Module 2: Manipulating Metabolism

DNA engineering

10/13/15

Wrapping up Mod 1

- Data summary draft submitted!!
  -

  - Comments to be returned on Oct. 18
  - Revision due by 5 pm on Oct. 24
- Mini presentation due by 10 pm on Oct. 15
- Blog post due by 5 pm on Oct. 25

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:

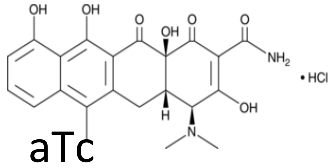
Increase production of ethanol or lactate in *E. coli* MG1655 by manipulating the fermentation pathway

WHENEVER I'M NEAR YOU
I UNDERGO ANAEROBIC
RESPIRATION

BECAUSE YOU TAKE
MY BREATH AWAY

quickmeme.com

CRISPRi system overview



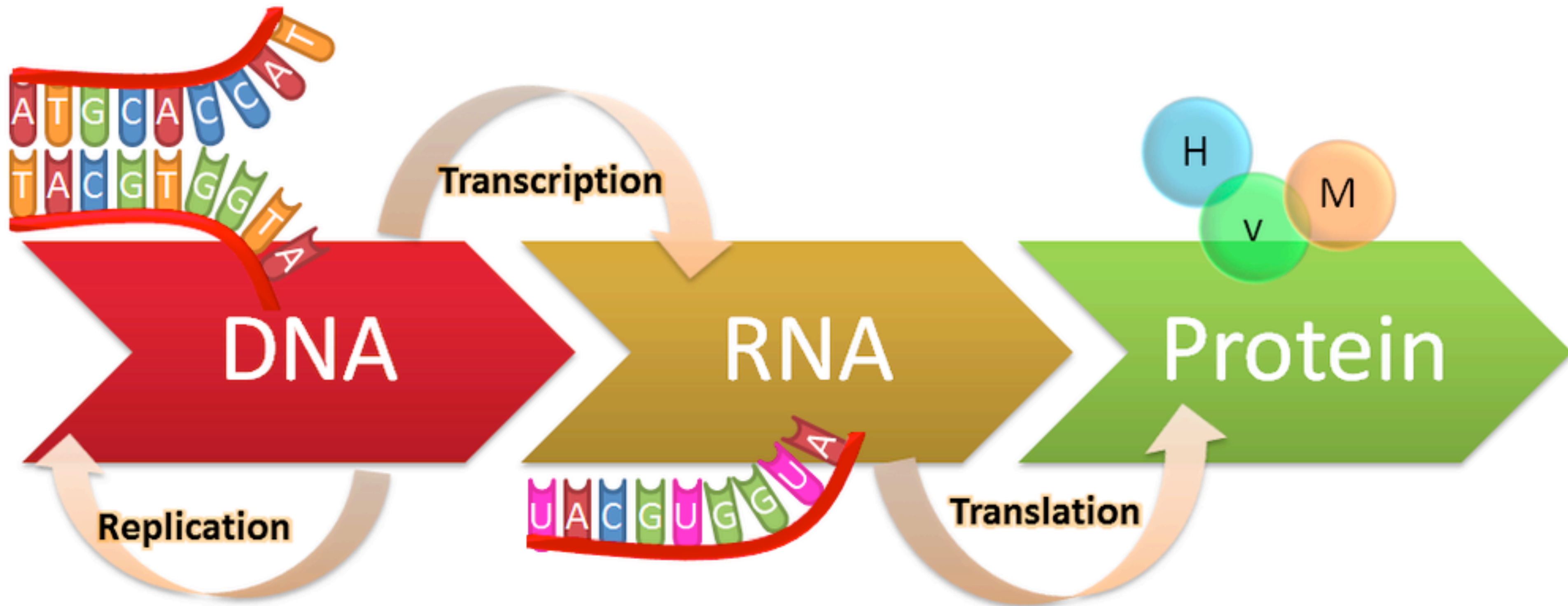
pdCas9

pgRNA_target

E. coli MG1655 genome

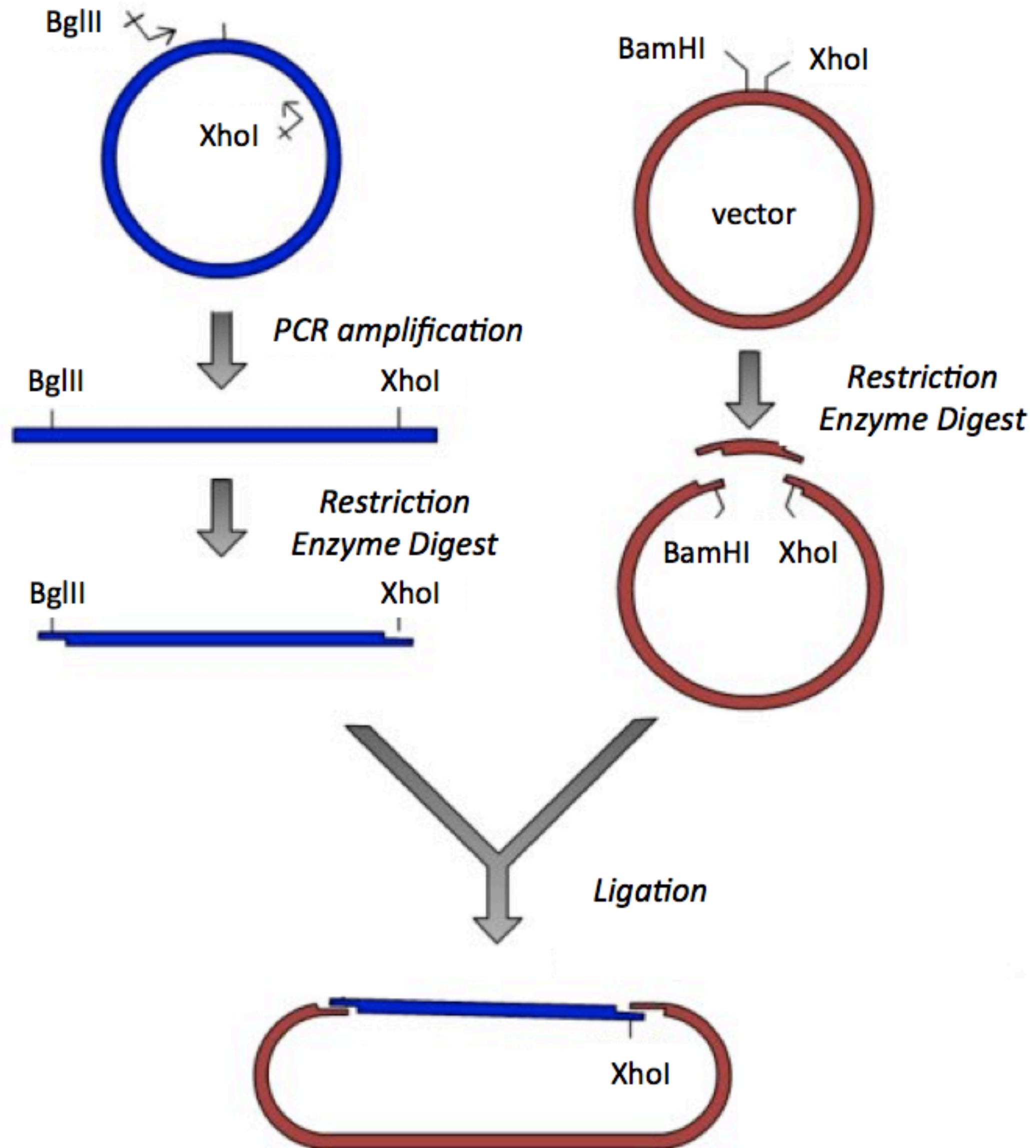
- Target gene
- pgRNA_target
- pdCas9

The central dogma



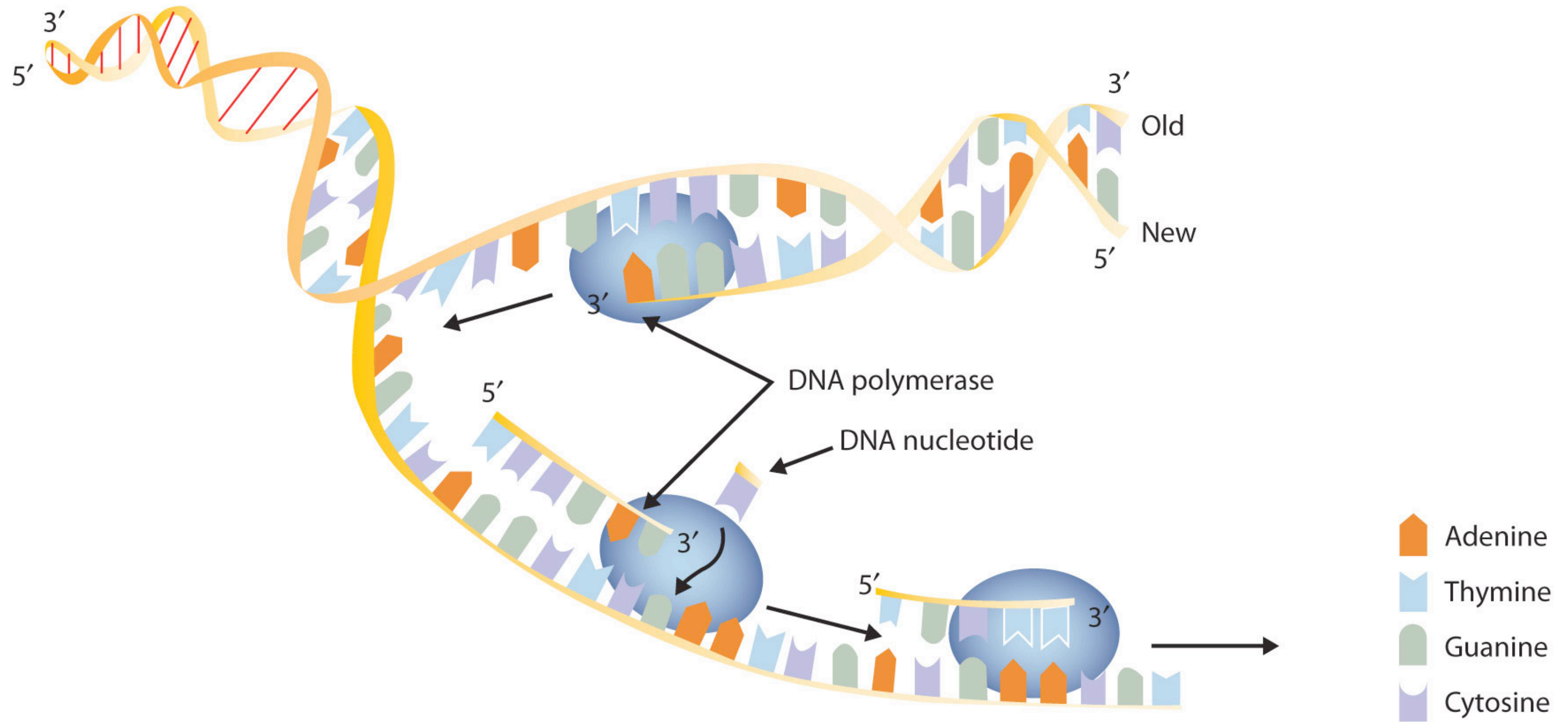
http://genius.com/Biology-genius-the-central-dogma-annotated

How do we engineer DNA?



