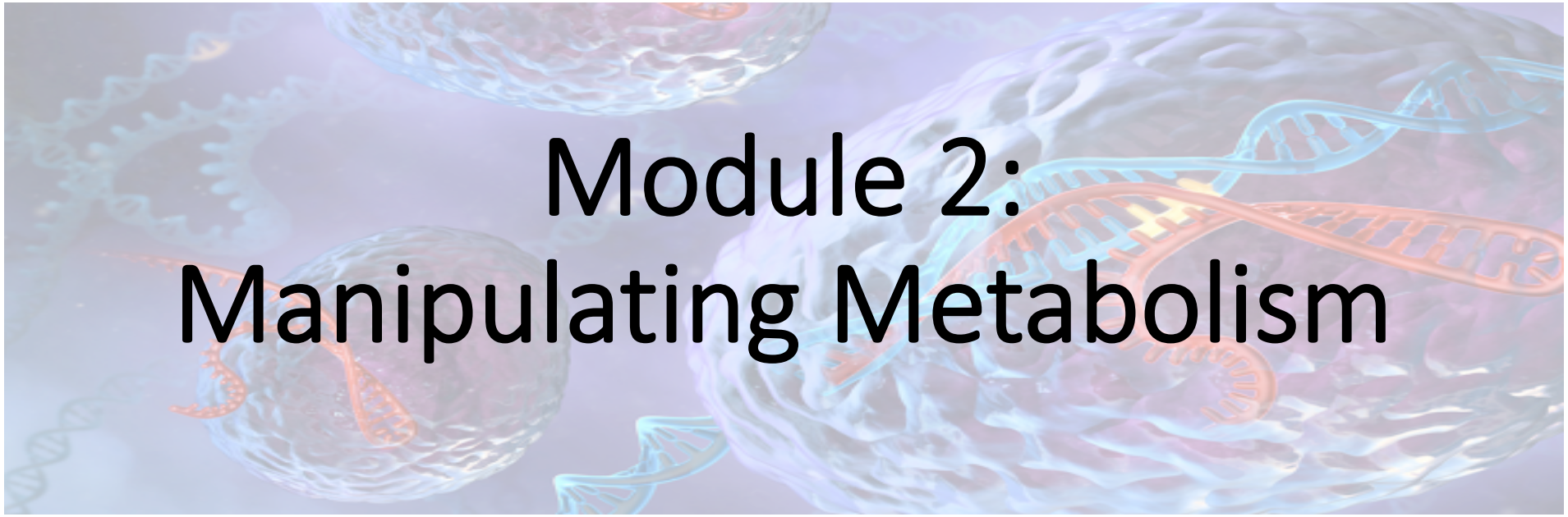


Module 2: Manipulating Metabolism

Engineering DNA

10/08/19

Wrapping up Mod 1

- Mod 1 experiments completed!!
  - 

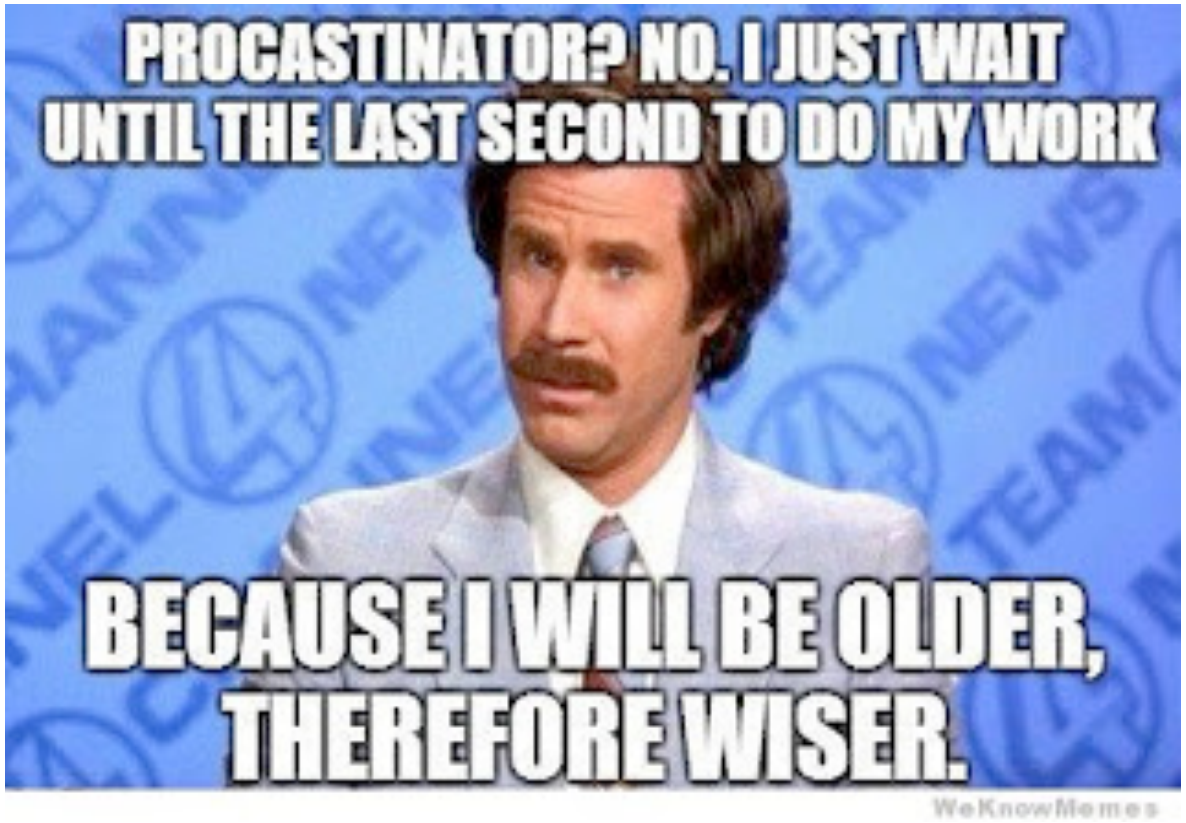
  - Draft due by 10 pm on Oct. 14
  - Comments to be returned on Oct. 21
  - Revision due by 10 pm on Oct. 26
- Mini presentation due by 10 pm on Oct. 19
- Blog post due by 10 pm on Oct. 15

Extra Office hours:

- See wiki for regularly scheduled days / times
- Friday, October 11 from 8a – 12p in 16-743c with Bevin
- Saturday, October 12 from 10a – 4:30p in 56-302 with Instructors

