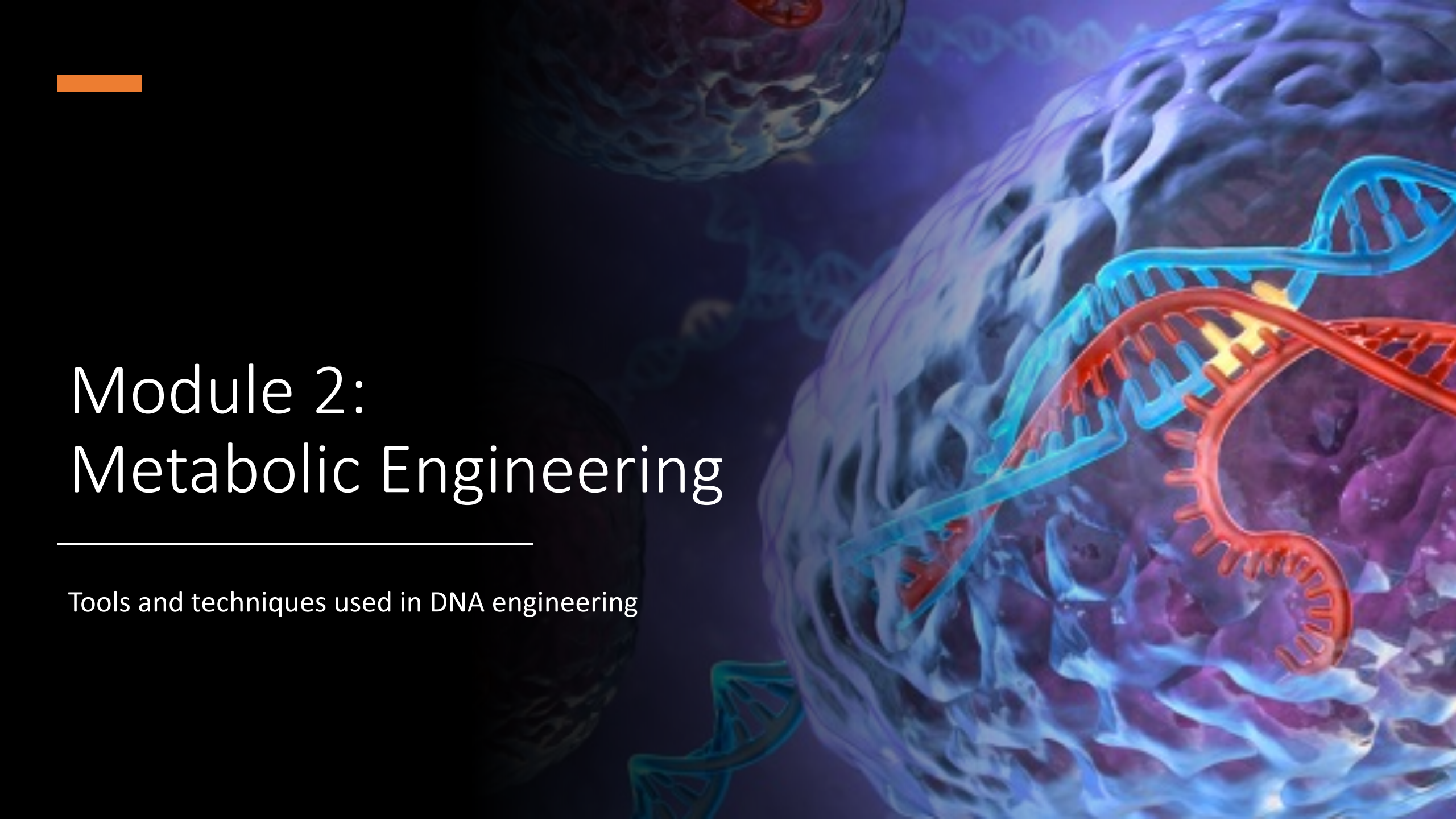
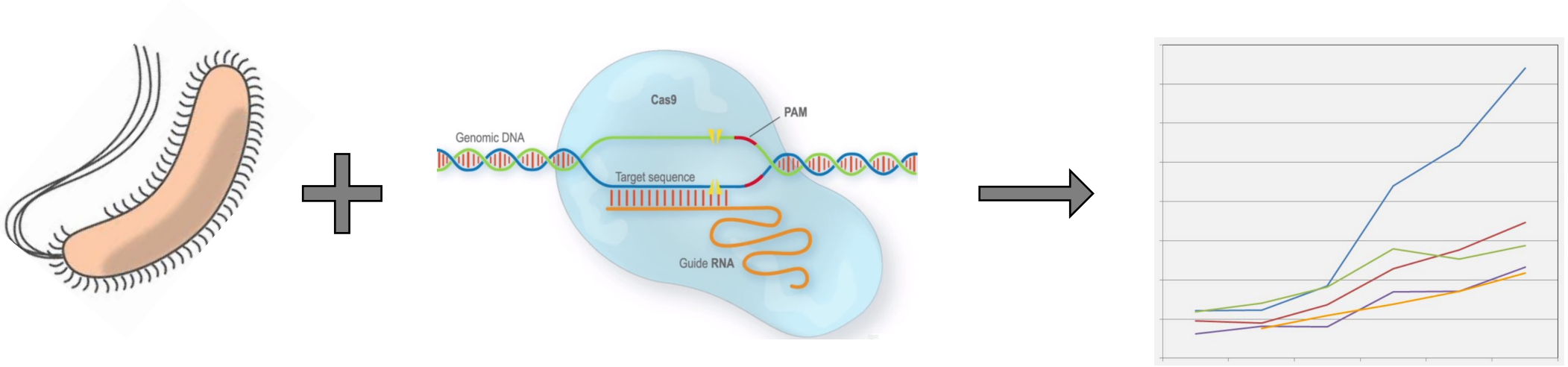


Module 2: Metabolic Engineering

Tools and techniques used in DNA engineering

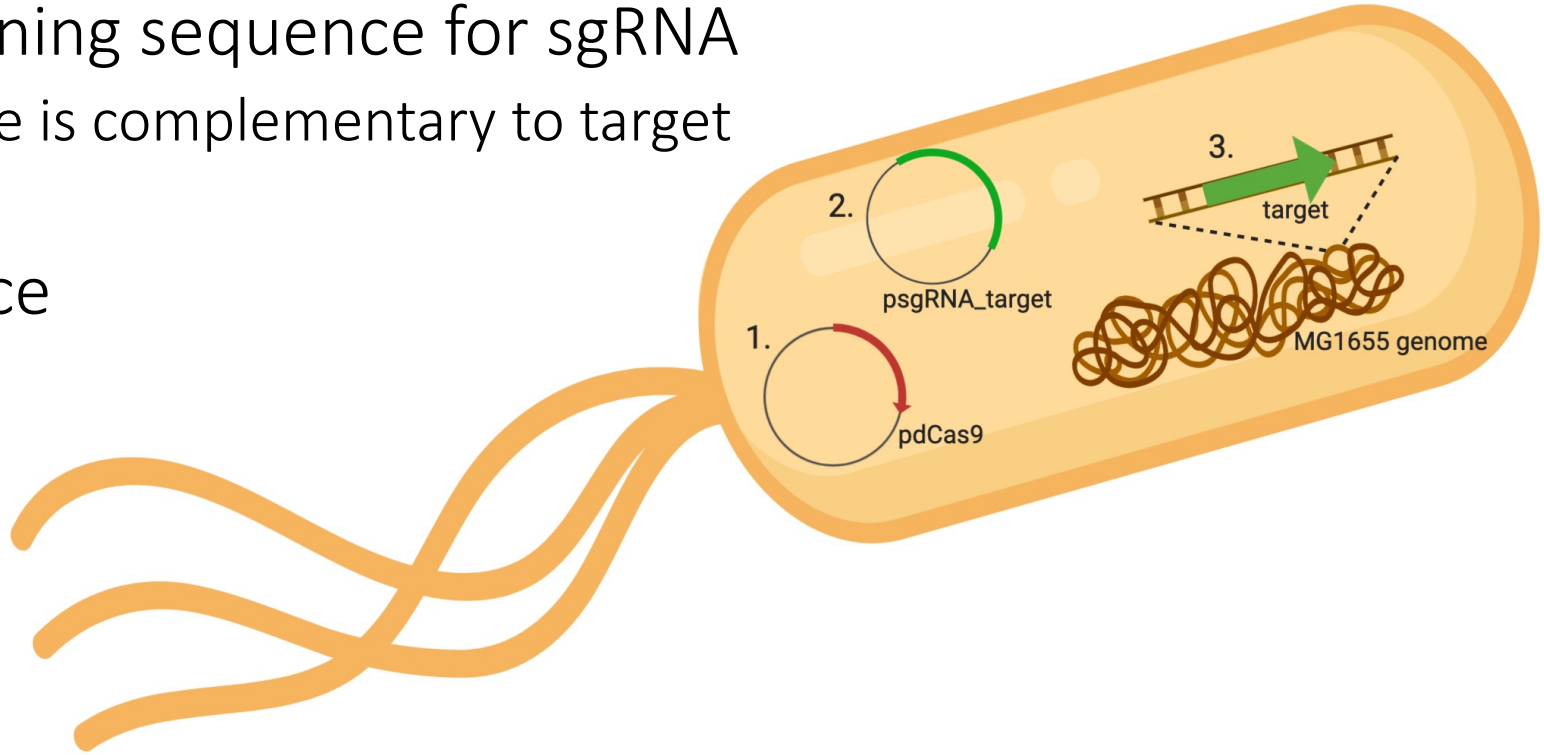
What is your biological engineering task in Mod 2?