1. Amplification
2. Digestion
3. Ligation

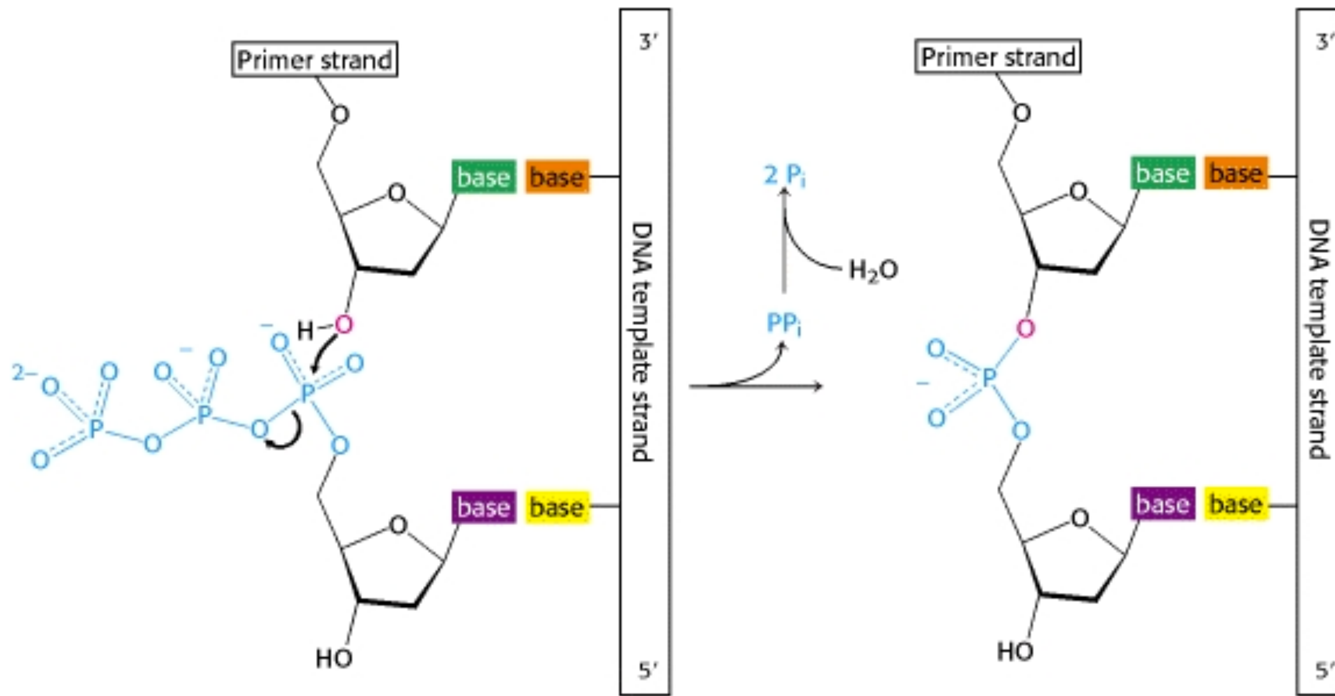
Amplification



Who are the key players?

