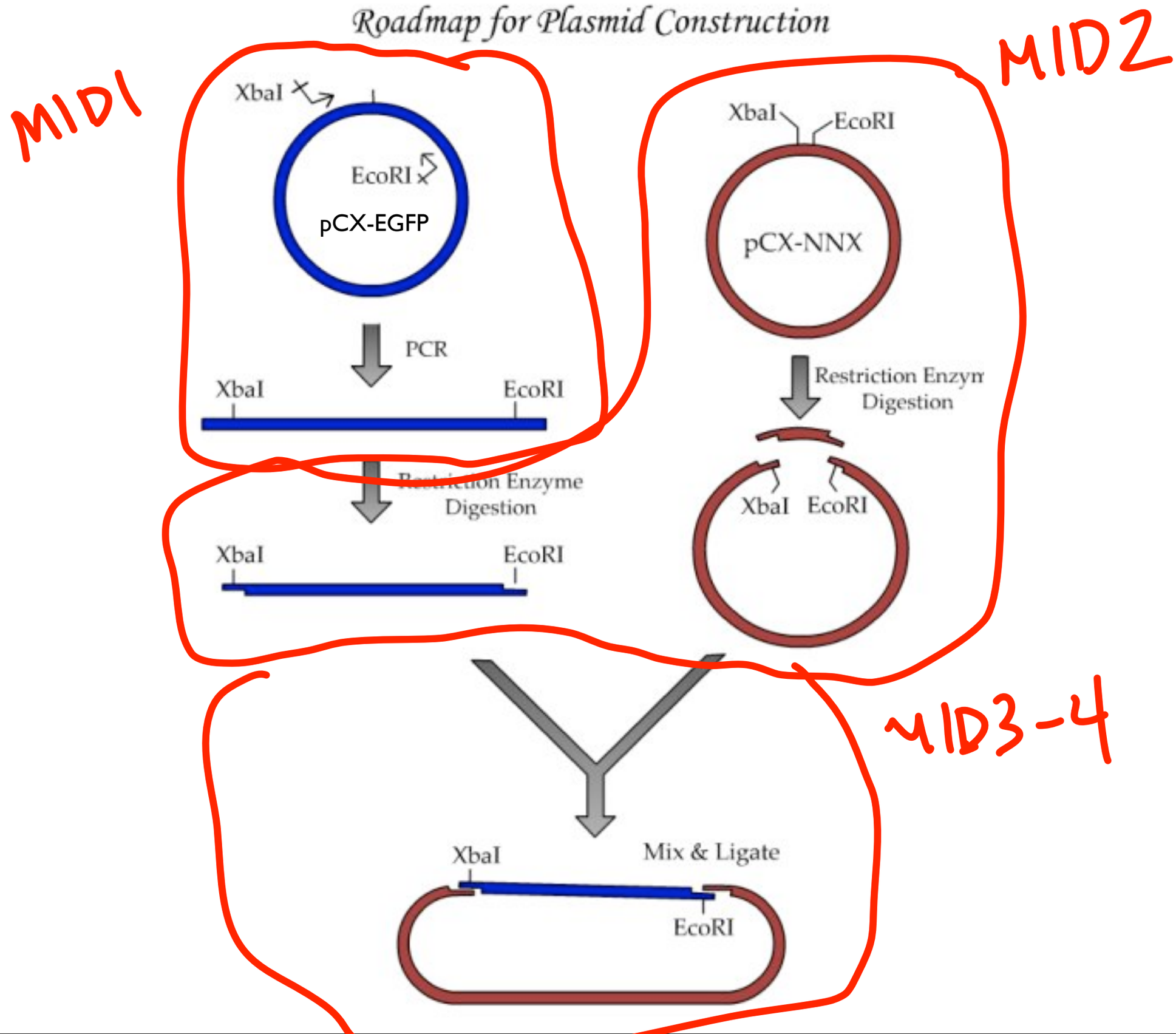
Module I Overview:



