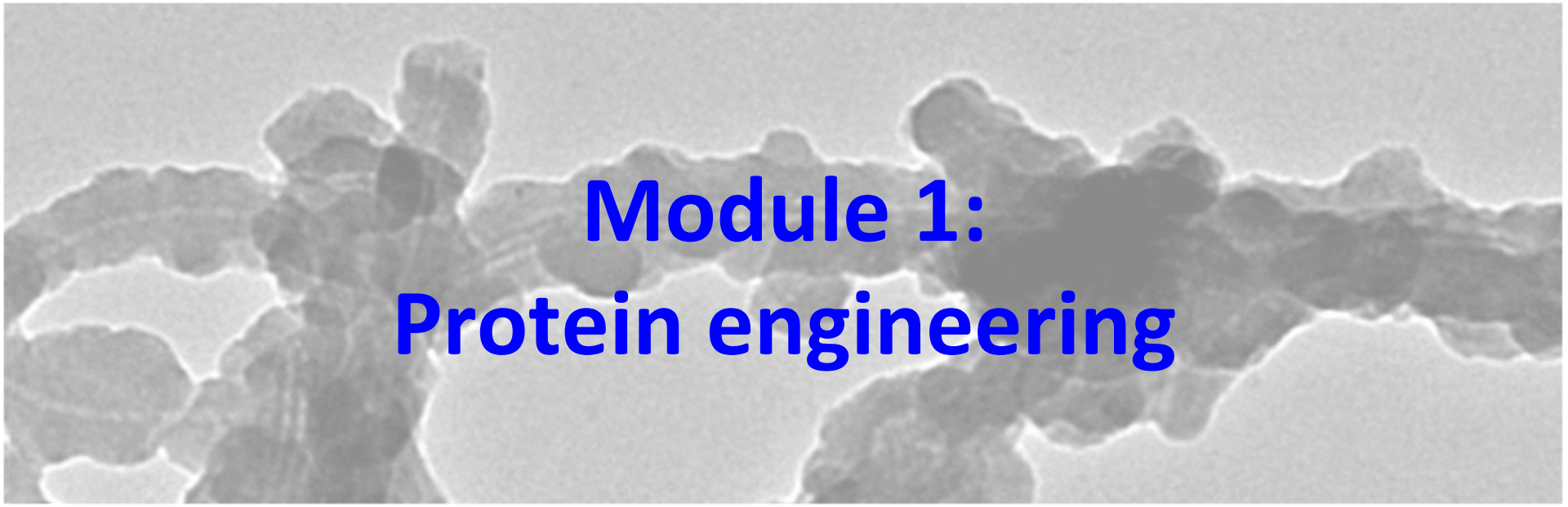


Module 1:
Protein engineering

DNA engineering

2/4/16

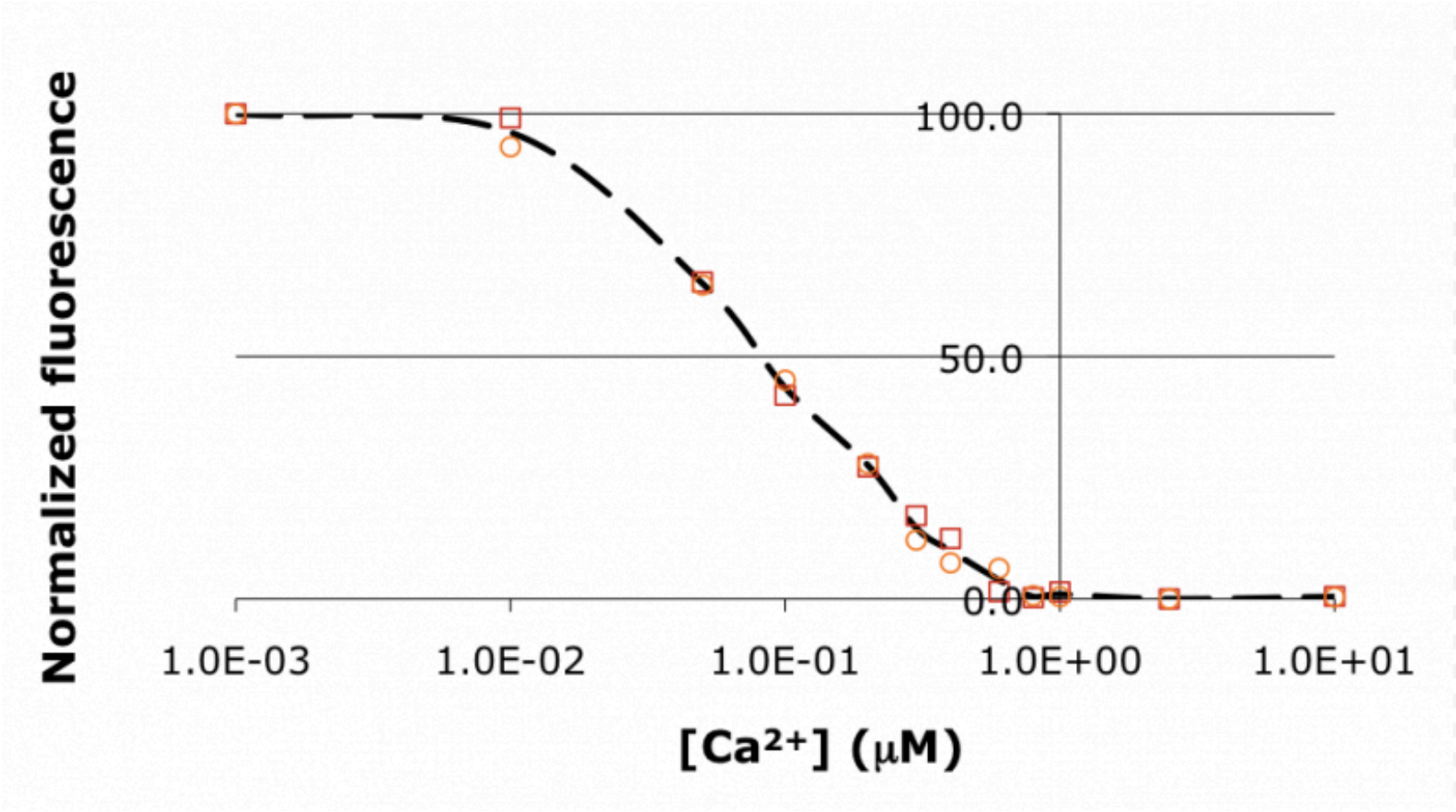
Mod 1 assignment overview

- Protein engineering summary
  - Abbreviated written article that details your experimental results
  - Opportunity for revision
  - Completed with laboratory partner
- Protein engineering mini-presentation
  - Short ‘elevator pitch’ that relays the key results and impact of your project
  - Completed individually

Your engineering task in Mod 1:

