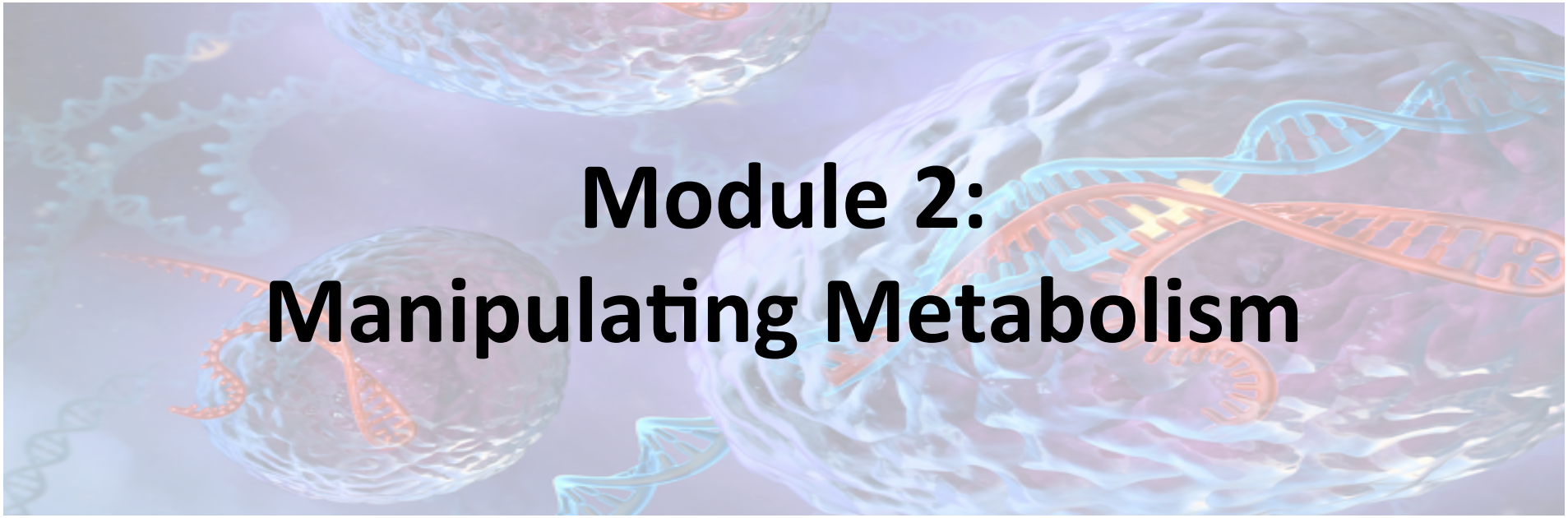


# Module 2: Manipulating Metabolism

DNA engineering

10/11/18

# Wrapping up Mod 1

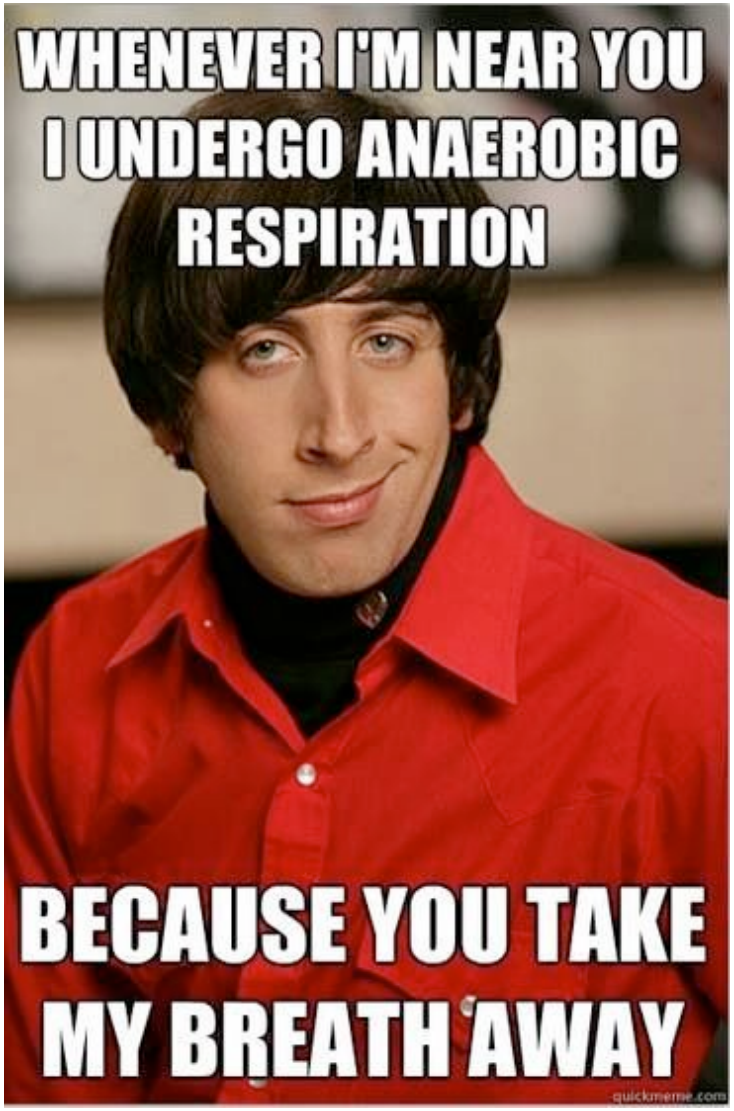
- Data summary draft submitted!!
  - 
    
  - Comments to be returned on Oct. 15
  - Revision due by 10 pm on Oct. 20
- Mini presentation due by 10 pm on Oct. 13
- Blog post due by 10 pm on Oct. 11

# Mod 2 assignment overview

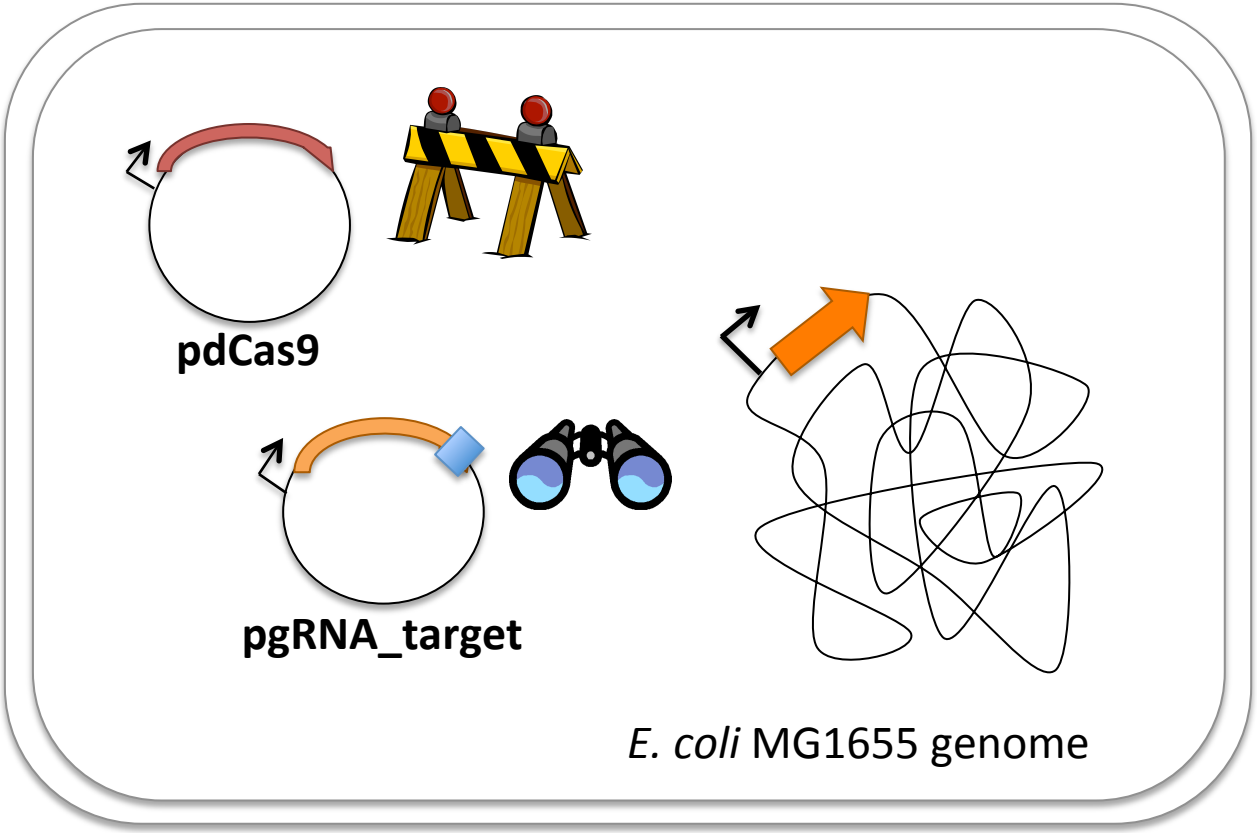
- Journal club presentation
  - Communicate the key findings from a peer-reviewed published article
  - Completed individually
- Research article
  - Describe your results and analysis in a written 'formal' document
  - Completed individually

