

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

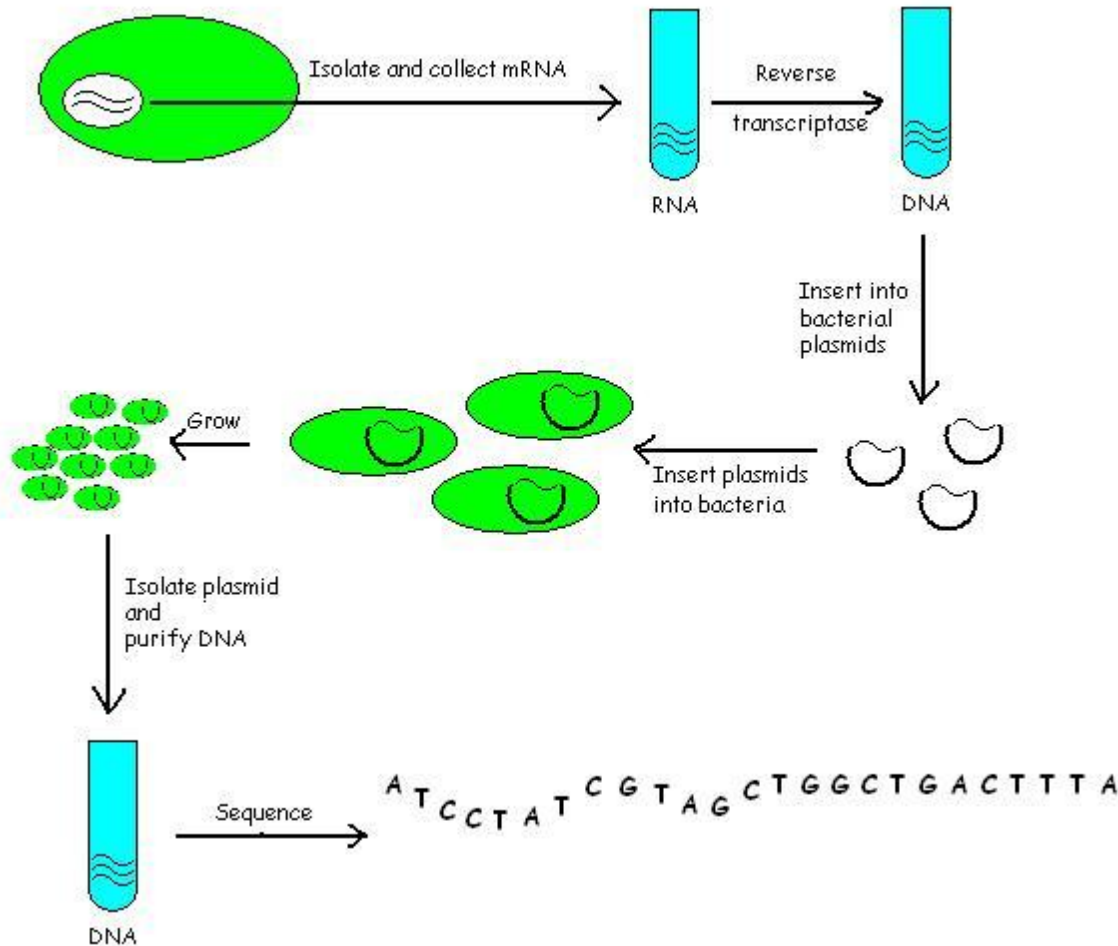
Decoding DNA

Genes

- Segment of DNA encoding mRNA, tRNA, or rRNA
- Produce proteins, not lipids or carbohydrates
- Size: 100 to 1 million bp

