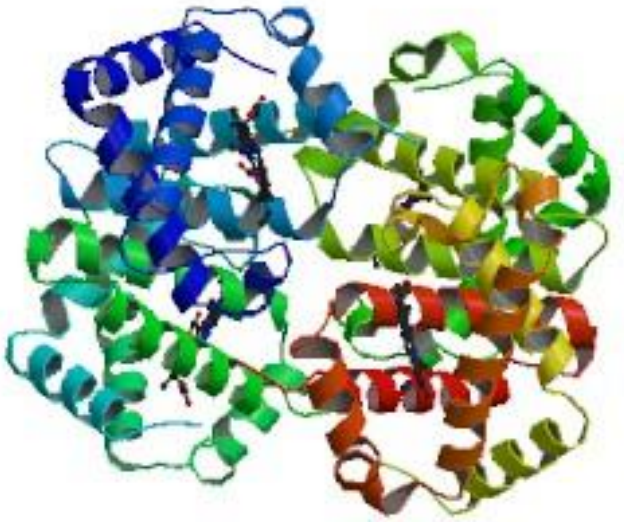
ME 498 / ME 599

Biological Frameworks for Engineers

Class Organization

- Lab 1 – Protein Structure
  - MEB 231
  - Friday



Biological Frameworks for Engineers