Mod 2 assignment overview

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



Mod 2 assignment overview

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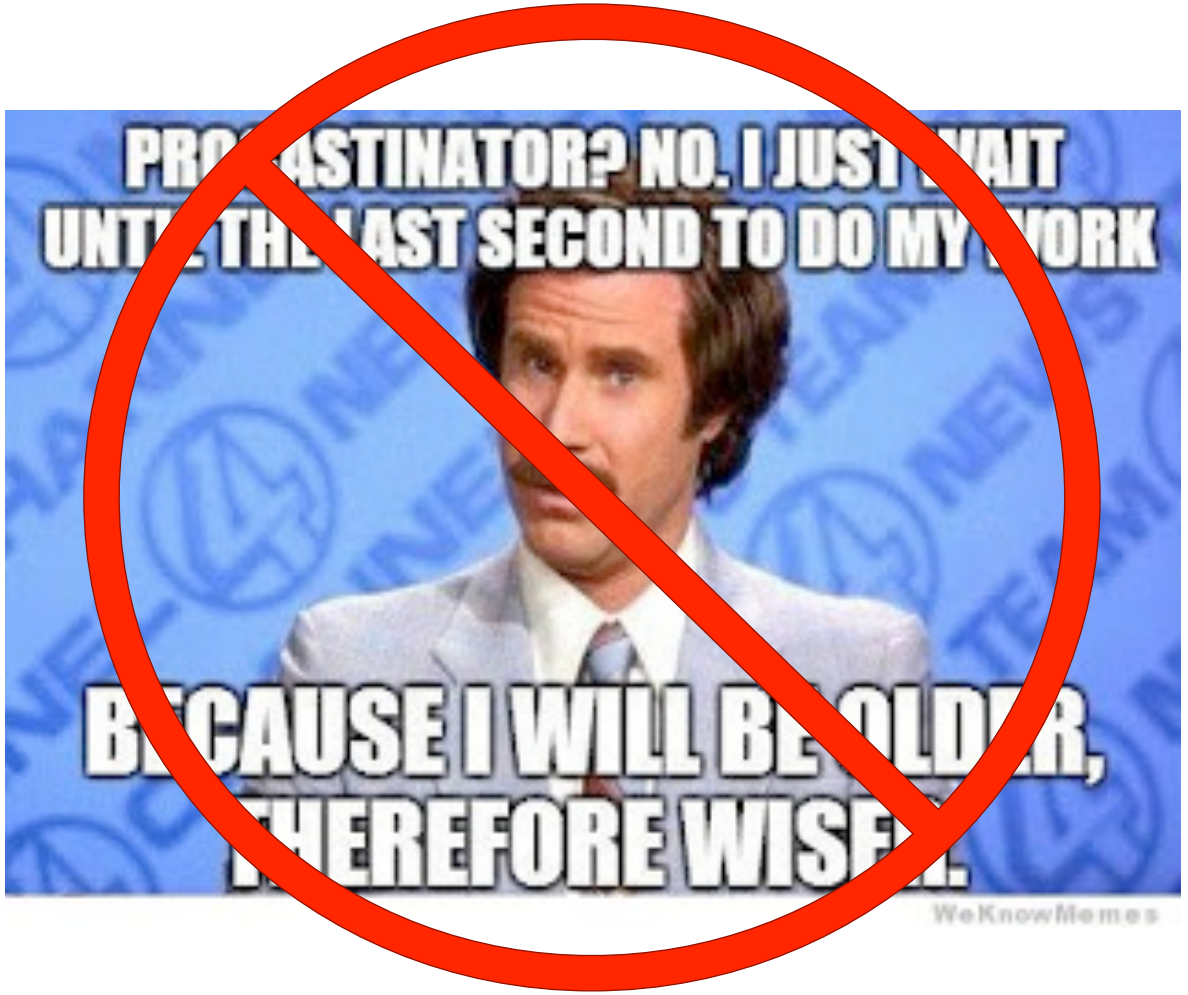
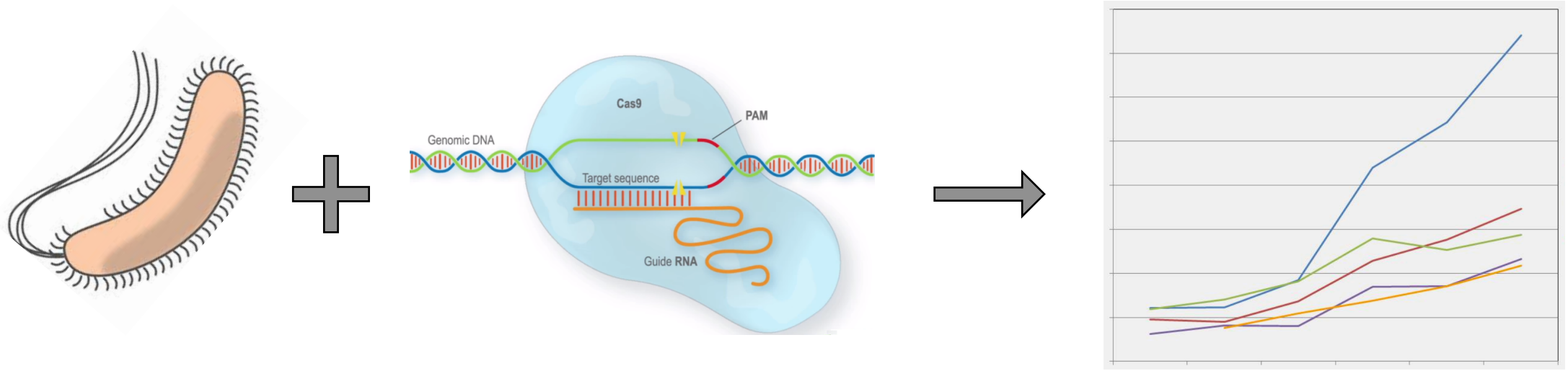
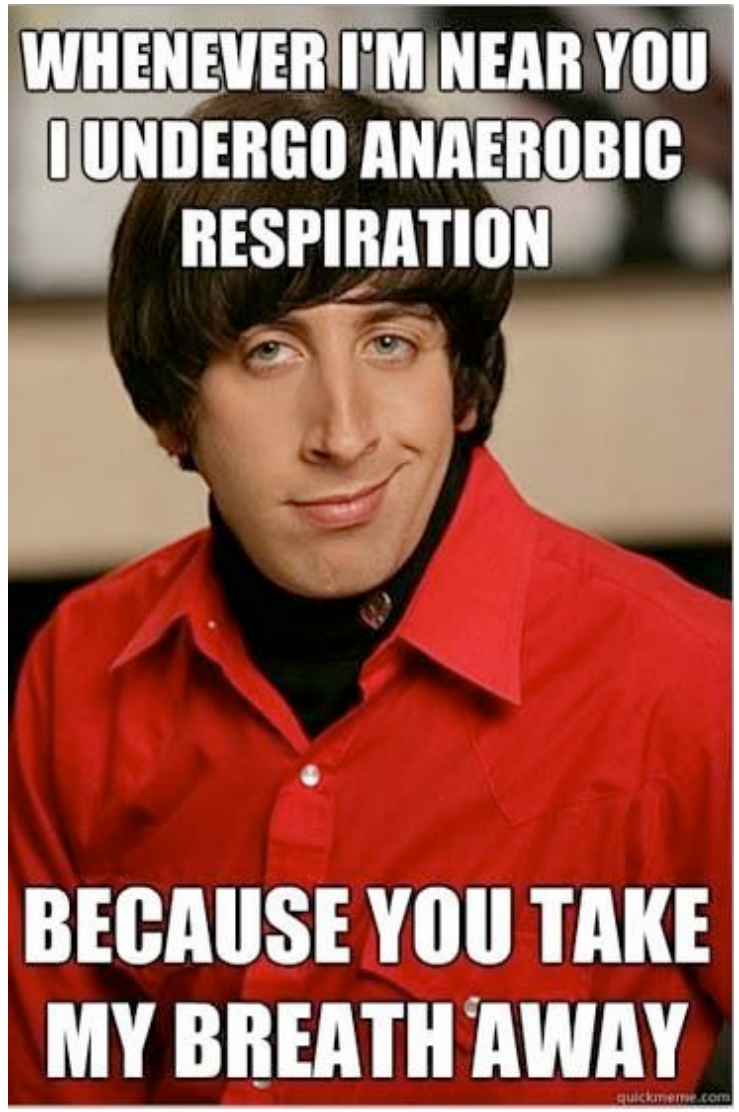


Don’t be this person!

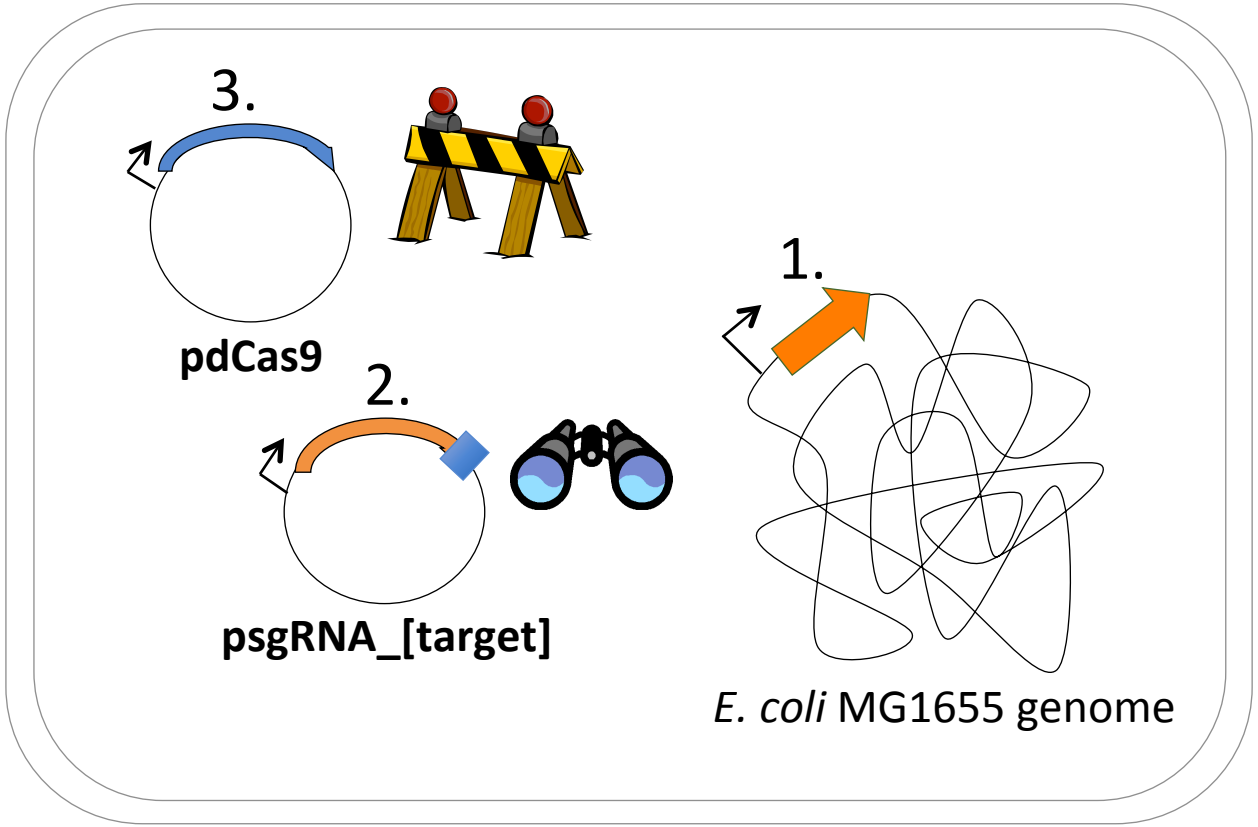
Your biological engineering task in Mod 2:

Increase production of ethanol or acetate in *E. coli* MG1655 by manipulating the fermentation pathway using CRISPR-based editing technology