2012books.lardbucket.org

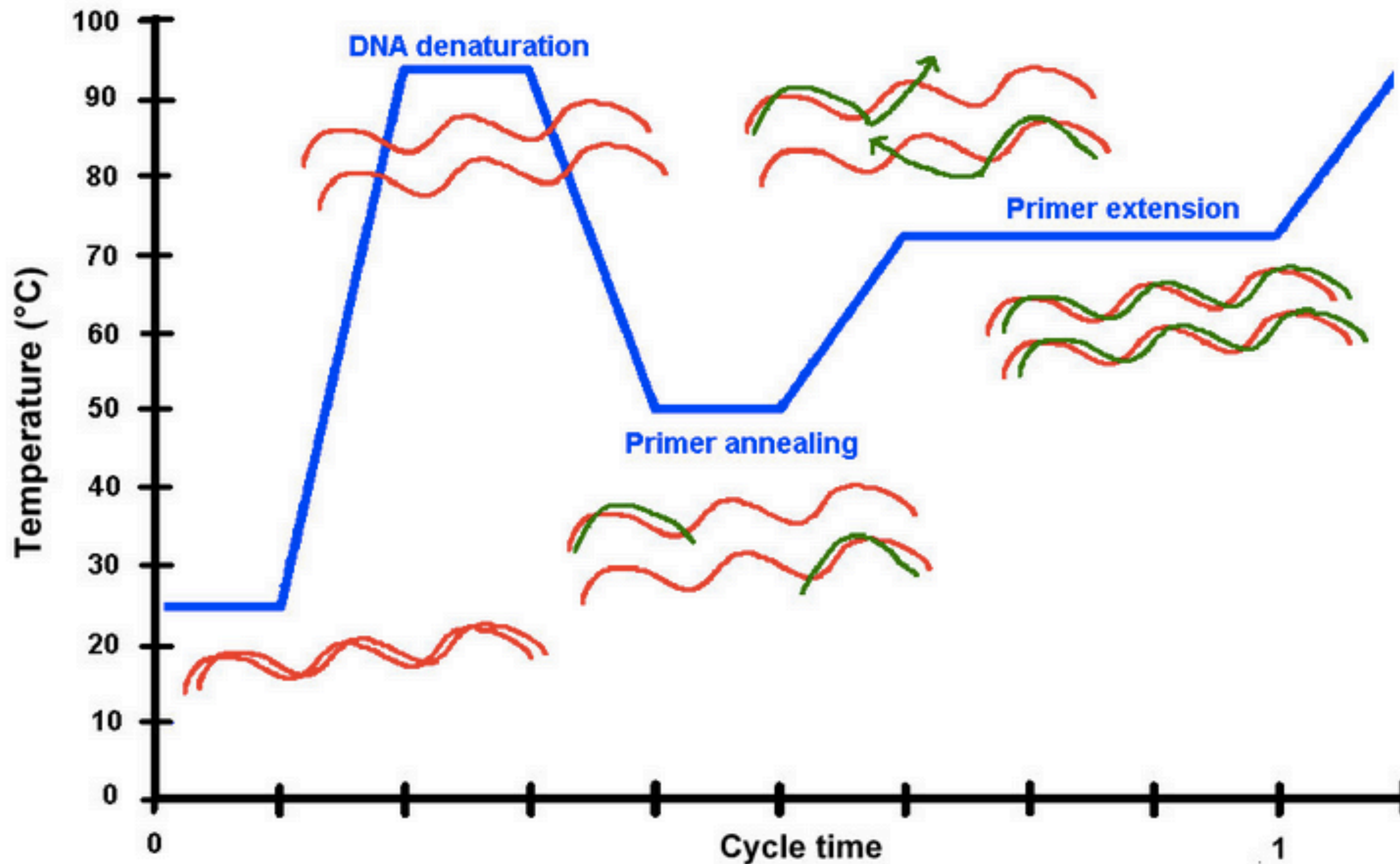
DNA polymerase



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

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

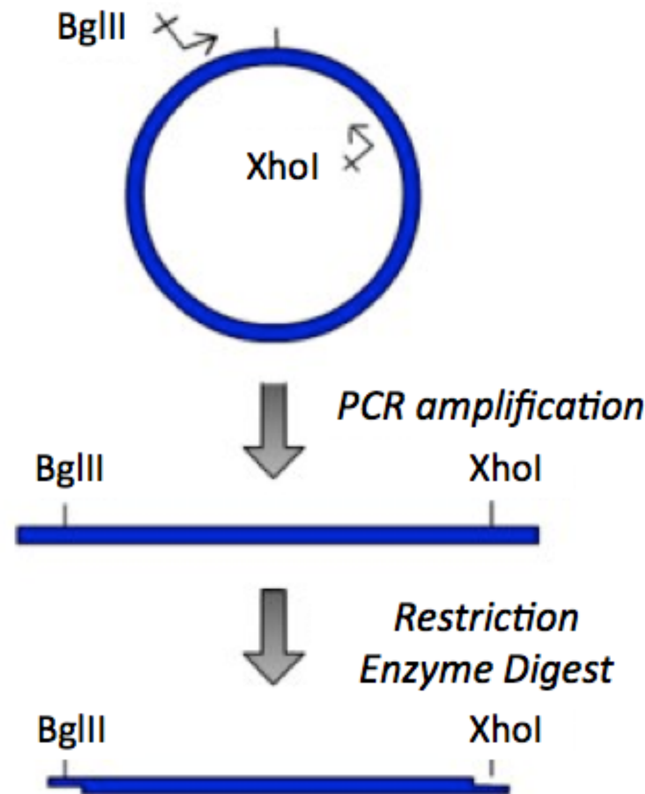
Polymerase chain reaction (PCR)



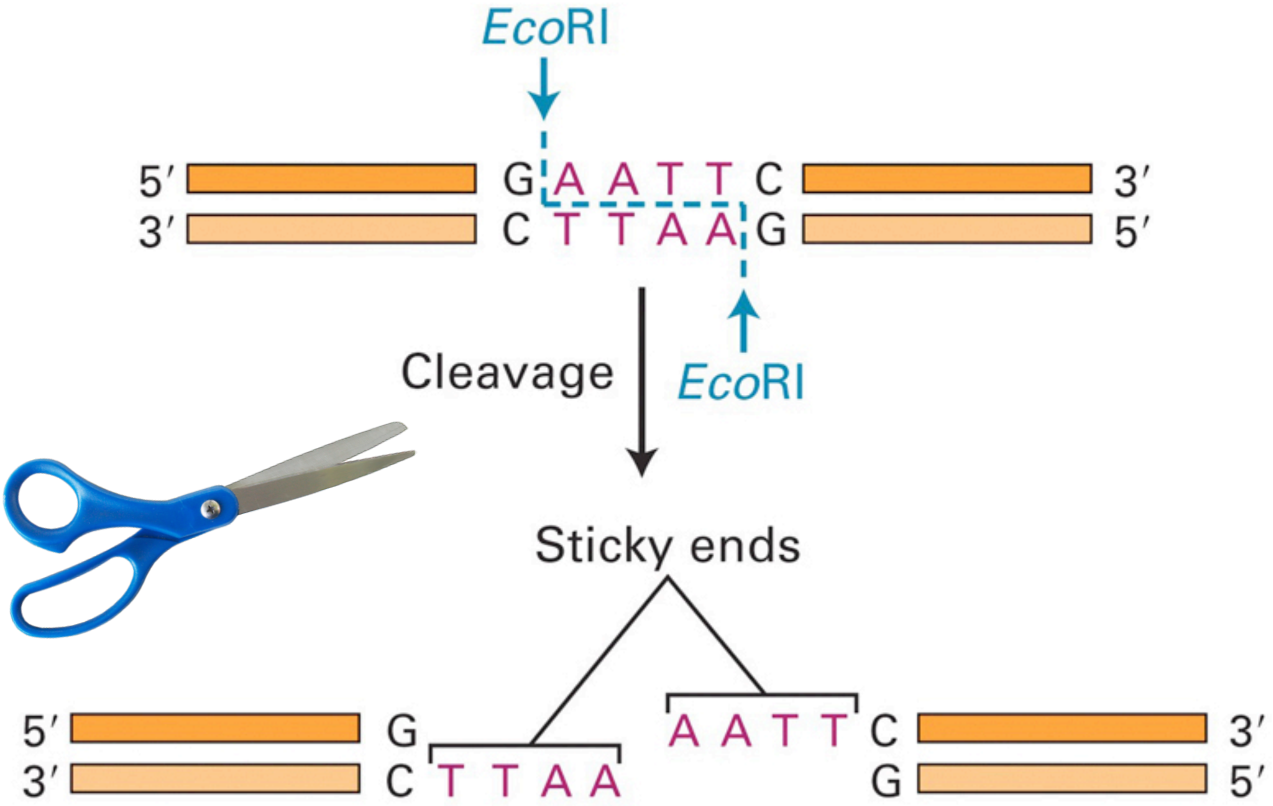
How many cycles until your product is generated?

What are we amplifying?