ME 498 / ME 599

Decoding DNA and Proteins

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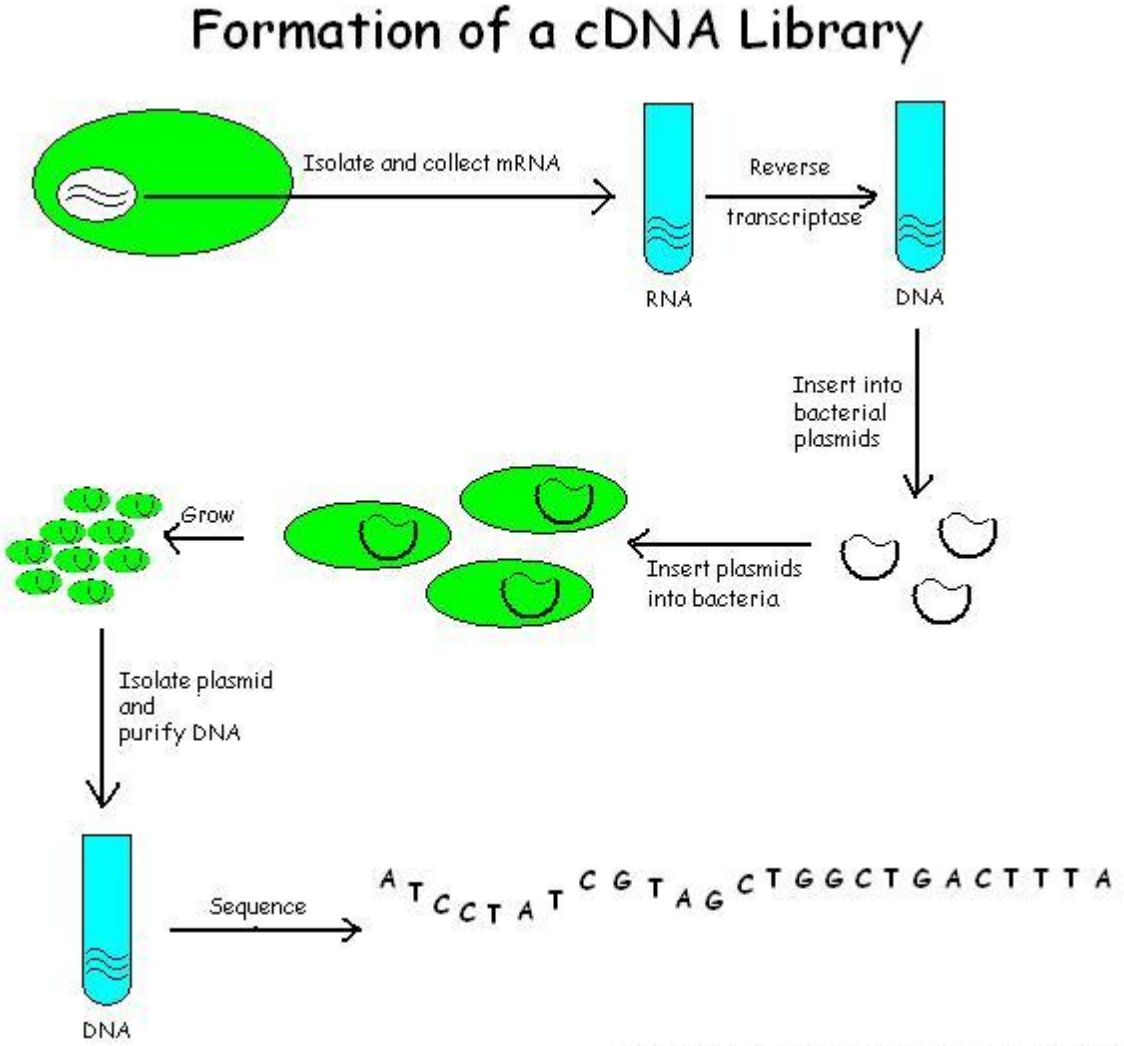
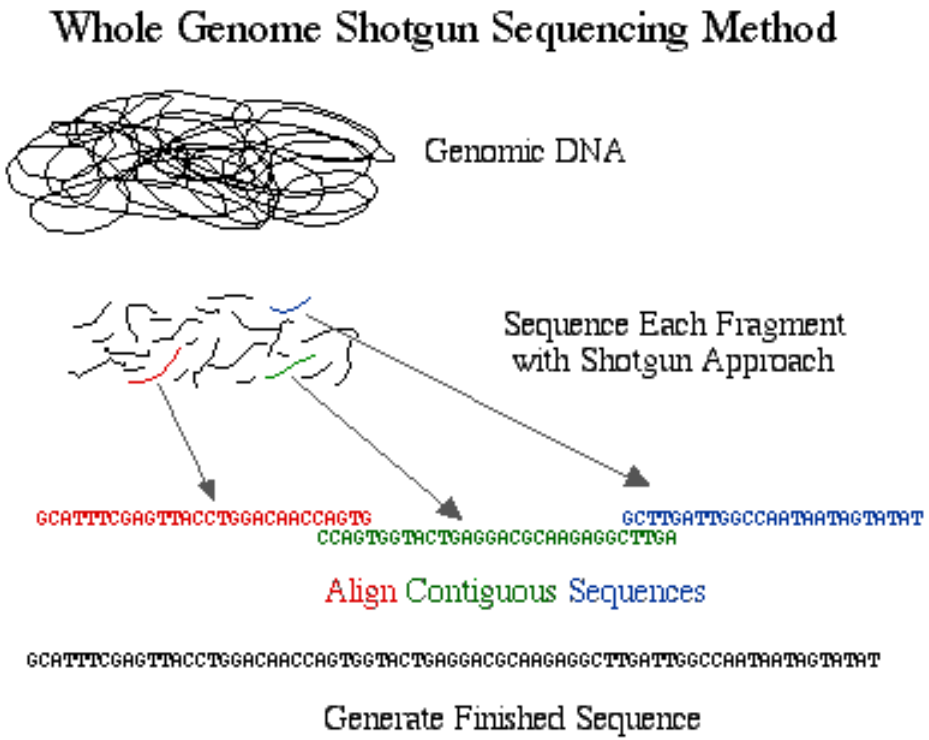
Genes

