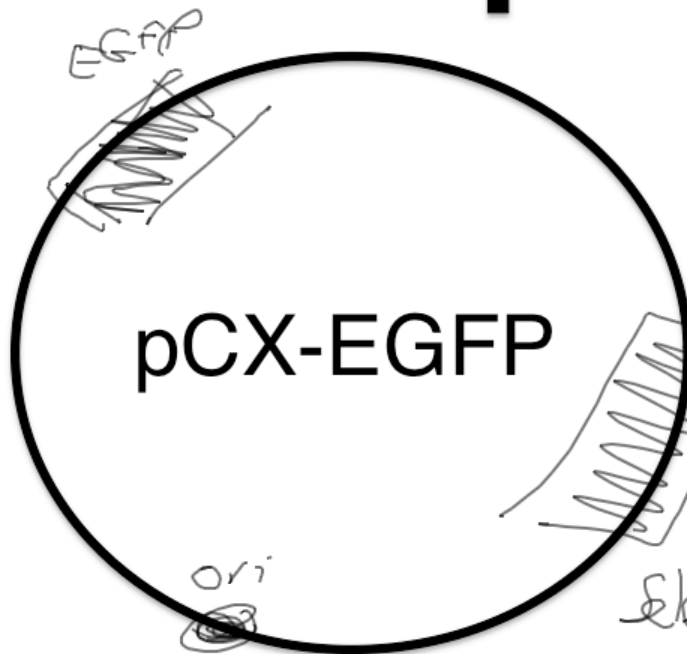
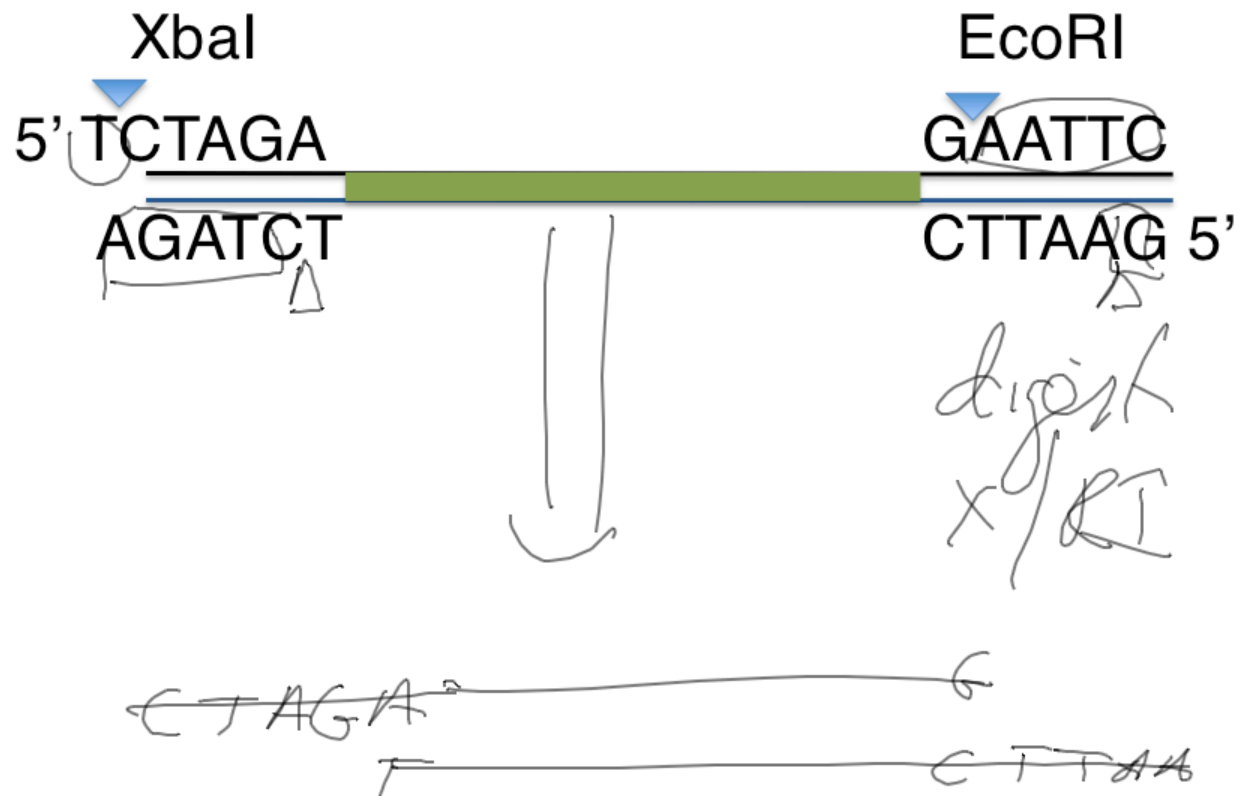


“Last time in 20.109...”

