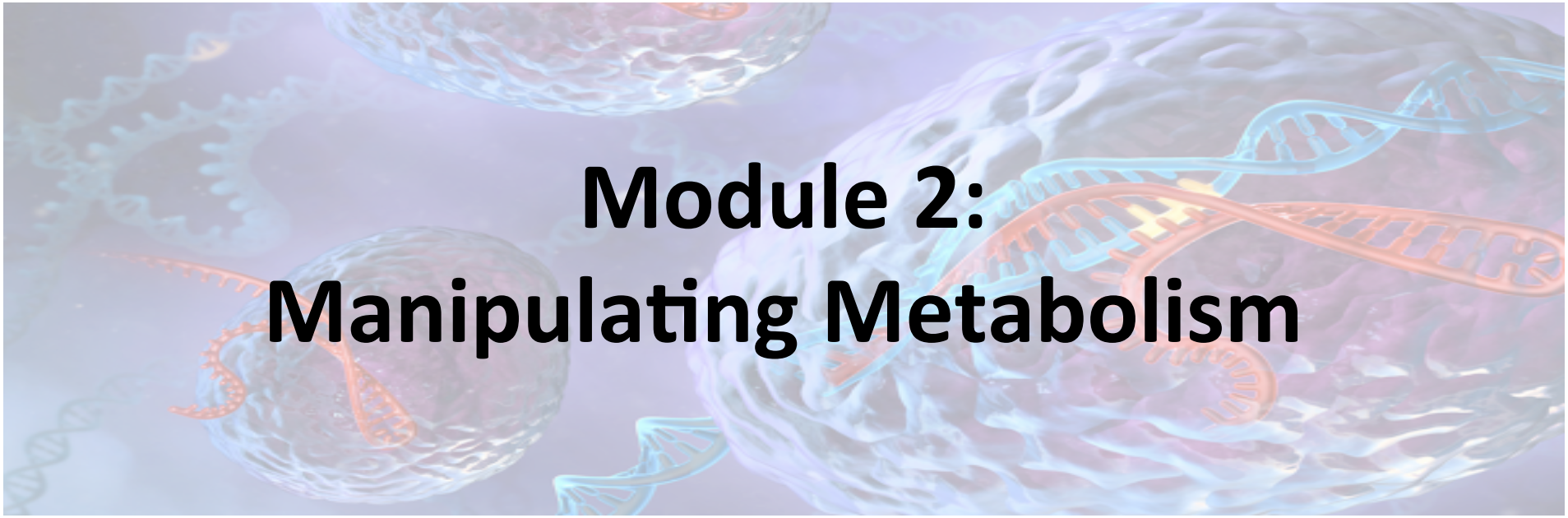


Module 2: Manipulating Metabolism

DNA engineering

10/12/17

Wrapping up Mod 1

- Data summary draft submitted!!
  - 

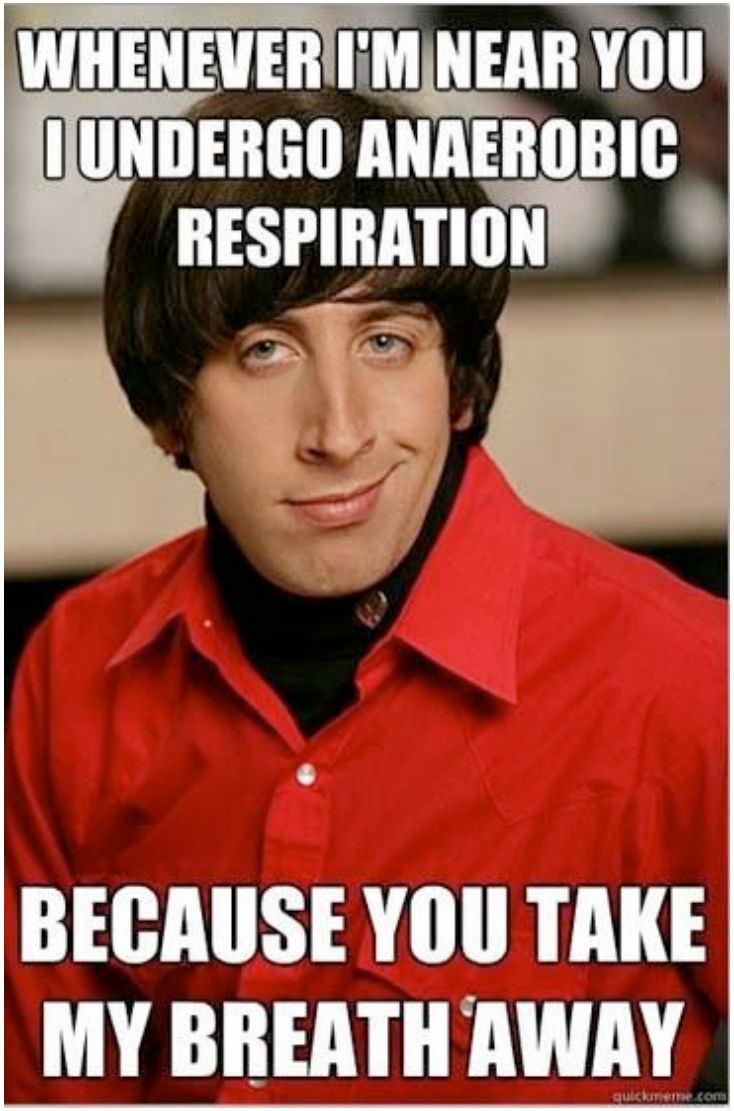
  - Comments to be returned on Oct. 16
  - Revision due by 10 pm on Oct. 22
- Mini presentation due by 10 pm on Oct. 14
- Blog post due by 10 pm on Oct. 23

Mod 2 assignment overview

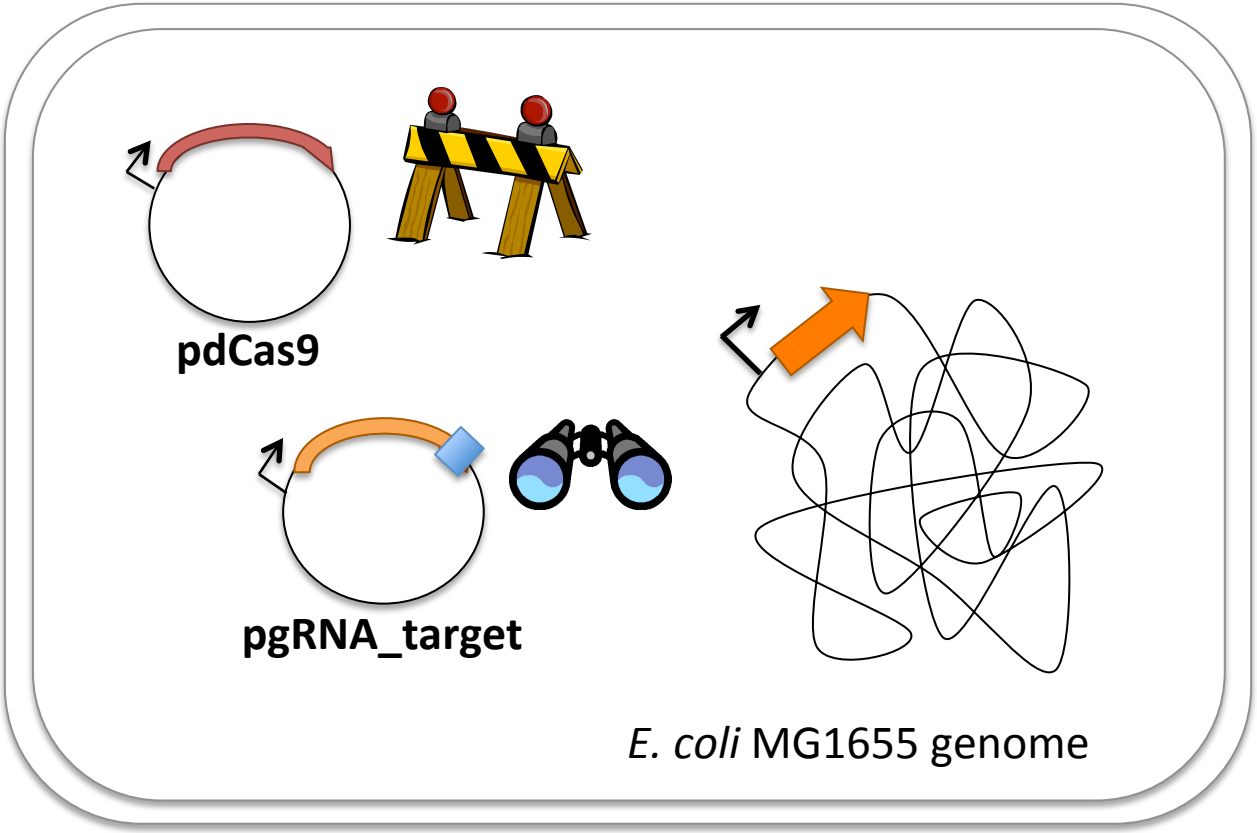
- Journal club presentation
  - Communicate the key findings from a peer-reviewed published article
  - Completed individually
- Research article
  - Describe your results and analysis in a written 'formal' document
  - Completed individually