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



Components of CRISPRi system

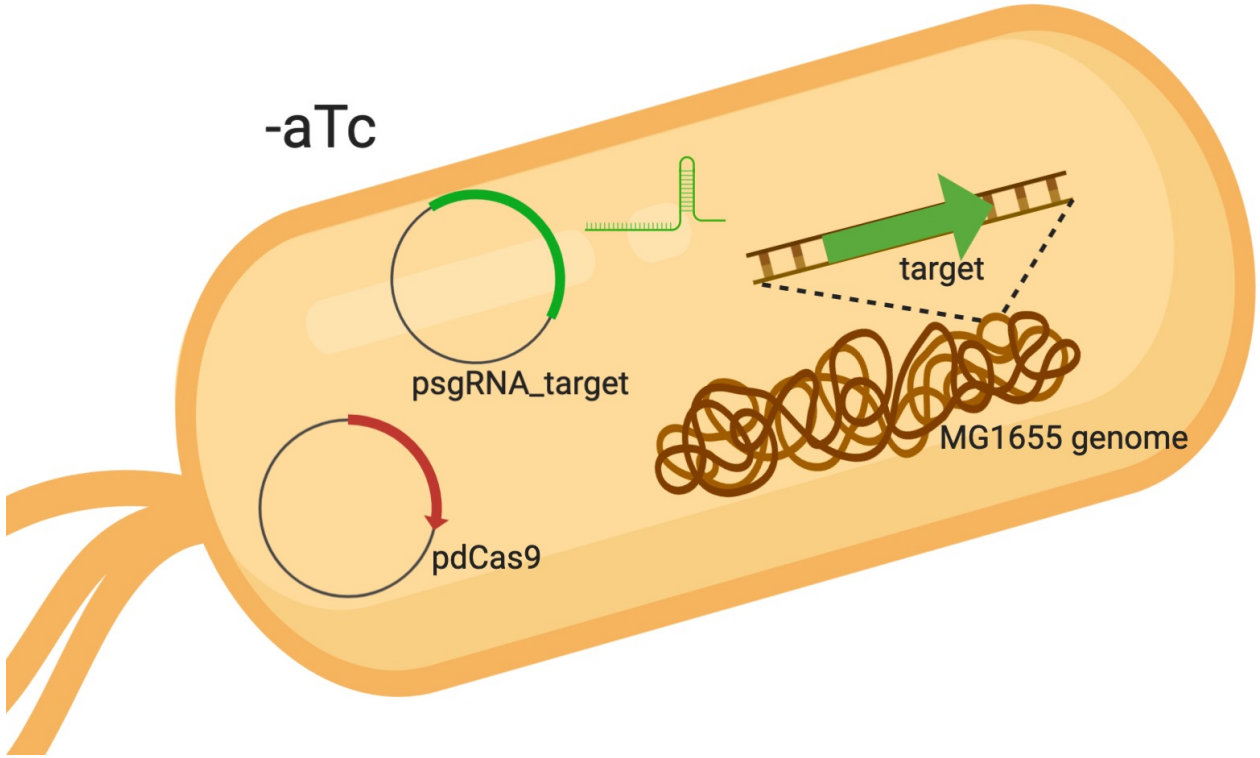
1. Plasmid containing gene that encodes dCas9
2. Plasmid containing sequence for sgRNA
   - sgRNA sequence is complementary to target sequence
3. Target sequence



CRISPRi inactive in absence of inducer

pgRNA_target expressed constitutively

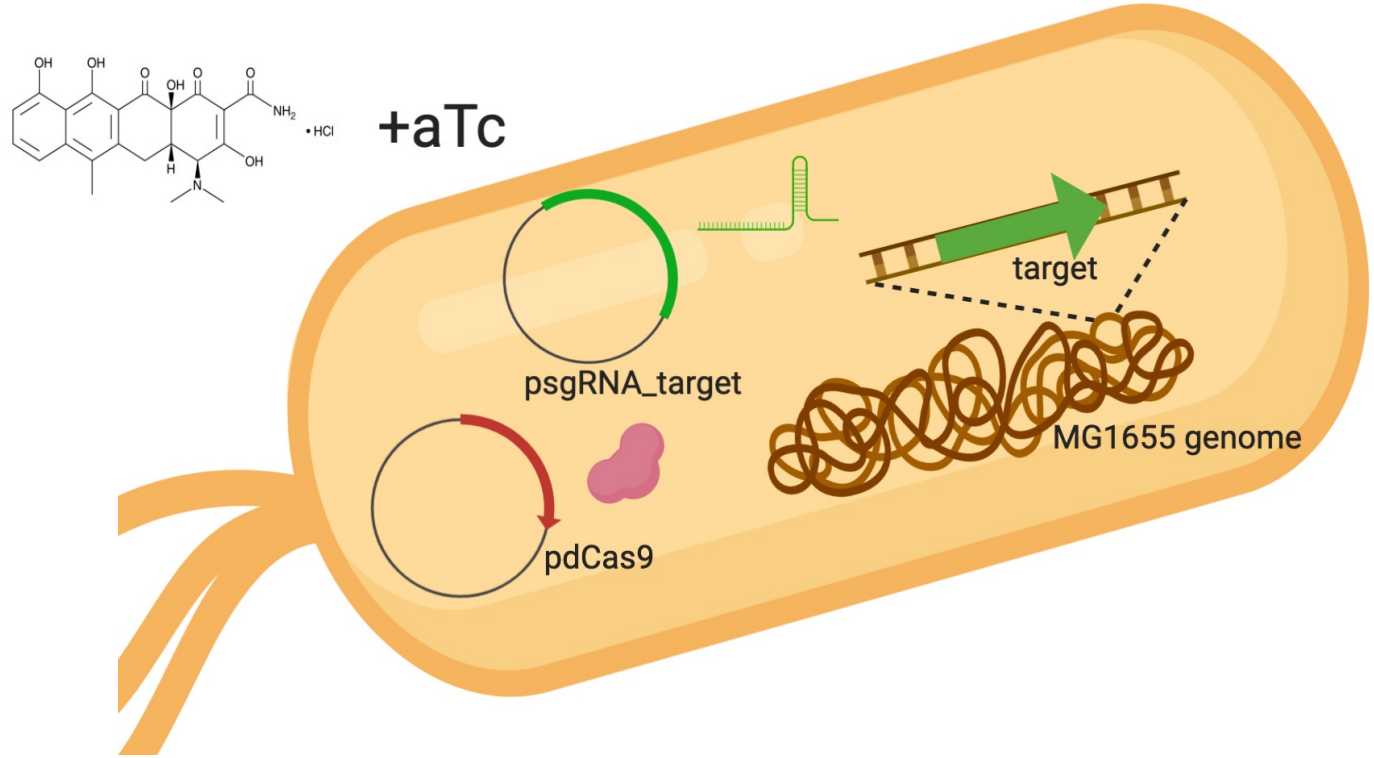
- Always transcribed and binding to target gene



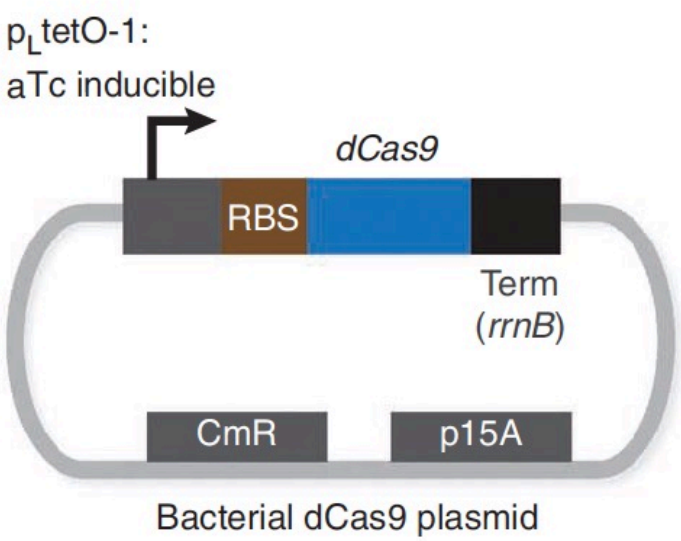
CRISPRi inhibits gene expression in presence of inducer

