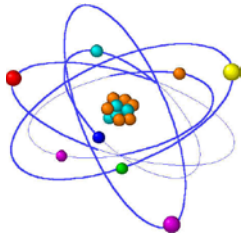
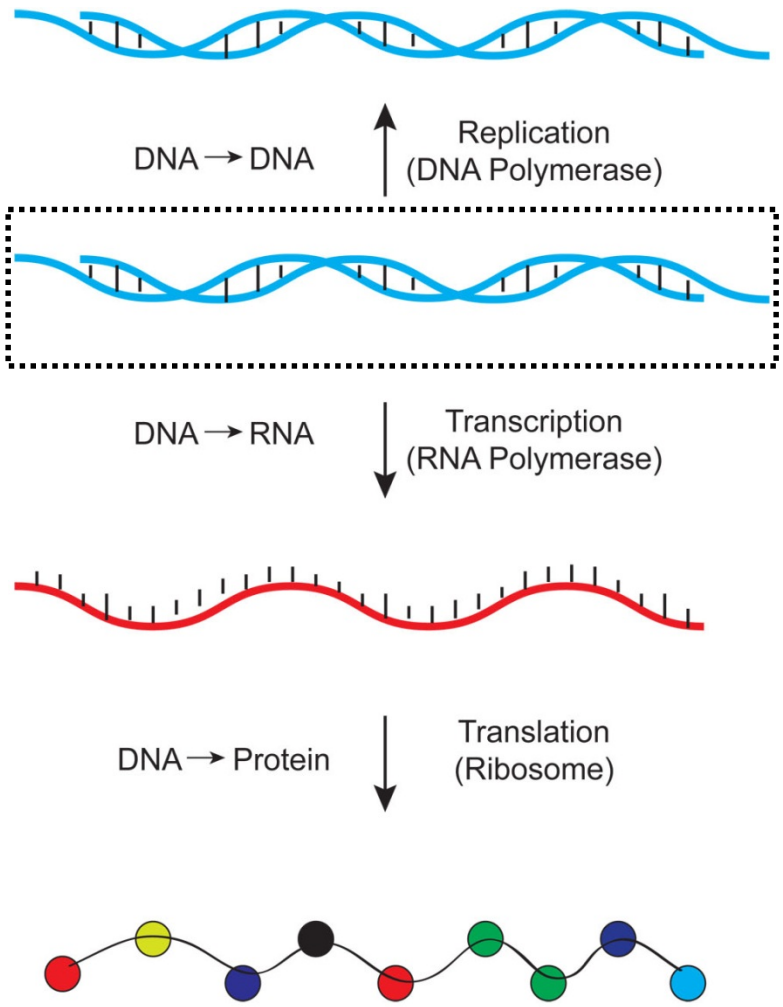
Biochemistry Lab



Molecular Cloning

Weeks 1 and 2…combined

Basics – the Central Dogma

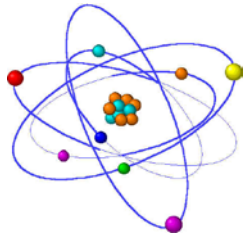


Genetic Information is the blueprint for how to respond to any situation

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?

