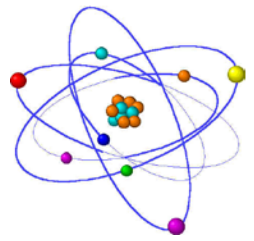
Biochemistry Lab



Cloning-1

Polymerase Chain Reaction

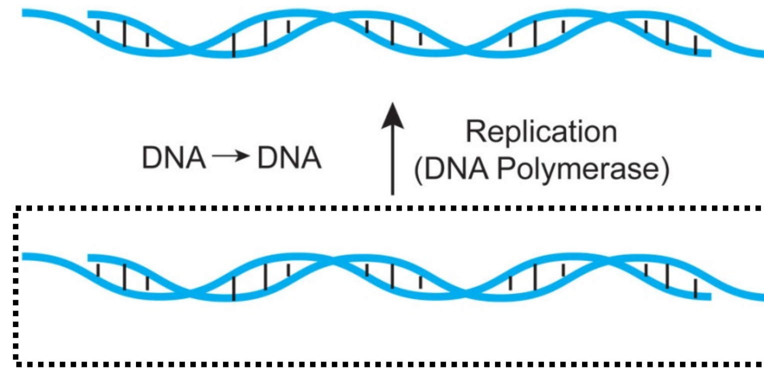
and

Endonucleases

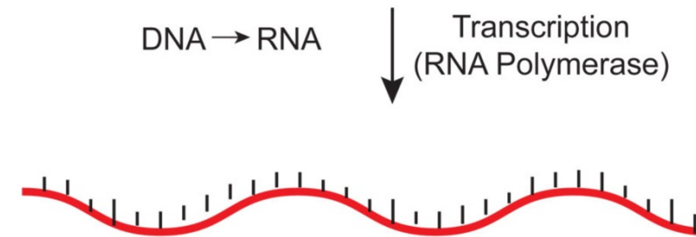
(Restriction Enzymes)

Basics – the Central Dogma

Genetic Information is the blueprint for how to respond to any situation

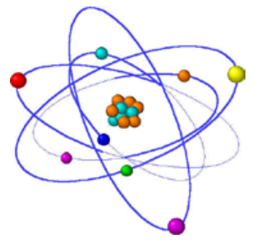


RNA is a short-lived intermediate that translates the genetic code



Proteins are the workhorse of the cell - carry out cellular functions.

Molecular Cloning



- What do we mean by “cloning”?

  Manipulating the genetic information present in an organism to give it new characteristics or function

- As a society, why would we want to do this?