Your engineering task in Mod 2:

Increase production of ethanol or acetate in *E. coli* MG1655 by manipulating the fermentation pathway

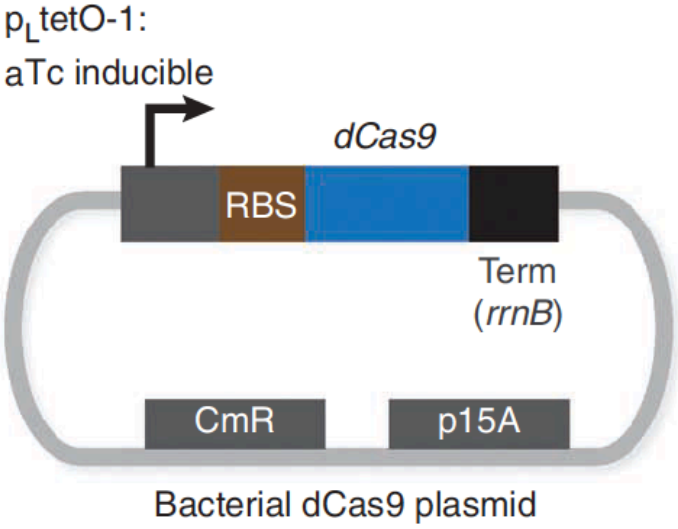

WHENEVER I'M NEAR YOU
I UNDERGO ANAEROBIC
RESPIRATION
BECAUSE YOU TAKE
MY BREATH AWAY
quickmeme.com

CRISPRi system overview



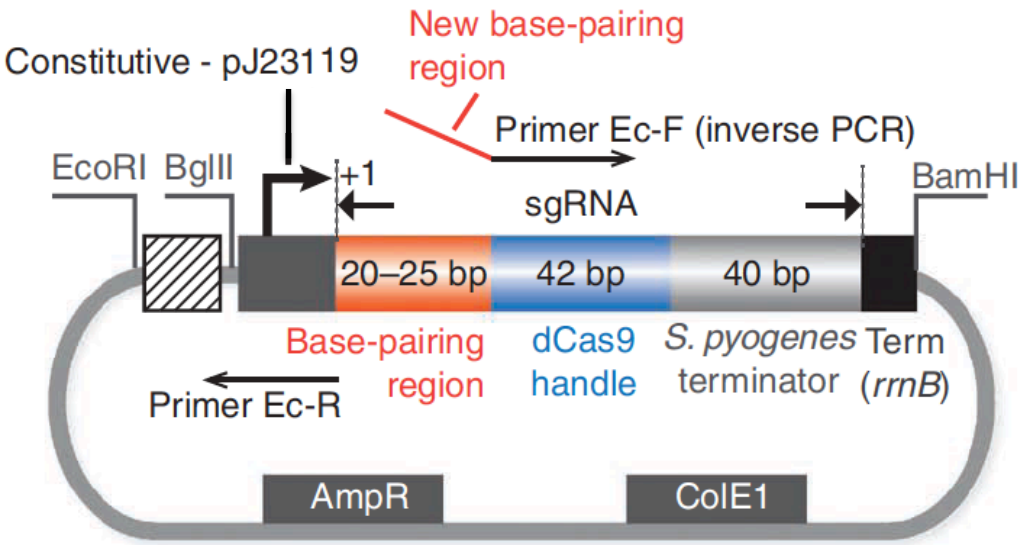
- Target gene
- pgRNA_target
- pdCas9

Closer look at pgRNA and pdCas9



Bacterial dCas9 plasmid

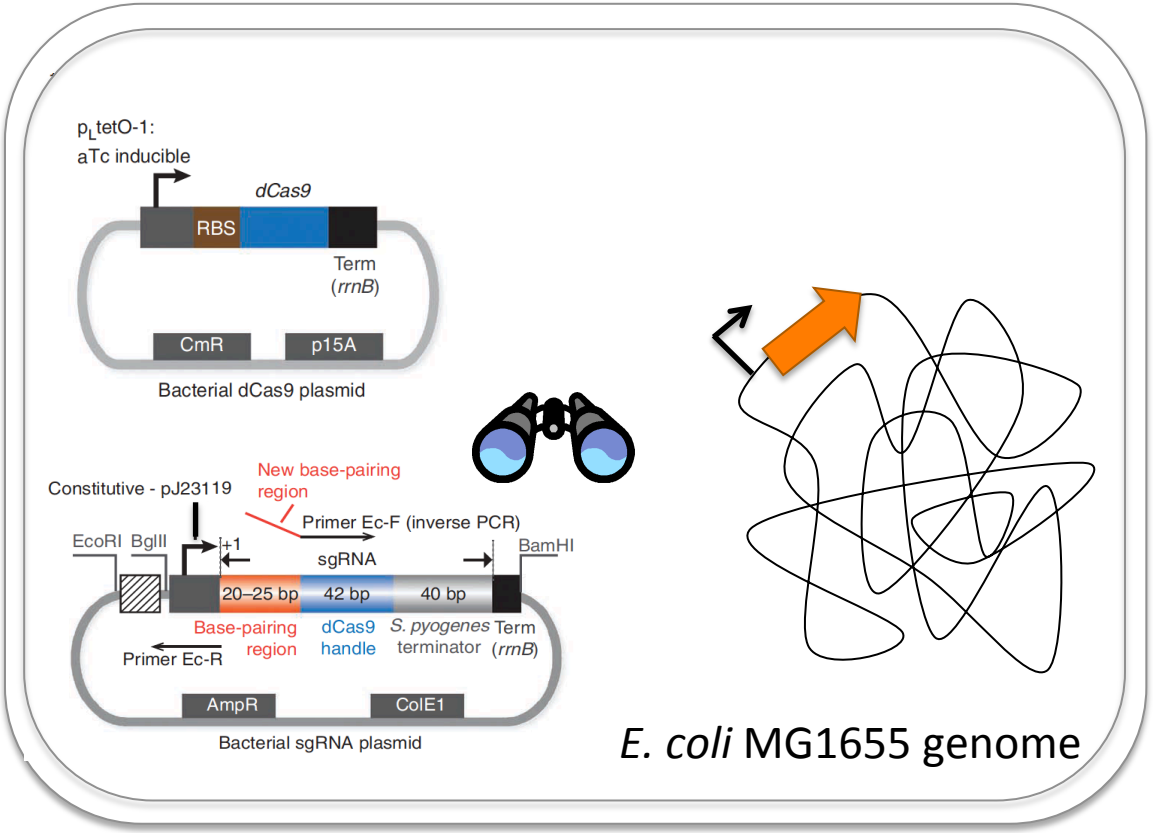
- Confirmation digest prepared on M2D1



Bacterial sgRNA plasmid

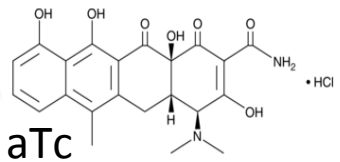
- Insert (gRNA target sequence) designed on M2D2