Alter Ca^{2+} binding properties of inverse pericam protein

Inverse pericam (IPC) dims with Ca^{2+}




GENETIC ENGINEERS FINALLY CREATE THE PERFECT CHICKEN
EGGS AND BACON!
4c X (R43 - b)
73ab < (56
=22%
7-2
©TUNDRA 2009
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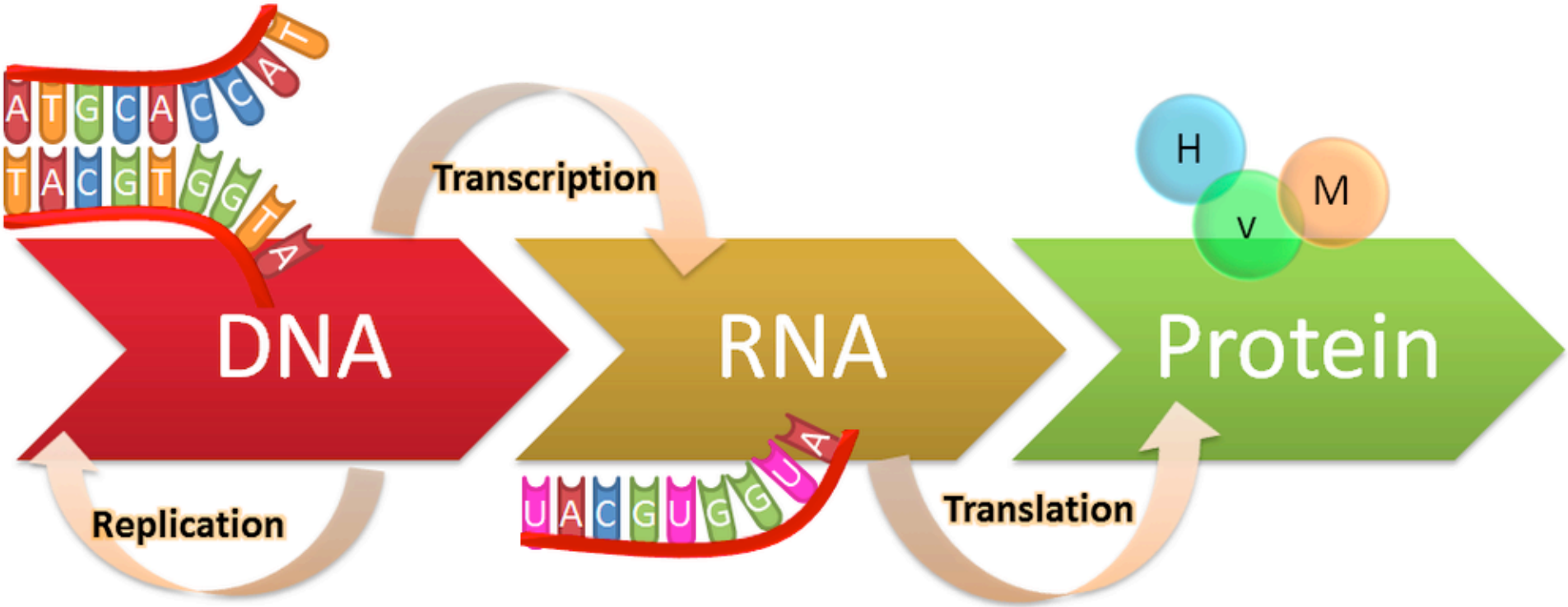
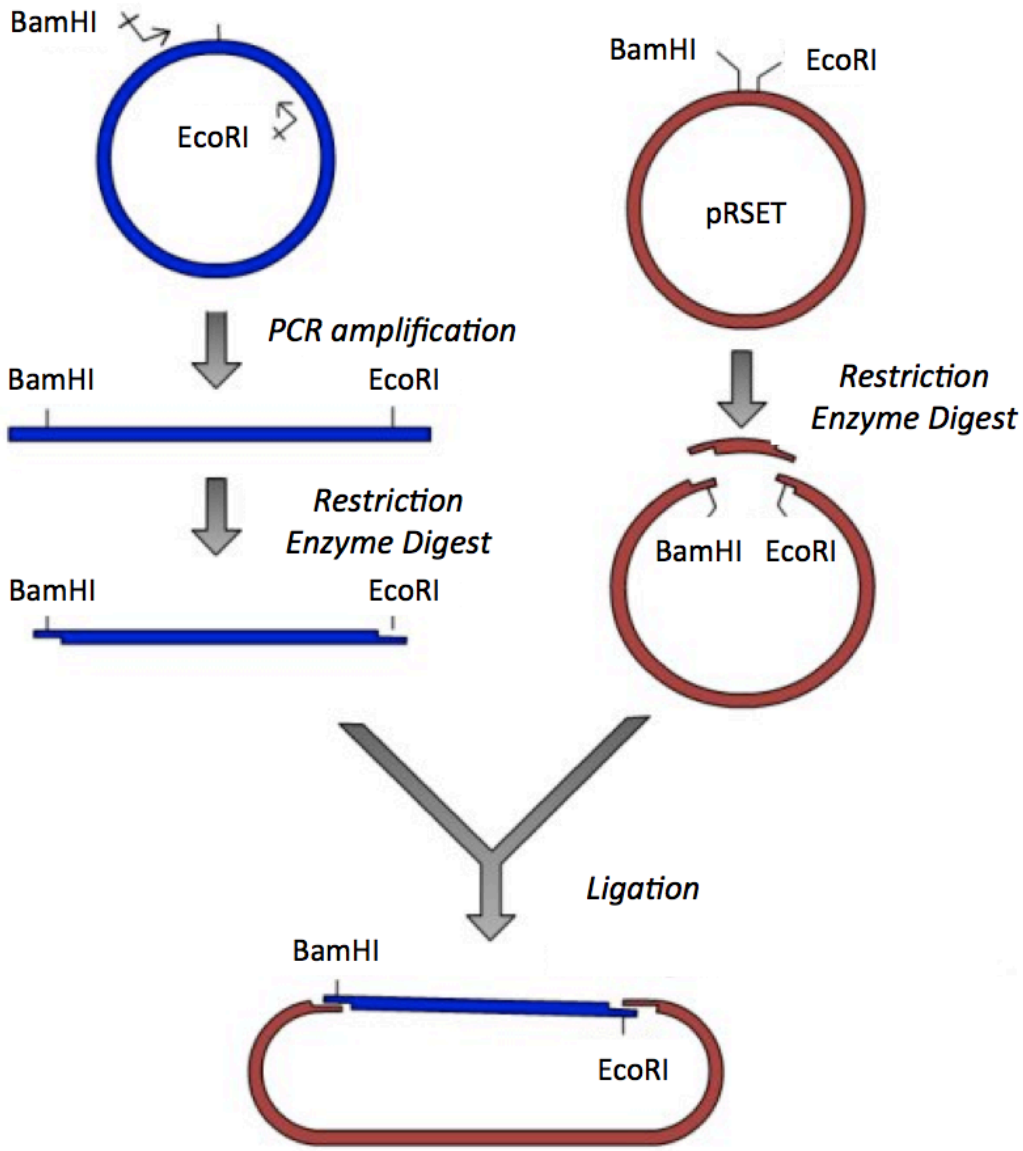
The central dogma