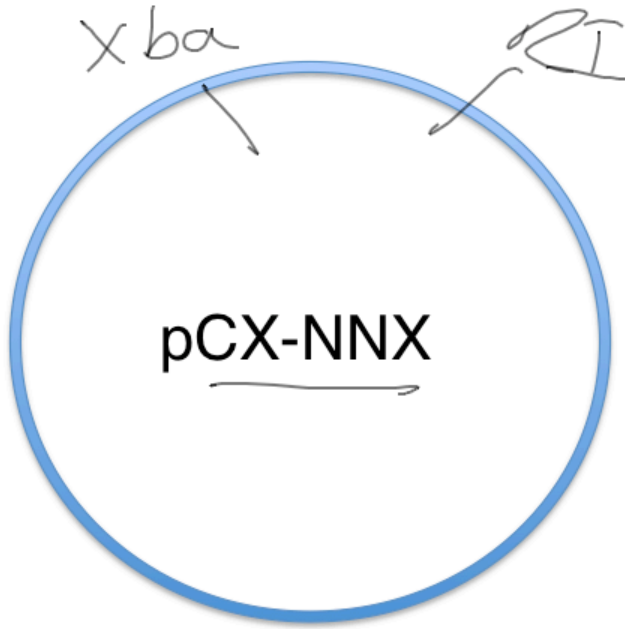


- $R_1$  XbaI
  - $R_2$  BamHI
  - $R_3$  EcoRV
  - $R_4$  EcoRI
- Selectable marker

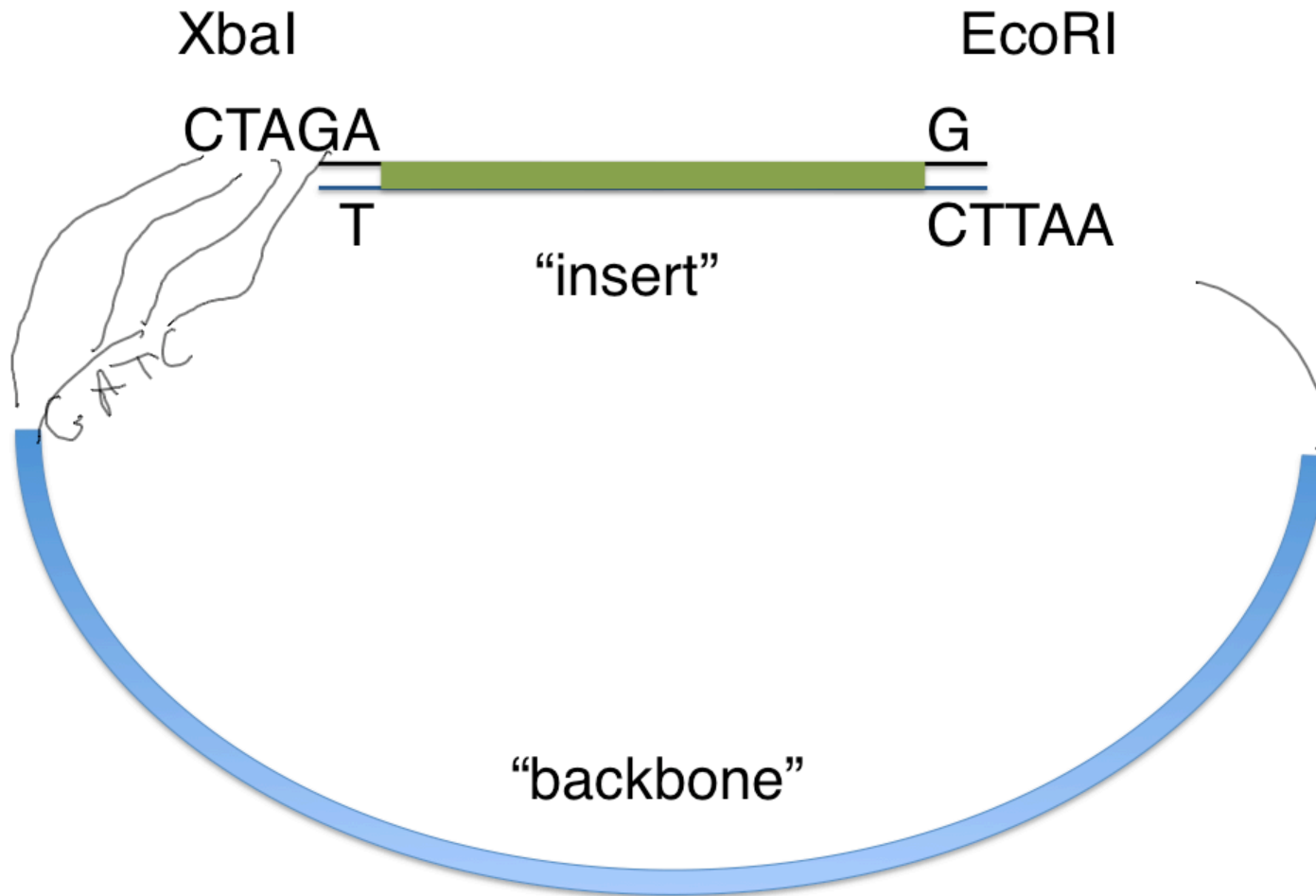
# Digesting your PCR product



(Sub)Cloning PCR product into new plasmid



# (Sub)Cloning PCR product into new plasmid



But first, clean up your PCR product

Why?

1. dNTPs, Top /  $X_{2+125}$
2. Buffer

But first, clean up your PCR product

How?



resin  
Qiagen

1. Bind DNA to resin on column (= silica)

High salt  
Low pH

2. Wash in presence of EtOH

3. Elute DNA in small volume

← keeps DNA bound  
out of sol'n

(w/ EtOH) High pH  
Low salt

## Your reactions

Component	Details
( <del>3</del> ) DNA (2)	PCR Prod (cleaned) PCR-VNX
(4) Enzyme	( $\times 60$ ) ( $\times 60$ ) (R1)
10XNEB (3)	10X $\rightarrow$ <u>1X</u>
( <del>2</del> ) H <sub>2</sub> O (1)	final volume of 25 $\mu$ l
(5) Temperature	<u>37°C</u>

Be careful with stock solutions and order of addition!!



In lab today and next week

(R) PCR product → Beware 5' from each  
→ Clean + cut  
→ 3:30 WAC

Next  
week : Run Gel  
Excise bands of DNA  
Ligate + transform