

ME 411 / ME 511

# Biological Frameworks for Engineers

# Class Organization

- Hw 2 due today
- Hw 3 online, due in 1 week

ME 411 / ME 511

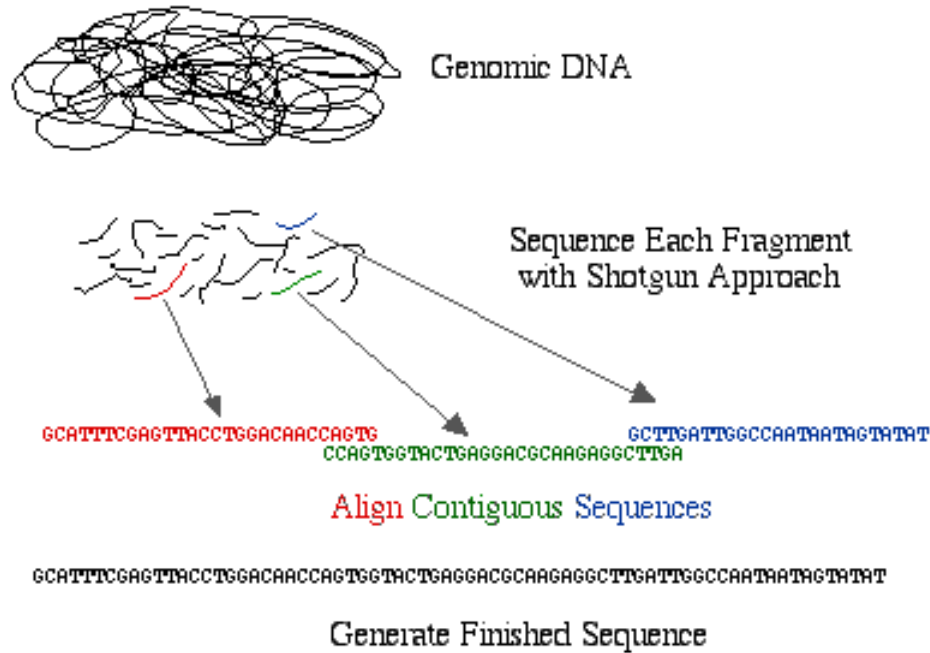
# Decoding DNA

# Genes

- Segment of DNA encoding mRNA
- Produce proteins, not lipids or carbohydrates
- Size: 100 to 1 million bp
- 98.5% of genomic DNA does not code for proteins

