

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

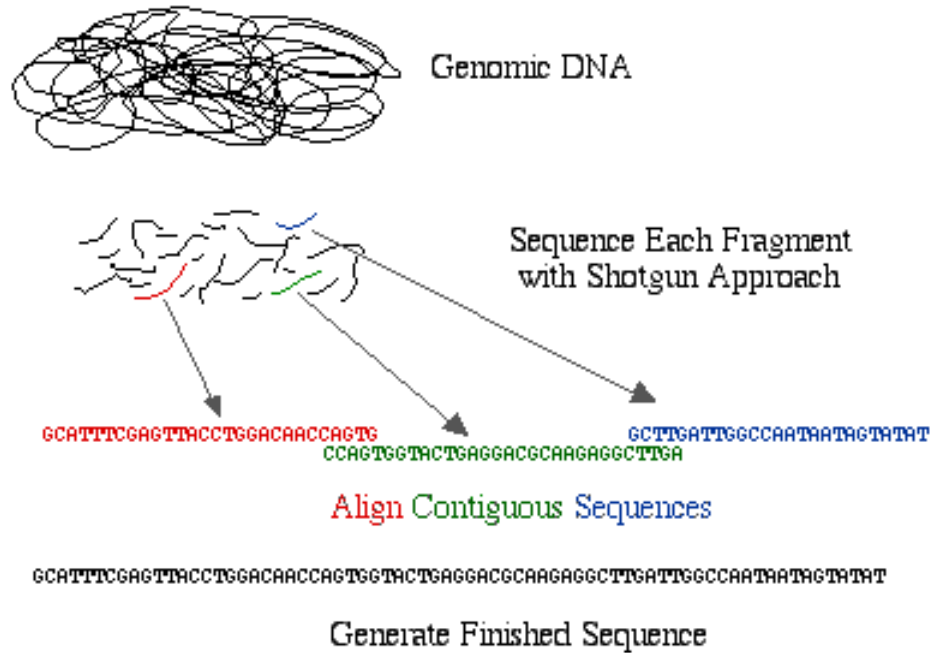
Decoding DNA

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

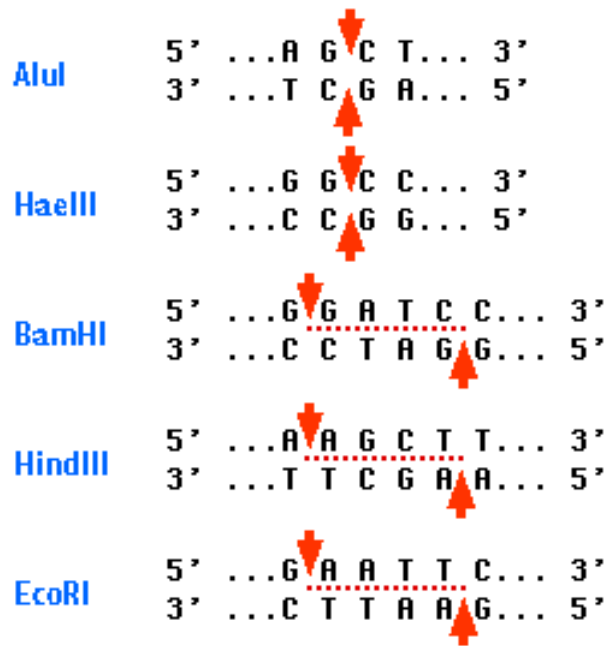
Decoding the Genome

Whole Genome Shotgun Sequencing Method



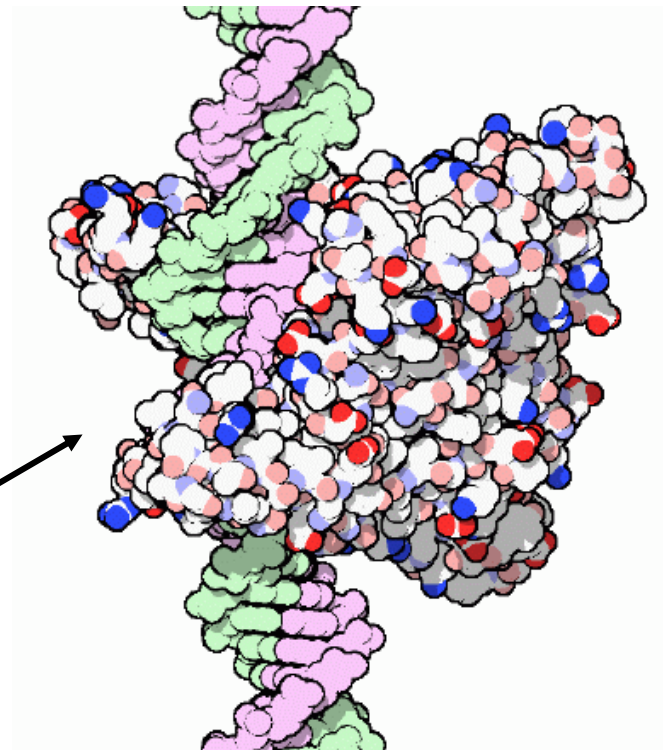
Restriction Endonucleases

- Restriction Enzymes