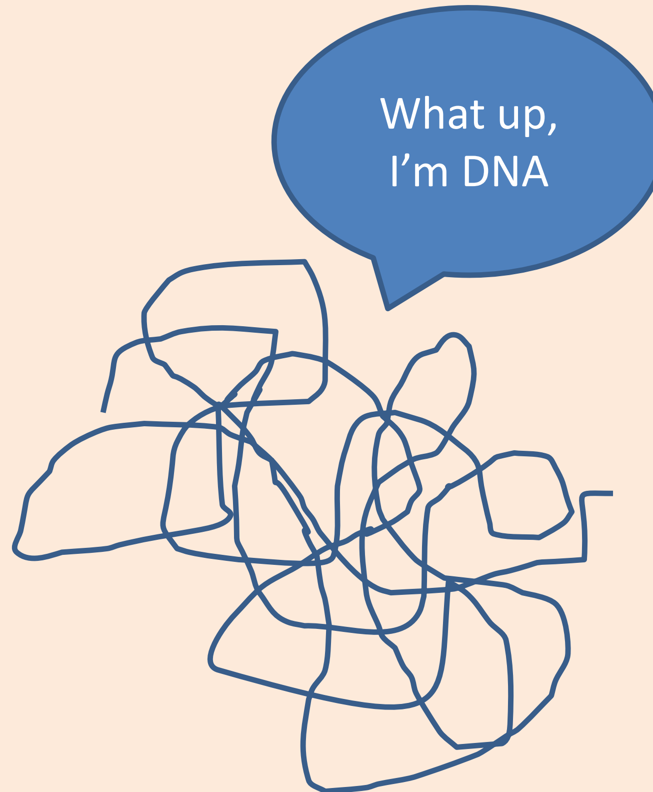
  Make farming easier/more efficient

  Enhance nutrition value of food

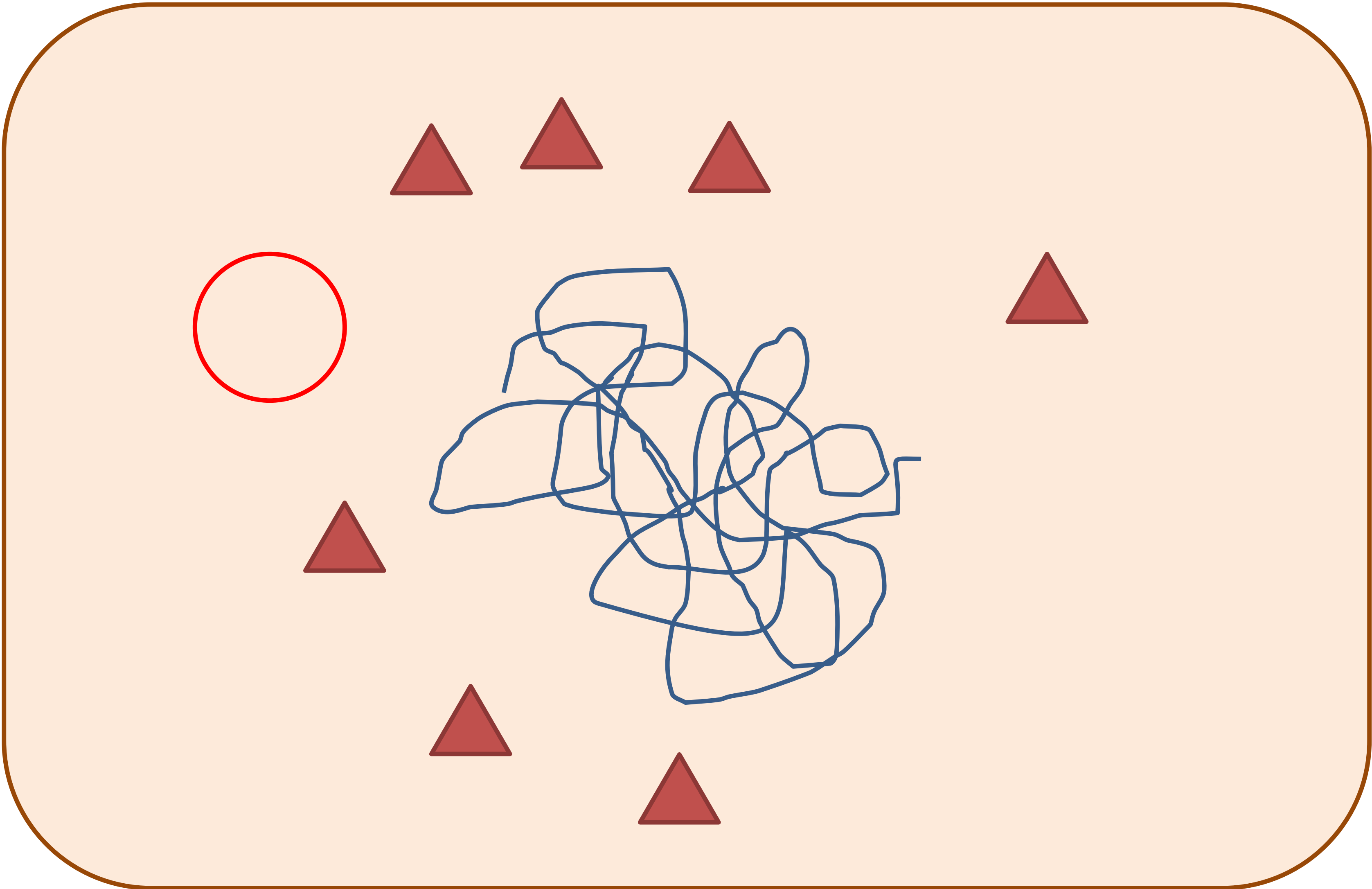
- As Biochemists, why would we want to do this?

  Take advantage of easily manipulated cells to increase protein yield.

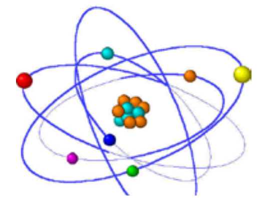
Overall Goal



Overall Goal



Plasmid DNA



pET-28a(+) sequence landmarks

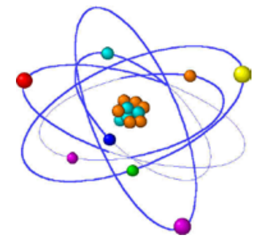
T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

