DNA Engineering: M1D2 Lab Talk

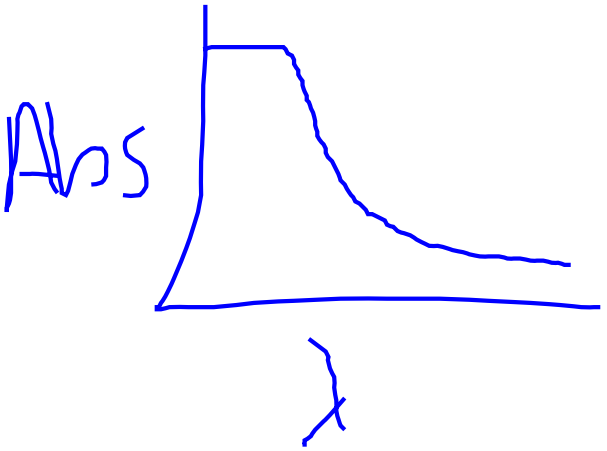
20.109 (F12)

09.13.12

Quick review of lab practical glitches…

50X

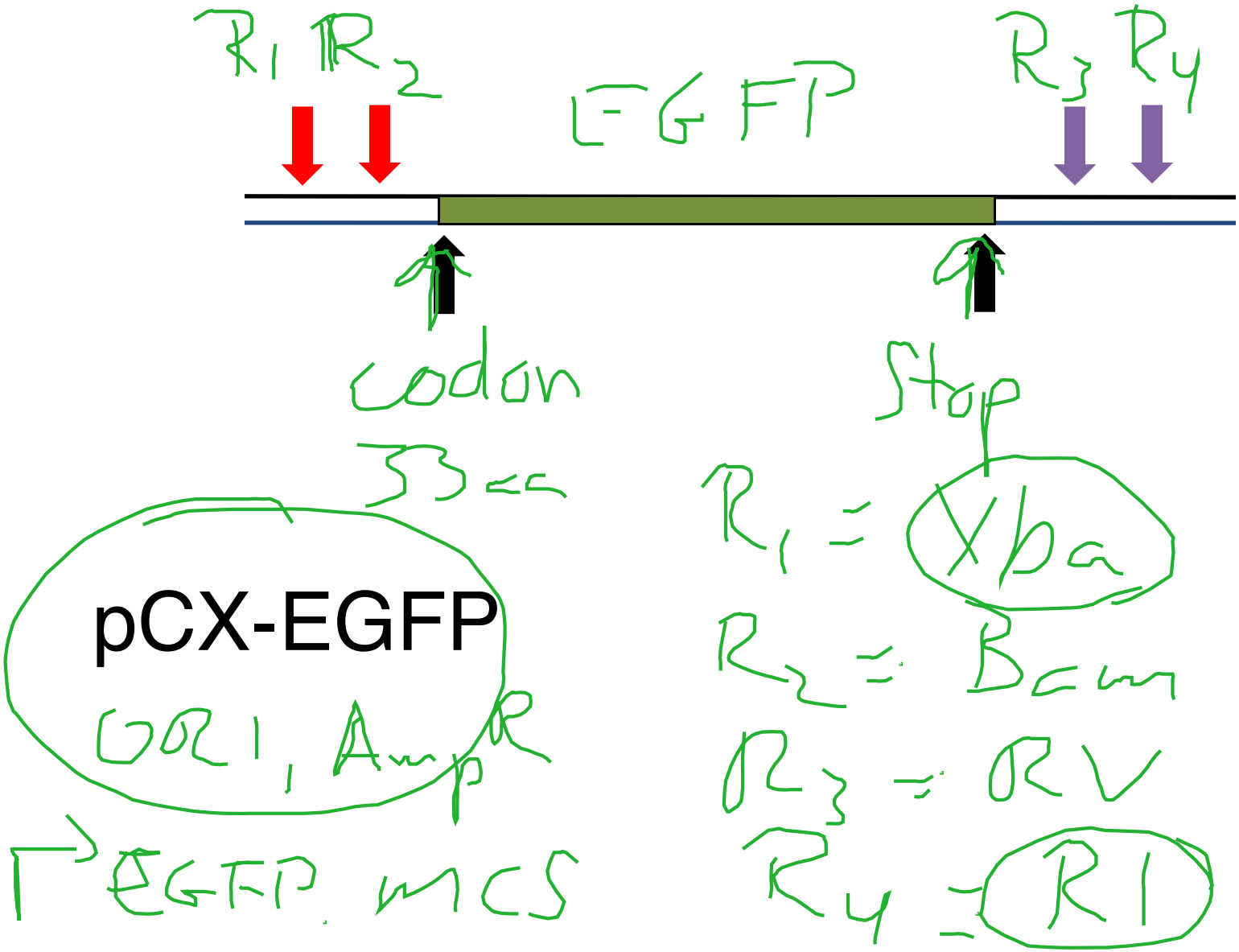
? throw gloves



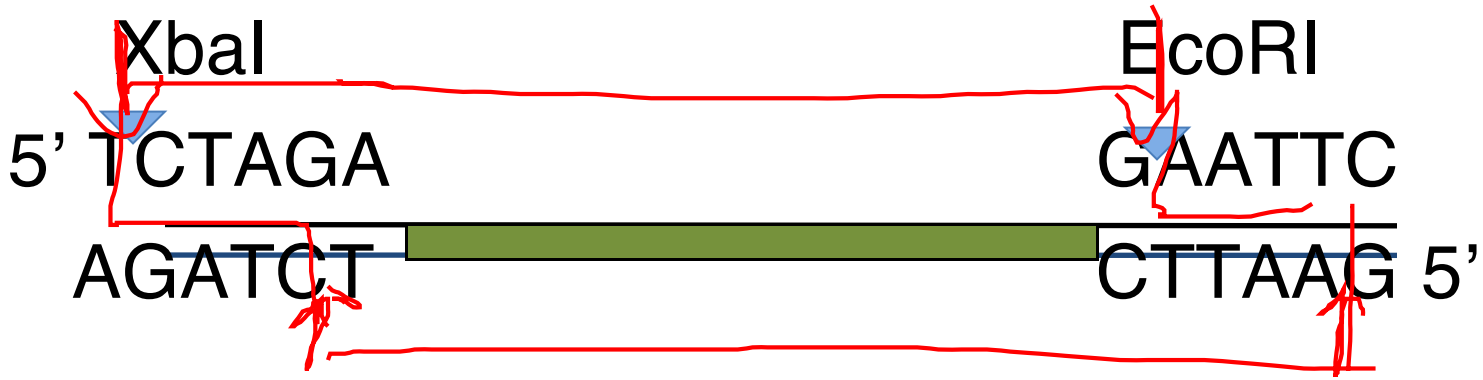
