

# DESIGNER GENES NOTES

**By: GuyFromNowhere**

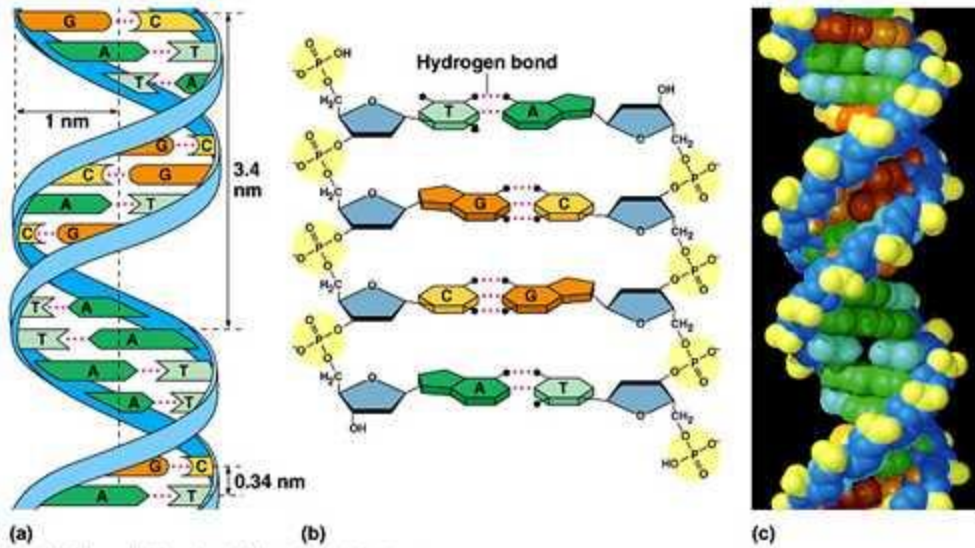
**Nota Bene:**

**For those with basic Heredity knowledge, proceed.**

**For those without basic Heredity knowledge, it is recommended that you review that sectionn first.**

## DNA Structure and Function

- DNA: Deoxyribose Nucleic Acid. Genetic Hereditary Material.
- Three key properties required for DNA's function
  - The structural features of DNA must *allow faithful replication*.
  - The genetic material must *have informational content*.
    - Sequence of nucleotide pairs dictates the sequence of amino acids in the protein specified by the gene.
  - The genetic material must *be able to change*.
- Watson and Crick discovered the double helix model through “model building” (assembly of previous and ongoing experiments) process in 1953.
- Rosalind Franklin's X-ray diffraction allowed for determination of the double-helix model.
- Building Blocks of DNA
  - Phosphate Group
  - Deoxyribose-Sugar with only a hydrogen atom at 2' carbon as opposed to ribose which has hydroxyl group attached to 2'.
  - Four nitrogenous bases-Adenine, Guanine, Cytosine, and Thymine.
    - Adenine and Guanine have a double-ring structure: they are purine.
    - Cytosine and Thymine have a single-ring structure: they are pyrimidine.
  - The chemical components of DNA are arranged into groups called nucleotides.
    - Nucleotide=phosphate, deoxyribose sugar, and any of the four bases.
  - Chargaff's rule shows the relationship regarding the amount of nucleotides in DNA.
    - The total amount of pyrimidine nucleotides equals the total amount of purine nucleotides.
    - The amount of T always equals the amount of A while amount of C always equals the amount of G.
    - A=T, C=G. A pairs with T and C pairs with G
      - A and T pair have two hydrogen bonds while C and G have three; thus, DNA with many G-C pairs is more stable.
- The Double Helix
  - Two nucleotide strands are held together by hydrogen bonds between the bases of each strand, forming a structure like a spiral staircase.
  - The backbone of each strand is formed by alternating phosphate and deoxyribose sugar units connected by phosphodiester bonds.
  - Two backbones are antiparallel, or facing opposing directions.
    - One backbone goes from 5' to 3' while the other goes from 3' to 5'. (3' and 5' denote the sugars in the ring.)
  - Each base is attached to 1' carbon of the carbon ring.

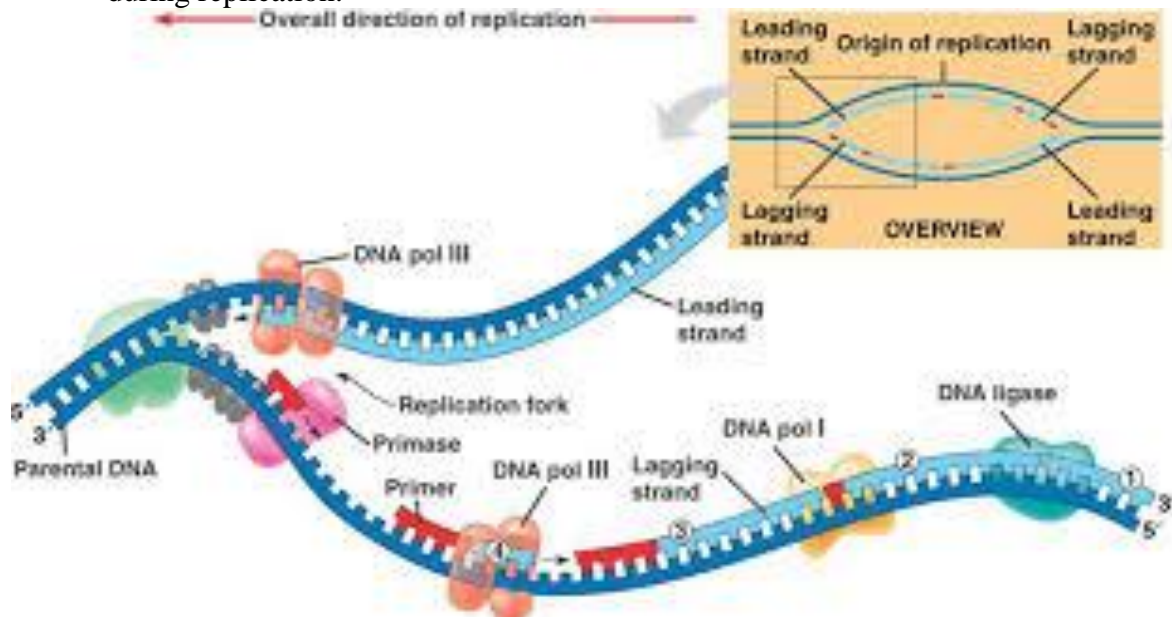


- Nucleosomes
  - Balls of double helix DNA wrapped around histone proteins
  - Eight histone proteins compose a nucleosome-nucleosomes make up basic structures of **chromatin**.

## Semiconservative Replication

- Analogous to a zipper: Unwinding of two joined strands to expose elements on a single strand.
- The separated strands have exposed bases with a potential to bind to complementary bases. (A to T, C to G)
- Two single strands after separation acts as a template for the assembly of complementary bases in order to create a double helix *identical* to the original.
- Three models were proposed after the discovery of double helix by Watson and Crick.
  - Semiconservative replication: double helix of each daughter DNA molecule contains one strand from the parent and one new synthesized strand.
  - Conservative replication: parent DNA molecule is conserved and a single daughter double helix is produced.
  - Dispersive replication: daughter DNA molecule consists of strands each containing segments of both parental DNA and newly synthesized DNA.
- Meselson-Stahl Experiment supported the Watson-Crick's *semiconservative* replication model.
- The replication fork
  - Location in which the double helix is unwound to produce the two single strands for template.
- DNA polymerase
  - Enzyme that adds deoxyriboses to 3' end of a growing nucleotide chain.
  - Triphosphate with deoxyribonucleotides.