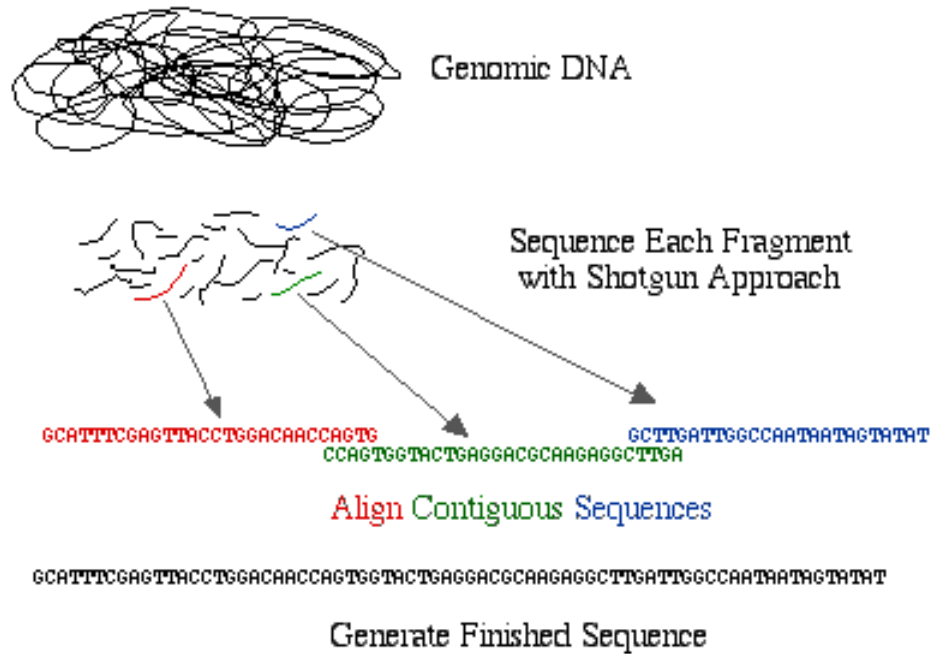
Decoding

Formation of a cDNA Library



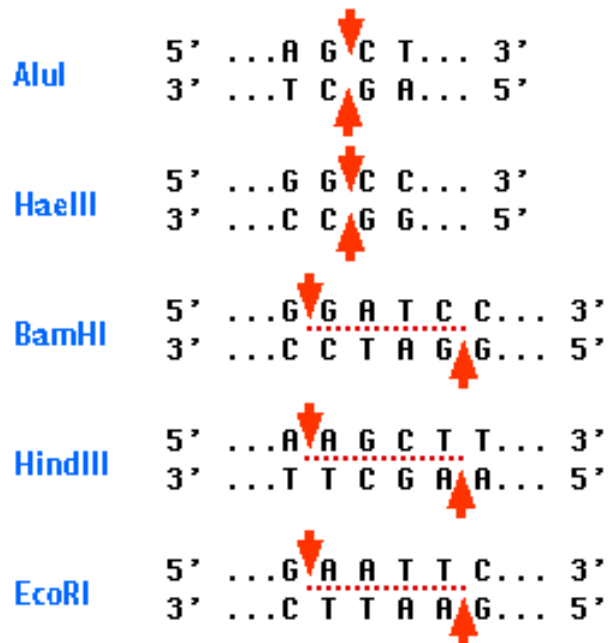
Decoding

Whole Genome Shotgun Sequencing Method



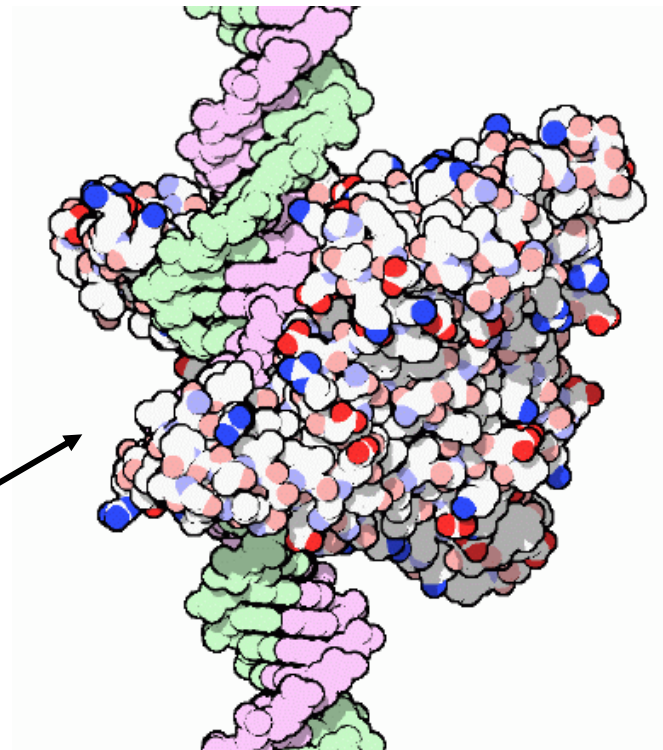
Restriction Endonucleases

- Restriction Enzymes



AluI and **HaeIII** produce blunt ends