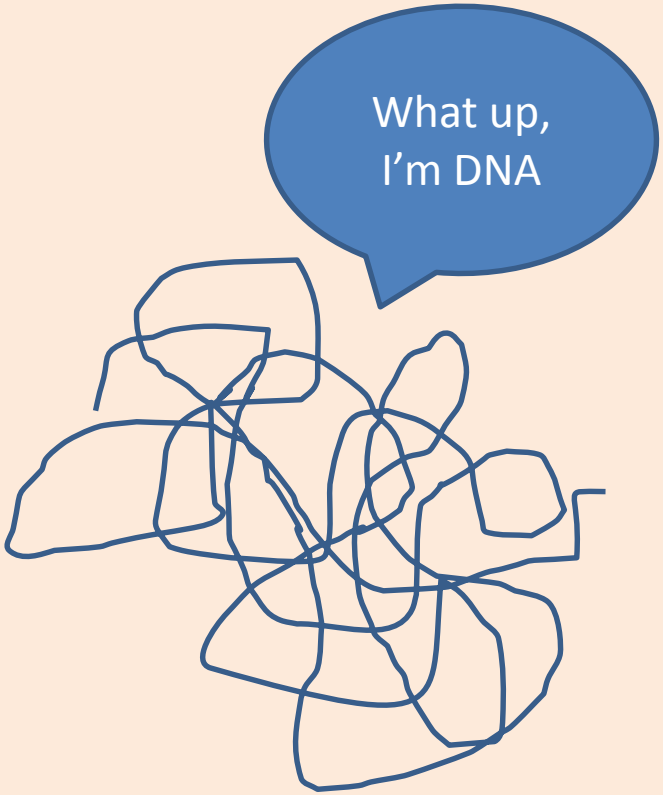
  Make farming easier/more efficient

  Enhance nutrition value of food

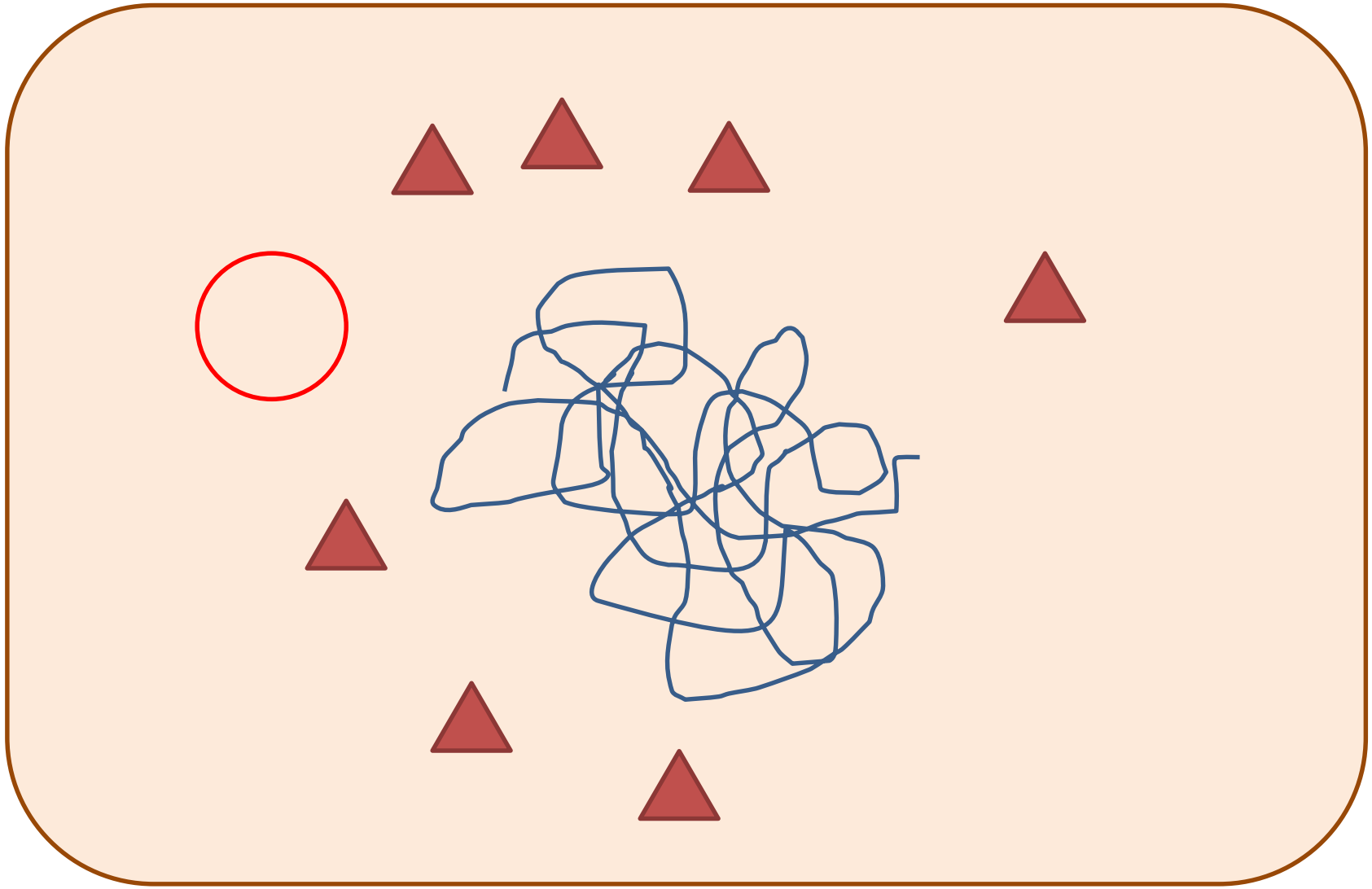
- As Biochemists, why would we want to do this?

  Take advantage of easily manipulated cells to increase protein yield.