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

Decoding

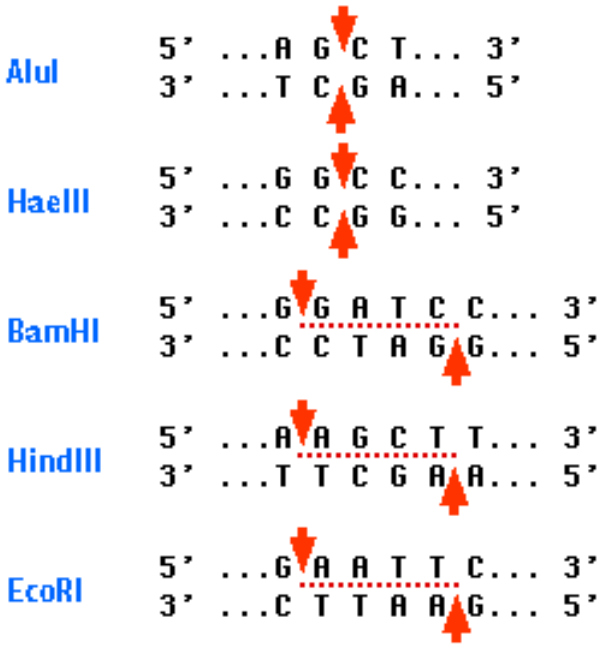

Formation of a cDNA Library
Isolate and collect mRNA
RNA
Reverse transcriptase
DNA
Insert into bacterial plasmids
Insert plasmids into bacteria
Grow
Isolate plasmid and purify DNA
DNA
Sequence
ATCCTATCGTAGCTGGCTGACTTTA

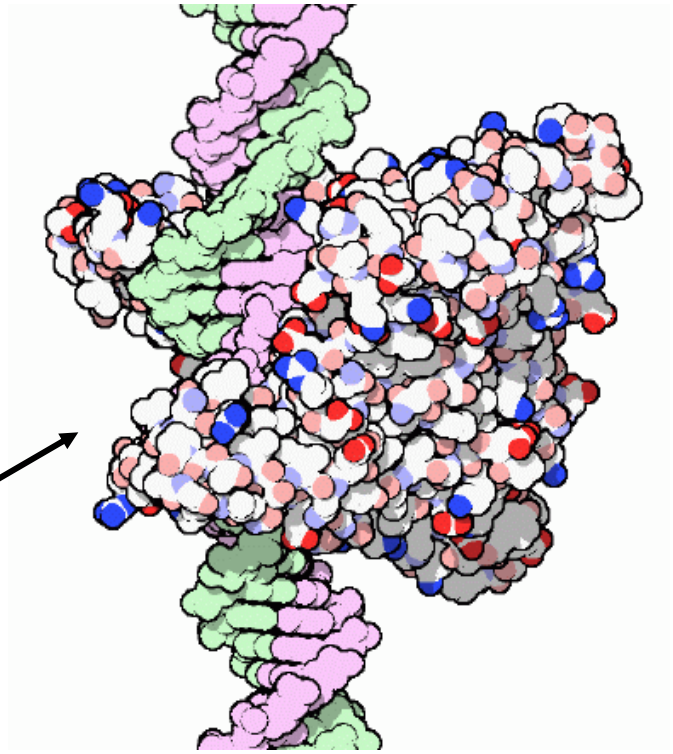
Decoding


Whole Genome Shotgun Sequencing Method
Genomic DNA
Sequence Each Fragment with Shotgun Approach
GCATTTCGAGTTACCTGGACAACCAGTG
CCAGTGGTACTGAGGACGCAAGAGGCTTGA
GCTTGATTGGCCAATAATAGTATAT
Align Contiguous Sequences
GCATTTCGAGTTACCTGGACAACCAGTGGTACTGAGGACGCAAGAGGCTTGATTGGCCAATAATAGTATAT
Generate Finished Sequence