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

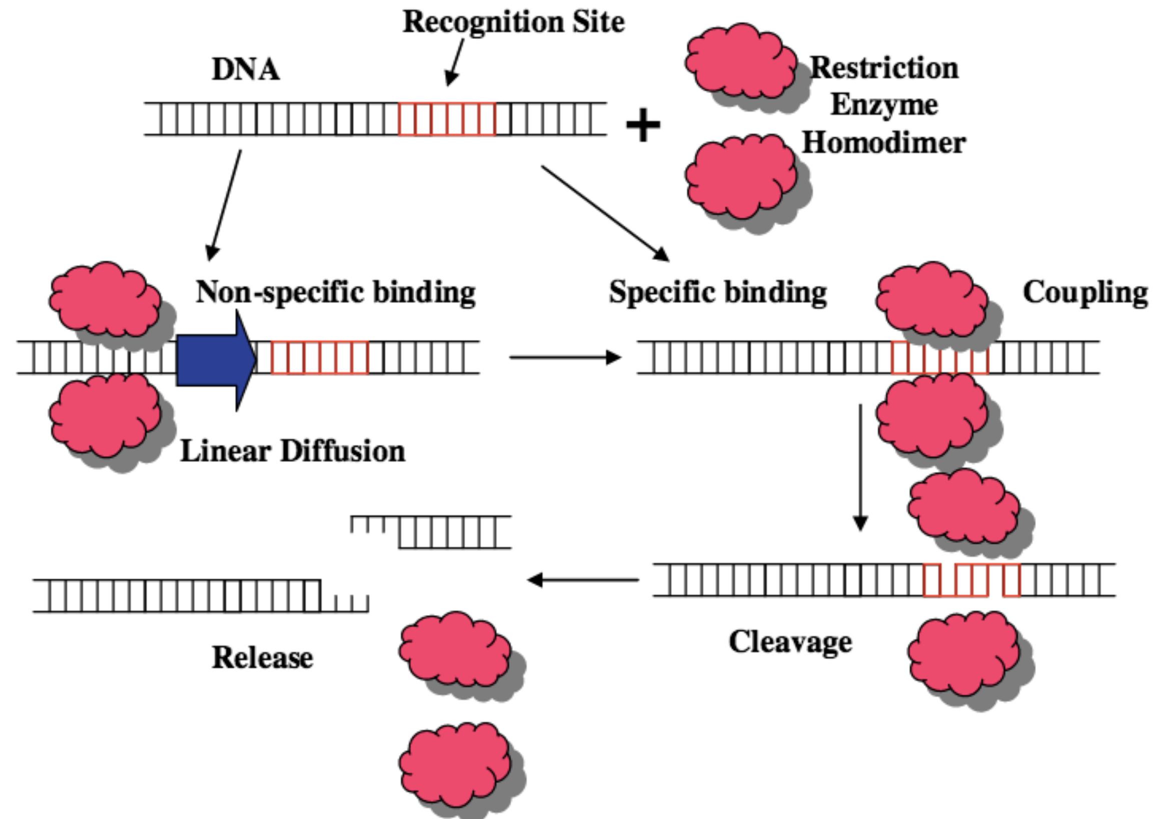


Digestion



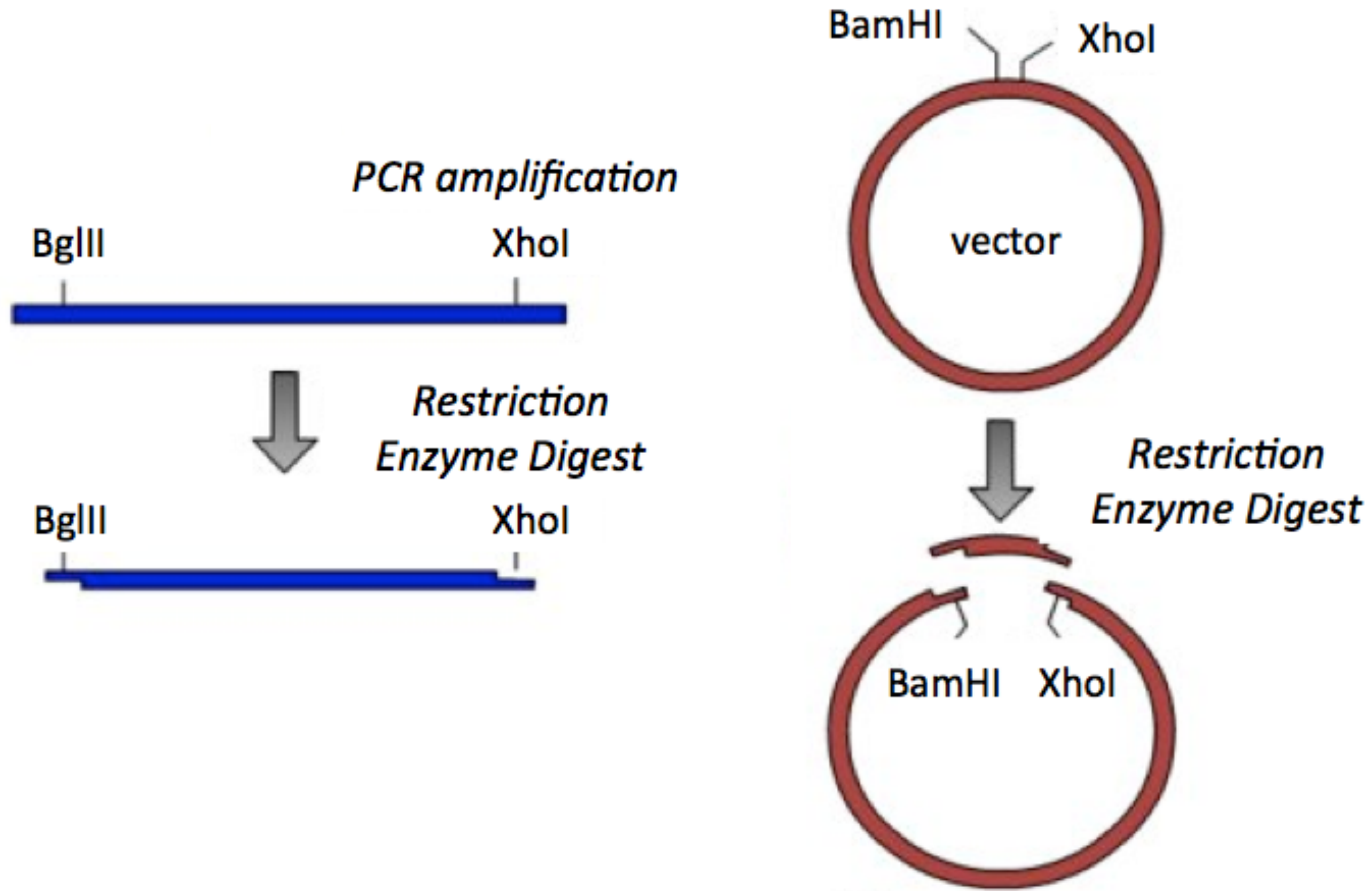
Restriction enzymes

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