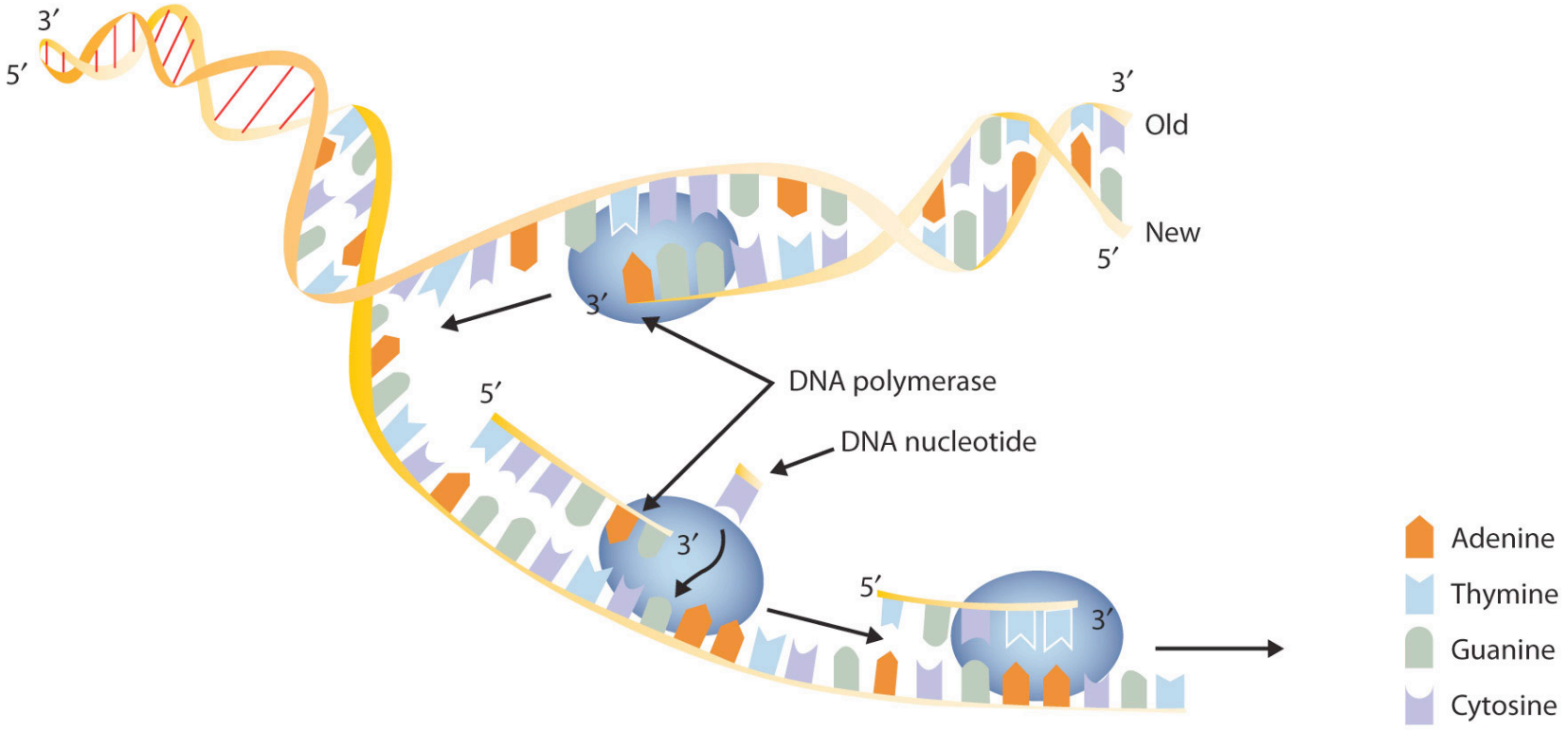
http://genius.com/Biology-genius-the-central-dogma-annotated

How do we engineer DNA?



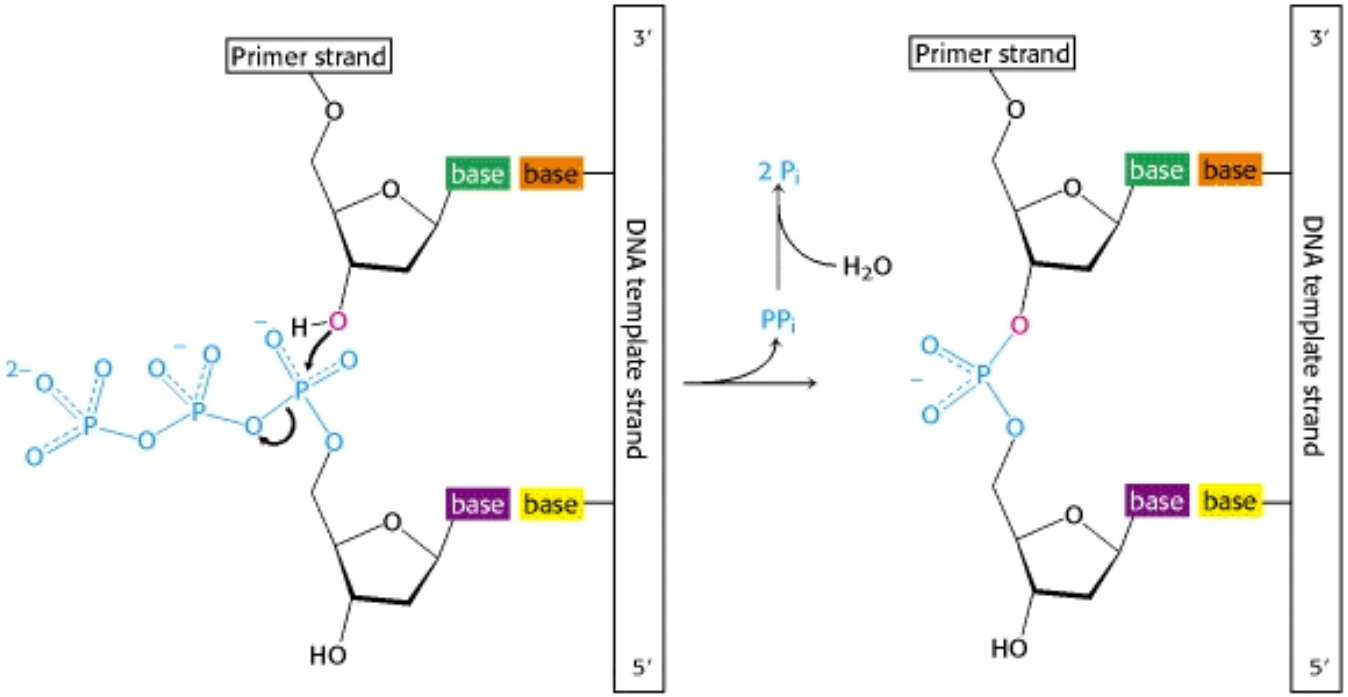
1. Amplification
2. Digestion
3. Ligation

Amplification



Who are the key players?