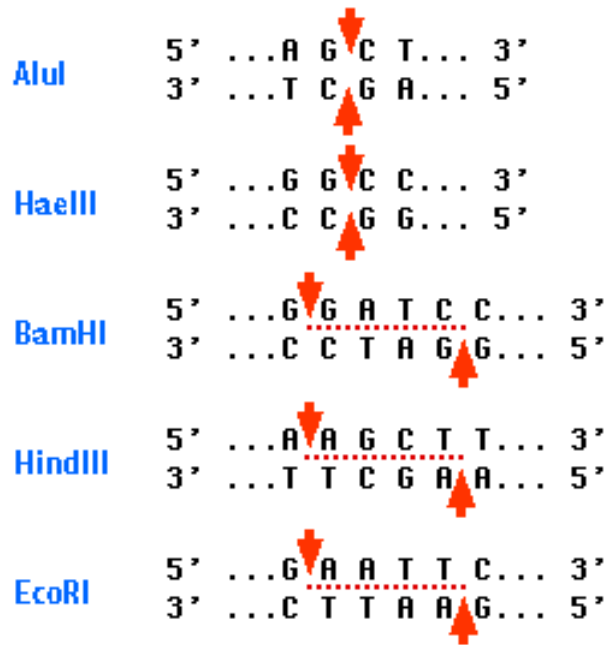
# Decoding the Genome

## Whole Genome Shotgun Sequencing Method



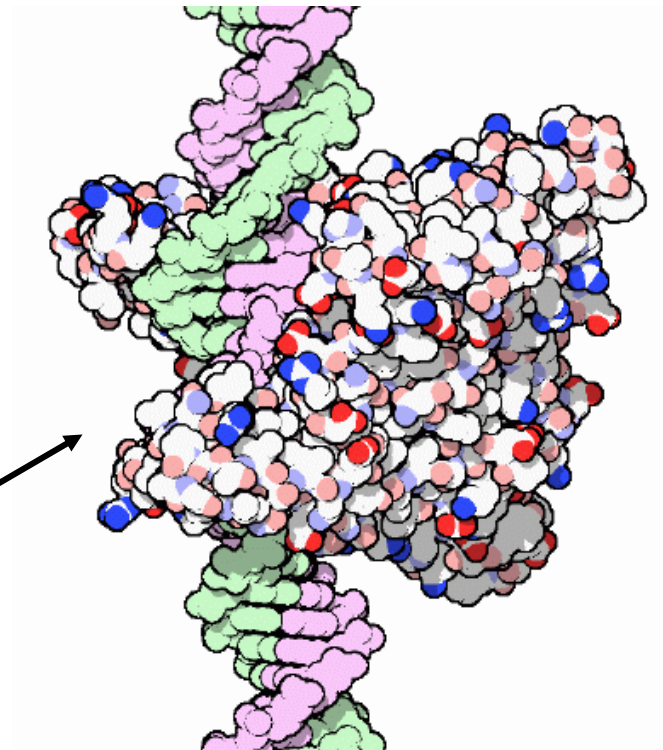
# Restriction Endonucleases

- Restriction Enzymes



**AluI** and **HaeIII** produce blunt ends

**BamHI** **HindIII** and **EcoRI** produce "sticky" ends



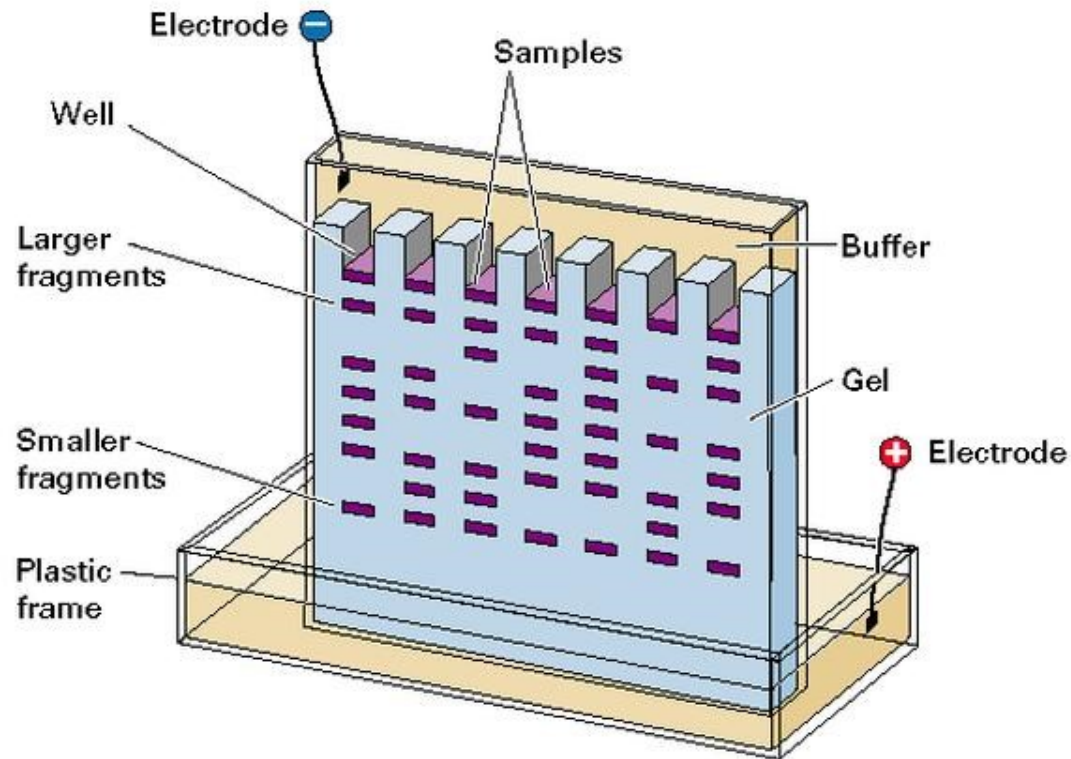
# Sanger Method

- Deoxynucleotides (dNTP)
  - dATP, dGTP, dCTP, dTTP
- Dideoxynucleotides (ddNTP)
  - H-group instead of OH-group (DNA chain terminators)
- DNA + primer + 4 dNTP + ddGTP + DNA polymerase:



# Electrophoresis

- Separation of molecules by size

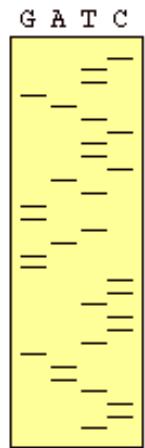
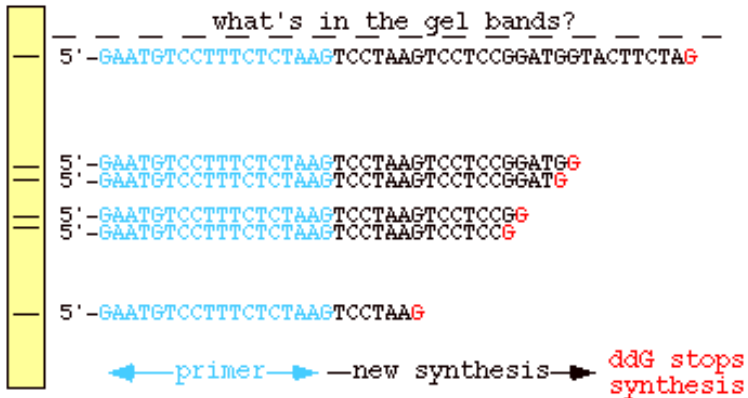




# Reading the Gel



Polyacrylamide gel electrophoresis of the "G" reaction



Line up all four reactions, and you can "read" the sequence ladder 5' to 3' as TCCTAAG...etc. in this example:



AGGATTC ...

↓

3' - GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG - 5'

