# MOD1 – DNA ENGINEERING

Engelward, Spring 2008

**Day 1**

**About this Module**

Goals for this Module

Brief background: Homologous recombination is not just for meiosis!

Overview of the Experiment

The Plasmid Construction Roadmap

**Today's Experiment**: **Design Primers and Perform PCR**

Chemistry of nucleotide addition (5' vs 3' end)

PCR - Cycling

What is a Restriction Enzyme Site?

How can you use PCR to add a restriction site to your PCR product?

# What you will gain from the opportunity to do these experiments:

-Confidence in your ability to work with plasmids

-Know-how for using plasmids to express a gene in a mammalian cell

-Ability to independently design primers and set up a PCR reaction

-Ability to culture mammalian cells

-Ability to use a flow cytometer and a basic understanding of how it works

# What you will learn about the science behind your experiments:

**By the end of this module, you will have a basic understanding of:**

**-where mutations come from**

**-how DNA is repaired**

**-the relationship between mutations and cancer**

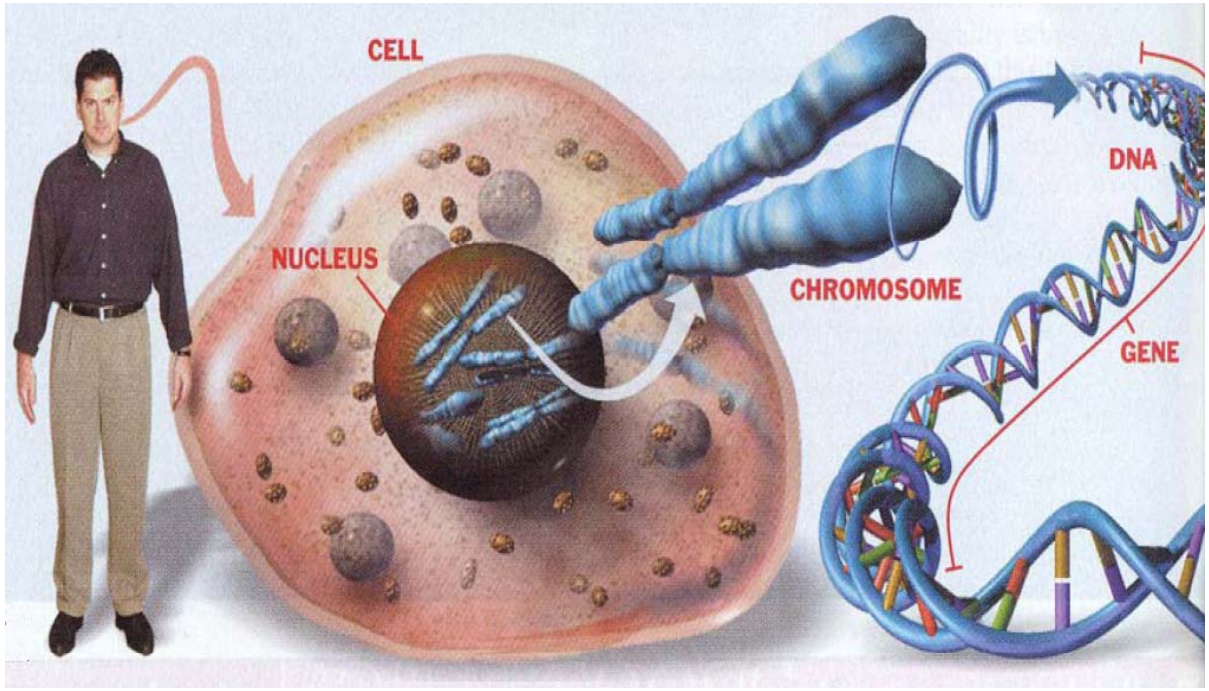
**-how homologous recombination works**

**-why companies are designing drugs to disable homologous recombination in tumor cells**

# What you will do :

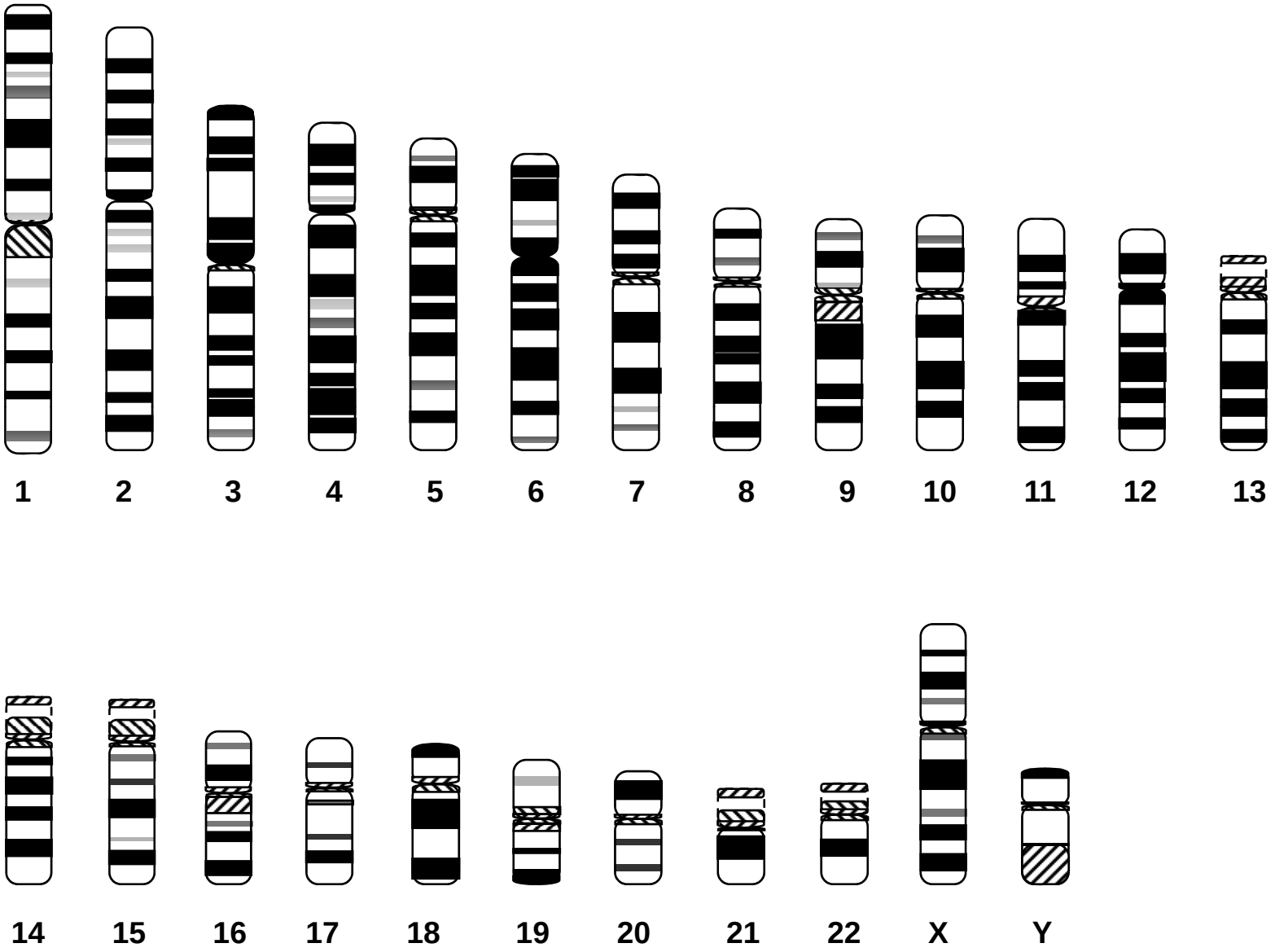
**In this module, you will create a plasmid that will be used in an assay to measure homologous recombination activity in mammalian cells.**