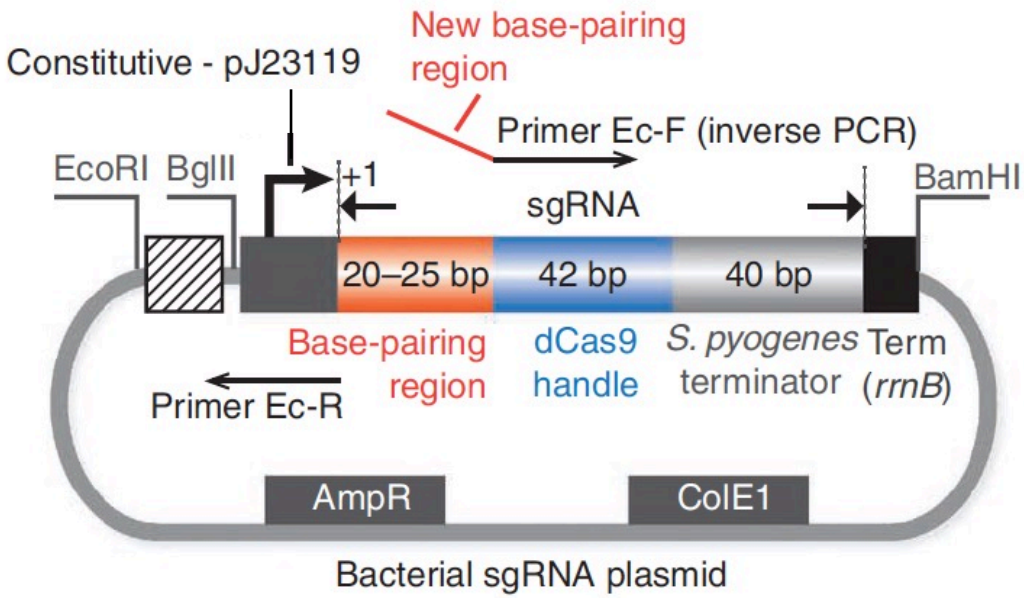
pdCas9 expressed when aTc added

- When transcribed associates with pgRNA_target / target gene



Closer look at pdCas9 and psgRNA

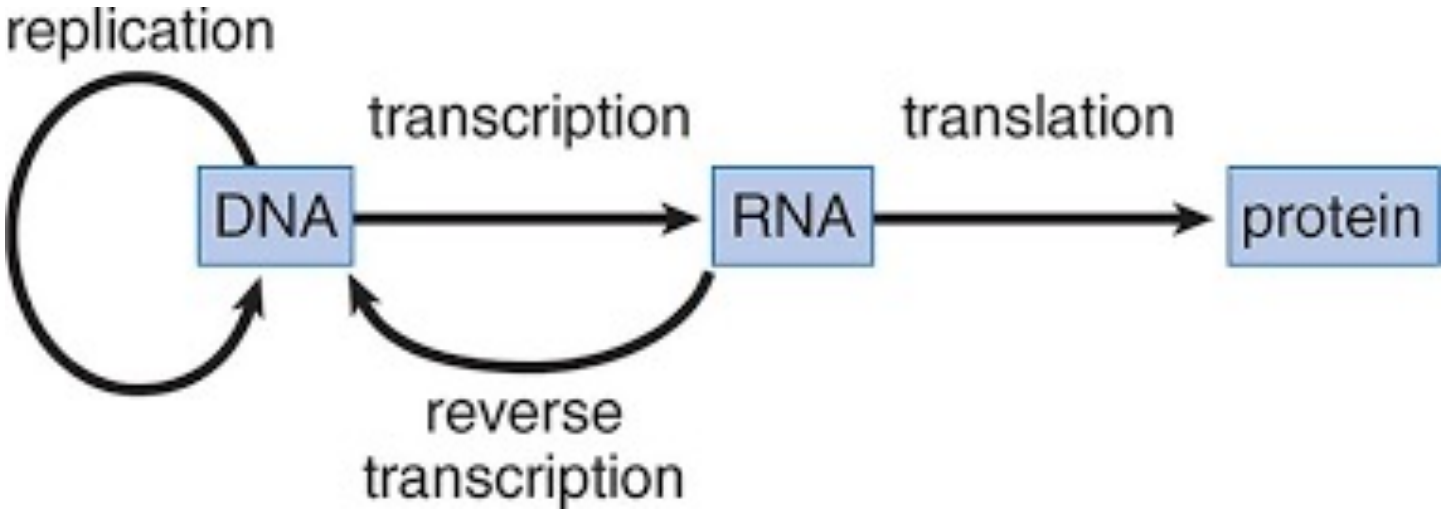




Prepare confirmation digest to check pdCas9 construct on M2D1

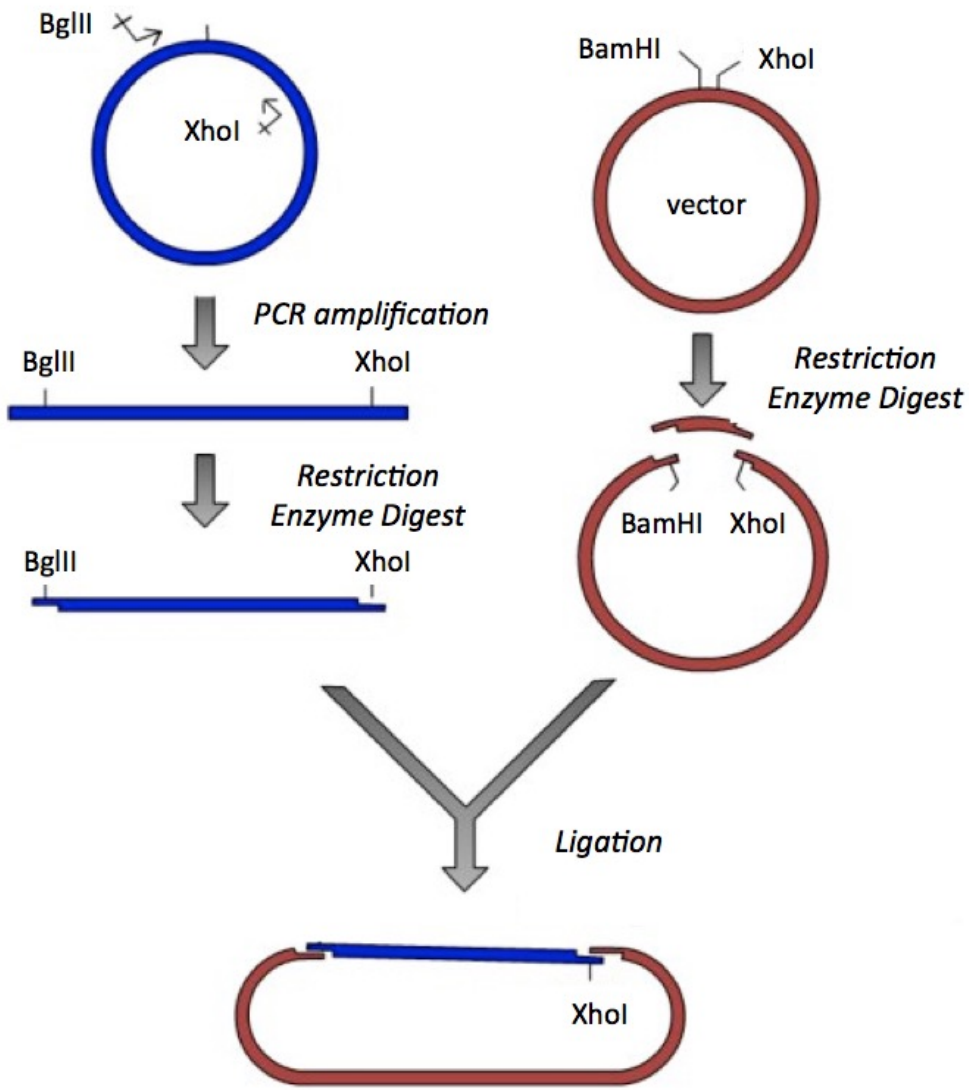
Design gRNA target sequence for psgRNA_[target] construct on M2D2