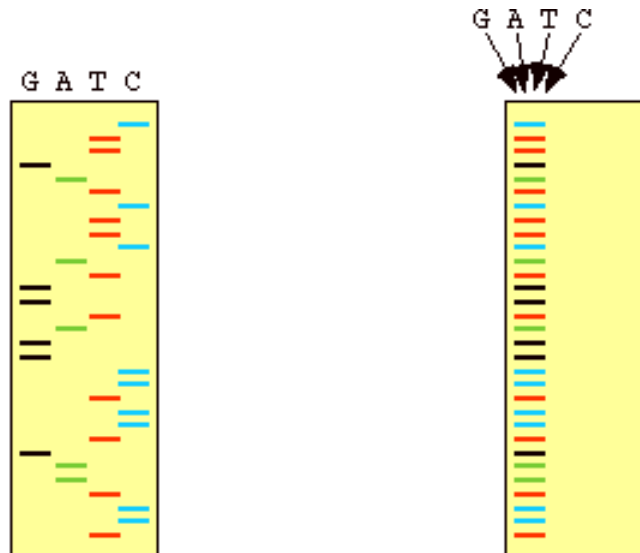
Primer sequence

# Automatic Sequencing

- Primer labeled with fluorescent dyes

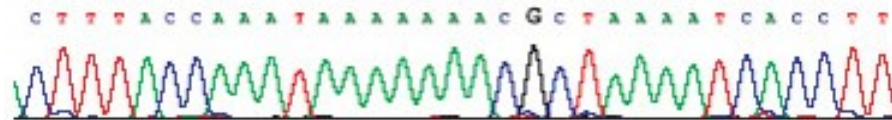


# Automatic Sequencing

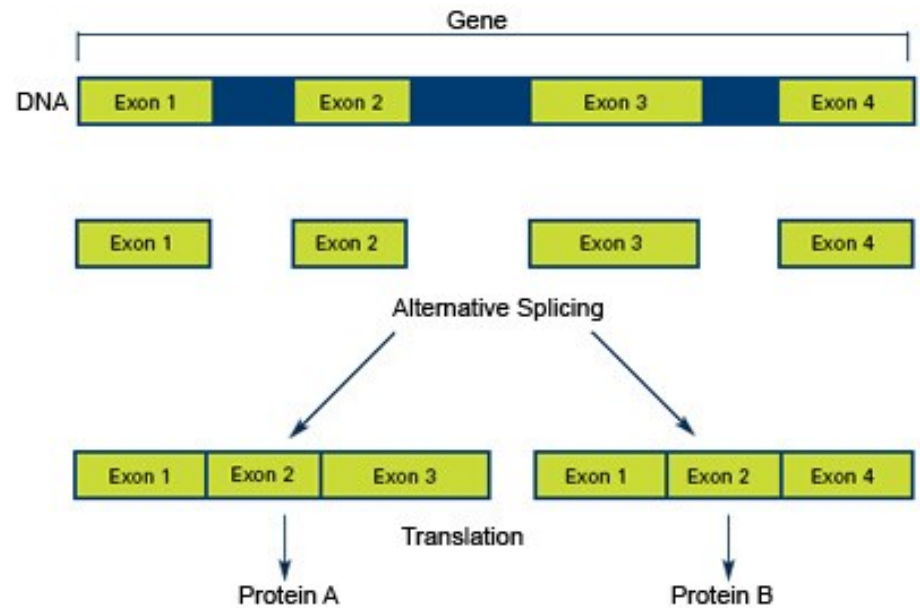
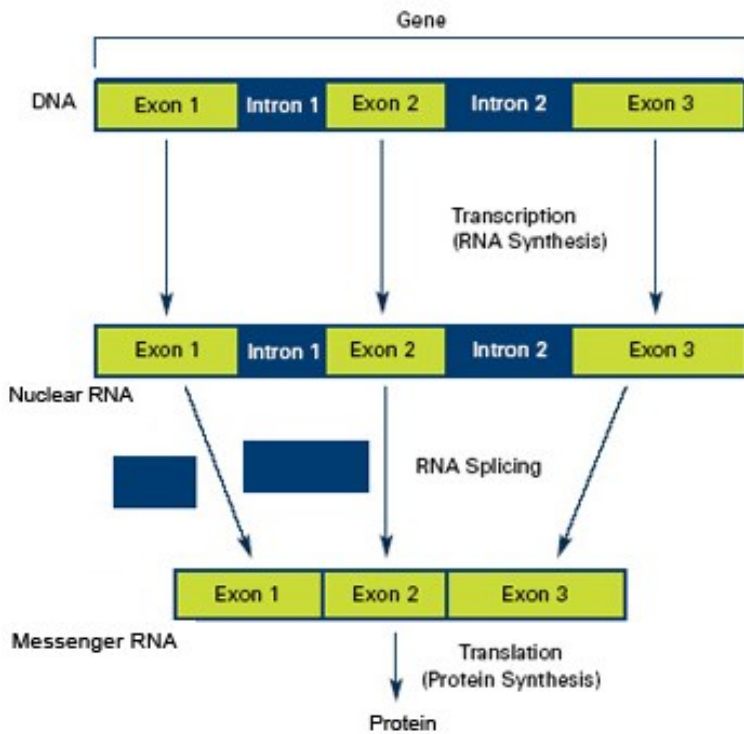


Here's what the products would look like in separate gel lanes.

Here's what the products would look like in a single gel lanes.