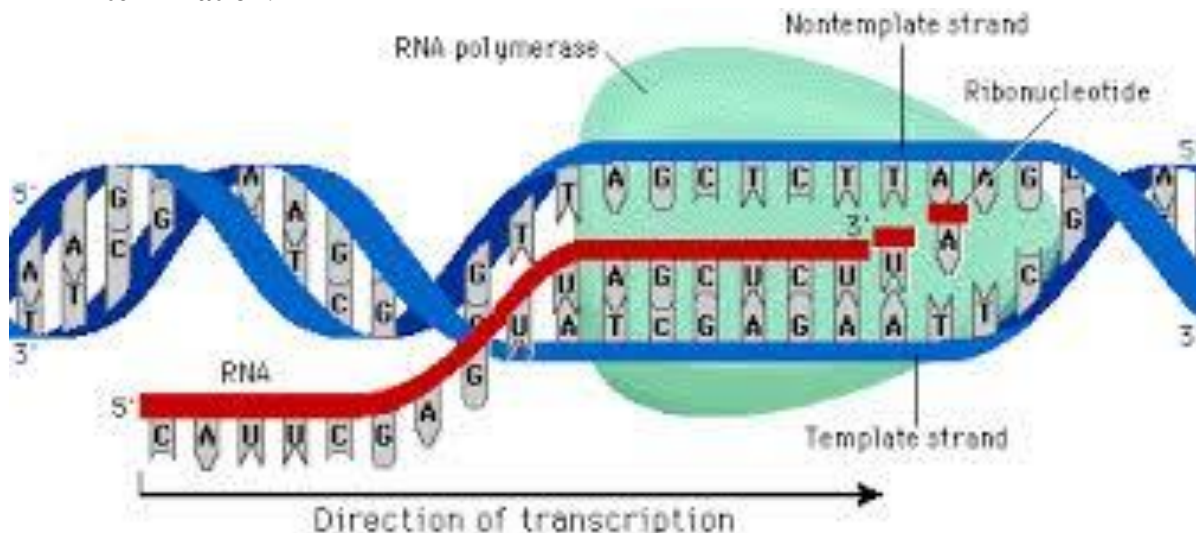
- Two inorganic phosphate molecules are removed after attaching the deoxyribose nucleotide-energy produced by the removal and hydrolysis helps drive the process of adding nucleotide bases.
- **Pol I**
  - Type of DNA polymerase that has three main activities:
    - Catalyzes chain growth in the 5'-3' direction.
    - 3' to 5' exonuclease activity to remove mismatched bases.
    - 5'-3' activity to degrade single strands of DNA or RNA-removal of primers.
- **Pol III**
  - Type of DNA polymerase: acts as **a replication fork**.
    - Since replication only occurs in 3' growing tip, the antiparallel strands replicate and grow in different directions.
    - Leading strand grows toward the replication fork; lagging strand grows away from the fork.
    - Lagging strand is made possible through **Okazaki fragments**-lagging strands is produced in short stretches called Okazaki fragments of 1000-2000 nucleotides and later joined by primer, a short chain of nucleotides
    - Primosome creates the RNA primers, which serve to link the Okazaki fragments together like glue. Primase, a type of RNA polymerase, is a main component of primosome.
    - Pol I removes the primers.
    - Ligase brings the fragments together.
- Pol I and Pol III's exonuclease 3' to 5' "proofreading" allows for extreme accuracy during replication.



REPLICATION

# Transcription

- Properties of RNA
  - RNA is a **single-stranded** nucleotide chain, not a double helix; however, some of its bases may pair up with other bases in the RNA chain, providing it an unique shape.
  - RNA contains a ribose sugar with a hydroxyl group as opposed to **deoxyribose**, which contains simply hydrogen.
  - RNA nucleotides have Adenine, Guanine, and Cytosine, but instead of thymine contains Uracil. **Uracil** pairs up with Adenine, and Guanine pairs up with Cytosine during transcription. (Uracil may pair up with Guanine through very weak hydrogen bonds for complicated structures)
  - RNA can catalyze biological reactions.
- Transcription process
  - RNA is produced by a process that produces a complementary sequence to the DNA; DNA serves as a **transcription template**.
  - A pairs with T in the DNA, G with C, C with G, and U with A.
  - For Ex.
    - DNA: GGTAACAGTCTG
    - RNA: CCAUUGUCAGAC
  - RNA also has a 5' and a 3' end; just like DNA, RNA always grows in 5' end to 3' end.
  - **RNA polymerase** attaches to the DNA and links the aligned ribonucleotides to the RNA molecule.
- There are three distinct stages of transcription for eukaryotes: **initiation, elongation, and termination**.



- **Initiation**
  - Proteins called activators bind to regions in the DNA called distal control elements-regions placed before the promoter.
  - General Transcription factors bind to regions in the promoter, a specific DNA sequence marking initiation.
  - RNA polymerase binds to the promoter region.

- The transcription factors and RNA polymerase II bound to the promoter region is called transcription initiation complex.
- TATA box, a region of nucleotides TATA about 25-30 nucleotides upstream from the transcriptional start point, mark an important initiation point for the promoter region.
- **Elongation**
  - RNA polymerase untwists the double helix and exposes 10-20 nucleotides for transcription with RNA nucleotides.
  - RNA are produced and detached from double helix template afterward.
  - Growing strand of RNA tails off from the RNA polymerase.
- **Termination**
  - The RNA finally codes for the polyadenylation signal (AAUAAA).
  - The proteins associated with the RNA stops about 10-35 nucleotides down except RNA polymerase, which continues for hundreds of nucleotides afterward producing adenosine nucleotides.
  - Spare RNA produced by the continued transcription may be used by the enzymes.
- **Post-transcriptional** (technically speaking cotranscriptional) processes
  - RNA undergoes many changes during/after transcription.
    - **Nota Bene:** these processes were believed to be post-transcriptional; however, the experimental evidence now indicates that these processes are rather cotranscriptional, occurring during the transcription process simultaneously as the RNA is produced.
  - Three main post-transcriptional processes are **processing of 5' and 3' ends, RNA splicing/removal of introns, and alternative splicing.**
  - 5' end gains a **cap**, a 7-methylguanosine residue (altered guanosine) connected by three phosphates.
    - The cap serves for protection of the RNA from degradation and is a crucial component for translation.
  - 3' end gains lots of spare RNA due to the continual of the RNA polymerase II- RNA polymerase adds a tail of 50-250 adenosine nucleotides to the RNA called the **Poly-A tail.**
  - RNA is spliced- introns are removed and exons are joined.
    - Introns-parts of RNA that are not used for translation and protein production.
    - Exons-part of RNA that are used for translation for protein production.
    - Splicing process brings together the expressed region of exons.
      - Small nuclear ribonuclearproteins, called snRNPS, recognize splice sites for introns.
      - snRNPS join additional proteins to form a large assembly called the **spliceosome.** The spliceosome is responsible for the removal of introns into a lariat-shaped loop.
  - Alternative splicing produces different RNAs from the same primary transcript.
    - Exons are mixed and matched to different exons in different combinations and lengths to produce different proteins from the same mRNA.
    - Alternative splicing, or exon shuffling, is responsible for the variety of proteins in humans despite the relatively small number of genes.

# Translation

- The Genetic Code
  - The triplet code, or three letter code, make up each codon.
  - Each **codon** is analogous to a word, while each nucleotide is analogous to a letter. Each codon codes for a amino acid.
  - 64 amino acids are possible with four existing RNA nucleotides (A, U, G, C); there are only 20 amino acids.
    - Amino acids are components of proteins; they are characterized by their nitrogen amino group and the carboxyl group.
  - The genetic code is **nonoverlapping**-that is, it has a defined start for a codon based on the triplet. For example, the genetic code would not code for UUG with AUUGCC.
  - Each codon codes for following abbreviated amino acids.

	U	C	A	G
U	UUU = phe UUC = phe UUA = leu UUG = leu	UCU = ser UCC = ser UCA = ser UCG = ser	UAU = tyr UAC = tyr UAA = stop UAG = stop	UGU = cys UGC = cys UGA = stop UGG = trp
C	CUU = leu CUC = leu CUA = leu CUG = leu	CCU = pro CCC = pro CCA = pro CCG = pro	CAU = his CAC = his CAA = gln CAG = gln	CGU = arg CGC = arg CGA = arg CGG = arg
A	AUU = ile AUC = ile AUA = ile AUG = met	ACU = thr ACC = thr ACA = thr ACG = thr	AAU = asn AAC = asn AAA = lys AAG = lys	AGU = ser AGC = ser AGA = arg AGG = arg
G	GUU = val GUC = val GUA = val GUG = val	GCU = ala GCC = ala GCA = ala GCG = ala	GAU = asp GAC = asp GAA = glu GAG = glu	GGU = gly GGC = gly GGA = gly GGG = gly