# Your engineering task in Mod 2:

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

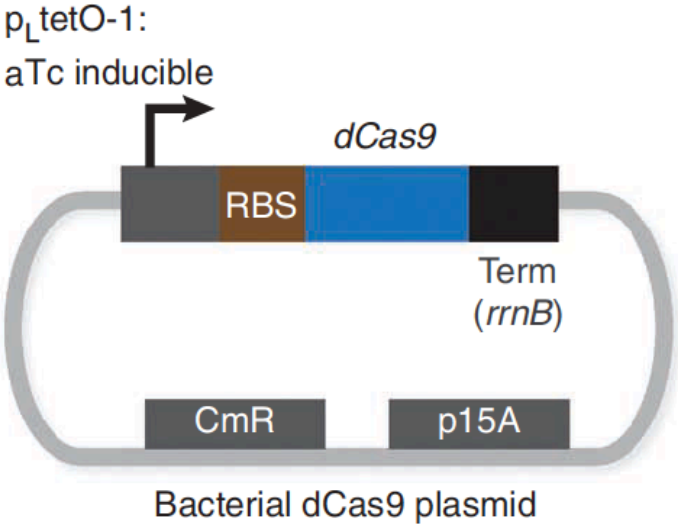

WHENEVER I'M NEAR YOU
I UNDERGO ANAEROBIC
RESPIRATION
BECAUSE YOU TAKE
MY BREATH AWAY
quickmeme.com

# CRISPRi system overview



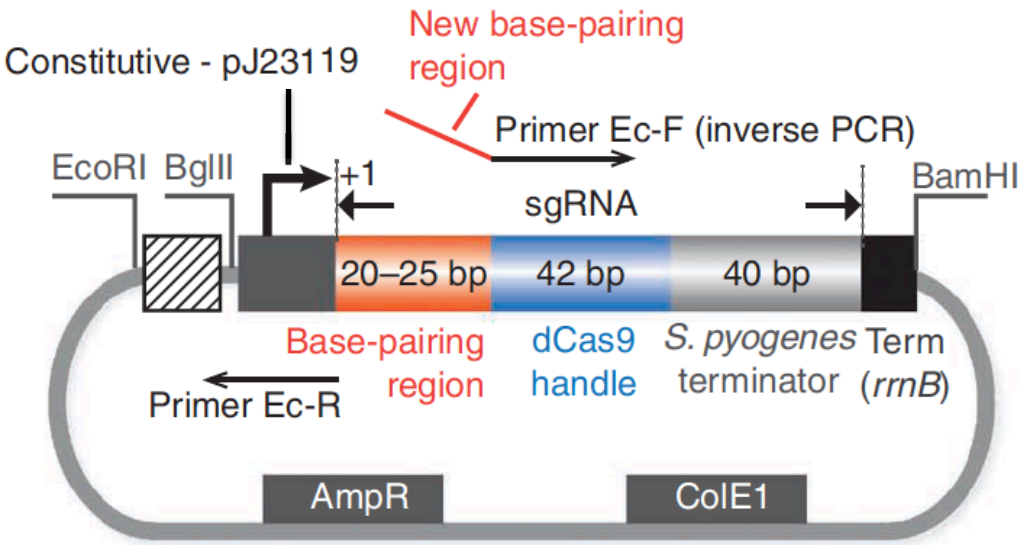

- Target gene
- pgRNA_target
- pdCas9

# Closer look at pgRNA and pdCas9



Bacterial dCas9 plasmid

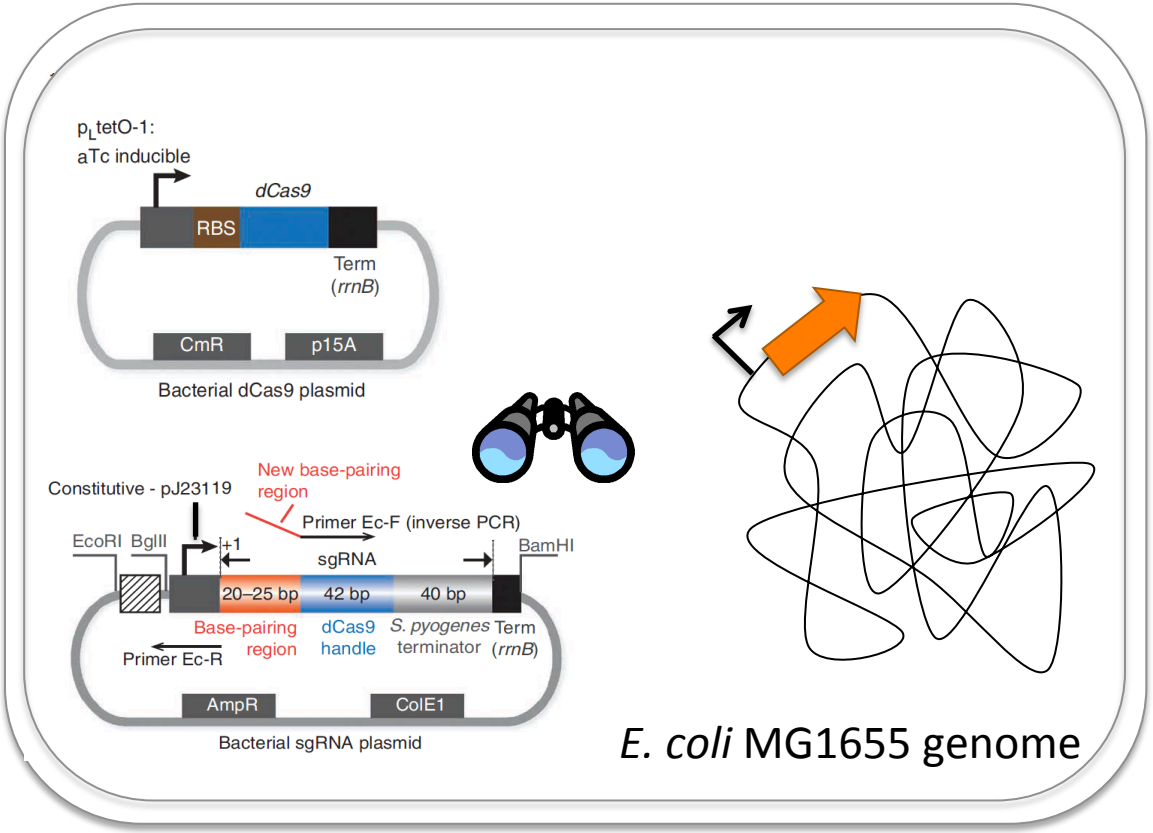
- Confirmation digest prepared on M2D1



Bacterial sgRNA plasmid

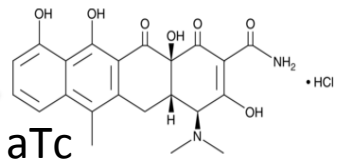
- Insert (gRNA target sequence) designed on M2D2