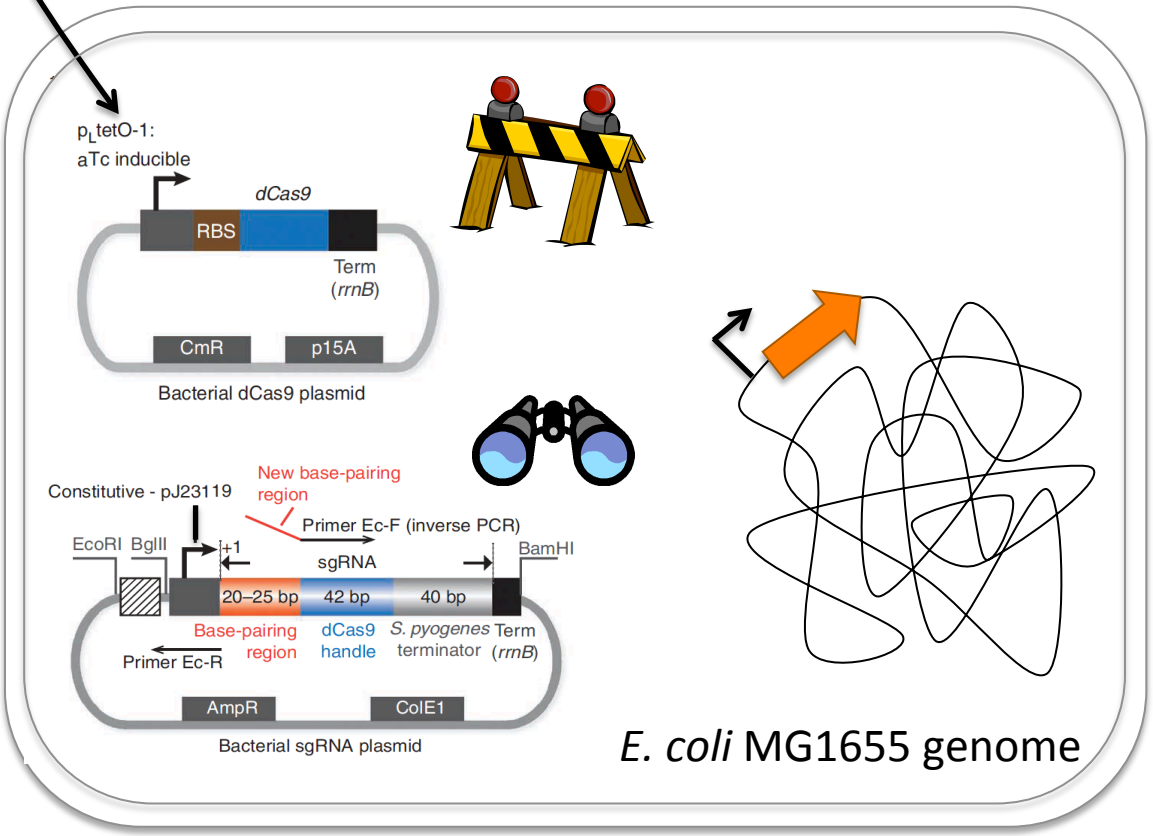
CRISPRi 'inactive' in absence of inducer



- pgRNA_target expressed constitutively
  - Always transcribed and binding to target gene

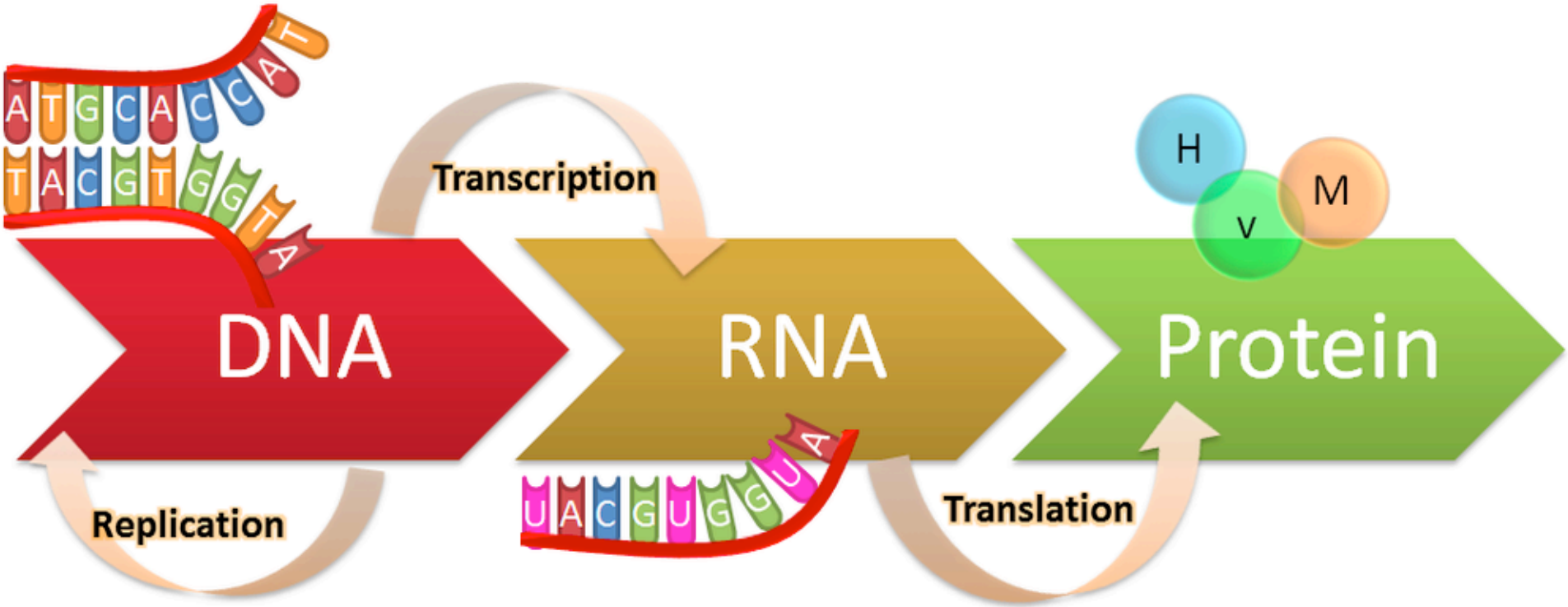
CRISPRi ‘blocks’ gene expression in presence of inducer





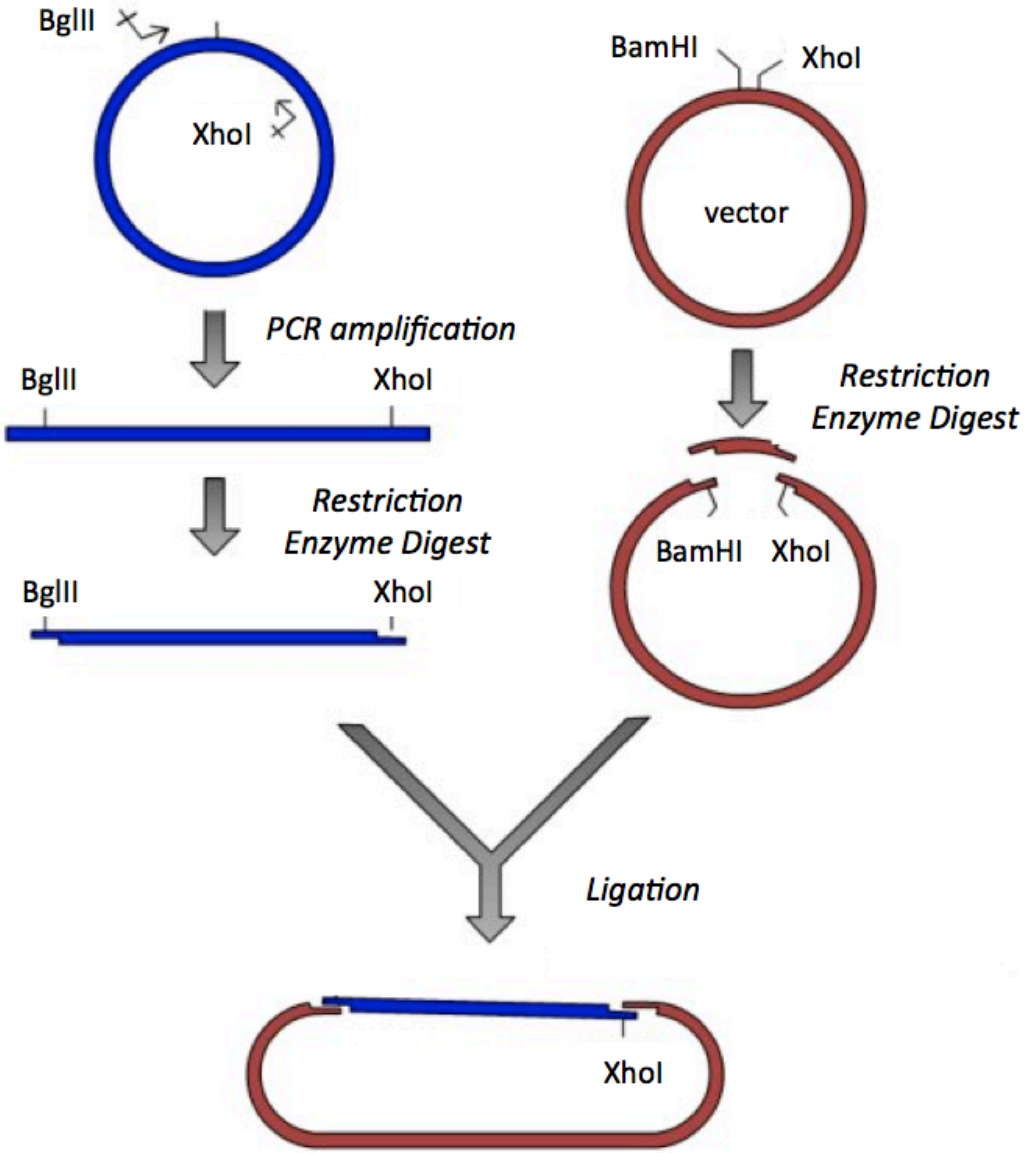
- pdCas9 expressed when aTc added
  - When transcribed associates with pgRNA_target / target gene