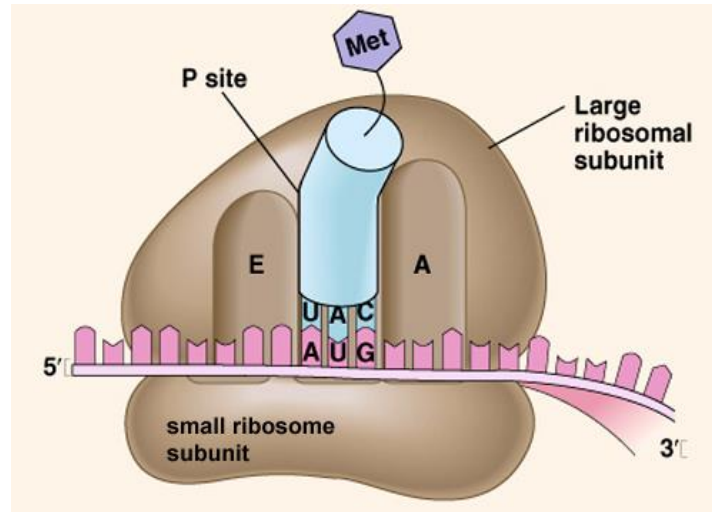
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Name	Abbreviation	Linear Structure
<u>Alanine</u>	ala A	CH <sub>3</sub> -CH(NH <sub>2</sub> )-COOH
<u>Arginine</u>	arg R	HN=C(NH <sub>2</sub> )-NH-(CH <sub>2</sub> ) <sub>3</sub> -CH(NH <sub>2</sub> )-COOH
<u>Asparagine</u>	asn N	H <sub>2</sub> N-CO-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Aspartic Acid</u>	asp D	HOOC-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Cysteine</u>	cys C	HS-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Glutamic Acid</u>	glu E	HOOC-(CH <sub>2</sub> ) <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Glutamine</u>	gln Q	H <sub>2</sub> N-CO-(CH <sub>2</sub> ) <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Glycine</u>	gly G	NH <sub>2</sub> -CH <sub>2</sub> -COOH
<u>Histidine</u>	his H	NH-CH=N-CH=C-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Isoleucine</u>	ile I	CH <sub>3</sub> -CH <sub>2</sub> -CH(CH <sub>3</sub> )-CH(NH <sub>2</sub> )-COOH

<u>Leucine</u>	<b>leu L</b>	(CH <sub>3</sub> ) <sub>2</sub> -CH-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Lysine</u>	<b>lys K</b>	H <sub>2</sub> N-(CH <sub>2</sub> ) <sub>4</sub> -CH(NH <sub>2</sub> )-COOH
<u>Methionine</u>	<b>met M</b>	CH <sub>3</sub> -S-(CH <sub>2</sub> ) <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Phenylalanine</u>	<b>phe F</b>	Ph-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Proline</u>	<b>pro P</b>	NH-(CH <sub>2</sub> ) <sub>3</sub> -CH-COOH
<u>Serine</u>	<b>ser S</b>	HO-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Threonine</u>	<b>thr T</b>	CH <sub>3</sub> -CH(OH)-CH(NH <sub>2</sub> )-COOH
<u>Tryptophan</u>	<b>trp W</b>	<b>Ph</b> -NH-CH=C-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Tyrosine</u>	<b>tyr Y</b>	HO-Ph-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH
<u>Valine</u>	<b>val V</b>	(CH <sub>3</sub> ) <sub>2</sub> -CH-CH(NH <sub>2</sub> )-COOH

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- Methionine(AUG) is the “start” codon that initiates translation
- **tRNA**
  - tRNA, transfer RNA, transfers amino acids from the cytoplasm onto the ribosome for assembly of proteins.
  - tRNA is a molecule of about 80 RNA nucleotides arranged in a cloveleaf shape; it possesses a 3’ end and a 5’ end. The amino acid attaches to the 3’ end, and the anticodon attaches to the mRNA.
  - Transfer RNA attaches itself to the mRNA, or messenger RNA (RNA produced from the DNA), using anticodon, a complementary triplet codon. For example, the codon for alanine, GCA, would be attached to tRNA CGU.
  - The amino acids are attached to tRNAs by enzymes called **aminoacyl-tRNA-synthetases** with usage of ATP.
  - There are only 45 tRNAs-this implies that some tRNAs are able to bind to more than one codon.
  - tRNAs attached to an amino acid is said to be charged.
- **Ribosomes**
  - Ribosome is an organelle responsible for translation-production of protein.
  - Ribosomes are constructed from ribosomal RNAs and proteins.
  - Ribosome has two subunits-large and small subunits.
  - Ribosome has four binding sites
    - mRNA binding site binds and holds the mRNA.
    - **A** (Aminoacyl-tRNA binding site) attaches to the charged tRNA.
    - **P** (Peptidyl-tRNA binding site) attaches to the tRNA holding the growing polypeptide chain.
    - **E** (Exit site) attaches and releases the discharged tRNA.



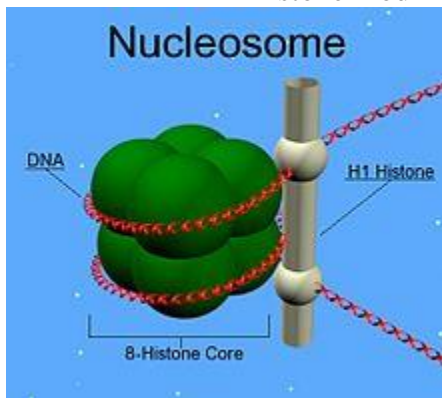


- There are three stages of translation: initiation, elongation, and termination.
- **Initiation**
  - The small subunit attaches to the mRNA.
  - The initiator methionine tRNA binds to the start codon, AUG.
  - The large subunit arrives and completes the translation initiation complex with the aid of proteins called initiation factors.
- **Elongation**
  - Amino acids are added one by one to the preceding amino acid.
  - First, anticodon of an incoming aminoacyl tRNA base binds to the mRNA in the A site of the ribosome.
  - An rRNA molecule of the large ribosomal unit on top catalyzes the formation of peptide bond between the amino acid on the A site and the peptide chain at the P site.
  - The ribosome translocates, or moves, the tRNA from the P site to the E site. The tRNA is released.
- **Termination**
  - Stop Codon reaches the A site of the ribosome.
  - Proteins called release factors bind directly to the stop codon on the A site.
  - Water molecule is added instead of amino acid to the end of the polypeptide bond; hydrolysis releases the completed protein.
- **Post-translational** modification (Not to be confused with equally important post-transcriptional modification)
  - Protein is folded, forming hydrogen bonds between nucleotides to form structures.
    - Chaperonin aids in the folding of the polypeptide
  - Attachment of sugars, lipids, and phosphate groups.
  - Enzymes may remove some amino acids from the polypeptide.
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## Control and Detection of Gene Expression (Eukaryotes)

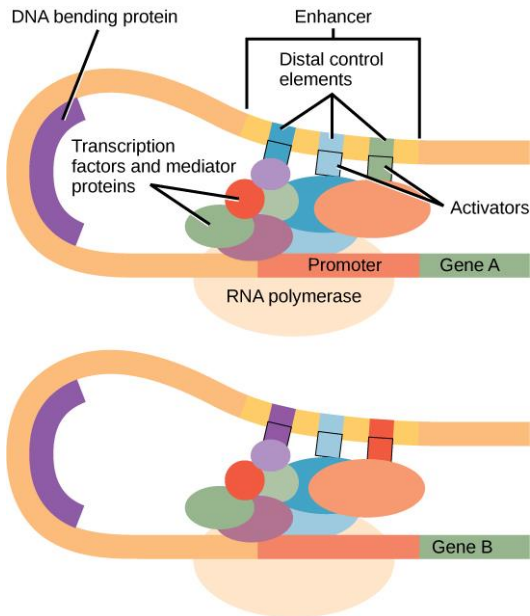
- Prior to differentiation, all the cells have almost identical genome.
- Differential gene expression is responsible for the variety of differentiated cells for different functions.

- Regulation of gene expression occurs in many levels: for ex, transcription, translation.
- Most regulation occurs at transcriptional level.
- Chromatin structure modification
  - The structure of the chromatin can be modified to affect the transcription.
  - **Histone Modification**
    - Recall that DNA is wrapped around eight histones-ordinarily, the histone proteins have ends that bind to neighboring histones.
    - Addition of acetyl group to the ends, or tails, of histone proteins loosens up the compact chromatin and allows for transcription.
    - Acetyl groups are  $\text{COCH}_3$
    - Methylation of histone tails can promote condensation and prevent transcription. (Addition of methyl group)
    - Phosphorylation of histones adjacent to methylated histones can aid transcription. (Addition of phosphate group)
    - Histone modifications can be **carried on** from one generation to the next.