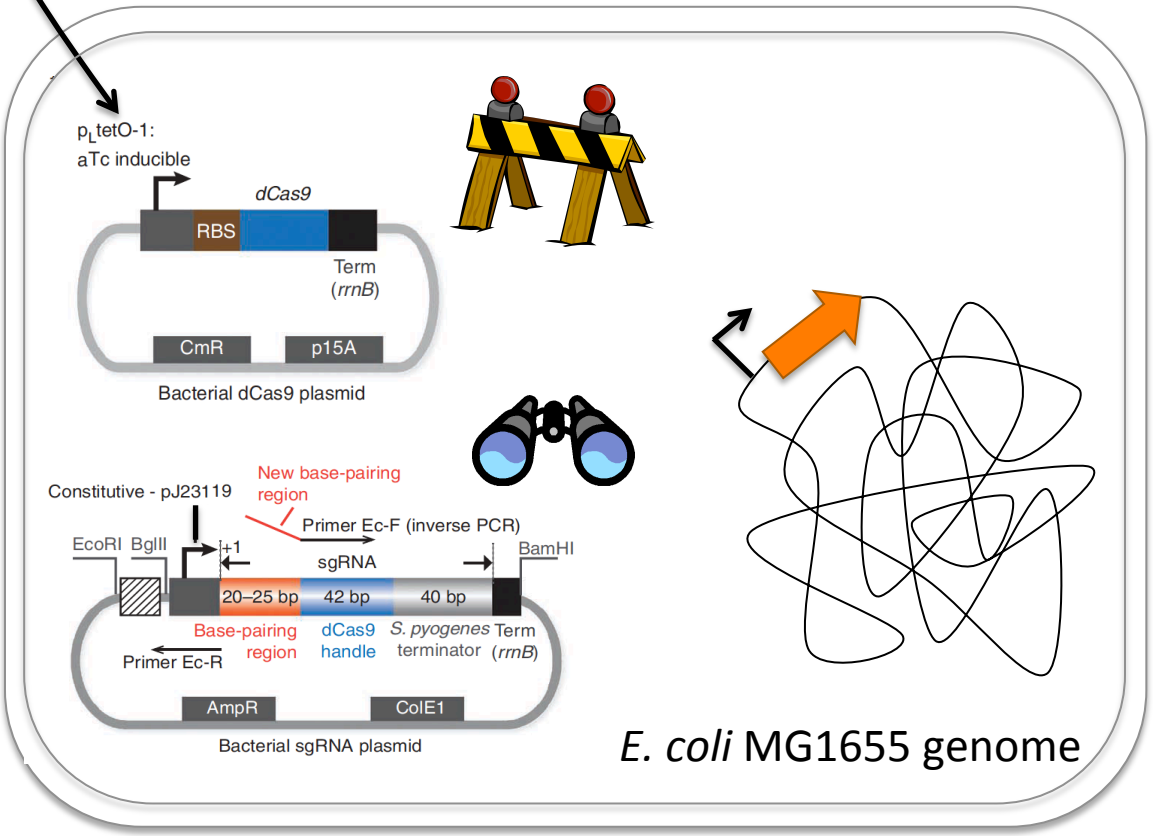
# CRISPRi 'inactive' in absence of inducer




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

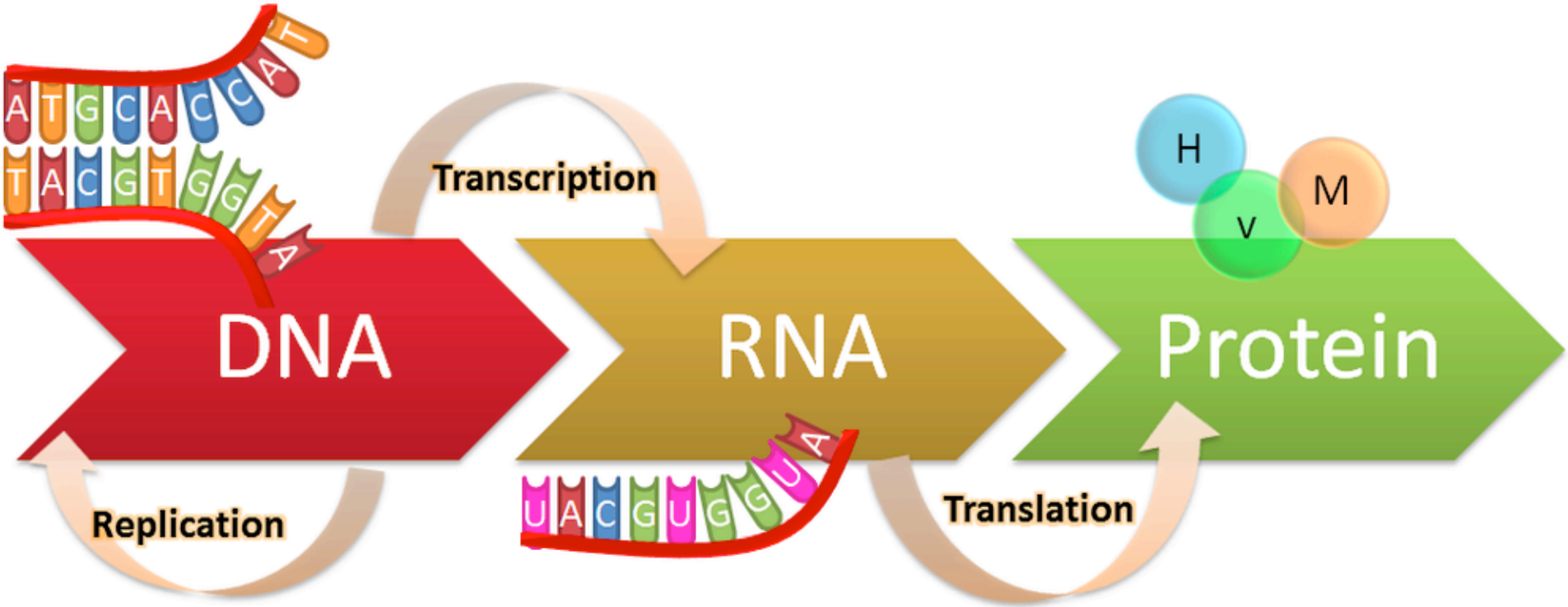
# CRISPRi ‘blocks’ gene expression in presence of inducer



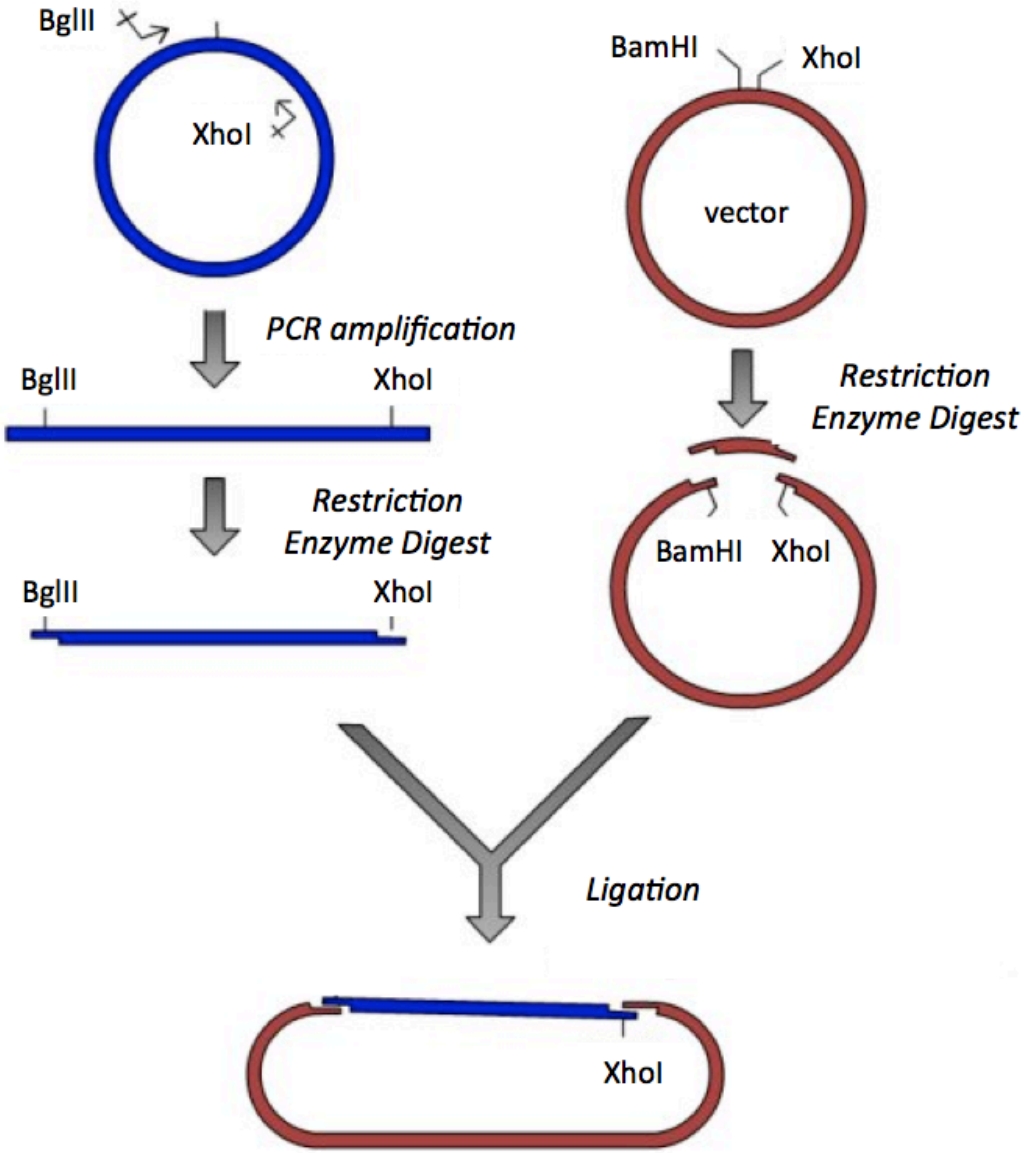

- pdCas9 expressed when aTc added
  - When transcribed associates with pgRNA_target / target gene