Restriction Endonucleases

- Restriction Enzymes





AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

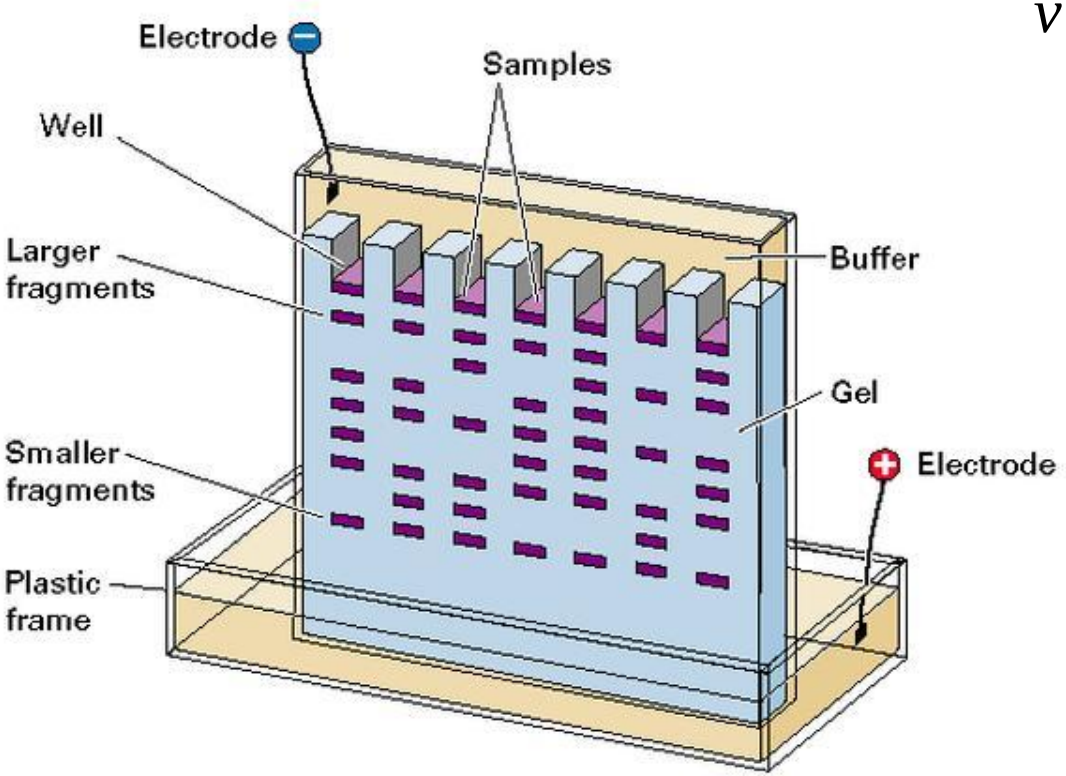
Sanger Method

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

```
     5'-GAATGTCCTTTCTCTAAGTCCTAAG
3'-GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG-5'

     5'-GAATGTCCTTTCTCTAAGTCCTAAGTCCTCCG
3'-GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG-5'

     5'-GAATGTCCTTTCTCTAAGTCCTAAGTCCTCCGG
3'-GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG-5'

     5'-GAATGTCCTTTCTCTAAGTCCTAAGTCCTCCGGATG
3'-GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG-5'

     5'-GAATGTCCTTTCTCTAAGTCCTAAGTCCTCCGGATGG
3'-GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG-5'

     5'-GAATGTCCTTTCTCTAAGTCCTAAGTCCTCCGGATGGTACTTCTAG
3'-GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG-5'
```

Electrophoresis

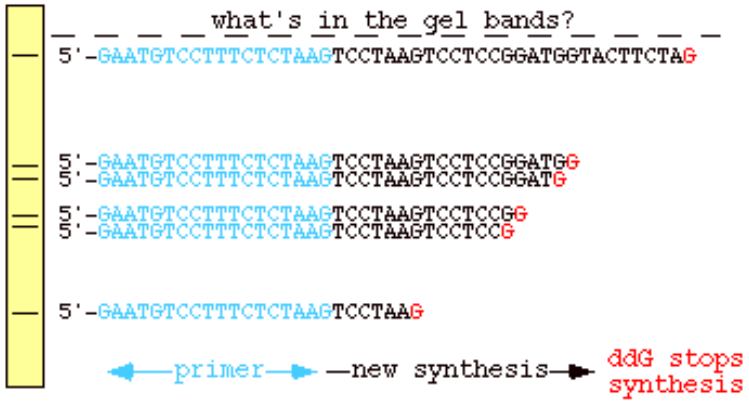
- Separation of molecules by size

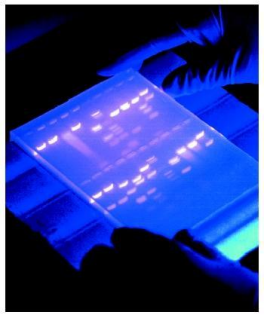


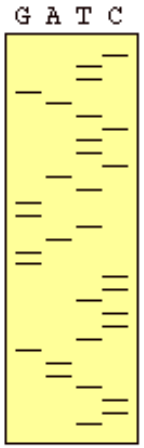
$$v = \frac{z}{f} E = \mu_e E$$

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:

A G G A T T C ...