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



Sanger Method

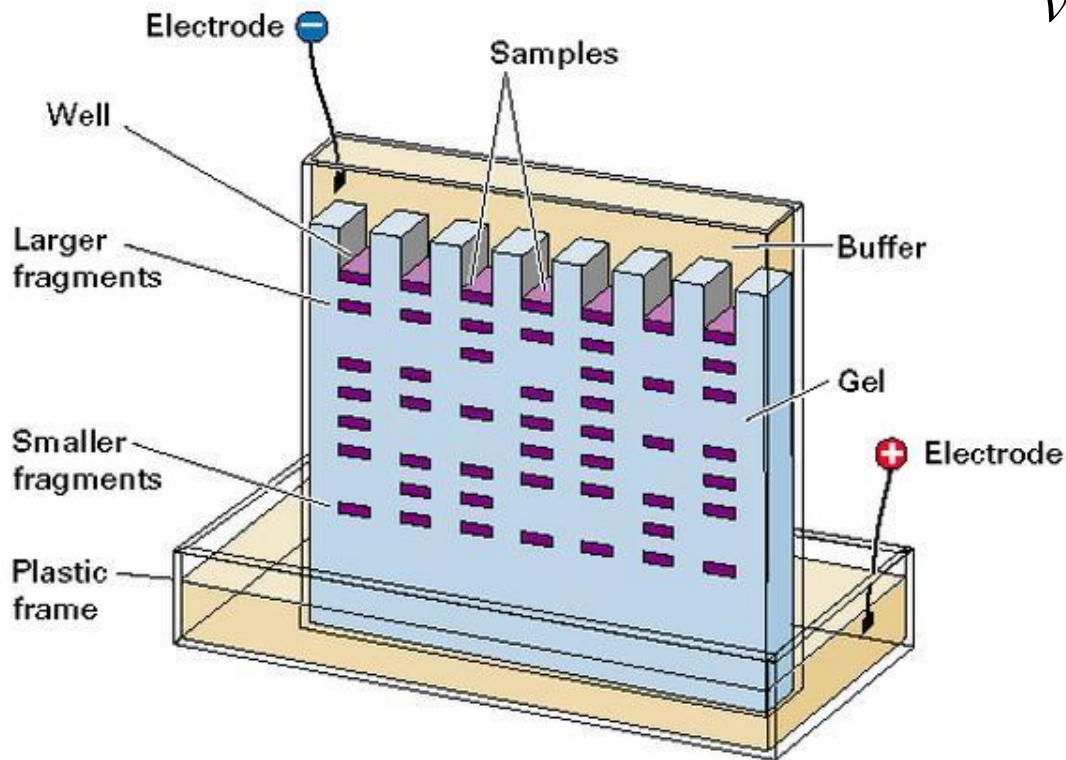
- Deoxynucleotides (dNTP)
 - dATP, dGTP, dCTP, dTTP
- Dideoxynucleotides (ddNTP)
 - H-group instead of OH-group (DNA chain terminators)
- DNA + primer + 4 dNTP + ddGTP + DNA polymerase:



Electrophoresis

- Separation of molecules by size

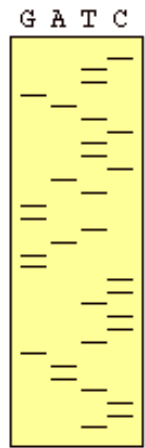
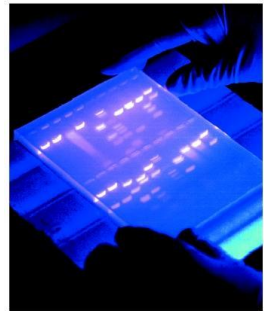
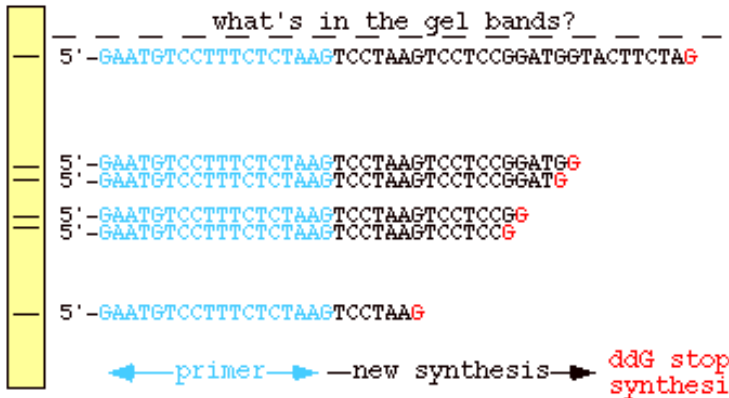
$$v = \frac{z}{f} E = \mu_e E$$



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'