- **DNA Methylation**
  - Methyl group is  $\text{CH}_3$
  - A methyl group can be attached to the 5 carbon of a nucleotide to prevent transcription of the DNA for that specific nucleotide.
  - Methylation patterns are passed down from generation to generation and are important for long term inactivation of a gene.
- The traits transmitted by mechanisms not directly involving the sequence but the expression of the sequence is called **epigenetic inheritance**, ex. Histone Modification and Methylation.
- **Gene silencing**
  - DNA “cassettes,” or areas of silent and active expression, may perform recombination for long term inactivation.
    - Example is  $\text{HMR}_\alpha$  and  $\text{HMR}_\alpha$ ; though the *S. Cerevisiae* has three regions for determining mating type, but  $\text{HMR}_\alpha$  and  $\text{HMR}_\alpha$  are silent and act to repress each other and keep the  $\text{MAT}_\alpha$  active through recombination.
- **Regulation of transcription initiation.**
  - Proteins called transcription factors affect transcription initiation.
  - Quick review of steps in transcription initiation
    - Activators bind to the distal control elements, also acting as an enhancer.

- DNA-bending protein bends the DNA so that the activators can touch the promoter region with the help of general transcription factors and mediator proteins.
- The general transcription factors such as RNA Polymerase II attaches to the promoter region
- Thus, the presence of certain activators can affect the expression of the gene
  - Certain cells have certain activators that can change which distal control elements will affect which promoter region.



- **Post-transcriptional regulation/control**
  - RNA Splicing (Refer to transcription section)
  - mRNA degradation
    - For bacteria, mRNA degrades rapidly and naturally.
    - For eukaryotes, certain enzymes break down the poly-A tail and the cap and the nuclease enzymes breaks down the mRNA.
- **Translation Initiation**
  - Translation can be prevented by attachments of regulatory proteins at the 5' end.

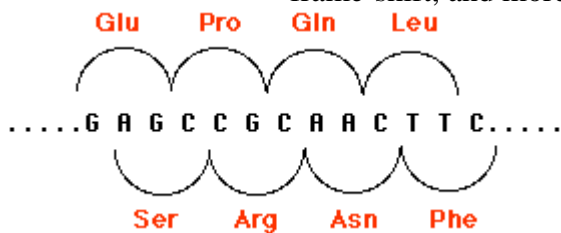
## Mutation and Repair

- **Mutations**
  - Changes to the genetic information due to change in the genetic code.
  - Ultimate source of new genes.
- **Three outcomes of mutations**
  - **Synonymous Mutations:** The mutation changes one codon for amino acid into another codon for that same amino acid. Synonymous mutations are also referred to as silent mutations.
  - **Missense mutations:** The codon for one amino acid is changed into a codon for another amino acid. Missense mutations are also referred to as nonsynonymous mutations.
  - **Nonsense mutations:** The codon for one amino acid is changed into a translation-termination codon.

- Synonymous or missense mutation may result in conservative substitution-that is, the amino acids are chemically similar even after the mutation and function isn't affected.

- **Point mutations**

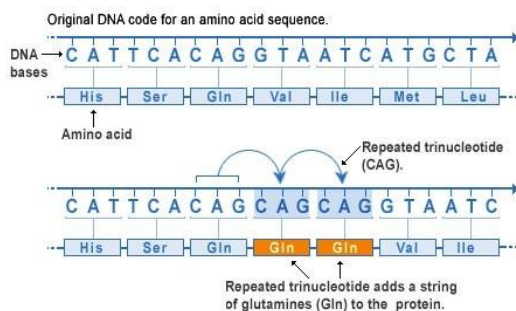
- Chemical changes in a single base pair of a gene.
- Base-pair substitution
  - Replacement of one nucleotide and its partner with another pair of nucleotides.
  - Usually missense mutations.
- Insertion and Deletions (Also called **Indel** mutations)
  - Addition or losses of nucleotide pairs in a gene.
  - Much more damaging than substitution
    - May alter the reading frame-the entire triplet codon is shifted one nucleotide forward or backward.
  - All the nucleotides after an insertion or deletion may experience the frame-shift, and more than often the protein will be nonfunctional.



- Mechanisms of mutation

- One of the nucleotide bases may become electronically ionized during tautomerization (conversion of isomers). This may result in incorrect pairing.
- **Transversions** may occur-it is possible for mispairing of purine with purine, or pyrimidine with pyrimidine though unstable.
- **Frameshift** mutations can occur due to the failure of a base “slipping” out from the loop during replication-the polymerase ignores the one nucleotide.
  - This may occur for entire separate loops to cause trinucleotide-repeat diseases.

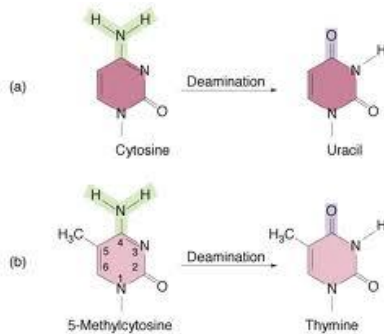
Repeat expansion mutation



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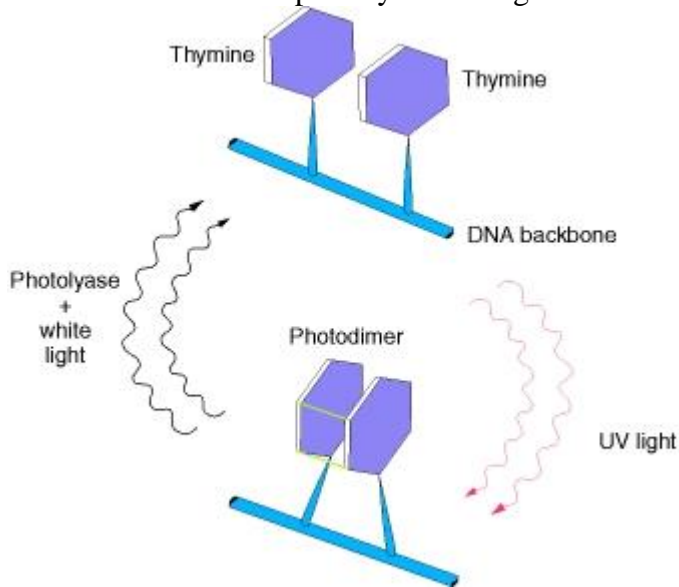
- Mutagens
  - Chemical and physical agents that cause mutations.
    - Ex. UV light may create thymine photodimers that are nonfunctional.

- Spontaneous Lesions-Naturally occurring damage to DNA.
  - **Depurination**-Loss of purine base due to the weakening of the chemical link between deoxyribose and guanine or adenine.
  - **Deamination**-Loss of amine group from Cytosine will result in Uracil.



- Mutation Repairs

- DNA Pol III during replication
- Direct reversal and fixing of damaged DNA.
  - For ex. thymine photodimers can be fixed through protein called CPD photolyase and light.