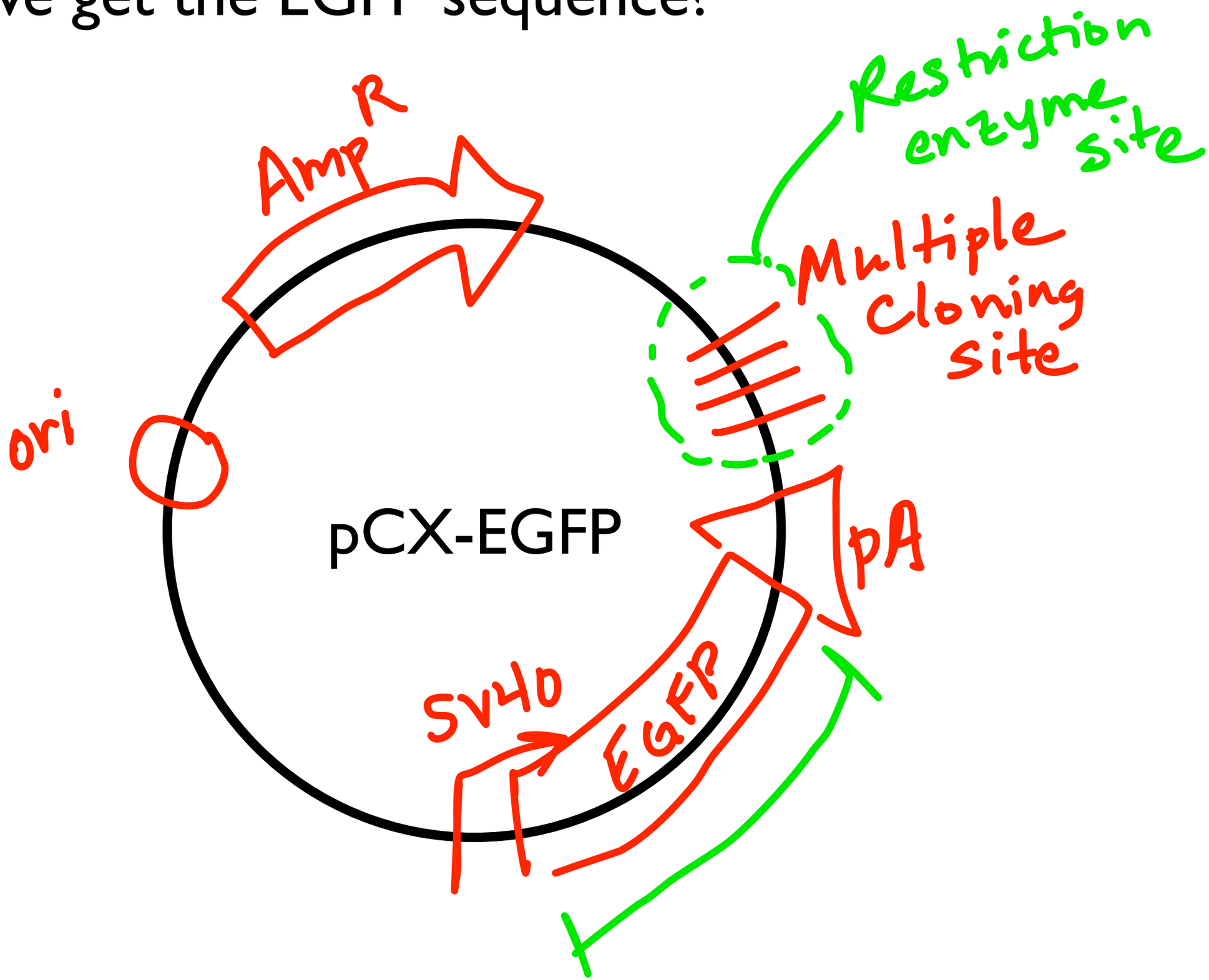


Julian Voss-Andreae
Steel Jellyfish (Green Fluorescent Protein), 2006
Stainless steel, 4' x 3' x 3' (1.20 x 0.90 x 0.90 m)
Location: Friday Harbor Laboratories (San Juan Island, WA)

Step 1: Build the system!

