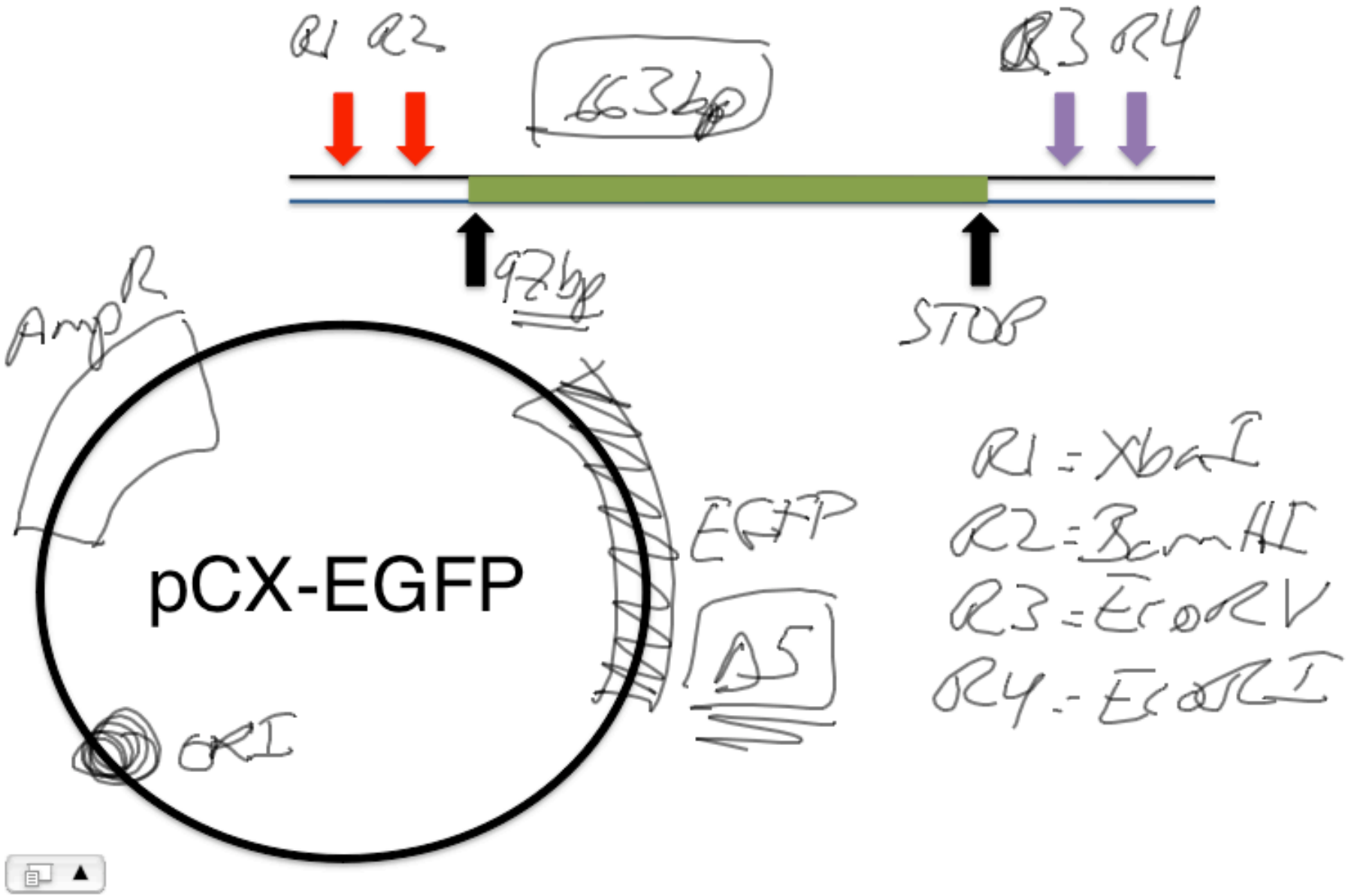
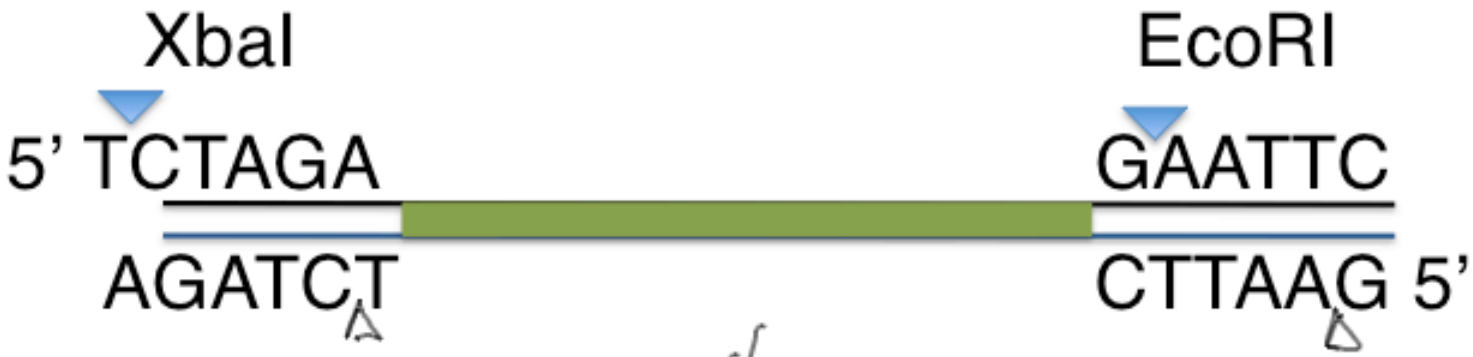
"Last time in 20.109…"