The central dogma



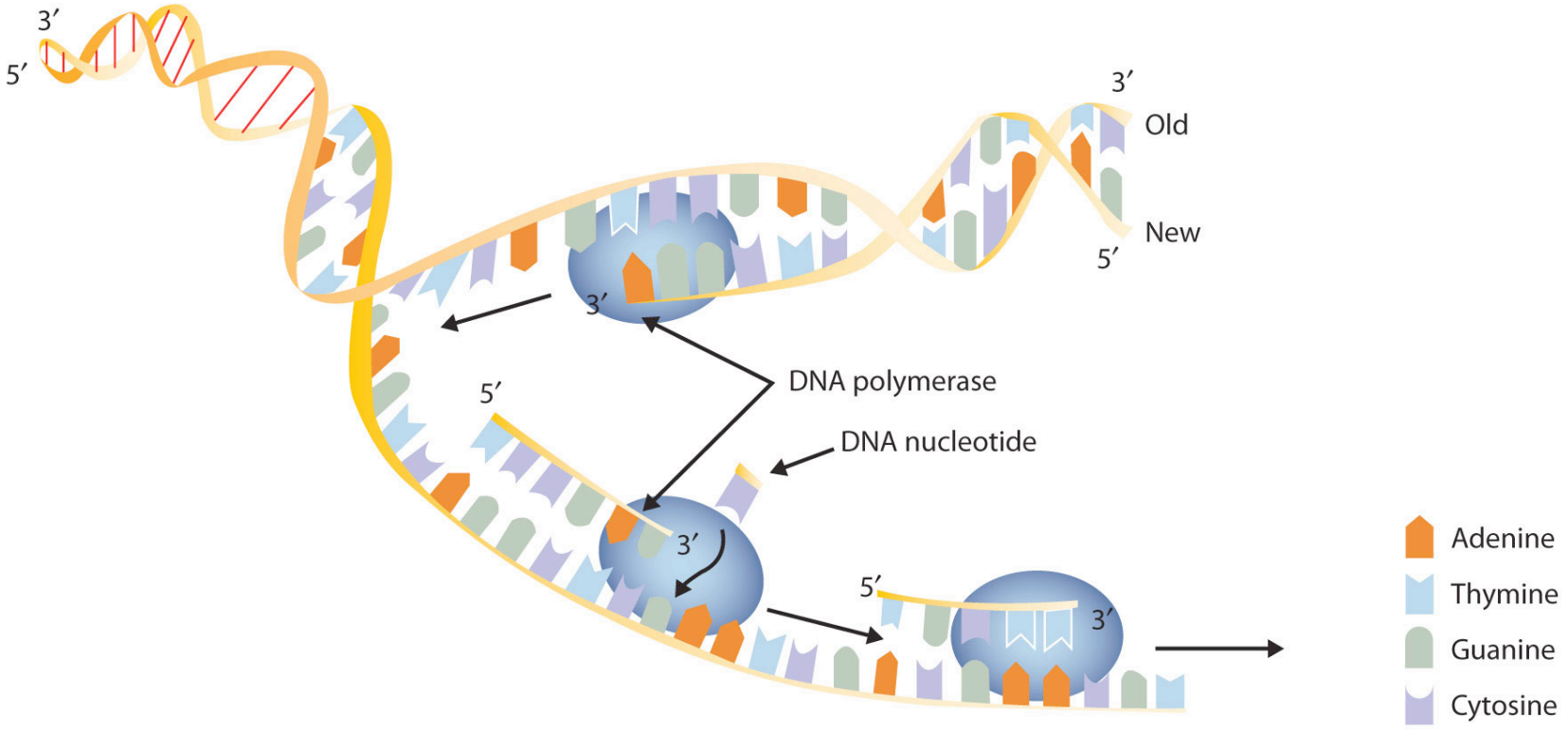
http://genius.com/Biology-genius-the-central-dogma-annotated

How do we engineer DNA?



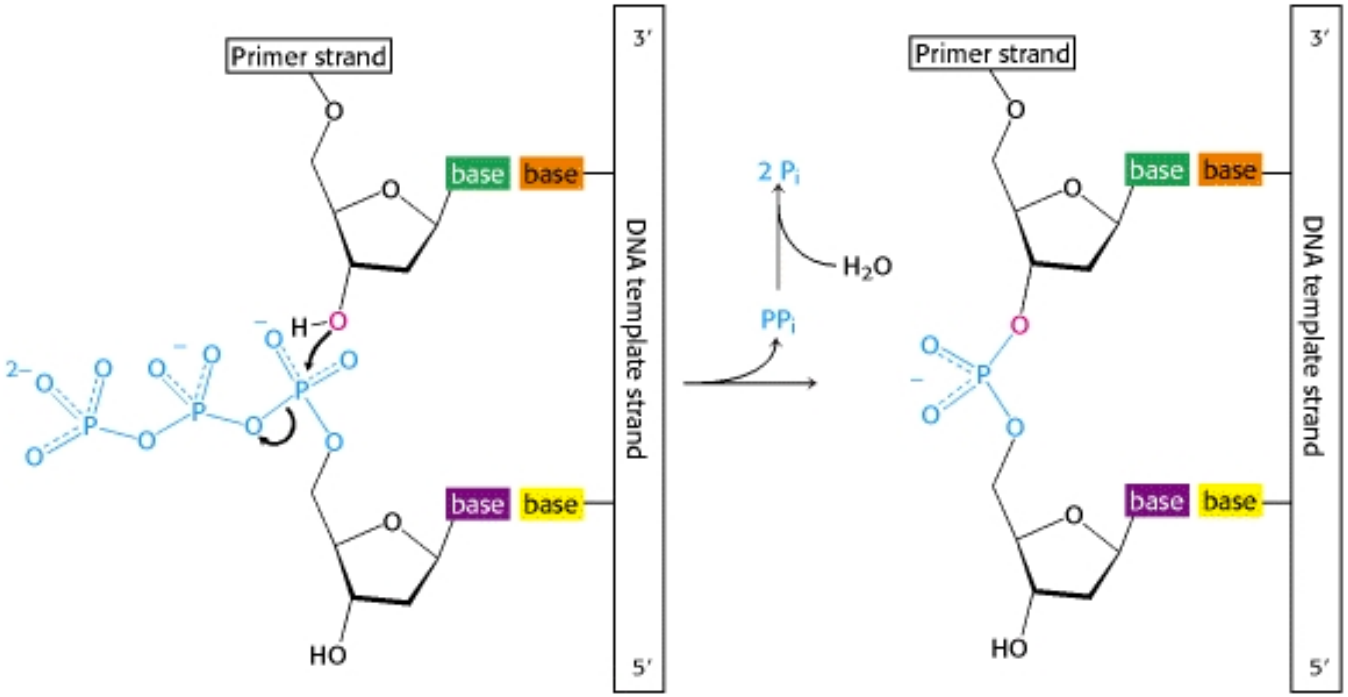
1. Amplification
2. Digestion
3. Ligation

Amplification



Who are the key players?