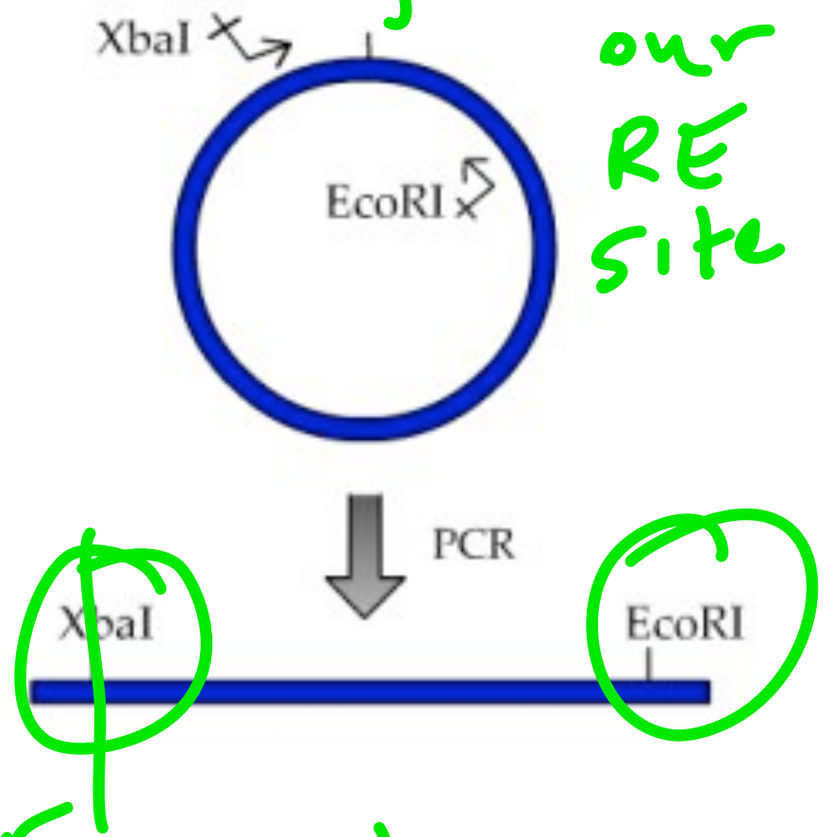


Where to we get the EGFP sequence?



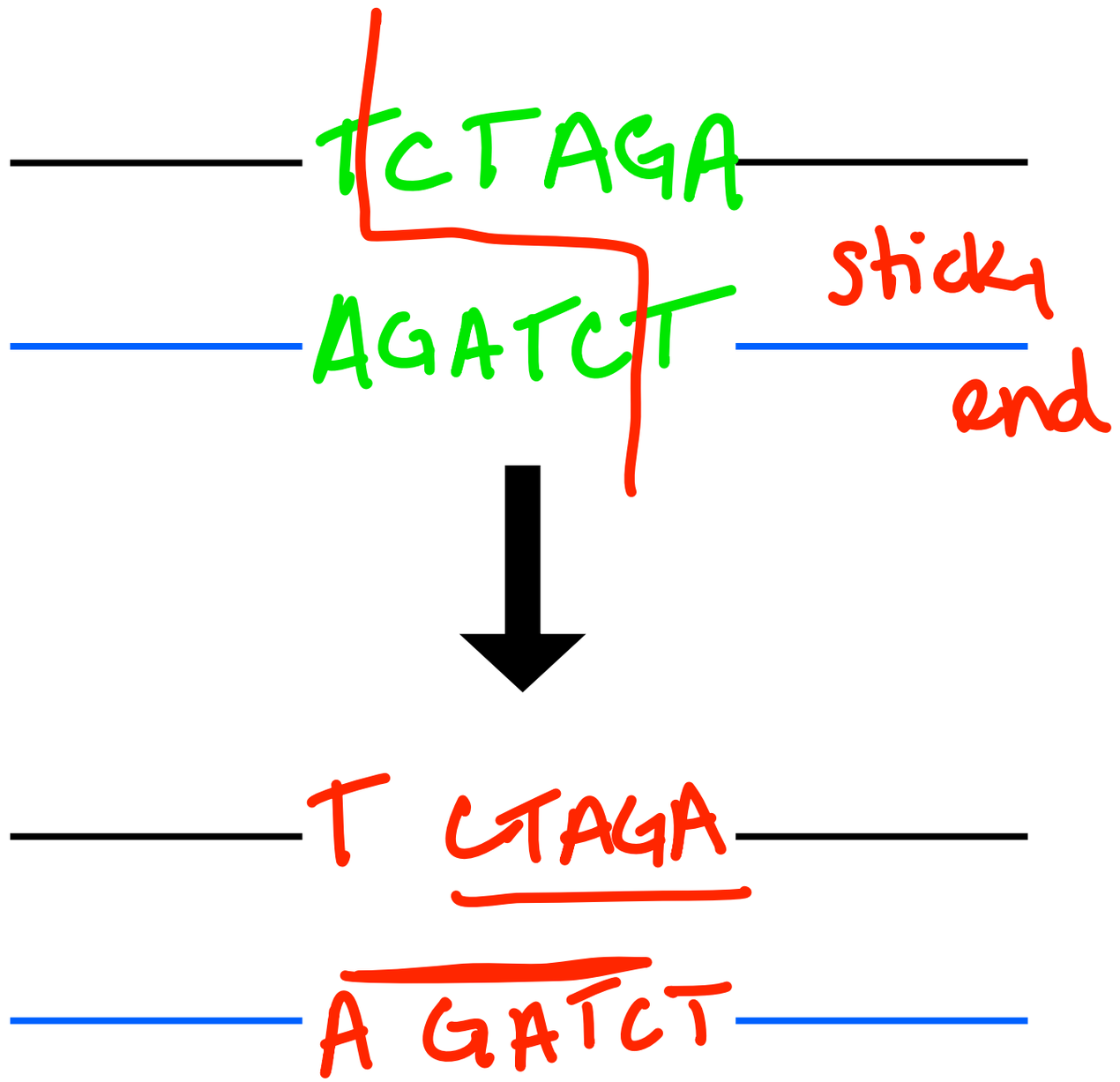
Restriction enzyme sites are the glue for our project.