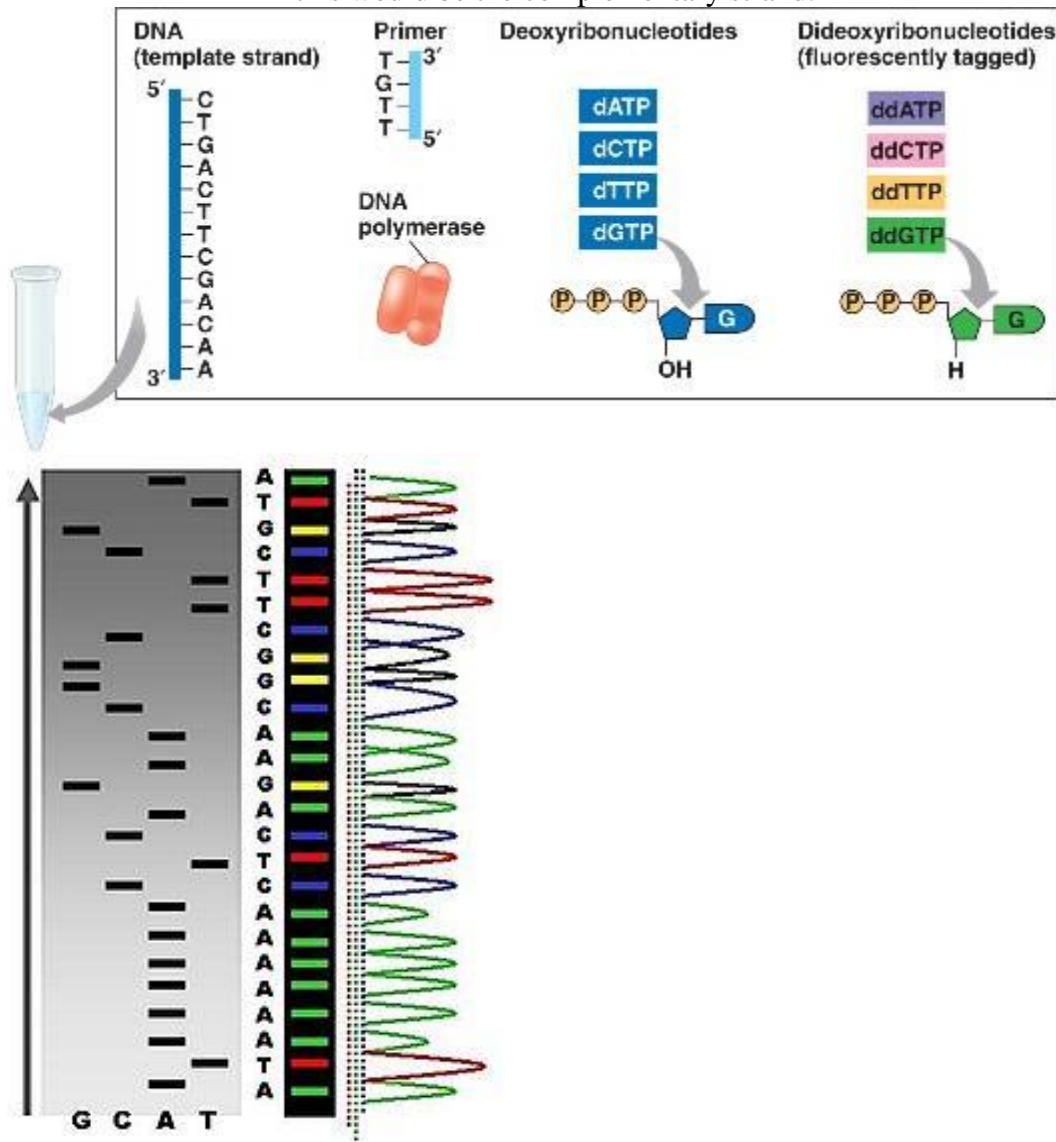


- Base-excision repair can repair minor base damage.
  - Removal and addition of new DNA bases
    - DNA glycosylase cleaves base-sugar bond.
    - AP endonuclease makes the cut.
    - dRpase removes stretch of DNA.
    - Polymerase synthesizes new DNA.
    - Ligase seals the connection between the new DNA and the new DNA.

## DNA Sequencing

- Dideoxy Chain Termination Method (Sanger Method)
  - Three Step Process

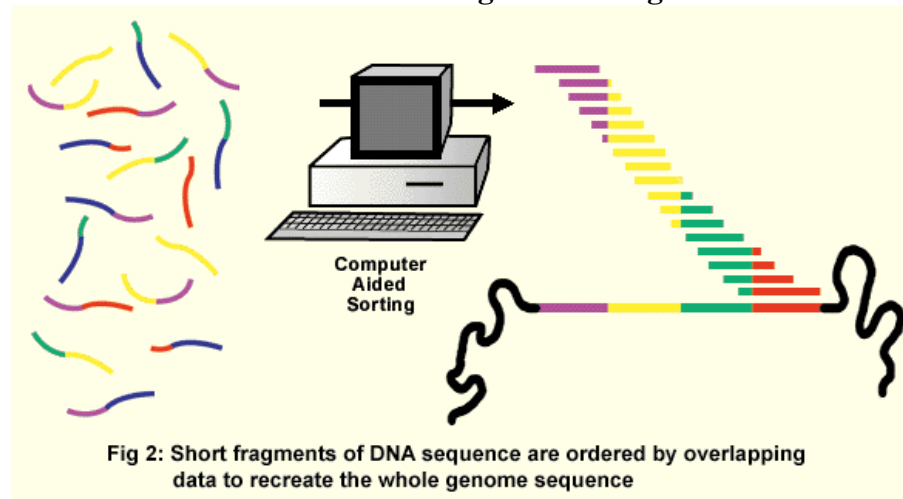
- The fragment of DNA is denatured into a single strand and incubated into a test tube. PCR (see section) amplifies the section of the DNA. A primer and a DNA polymerase are added along with ordinary and fluorescent deoxyribonucleotides.
- The DNA polymerase adds deoxyribonucleotides to appropriate sites of the template DNA. Once the fluorescent deoxyribonucleotide is added to the single stranded DNA, the elongation can no longer continue.
- There are now numerous strands of DNA of different lengths with an end of fluorescent deoxyribonucleotides. Separate the fragments by length using polyacrylamide gel—shorter fragments move faster and farther. Use laser and detector to show which nucleotide is where. Note, however, that this would be the complementary strand.



## DNA Sequencing (Entire Genome)

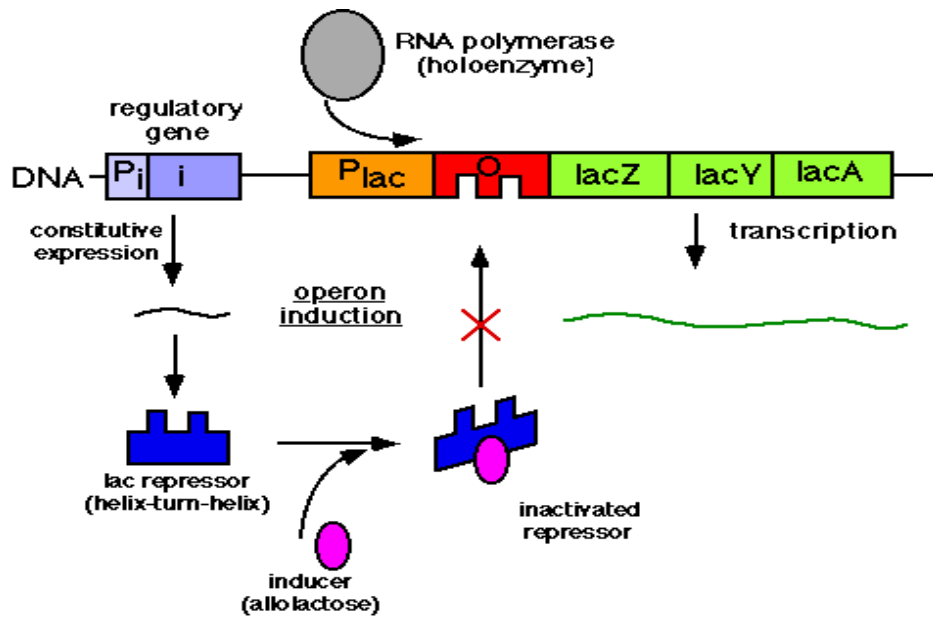
- Four step process

- Cut many copies of the genome into thousand to millions more or less random small fragments.
- Read the sequence of each small segment
- Compute overlaps in the segments.
- Connect the segments through the overlaps to create one comprehensive sequence.
- The human genome project has sequenced the entire human genome of 22 autosomes and X and Y chromosomes.
- This method is called **whole genome shotgun method**.

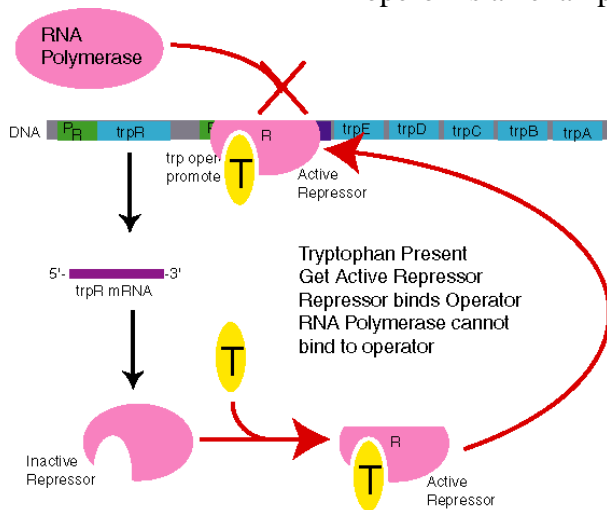


## Lac and TRP Operons

- Operons
  - Group of genes linked together by a single promoter region.
  - Lac operon
    - First to be discovered
    - Responsible for production of enzymes for digestion and usage of lactose for energy such as B-Galactosidase, permease, and transacetylase.
    - Under the command of a single **operator** and **promoter**.
    - Three genetic parts: lacA codes for transacetylase, lacY codes for Permease, and lacZ codes for B-Galactosidase.
    - If the lactose is not present, then no transcription occurs because of the presence of the repressor on the **operator** placed slightly downstream of the promoter region.
    - If lactose is present, then transcription occurs because the lactose's isomer, allactose, acts an **inducer** that takes off the **repressor** and allows for transcription of the lac operon.
    - Lac operon is a type of negatively-induced operon.