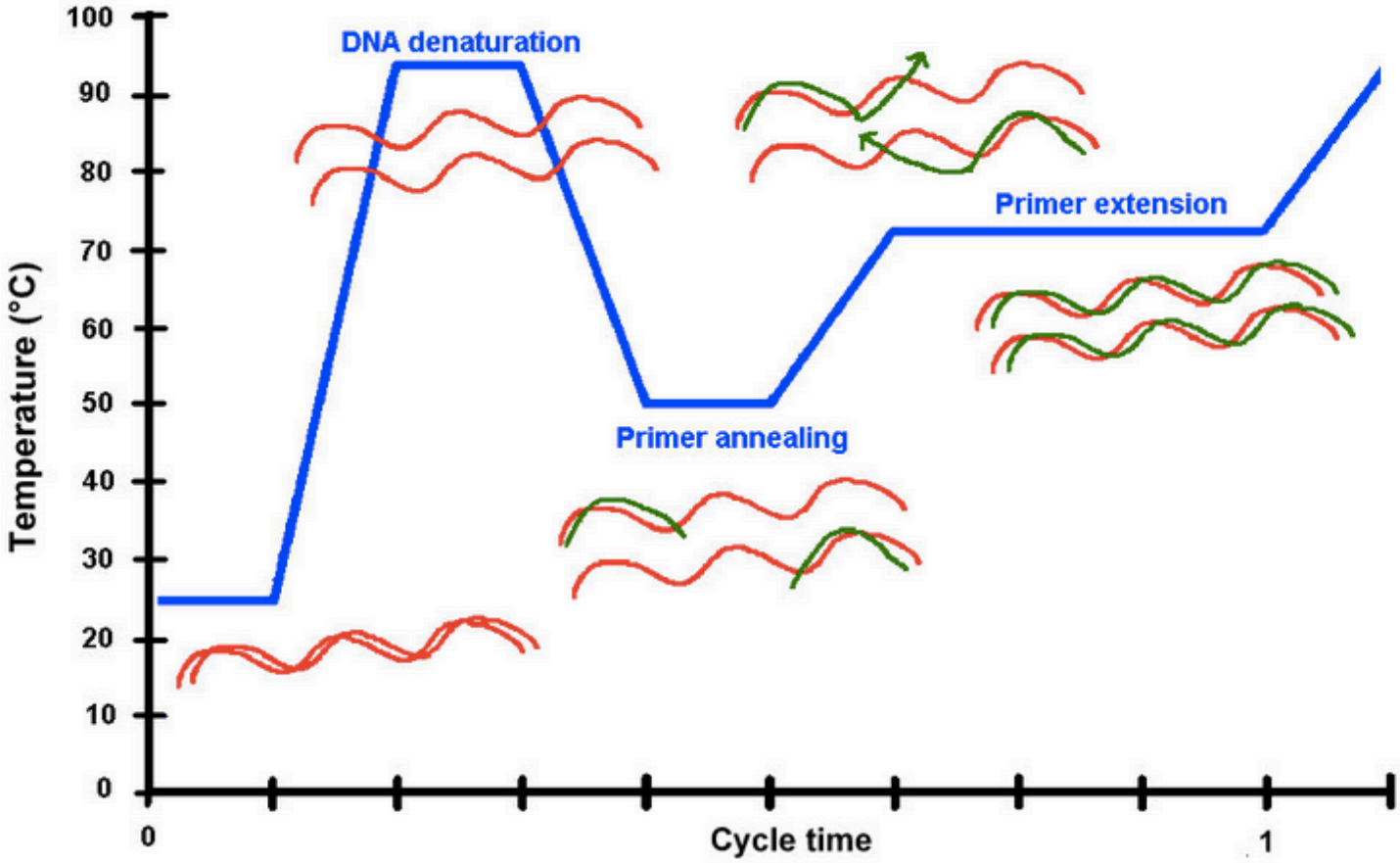
DNA polymerase



- Catalyzes formation of polynucleotide chains
- Requires a primer base-paired to template

http://www.ncbi.nlm.nih.gov/books/NBK22374/

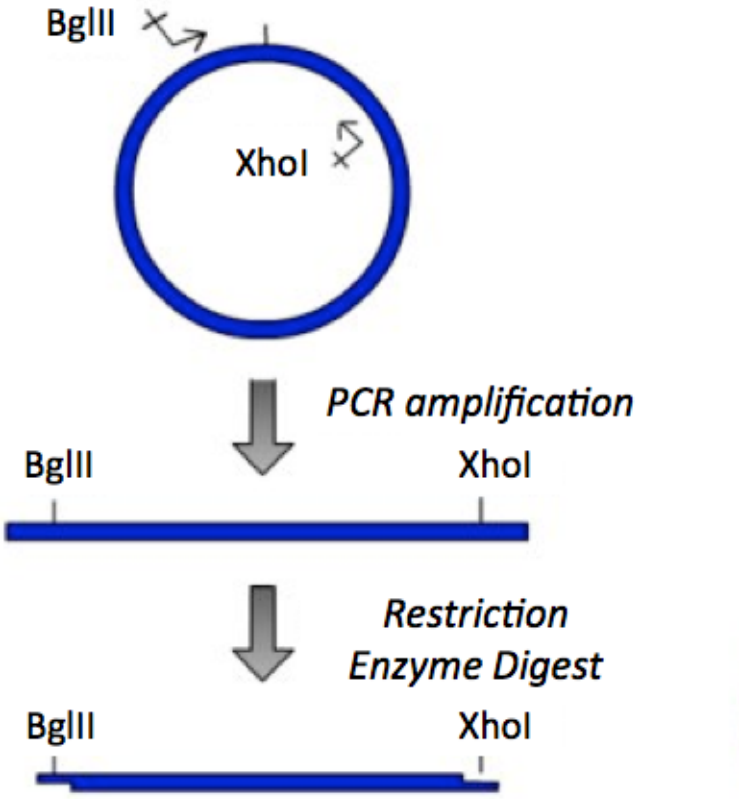
Polymerase chain reaction (PCR)



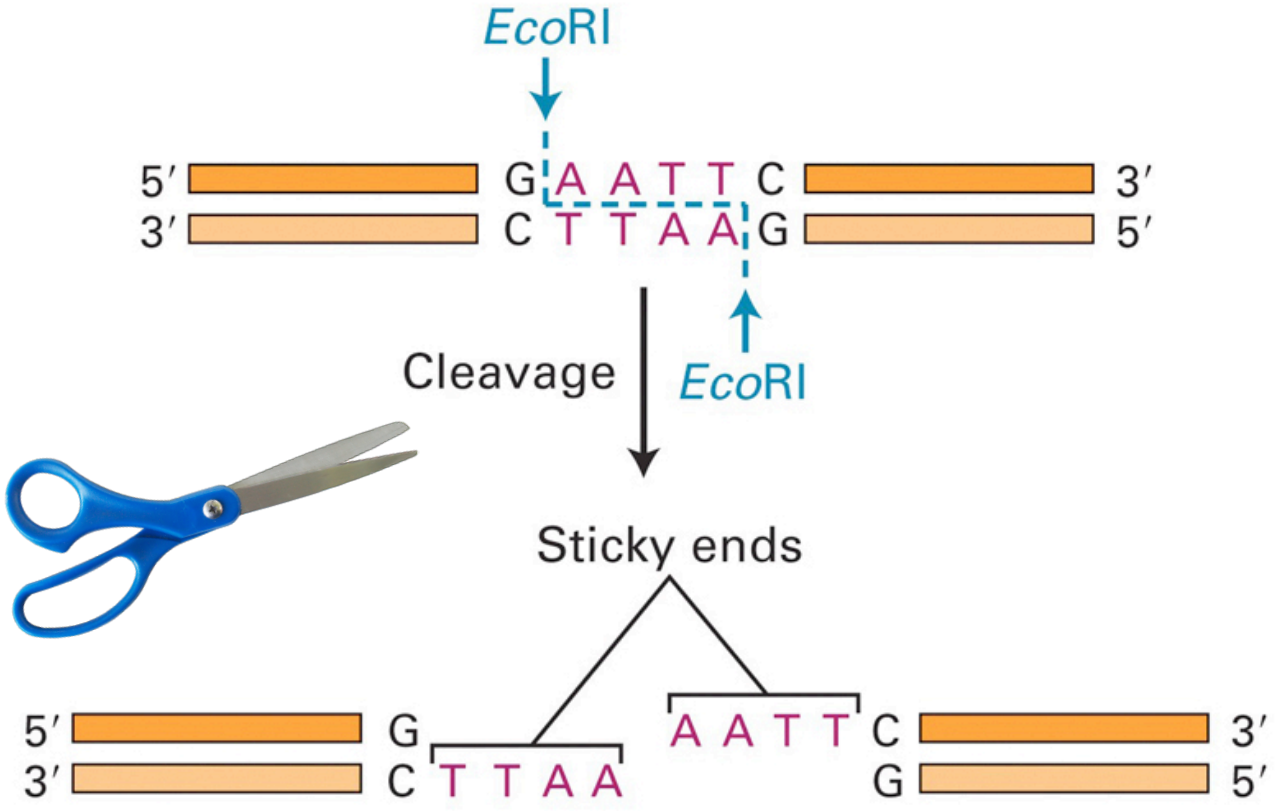
How many cycles until your product is generated?

What are we amplifying?