Digesting your PCR product



digest w/ XbaI, EcoRI

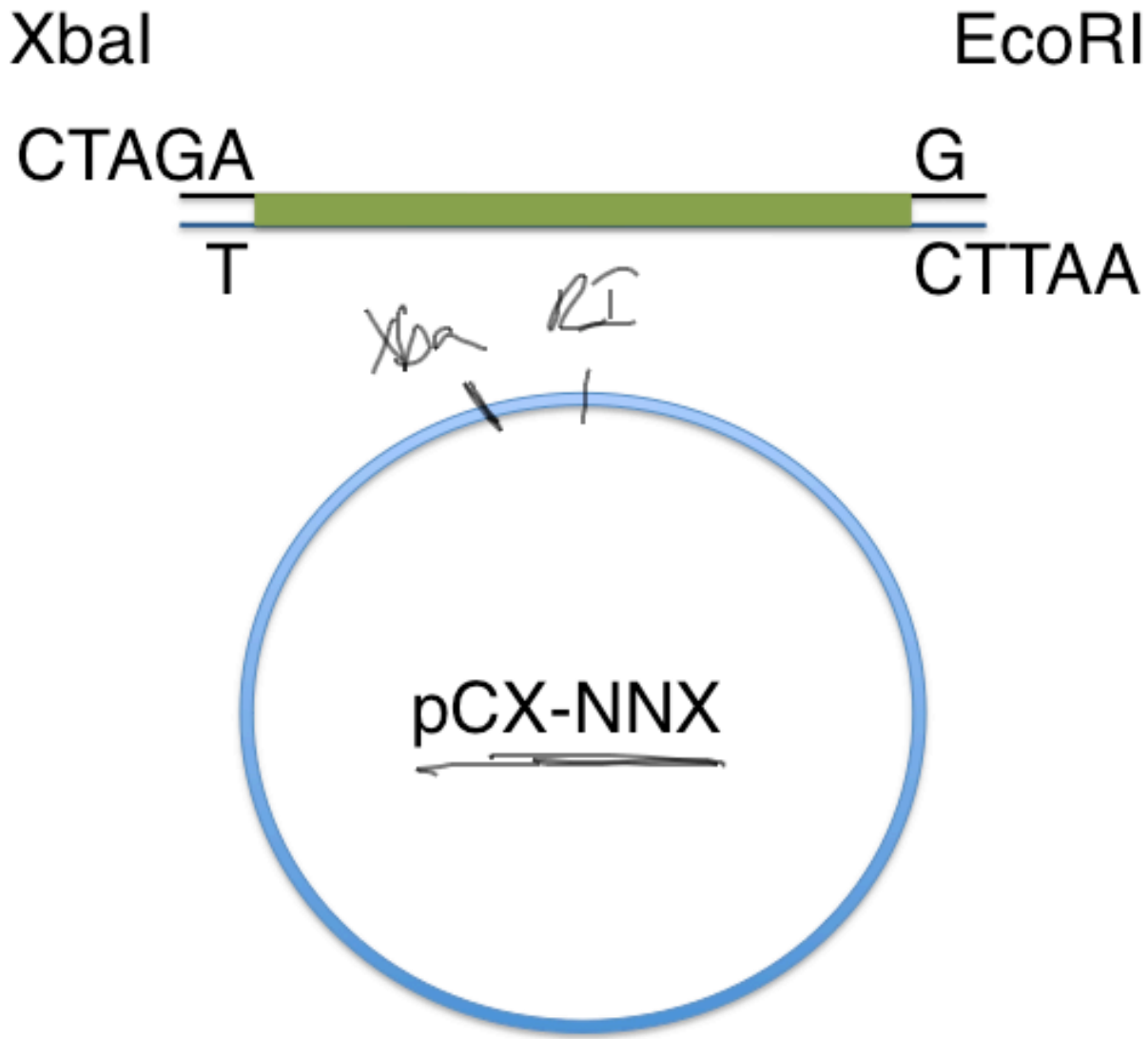
CTAG-A

T

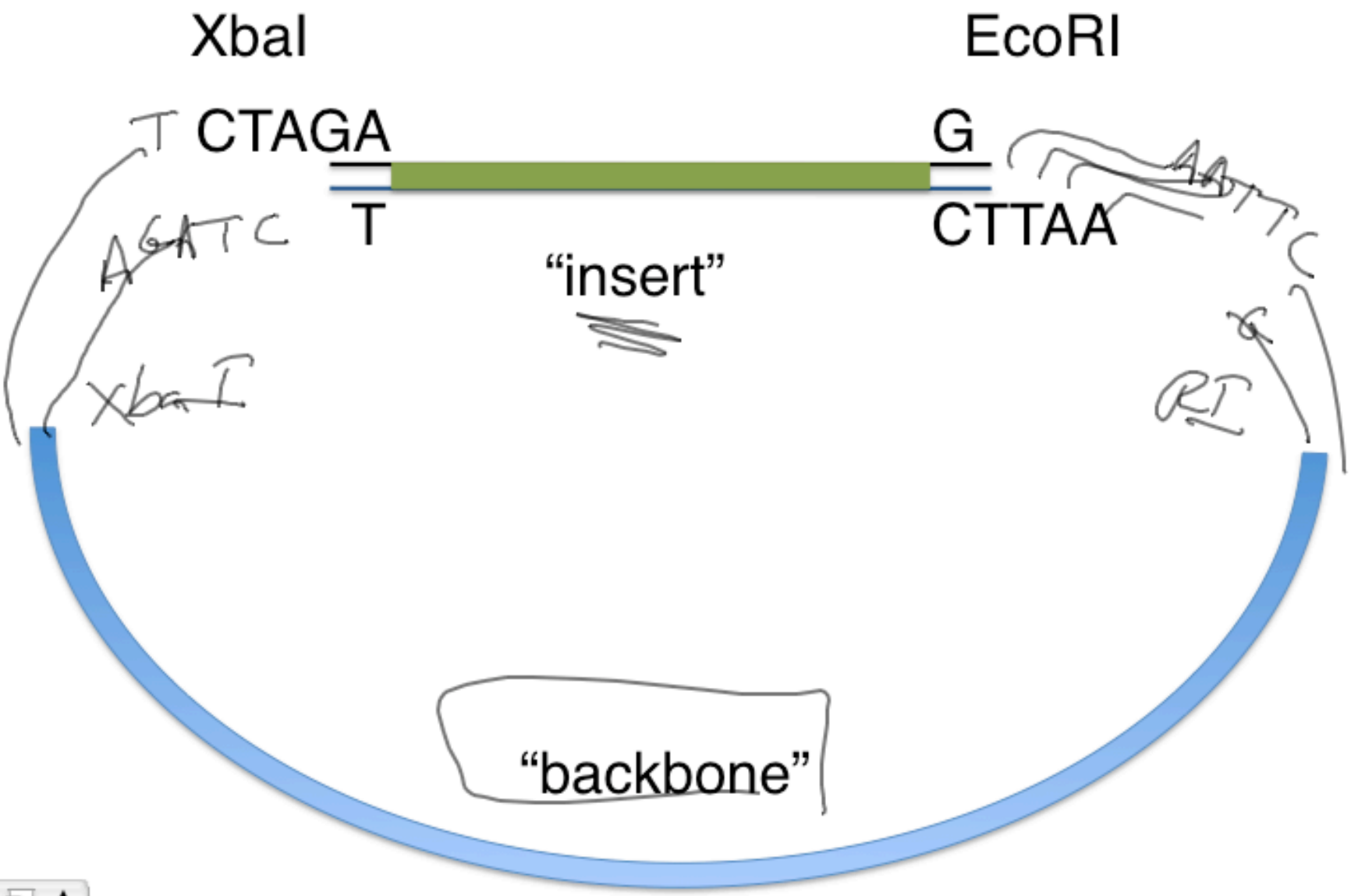
G

CTTAA

(Sub)Cloning PCR product into new plasmid



(Sub)Cloning PCR product into new plasmid



But first, clean up your PCR product

Why?

1. Buffer
2. Get Rid of dNTPs

But first, clean up your PCR product

How?

1. Bind DNA to resin on column (= silica)
   High Salt, low pH
2. Wash in presence of EtOH
   ~~Keeps~~ DNA ppt'd
3. Elute DNA in small volume
   Low Salt; High pH

Your reactions

	Component	Details
2	DNA	insert = PCR Product bkb = PCR w/ RVX
4	Enzyme	Xba I EcoRI ← indiv. + double
3	10XNEB?	10X → 1X
1	H_2O	bring to a final volume ~25 µl.
5	Temperature	37°C

Be careful with stock solutions and order of addition!!

In lab today and next week