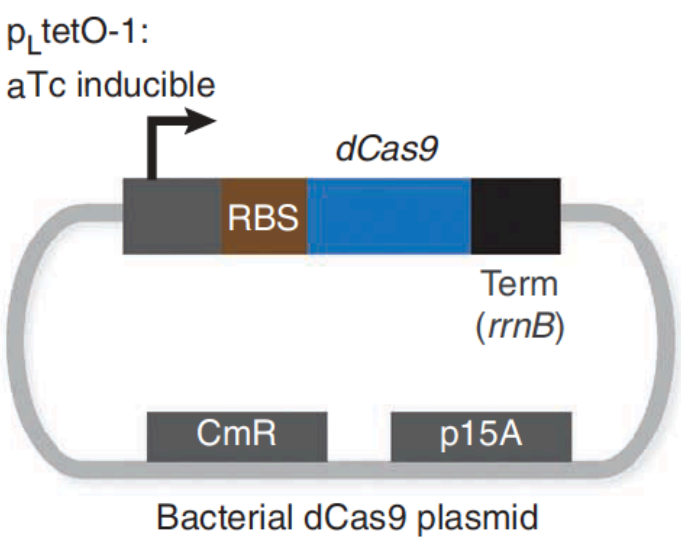

WHENEVER I'M NEAR YOU
I UNDERGO ANAEROBIC
RESPIRATION
BECAUSE YOU TAKE
MY BREATH AWAY
quickmeme.com

CRISPRi system involves three key players

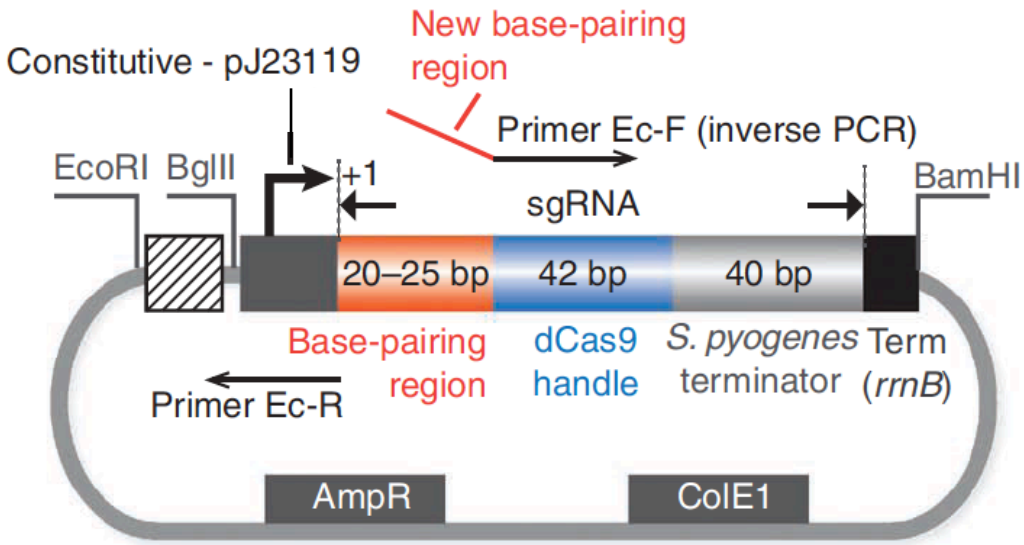


1. Target gene
2. psgRNA_[target]
3. pdCas9

Closer look at psgRNA and pdCas9



Bacterial dCas9 plasmid

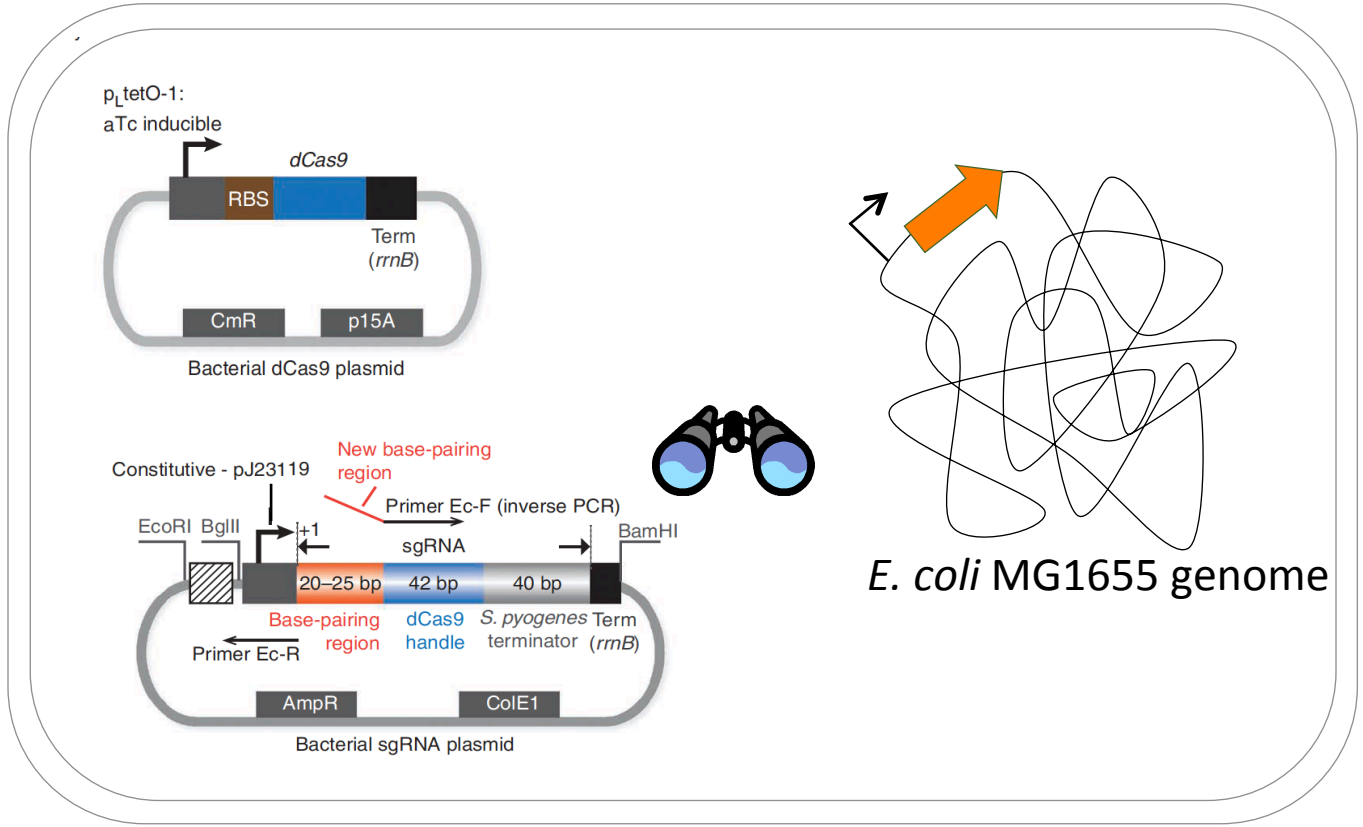


Bacterial sgRNA plasmid

Prepare confirmation digest to check pdCas9 construct on M2D1

Design gRNA target sequence for psgRNA_[target] construct on M2D2

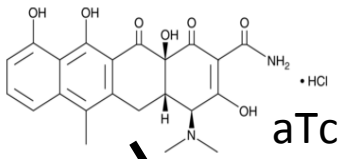
CRISPRi ‘inactive’ in absence of inducer