It all starts with DNA…



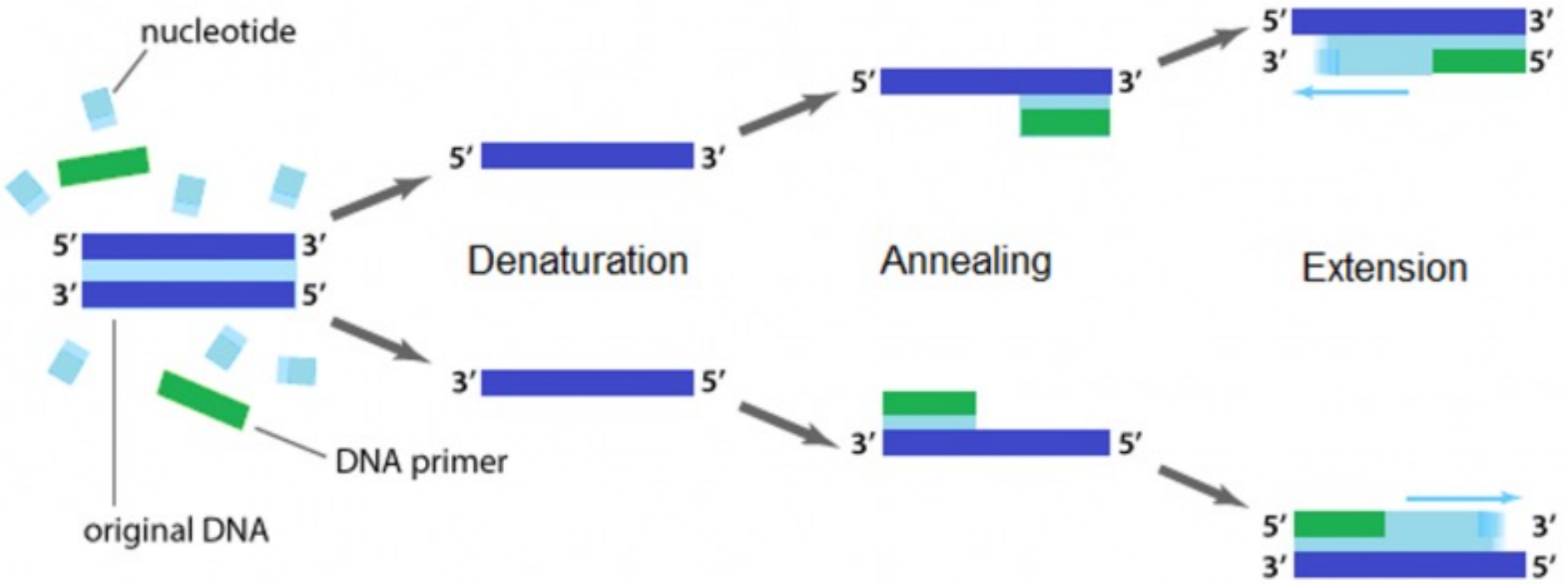
How do we engineer DNA?

1. Amplification
2. Digestion
3. Ligation



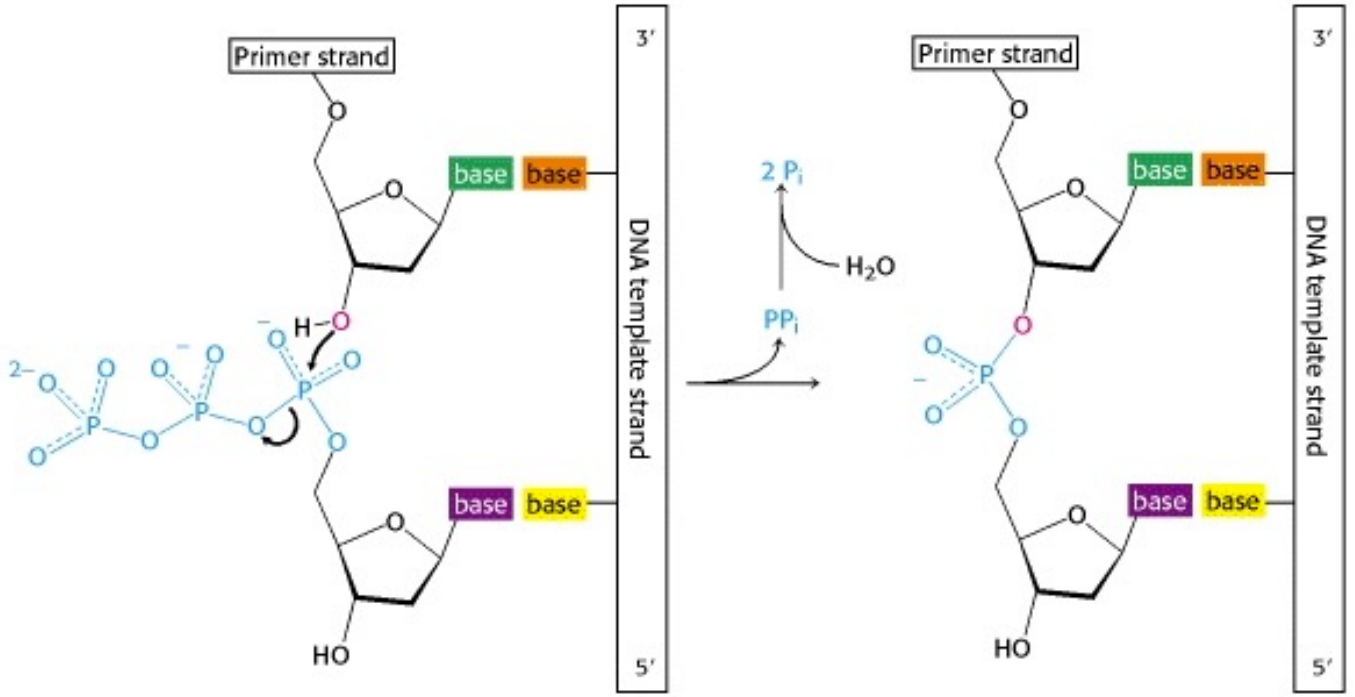
1. Amplification is used to copy DNA sequences

Who are the key players?



DNA polymerase catalyzes formation of polynucleotide chains

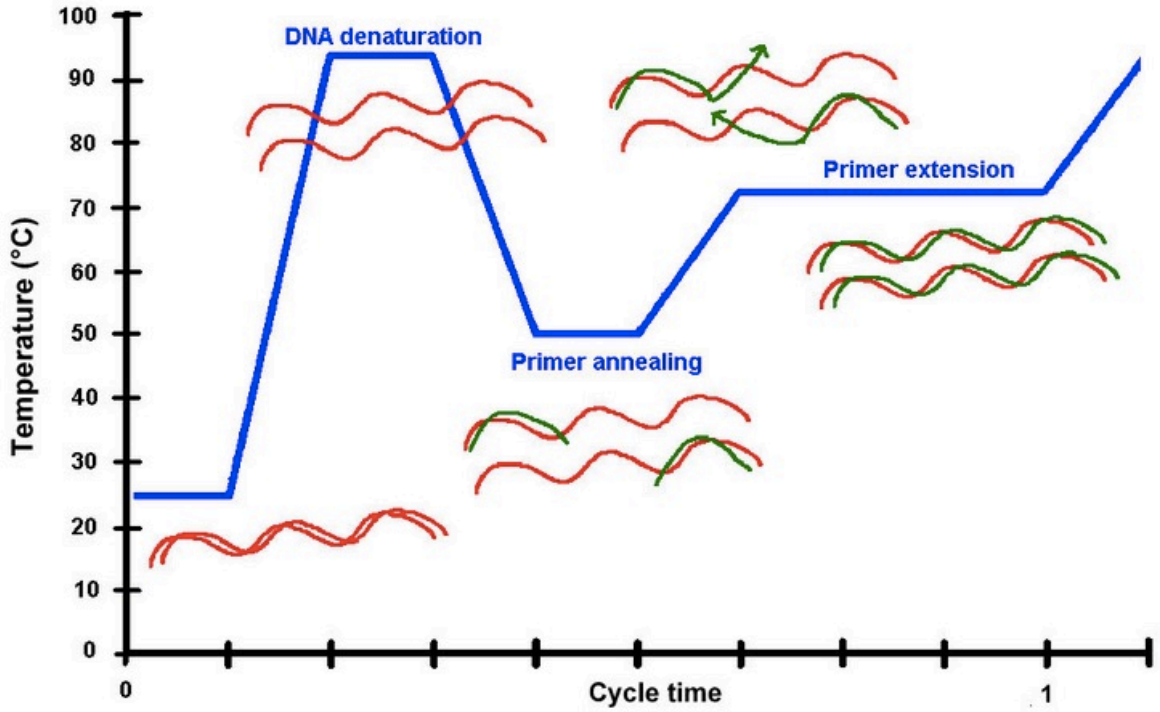
Requires a primer base-paired to template



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

Polymerase chain reaction (PCR) technology amplifies specific DNA sequence

How many cycles until the desired product is generated?