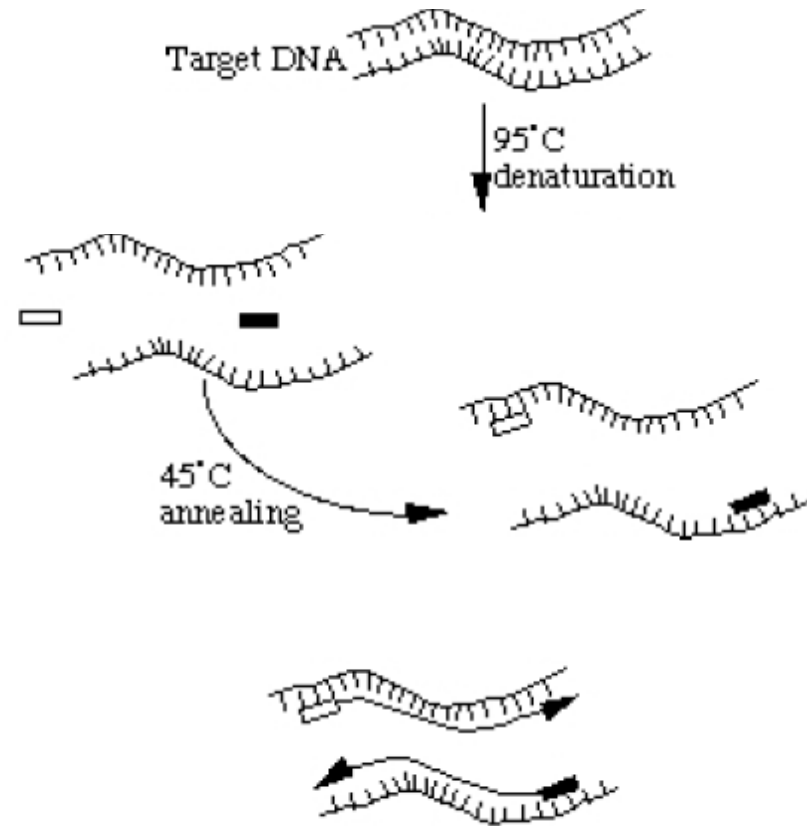


# Splicing

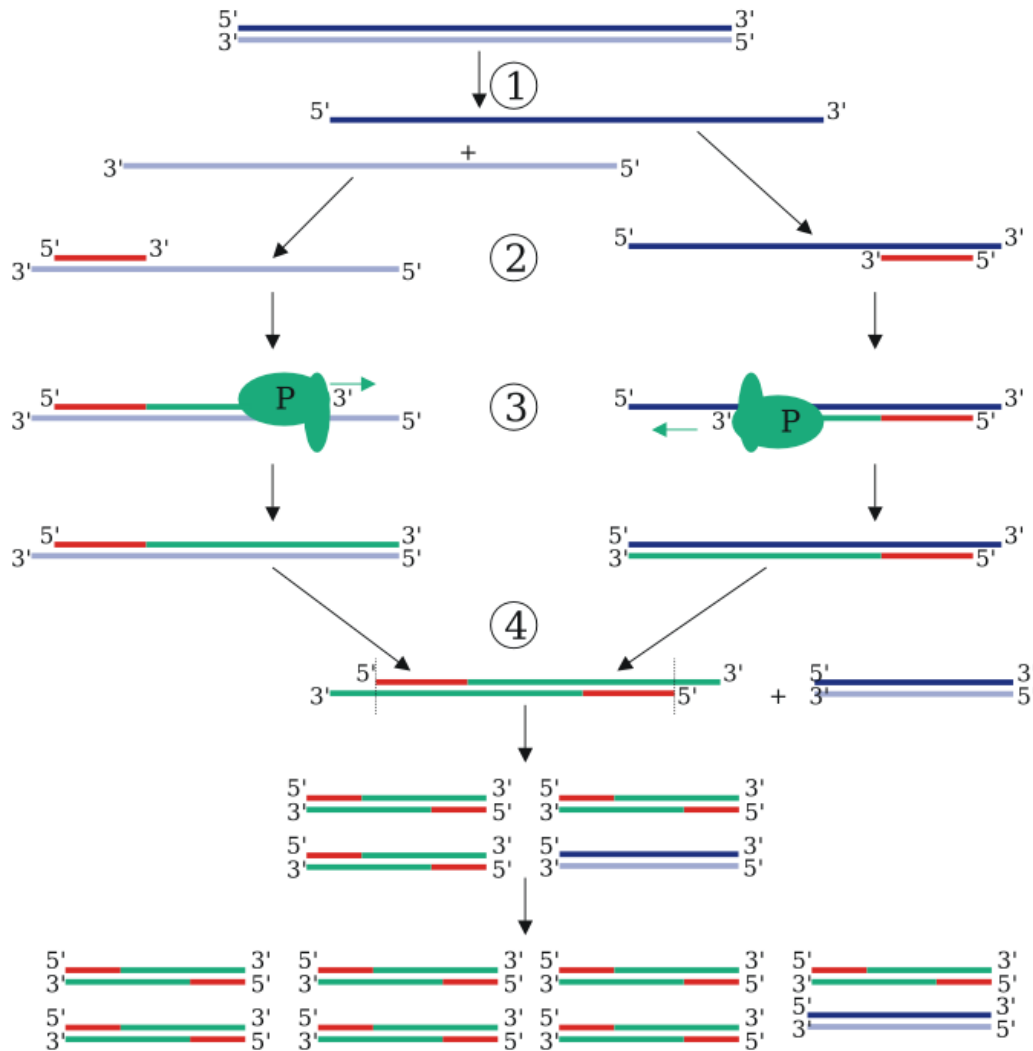


# Polymerase Chain Reaction

- Denature DNA  
(94-96°C)
- Anneal Primers  
(50-65°C)
- Extend complimentary strands  
Taq DNA polymerase  
(72°C)
- Repeat