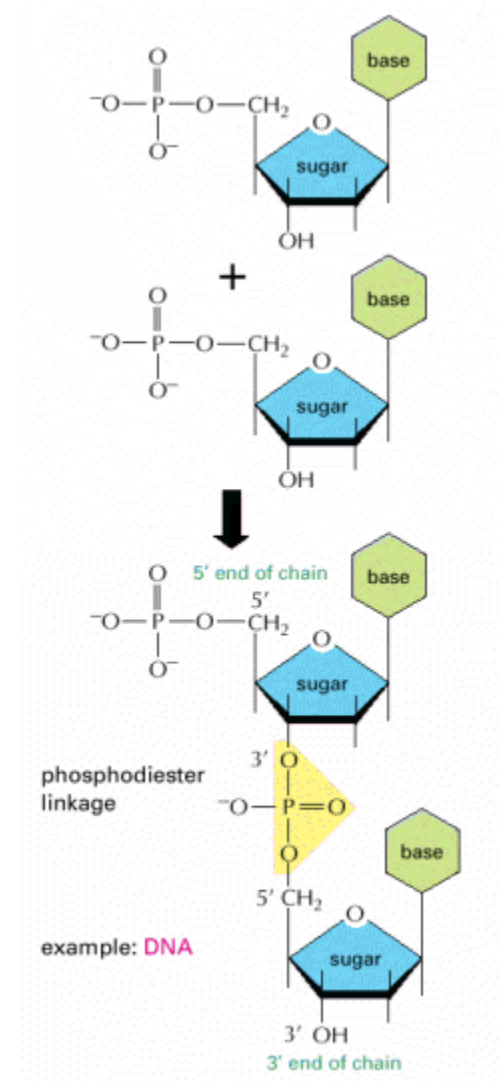
AluI and **HaeIII** produce blunt ends

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



Sanger Method

- Deoxynucleotides (dNTP)
 - dATP, dGTP, dCTP, dTTP



Sanger Method

- Deoxynucleotides (dNTP)
 - dATP, dGTP, dCTP, dTTP
- Dideoxynucleotides (ddNTP)
 - H-group instead of OH-group

ddNTPs terminate DNA synthesis.