- Primers enable you to specify which region of DNA is amplified by polymerase

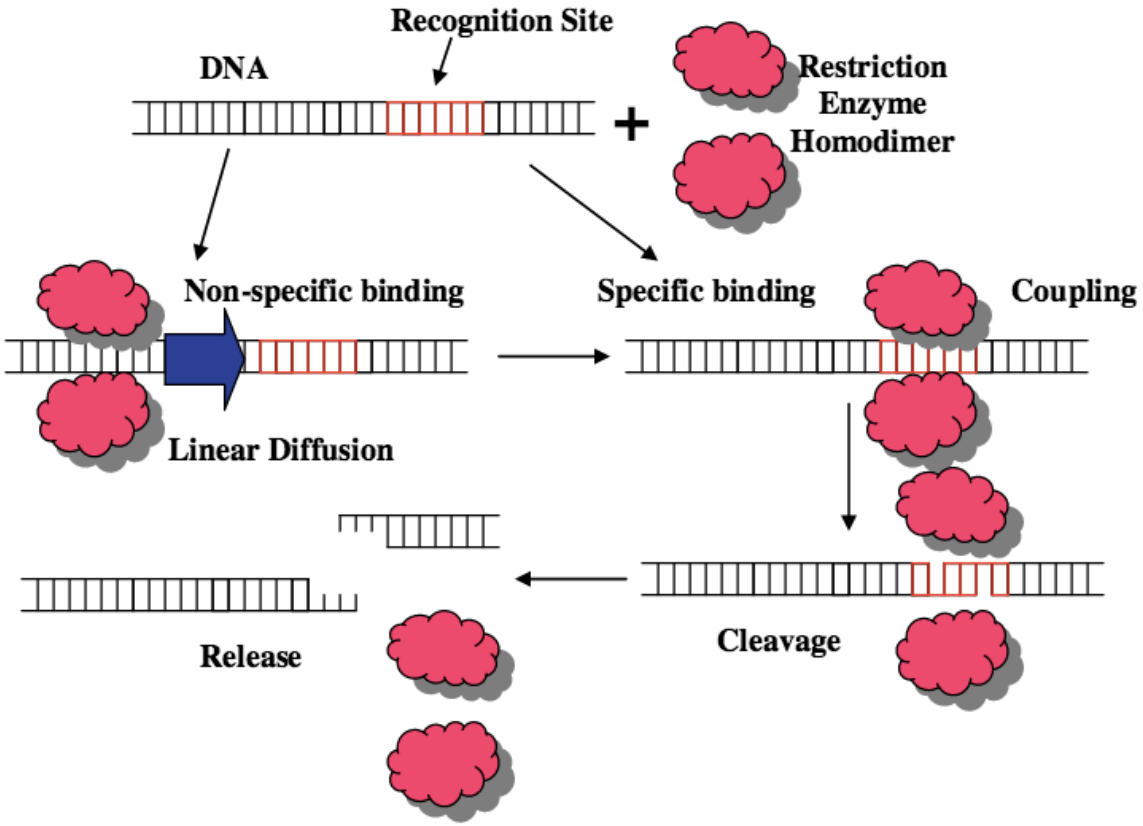


Digestion

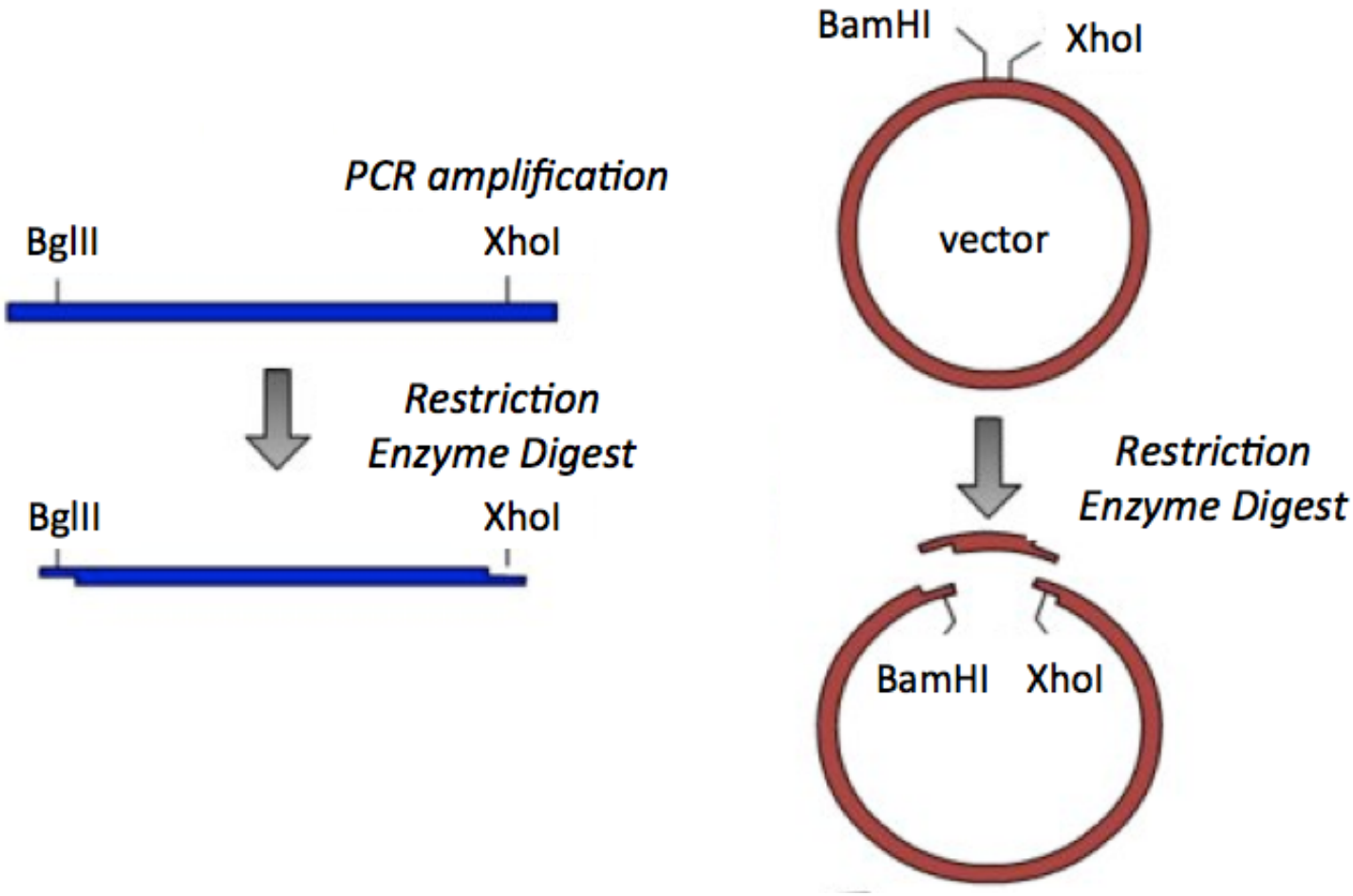

EcoRI
5′
3′
G A A T T C
C T T A A G
3′
5′
Cleavage
EcoRI
Sticky ends
5′
3′
G
C T T A A
A A T T C
G
3′
5′

Restriction enzymes

- Function as homodimers
  - Each dimer cleaves backbone at site of palindromic recognition sequence



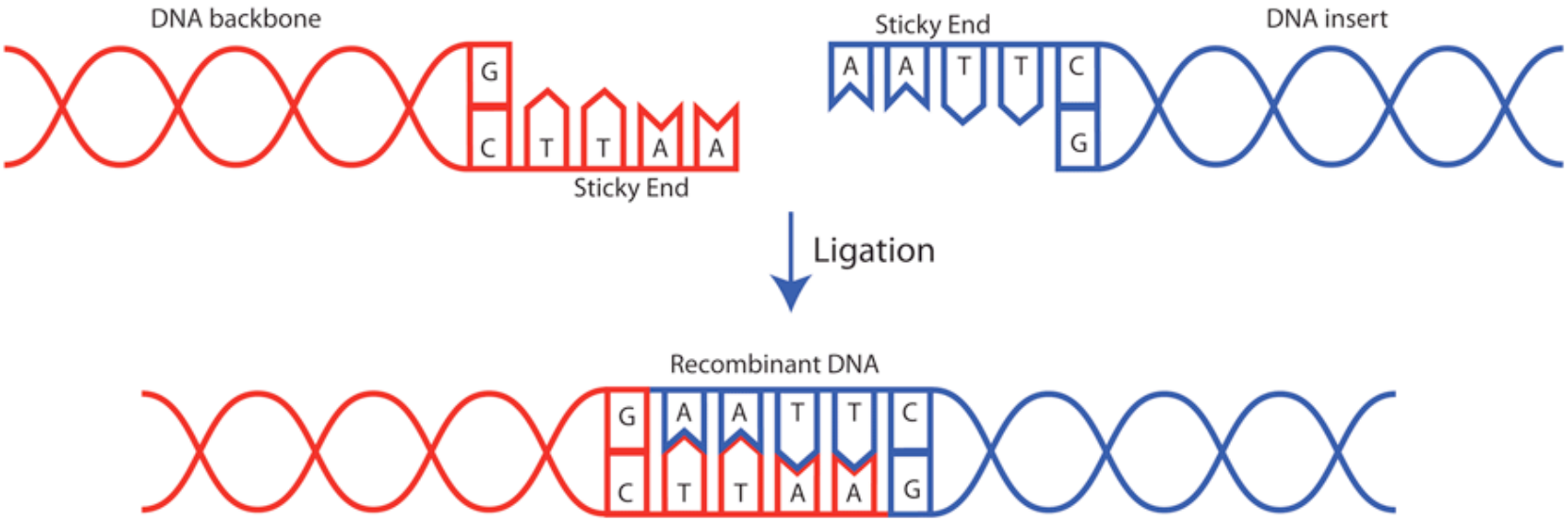
What are we digesting?