Overall Goal



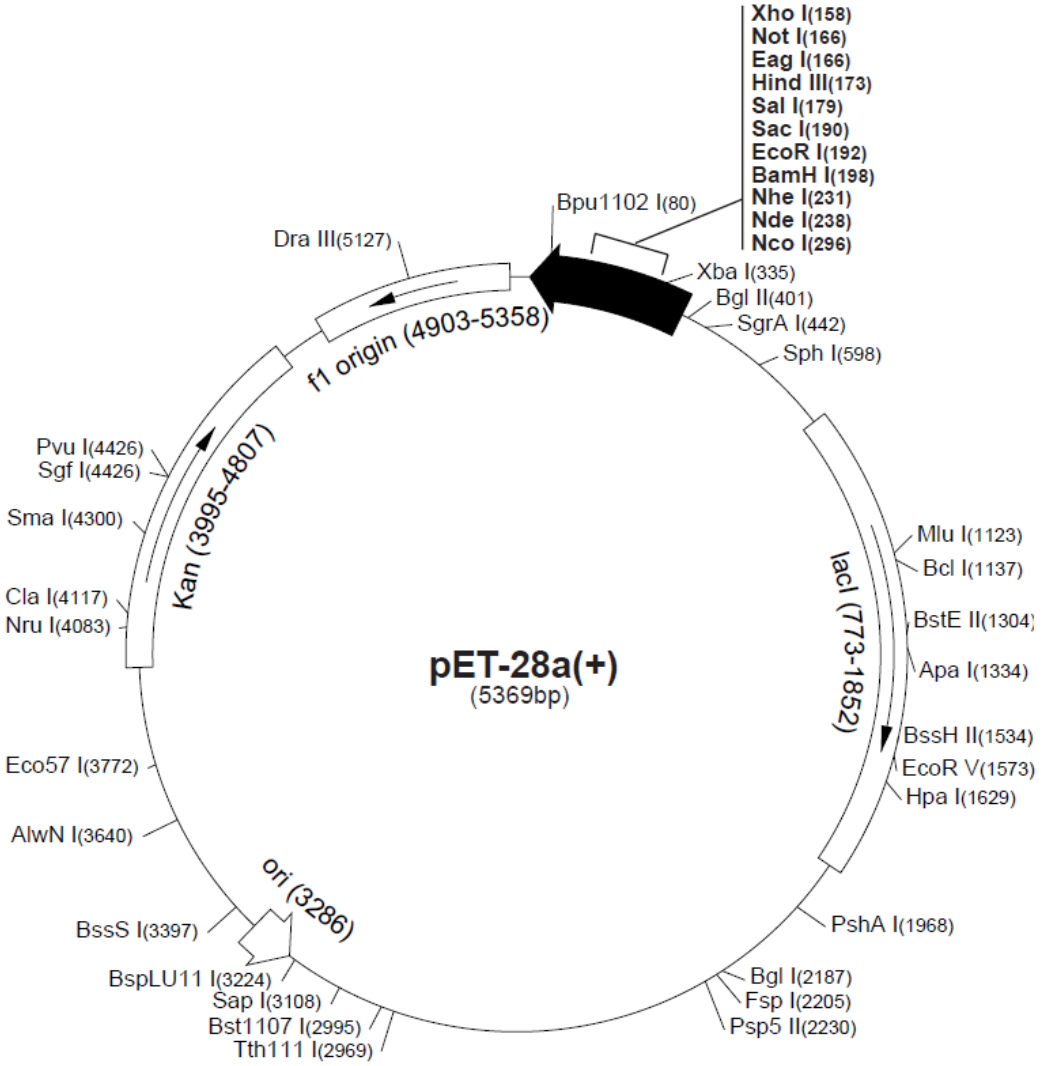
Overall Goal



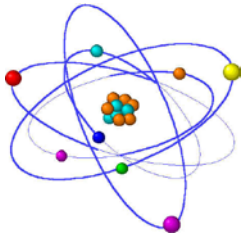
Plasmid DNA

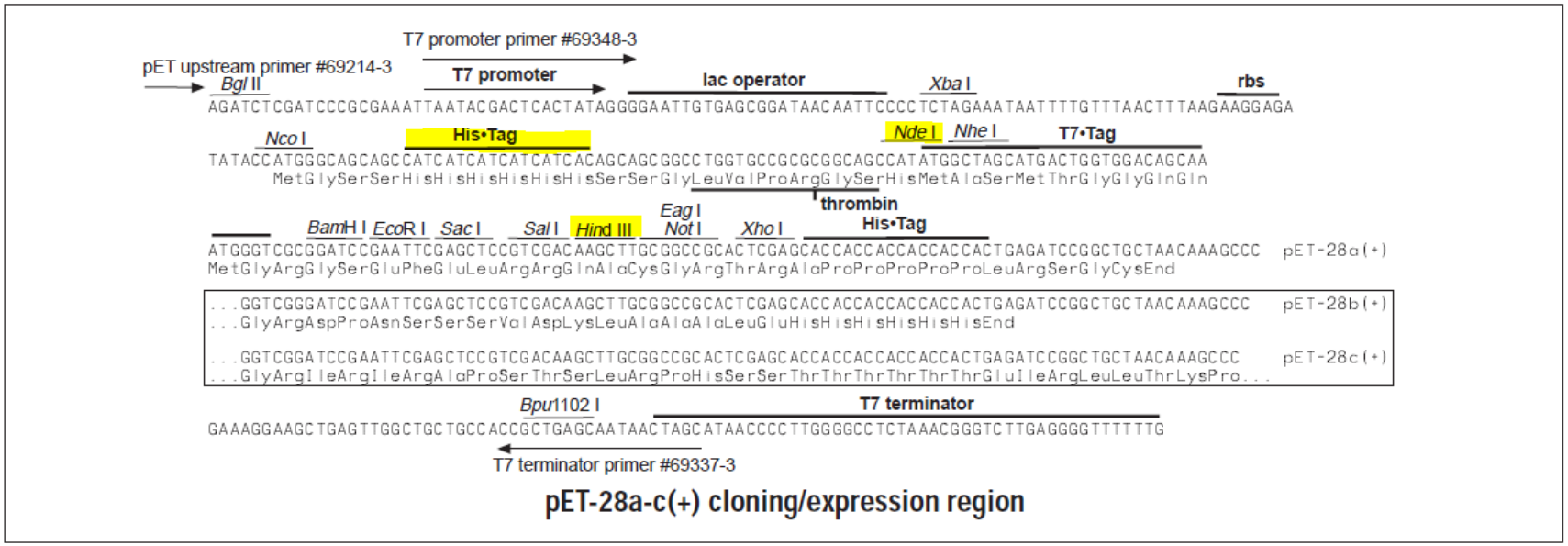
pET-28a(+) sequence landmarks	
T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding 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.

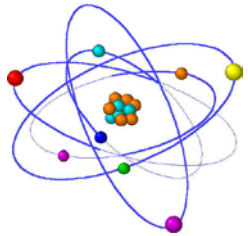


Plasmid DNA




T7 promoter primer #69348-3
pET upstream primer #69214-3
T7 promoter
lac operator
rbs
BglII
XbaI
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
NcoI
His•Tag
NdeI
NheI
T7•Tag
TATACCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAA
MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetAlaSerMetThrGlyGlyGlnGln
thrombin
EagI
BamHI EcoRI SacI SalI HindIII NotI XhoI
His•Tag
ATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCC pET-28a(+)
MetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeuArgSerGlyCysEnd
...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCC pET-28b(+)
...GlyArgAspProAsnSerSerSerValAspLysLeuAlaAlaAlaLeuGluHisHisHisHisHisEnd
...GGTCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCC pET-28c(+)
...GlyArgIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrThrGluIleArgLeuLeuThrLysPro...
Bpu1102I
T7 terminator
GAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
T7 terminator primer #69337-3
pET-28a-c(+) cloning/expression region