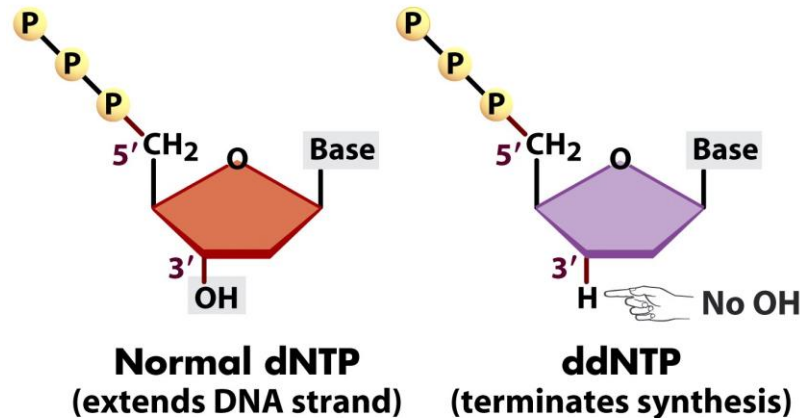
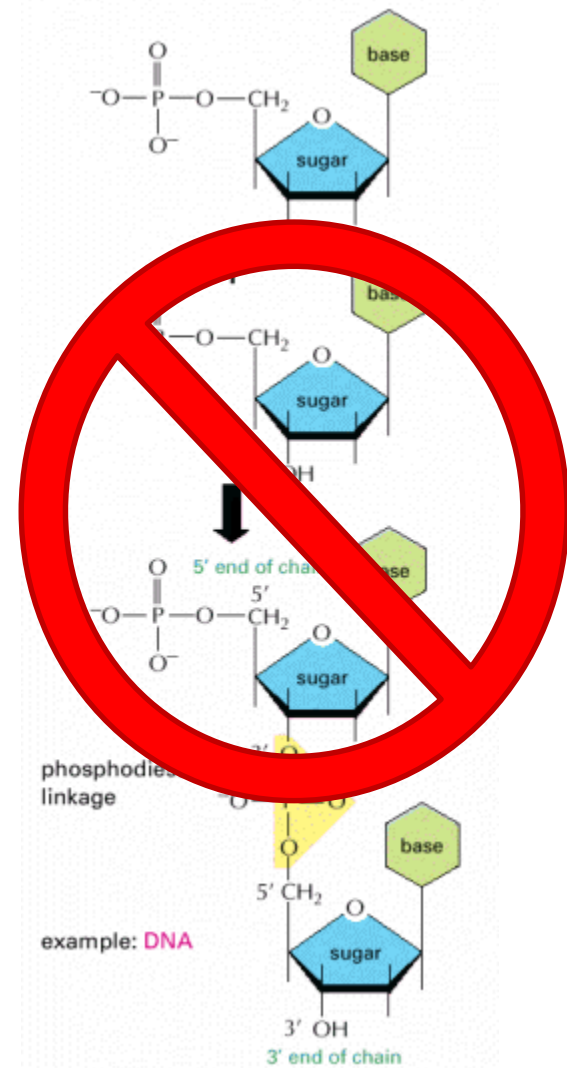


Figure 19-6a Biological Science, 2/e

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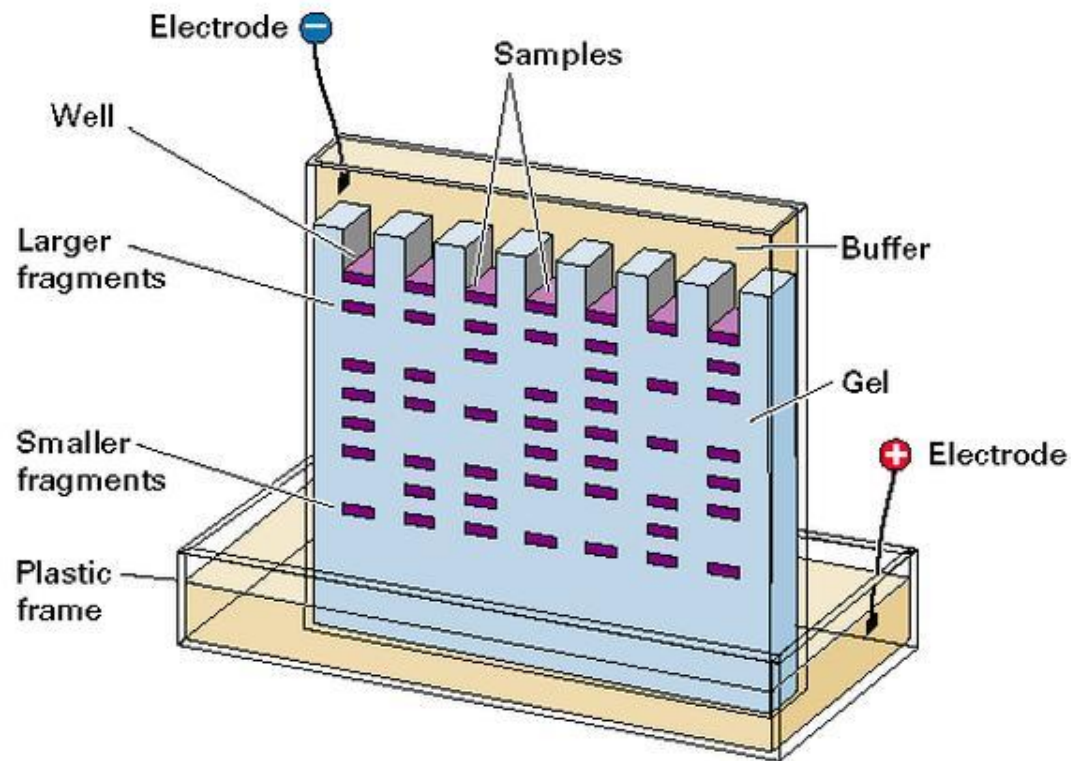
Sanger Method