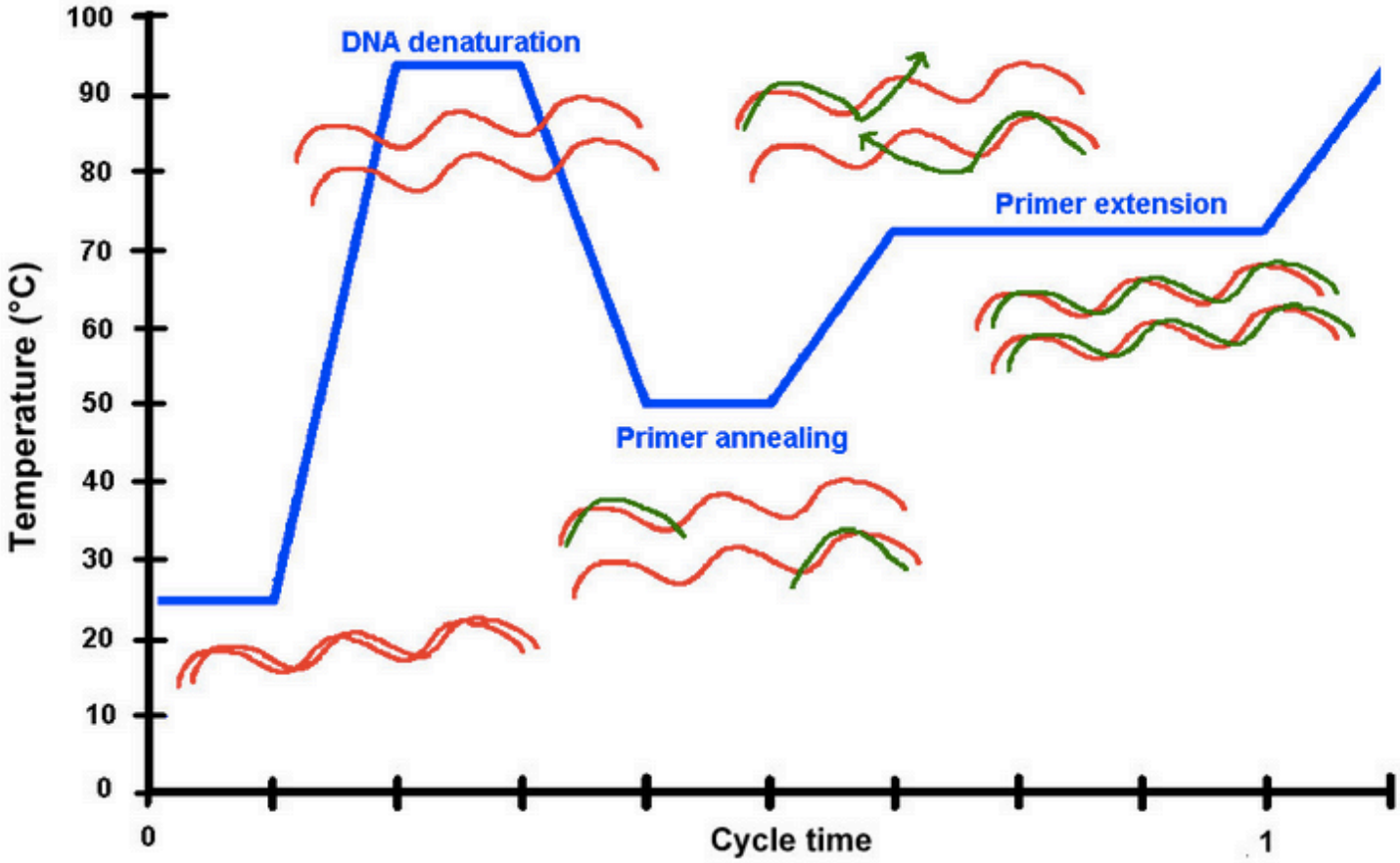
DNA polymerase



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

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

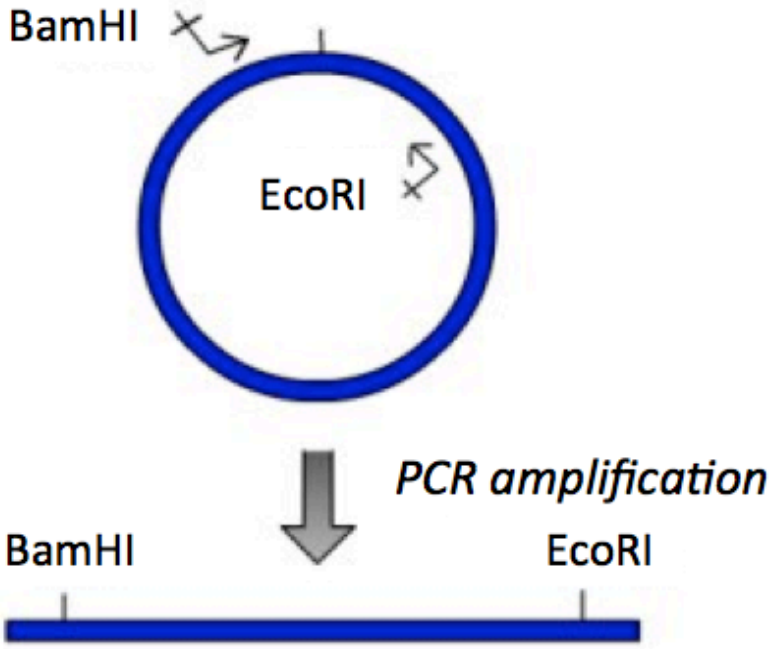
Polymerase chain reaction (PCR)