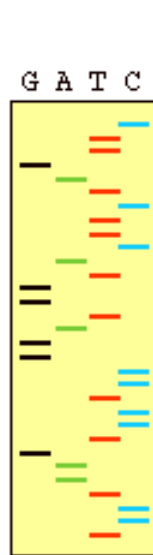
Primer sequence

Automatic Sequencing

- Primer labeled with fluorescent dyes



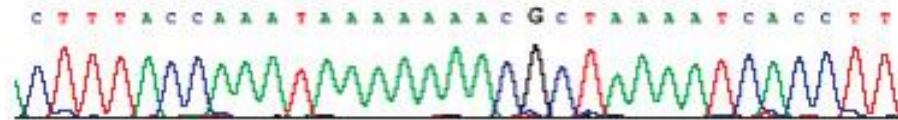
Automatic Sequencing



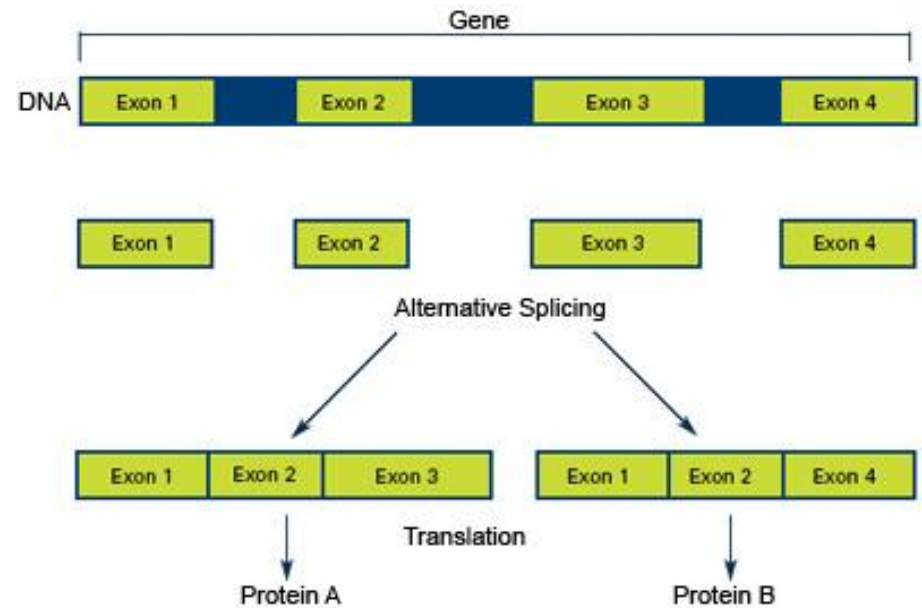
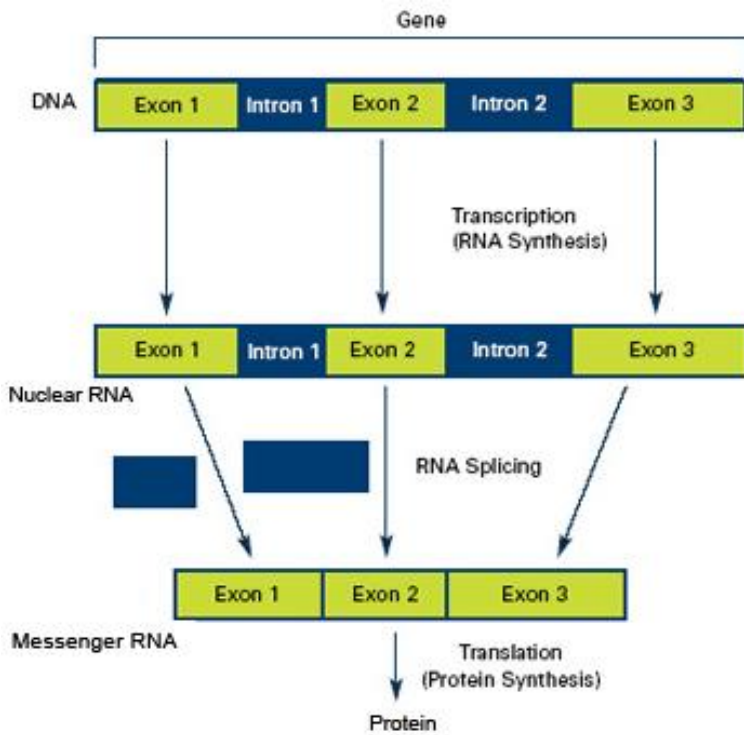
Here's what the products would look like in separate gel lanes.