What should we consider when performing a double digest?

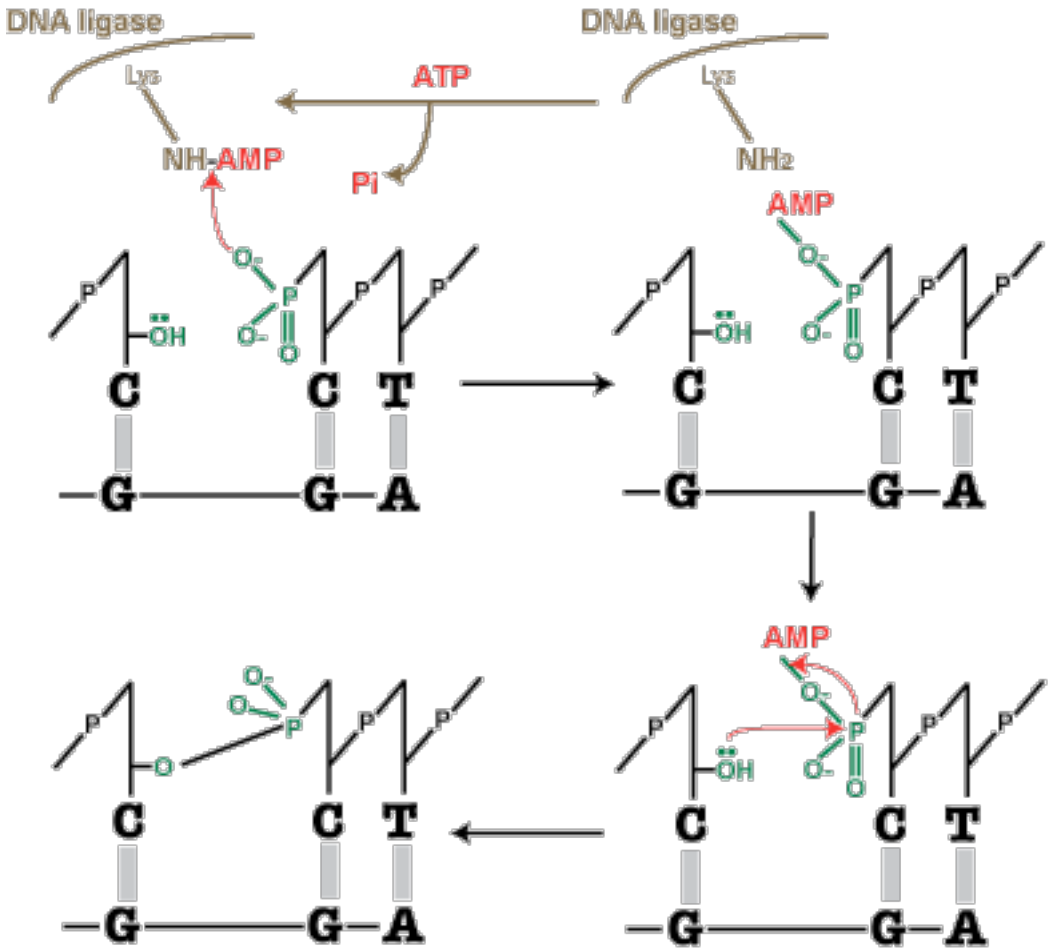
Ligation


Glue-All
ELMER'S

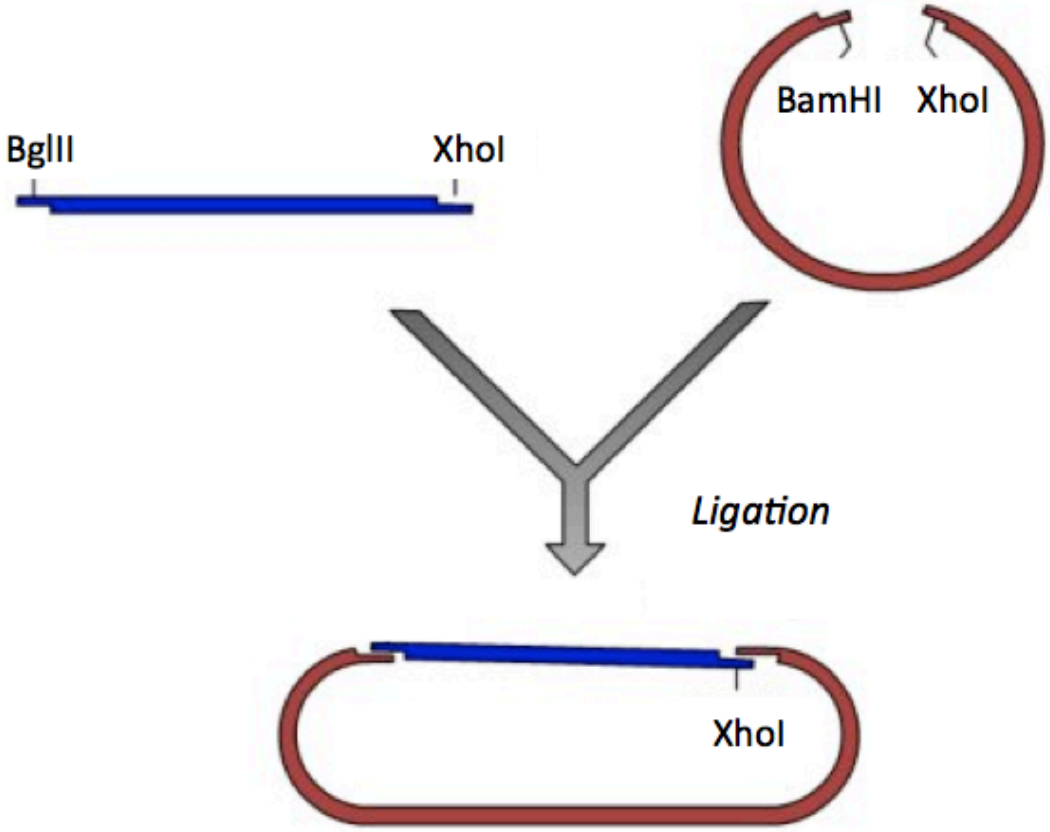

DNA backbone
G
C
T
T
A
A
Sticky End
Sticky End
DNA insert
A
A
T
T
C
G
Ligation
Recombinant DNA
G
A
A
T
T
C
C
T
T
A
A
G

DNA ligase

- Forms covalent phosphodiester bond between 3’ OH acceptor and 5’ phosphate donor
- Requires ATP

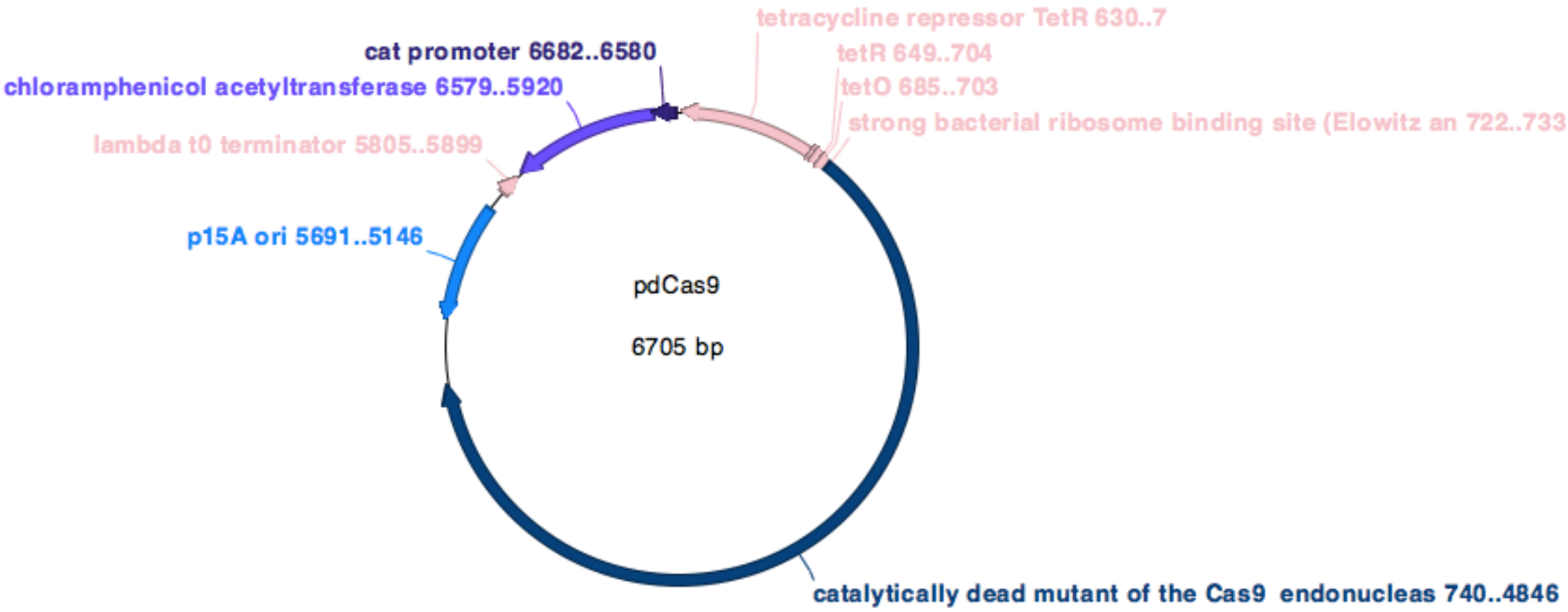


What are we ligating?