# Polymerase Chain Reaction



# The PCR Song

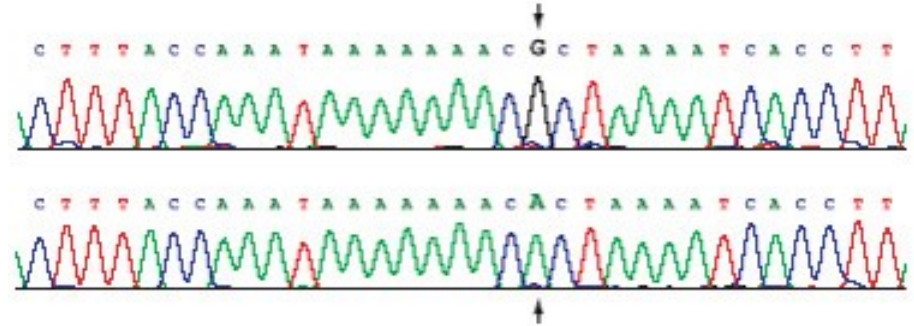


Youtube: [Click here](#)

<http://www.youtube.com/watch?v=x5yPkxCLads>

# Mutations

- Point Mutation



- Silent : codon for same amino acid
- Missense : codon for different a.a.
- Nonsense : stop codon

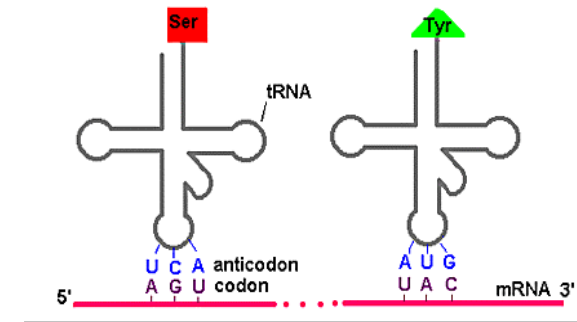
- Rearrangement Mutation





# Mutation Exercise

- (5') GGATAGCATGAAACCCGCATAA (3')
- amino acid
- (5') GGATAGCATGAAACCAAGCATAA (3')
- amino acid
- (5') GGATAGCATGAAACCCCCATAA (3')
- amino acid

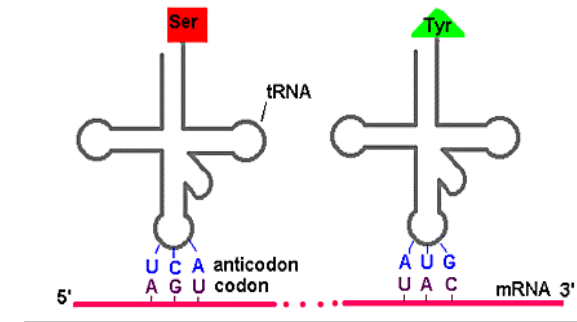


		2nd base in codon					
		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr <b>STOP</b> <b>STOP</b>	Cys Cys <b>STOP</b> Trp	U C A G	3rd base in codon
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

# Mutation Exercise

- (5') GGATAGCATGAAACCCGCATAA (3')
- Antisense (3') TACTTTGGGCGTATT (5') ←
- mRNA → (5') AUGAAACCCGCAUAA (3')
- amino acid Met Lys Pro Ala Stop
- (5') GGATAGCATGAAACCAAGCATAA (3')
- amino acid
- (5') GGATAGCATGAAACCCCCATAA (3')
- amino acid