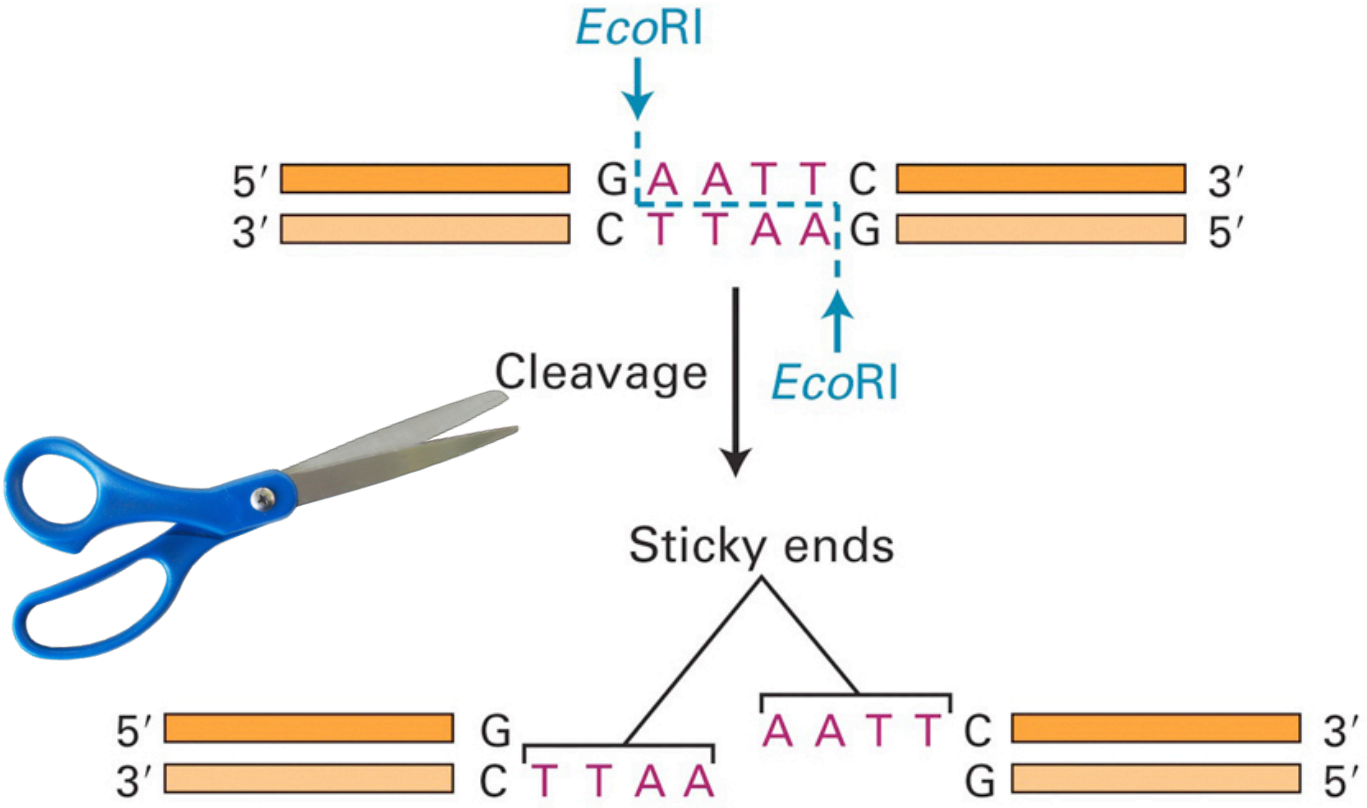
How many cycles until your product is generated?

What are we amplifying?