Polymerase Chain Reaction



Role: Amplify a DNA sequence

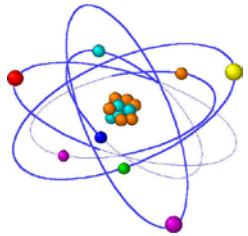
Melting

Annealing

Elongation

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

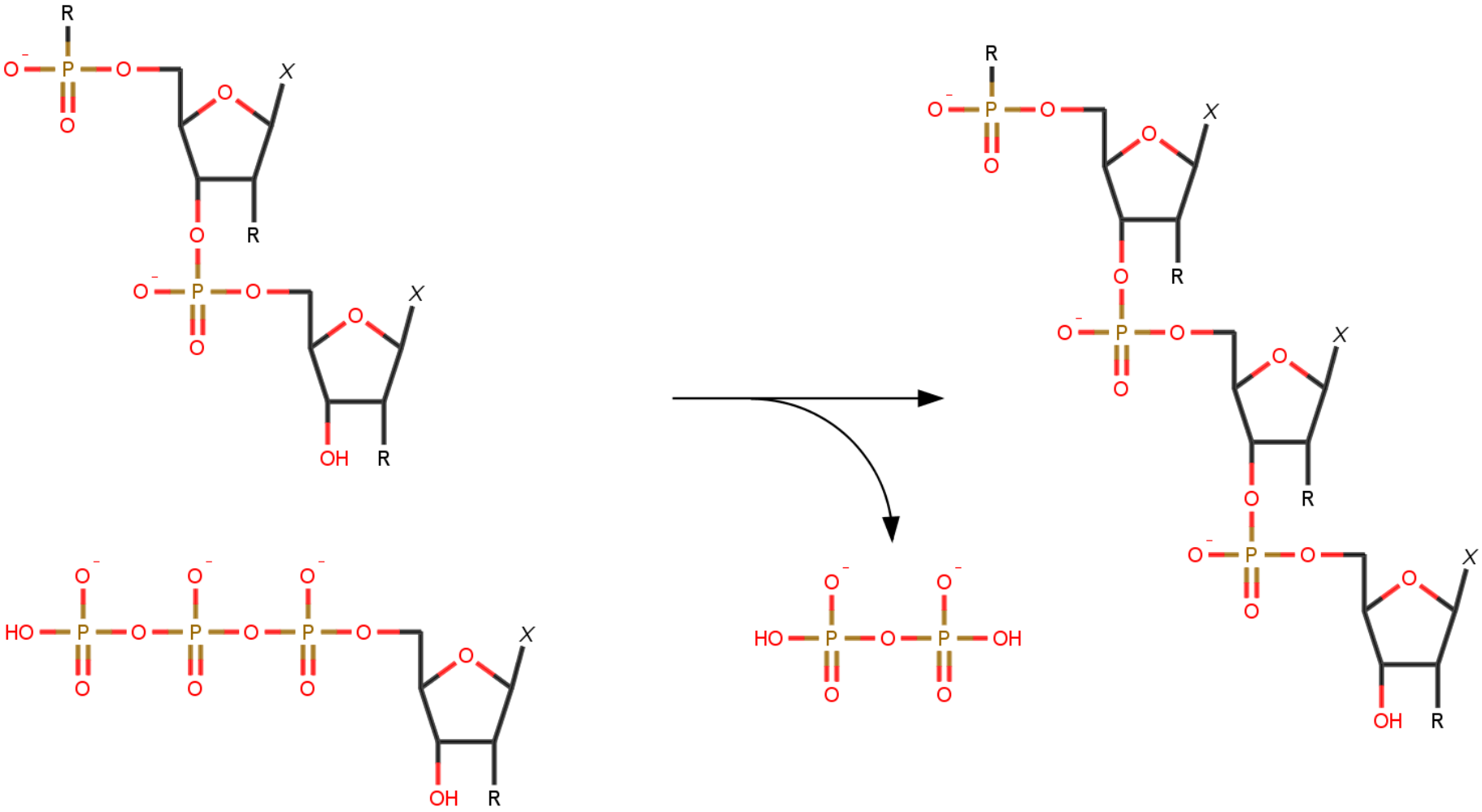
Polymerase Chain Reaction



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.)

Polymerase Chain Reaction



Digestion Overview

- HindIII

  5’…A|A G C T T …3’

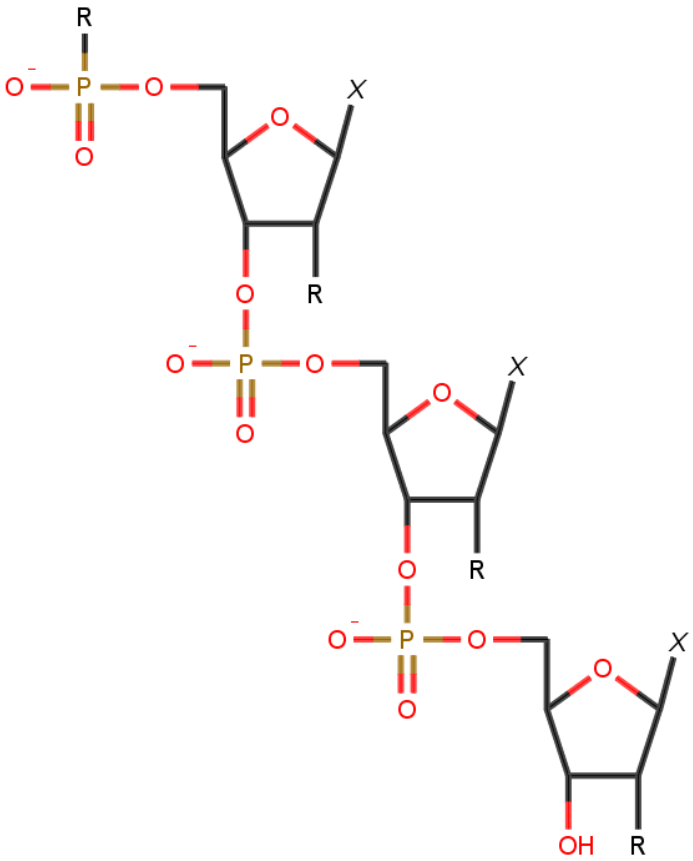
  3’…T T C G A|A …5’