# The central dogma



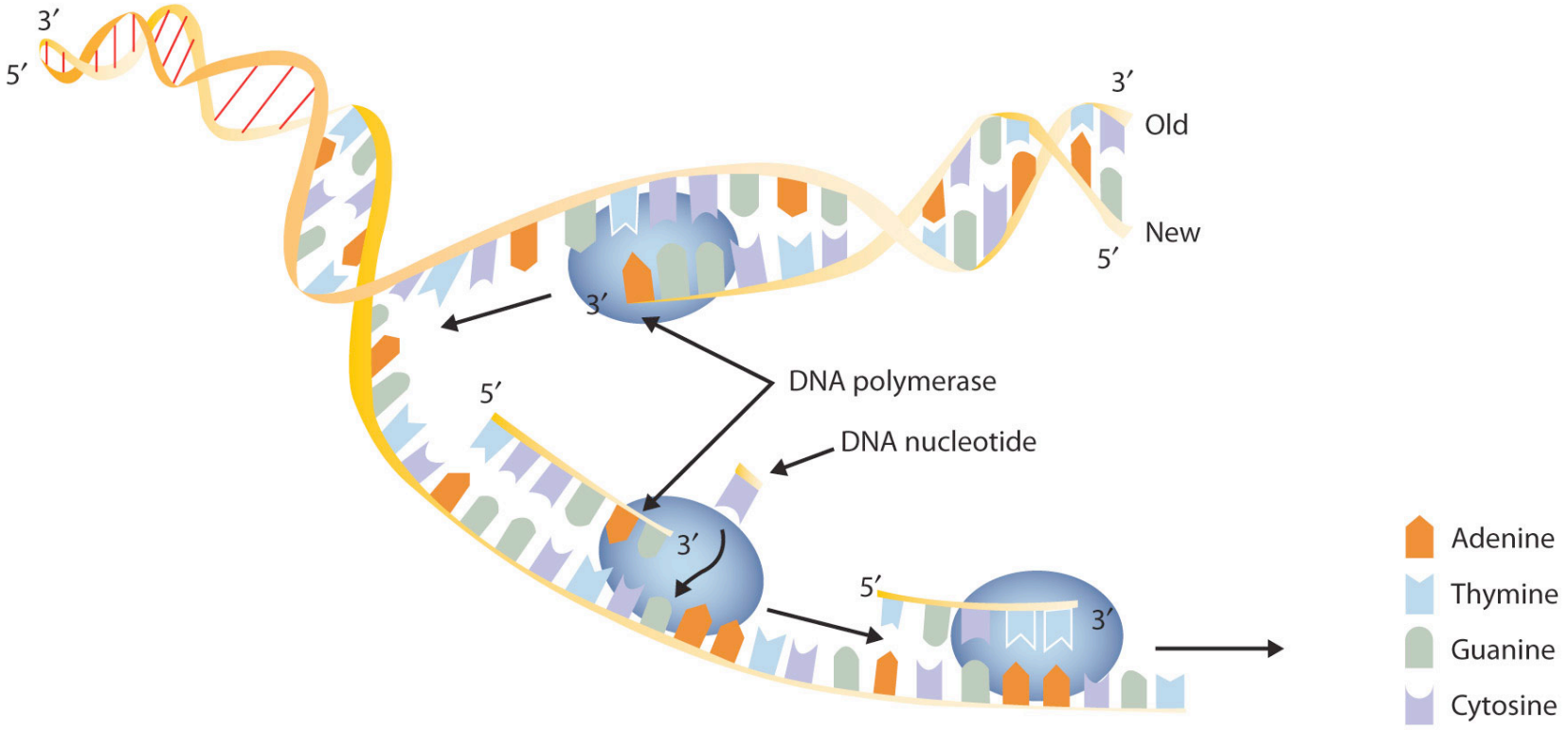

http://genius.com/Biology-genius-the-central-dogma-annotated

# How do we engineer DNA?



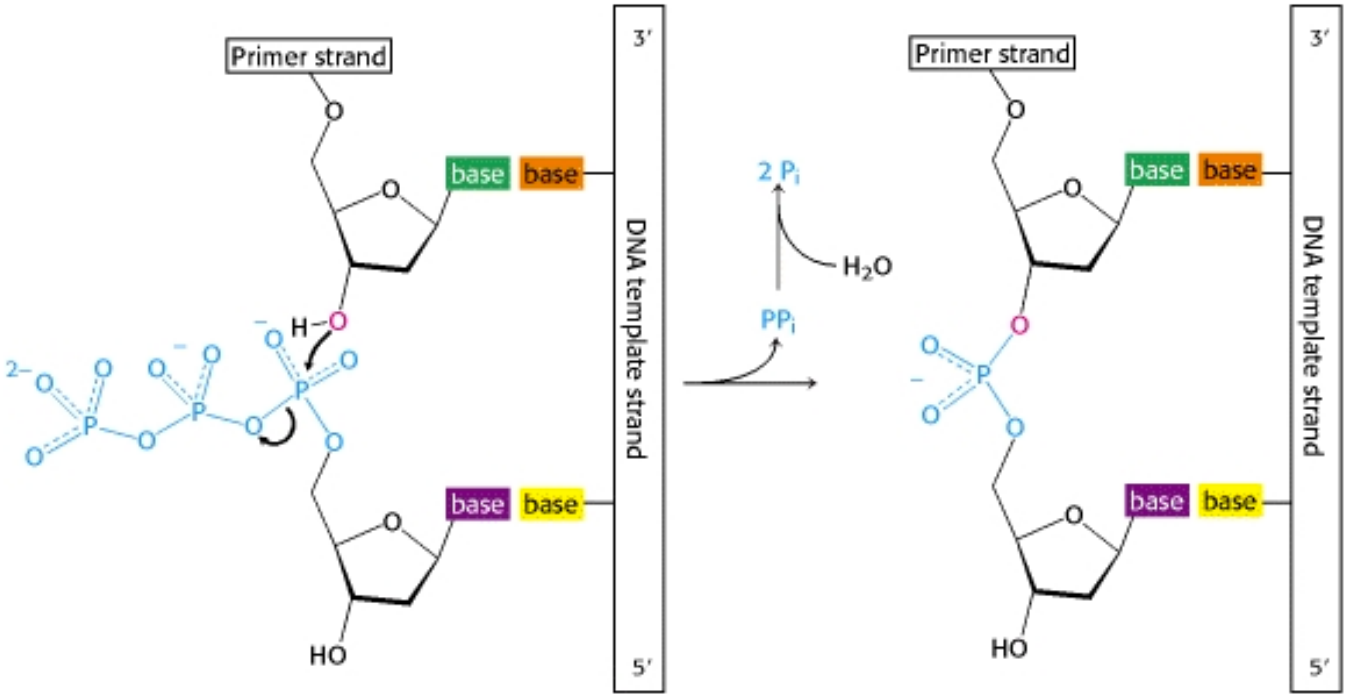

1. Amplification
2. Digestion
3. Ligation

# Amplification




Who are the key players?