3'-GGAGACTTACAGGAAAGAGATTCAGGATTCAGGAGGCCTACCATGAAGATCAAG-5'

Primer sequence

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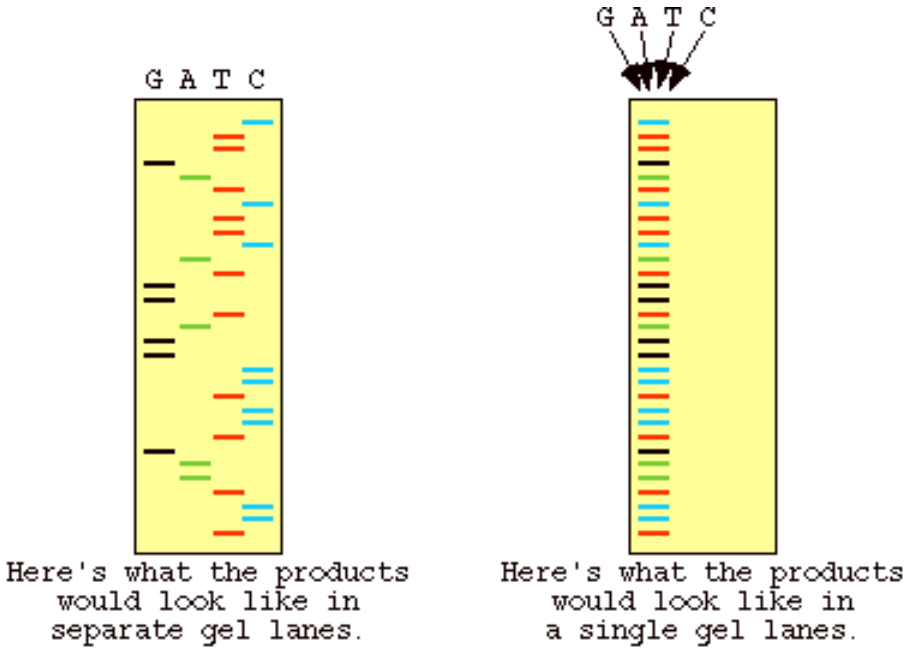
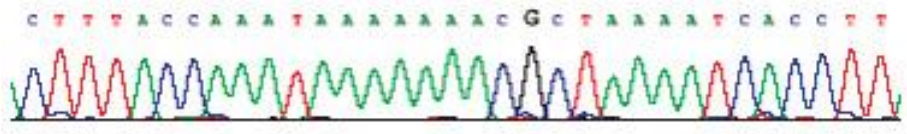
Automatic Sequencing