- TRP operon
  - Similar to Lac operon
  - However, rather inducing, this operon is an example of repressible operon.
  - Responsible for production of enzymes for metabolizing tryptophan.
  - Usually transcribed-however, excess of tryptophan presence may cause repression.
  - Tryptophan acts as a **corepressor**; it binds to the **repressor** which in turn binds to the **operator** on the **promoter** region, preventing transcription.
  - TRP operon is an example of negatively repressed operon.



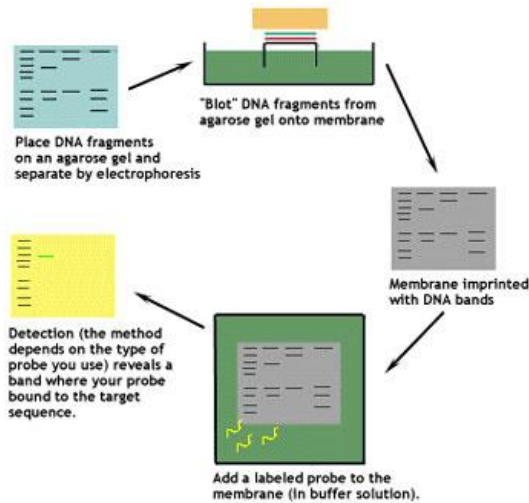
- The Lac and TRP operons are negative operons because they affect the behavior of repressor protein.
- There are also positively induced and positively repressed operons that affect the behavior of the activator protein.

## Southern Blotting and Other Techniques

- Southern Blotting
  - First type of blotting

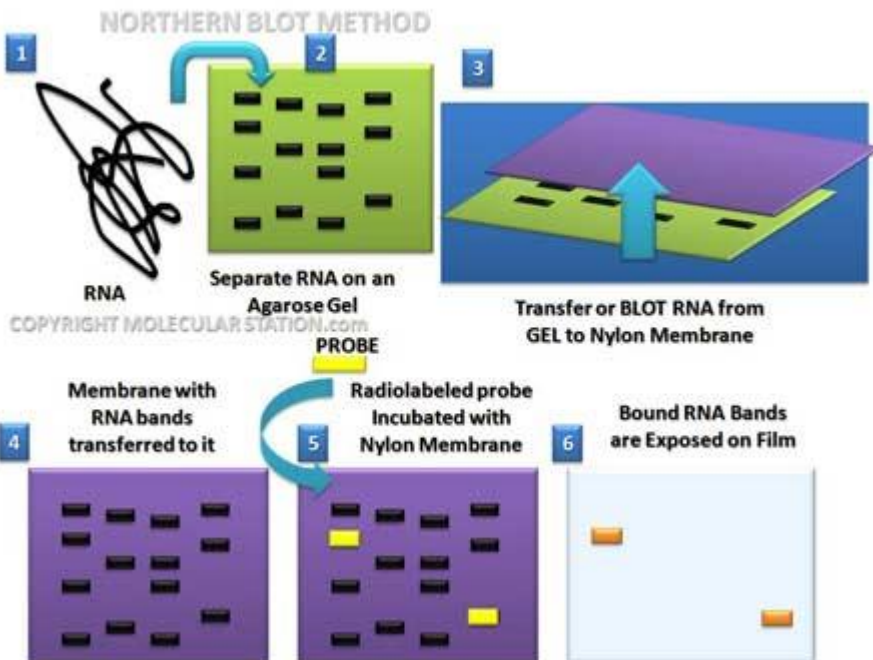


- Named after inventor Edward Southern
- Detects and profiles DNA fragments based on their size
  - Essentially, DNA fragments are passed through a gel; smaller fragments will travel farther through the gel when the gel is conducted with positive and negative poles.
  - Restriction enzymes cut the DNA at appropriate sites prior to passing through the Gel
  - The procedure is called gel electrophoresis.
  - RFLP is done through southern blot and targets SNPs. (Refer to RFLP section for more details)



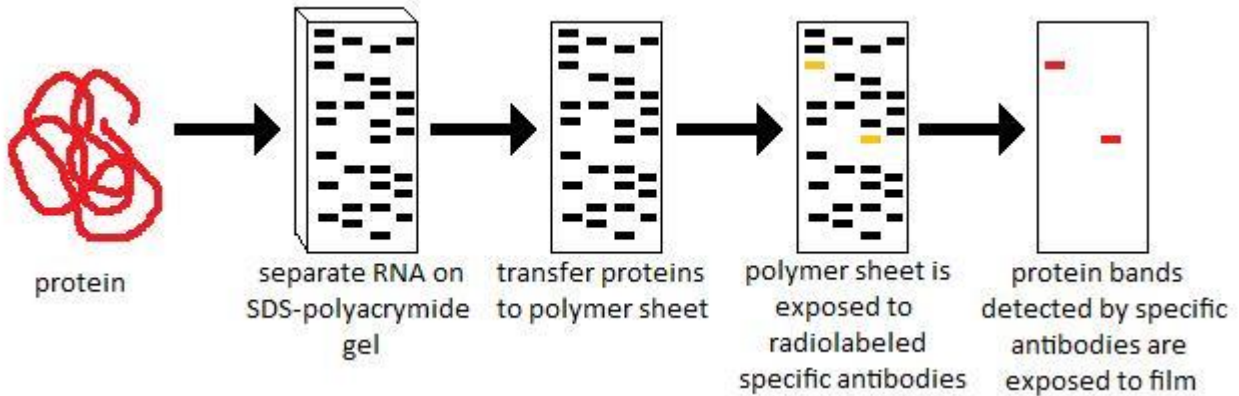
- **Northern Blotting**

- Similar to southern blotting in procedure.
- However, targets RNA.
- Again, similarities in the banding patterns signify relationship.



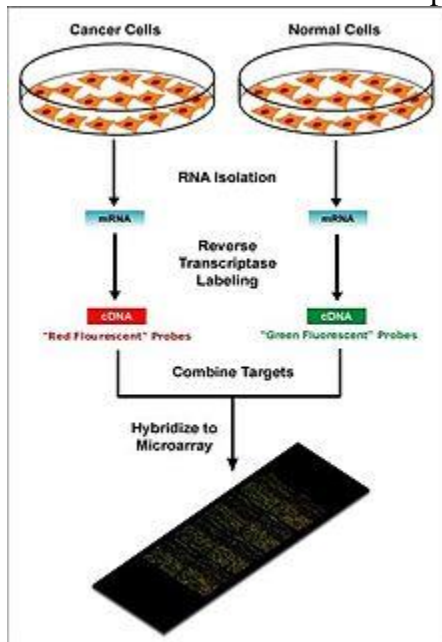
- **Western Blotting**

- Similar to Southern blotting in procedure.
- However, targets proteins based on their different polypeptide lengths.



- **DNA microarray**

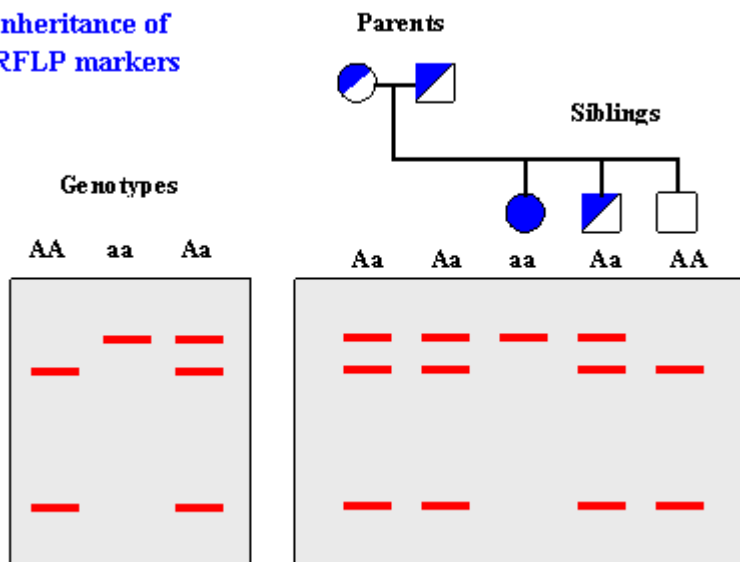
- Shows amount of gene expression in mRNA.
- Six Step Process
  - Extract mRNA
  - Make cDNA (complementary DNA) transcript with reverse transcriptase. Prepare a microarray filled with short synthetic oligonucleotides representing most or all of the genes in a genomes.
    - Oligonucleotides: Short synthetic single-stranded RNA molecules.
  - Usually, two sets of cDNA are used; a control for organism without exposure to a certain condition and an experimental for organism with exposure to a certain condition. Both cDNA sets are labeled with fluorescent dyes.
  - Laser emission shows which cDNA binds with which microarrays; thus, level of expression is shown.