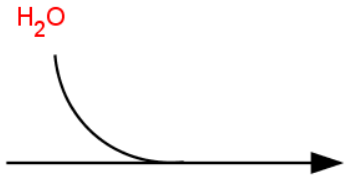
- NdeI

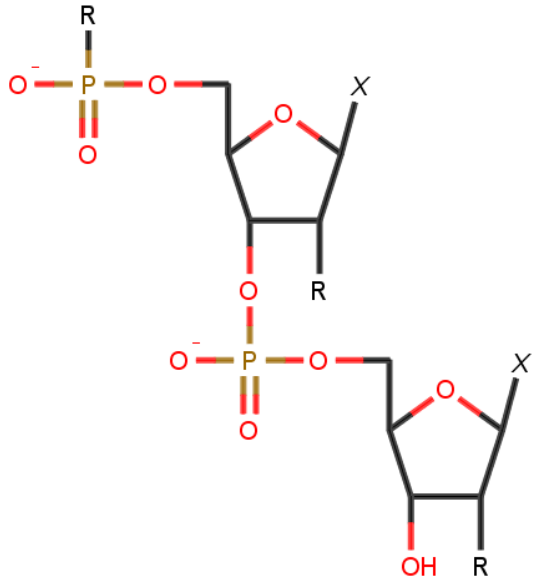
  5’… C A|T A T G …3’

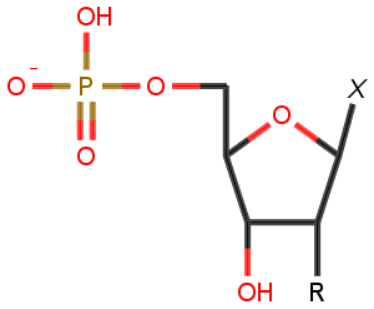
  3’… G T A T|A C …5’