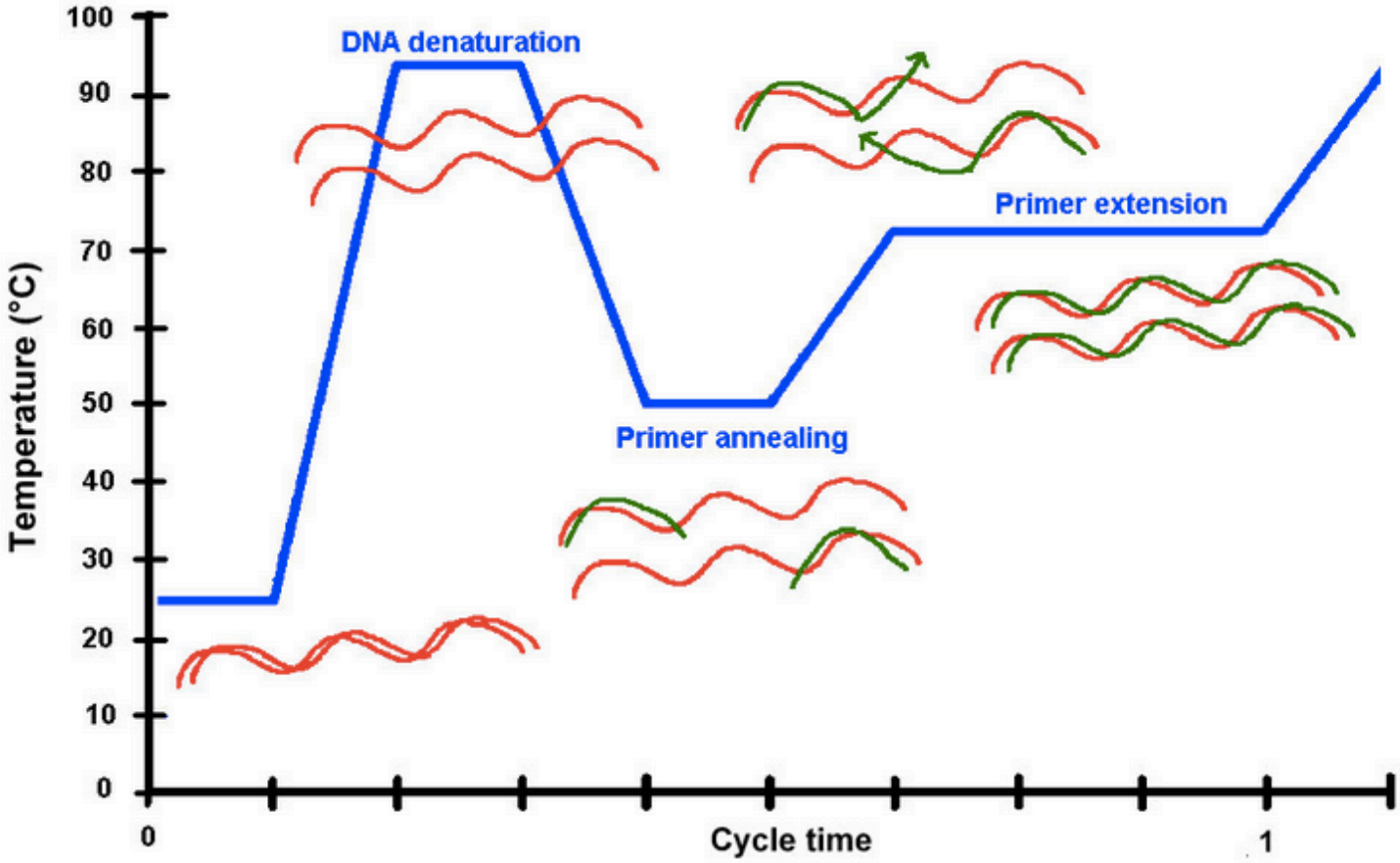
# DNA polymerase




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

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

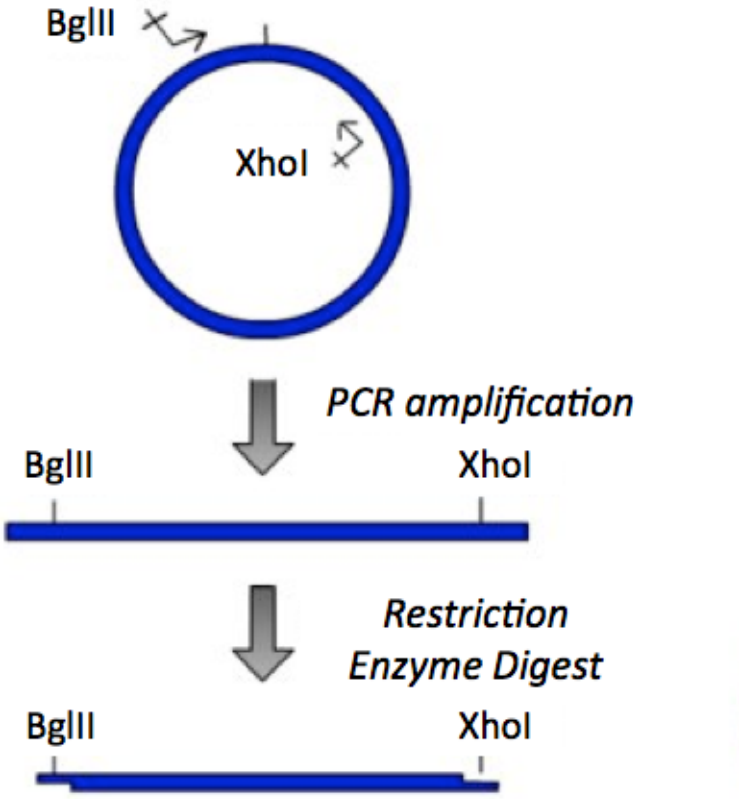
# Polymerase chain reaction (PCR)



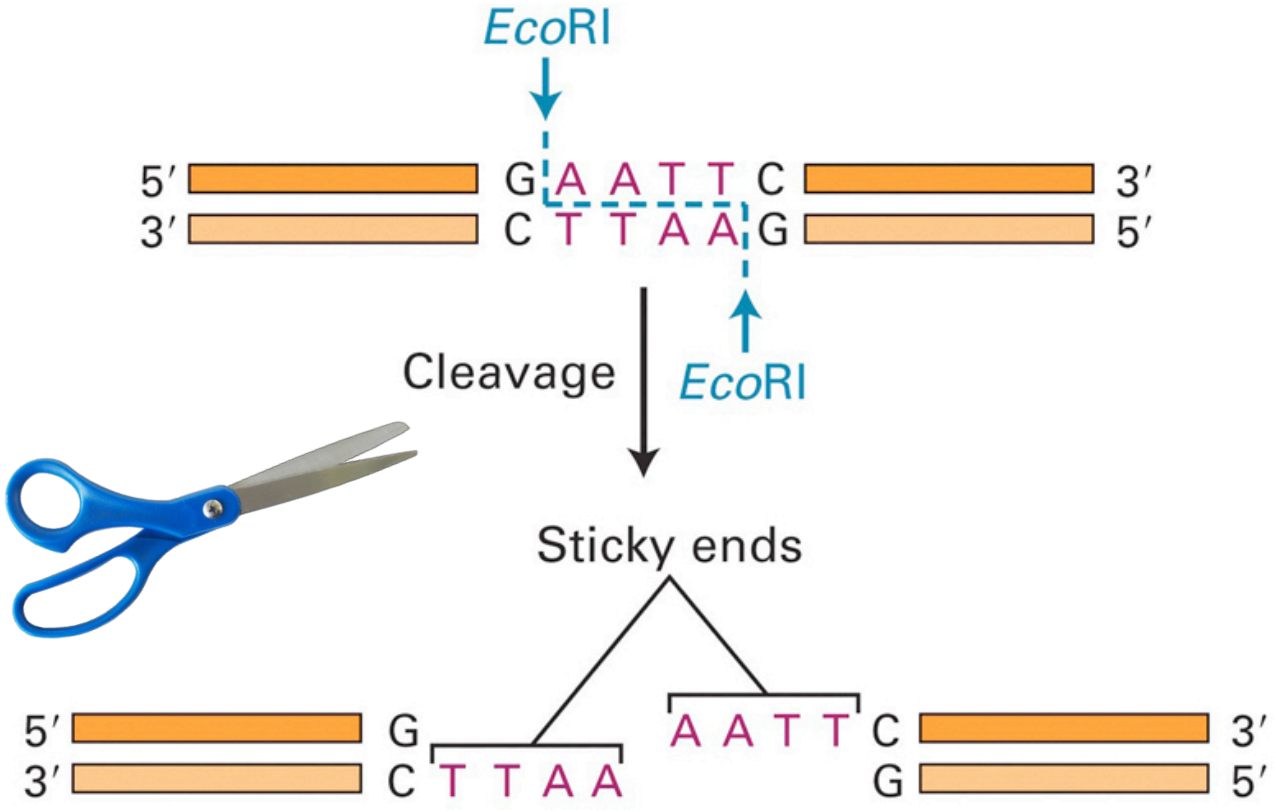

How many cycles until your product is generated?