## RFLP/DNA Fingerprinting

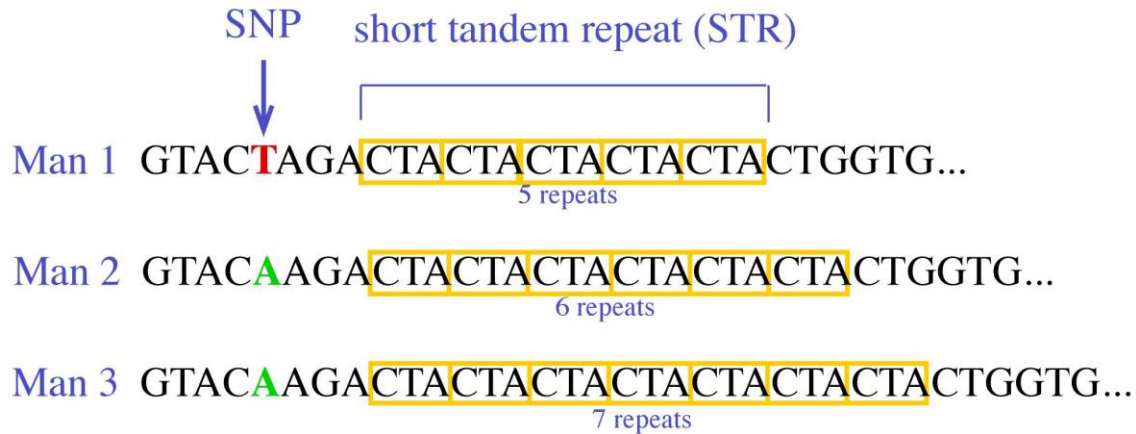
- DNA Fingerprinting is used to assist in identification and profiling of an individual in a crime scene, paternal testing, etc.
- Full genome sequence is **not** DNA fingerprinting.
- **RFLP** and **STR** analysis are two most commonly used for DNA fingerprinting; STR used with PCR (see section) is the most commonly used recently.
- RFLP
  - Restriction fragment length polymorphism
  - Works on the principle that each individuals have single nucleotide polymorphisms, abbreviated SNPs.
    - Genome sequences are about 99.9% identical; however, the .1% varies per individual.
    - Approximately 1 in every 300 to 1000 bases are different for an individual; this is a **single nucleotide polymorphism**.
    - SNPs are inherited per generation.
    - The SNPs can be targeted by restriction enzymes, which will make a cut at the specific nucleotides.
    - Thus, some DNAs will be cut into small fragments at the presence of certain SNPs; others without the SNPs will not be cut.
  - The different sized fragments are then subjected to Southern Blotting; the samples with similar blot patterns are essentially related because they contain similar SNPs.
  - RFLP is a laborious technique.

### Inheritance of RFLP markers



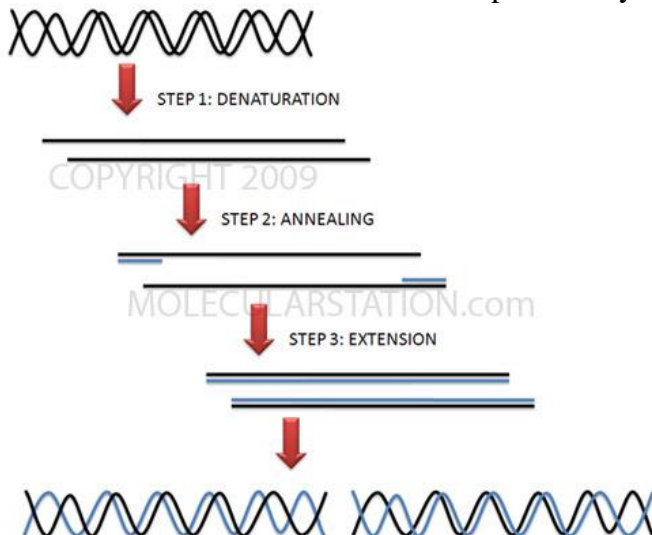
- **STR** analysis
  - VNTR: Variable Number Tandem Repeats
    - Human gene is filled with repetitive DNAs.
    - However, the amount of repetitive DNAs differs per individual and is inherited.
    - The repetitive DNAs are called tandem repeats.

- Short Tandem Repeats (STR), or also referred to as **microsatellite** marker, are repeats of simple sequences usually about 4-5 bases long.
- These tandem repeats can be amplified with PCR and ran through gel electrophoresis for analysis and identification.



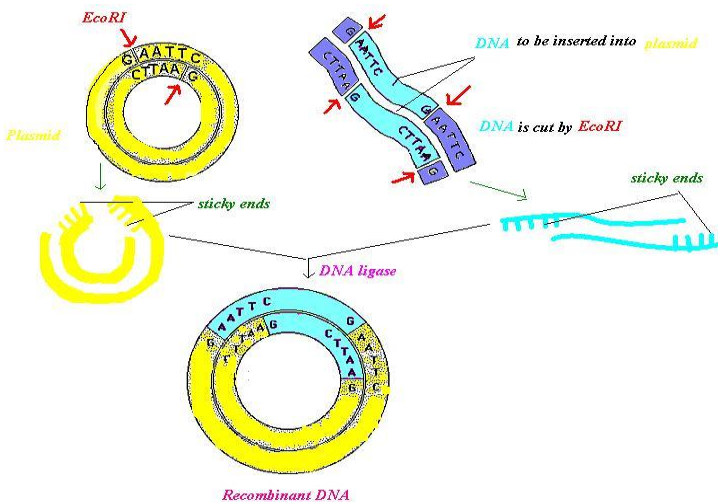
## PCR

- Polymerase Chain Reaction
- Quick way to make numerous copies of desired DNA or to amplify the sample of DNA.
- Before PCR, gene had to be amplified through in vivo method; that is, the desired gene was added to a plasmid and numerous cells were grown.
- PCR is done in vitro without having to grow cells.
  - Oligonucleotide DNA primers are added to the DNA sample.
  - Strands are heated and separated at 95 degrees Celsius.
  - The strands are cooled and the primers attach to respective strands at the 5' end of the desired section.
  - The primer attached strands are heated again at 72 degrees Celsius to allow DNA synthesis.
  - The steps are repeated again and again, and DNA is amplified exponentially
    - By 25 cycles, the target sequence has been amplified about  $10^6$  fold.
- PCR is used for short tandem repeats analysis.



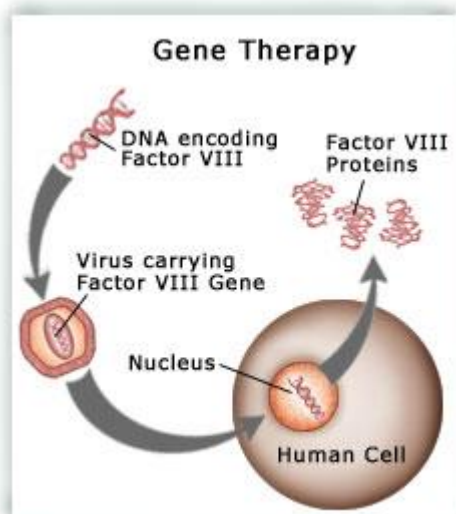
# Plasmid Selection and Isolation

- **Plasmids**
  - Small, double-stranded, circular DNA in bacteria.
  - Very useful for storing and to some degree, amplifying, genetic information.
    - Plasmid is physically separate from the bacterial chromosome and can replicate independently.
  - For that reason, plasmids are often used as “genomic libraries”
    - Desired genes are added, or **recombined**, to the plasmid, with the plasmid acting as a **vector** or **carrier**.
    - The desired genes are then amplified through reproduction of the bacteria and can be stored for future usage.
    - Viral bacteriophages can be also used for similar purpose.
- **Recombination or Transformation** of the bacterial plasmid.
  - Essentially, the plasmid is cut and the new DNA is inserted.
  - Protein called restriction enzyme makes a cut to both the Donor DNA and the plasmid.
    - An example of the restriction enzyme would be the EcoRI endonuclease.
    - The cut usually has a staggered (sticky) end; that is, there are some single-stranded regions at the end.
      - Without the staggered end, the recombination is much more difficult.
    - DNA ligase connects the cleaved plasmid to the cleaved DNA; this process is referred to as **hybridization**.
- Selection of Vector
  - Vectors must be small molecules for convenient manipulation.
  - If amplification is desired, prolific replication of the vector’s cell is also good.
  - Vectors must have convenient restriction sites for the cut made by the restriction enzyme.
  - Ideally, restriction site should be present just once in the vector.
  - The recombinant molecule should be easily recoverable.