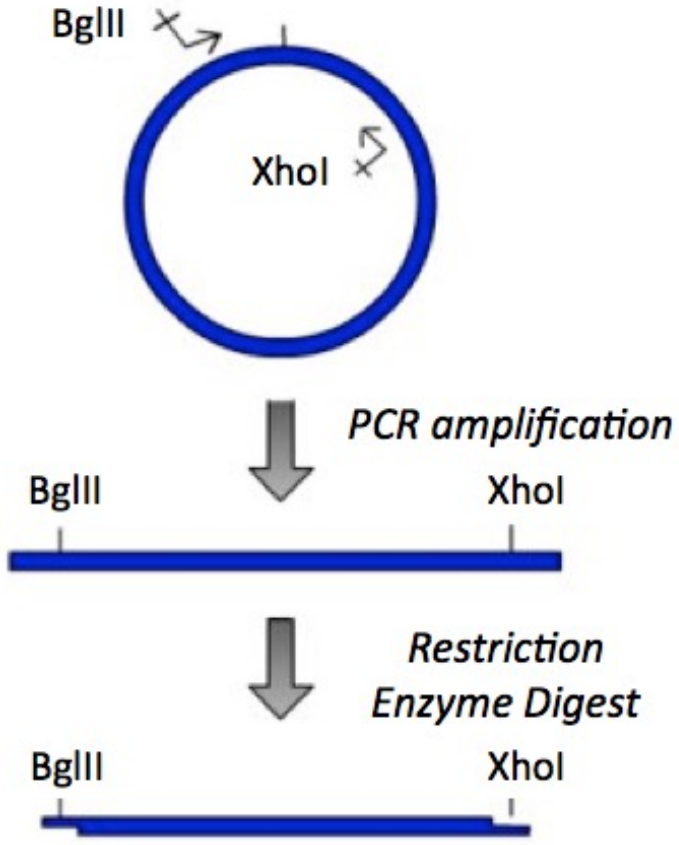


What are we amplifying?

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

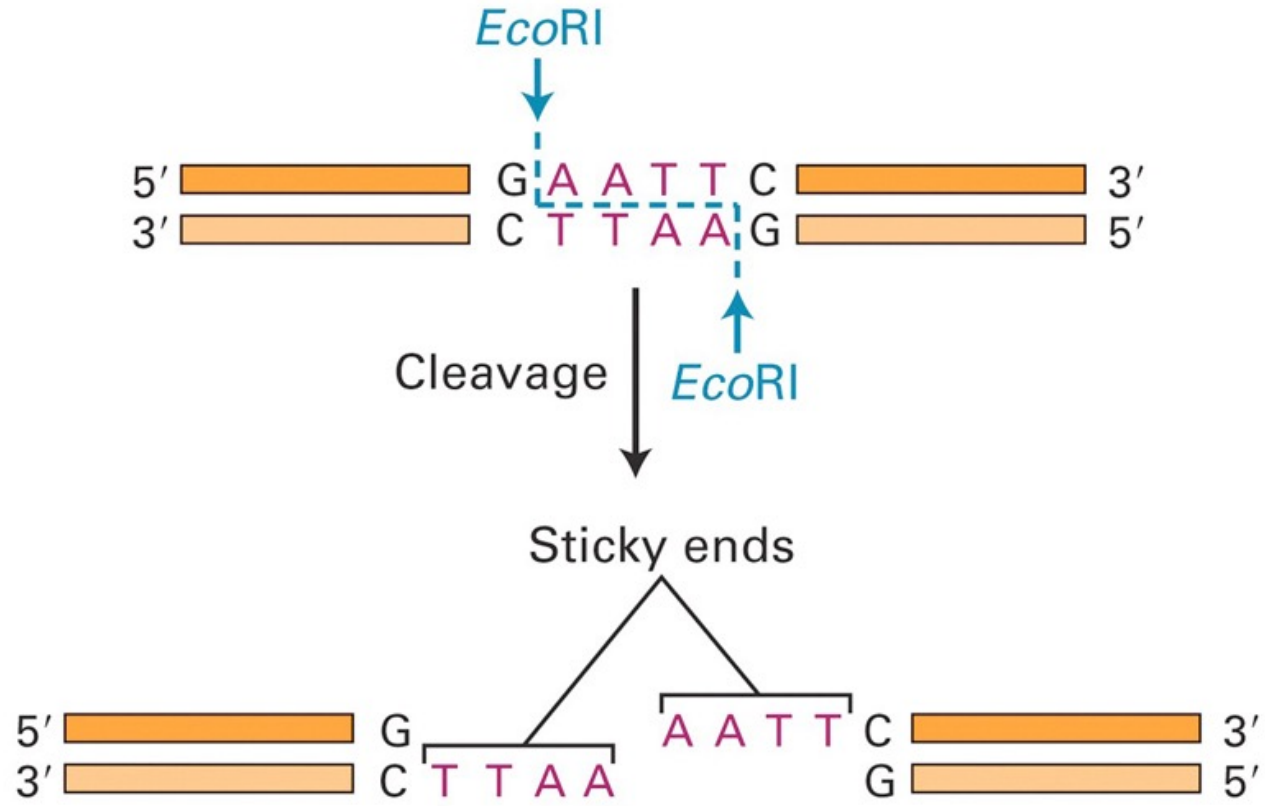
- Polymerase requires primer to add bases in sequence

Why do we need two primers?



2. Digestion generates compatible ends for joining DNA strands

Why are most restriction sites palindromes?