- 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

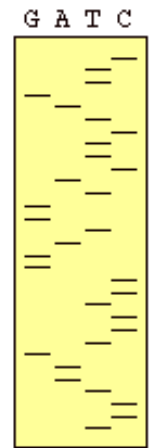
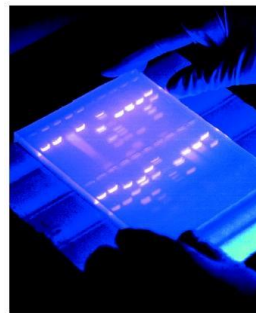
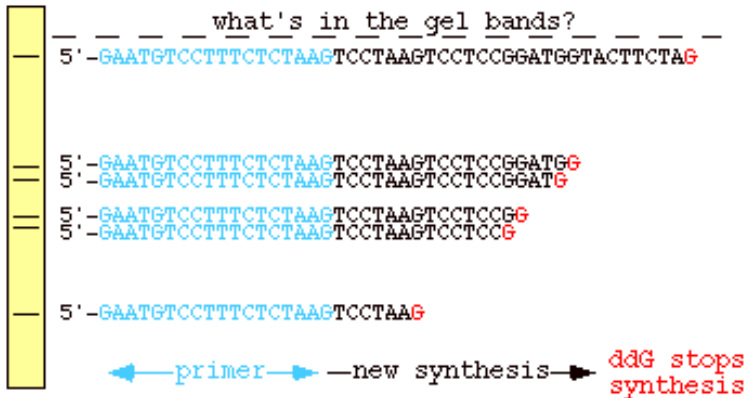
- Separation of molecules by size



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'