# What are we amplifying?

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

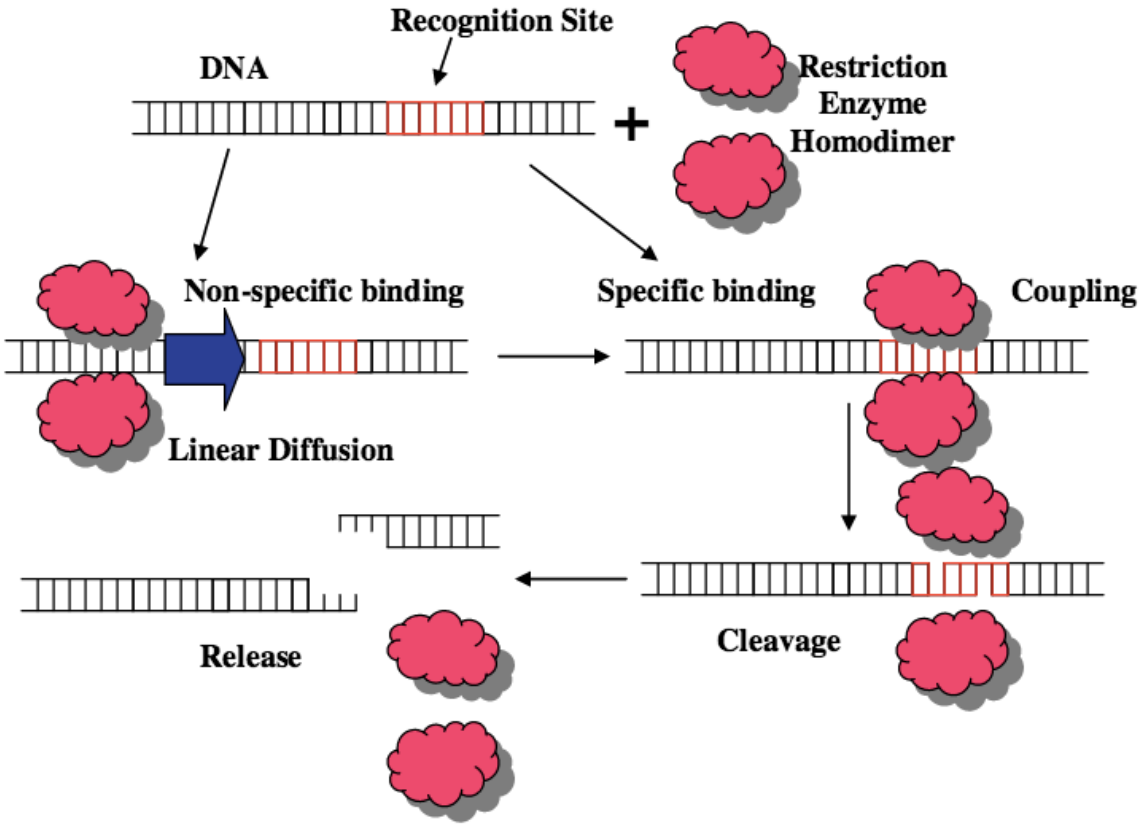


# Digestion

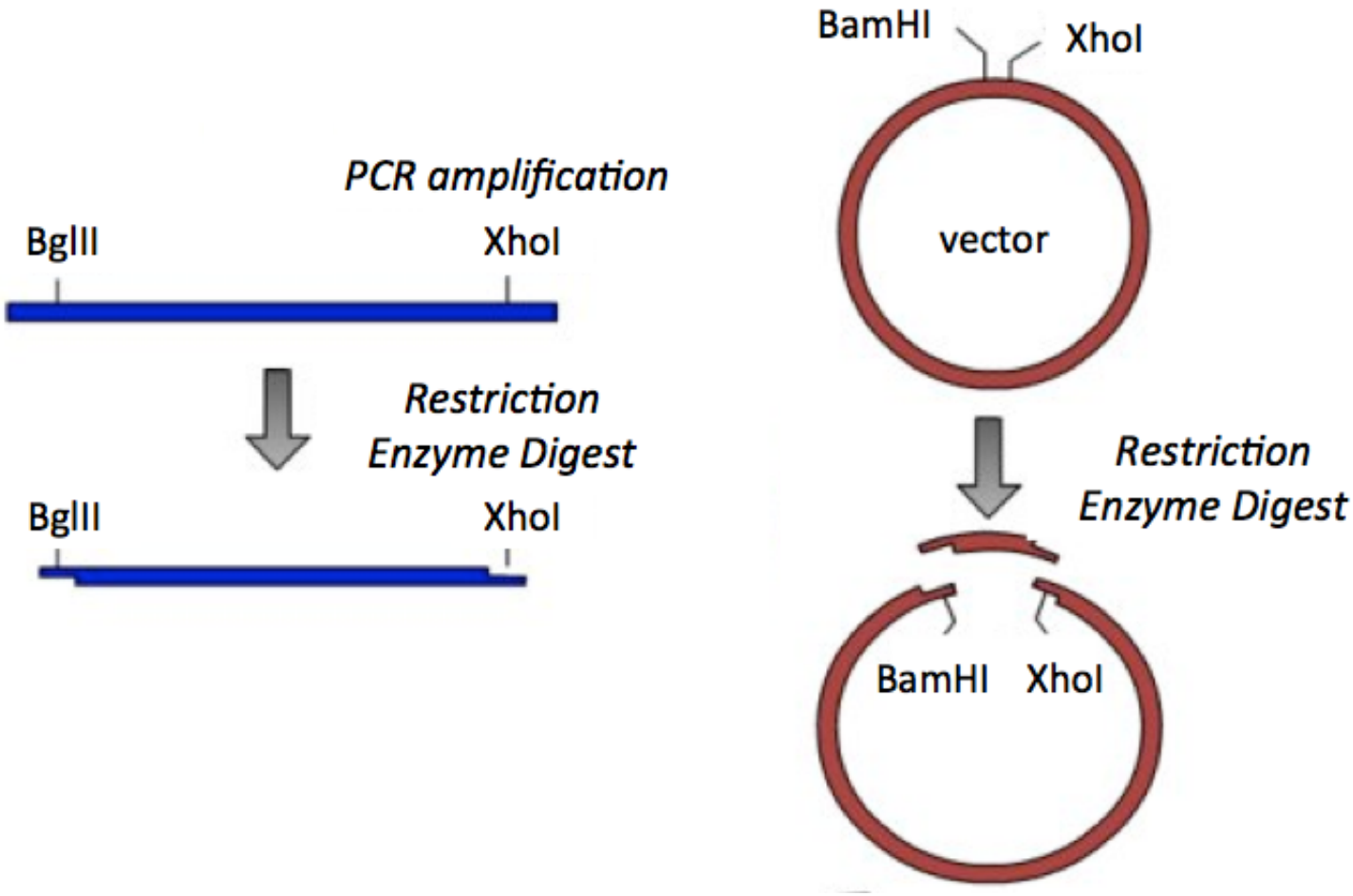

EcoRI
5′
3′
G A A T T C
C T T A A G
3′
5′
Cleavage
EcoRI
Sticky ends
5′
3′
G
C T T A A
A A T T C
G
3′
5′

# Restriction enzymes

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



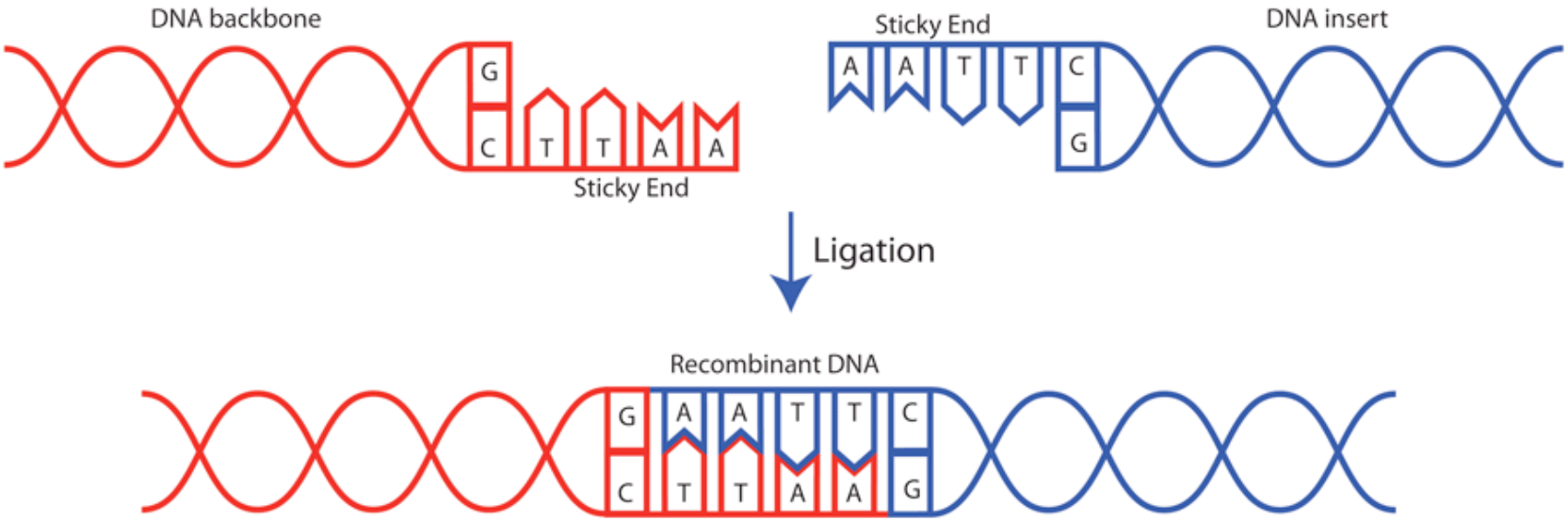
# What are we digesting?