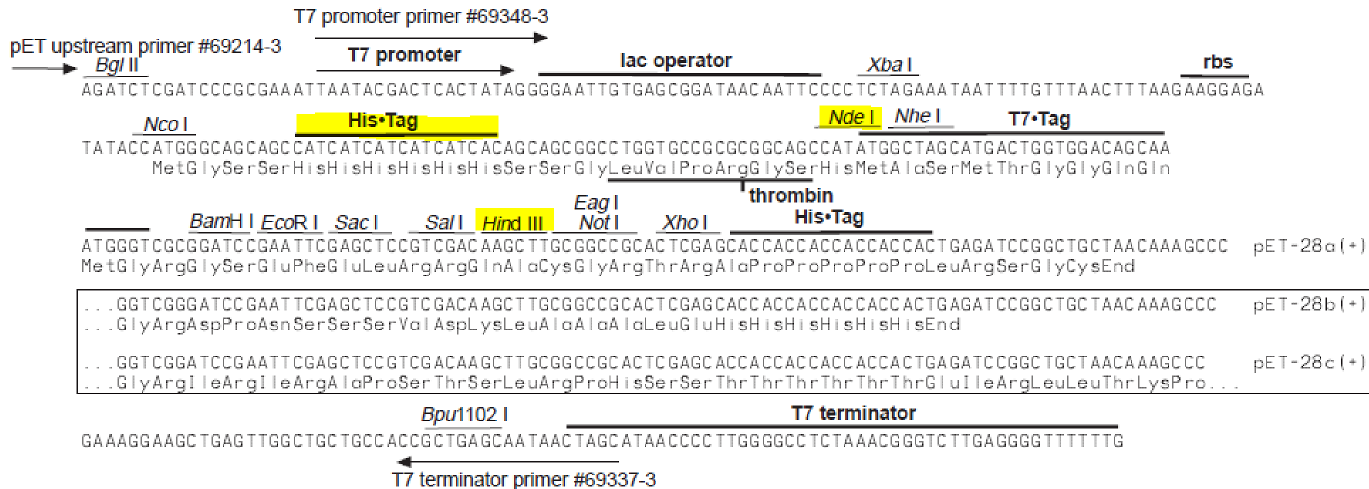
The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.

pET-28a(+)
(5369bp)

Xho I(158)
Not I(166)
Eag I(166)
Hind III(173)
Sal I(179)
Sac I(190)
EcoR I(192)
BamH I(198)
Nhe I(231)
Nde I(238)
Nco I(296)
Bpu1102 I(80)
Xba I(335)
Bgl II(401)
SgrA I(442)
Sph I(598)
Mlu I(1123)
Bcl I(1137)
BstE II(1304)
Apa I(1334)
BssH II(1534)
EcoR V(1573)
Hpa I(1629)
PshA I(1968)
Bgl I(2187)
Fsp I(2205)
Psp5 II(2230)
Tth111 I(2969)
Bst1107 I(2995)
Sap I(3108)
BspLU11 I(3224)
BssS I(3397)
AlwN I(3640)
Eco57 I(3772)
Nru I(4083)
Cla I(4117)
Sma I(4300)
Sgf I(4426)
Pvu I(4426)
Dra III(5127)
f1 origin (4903-5358)
Kan (3995-4807)
lacI (773-1852)
ori (3286)

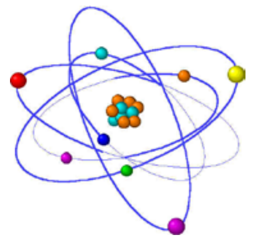
Plasmid DNA





pET-28a-c(+) cloning/expression region

Polymerase Chain Reaction



Role: Amplify a DNA sequence

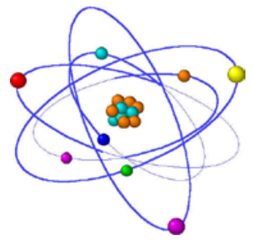
Melting

Annealing

Elongation

$$Total\ copies\ per\ unit\ of\ template = 2^n$$

Polymerase Chain Reaction



So what do we need to carry out a PCR Reaction"?

1. Template DNA
2. Oligonucleotides - Also called "primers" – the sequence of these are critical to select the proper region of the template to amplify
3. dNTPs (dATP, dGTP, dCTP, dTTP) – monomers that build the polymer
4. DNA Polymerase – the enzyme that catalyzes the polymerization reaction
5. Proper solution conditions (buffer, salt, etc.)