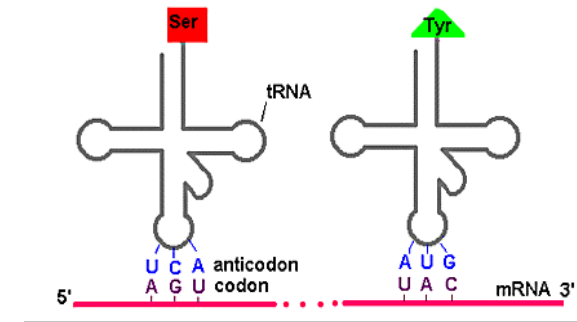
2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr <b>STOP</b> <b>STOP</b>	Cys Cys <b>STOP</b> Trp	U C A G	3rd base in codon
	C	Leu Leu Leu	Pro Pro Pro	His His Gln	Arg Arg Arg	U C A G	
	A	Ile Ile Met	Thr Thr Thr	Asn Asn Lys	Ser Ser Arg	U C A G	
	G	Val Val Val	Ala Ala Ala	Asp Asp Glu	Gly Gly Gly	U C A G	

The Genetic Code

# Mutation Exercise

- (5') GGATAGCATGAAACCCGCATAA (3')
- Antisense (3') TACTTTGGGCGTATT (5') ←
- mRNA → (5') AUGAAACCCGCAUAA (3')
- amino acid Met Lys Pro Ala Stop
- (5') GGATAGCATGAAACCC**A**GCATAA (3')
- Antisense (3') TACTTTGG**T**CGTATT (5')
- mRNA (5') AUGAAACCC**A**GCAUAA (3')
- amino acid Met Lys Pro Ala Stop
- (5') GGATAGCATGAAACCC**C**CATAA (3')
- amino acid



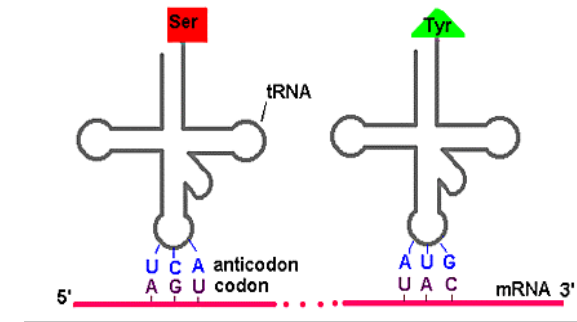
2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr <b>STOP</b> <b>STOP</b>	Cys Cys <b>STOP</b> Trp	U C A G	3rd base in codon
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

# Mutation Exercise

- (5') GGATAGCATGAAACCCGCATAA (3')
- Antisense (3') TACTTTGGGCGTATT (5') ←
- mRNA → (5') AUGAAACCCGCAUAA (3')
- amino acid Met Lys Pro Ala Stop
  
- (5') GGATAGCATGAAACCC**A**GCATAA (3')
- Antisense (3') TACTTTGG**T**CGTATT (5')
- mRNA (5') AUGAAACCC**A**GCAUAA (3')
- amino acid Met Lys Pro Ala Stop
  
- (5') GGATAGCATGAAACCC**C**CATAA (3')
- Antisense (3') TACTTTGGG**G**GTATT (5')
- mRNA (5') AUGAAACCC**C**CAUAA (3')
- amino acid Met Lys Pro **Pro** Stop



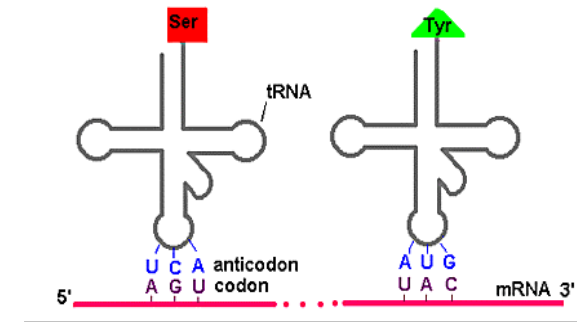
2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr <b>STOP</b> <b>STOP</b>	Cys Cys <b>STOP</b> Trp	U C A G	3rd base in codon
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

# Mutation Exercise

- (5') GGATAGCATGAAA . CCGCATAA (3')
- amino acid
- (5') GGATAGCATG**T**AACCAGCATAA (3')
- amino acid
- (5') GGATAGCATGAA**A****T**AACCAGCA (3')
- amino acid



2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr <b>STOP</b> <b>STOP</b>	Cys Cys <b>STOP</b> Trp	U C A G	3rd base in codon
	C	Leu Leu Leu	Pro Pro Pro	His His Gln	Arg Arg Arg	U C A G	
	A	Ile Ile Met	Thr Thr Thr	Asn Asn Lys	Ser Ser Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