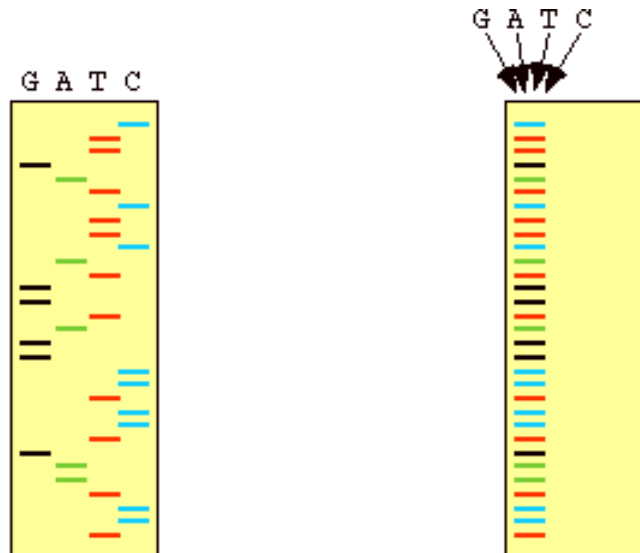
Primer sequence

Automatic Sequencing

- Primer labeled with fluorescent dyes

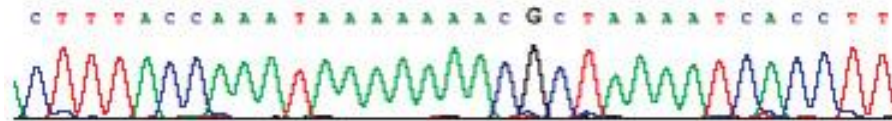


Automatic Sequencing

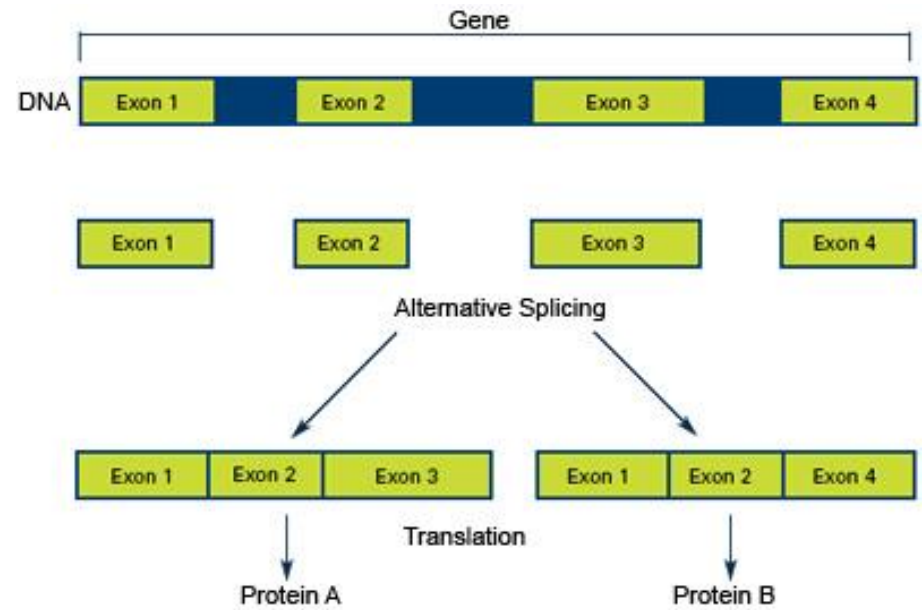
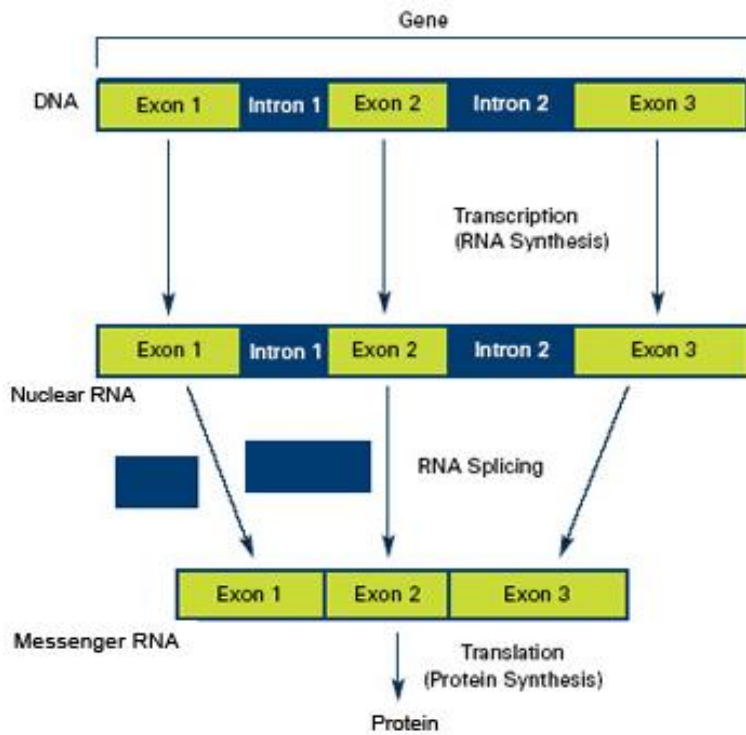


Here's what the products would look like in separate gel lanes.