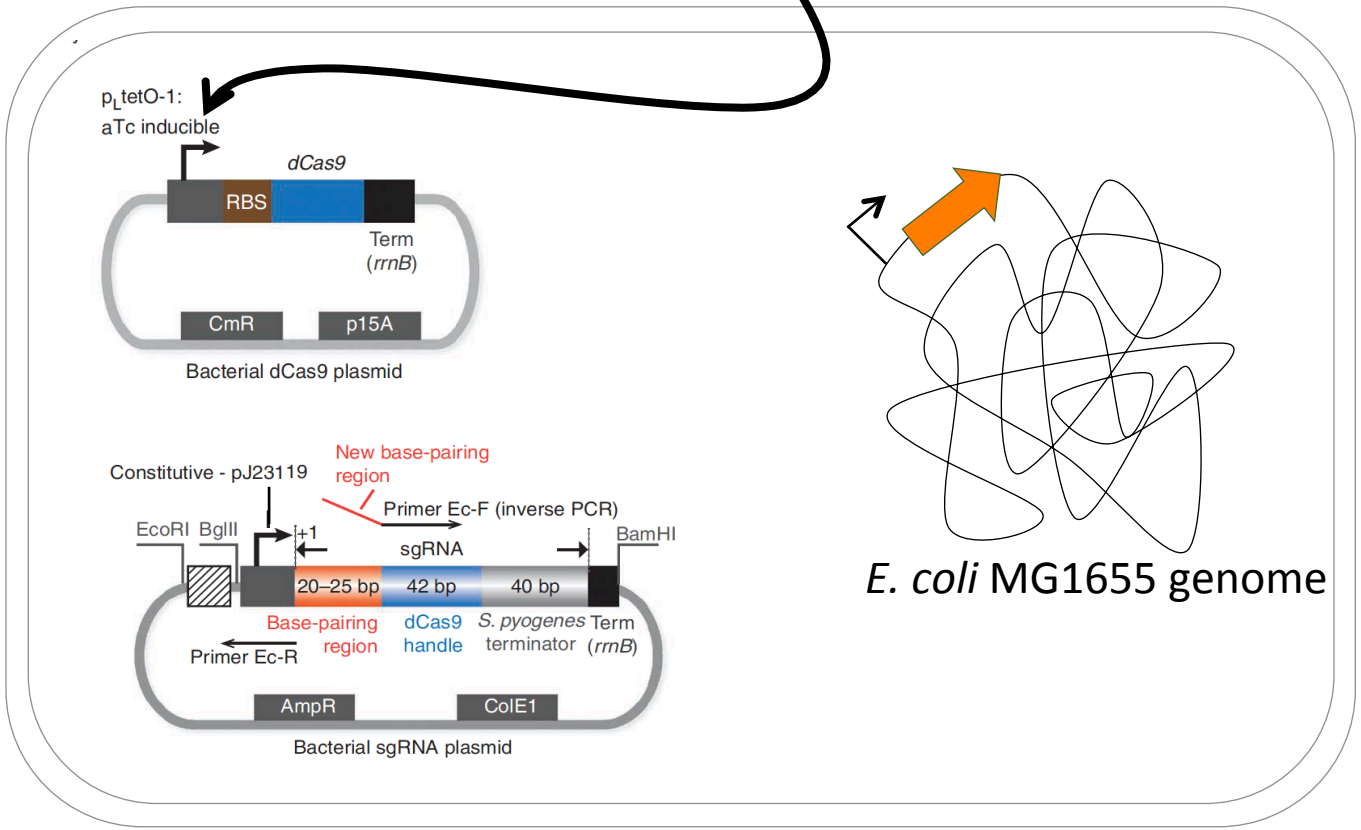


psgRNA_[target] is expressed constitutively

- sgRNA is continually transcribed

CRISPRi ‘blocks’ gene expression in presence of inducer

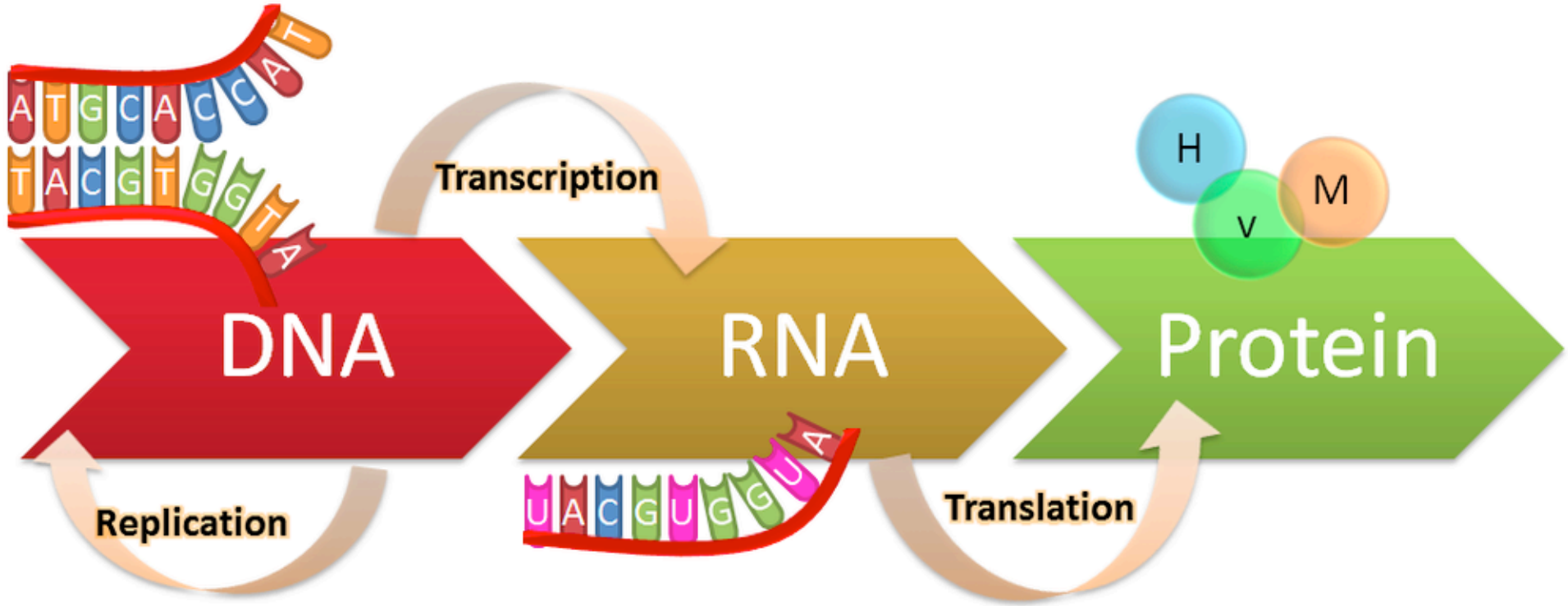




pdCas9 is expressed when inducer aTc added

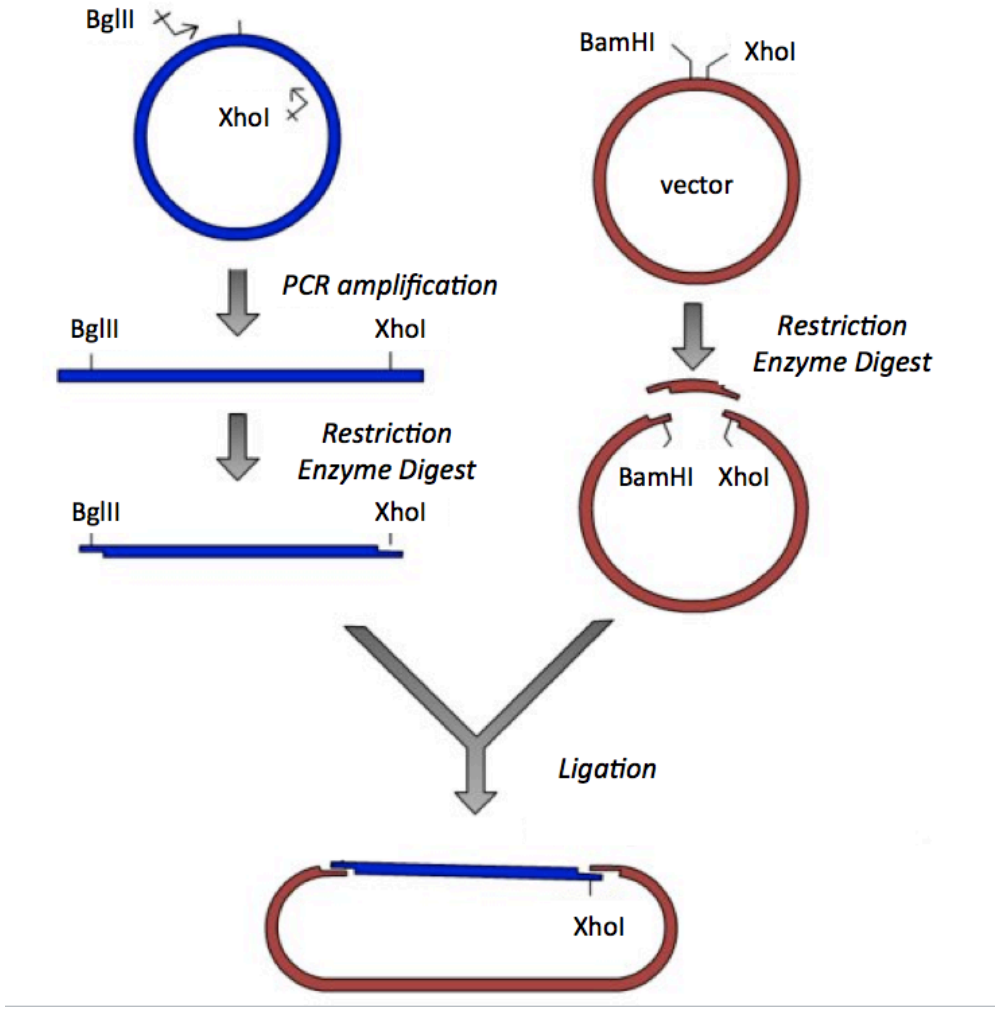
- when transcribed, associates with psgRNA_[target]
- Cas9 / psgRNA_[target] complex scans DNA for target gene

The central dogma



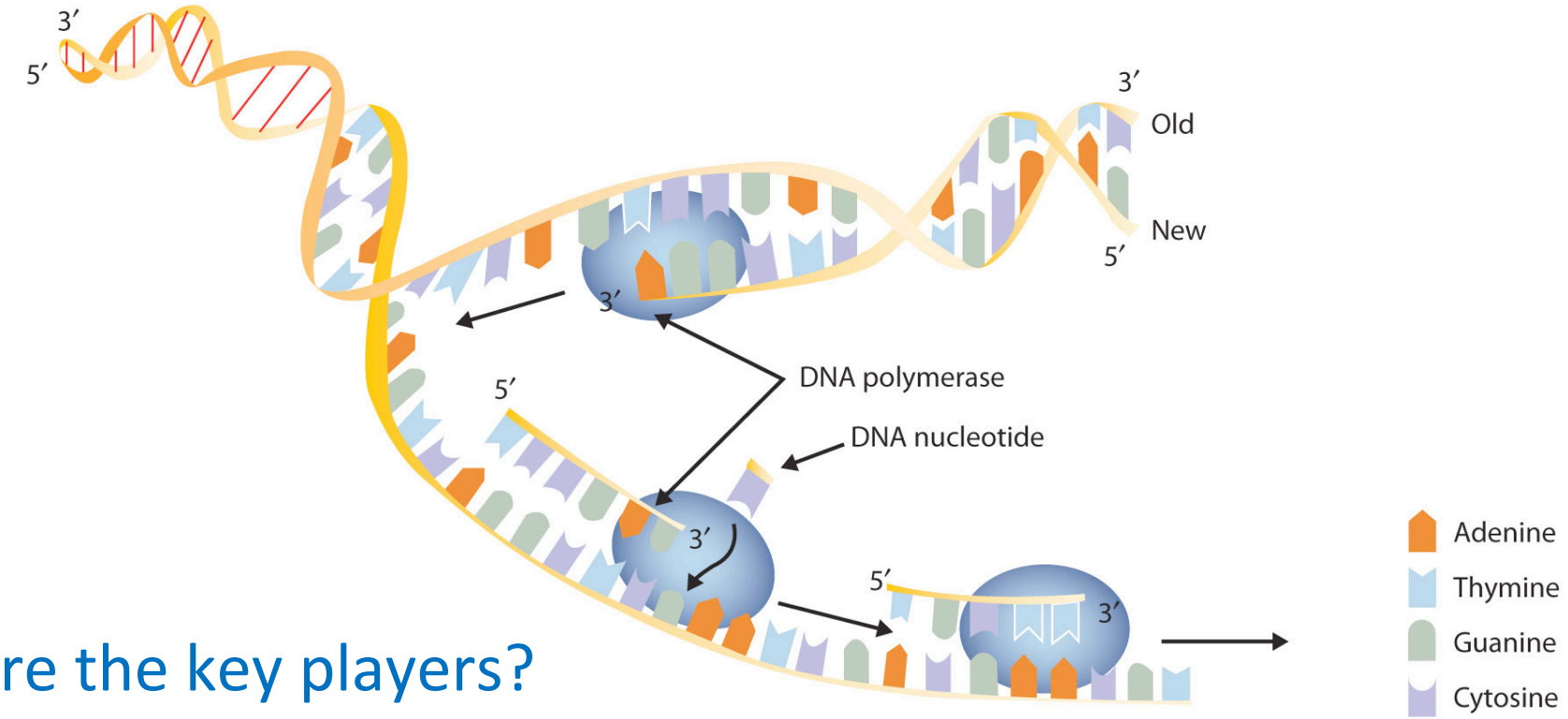
http://genius.com/Biology-genius-the-central-dogma-annotated

How do we engineer DNA?