Endonucleases



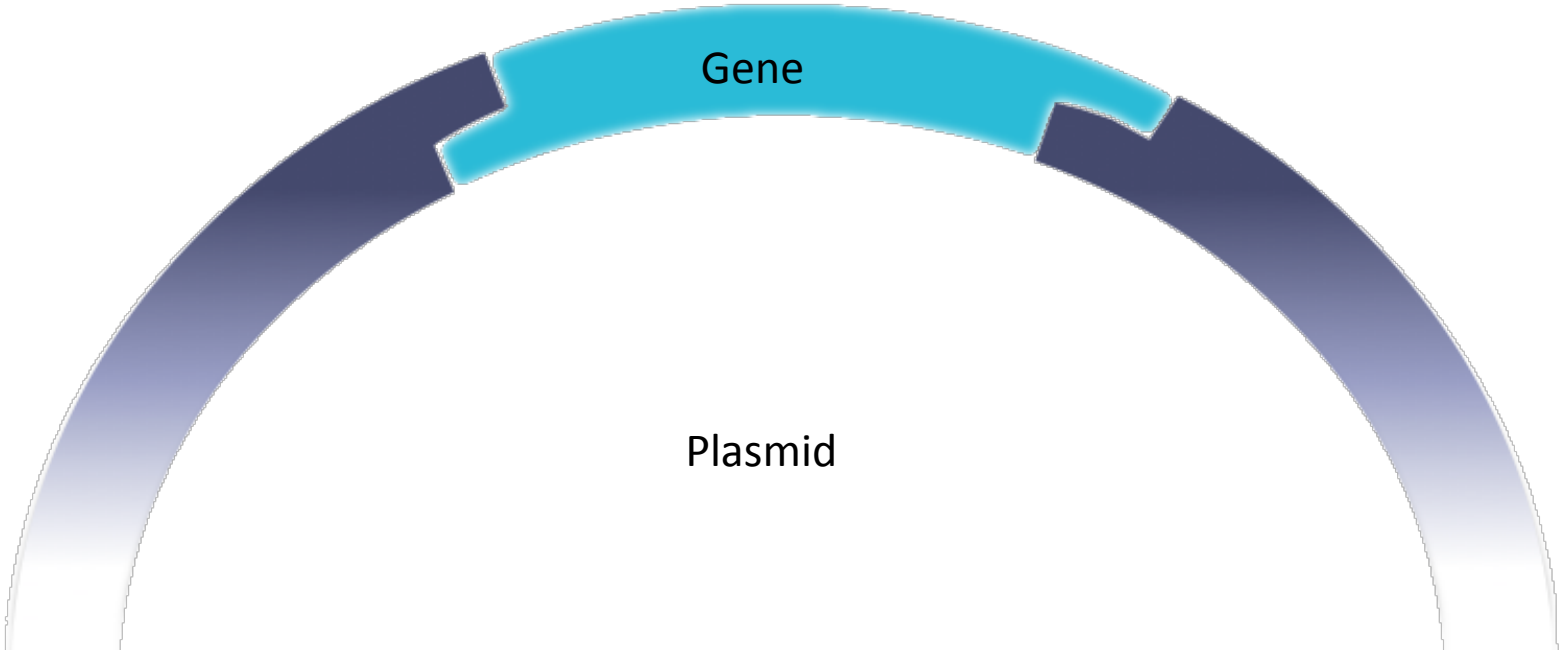






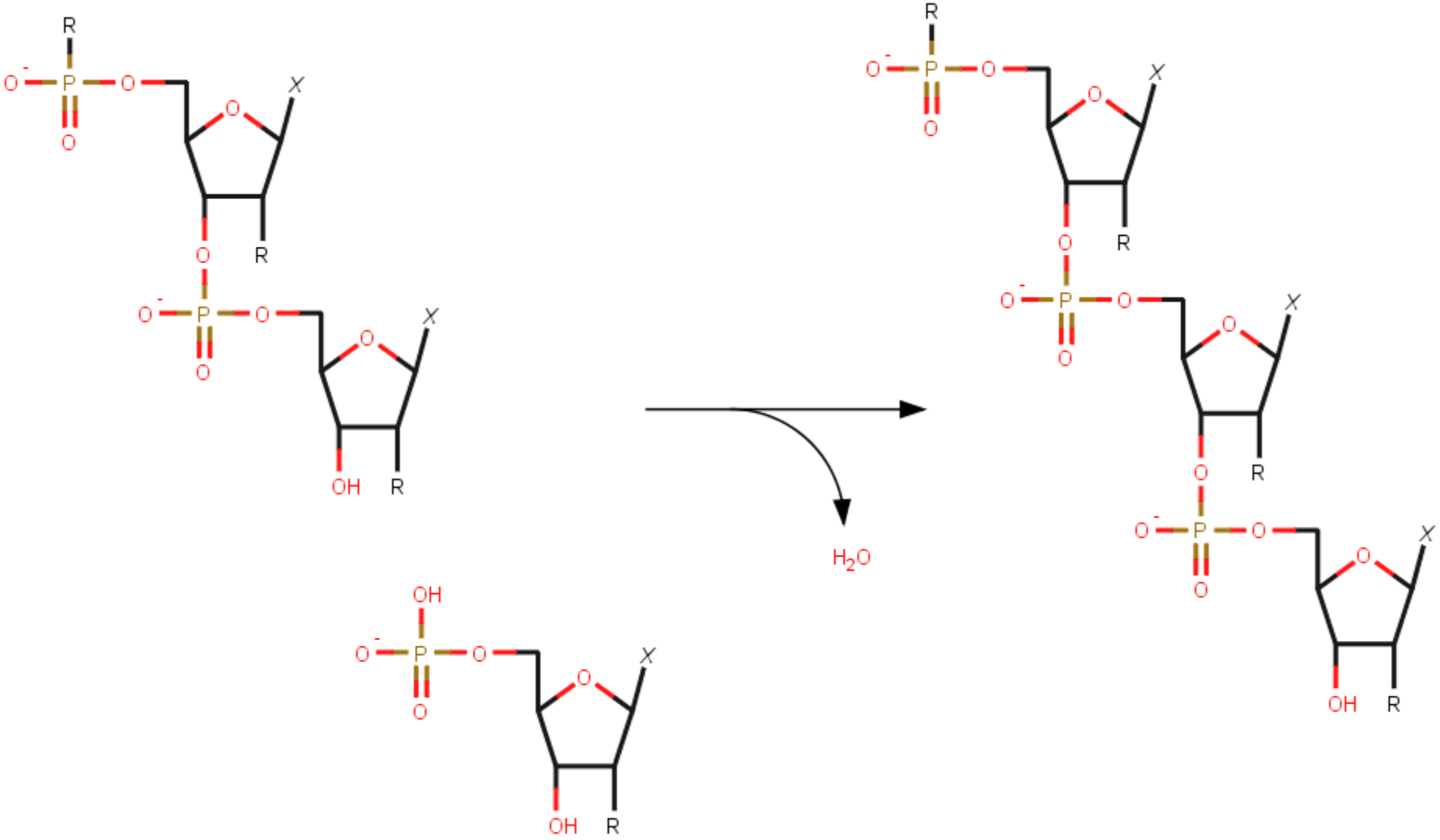
Ligation

- Insert DNA into plasmid

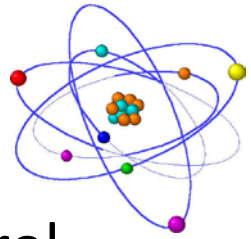


- Transformation of DH5α *E. coli* cells

Ligation


R
O
P
O
O
O
X
O
R
O
P
O
O
O
X
OH
R
OH
O
P
O
O
O
X
OH
R
H_2O
R
O
P
O
O
O
X
O
R
O
P
O
O
O
X
O
R
O
P
O
O
O
X
OH
R

What's in a Primer?



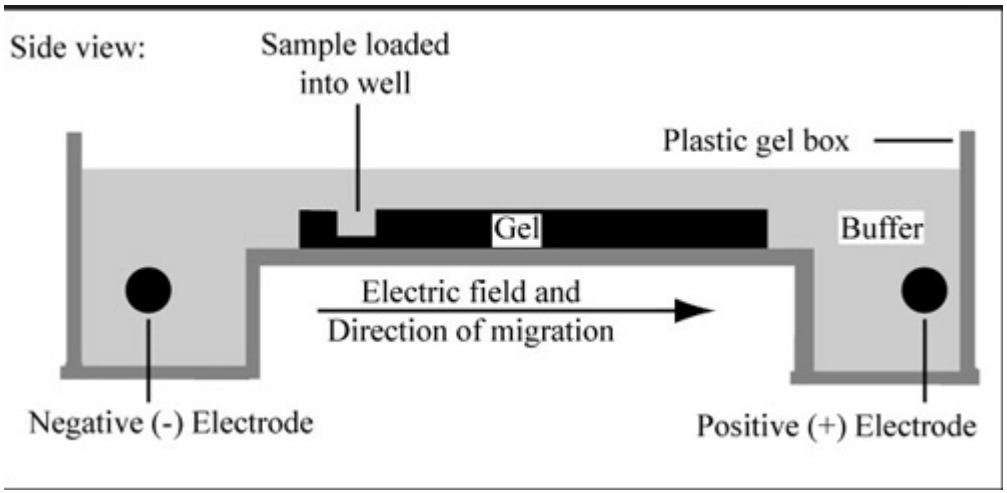
This is the part of the PCR reaction that you get to control – and the easiest way to make a PCR reaction fail!