What should we consider when performing a double digest?

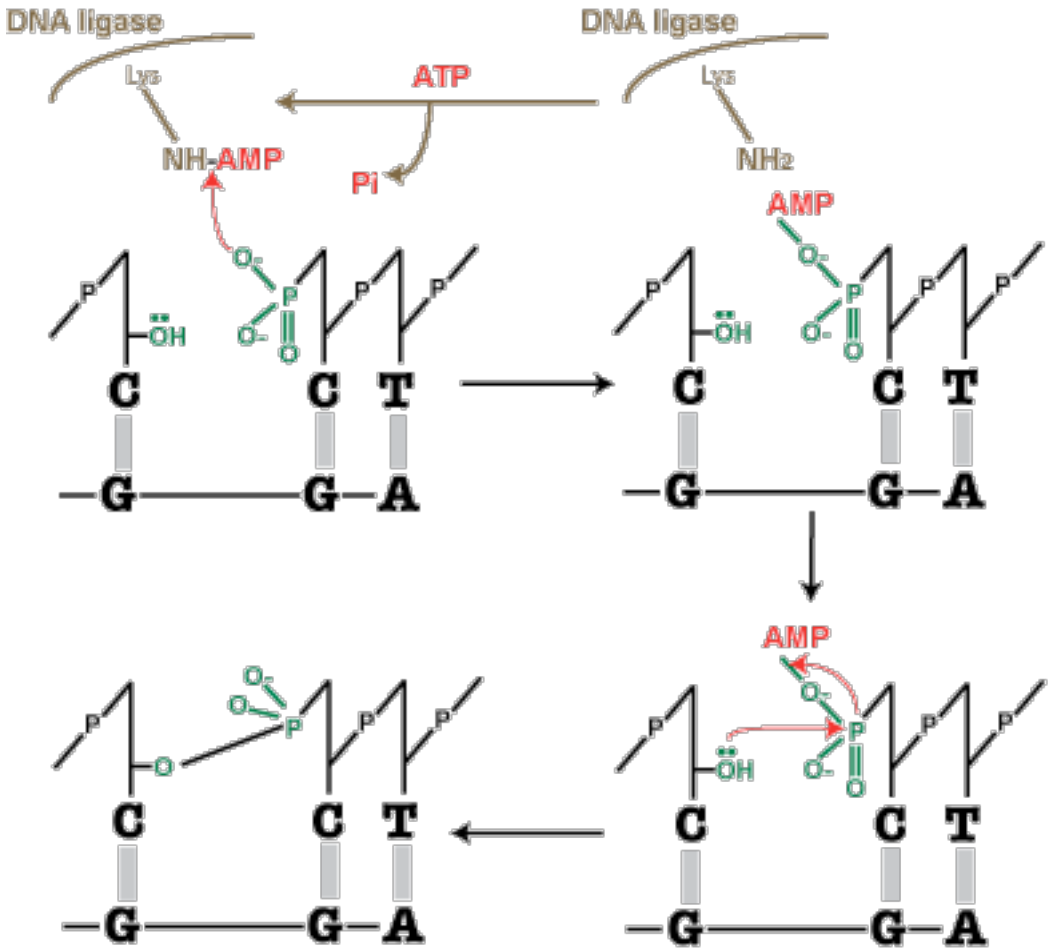
# Ligation


Glue-All
ELMER'S

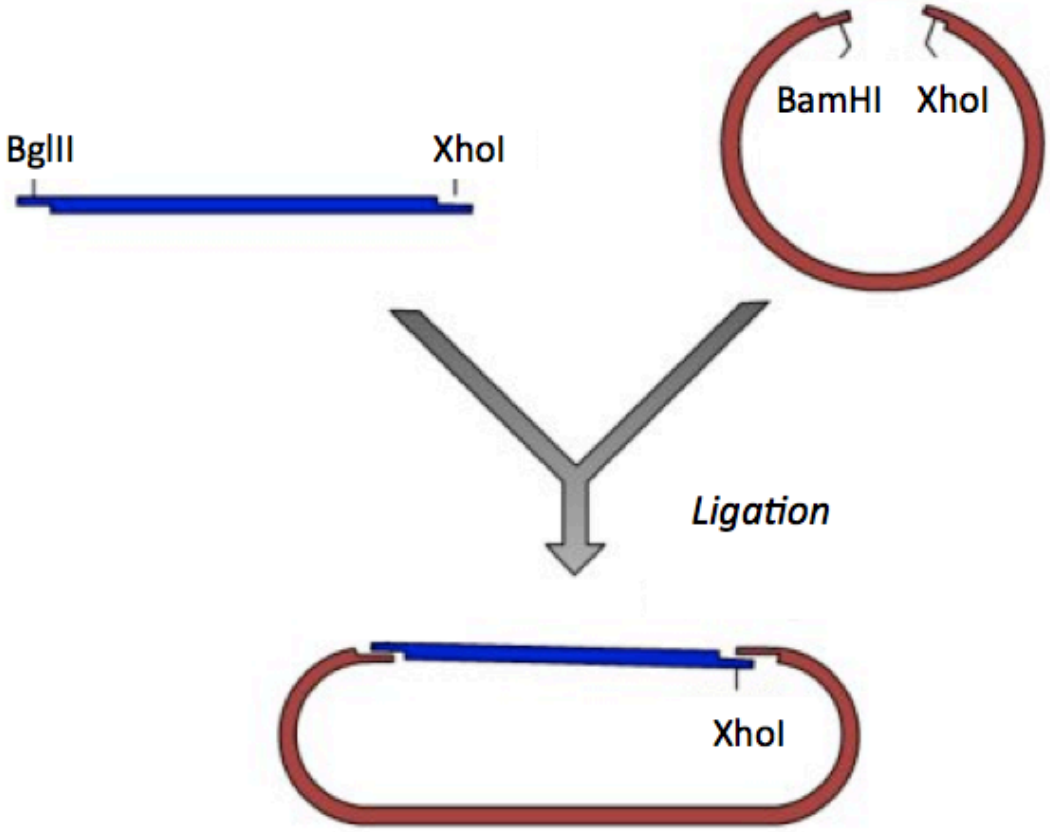

DNA backbone
G
C
T
T
A
A
Sticky End
Sticky End
DNA insert
A
A
T
T
C
G
Ligation
Recombinant DNA
G
A
A
T
T
C
C
T
T
A
A
G