# Mutation Exercise

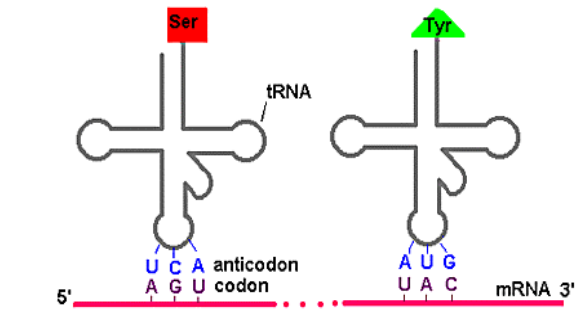
- (5') GGATAGCATGAAA . CCGCATAA (3')
- Antisense (3') TACTTT . GGCGTATT (5')
- mRNA (5') AUGAAA . CCGCAUAA (3')
- amino acid Met Lys Pro His ? ?

• (5') GGATAGCATG**T**AACCAGCATAA (3')

• amino acid

• (5') GGATAGCATGAA**A****T**AACCAGCA (3')

• amino acid



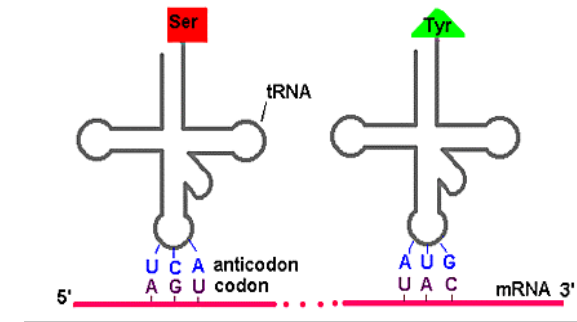
2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr <b>STOP</b> <b>STOP</b>	Cys Cys <b>STOP</b> Trp	U C A G	3rd base in codon
	C	Leu Leu Leu	Pro Pro Pro	His His Gln	Arg Arg Arg	U C A G	
	A	Ile Ile Met	Thr Thr Thr	Asn Asn Lys	Ser Ser Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

# Mutation Exercise

- (5') GGATAGCATGAAA . CCGCATAA (3')
  - Antisense (3') TACTTT . GGCGTATT (5')
  - mRNA (5') AUGAAA . CCGCAUAA (3')
  - amino acid Met Lys Pro His ? ?
- 
- (5') GGATAGCATGTAACCAGCATAA (3')
  - Antisense (3') TACA TTGGTCGTATT (5')
  - mRNA (5') AUGUAACCAGCAUAA (3')
  - amino acid Met Stop
- 
- (5') GGATAGCATGAAATAACCAGCA (3')
  - amino acid



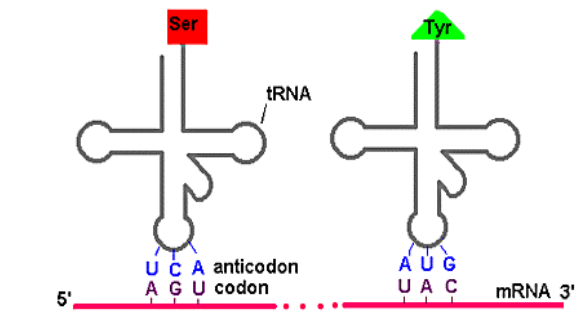
2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G	3rd base in codon
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

# Mutation Exercise

- (5') GGATAGCATGAAA . CCGCATAA (3')
  - Antisense (3') TACTTT . GGCGTATT (5')
  - mRNA (5') AUGAAA . CCGCAUAA (3')
  - amino acid Met Lys Pro His ? ?
- 
- (5') GGATAGCATGTAACCAGCATAA (3')
  - Antisense (3') TACA TTGGTCGTATT (5')
  - mRNA (5') AUGUAACCAGCAUAA (3')
  - amino acid Met Stop
- 
- (5') GGATAGCATGAAATAACCAGCA (3')
  - Antisense (3') TACTTT AT TGGT CGT (5')
  - mRNA (5') AUGAAA UAACCAGCA (3')
  - amino acid Met Lys Stop



2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr <b>STOP</b> <b>STOP</b>	Cys Cys <b>STOP</b> Trp	U C A G	3rd base in codon
	C	Leu Leu Leu	Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code



# Questions ?