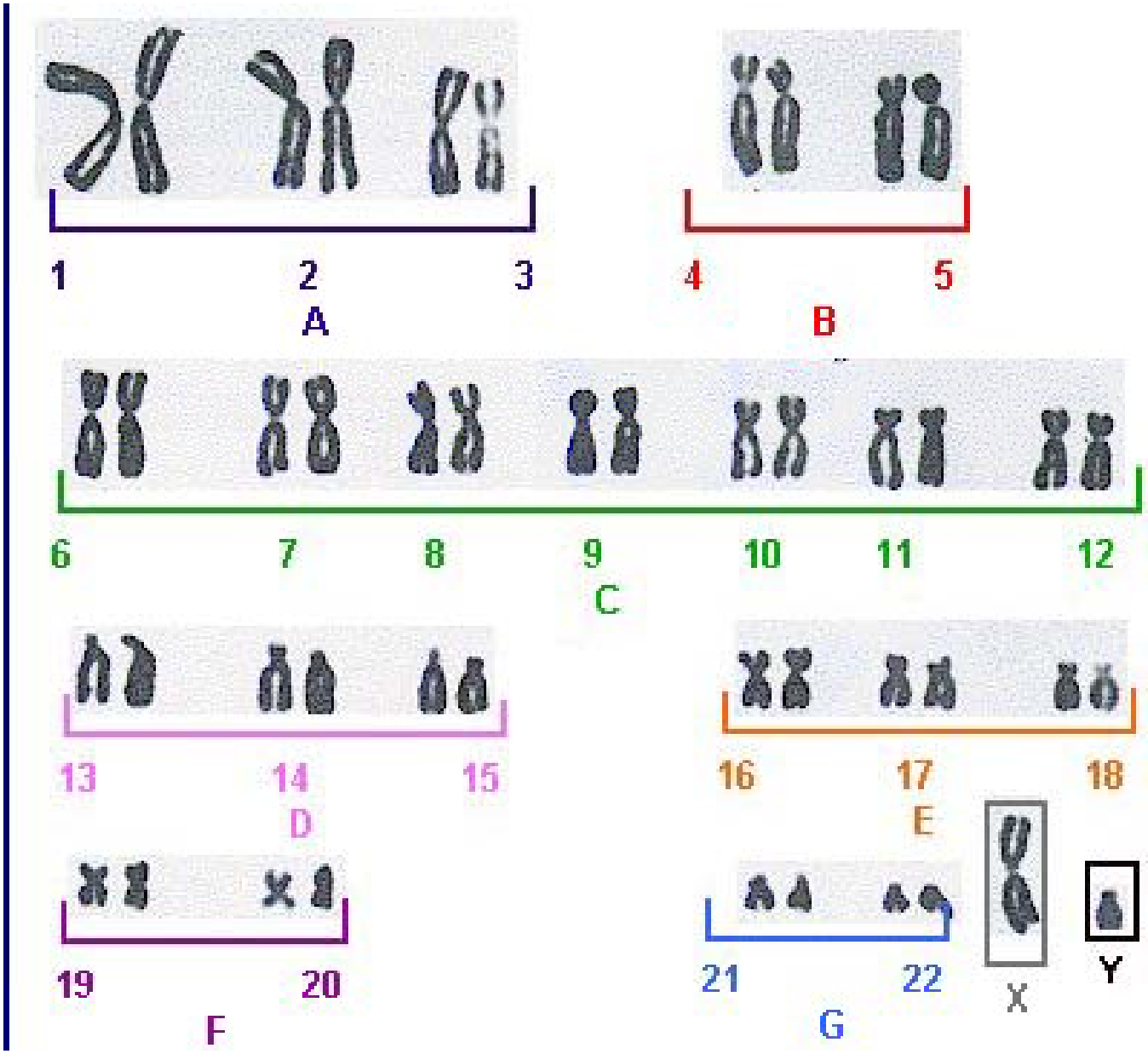
# Your DNA



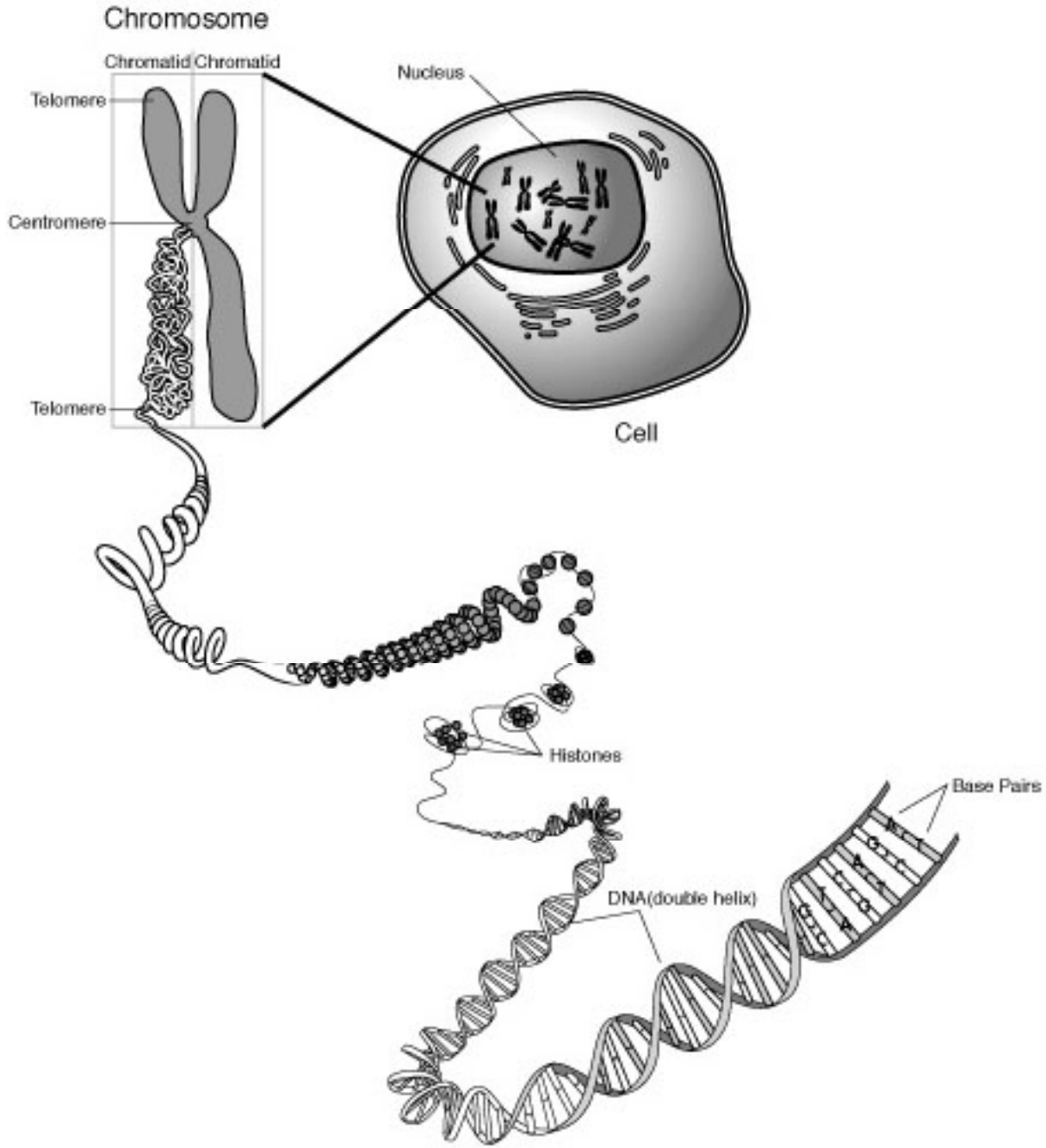

"*TIME*" Nov., 1999

# Human chromosomes

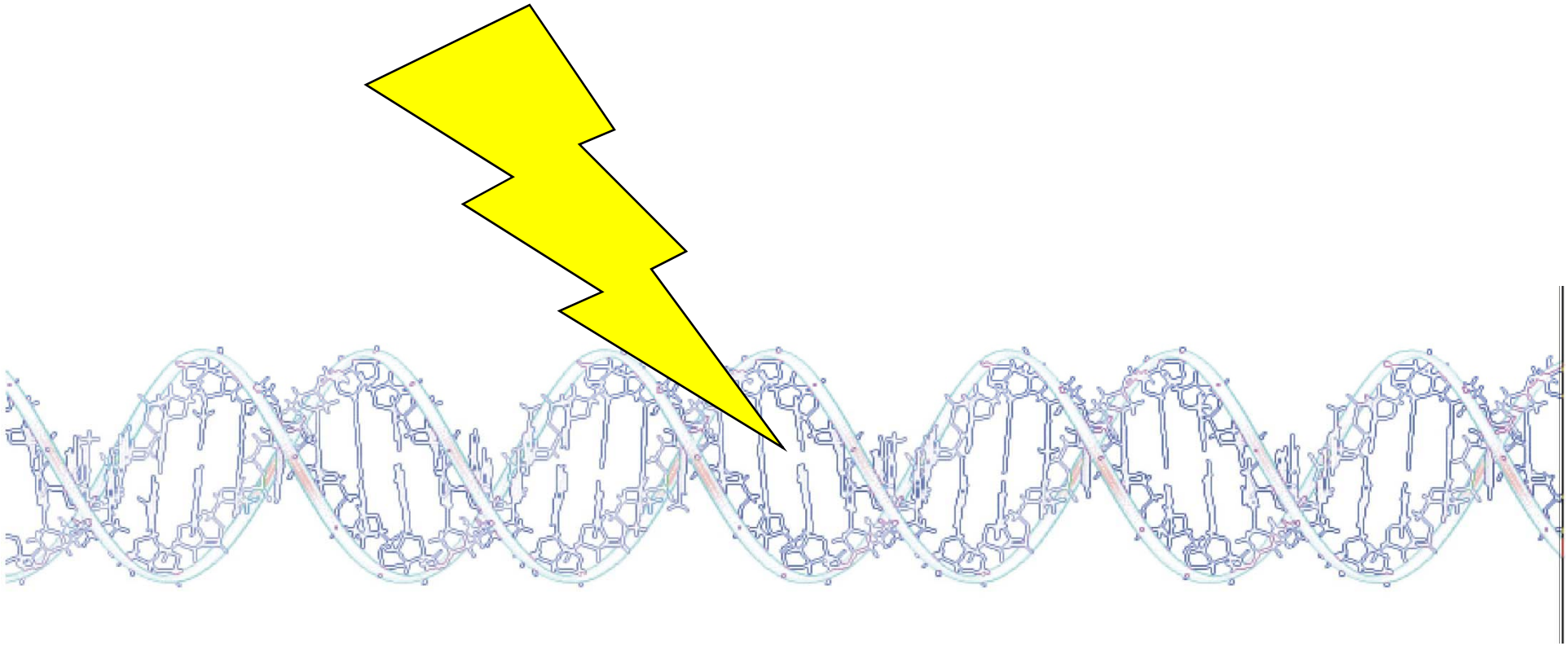


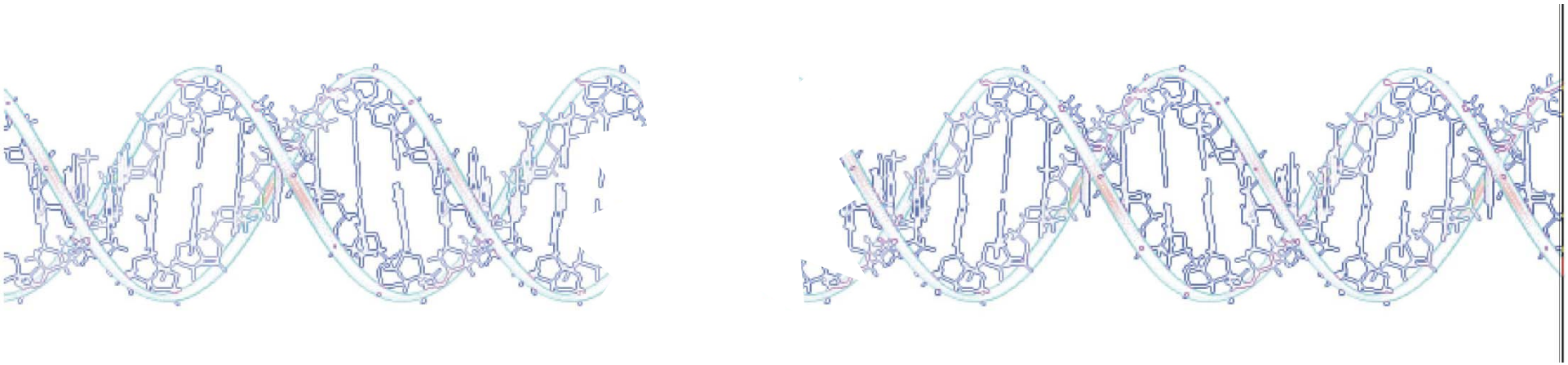



http://homepages.uel.ac.uk/V.K.Sieber/human.htm


Chromosome
Chromatid
Chromatid
Telomere
Centromere
Telomere
Nucleus
Cell
Histones
Base Pairs
DNA(double helix)

# Why should you care about Homologous Recombination?





# Gene Conversion
# SDSA
# Animation

# Homologous Recombination Protects Cells from the Lethal Effects of DNA Damage

# Your Experiment:

**Create a plasmid that will be part of a homologous recombination assay.**

**Measure the frequency of cells in which homologous recombination between two plasmids gives rise to a fluorescent cell.**

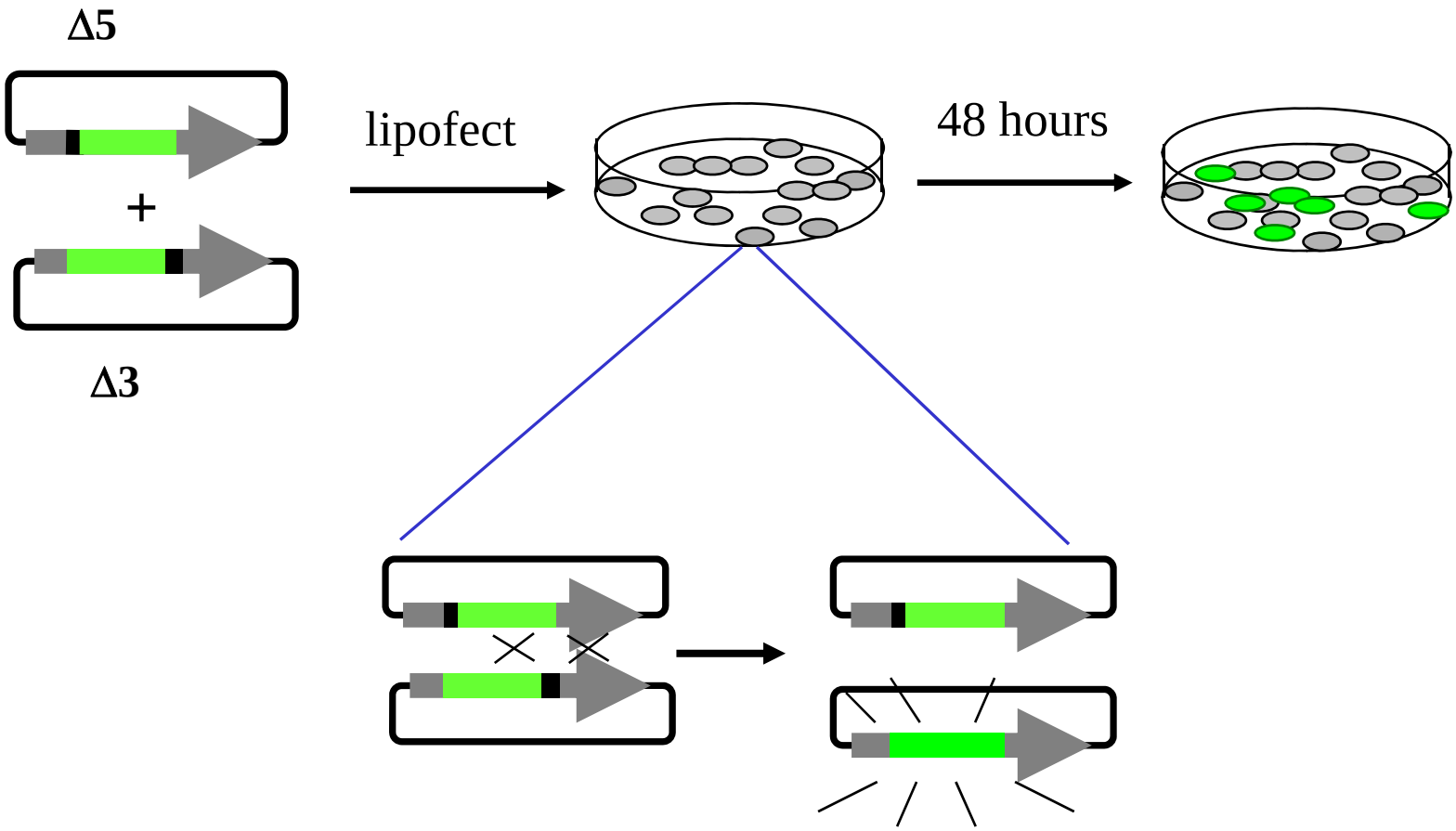
**Test conditions that might affect the frequency of green cells!**