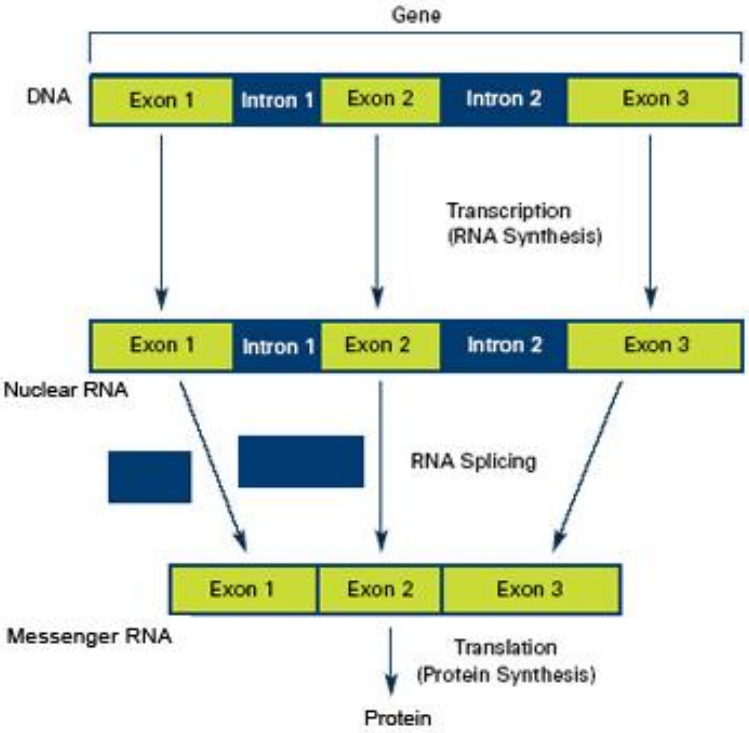
- Primer labeled with fluorescent dyes

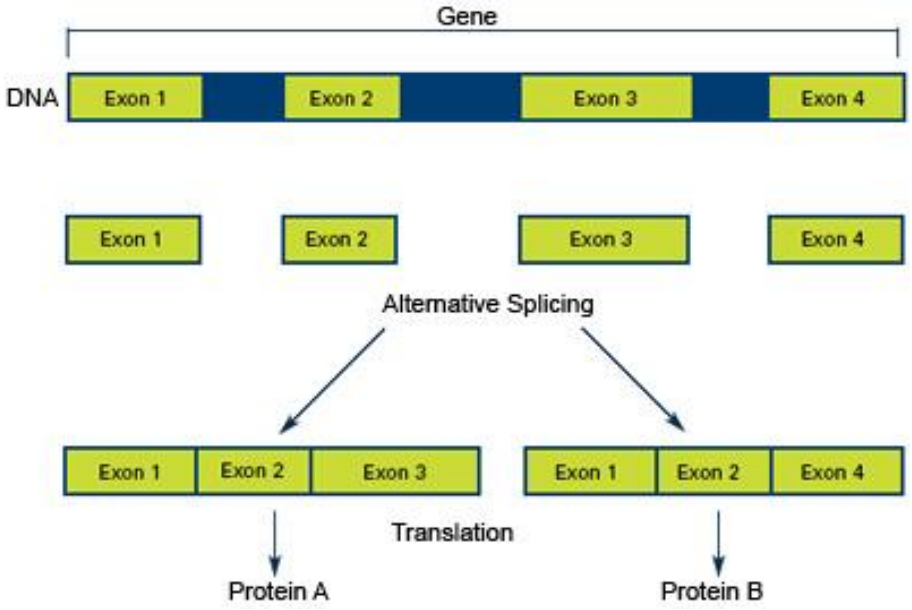
Automatic Sequencing





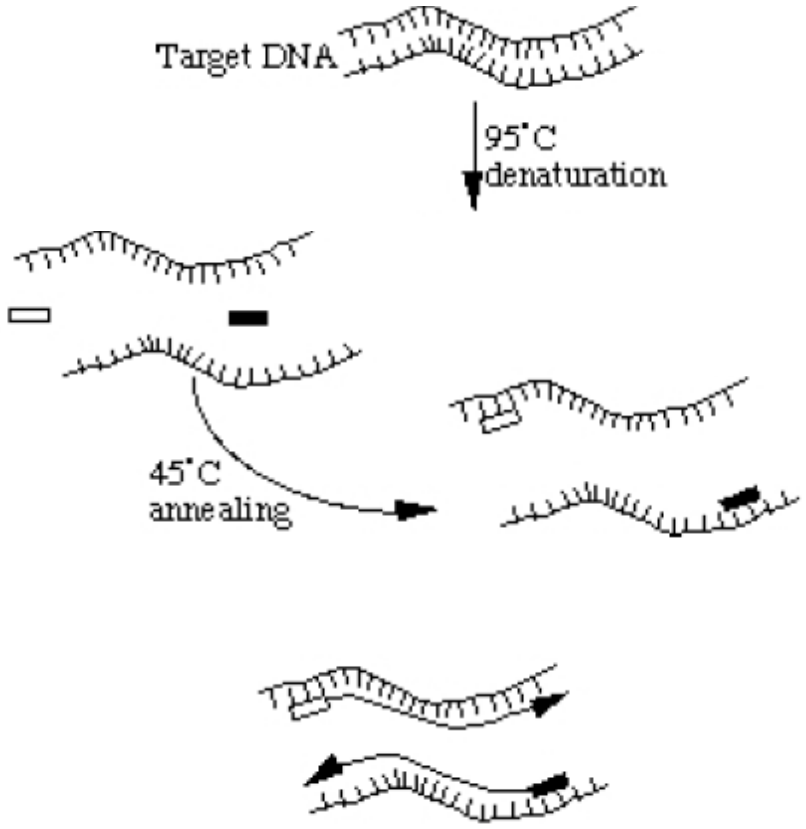
Splicing


Gene
DNA
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Transcription
(RNA Synthesis)
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Nuclear RNA
RNA Splicing
Exon 1
Exon 2
Exon 3
Messenger RNA
Translation
(Protein Synthesis)
Protein