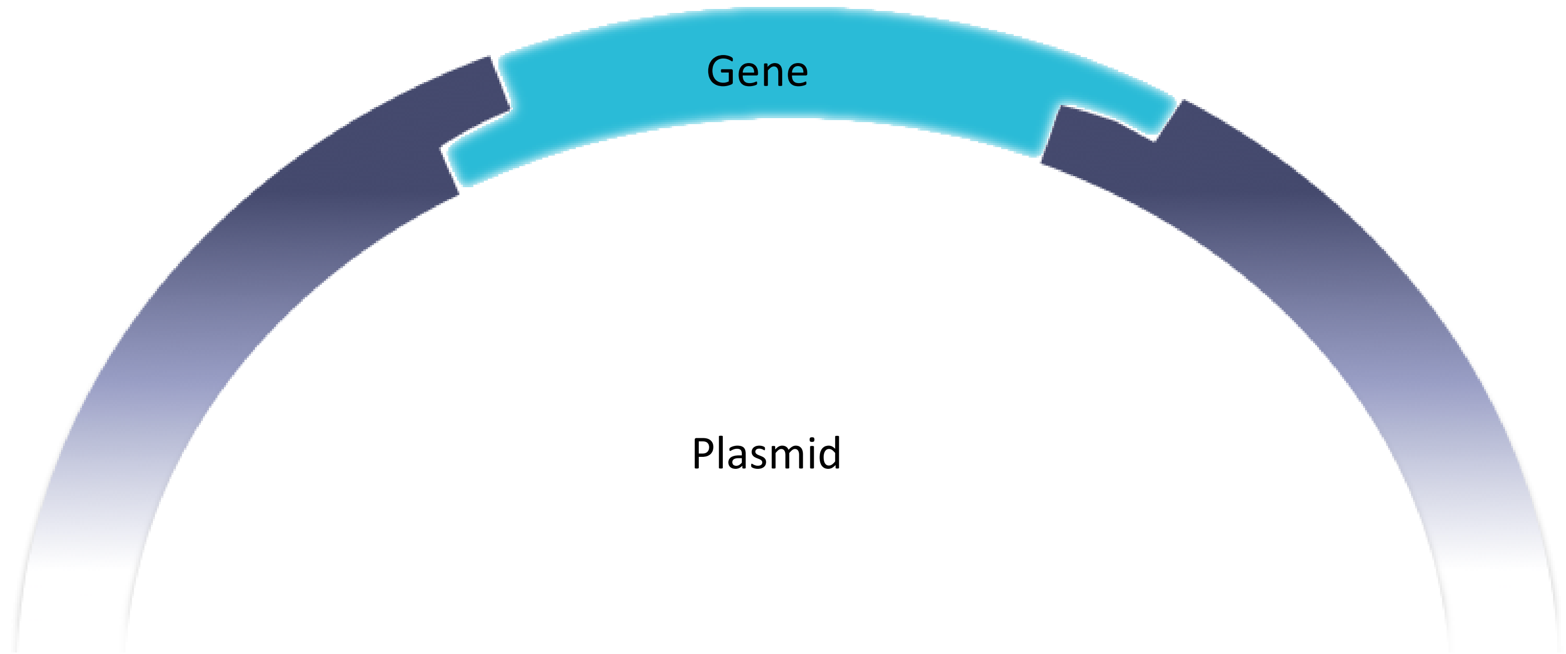
Digestion Overview

- HindIII

- NdeI

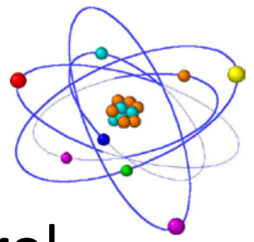
Ligation

- Insert DNA into plasmid



- Transformation of DH5α *E. coli* cells

What's in a Primer?



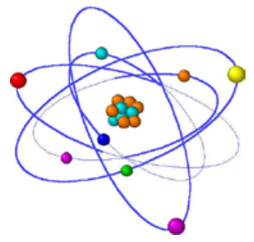
This is the part of the PCR reaction that you get to control – and the easiest way to make a PCR reaction fail!

Primers need to "Bracket" the target DNA

Requirements:

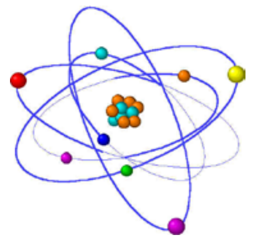
- Specificity –
  - Correct Sequence!
  - Aiming for Tm~60°C
- Directionality
- Include "add-ons".
  - Probably need to include restriction enzyme sequences
  - Start or stop codon?
  - Protein "tag"

In Lab



- Set up a PCR reaction to amplify the LDH gene
  - Be prepared to do this quickly – the PCR reaction takes 1.5 hours
  - Always keep enzymes on ice
- Column purify (mini-prep) pET28 from *E.coli:DH5α::pET28a*
- Column purify your PCR sample
- Determine the concentration of your two samples using the NanoDrop
- Save 5μL of your purified PCR
- Set up two separate digestion reactions (using BamHI and HindIII):
  - pET28a
  - LDH

Outside of Lab



Before Lab

- Complete the PreLab assignment – this will include exploring the sequence of the pET28 plasmid. I encourage you to download the ApE program. You'll also be designing a set of primers to amplify a target DNA sequence. You may want to use the tool here to help.
- Know exactly what you'll be doing – if you are unprepared, you will not finish on time.
- Download the protocols in the resources link on the webpage – *these need to be copied or pasted in your lab notebook!*

After Lab

- Think critically about what you did in lab. Understand the molecular underpinning of the reactions you conducted. This is all a very important part of your Introduction
- Begin writing your Introduction and Methods section – these are each upcoming assignments.