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

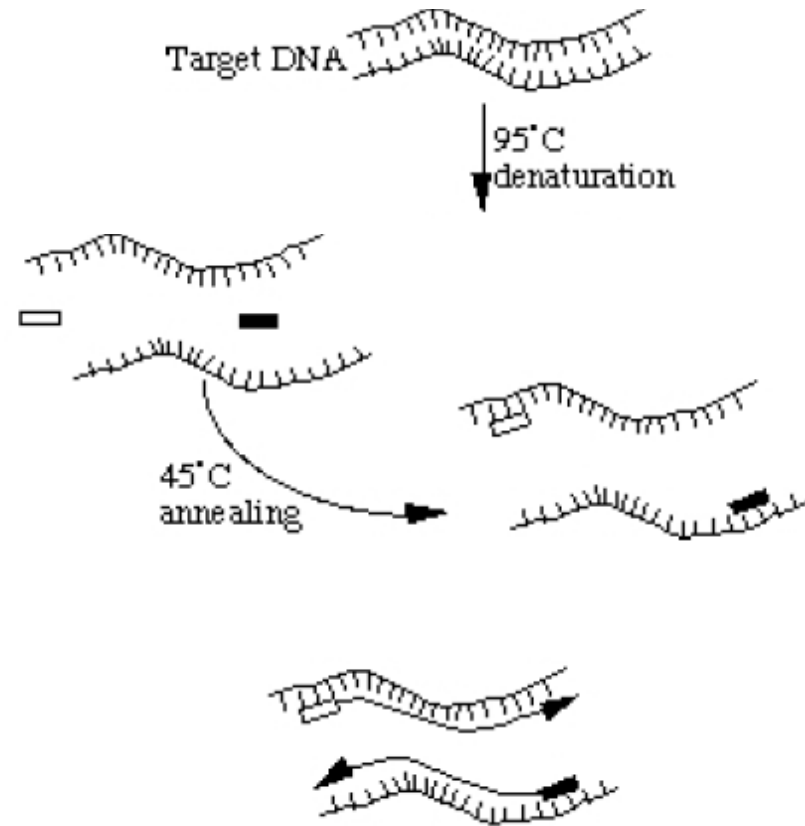


Splicing

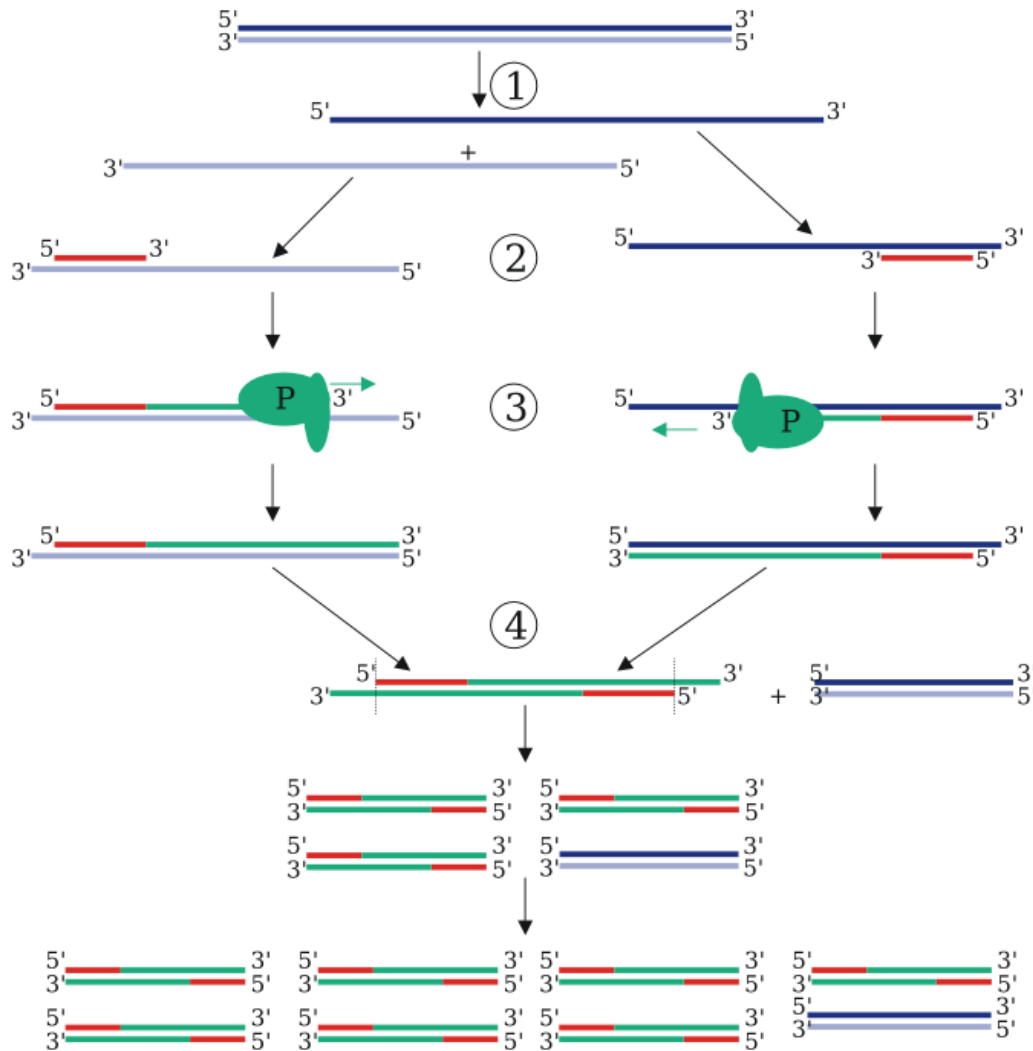


Polymerase Chain Reaction

- 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

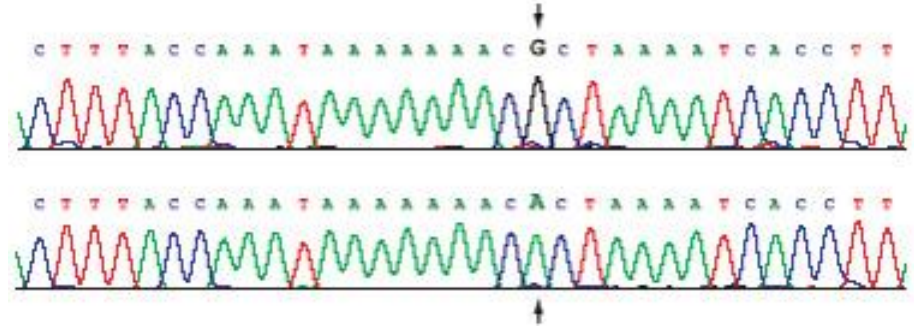


Youtube: [Click here](#)

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

Mutations

- Point Mutation



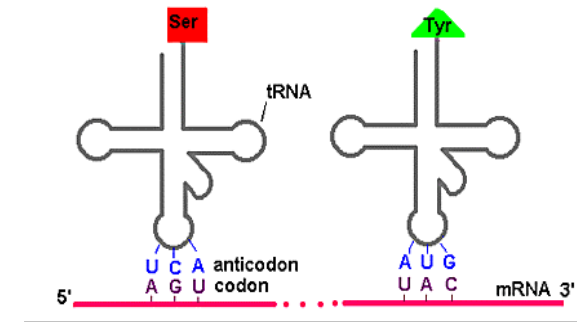
- Silent : codon for same amino acid
- Missense : codon for different a.a.
- Nonsense : stop codon

- Rearrangement Mutation