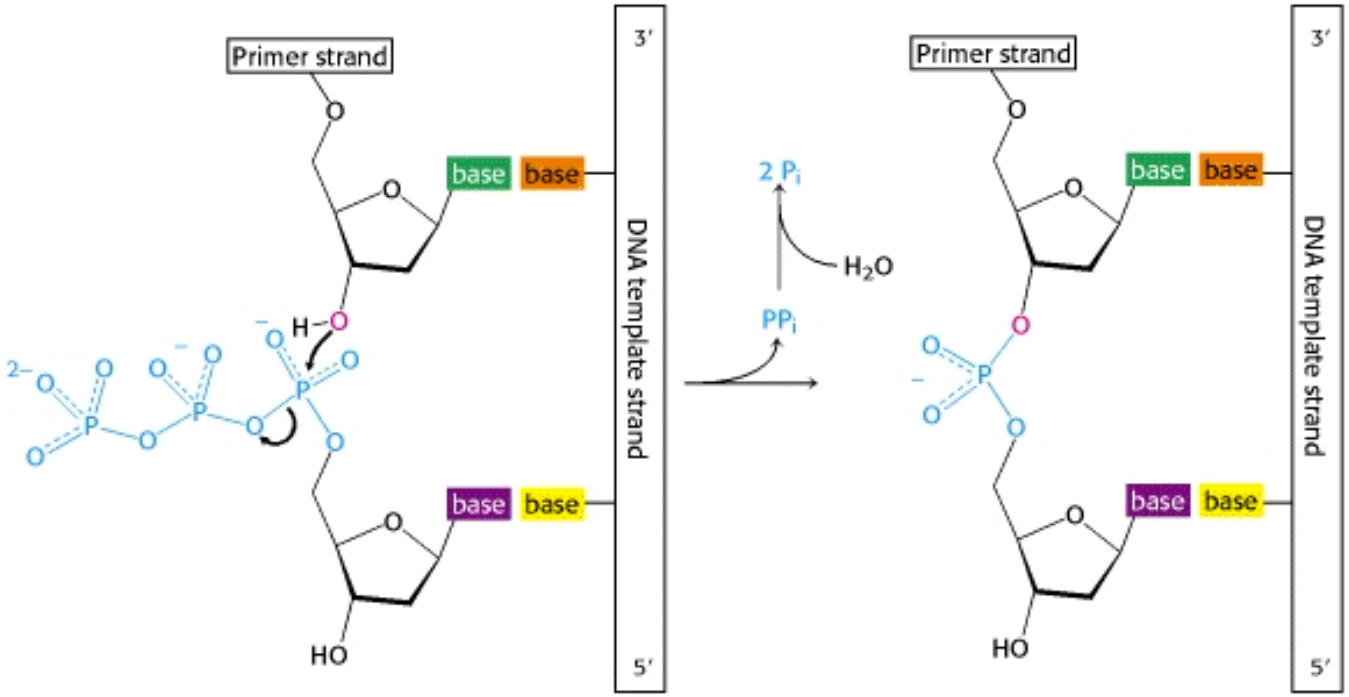
1. Amplification
2. Digestion
3. Ligation

1. Amplification



Who are the key players?

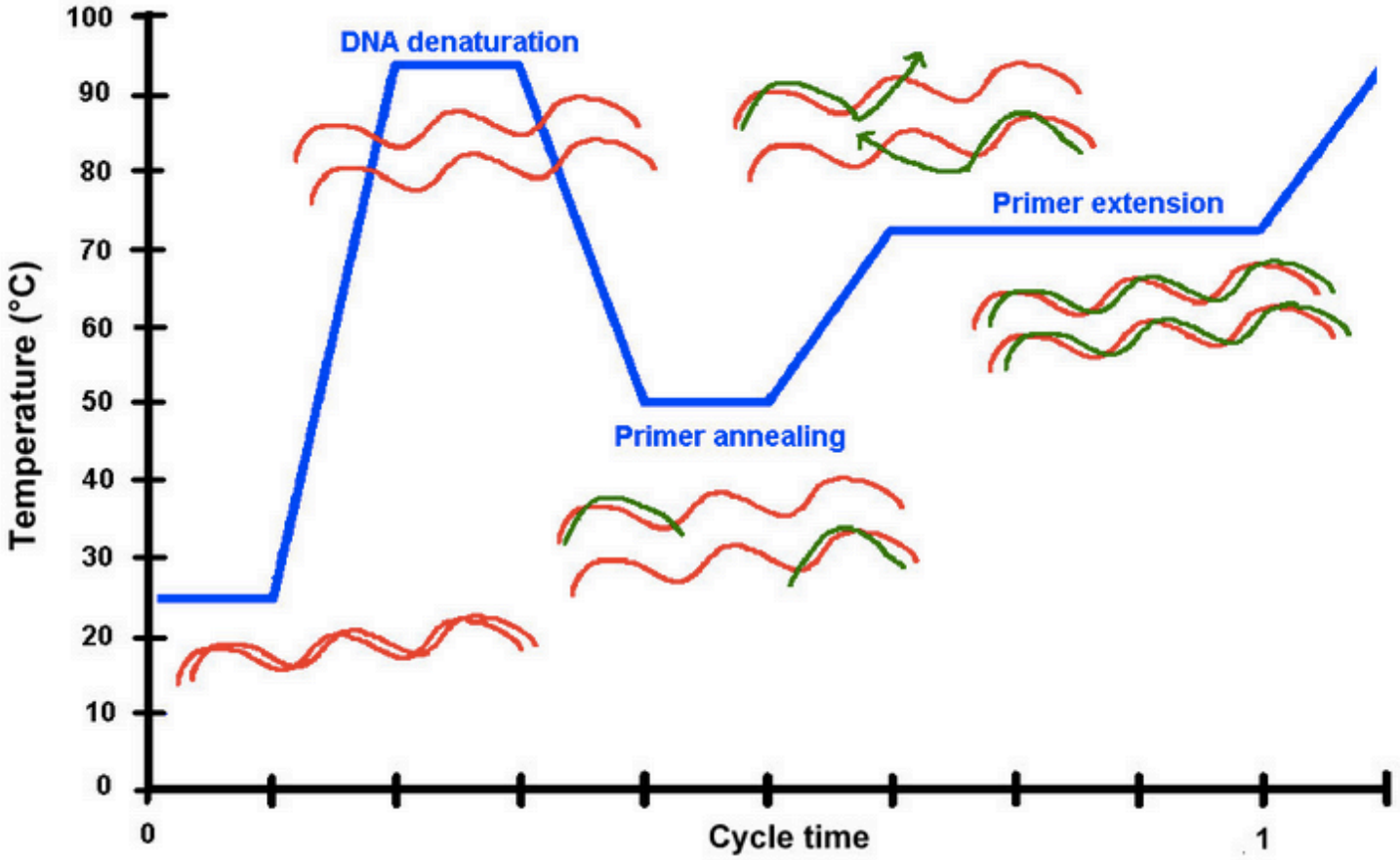
DNA polymerase



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

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

Polymerase chain reaction (PCR)



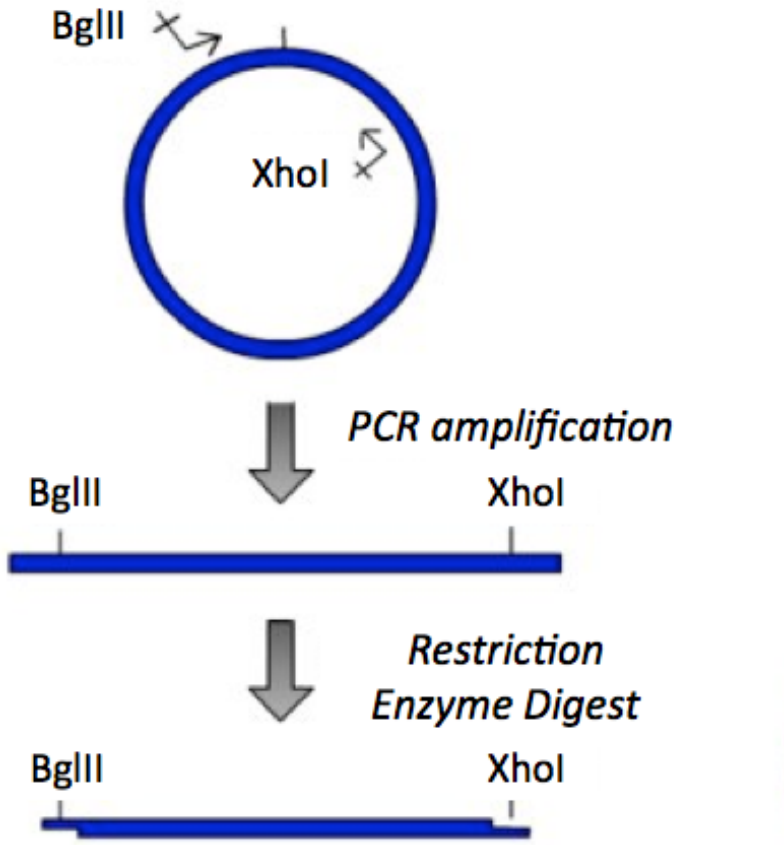
How many cycles until the desired product is generated?

What are we amplifying?