Engineer in our RE site



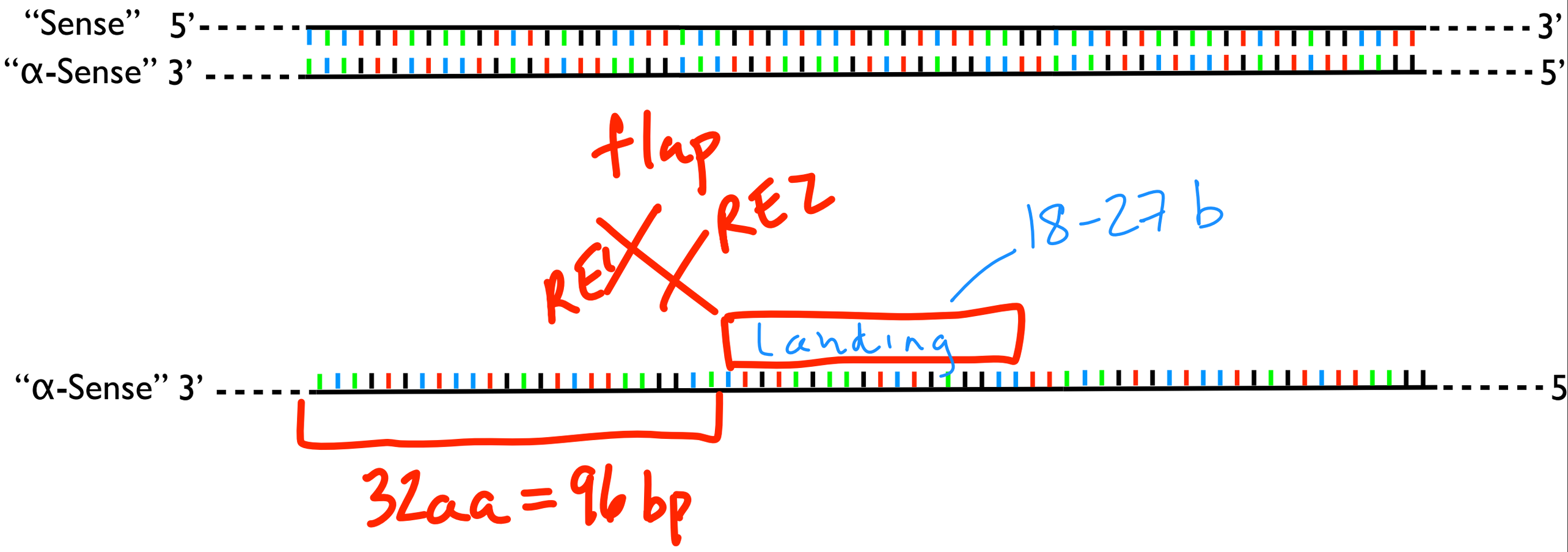
- Endonucleases
- palindromic
-

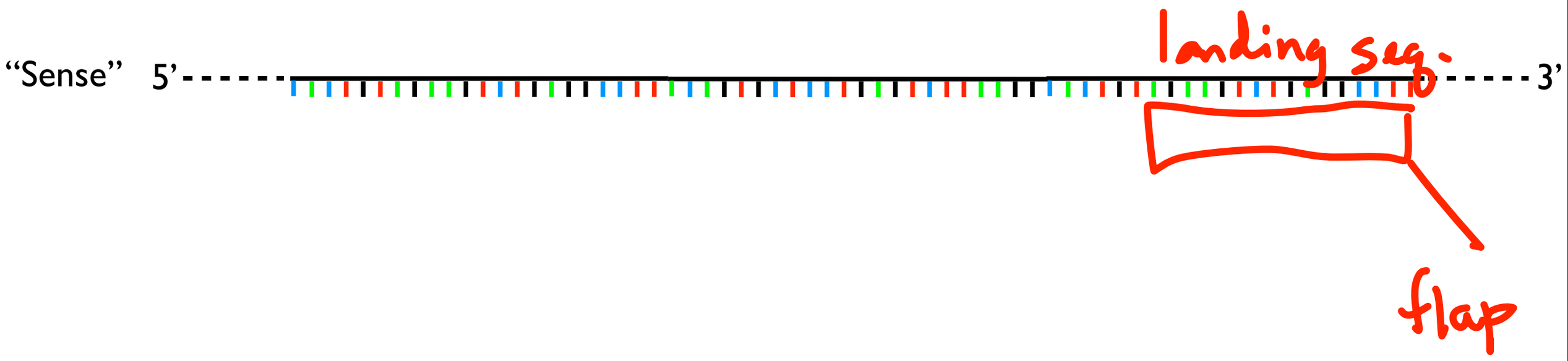
XbaI



Today you will engineer these sites into your PCR primers.

Primer design basics:



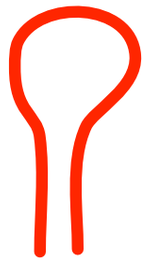