Mutation Exercise

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



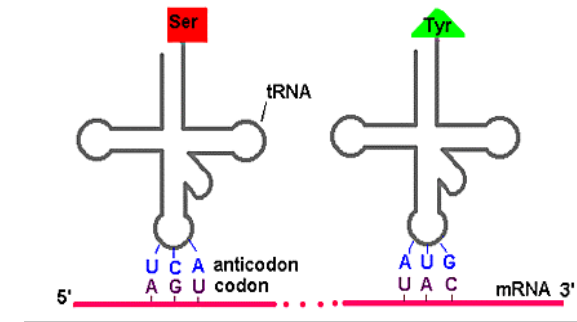
2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr 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') 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



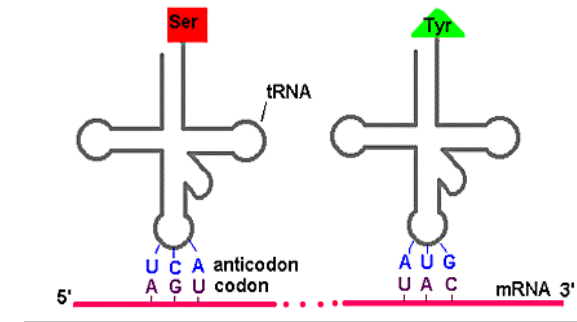
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	C	Leu Leu Leu	Pro Pro Pro	His His Gln	Arg Arg Arg	U C A G	
	A	Ile Ile Met	Thr Thr Thr	Asn Asn Lys	Ser Ser Arg	U C A G	
	G	Val Val Val	Ala Ala Ala	Asp Asp Glu	Gly Gly Gly	U C A G	