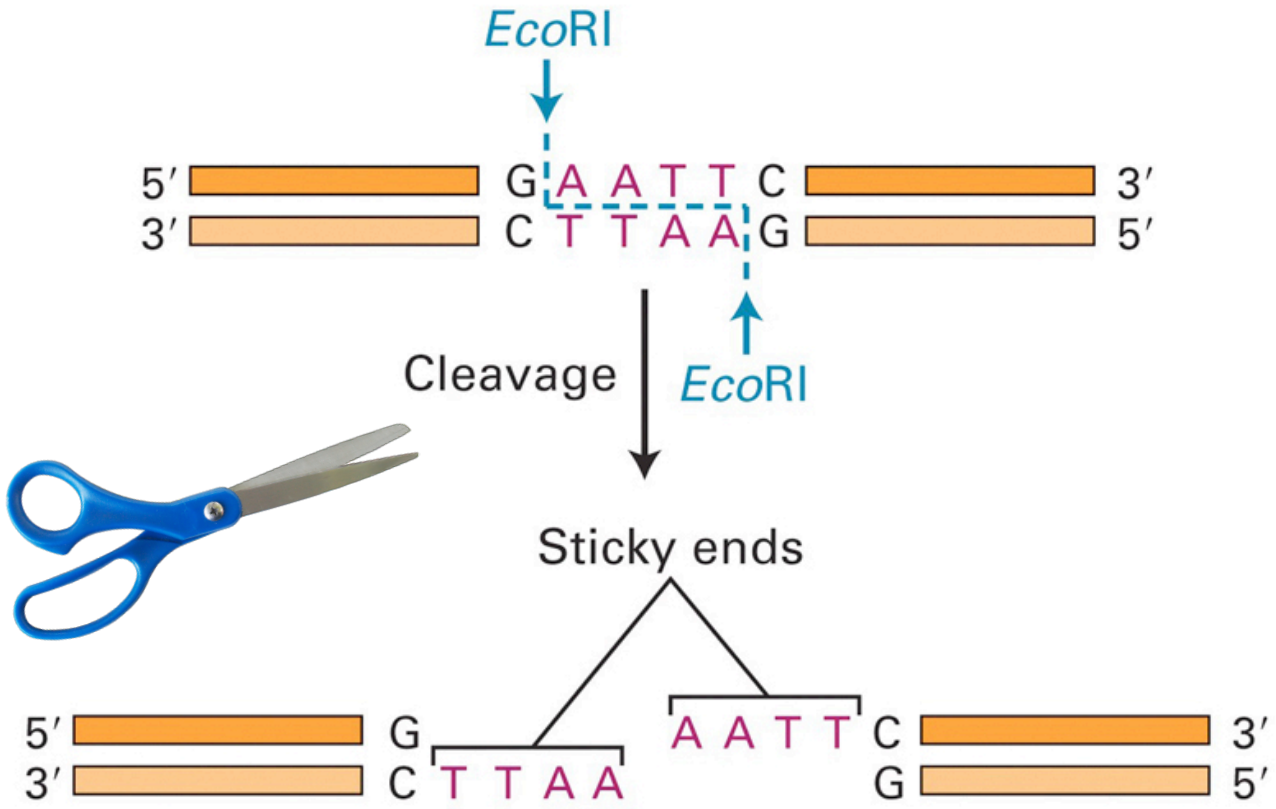
Primers enable you to specify which region of DNA is amplified by polymerase

- Polymerase requires primer to add bases in sequence

- Why do we need two primers?



2. Digestion

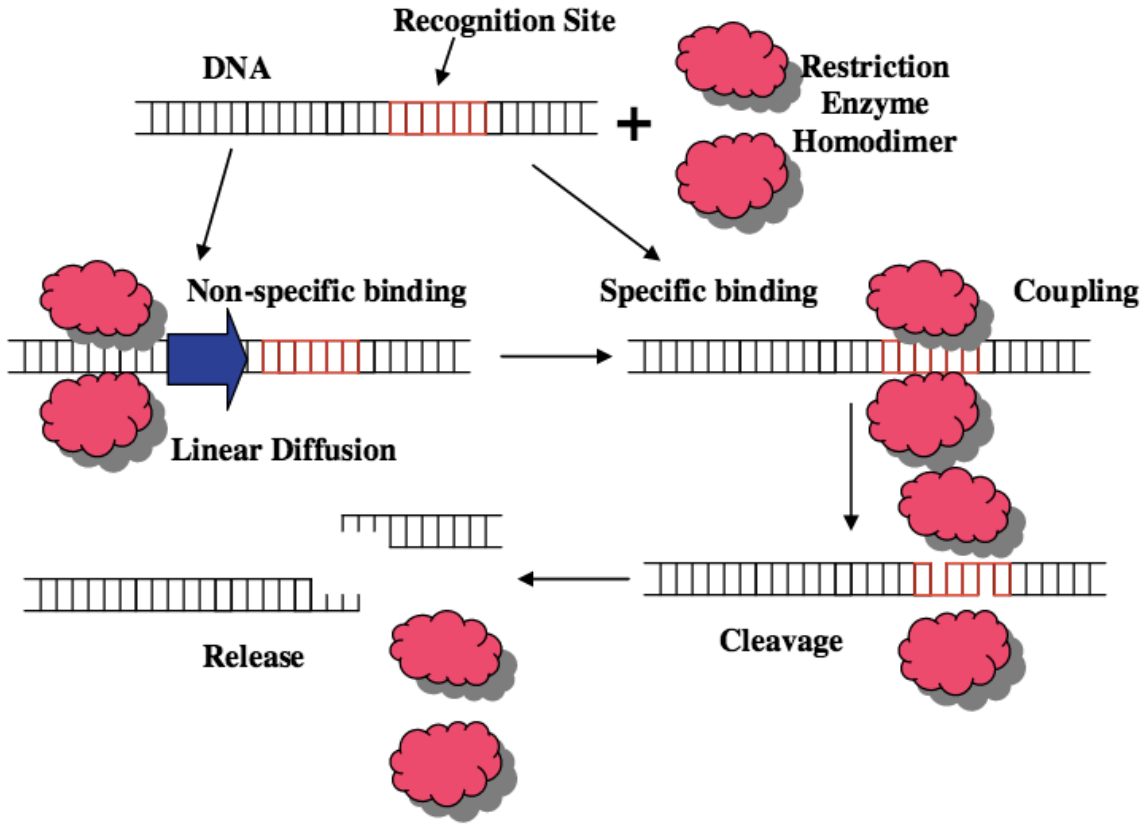

EcoRI
5′ G A A T T C 3′
3′ C T T A A G 5′
Cleavage
EcoRI
Sticky ends
5′ G
3′ C T T A A
A A T T C 3′
G 5′

Restriction enzymes

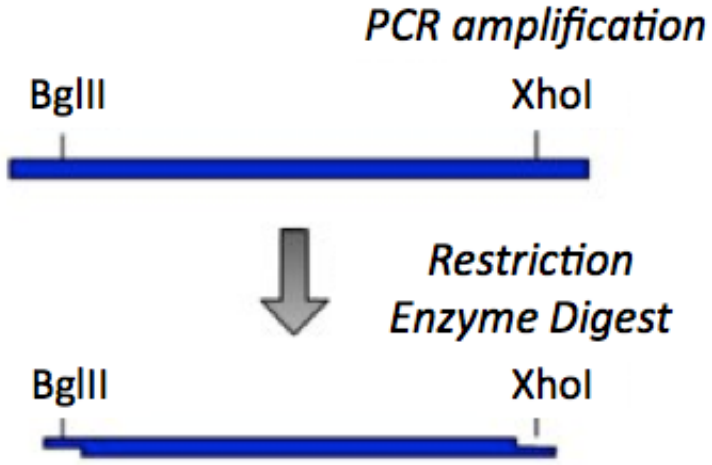
Function as homodimers

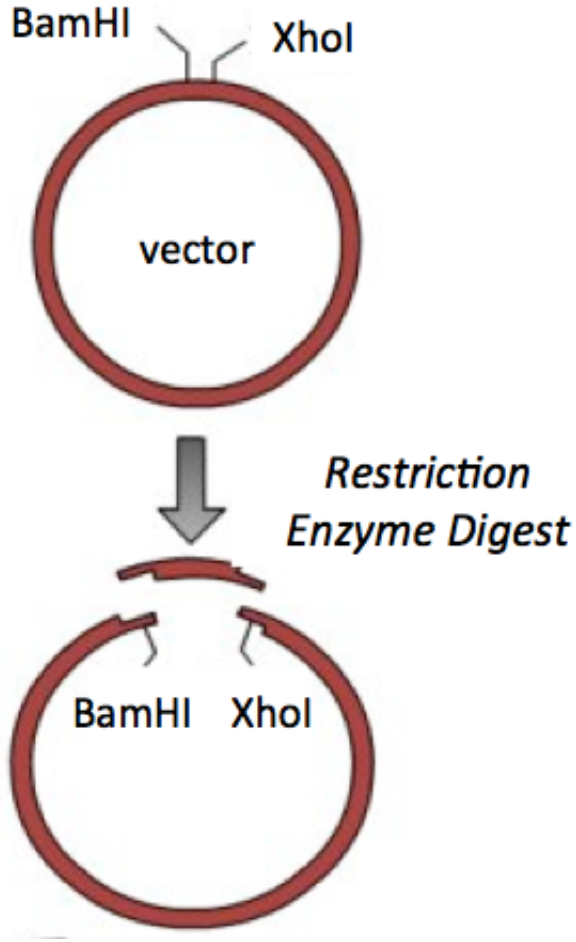
- Each dimer cleaves backbone at site of palindromic recognition sequence

- Why are most restriction sites palindromes?



What are we digesting?

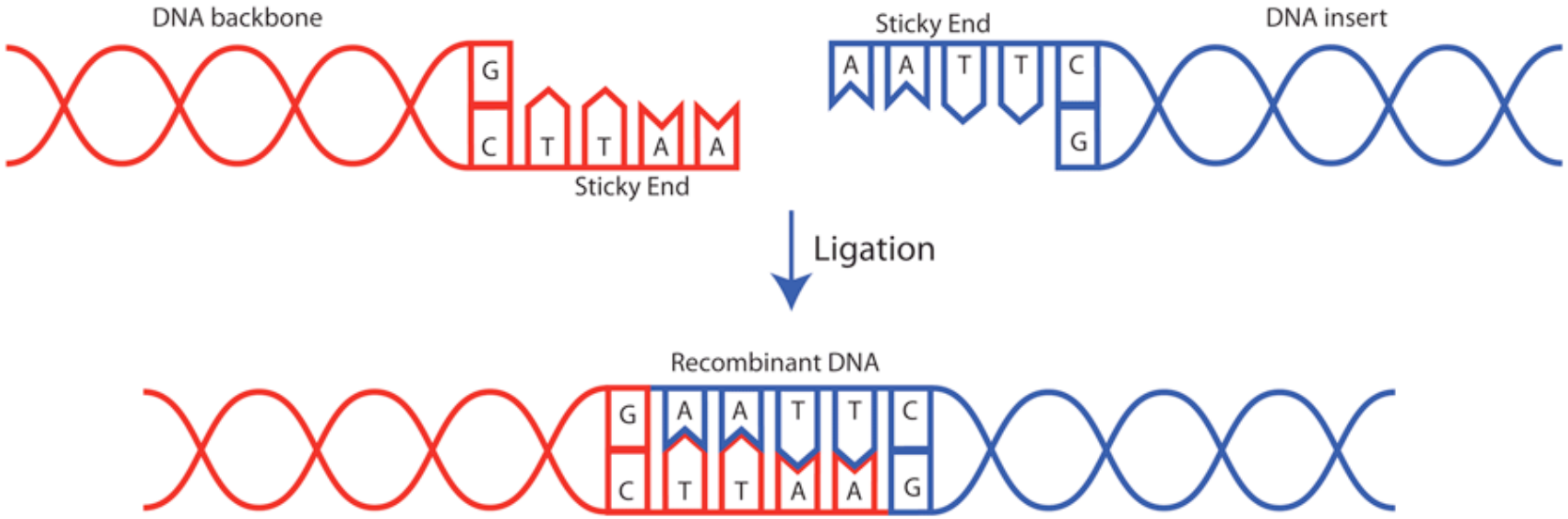




What should we consider when performing a double digest?