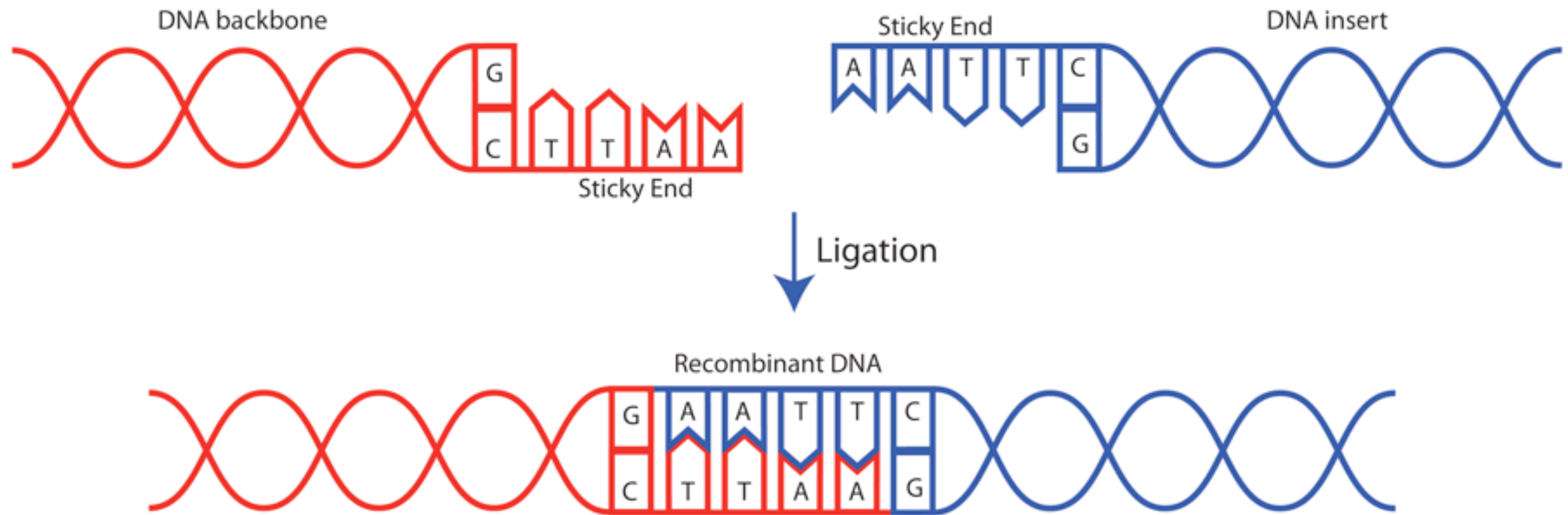
www.idtdna.com

What are we digesting?



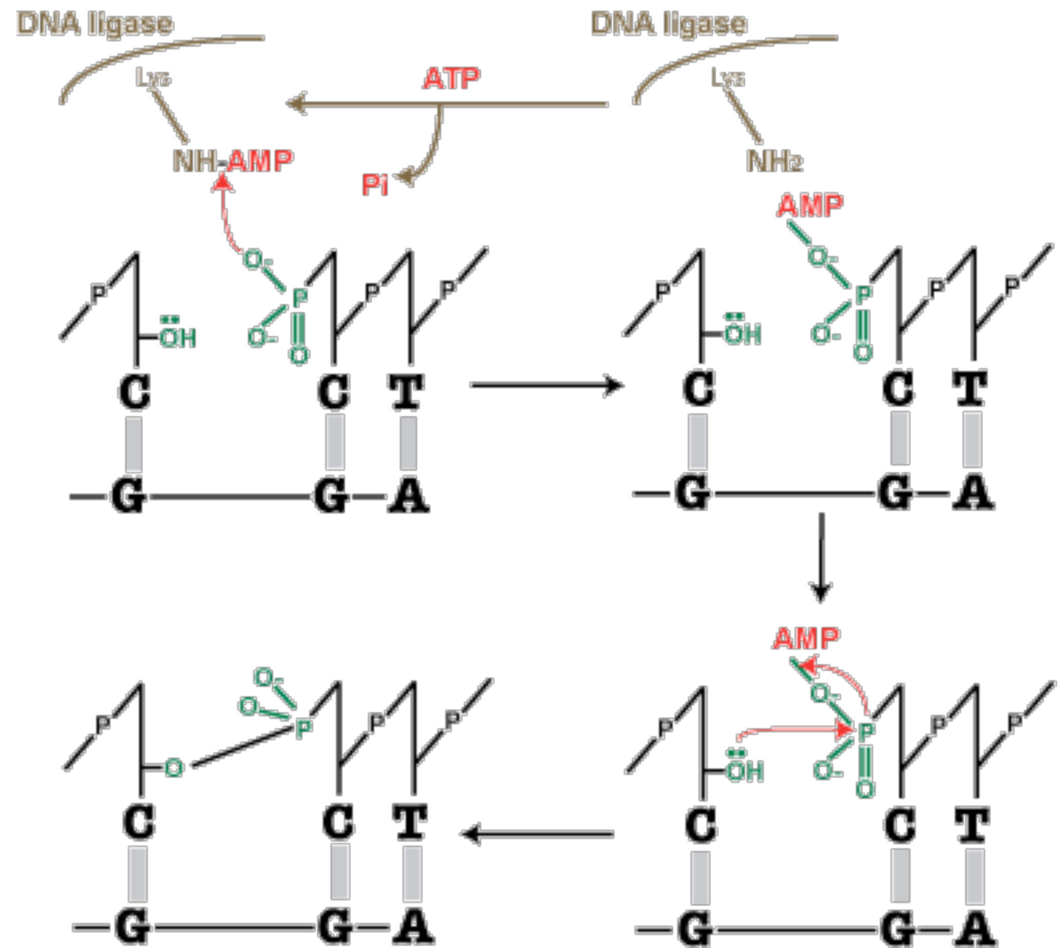
What should we consider when performing a double digest?