$1 + 1 \div 2$

$V_1C_1 = V_2C_2$

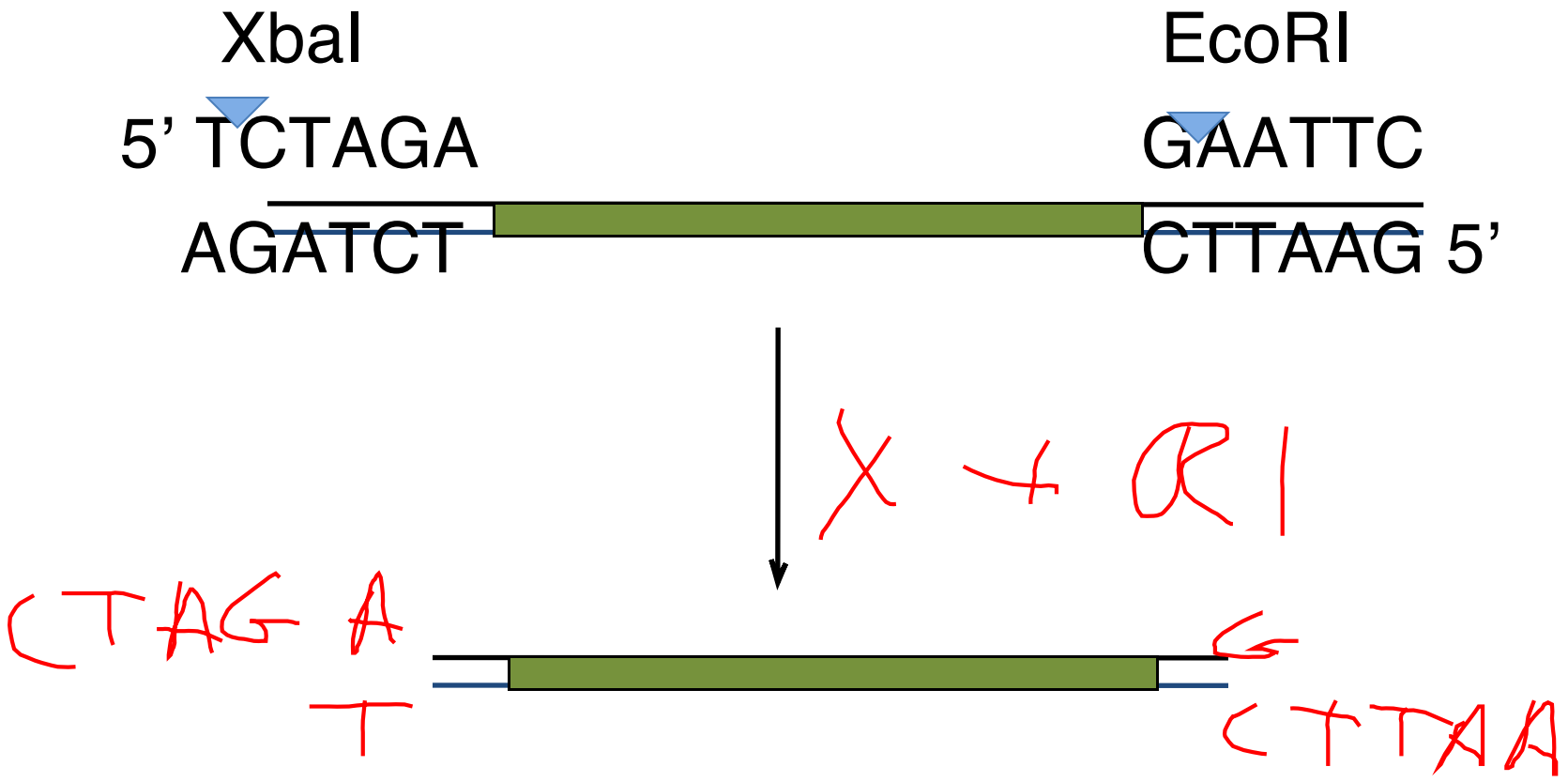
"Last time in 20.109…"