3. Ligation

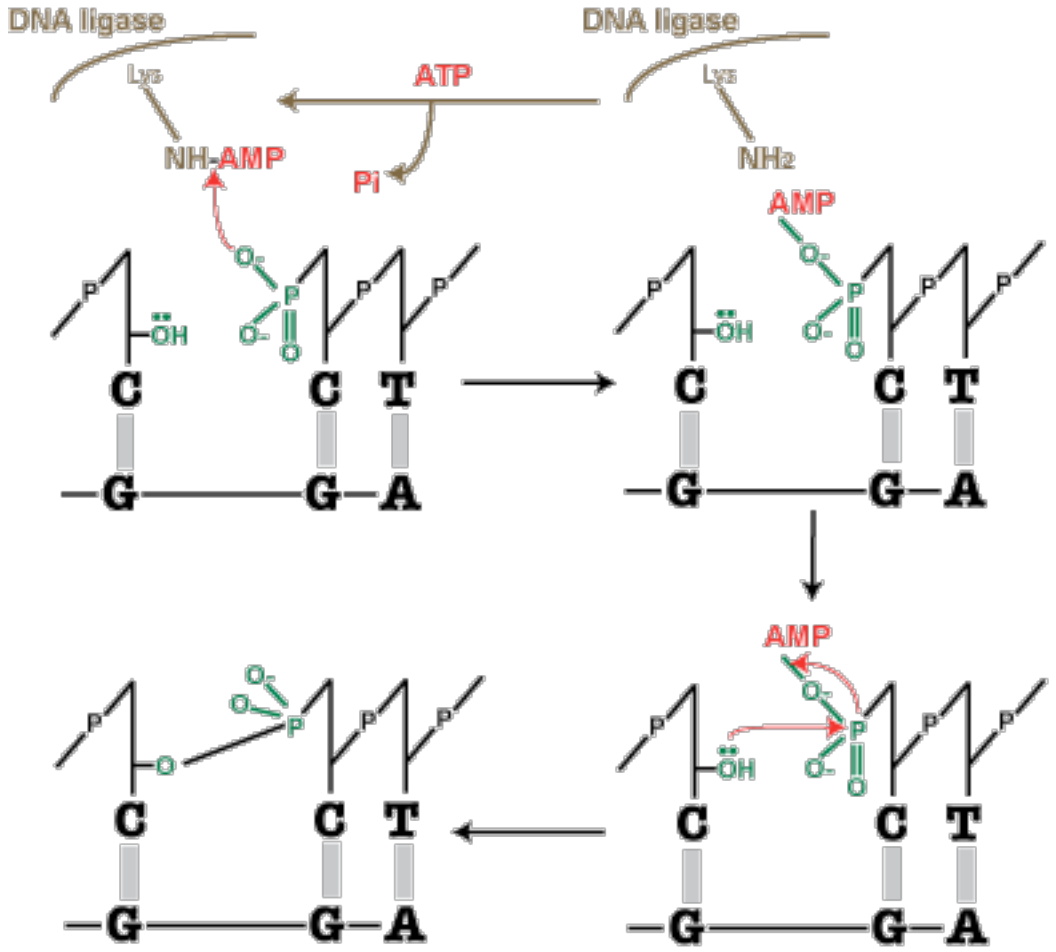

Glue-All
ELMER'S


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

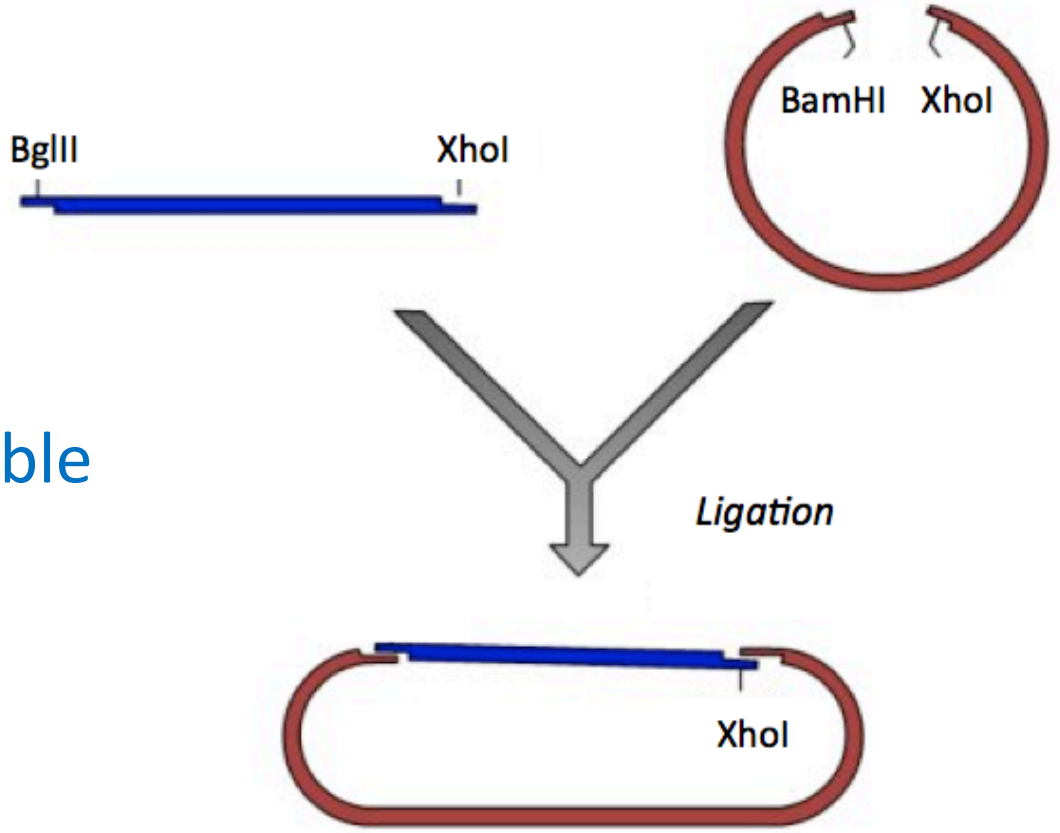
- Requires ATP for adenylation of lysine residue in active site of DNA ligase
- AMP then transferred to 5’ phosphate of DNA base



bitesizebio.com

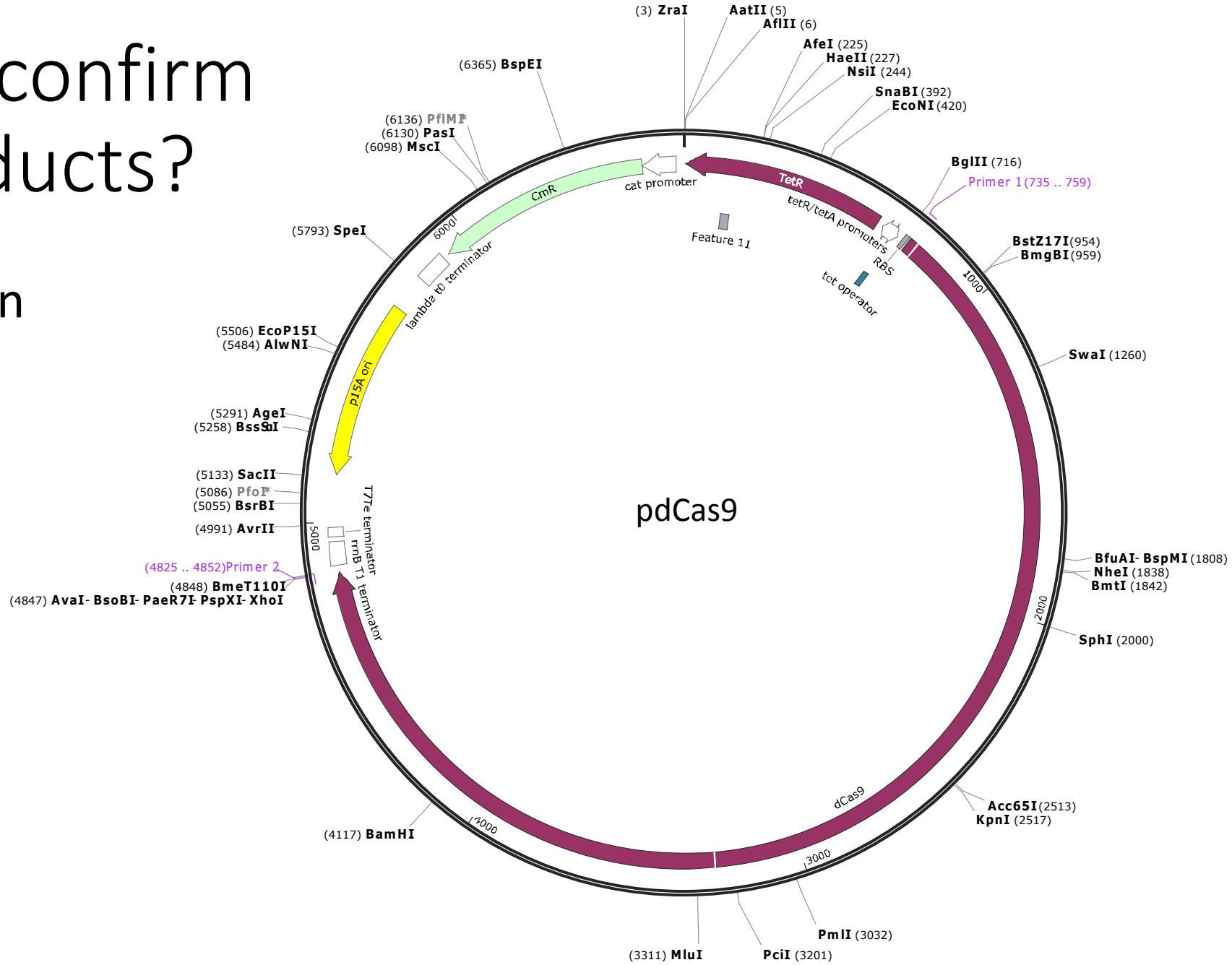
What are we ligating?