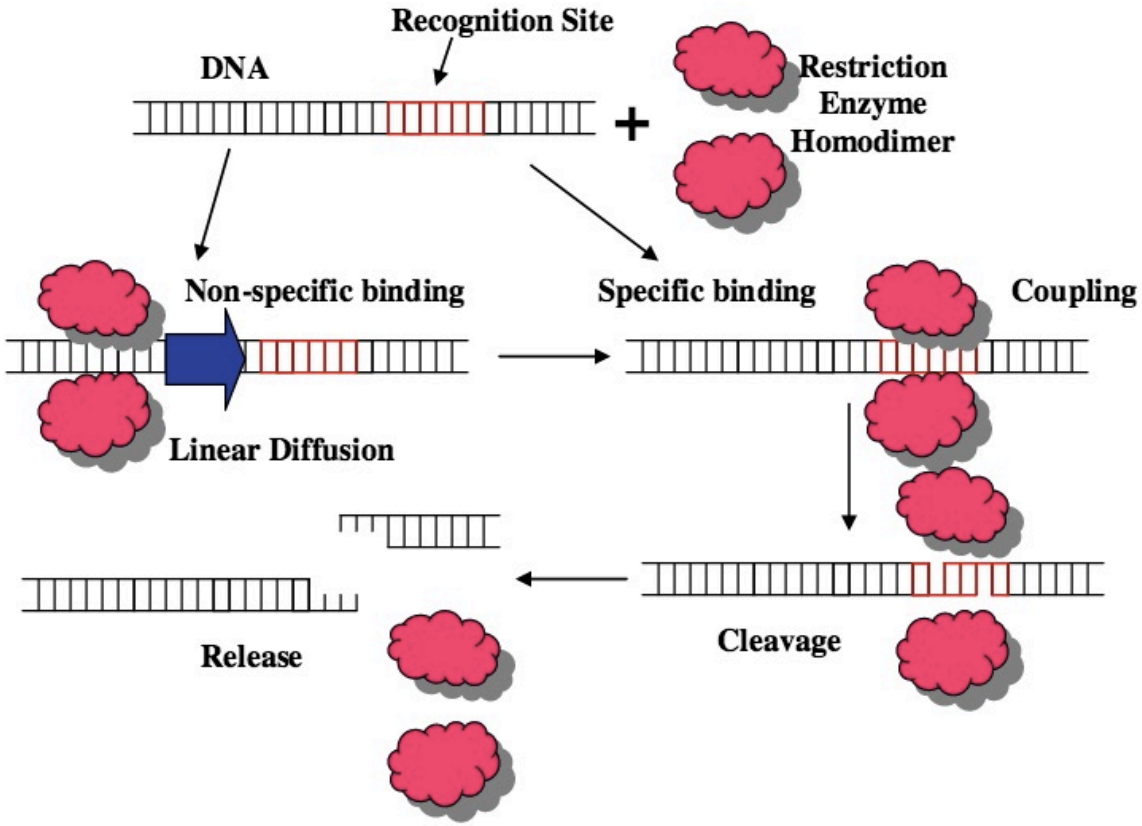


Restriction enzymes cleave DNA at specific sequences

Function as homodimers

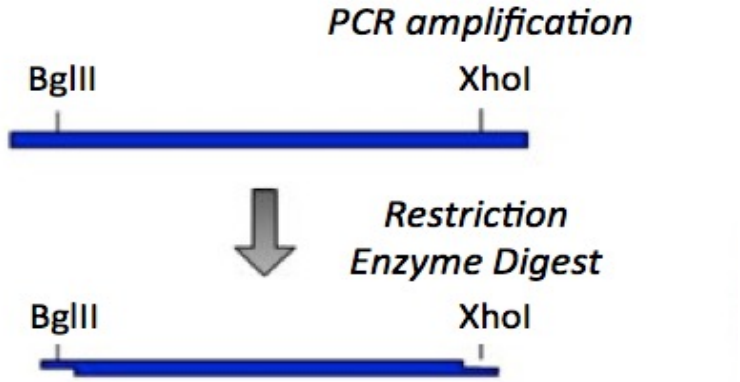
- Each dimer cleaves backbone at site of palindromic recognition sequence

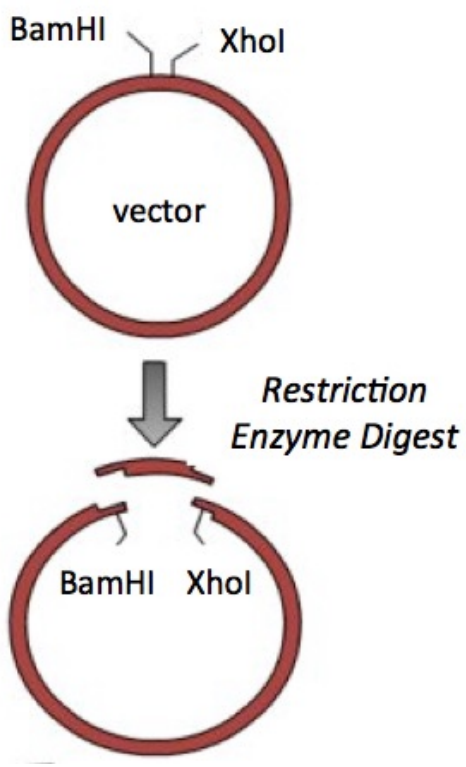
How do restriction enzymes recognize DNA sequences?



What are we digesting?

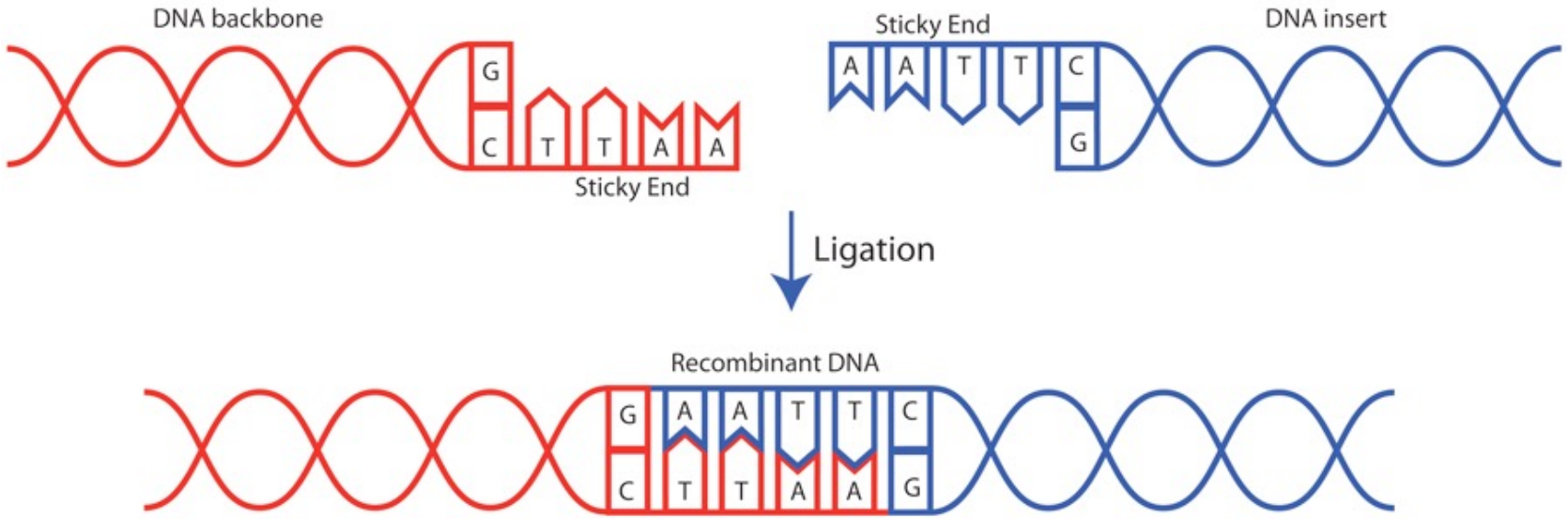
What should we consider when performing a double digest?





3. Ligation joins compatible DNA ends

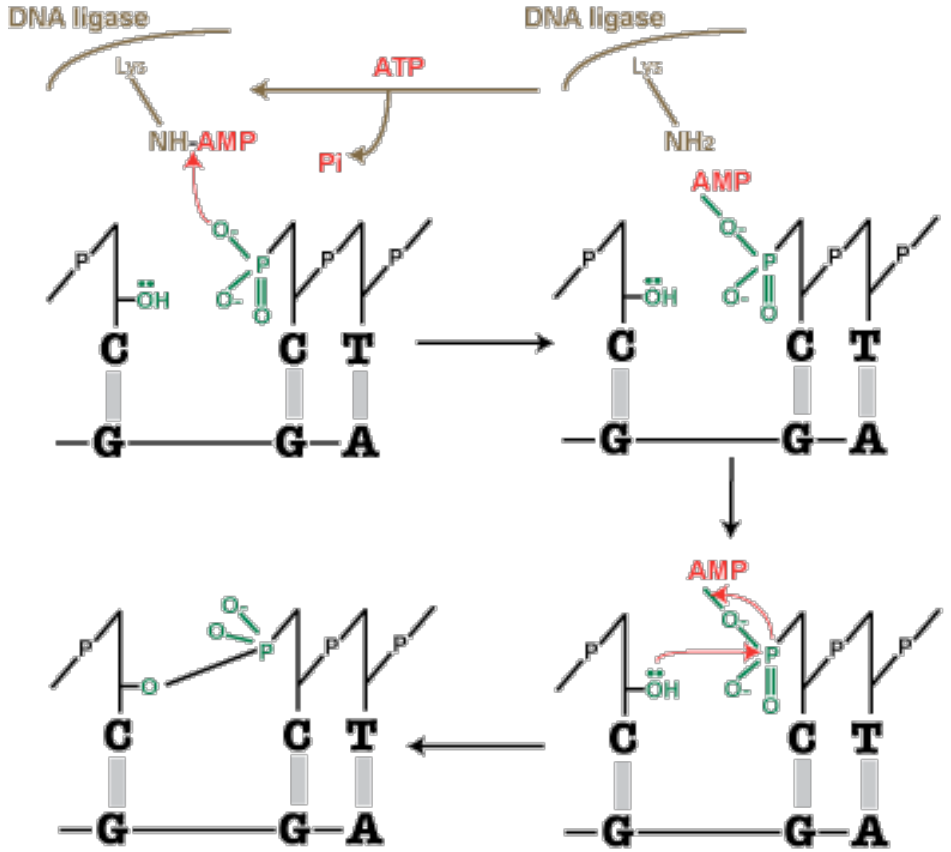
How are compatible DNA ends paired prior to ligation?