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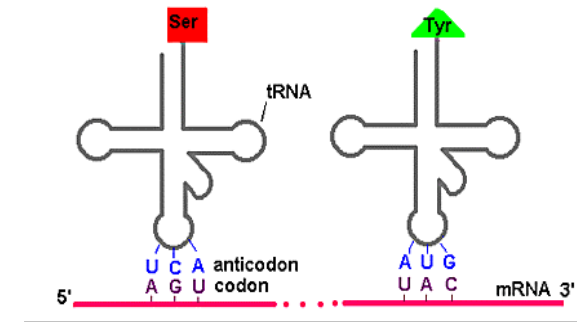
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	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

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 - Antisense (3') TACTTTGGTCGTATT (5')
 - mRNA (5') AUGAAACCAAGCAUAA (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



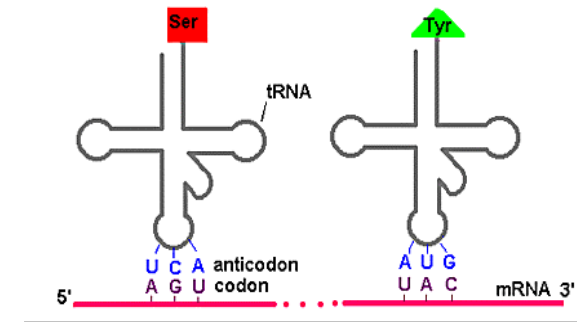
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	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

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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	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

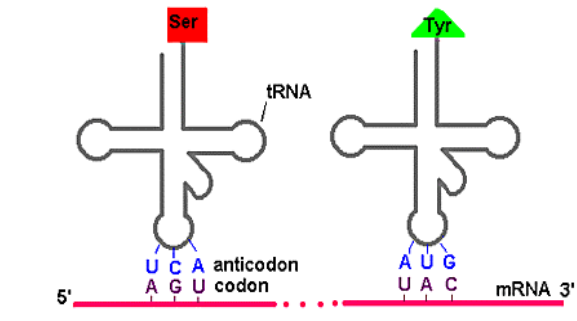
- (5') GGATAGCATGAAA . CCGCATAA (3')
- Antisense (3') TACTTT . GGCGTATT (5')
- mRNA (5') AUGAAA . CCGCAUAA (3')
- amino acid Met Lys Pro His ? ?

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

• amino acid

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

• amino acid



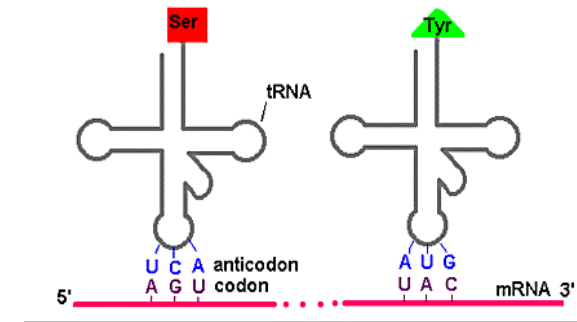
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	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

The Genetic Code

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- (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



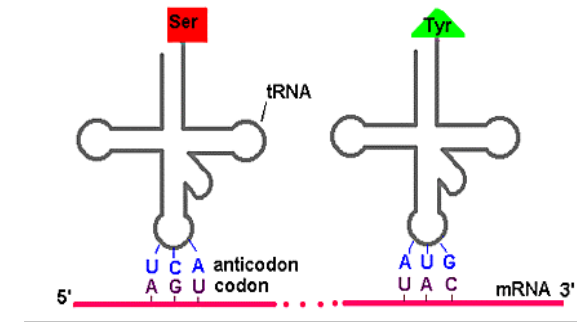
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	C	Leu Leu Leu	Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Met	Thr Thr Thr	Asn Asn Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

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 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

Questions ?