How is a BglII site compatible with a BamHI site?

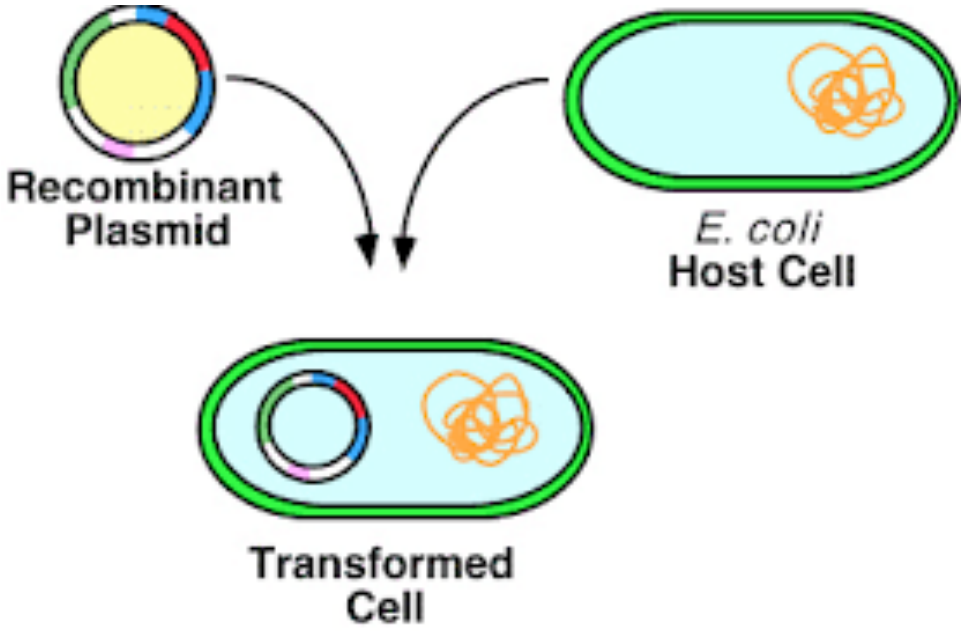


How do we confirm cloning products?

1. Transformation
2. Purification
3. Digestion



1. Transformation

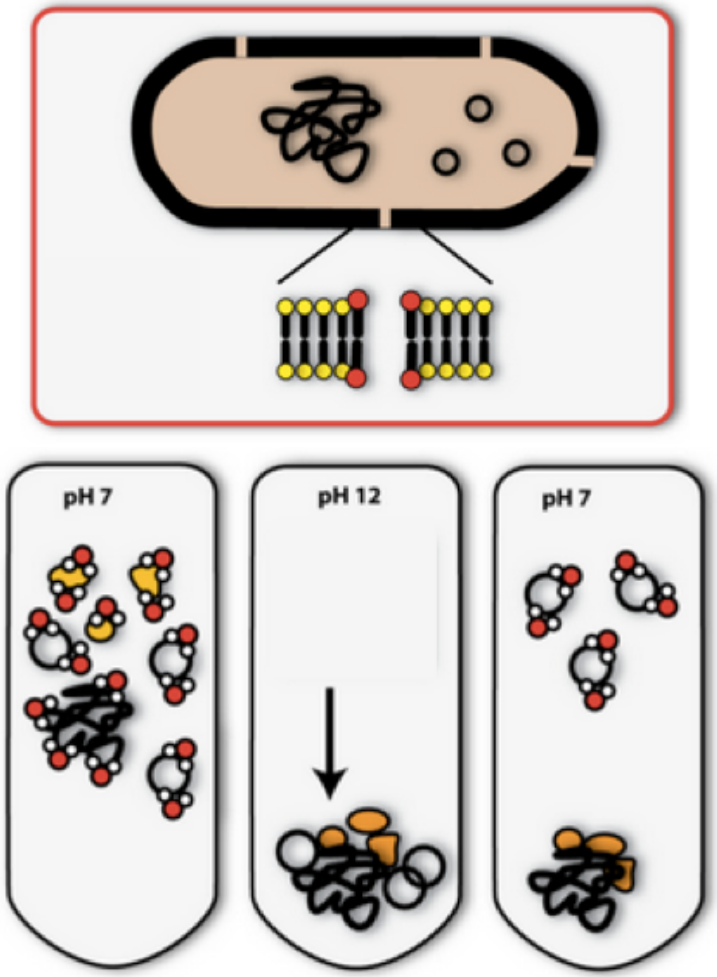


1. Incubation
2. Heat shock
   - DNA taken in by competent cells
3. Recovery
4. Selection

Why do we transform the ligation product?

2. Purification

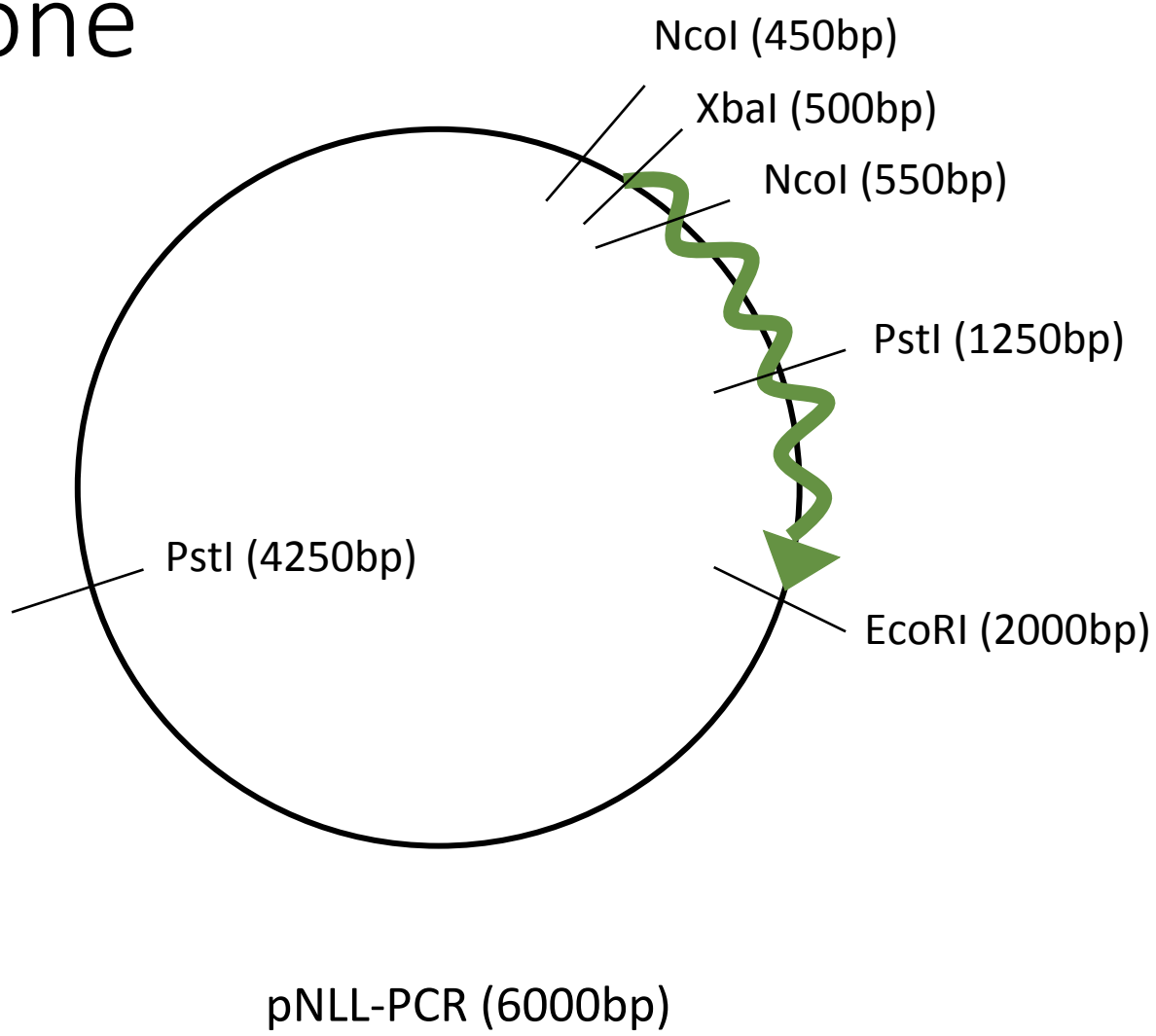
1. Resuspend cells
2. Lyse
3. Neutralize
   - Separates chromosomal DNA from plasmid DNA
4. Wash
5. Resuspend or elute DNA



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

3. Digestion, another one

- Initially a digest is required to prepare components for cloning reaction
- Confirmation digest is used to confirm cloning success
  - Ideally, will cut once in insert and once in vector
  - Should we digest with XbaI and EcoRI? PstI? NcoI?



In the laboratory…

1. Engineer pdCas9 construct
   - *In silico* ‘cloning’ of dCas9 insert into expression vector
2. Confirm pdCas9 construct
   - Actual digestion of pdCas9 to confirm successful cloning of dCas9 insert into expression vector