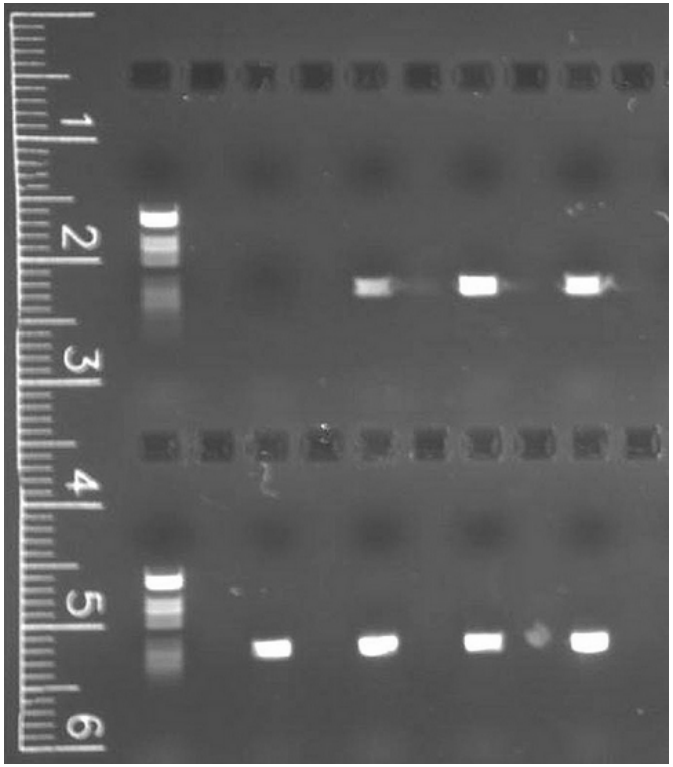
Primers need to "Bracket" the target DNA

Requirements:

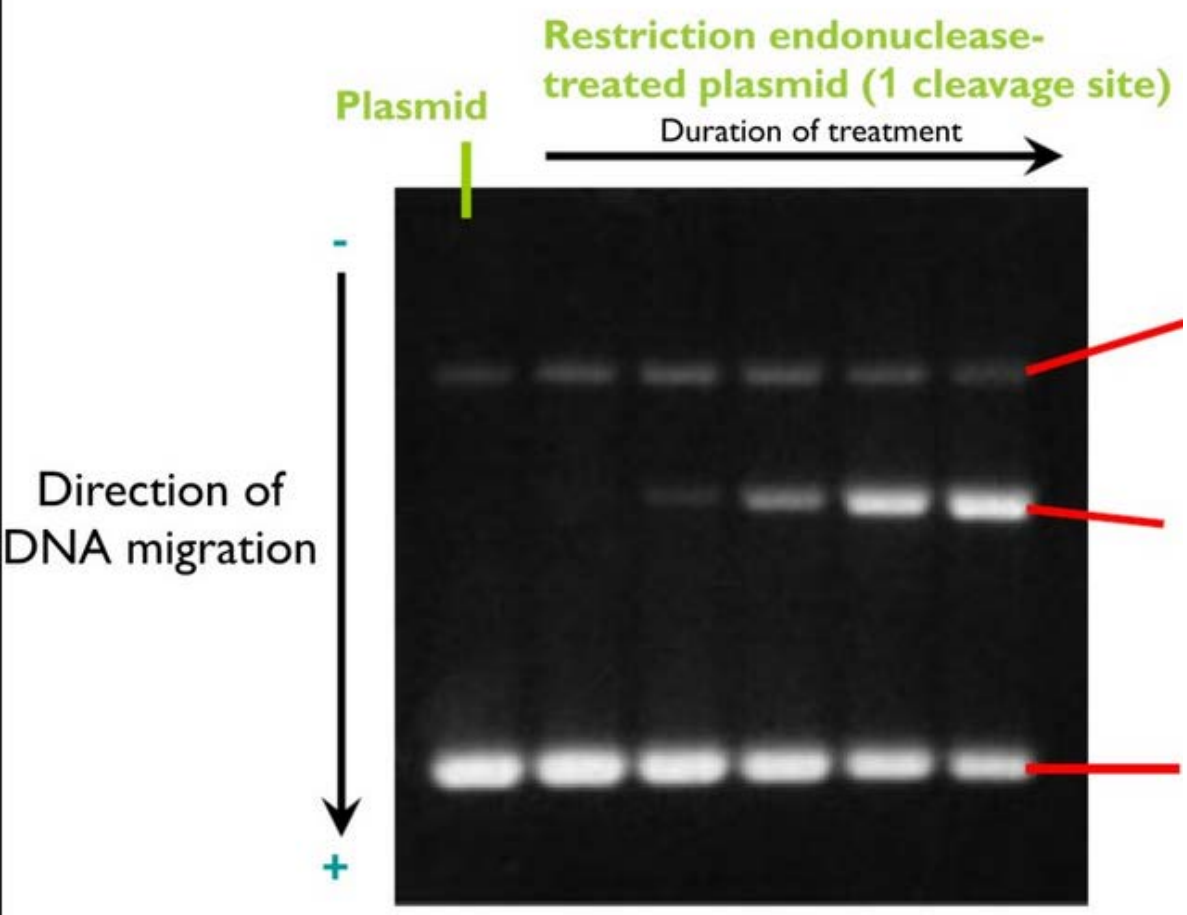
- Specificity –
  - Correct Sequence!
  - Aiming for Tm~60°C
- Directionality
- Include "add-ons".
  - Probably need to include restriction enzyme sequences
  - Start or stop codon?
  - Protein "tag"