Ligation



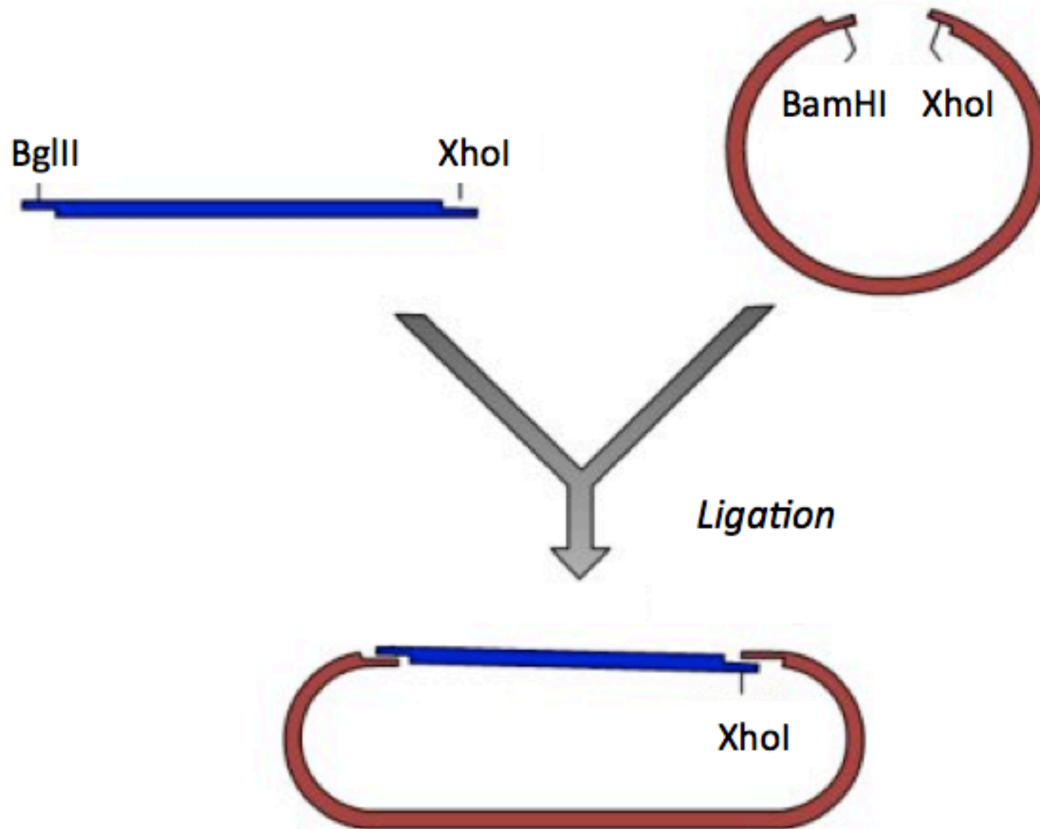
DNA ligase

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



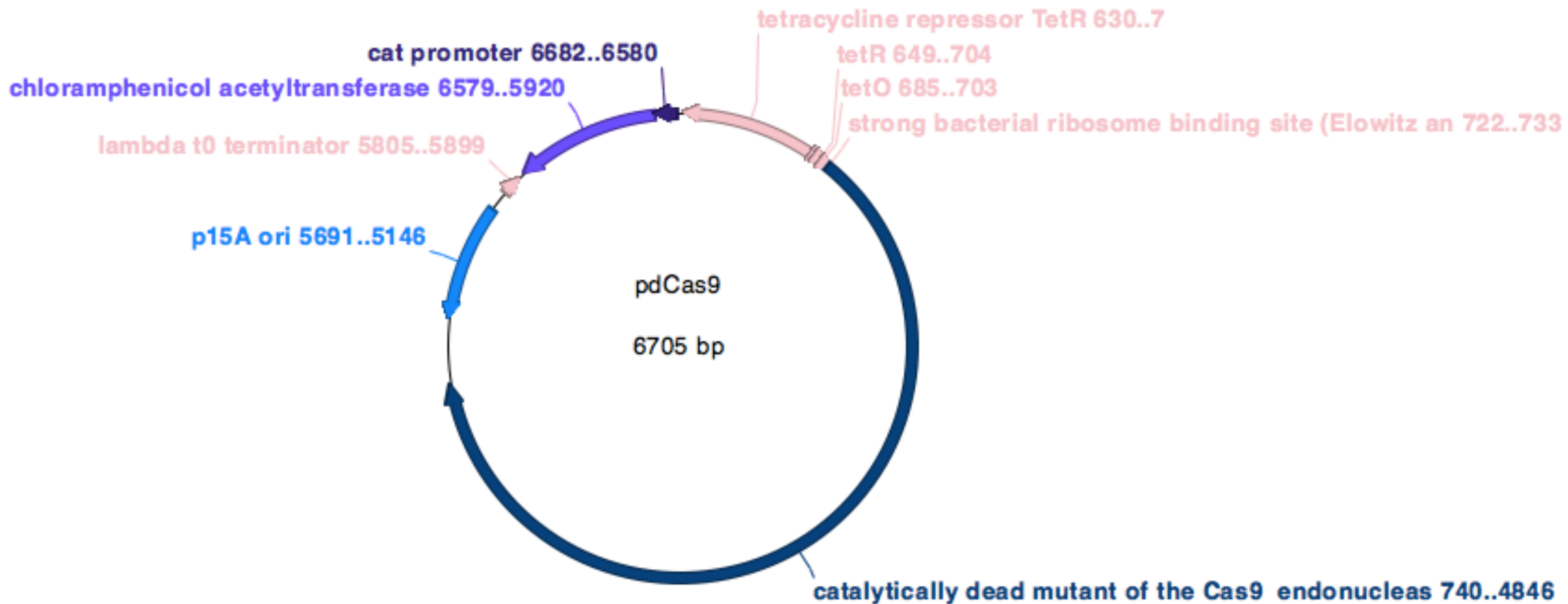
bitesizebio.com

What are we ligating?



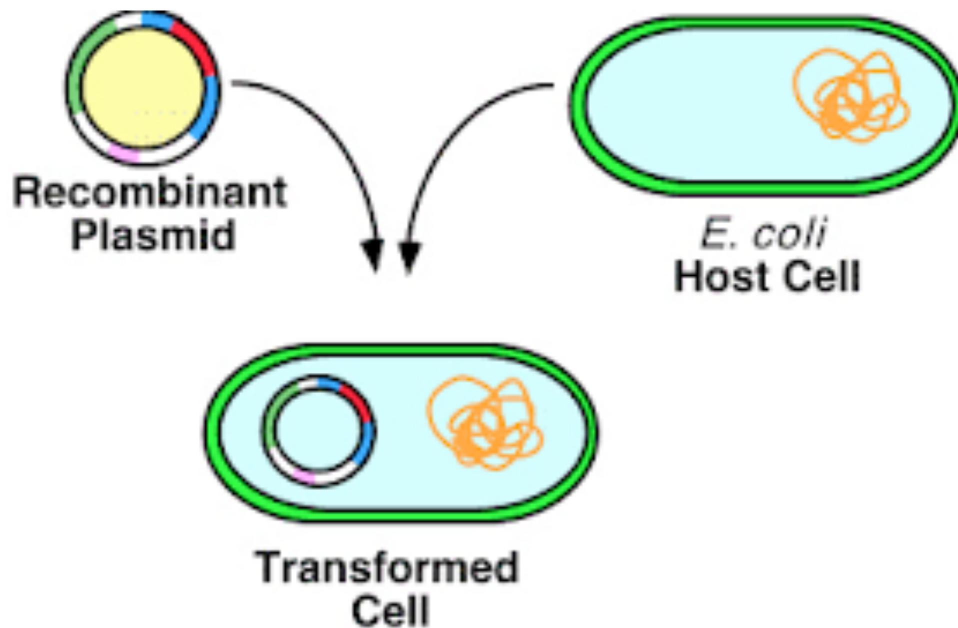
Note: in your laboratory exercise only the ‘top’ DNA strand is represented…remember this when determining basepair sites of digestion and ligation.