**What is a gene?**

**What is an expression cassette?**

**What is a plasmid?**

# A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



# Roadmap: Blueprint of Plasmid Construction Plan

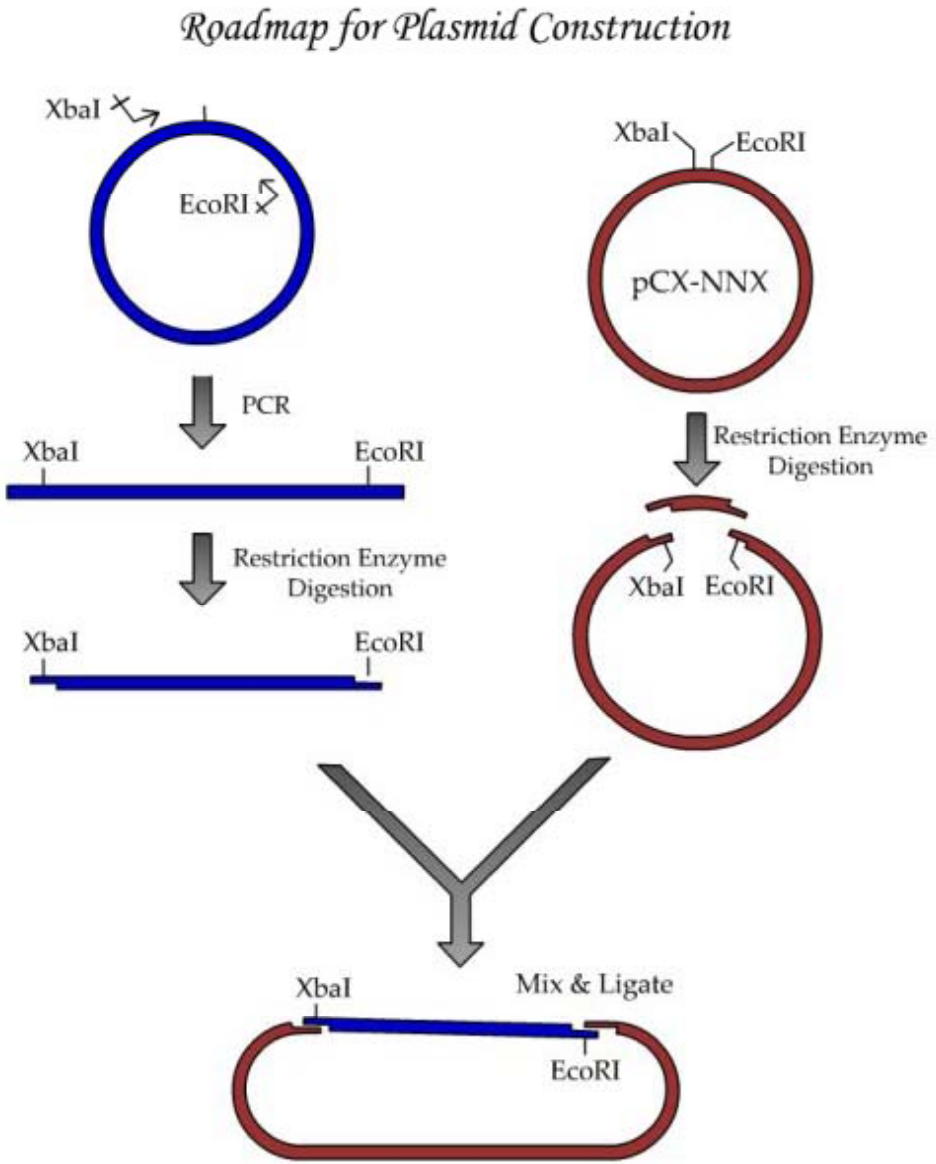
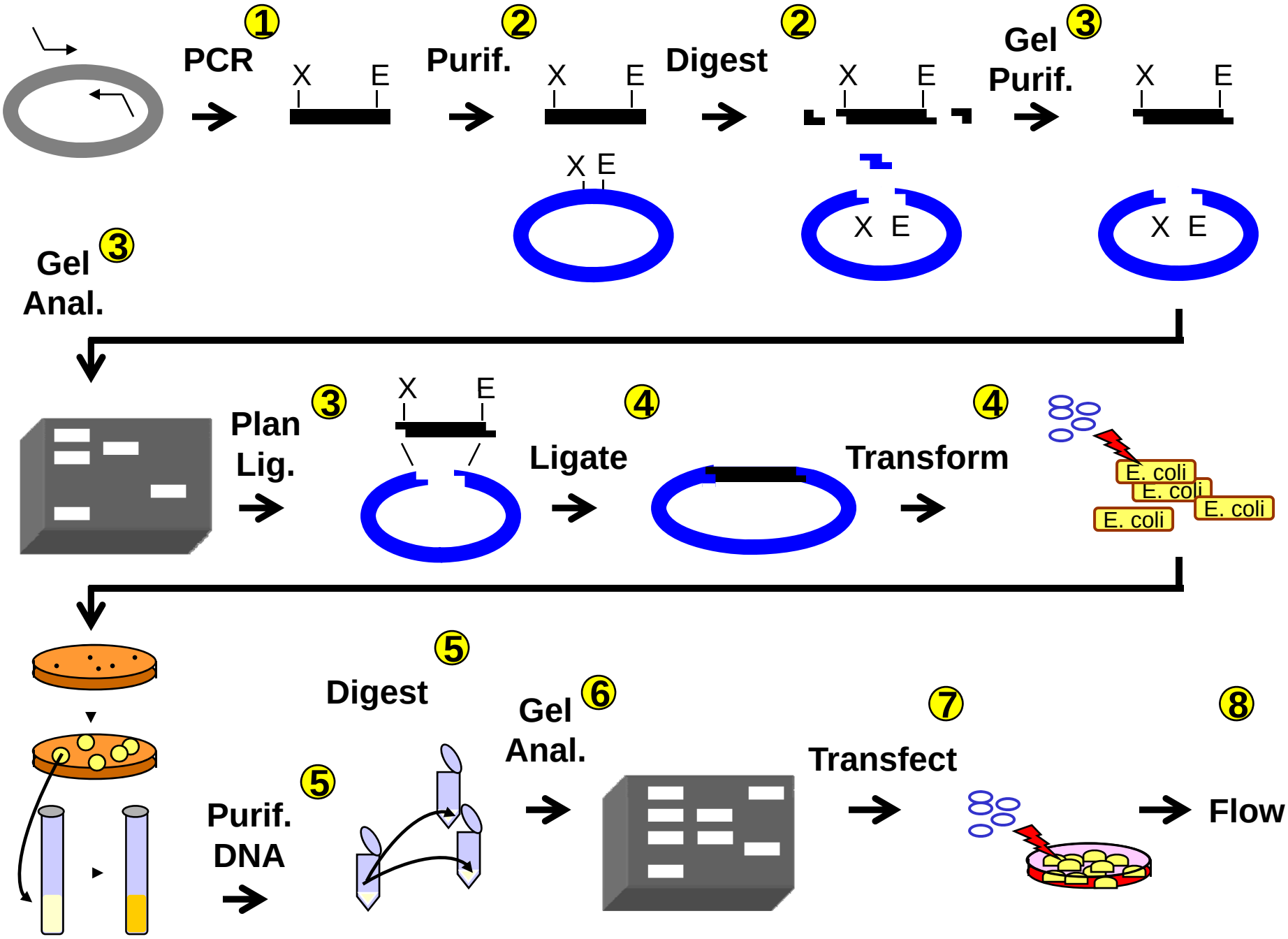
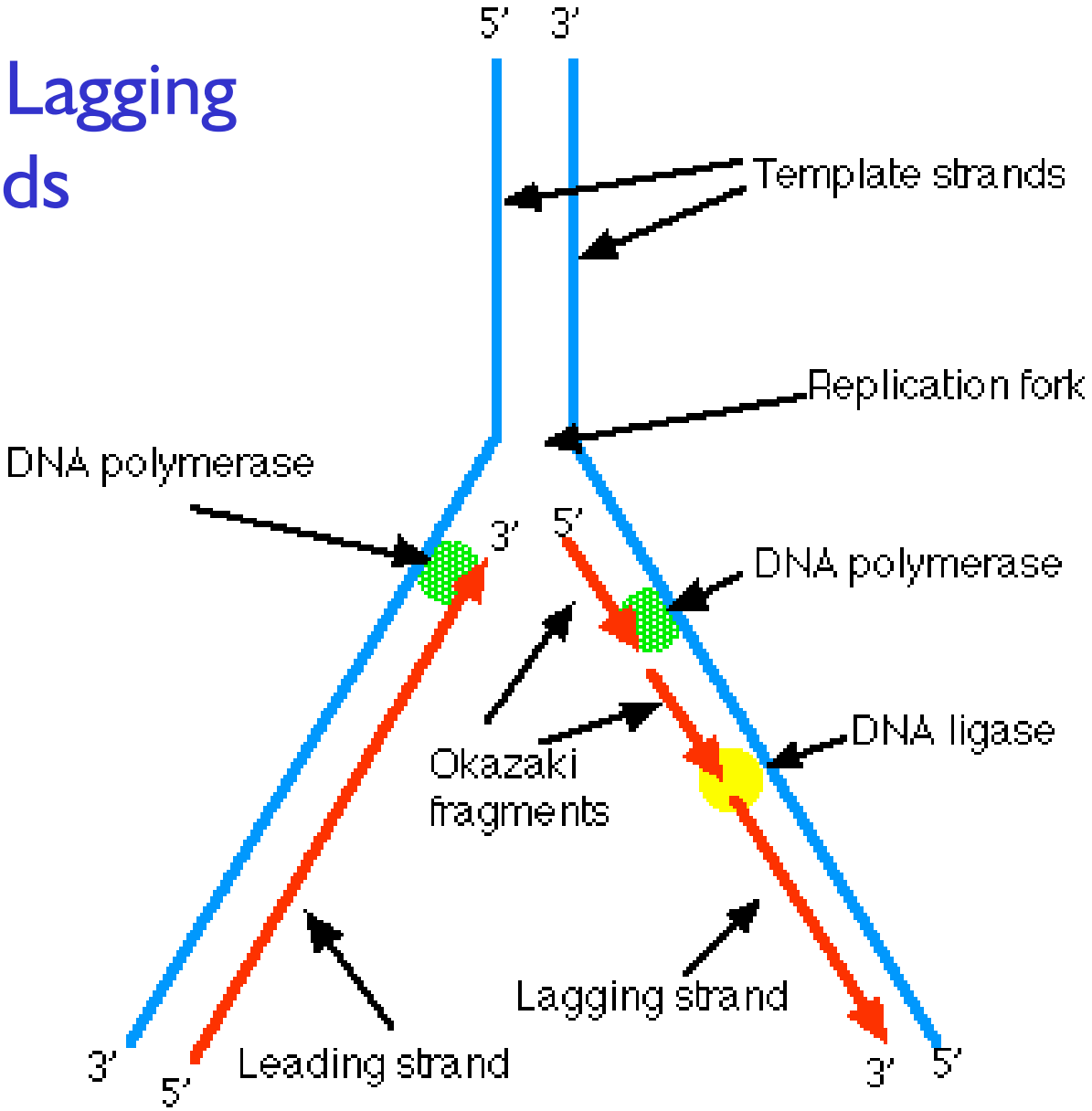



Figure by Justin Lo

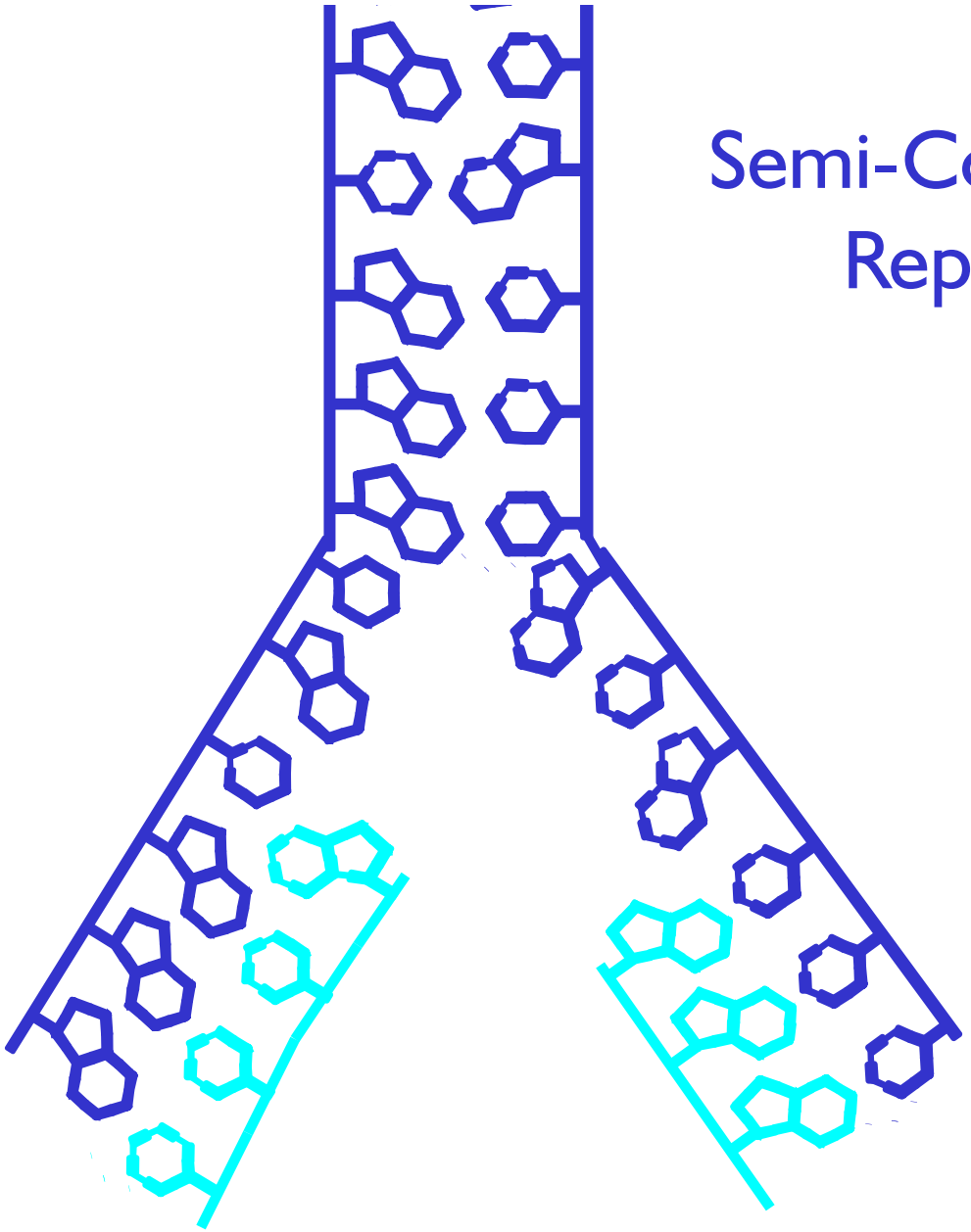

1
PCR
X
E
2
Purif.
X
E
2
Digest
X
E
3
Gel
Purif.
X
E
X E
X E
X E
3
Gel
Anal.
3
Plan
Lig.
X
E
4
Ligate
4
Transform
E. coli
E. coli
E. coli
E. coli
5
Digest
5
Purif.
DNA
6
Gel
Anal.
7
Transfect
8
Flow


PCR!