Digesting your PCR product



Digesting your PCR product



(Sub)Cloning PCR product into new plasmid

XbaI

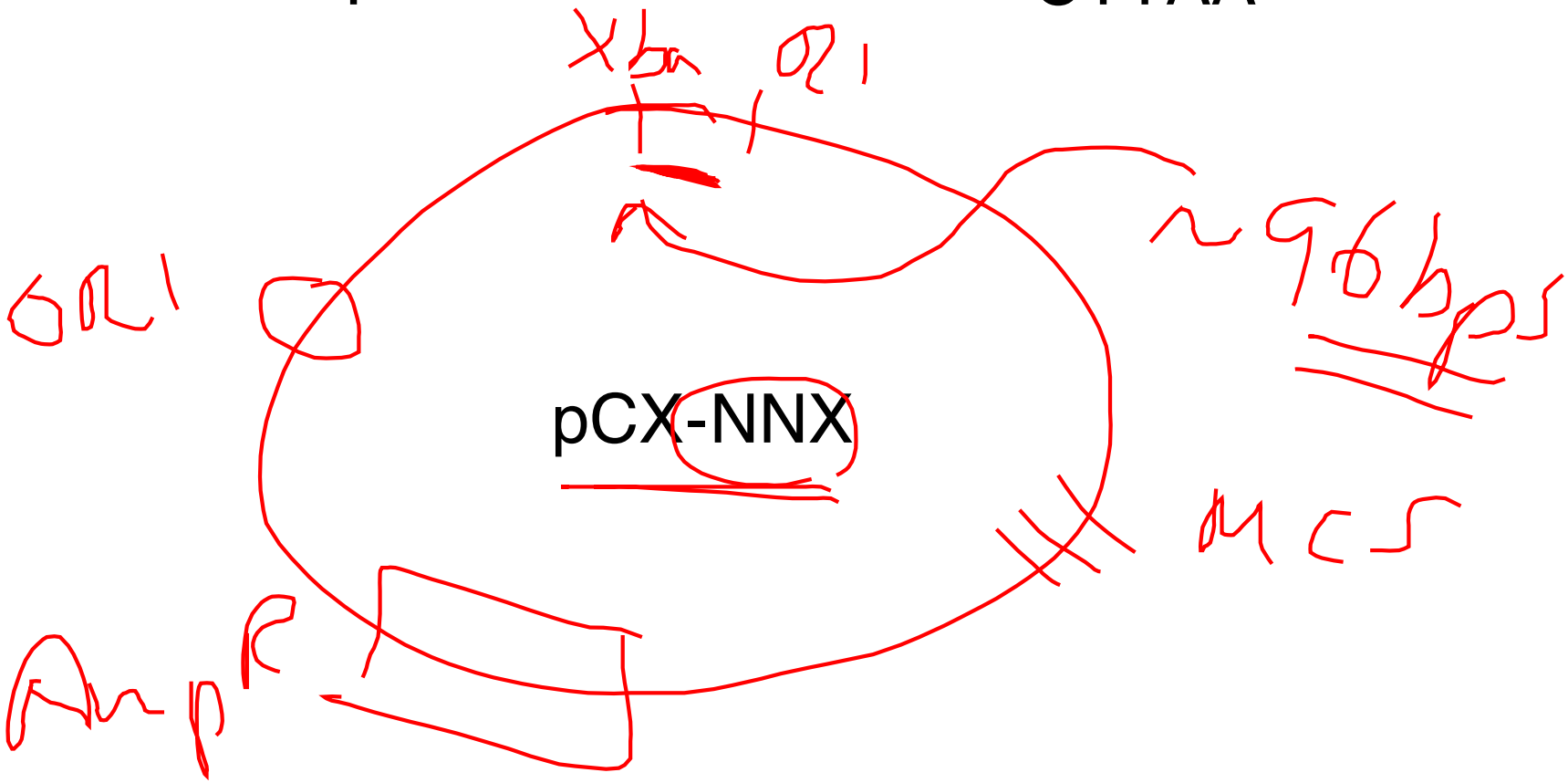
CTAGA

T

EcoRI

G

CTTAA



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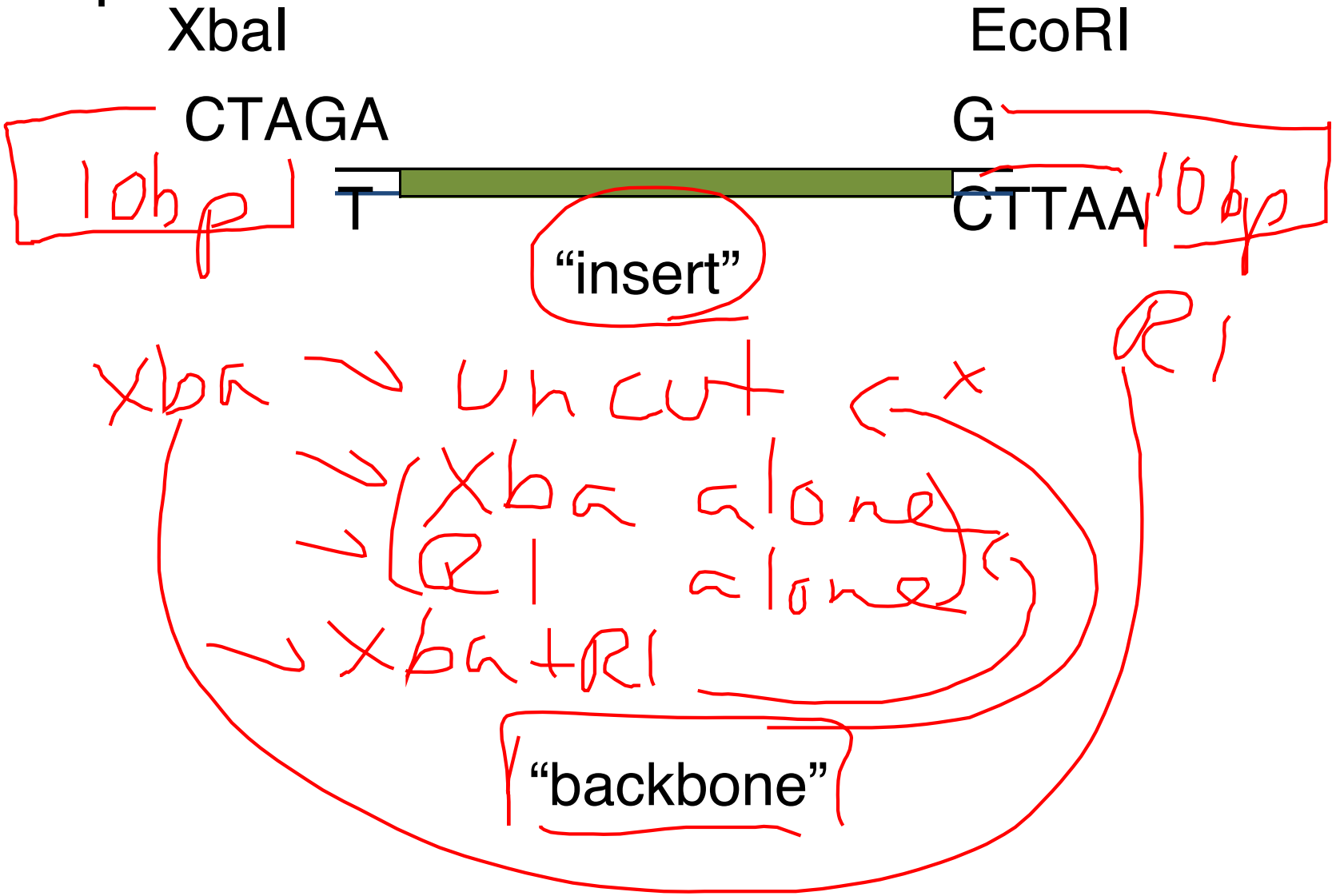

But first, clean up your PCR product

Why?

1. Remove dNTPs + Taq
2. ΔBuffer

But first, clean up your PCR product

How?

1. Bind DNA to resin on column (= silica) ↑salt ↓pH
2. Wash in presence of EtOH keeps DNA on membrane
3. Elute DNA in small volume ↓salt ↑pH

Your reactions

Component	Details
DNA	PCR prod/pCX-MMX
Enzyme	XbaI EcoRI
10XNEB	1x final
H_2O	to final 25
Temperature	32°

Be careful with stock solutions and order of addition!!

In lab today and next week