New Technique: Electrophoresis

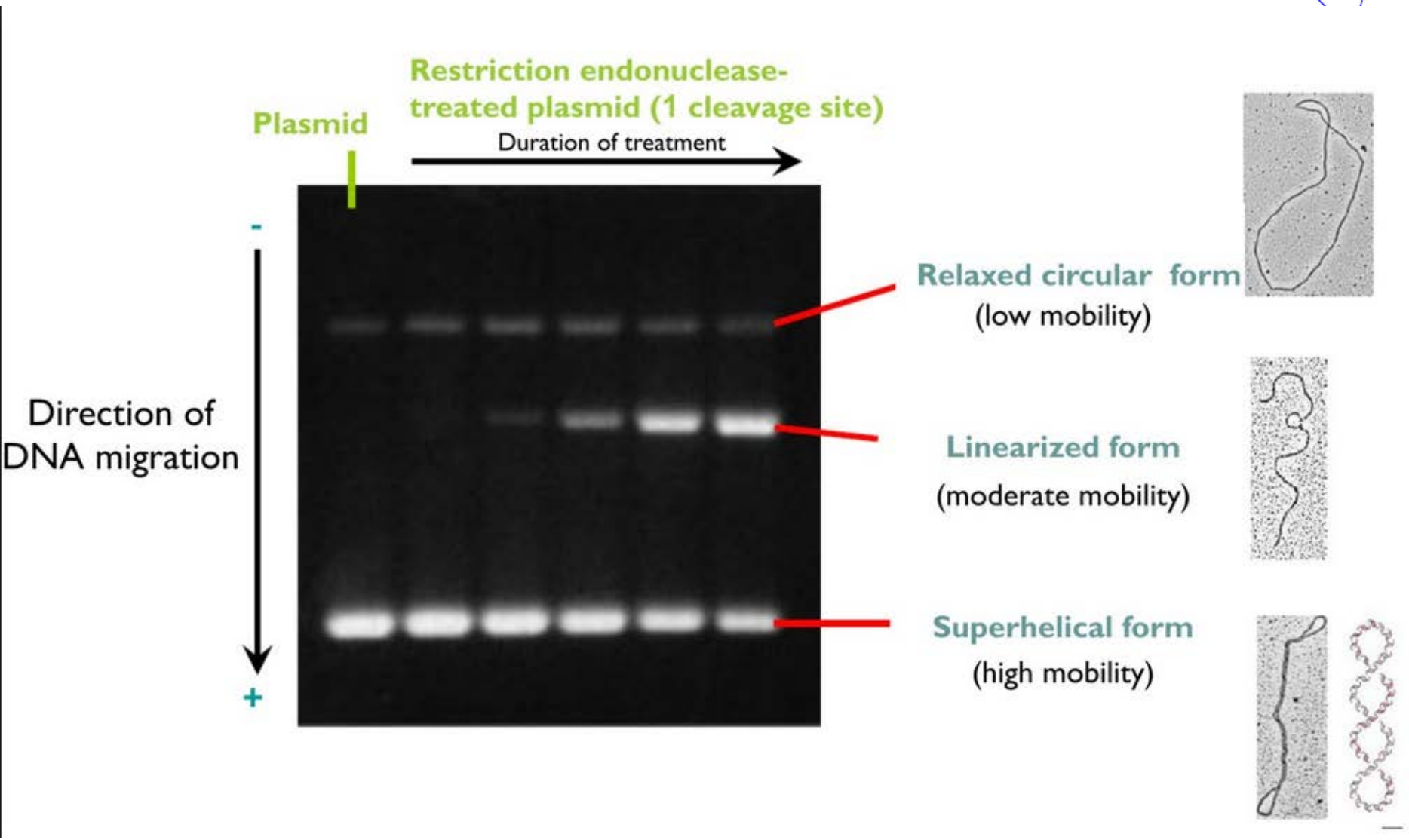




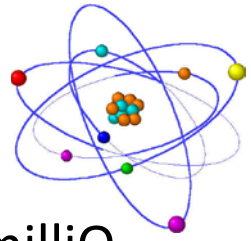
New Technique: Electrophoresis



New Technique: Electrophoresis

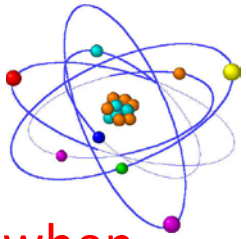


In Lab



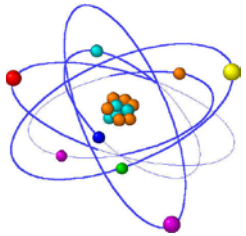
- Column purify the digestion reactions – elute with 30 μL of milliQ water.
- Concentrate samples on the SpeedVac
- Pour the agarose gel.
  - One gel will be shared between two groups.
  - Handle the ethidium bromide with care
- Samples to include on your gel:
  - Digested plamid
  - Digested PCR product
  - Undigested PCR reaction
  - Ladder
  - Undigested pET28
- Run the gel at 110V ~30 minutes
- Take a picture of your gel using the gel doc. You can fiddle with the image when you have some free time.

In Lab



- Gel extract – do not look directly at UV light and use caution when handling the razor blade!
- Column purify the gel fragment
- Measure the concentration on the nanoDrop
  - Concentrate on the speedVac if necessary
- Set up a ligation reaction

Outside of Lab



Before Lab

- Complete the PreLab assignment – this will include sample calculations for setting up your experiment
- Know exactly what you'll be doing – if you are unprepared, you will not finish on time.
- Make sure that you notebook contains all the appropriate protocols. If not, download them from the resources link on the webpage.

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
- Continue working on your Introduction and Methods section – these are each upcoming assignments.