# Leading & Lagging Strands

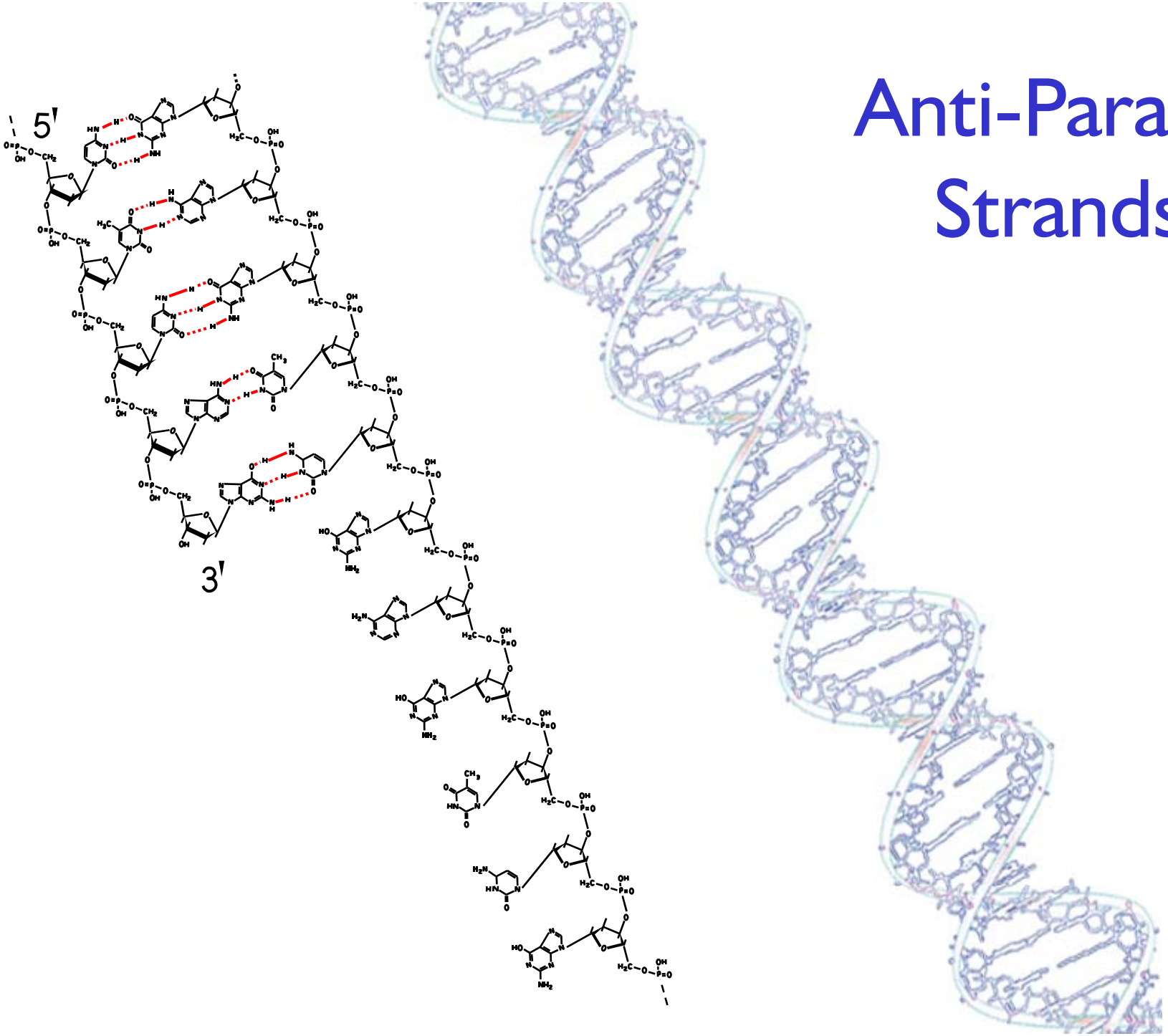



http://www.uic.edu/classes/bios/bios100/lecturesf04am/lect13.htm



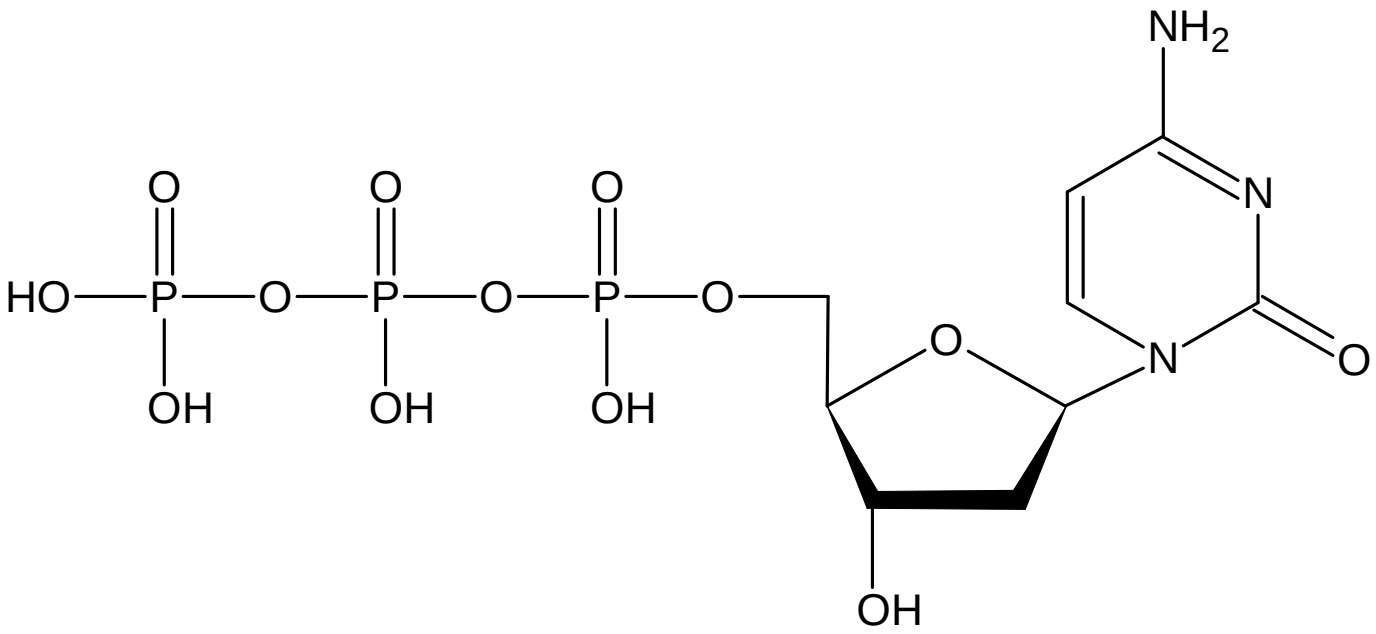
# Semi-Conservative Replication

# Anti-Parallel Strands



How do you design primers for PCR?

First, we need to review DNA replication...

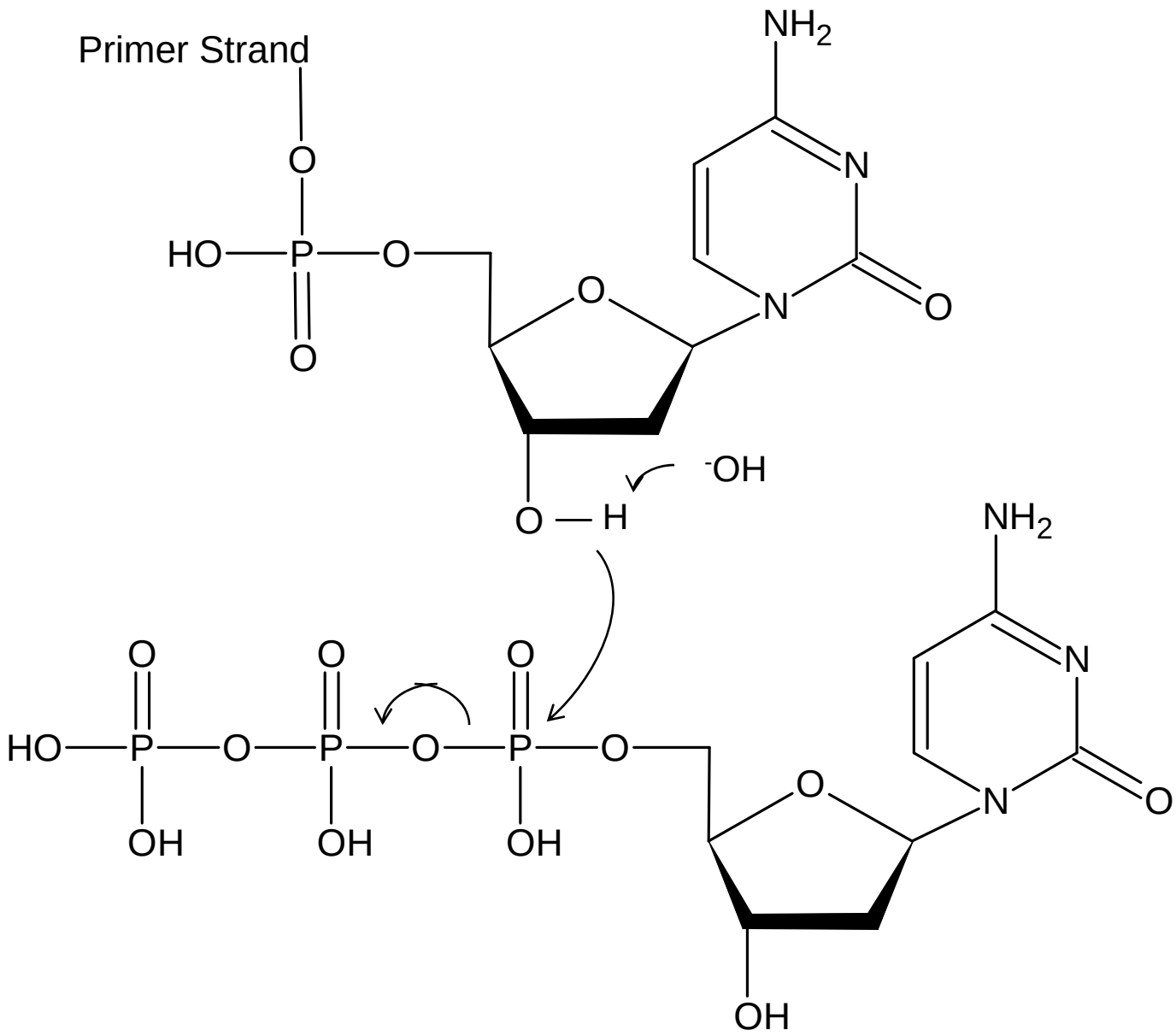



dCTP = 2'-deoxycytidine 5'-triphosphate

dNTPs = ?