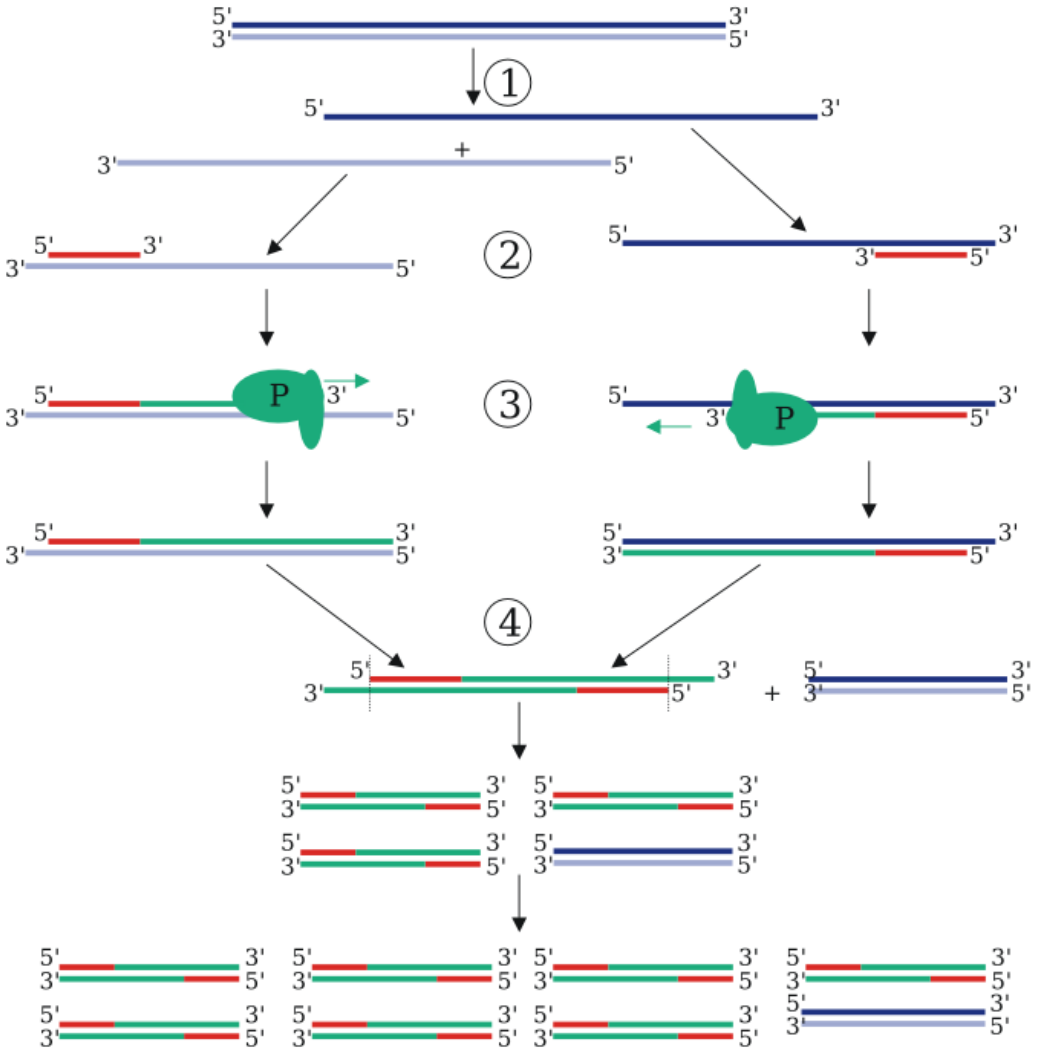

Gene
DNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 1
Exon 2
Exon 3
Exon 4
Alternative Splicing
Exon 1
Exon 2
Exon 3
Exon 1
Exon 2
Exon 4
Translation
Protein A
Protein B