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

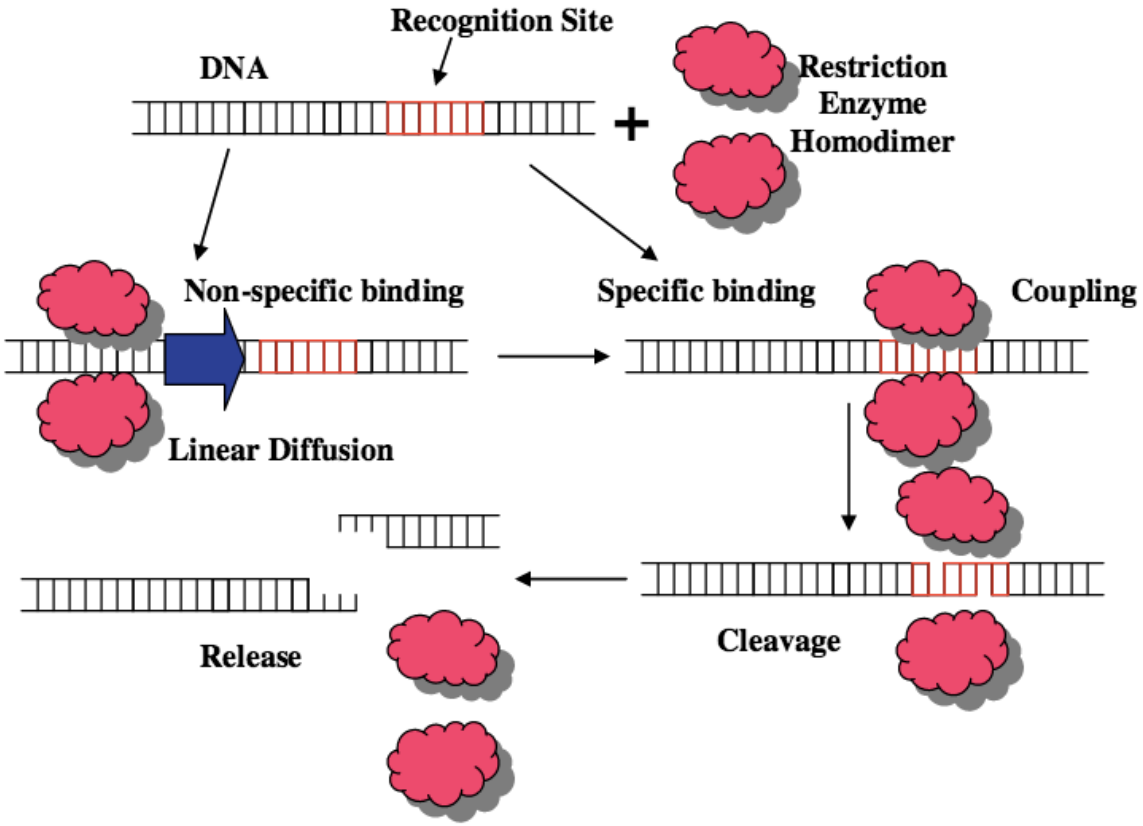


Digestion

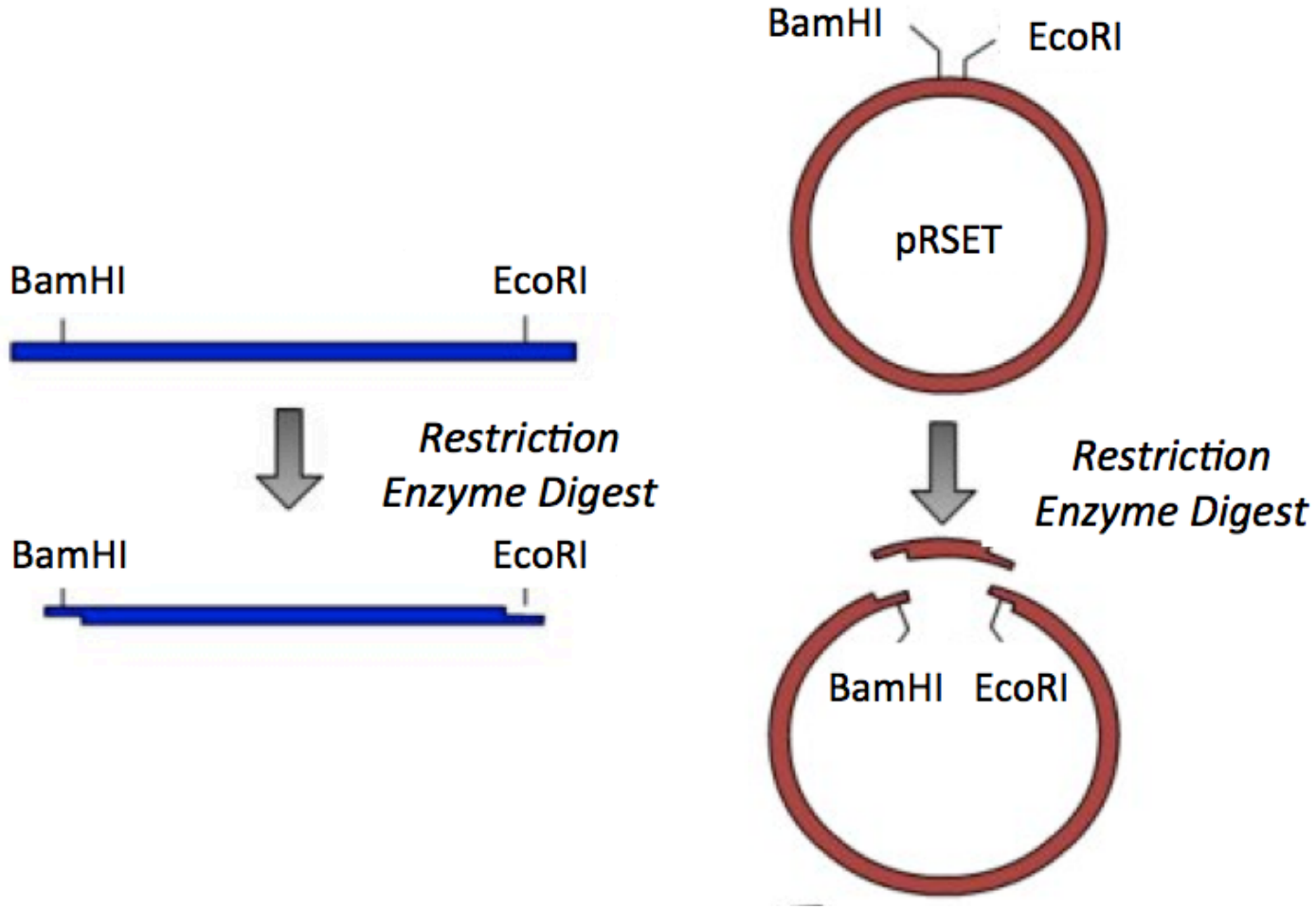

EcoRI
5′ G A A T T C 3′
3′ C T T A A G 5′
Cleavage
EcoRI
Sticky ends
5′ G
3′ C T T A A
A A T T C 3′
G 5′

Restriction enzymes

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



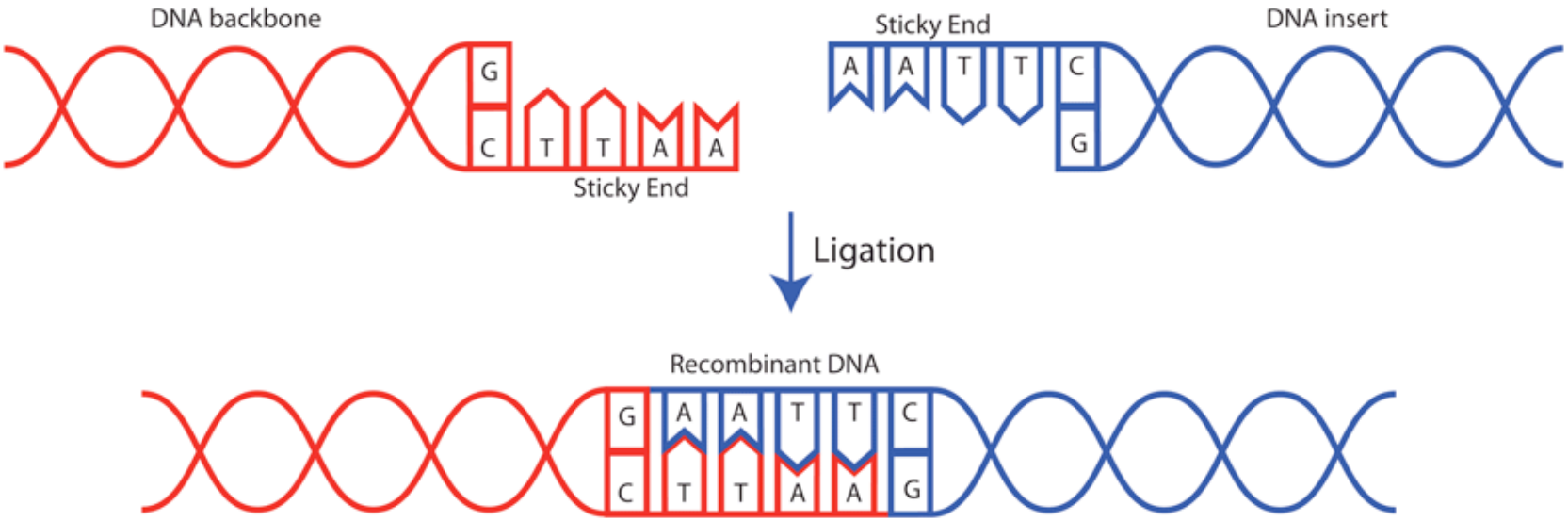
What are we digesting?



What should we consider when performing a double digest?