(the others being deoxyadenosine, deoxyguanosine, and deoxythymidine)

**Note: We generally refer to the bases when speaking about duplex DNA.. "Adenine, Guanine, Cytosine and Thymine"**


Primer Strand
NH2
O
HO
P
O
O
O
N
N
O
O
-OH
O — H
NH2
O
O
O
HO
P
O
P
O
P
O
N
N
O
O
OH
OH
OH
OH

# What are the components of a PCR reaction?

Polymerase

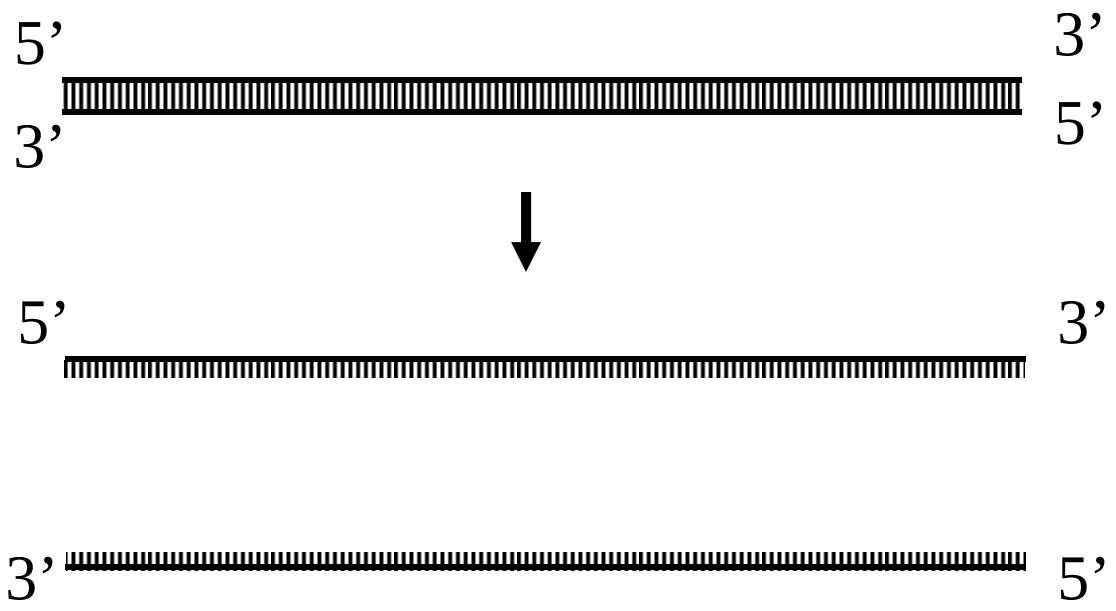
Template

Primer

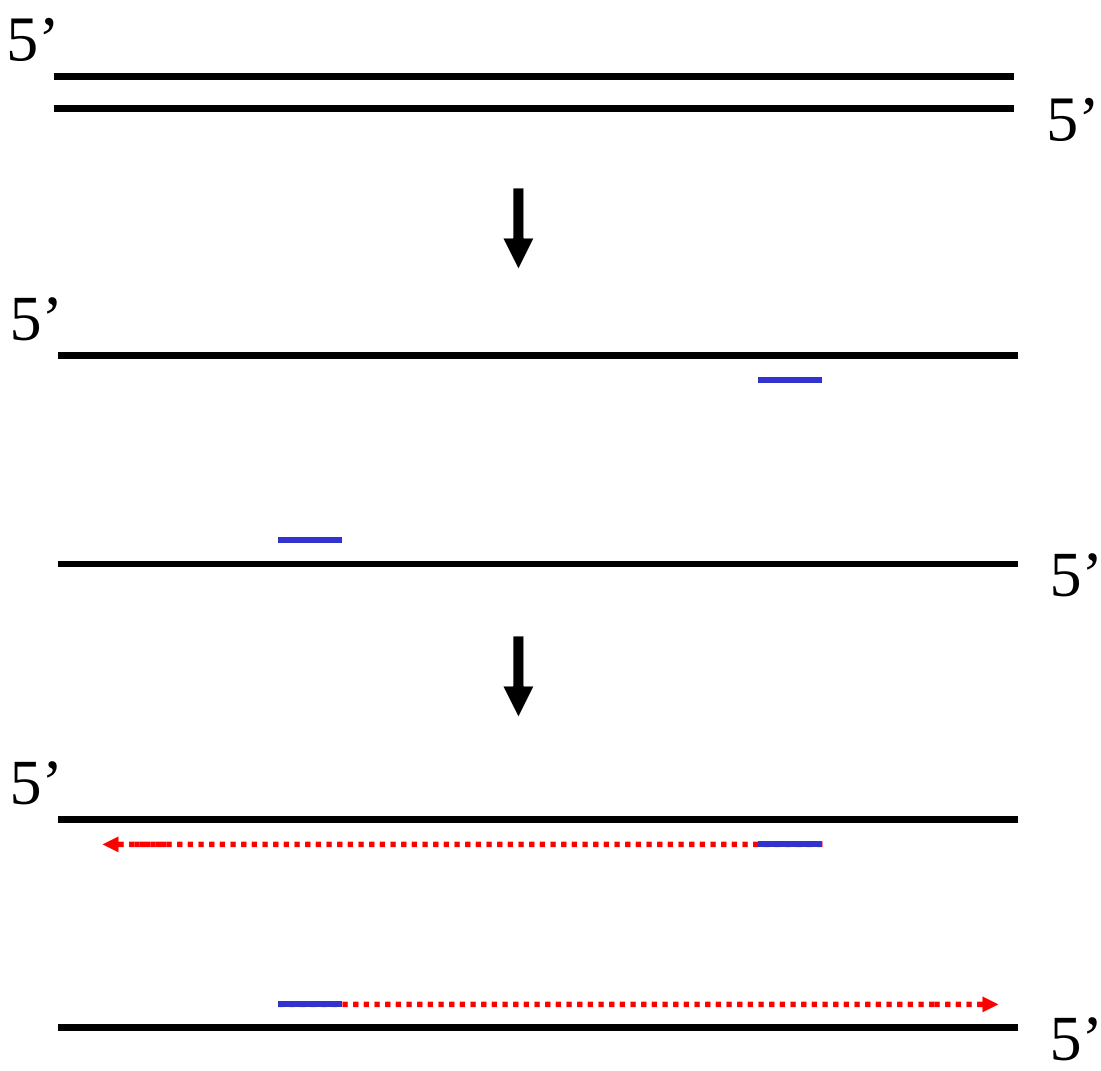
dNTPs

Mg++

# PCR



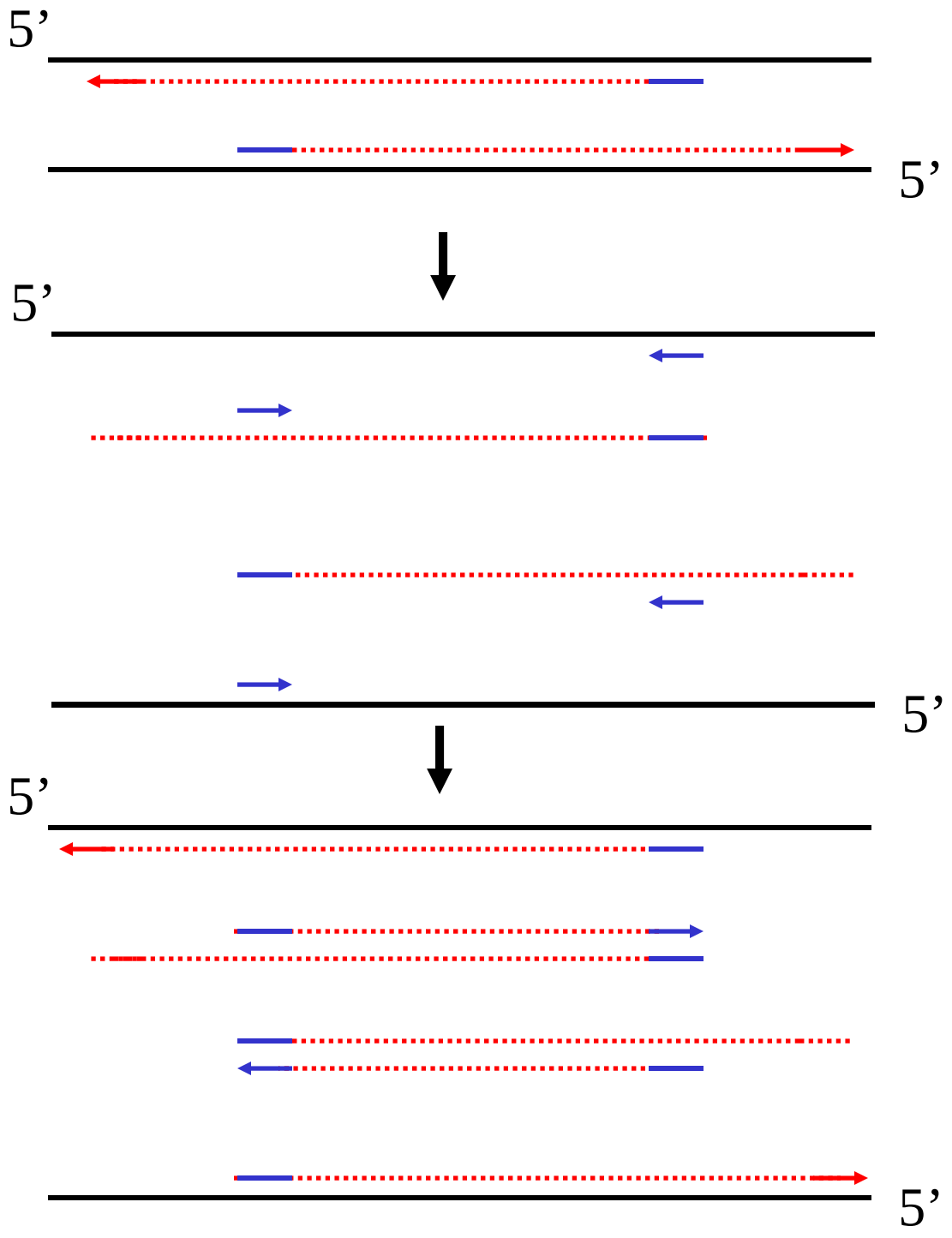
# PCR

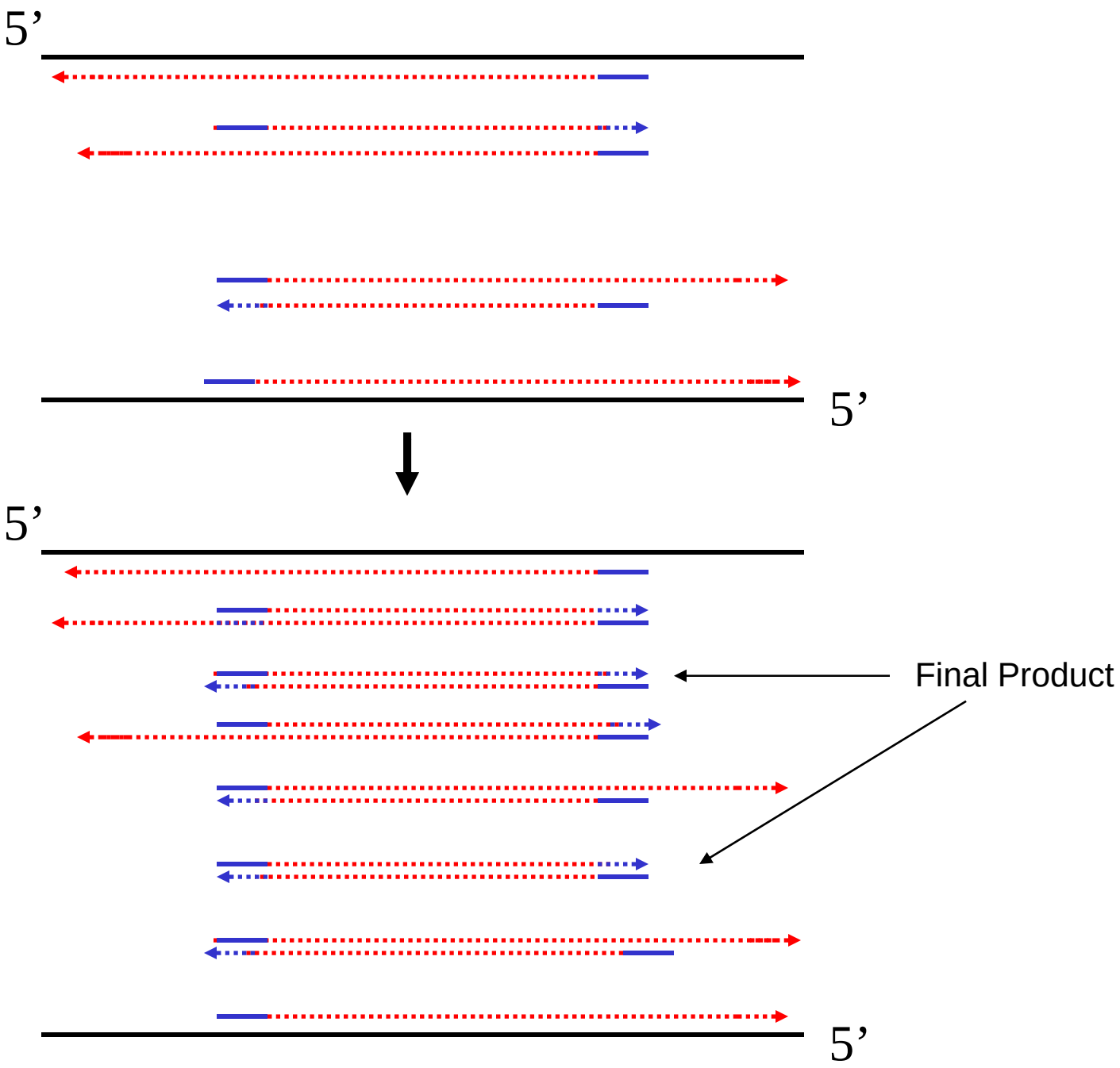


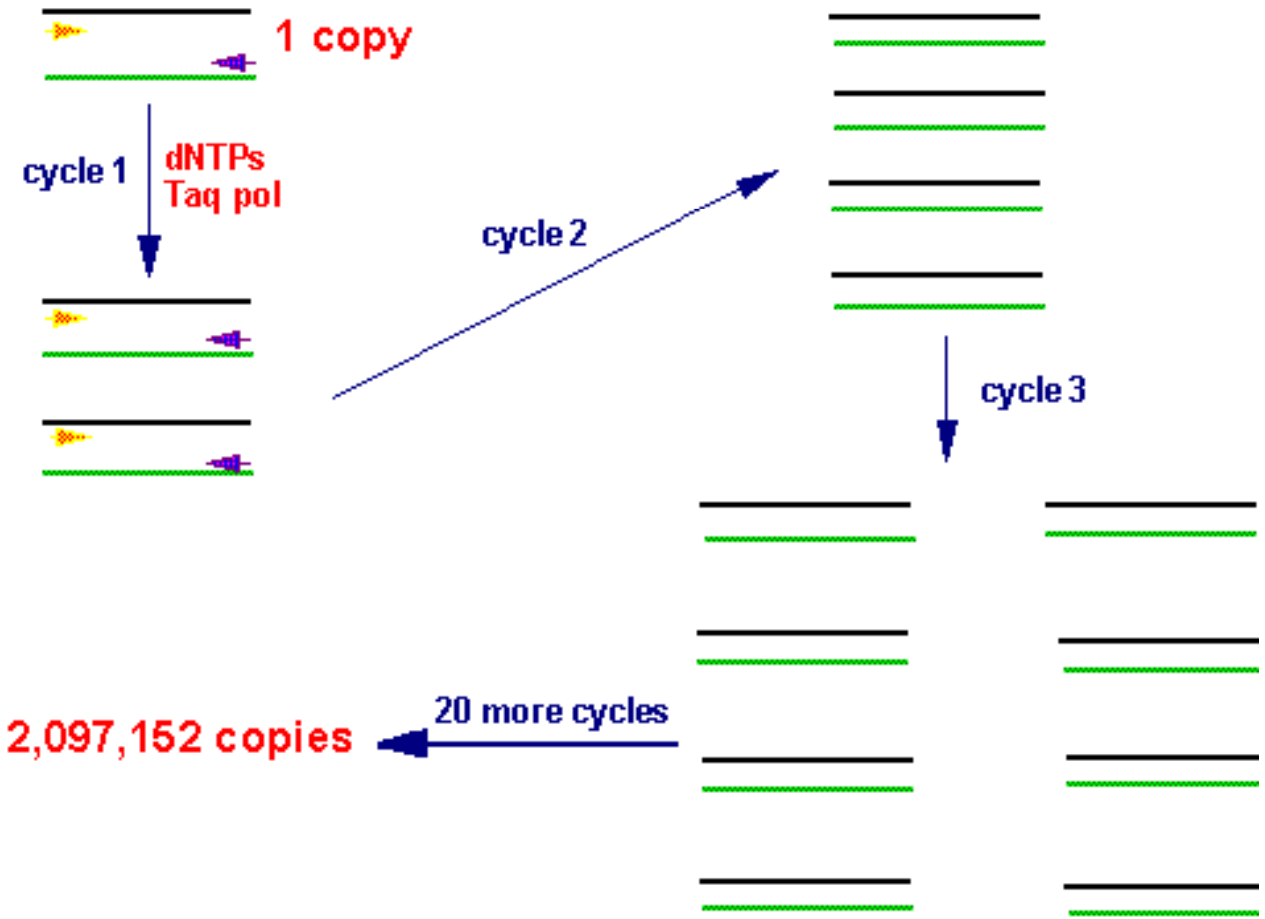