Primer design basics:

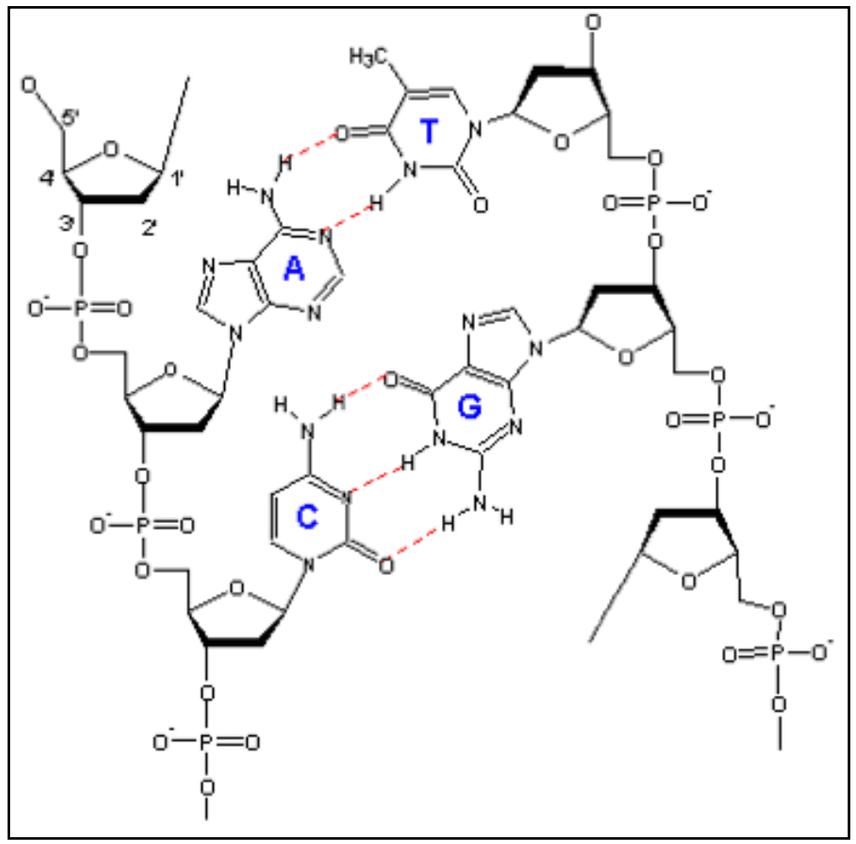
primer = 10 bp

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

- Length 17-28 b
- 50-60% G-C content $\lesssim$ 2° secondary structure / Melting Temp
- Avoid hairpin