DNA ligase catalyzes formation of covalent phosphodiester bond

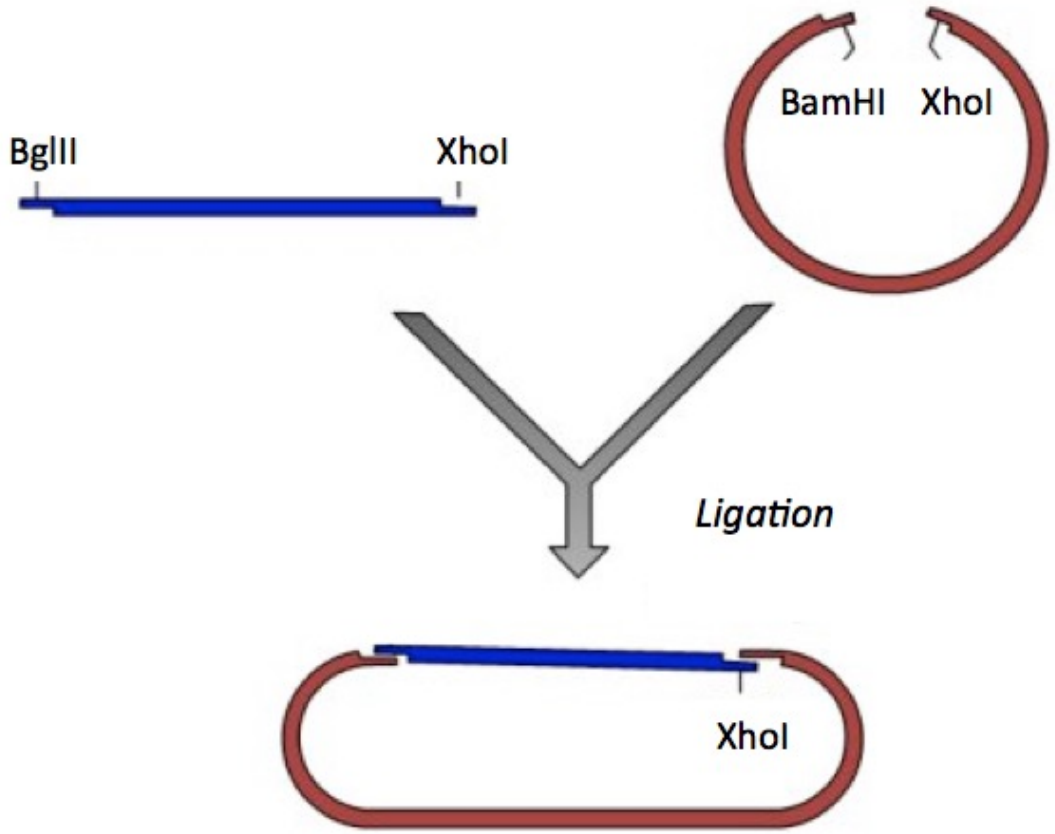
DNA ends from 3’ OH acceptor and 5’ phosphate donor joined

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



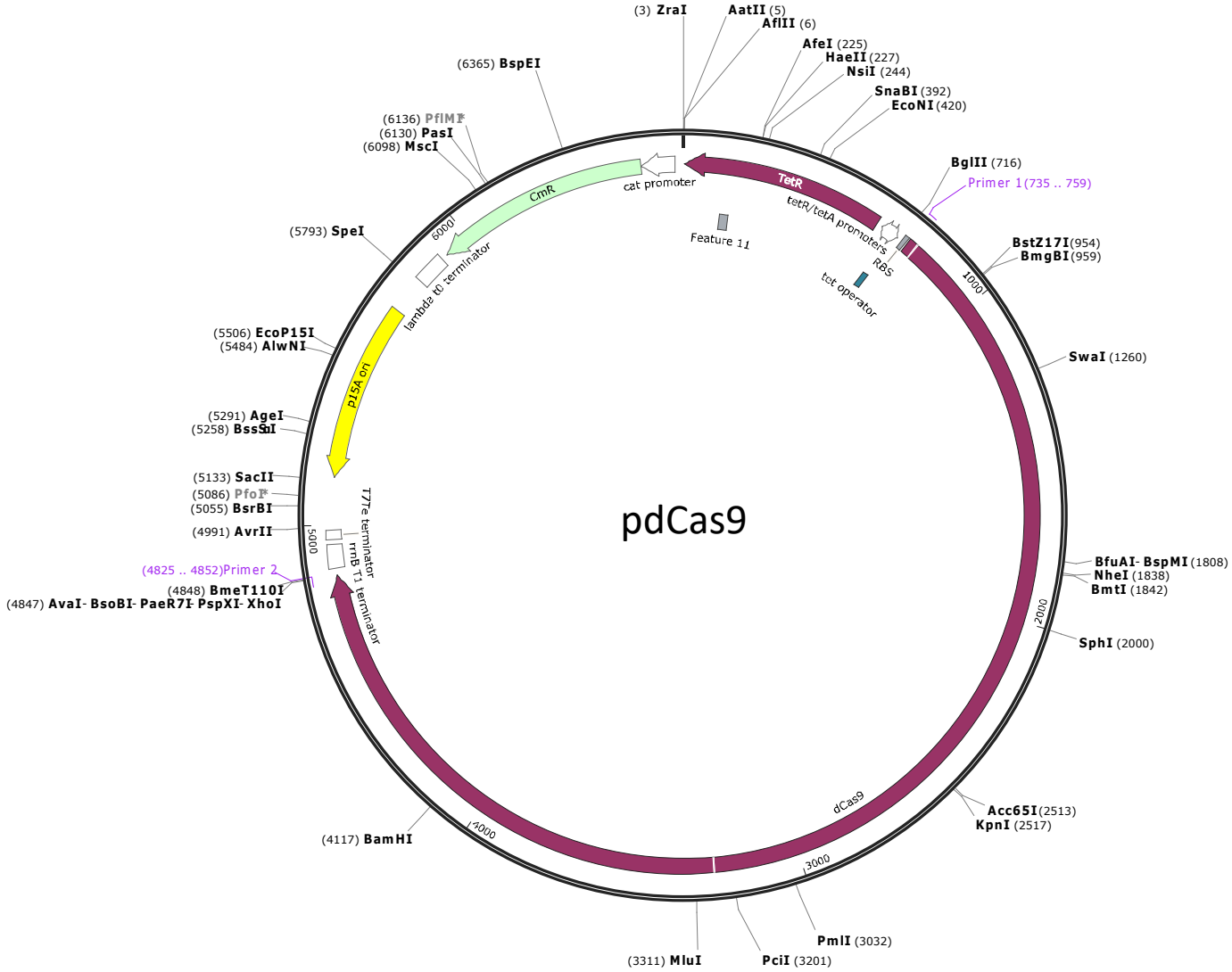
What are we ligating?

How is a BglII site compatible with a BamHI site?



How do we confirm cloning products?

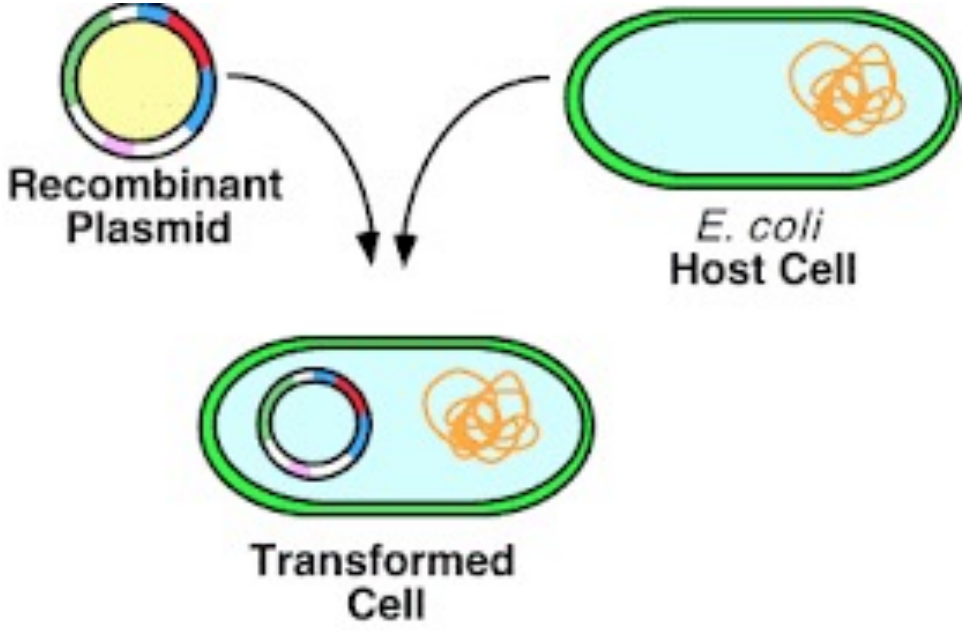
1. Transformation
2. Purification
3. Digestion



1. Transformation used to promote uptake of foreign DNA in bacteria

Why do we transform the ligation product?