**MELT**
**94ºC**

**ANNEAL**
**55ºC**

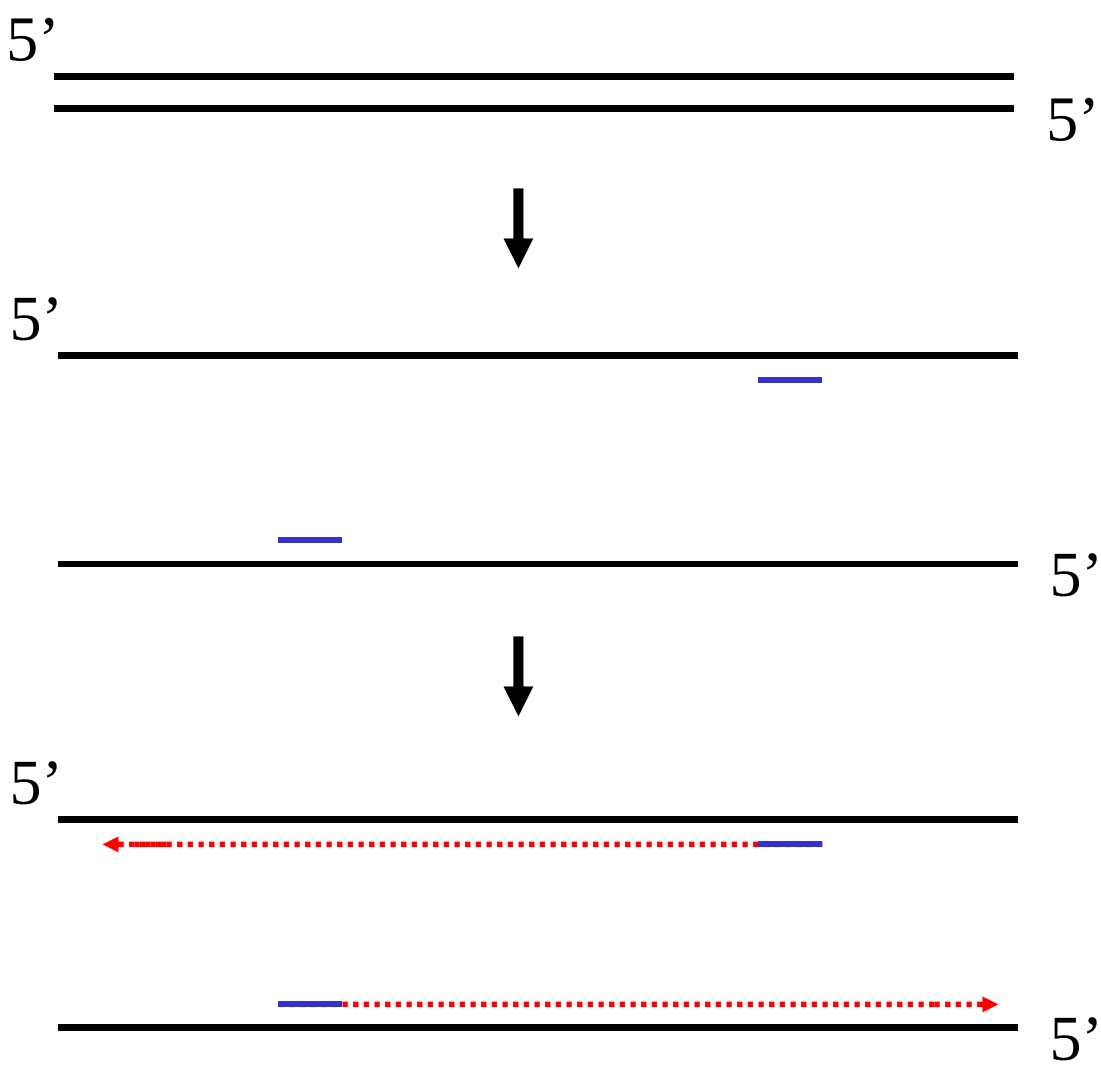
**EXTEND**
**72ºC**


5’
5’
5’
5’
5’
5’
5’


5’
5’
5’
Final Product
5’



# PCR

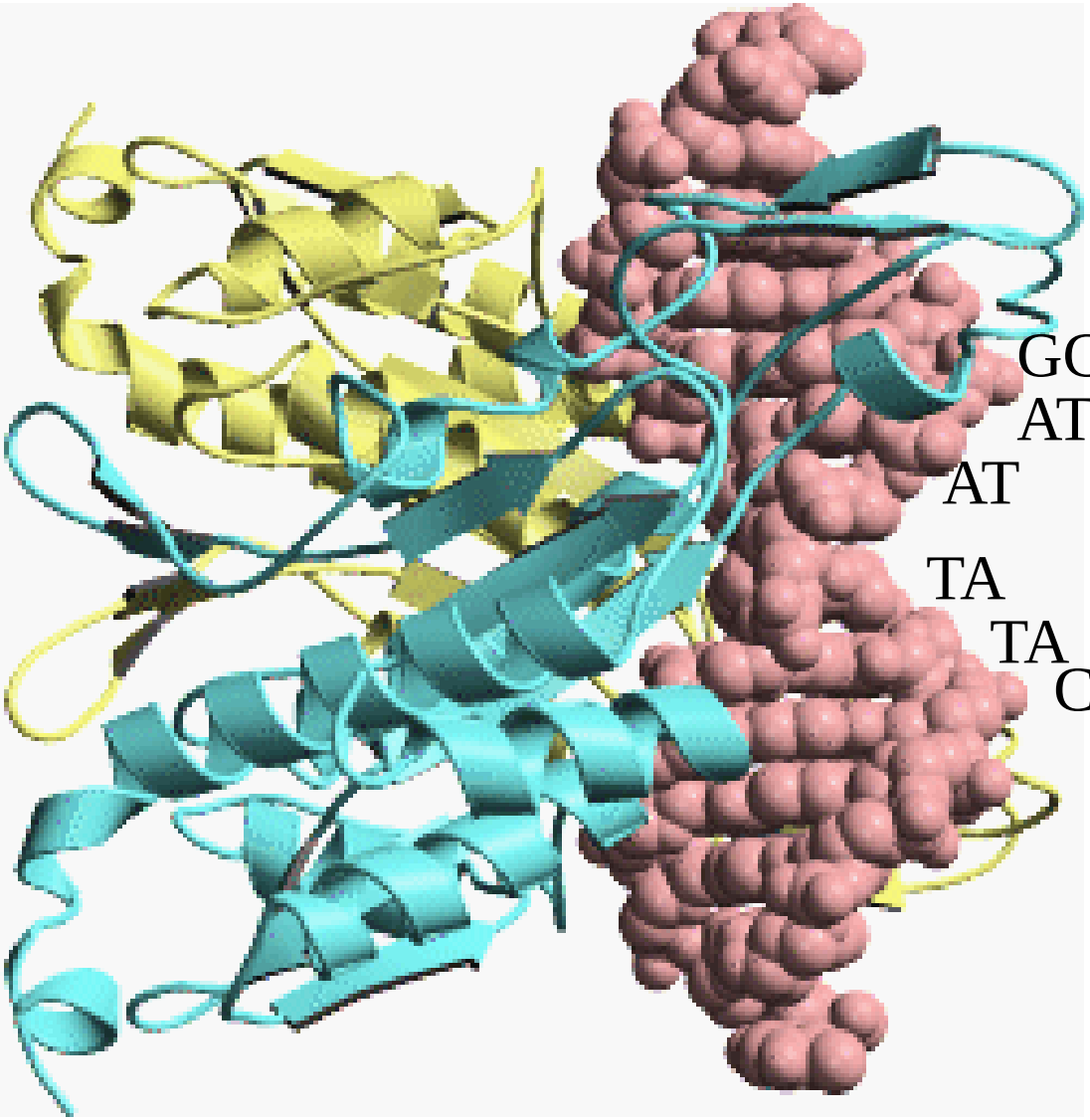



**MELT**
**94ºC**

**ANNEAL**
**55ºC**

**EXTEND**
**72ºC**

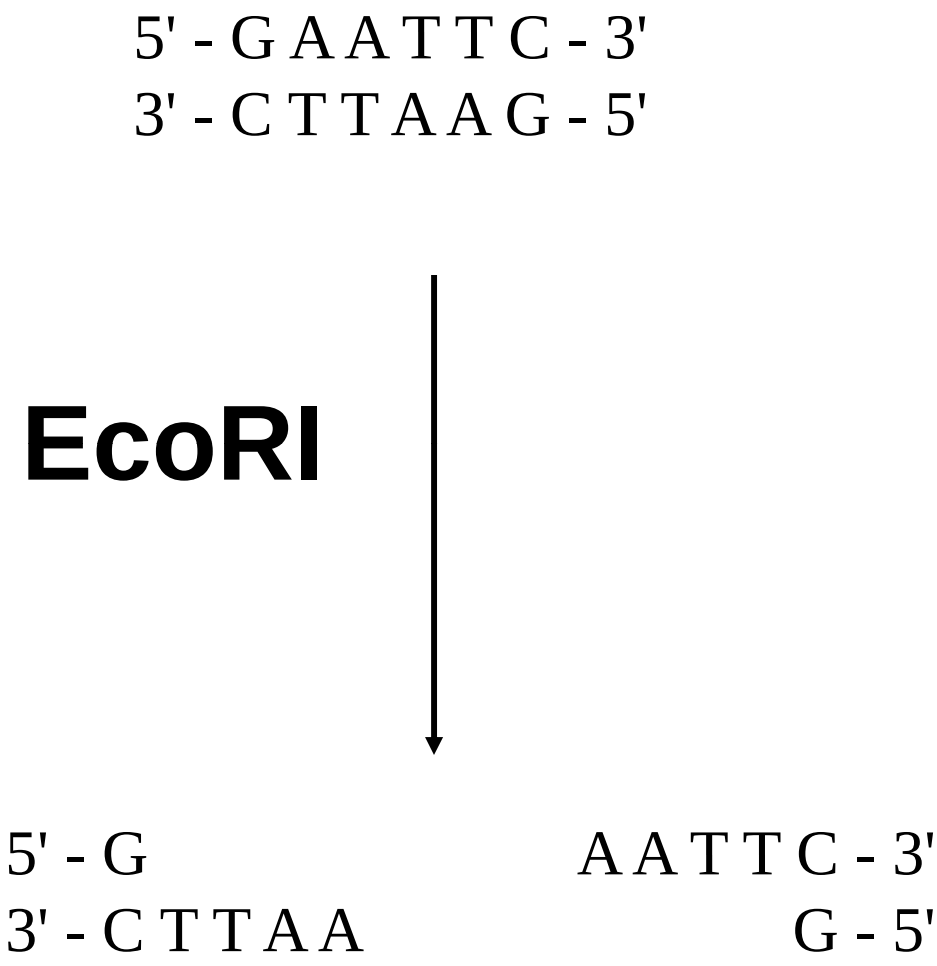
# Restriction Enzymes




5' - G A A T T C - 3'
3' - C T T A A G - 5'

**EcoRI**

Image from: Rosenberg, J. M. Curr. Opin. Struct. Biol. 1: 104-110 (1991)


5' - G A A T T C - 3'
3' - C T T A A G - 5'
EcoRI
5' - G
3' - C T T A A
A A T T C - 3'
G - 5'

“Old cloners never die, they just come to a sticky end.”