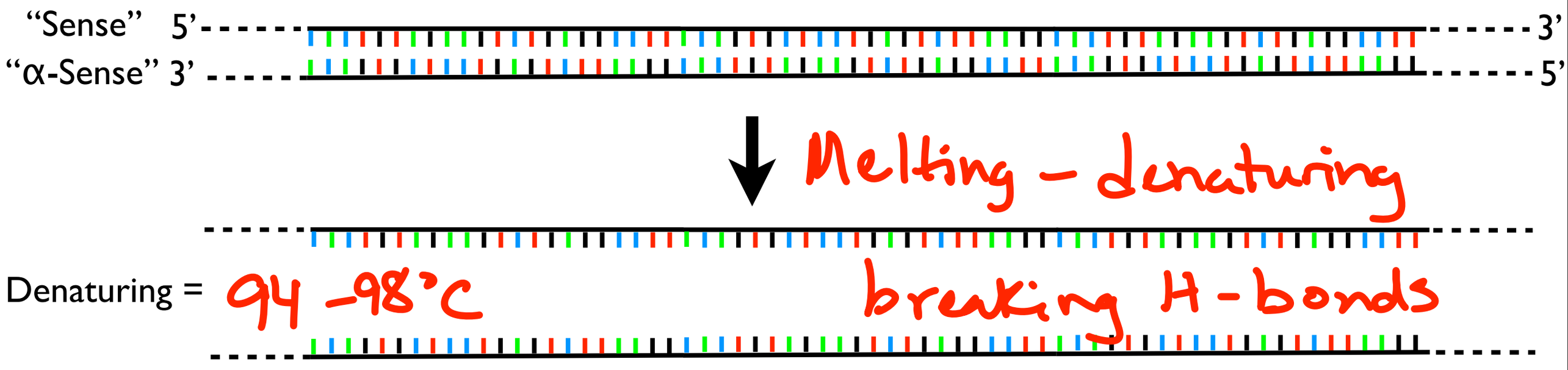


- Primer dimers - avoid complements
- AAAAAAAAGCTA
-

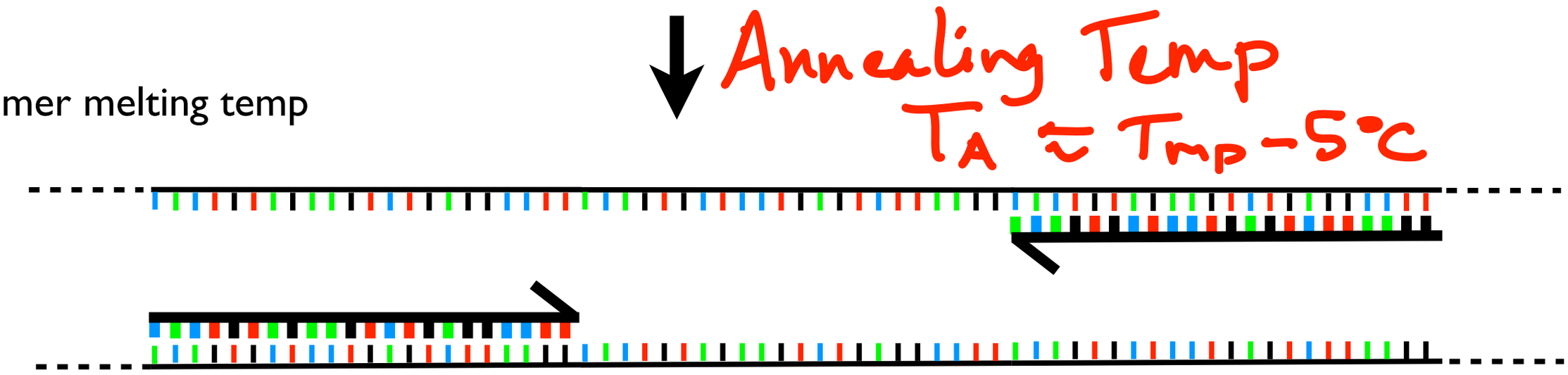


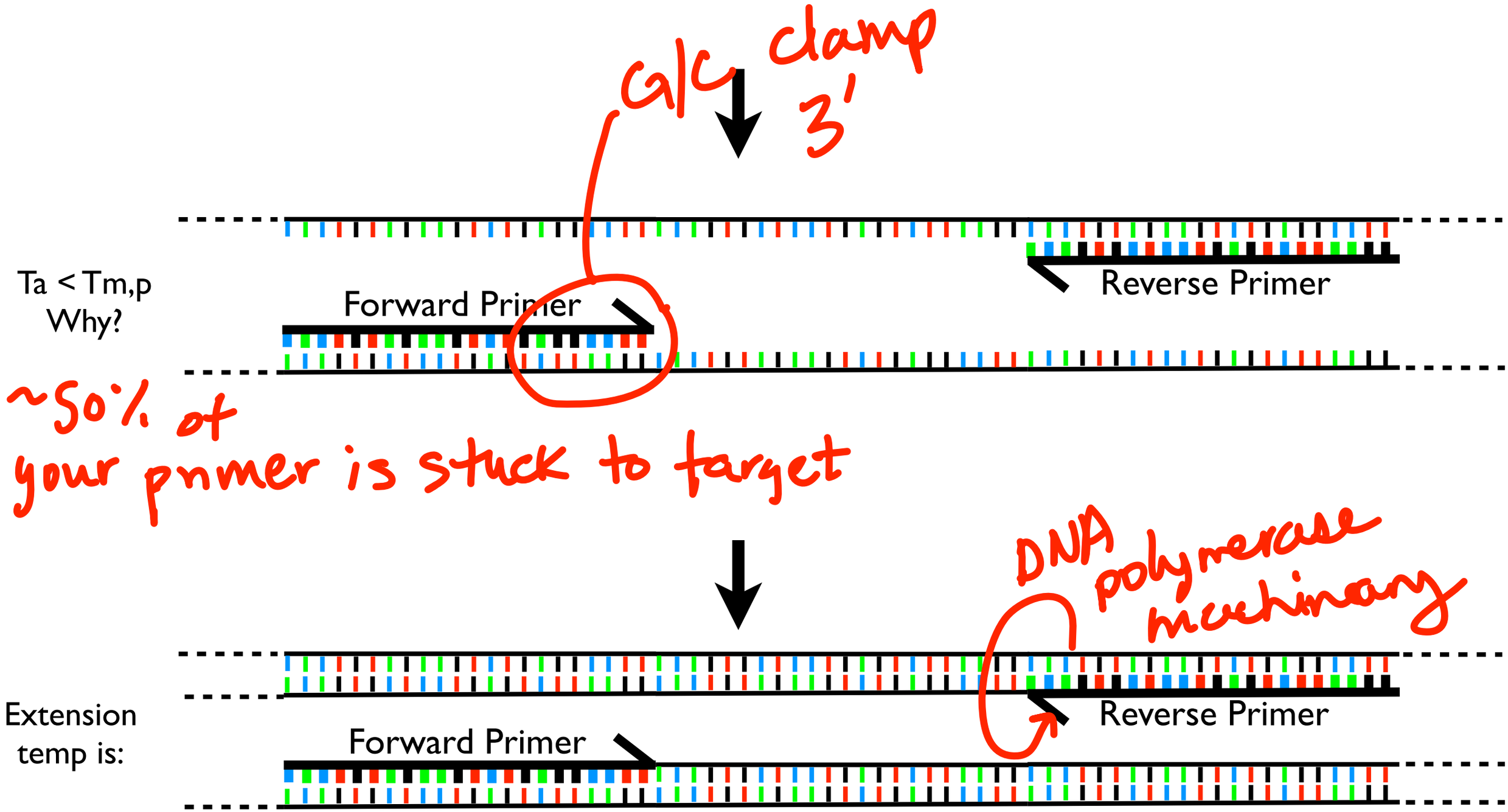
Thermodynamics of DNA Duplex, New Mexico State University

PCR – polymerase chain rxn



Tm,p = primer melting temp




clamp
3'
Ta < Tm,p
Why?
Forward Primer
Reverse Primer
~50% of
your primer is stuck to target
DNA
polymerase
machinary
Extension
temp is:
Forward Primer
Reverse Primer
~72°C
~1min/1000b

Your PCR:

Component	Purpose
Primers (2)	define target
Template	pCX-EGFP "pattern"
Master Mix	polymerase
	dNTPs
	buffers - 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:

- Safety training
- Clean up your PCR run
- RE digest