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

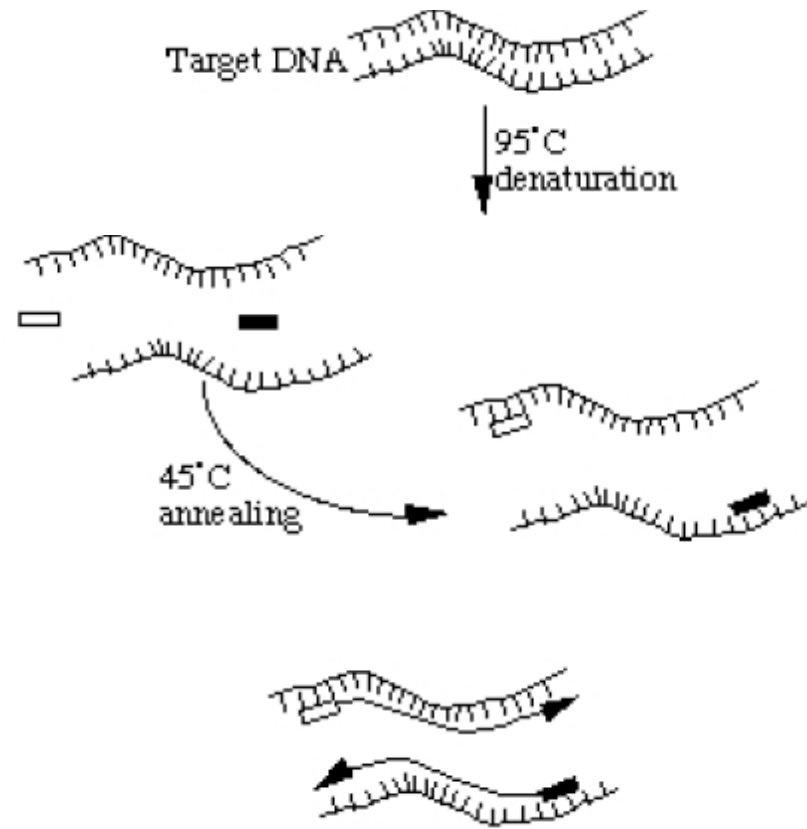


Splicing

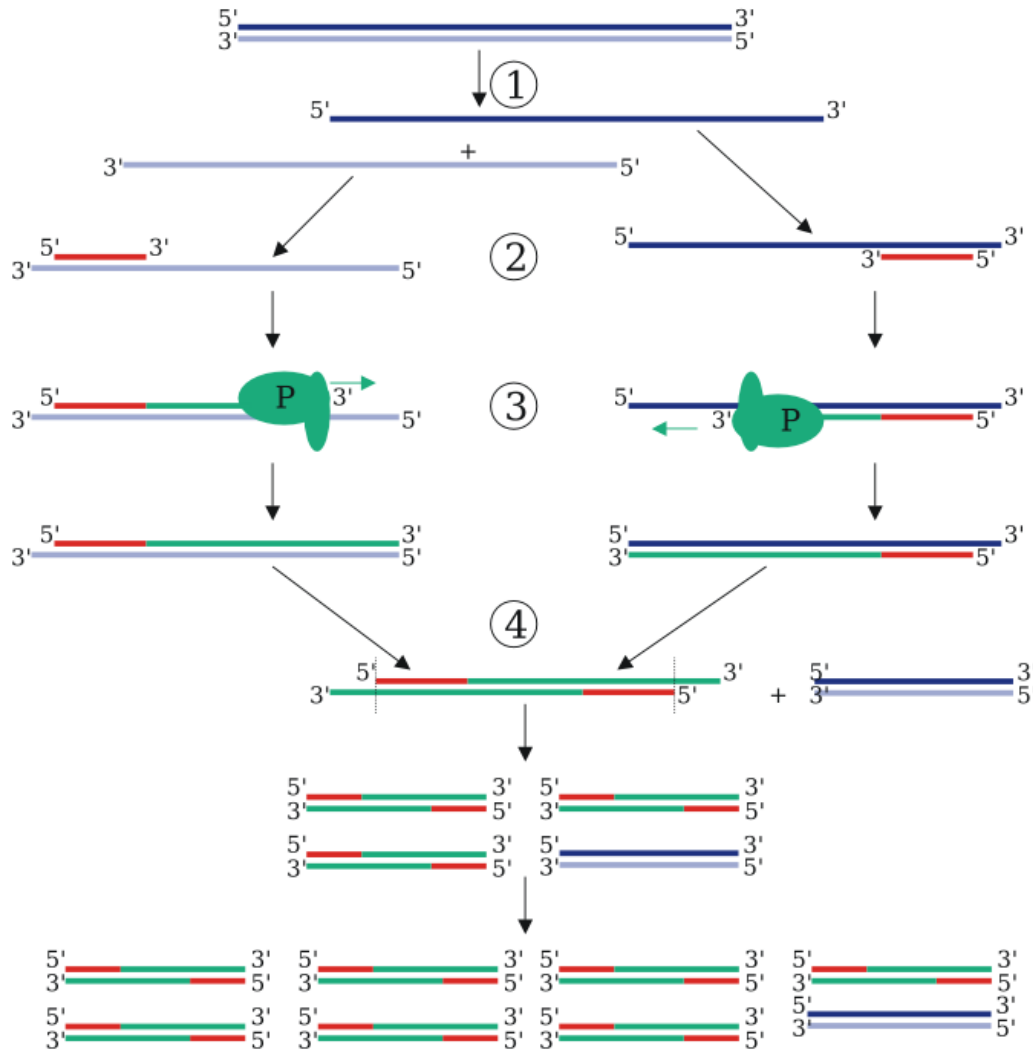


Polymerase Chain Reaction

- 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

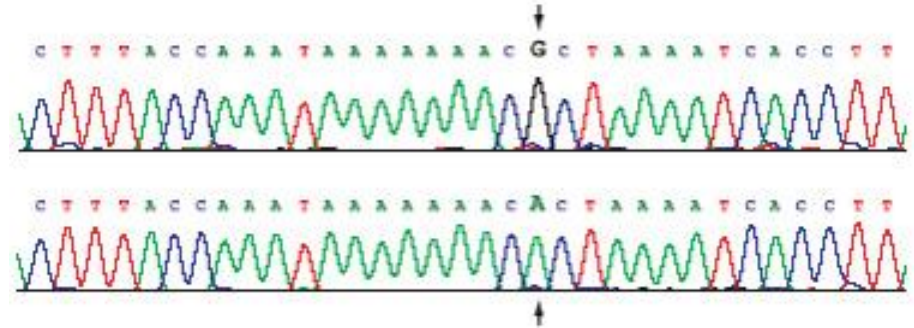


Youtube: [Click here](#)

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

Mutations

- Point Mutation



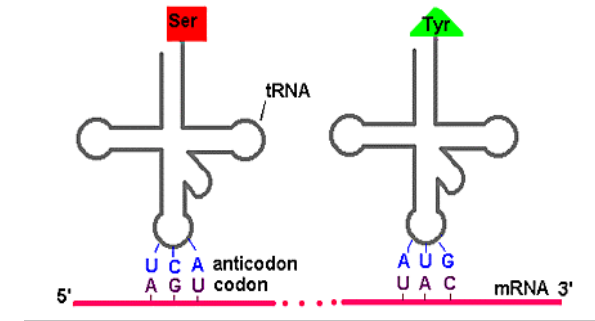
- Silent : codon for same amino acid
- Missense : codon for different a.a.
- Nonsense : stop codon

- Rearrangement Mutation



Mutation Exercise

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



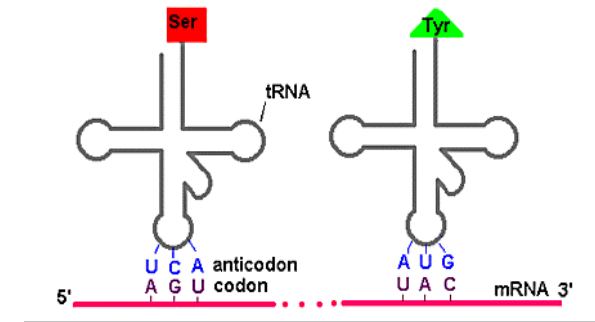
		2nd base in codon				
		U	C	A	G	