Why are sticky ends useful?

# Roadmap: Blueprint of Plasmid Construction Plan

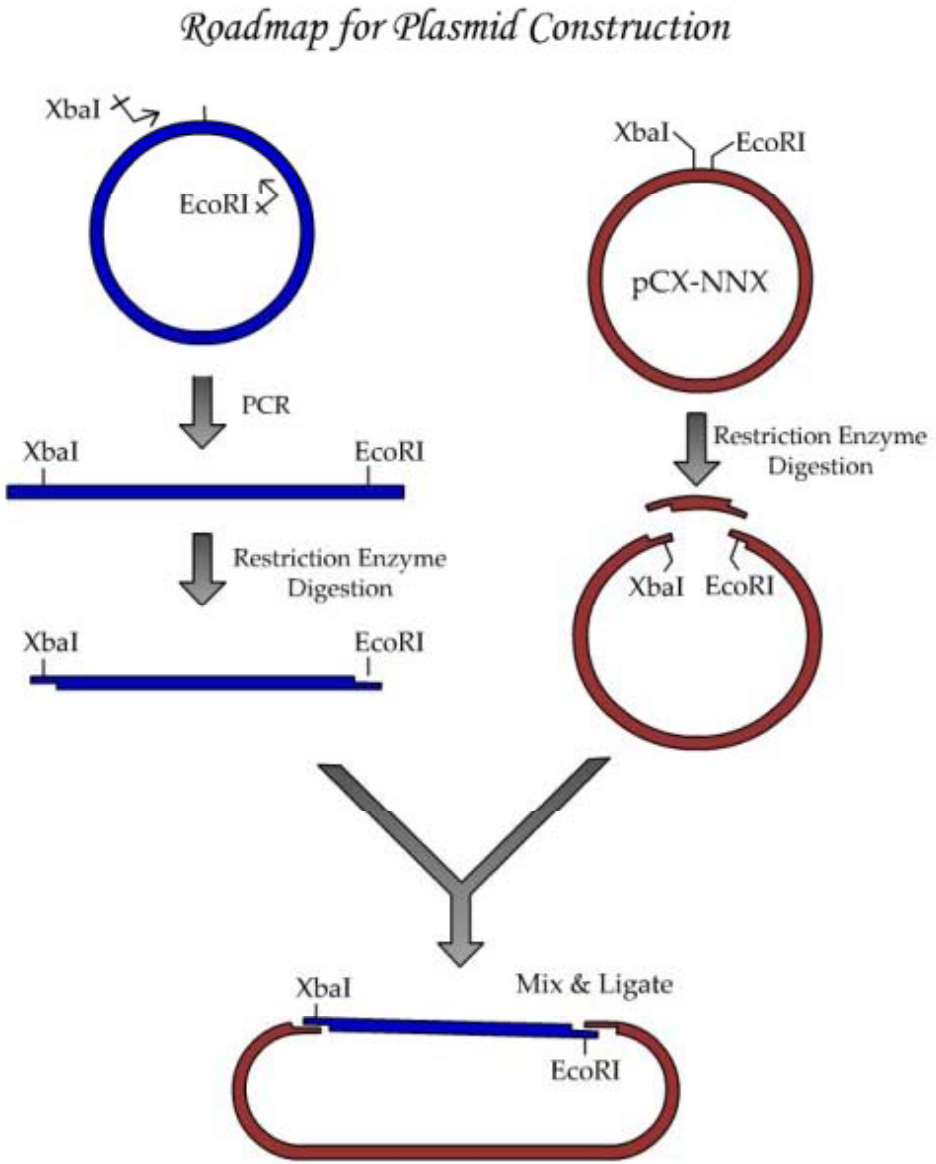
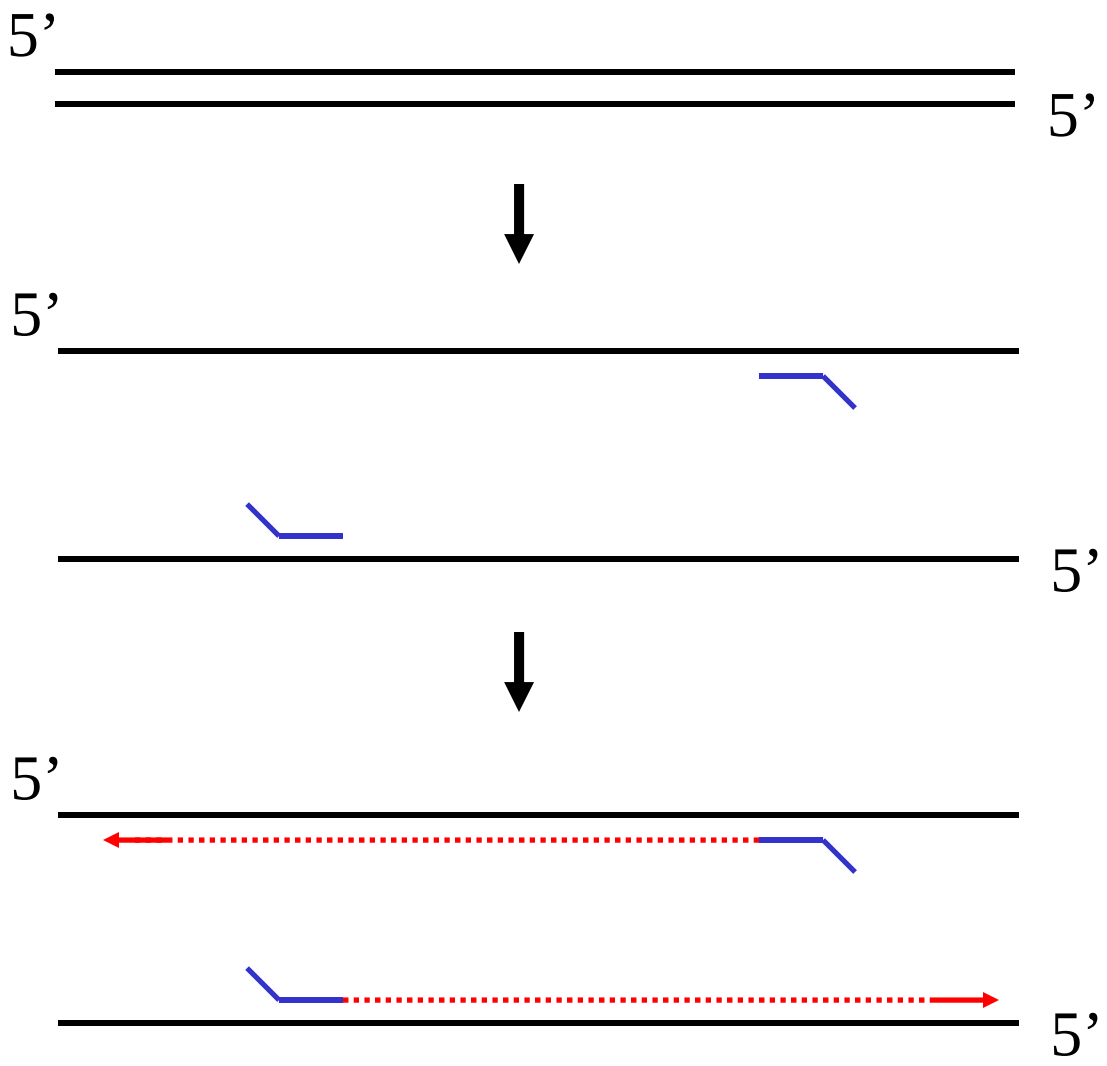


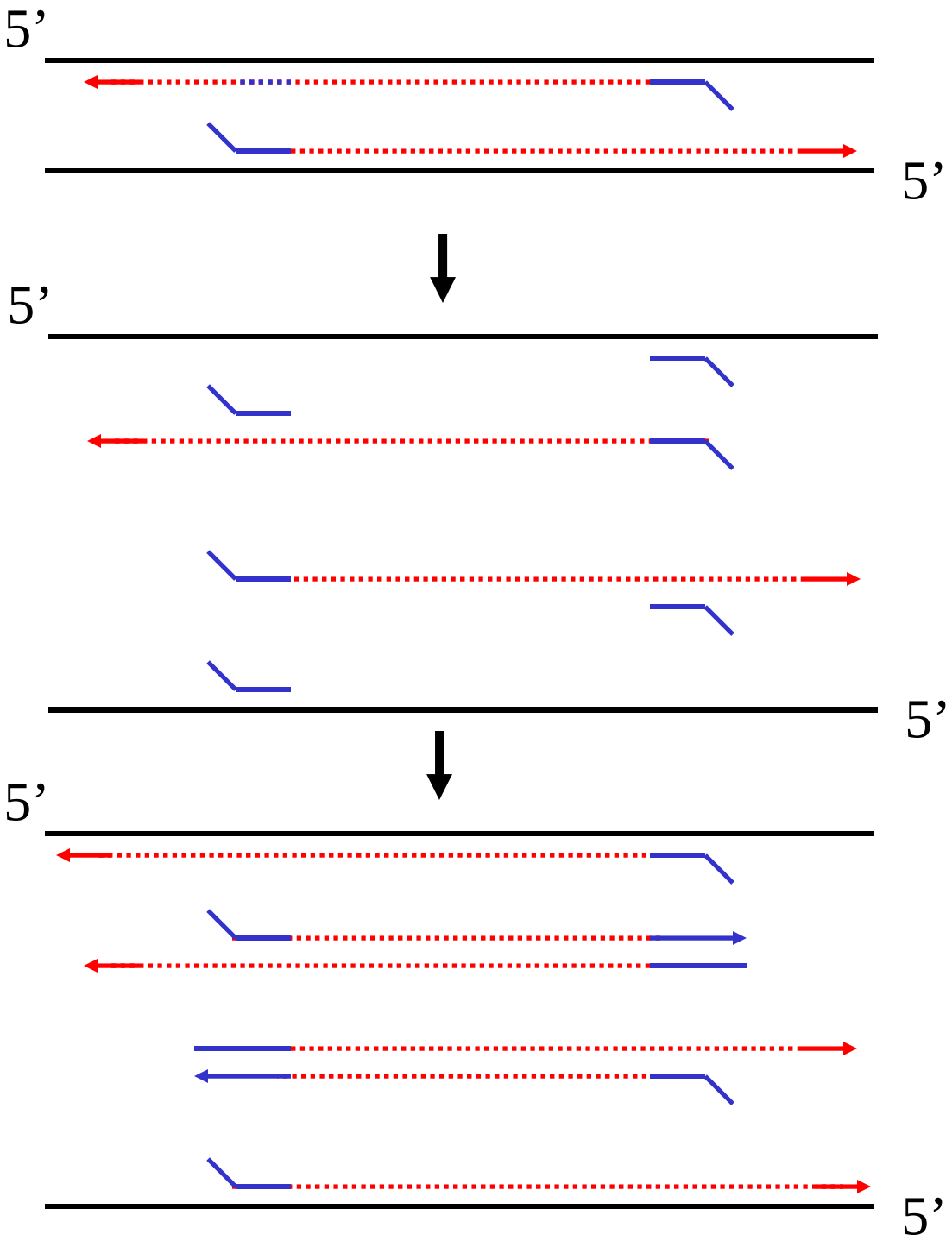

Figure by Justin Lo

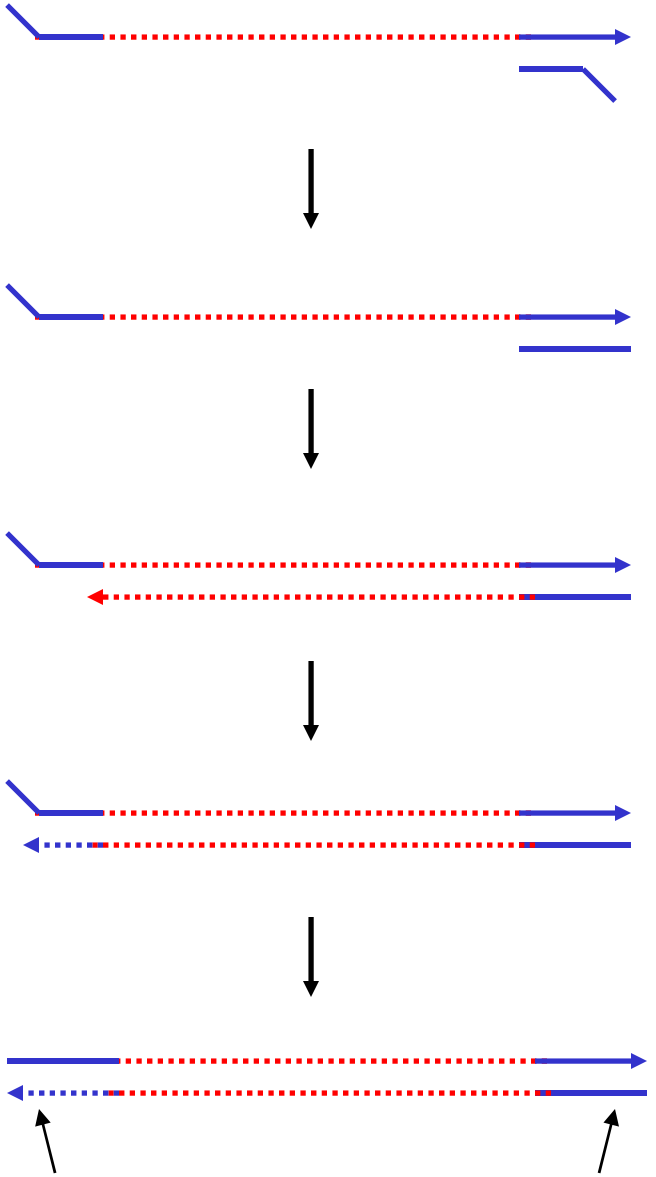
**PCR can be used to add sequences to the ends of a product.**