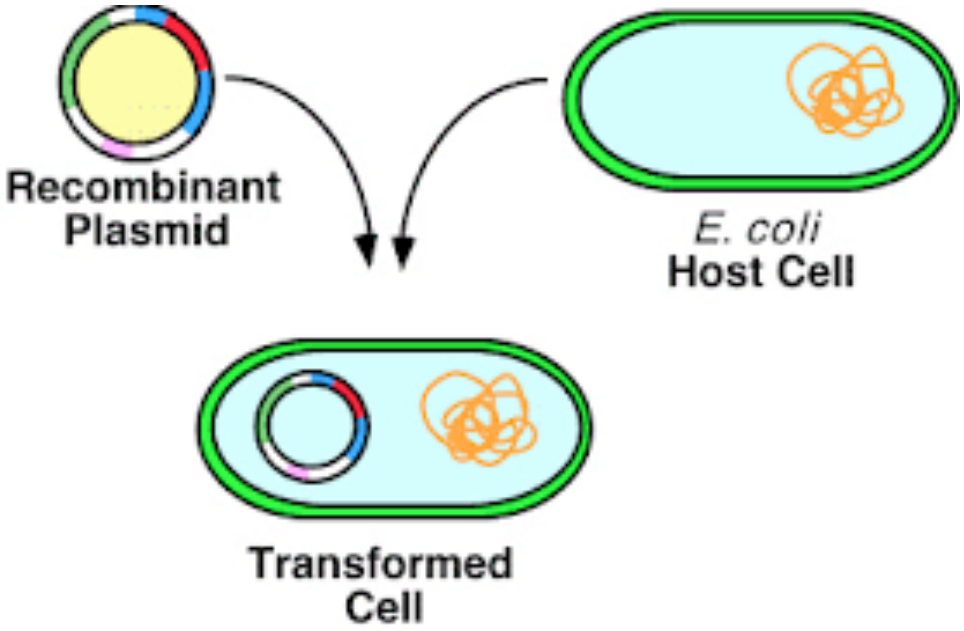
Note: in your laboratory exercise only the 'top' DNA strand is represented…remember this when determining basepair sites of digestion and ligation.

How do we confirm our product?



1. Transformation 2. Purification 3. Digestion

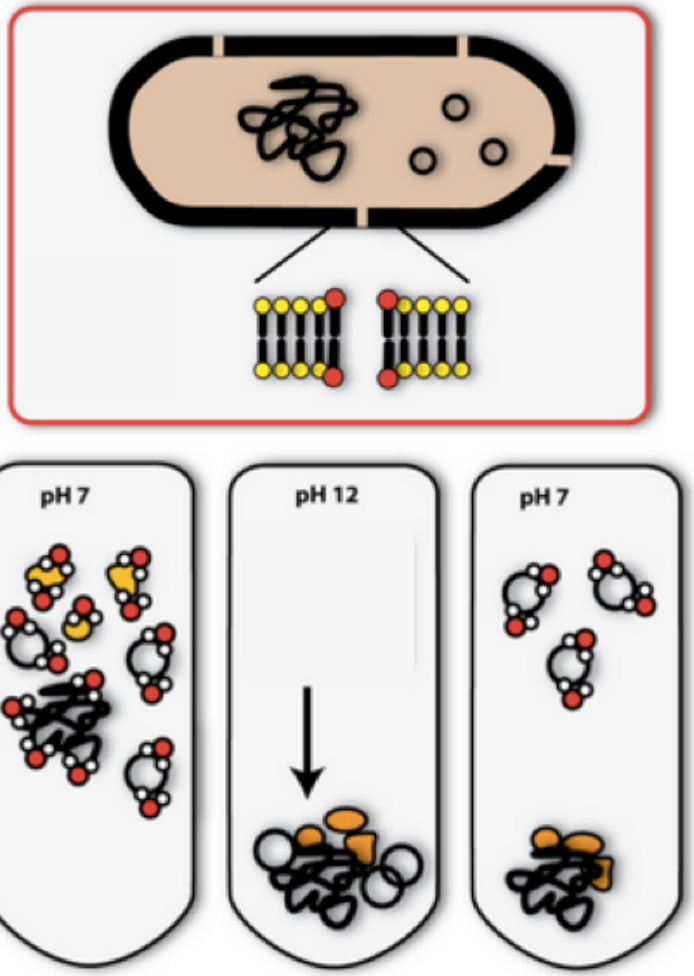
Transformation



1. Incubation
2. Heat shock
   - DNA taken in by competent cells
3. Recovery
4. Selection

Why do we transform the ligation product?

Purification

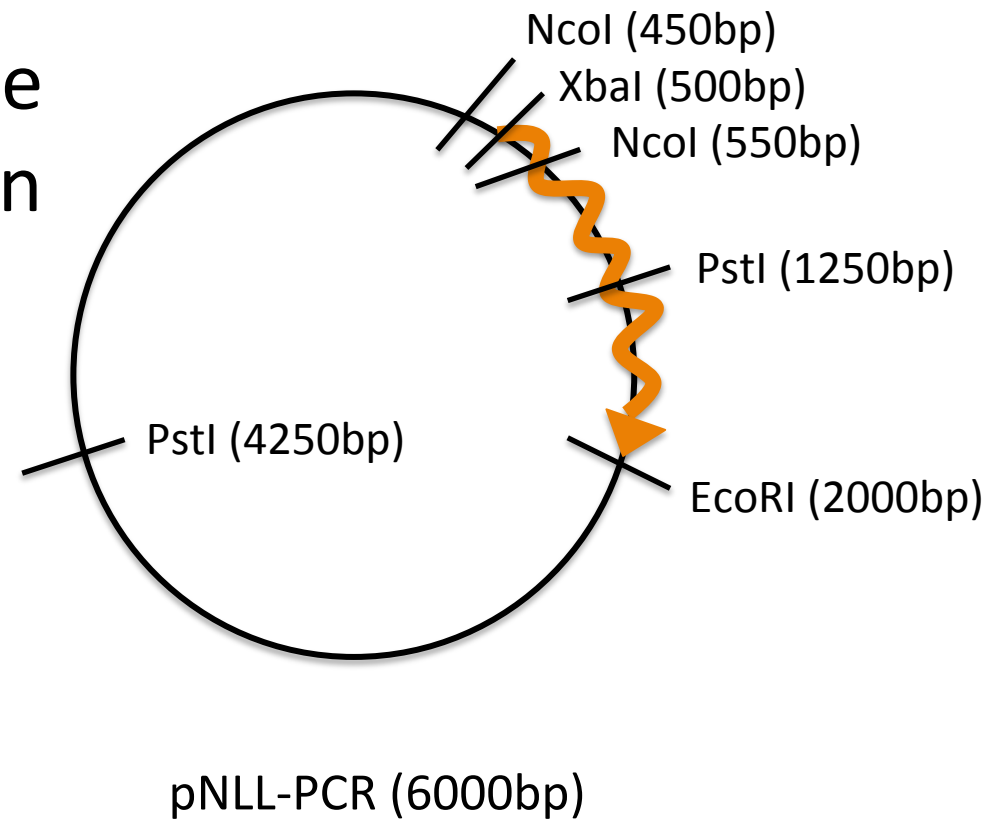


1. Resuspend cells
2. Lysis
3. Neutralization
   - Separates chromosomal DNA from plasmid DNA
4. Wash
5. Resuspend or elute DNA

Why do we purify (mini-prep) the ligation product?

Digestion, again

- Confirmation digests
- Ideally, will cut once in insert and once in vector
  - XbaI and EcoRI?
  - PstI?
  - NcoI?



In the laboratory…

1. Engineer pdCas9 construct
   - In silico ‘cloning’ of dCas9 gene insert into expression vector
2. Confirm pdCas9 construct
   - Digestion of pdCas9 to confirm cloning of dCas9 gene insert