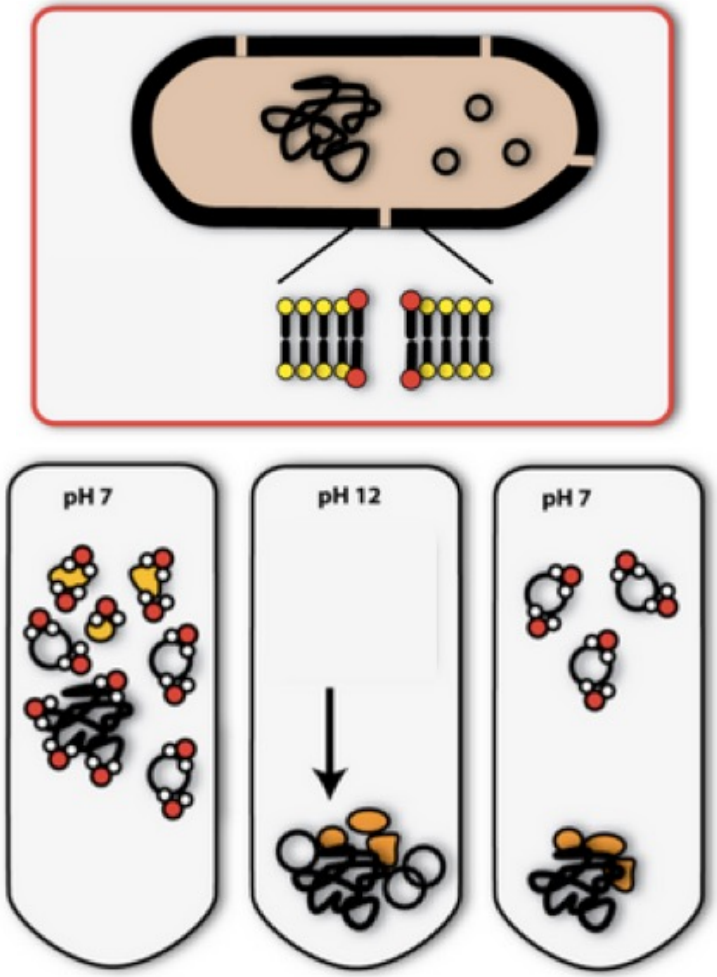
1. Incubation
2. Heat shock
   - DNA taken in by competent cells
3. Recovery
4. Selection



2. Purification used to isolate plasmid DNA

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

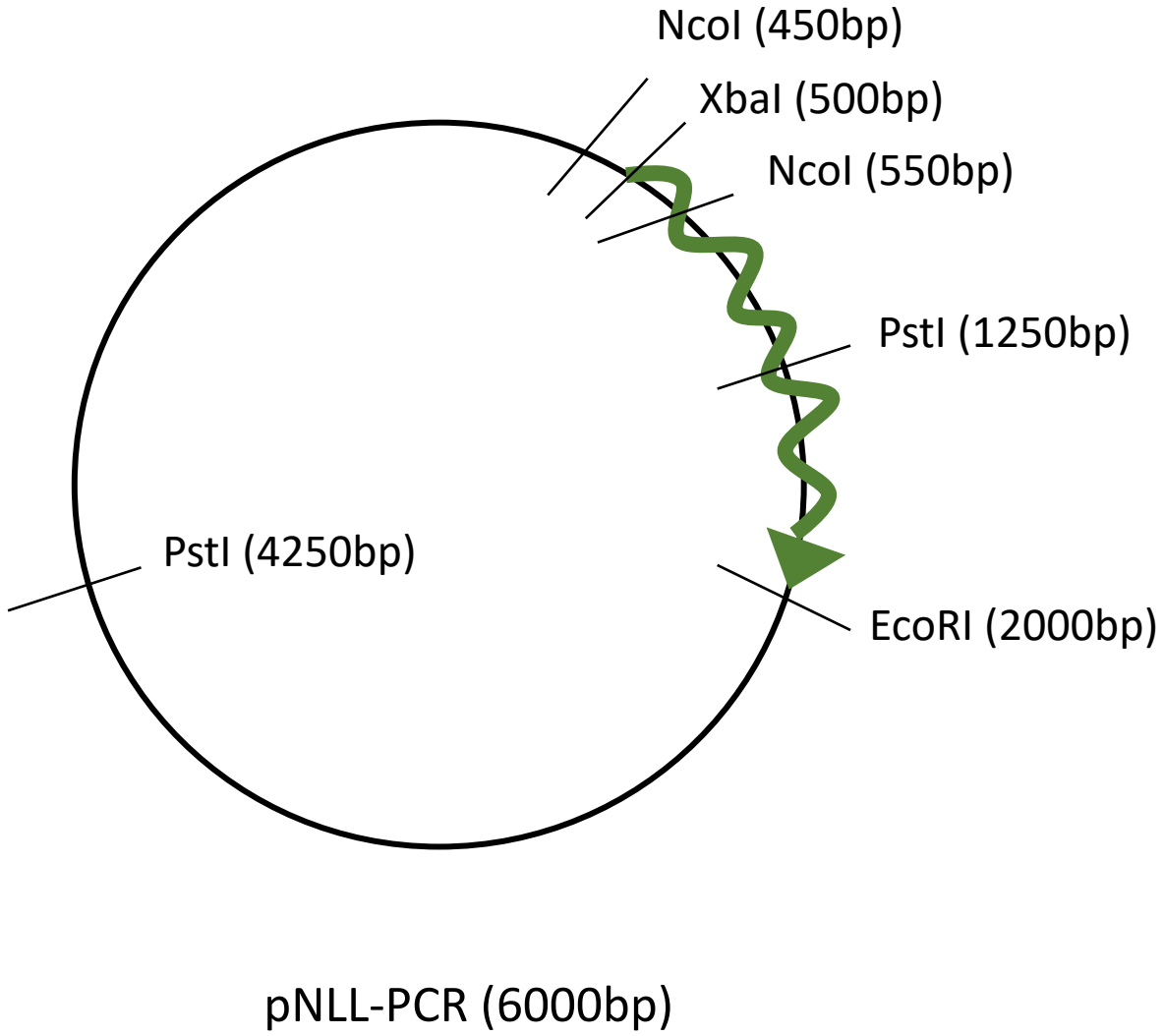
1. Resuspend cells
2. Lyse
3. Neutralize
   - Separates chromosomal DNA from plasmid DNA
4. Wash
5. Resuspend or elute DNA



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

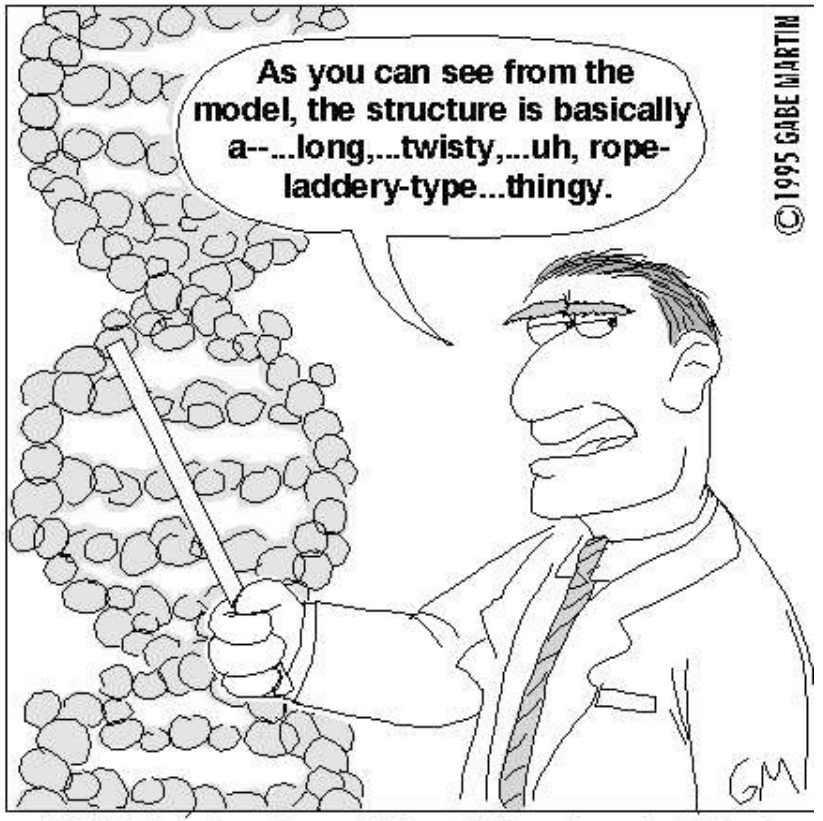
Should we digest with XbaI and EcoRI? PstI? NcoI?



What is the take-home message?

- DNA engineering tools and genetically tractable organisms are crucial to studying biological systems

- Many tools used to manipulate DNA are products of biological engineering!



1953: The structure of the DNA molecule is first described.