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala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

3rd base in codon

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

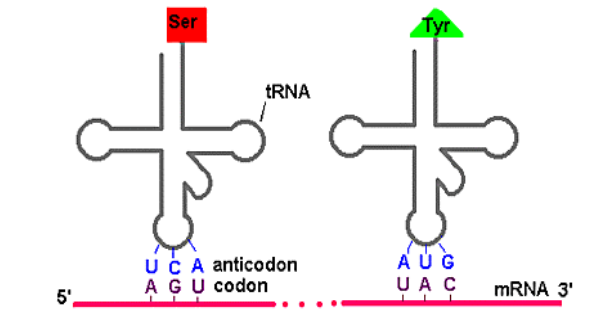
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- Antisense (3') TACTTTGGTCGTATT (5')
- mRNA (5') AUGAAACCAAGCAUAA (3')
- amino acid Met Lys Pro Ala Stop

- (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 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	

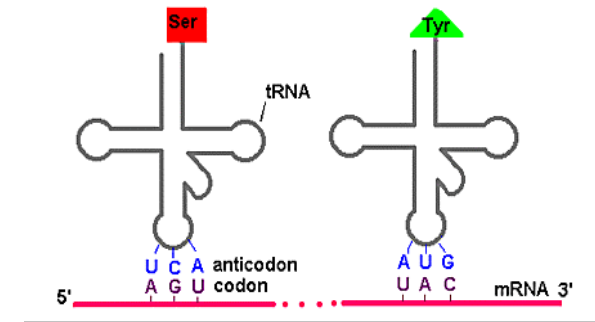
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- (5') GGATAGCATGAAACCC**A**GCATAA (3')
- Antisense (3') TACTTTGG**T**CGTATT (5')
- mRNA (5') AUGAAACCC**A**GCAUAA (3')
- amino acid Met Lys Pro Ala Stop