Polymerase Chain Reaction

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

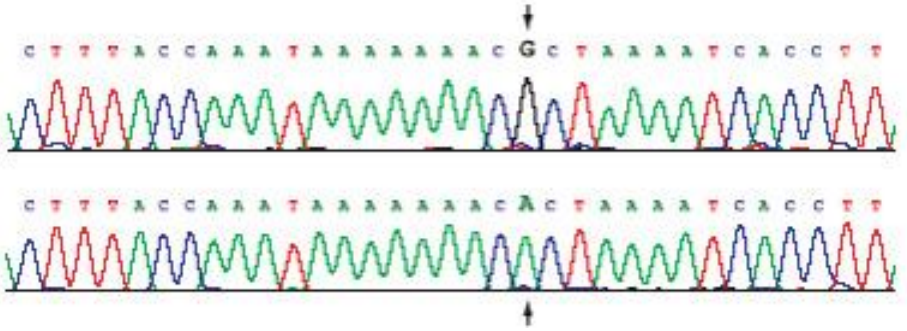


Polymerase Chain Reaction



Mutations

- Point Mutation



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

- Rearrangement Mutation

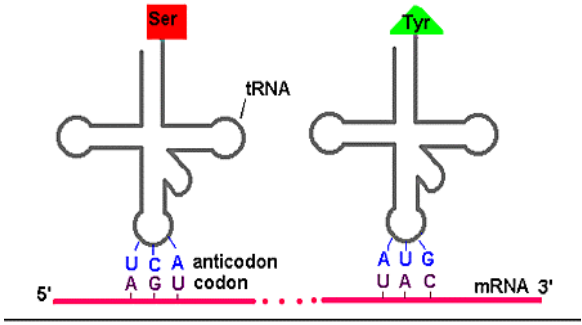
(5') G G A T A G C A T G A A A C C A G C A **T A A** (3')

Mutation Exercise

- (5') G G A T A G C A T G A A A C C C G C A T A A (3')
- Antisense (3') T A C T T T G G G C G T A T T **(5')←**
- mRNA **→(5')** A U G A A A C C C G C A U A A (3')
- amino acid Met Lys Pro Ala Stop

- (5') G G A T A G C A T G A A A C C **A** G C A T A A (3')
- Antisense (3') T A C T T T G G **T** C G T A T T (5')
- mRNA (5') A U G A A A C C **A** G C A U A A (3')
- amino acid Met Lys Pro Ala Stop

- (5') G G A T A G C A T G A A A C C C **C** C A T A A (3')
- Antisense (3') T A C T T T G G G **G** G T A T T (5')
- mRNA (5') A U G A A A C C C **C** C A U A A (3')
- amino acid Met Lys Pro **Pro** Stop



The Genetic Code

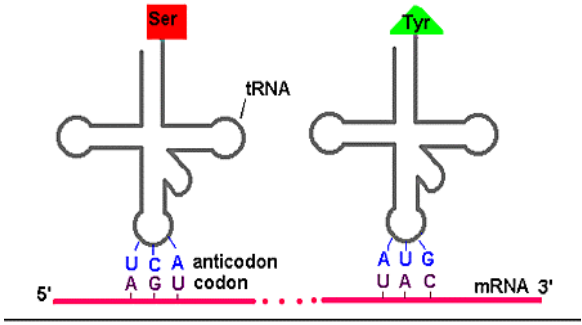
1st base in codon	2nd base in codon: U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val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

Mutation Exercise

- (5') G G A T A G C A T G A A A . C C G C A T A A (3')
- Antisense (3') T A C T T T . G G C G T A T T (5')
- mRNA (5') A U G A A A . C C G C A U A A (3')
- amino acid Met Lys Pro **His** **?** **?**

- (5') G G A T A G C A T G **T** A A C C A G C A T A A (3')
- Antisense (3') T A C **A** T T G G T C G T A T T (5')
- mRNA (5') A U G **U** A A C C A G C A U A A (3')
- amino acid Met Stop

- (5') G G A T A G C A T G A A A **T A A** C C A G C A (3')
- Antisense (3') T A C T T T **A T T** G G T C G T (5')
- mRNA (5') A U G A A A **U A A** C C A G C A (3')
- amino acid Met Lys Stop



2nd base in codon

1st base in codon	U	C	A	G	3rd 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

The Genetic Code

Questions ?