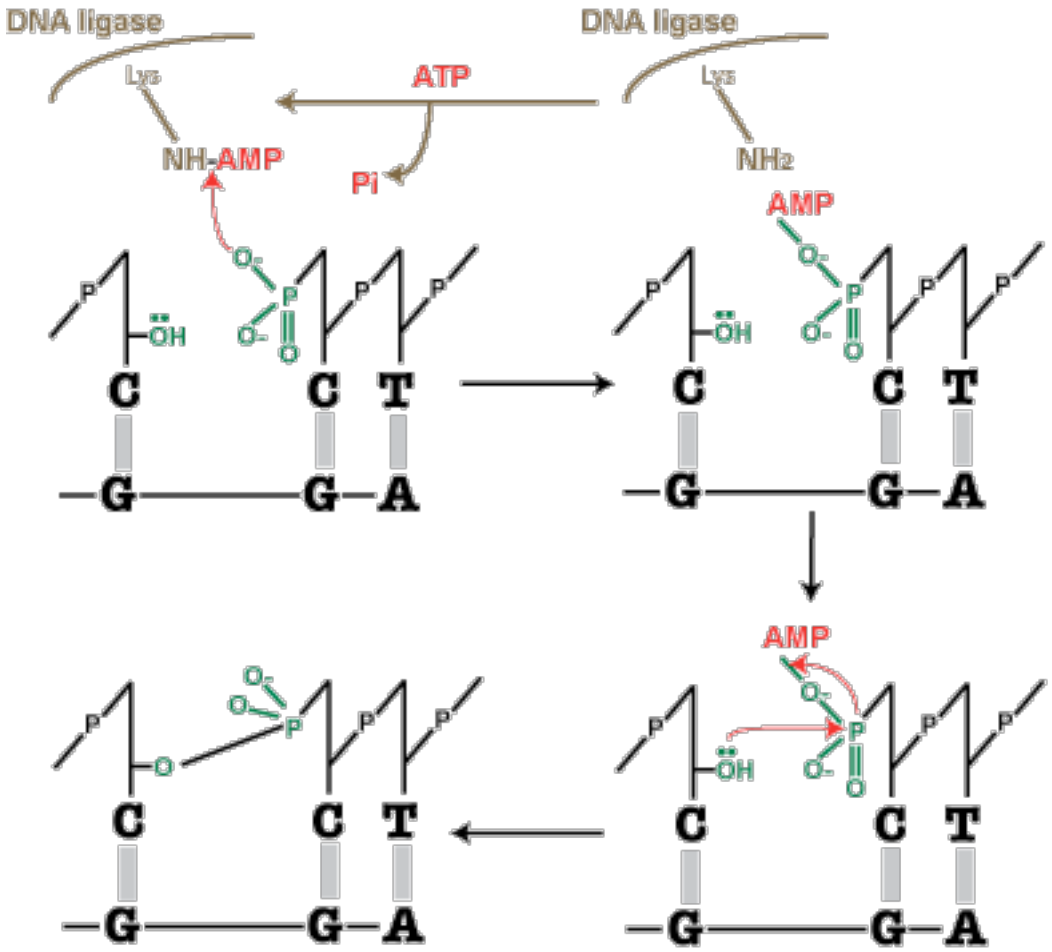
Ligation


Glue-All
ELMER'S

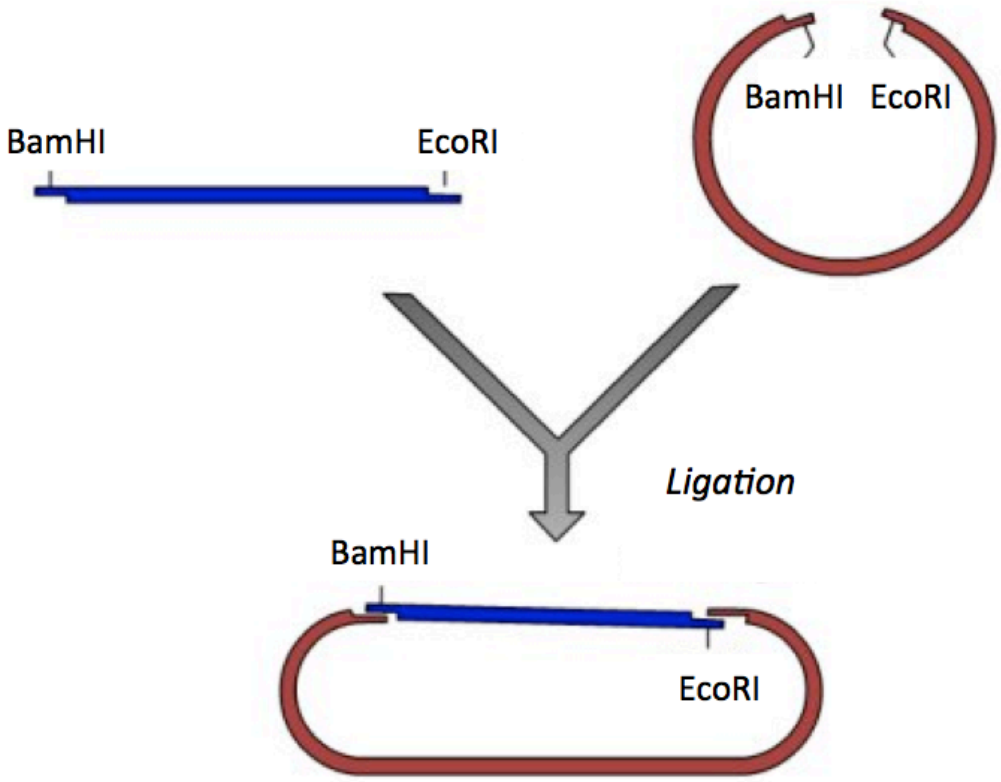

DNA backbone
G
C
T
T
A
A
Sticky End
Sticky End
DNA insert
A
A
T
T
C
G
Ligation
Recombinant DNA
G
A
A
T
T
C
C
T
T
A
A
G

DNA ligase

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



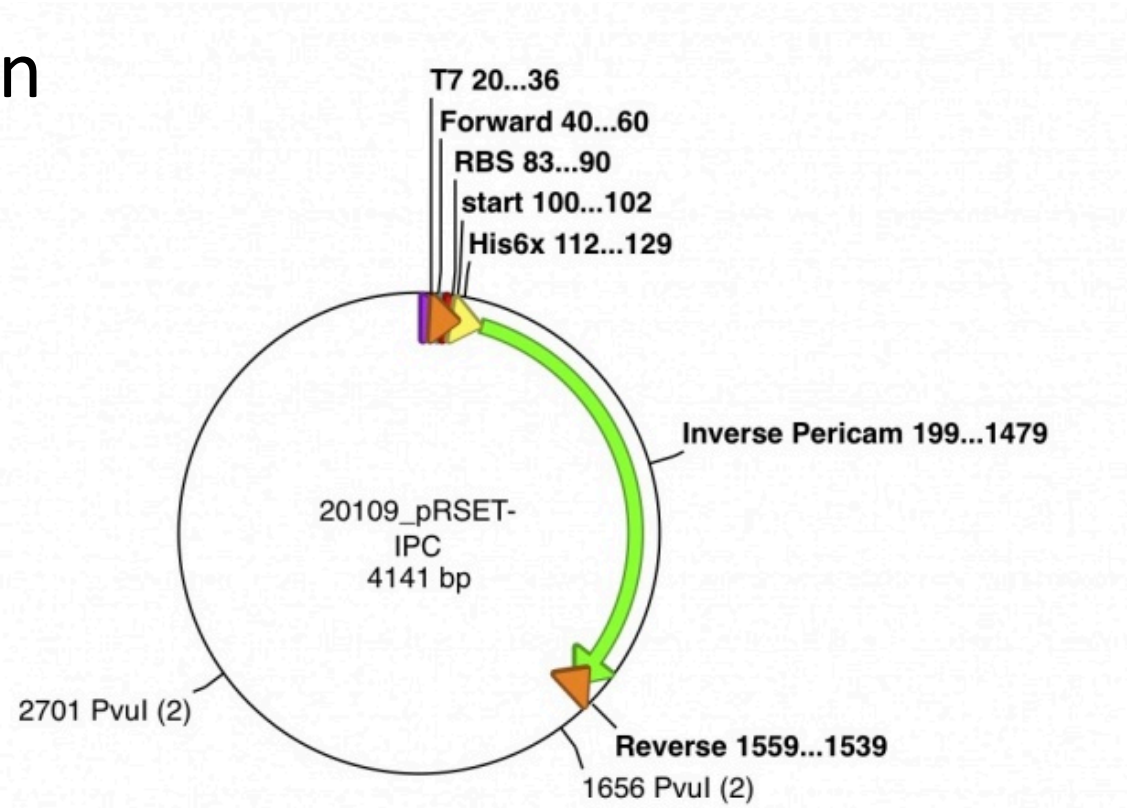
What are we ligating?