How do we confirm our product?



1. Transformation 2. Purification 3. Digestion

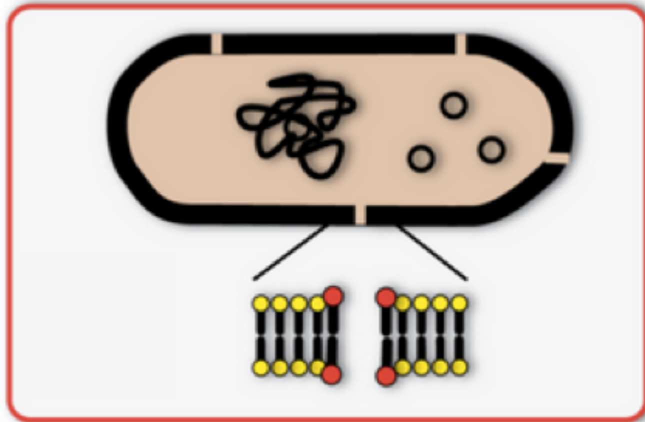
Transformation

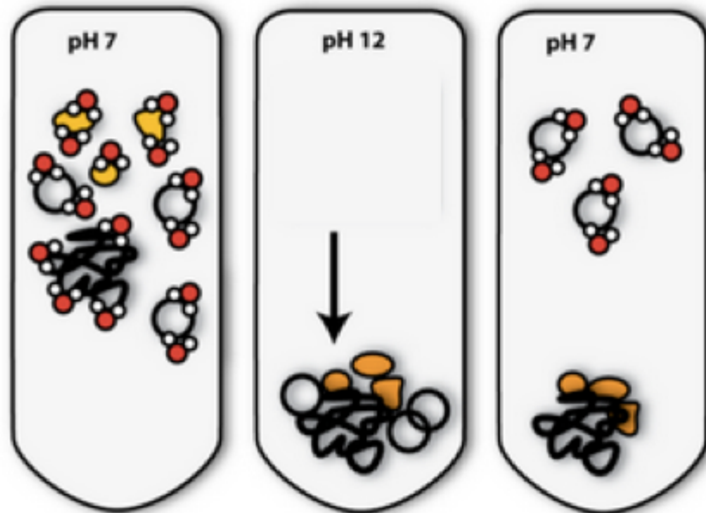


1. Incubation
2. Heat shock
   – DNA taken in by competent cells
3. Recovery
4. Selection

Why do we transform the ligation product?

Purification



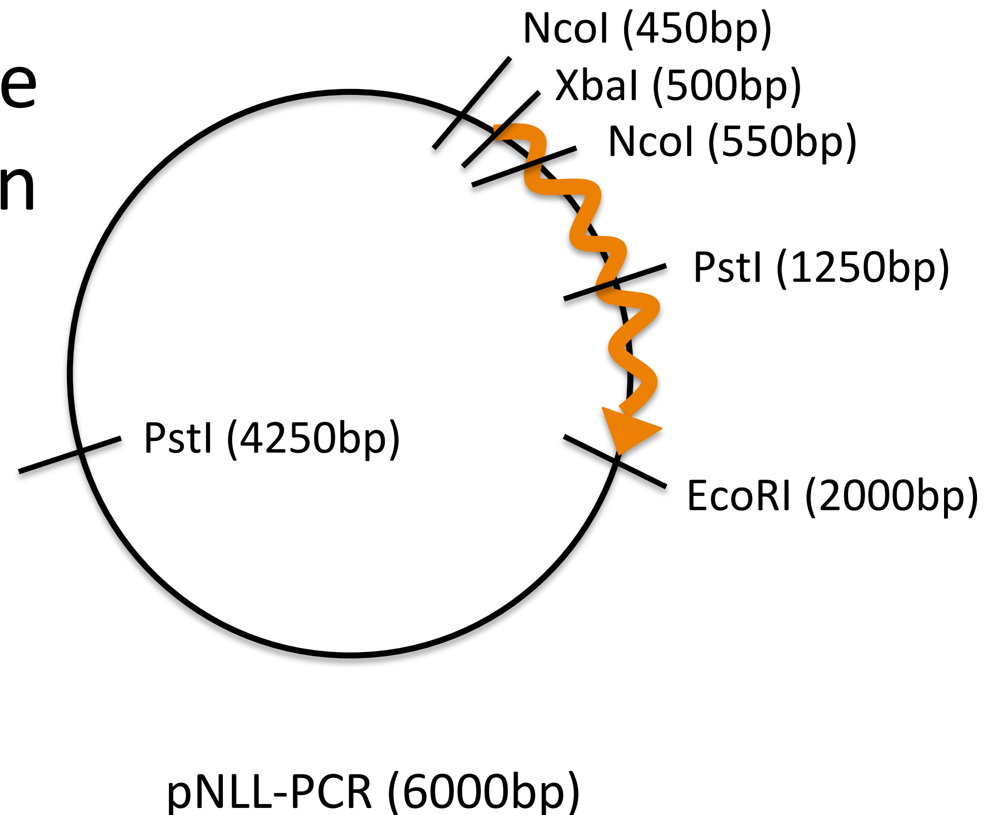


1. Resuspend cells
2. Lysis
3. Neutralization
   – Separates chromosomal DNA from plasmid DNA
4. Wash
5. Resuspend or elute DNA

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 expression vector and dCas9 gene insert
2. Confirm pdCas9 construct
   - Digestion of pdCas9 to confirm presence of dCas9 gene insert