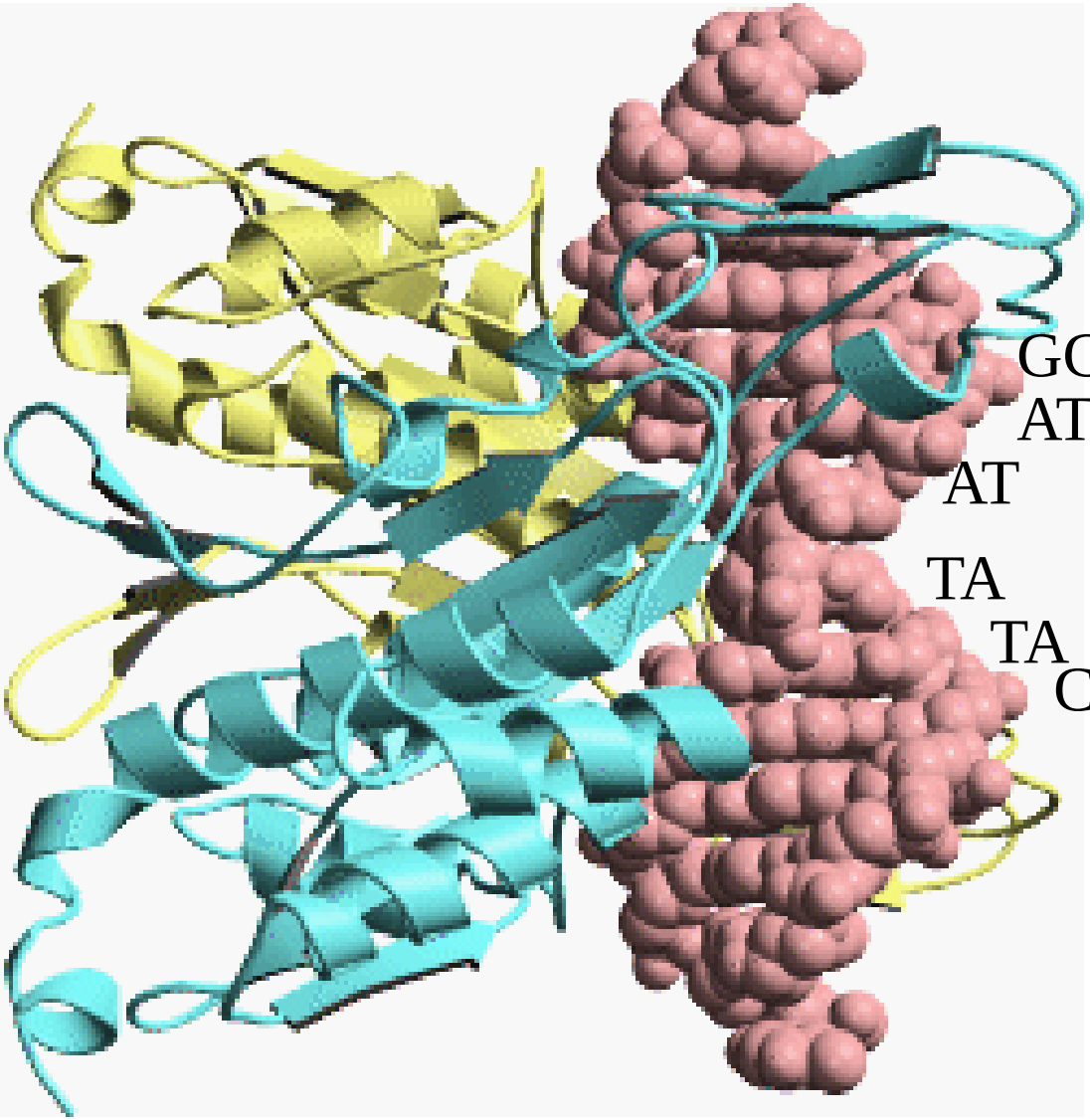
# PCR

Basic Principles:




5’
5’
5’
5’
5’
5’
5’


Restriction Enzyme Sites




5' - G A A T T C - 3'
3' - C T T A A G - 5'

# EcoRI

**Why do you need to add extra sequence to the 5' end of your primer?**

Image from: Rosenberg, J. M. Curr. Opin. Struct. Biol. 1: 104-110 (1991)

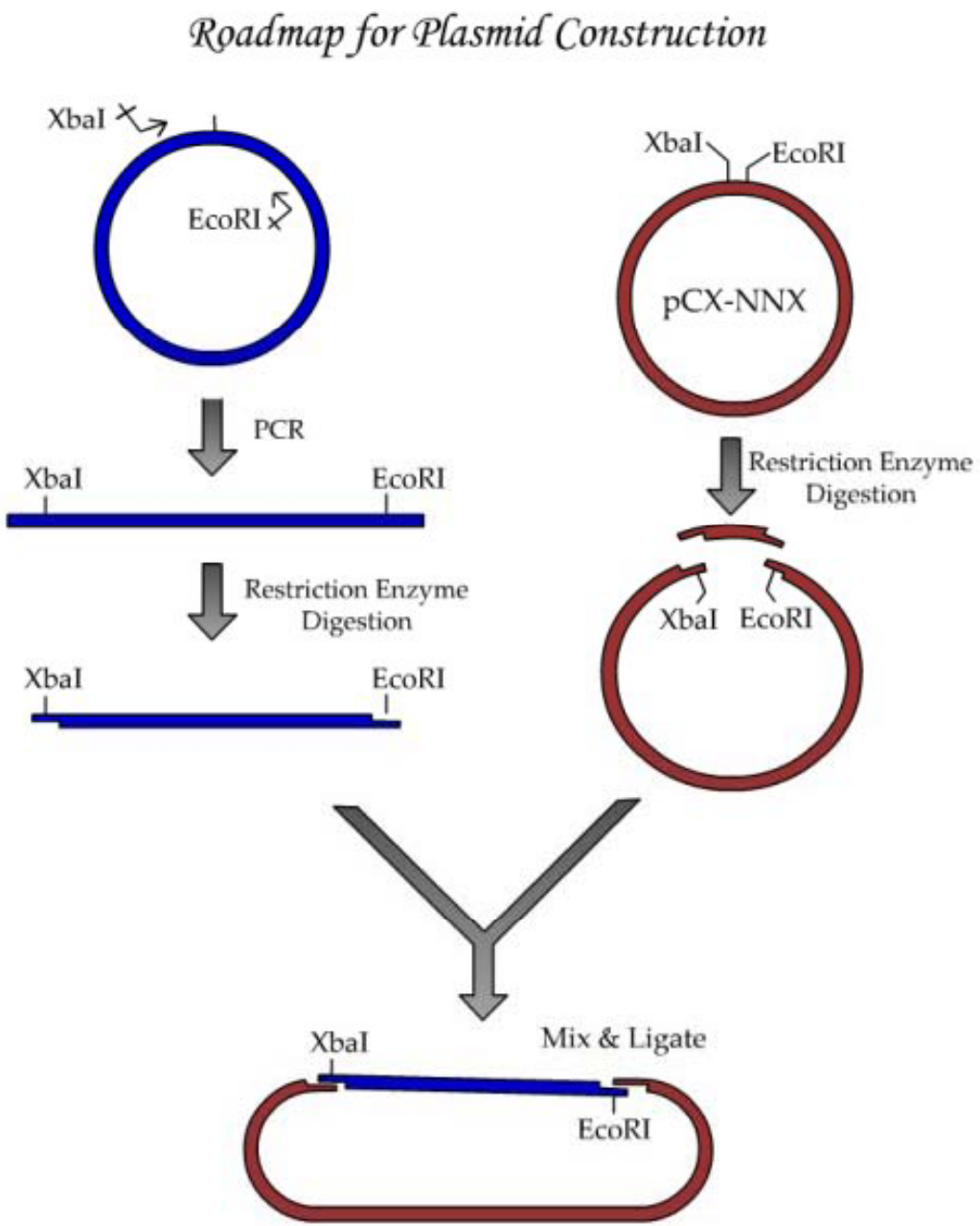
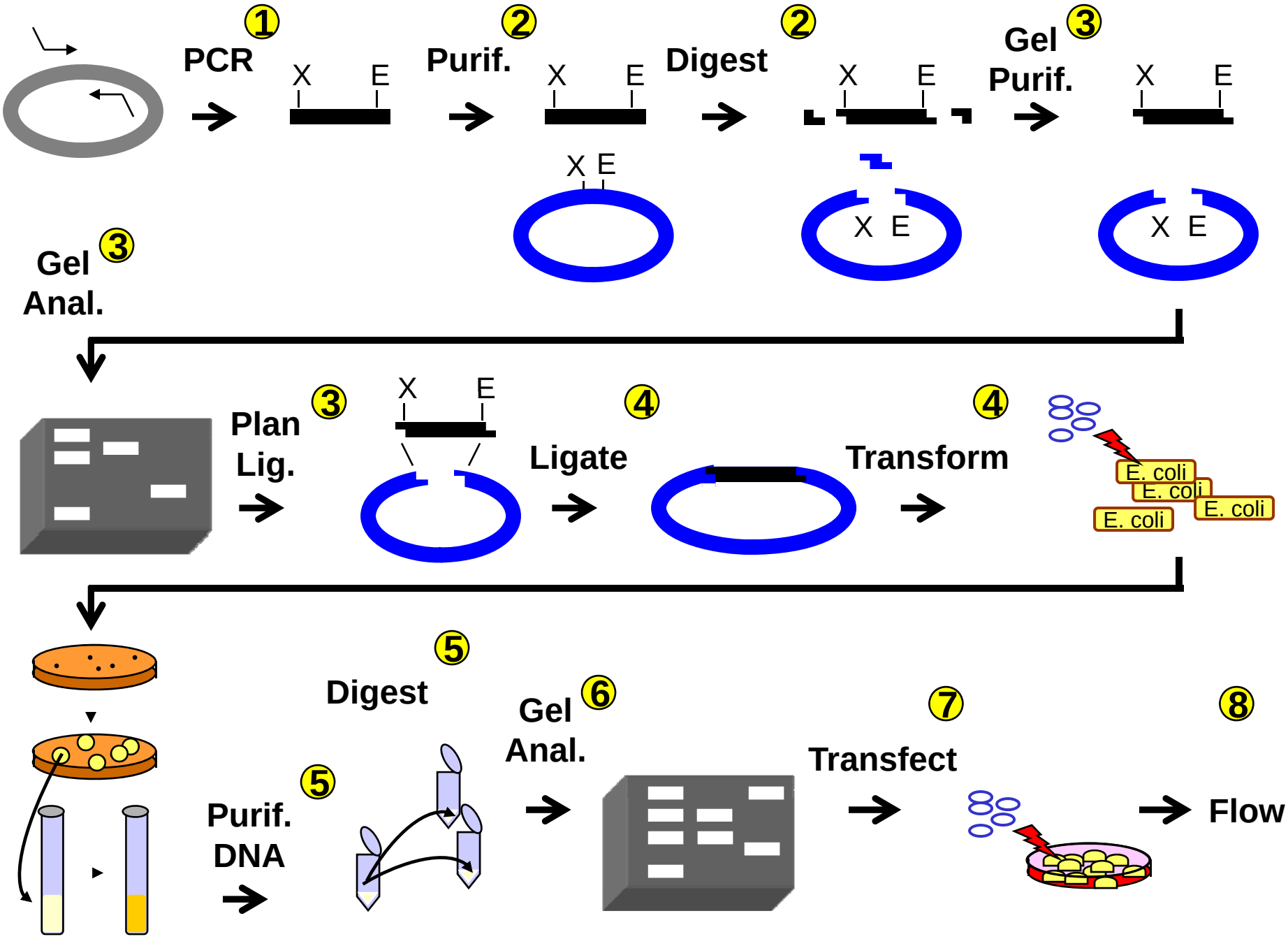



Figure by Justin Lo


1
PCR
X
E
2
Purif.
X
E
2
Digest
X
E
3
Gel
Purif.
X
E
X E
X E
X E
3
Gel
Anal.
3
Plan
Lig.
X
E
4
Ligate
4
Transform
E. coli
E. coli
E. coli
E. coli
5
Digest
5
Purif.
DNA
6
Gel
Anal.
7
Transfect
8
Flow

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