# DNA ligase

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

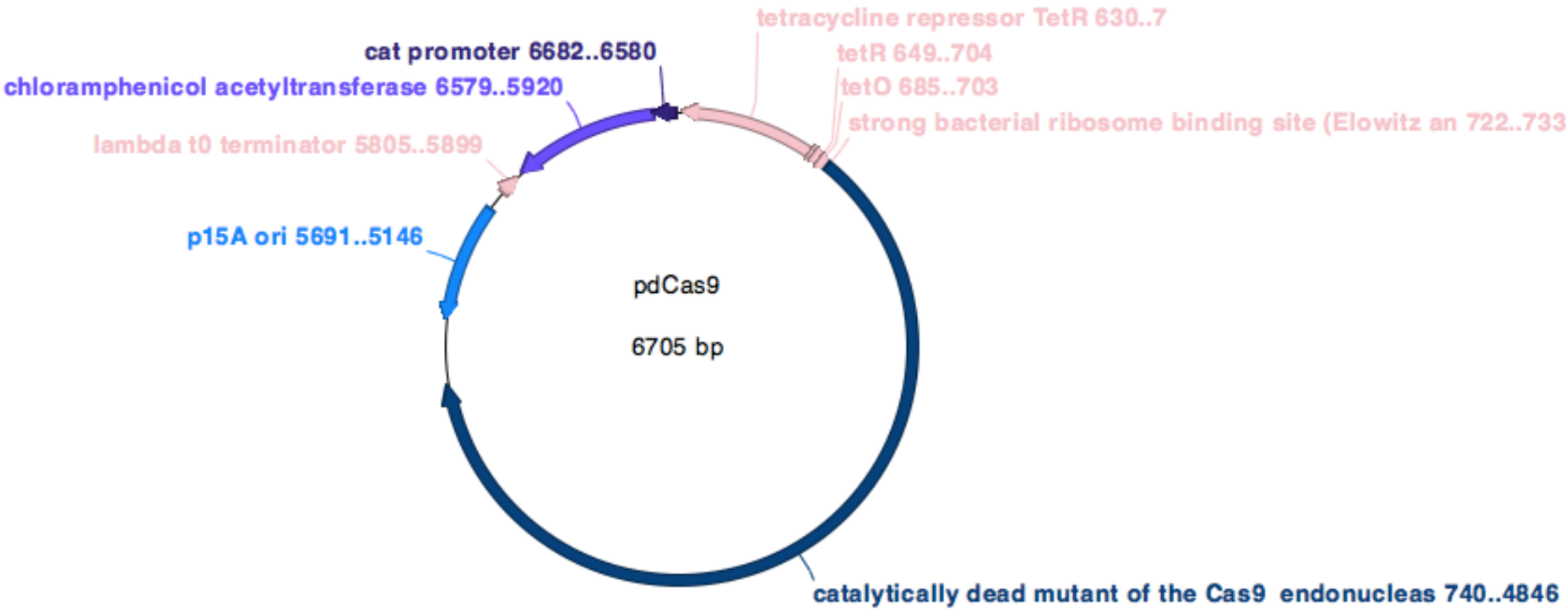


# What are we ligating?



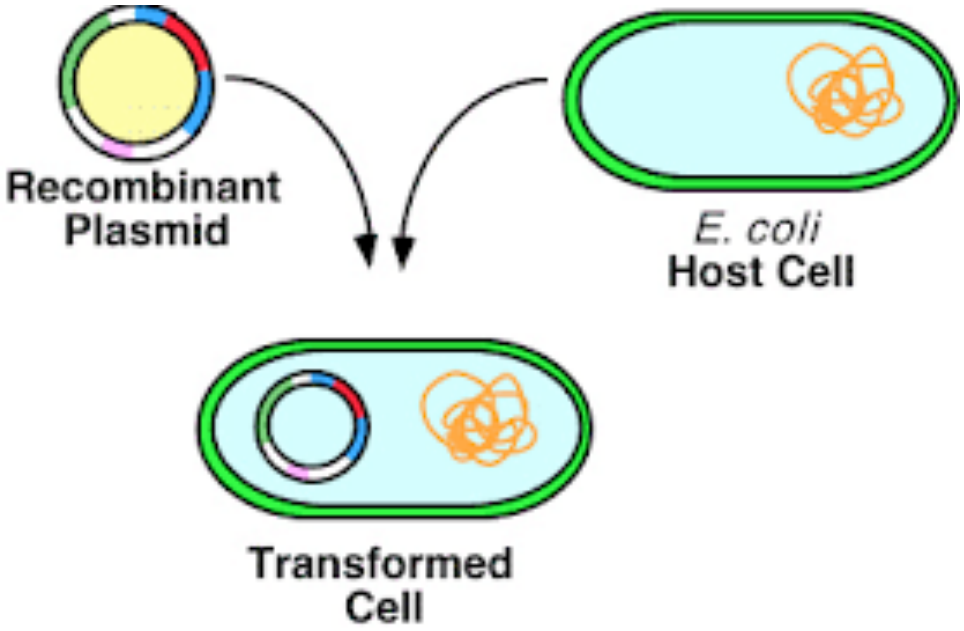

Note: in your laboratory exercise only the 'top' DNA strand is represented…remember this when determining basepair sites of digestion and ligation.

# How do we confirm our product?




1. Transformation 2. Purification 3. Digestion

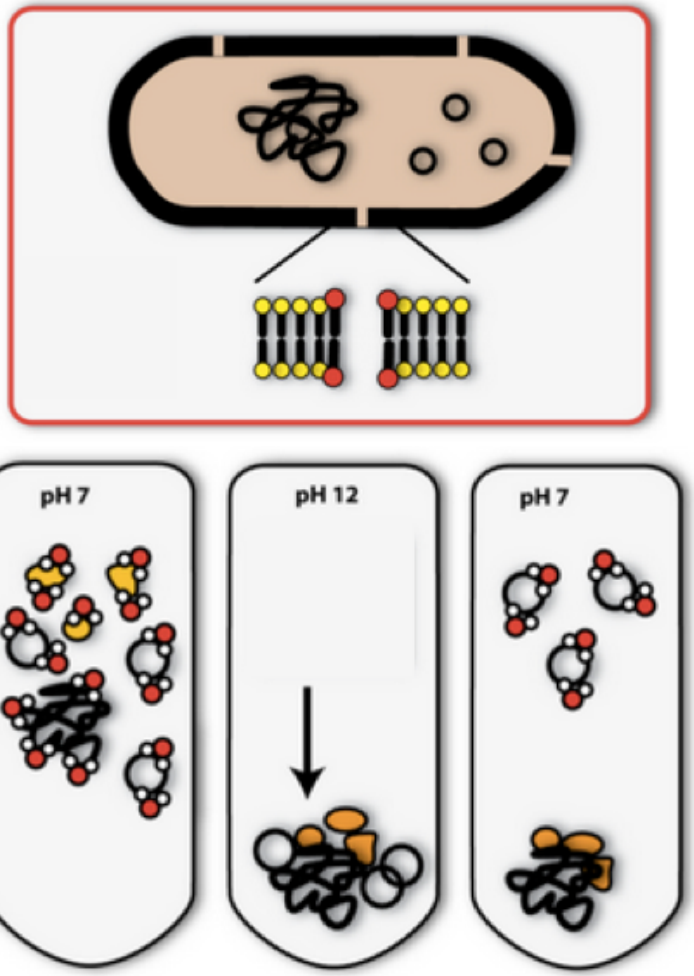
# Transformation




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

Why do we transform the ligation product?

# Purification

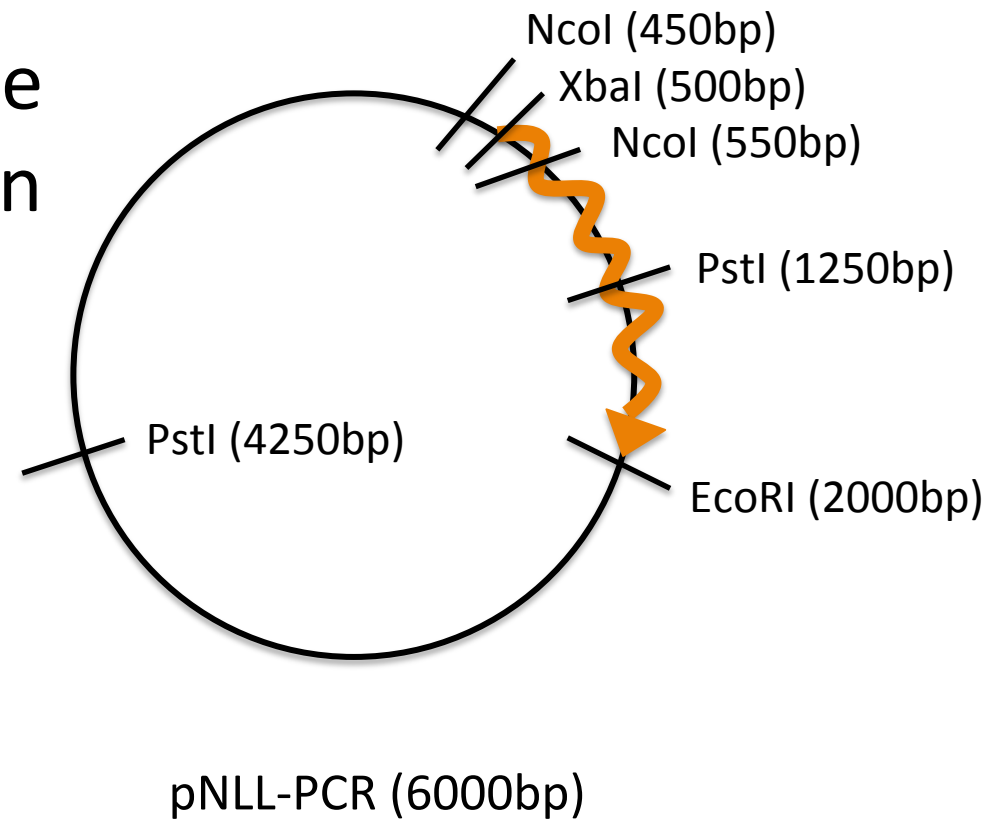



1. Resuspend cells
2. Lysis
3. Neutralization
   - Separates chromosomal DNA from plasmid DNA
4. Wash
5. Resuspend or elute DNA

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