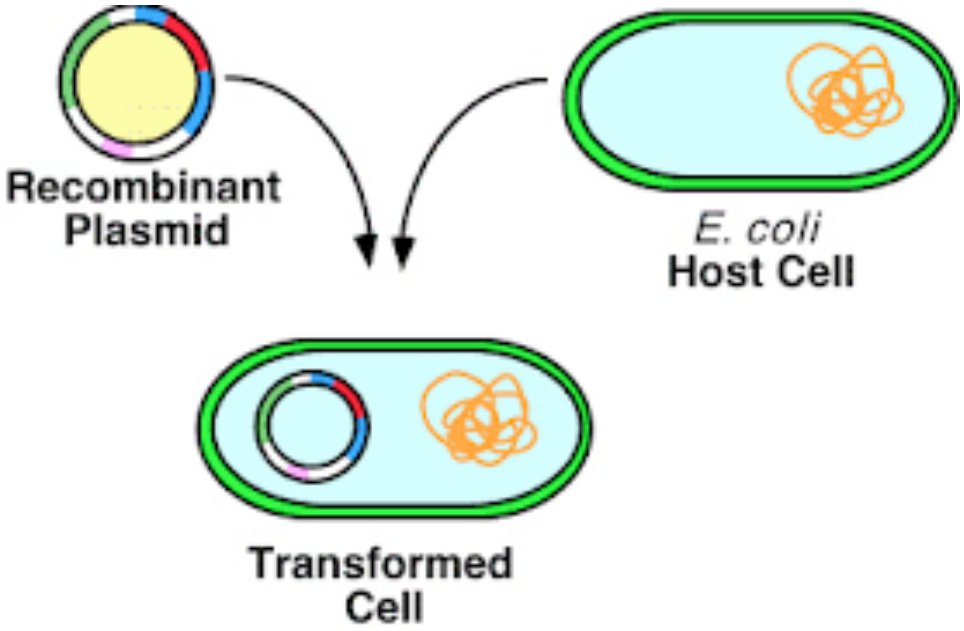
Note: in your laboratory exercise only the 'top' DNA strand is represented…remember this when determining basepair sites of digestion and ligation.

How do we confirm our product?

1. Transformation
2. Purification
3. Digestion



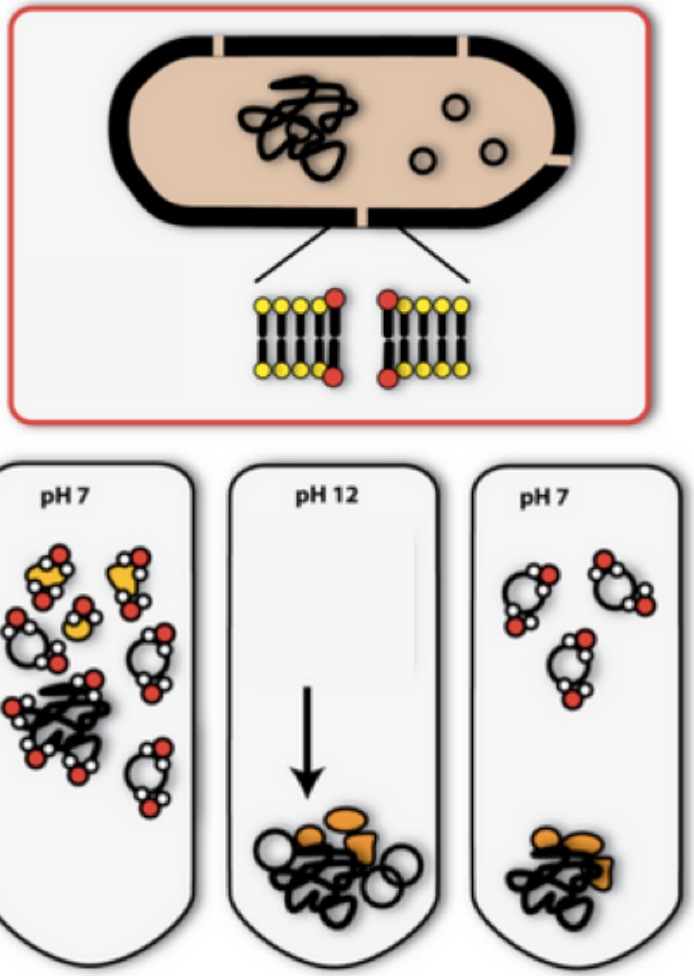
Transformation



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

Why do we transform the ligation product?

Purification

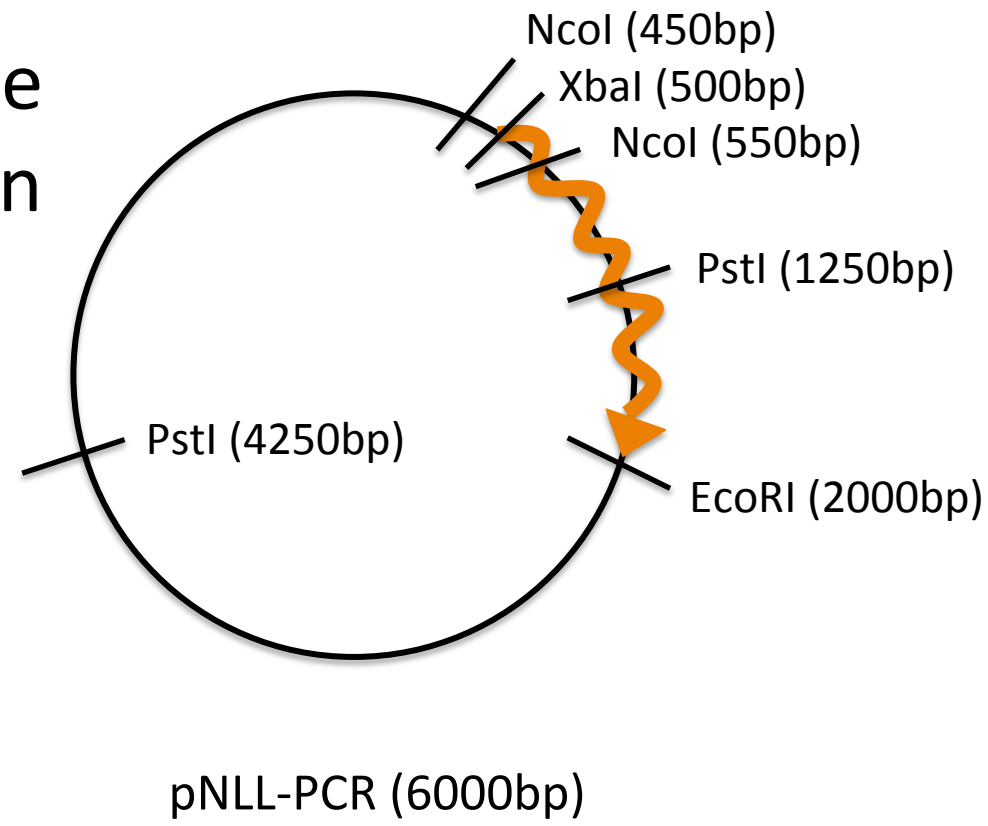


1. Resuspend cells
2. Lysis
3. Neutralization
   - Separates chromosomal DNA from plasmid DNA
4. Wash
5. Resuspend or elute DNA

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…

- Orientation laboratory quiz

- Engineer pRSET-IPC product
  - Template for protein engineering
- Confirm pRSET-IPC product