- (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

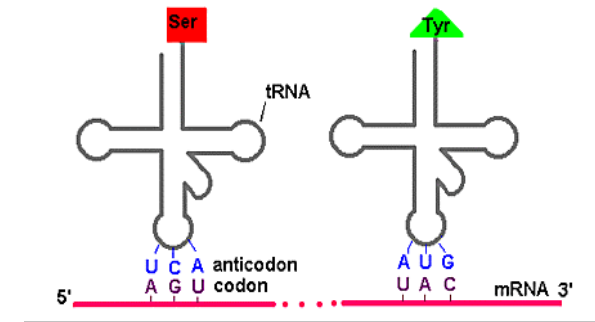
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	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

3rd base in codon

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



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	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

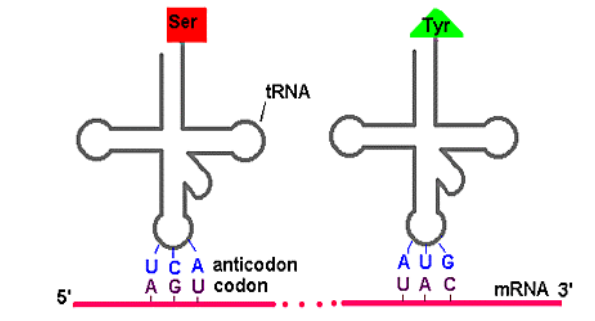
- (5') GGATAGCATGAAA . CCGCATAA (3')
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- mRNA (5') AUGAAA . CCGCAUAA (3')
- amino acid Met Lys Pro His ? ?

• (5') GGATAGCATGTAACCAGCATAA (3')

• amino acid

• (5') GGATAGCATGAAATAACCAGCA (3')

• amino acid



2nd base in codon

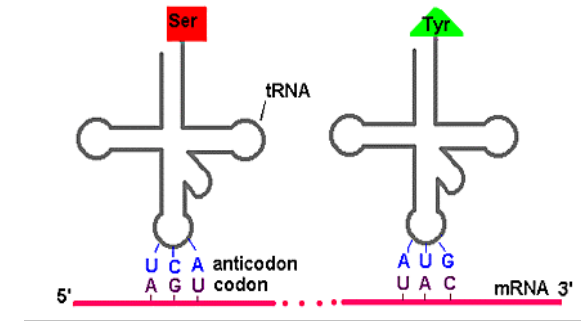
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3rd base in codon

The Genetic Code

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-
- (5') GGATAGCATGTAACCAGCATAA (3')
 - Antisense (3') TACA TTGGTCGTATT (5')
 - mRNA (5') AUGUAACCAGCAUAA (3')
 - amino acid Met Stop
-
- (5') GGATAGCATGAAATAACCAGCA (3')
 - amino acid

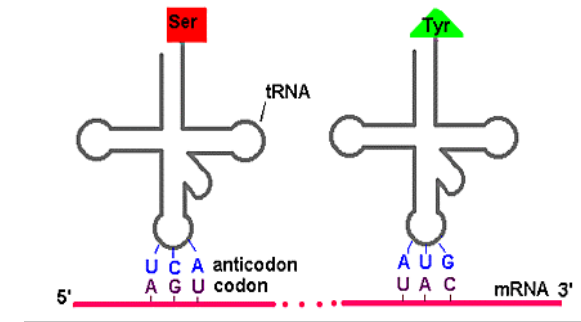


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	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

Mutation Exercise

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 - mRNA (5') AUGAAA . CCGCAUAA (3')
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 - 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 STOP STOP	Cys Cys STOP 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 ?