## Gene Therapy

- Introducing genes into an afflicted individual for therapeutic purposes
- In theory, normal allele can be inserted into a cell with defective gene.
- For permanent effect, the cells that reproduce must be the ones that receive the normal alleles.
  - Bone marrow cells are prime target.
- Example of a treatment:
  - RNA version of the normal allele is created through transcription and inserted into a retrovirus. (Viruses contain RNA, not DNA).
    - Retroviruses start out as RNA, insert themselves into the cell, and perform reverse transcription to become DNA and be incorporated into the cellular chromosome.
    - The retroviruses used for gene therapy are harmless.
  - Retroviruses are inserted into the desired cells; the retroviruses perform reverse transcription and insert their viral DNA, and the normal allele, into the cell's chromosome.
  - The cell gains normal allele.



- Such treatments have been proven effective against SCID.
- There are potential issues regarding the unknown functions of the inserted genes as well as difficulty in controlling the insertion; also, there are numerous ethical issues related to the treatment as well.

# REVIEW OF HEREDITY

## Quick Vocab:

**Basic concepts dealt with in Heredity. If you do not know some of these vocabs, consult your nearest Biology textbook, preferably Campbell Reece!**

**Gene:** Discrete unit of hereditary information consisting of a specific nucleotide sequence in DNA.

**Chromosome:** A cellular structure carrying genetic material. Found in the nucleus of eukaryotic cells. Each chromosome consists of one very long DNA molecule and associated proteins.

**Allele:** Any of the alternative versions of a gene that produce distinguishable phenotypic effects.

**Dominant Alleles** are expressed above **recessive alleles**. For ex, tall allele may be expressed despite the presence of the short allele because the tall allele is dominant.

**Character:** Heritable feature that varies among individuals.

**Traits:** Variant in character.

**Genotype:** The genetic makeup, or set of alleles, of an organism. For ex. Tt (If T stood for tall allele and t stood for short allele.) Not to be confused with Phenotype.

**Phenotype:** The physical and physiological traits of an organism, which are determined by its genetic makeup.

**Homozygous:** Genotype has identical alleles. Ex. TT

**Heterozygous:** Genotype has two different alleles. Ex. Tt

**Mitosis:** A process of nuclear division in eukaryotic cells conventionally divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.

**Meiosis:** Formation of Gametes.

**Gametes:** A haploid reproductive cell, such as an egg or sperm. Gametes unite during sexual reproduction to produce a diploid zygote.

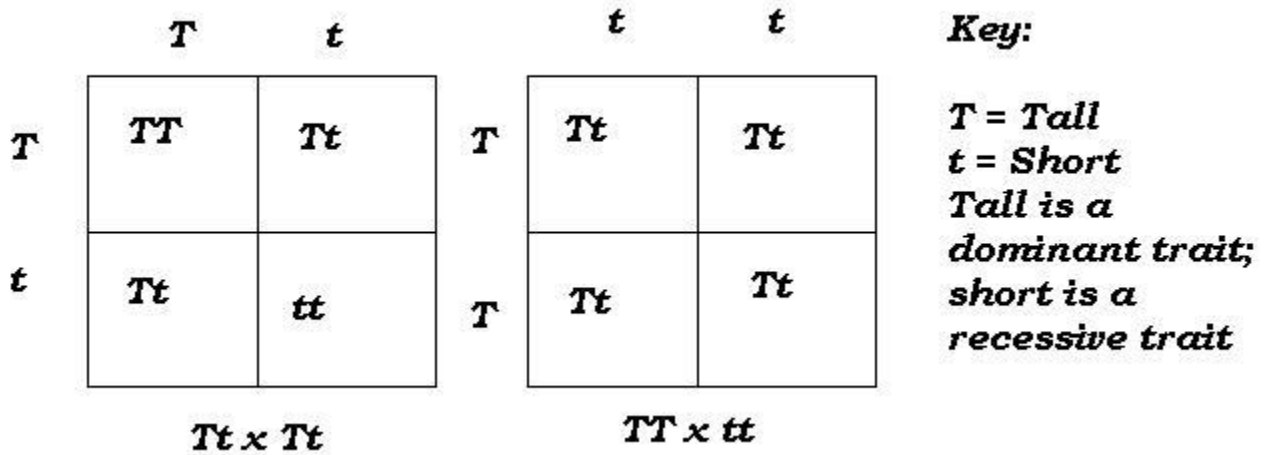
**Diploid:** Two sets of chromosome. Human cells are ordinarily diploid.

**Haploid:** One set of chromosome. Human gametes are ordinarily haploid.

## Monohybrid Cross

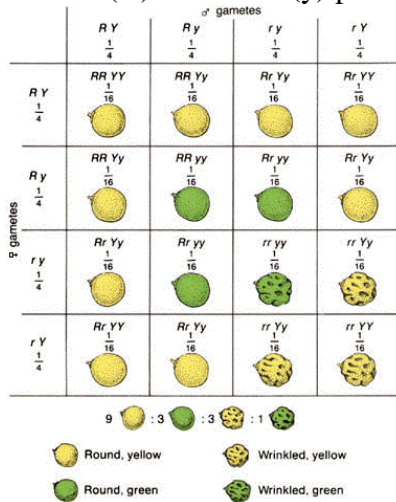
- Involves Punnet Squares
  - Named after Reginald Punnet.
  - Squares designed to show the four possible combinations of alleles that could occur when the gametes combine.
  - Top and the side of the boxes designate the alleles of the gametes.
- Monohybrid cross only cross one trait, or one pair of alleles. It is also referred to as single-factor cross.
- There are five possibilities for cross depending on the three initial genotypes of the gametes:
  - Homozygous Dominant and Homozygous Dominant (TT x TT)
  - Homozygous Dominant and Heterozygous (TT x Tt)
  - Homozygous Recessive and Heterozygous (tt x Tt)
  - Homozygous Recessive and Homozygous Recessive (tt x tt)
  - Homozygous Dominant and Homozygous Recessive (TT x tt)

- The genotypic ratio is the ratio of occurring genotypes; the phenotypic ratio is the ratio of occurring phenotypes.
- The following is the comprehensive genotypic and phenotypic ratio for a situation with dominant T (tall) allele and recessive t (short) allele:
  - Homozygous Dominant and Homozygous Dominant (TT x TT): 4/4 TT. 4/4 Tall.
  - Homozygous Dominant and Heterozygous (TT x Tt): 2/4 TT, 2/4 Tt. 4/4 Tall.
  - Homozygous Recessive and Heterozygous (tt x Tt): 2/4 Tt, 2/4 tt. 2/4 Tall, 2/4 Short.
  - Homozygous Recessive and Homozygous Recessive (tt x tt): 4/4 tt. 4/4 Short.
  - Homozygous Dominant and Homozygous Recessive (TT x tt): 4/4 Tt. 4/4 Tall.



## Dihybrid Cross

- Mating of parental varieties that differ in two characters.
- Similar to monohybrid cross except that two traits are crossed instead of one.
- The two traits and the four alleles of the gamete is shown on the top and side of a 4x4 box.
- The following is a genotypic and phenotypic ratio for Round (R) vs. Wrinkled (r) and Yellow (Y) vs. Green (y) punnet square, cross of both heterozygotes:





- 1/16 RRYy, 1/16 rryy, 4/16 RrYy, 2/16 RRYy, 2/16 rrYy, 1/16 RRyy, 1/16 rrYY, 2/16 TrYY, 2/16 Rryy
- 9/16 Round, Yellow. 3/16 Round, Green. 3/16 Wrinkled, Yellow. 1/16 Wrinkled, Green.

### Three-Factor Crosses (Trihybrid)

- Mating of parental varieties that differ in three characters.
- Similar to monohybrid cross except that two traits are crossed instead of one.
- The three traits and the six alleles of the gamete are shown on the top and side of a 8x8 box.
- The following is a genotypic and phenotypic ratio for A vs. a, B vs. b, and C vs. c punnet square, cross of both heterozygotes (AaBbCc x AaBbCc)

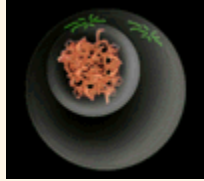
<u>SsYyAa x SsYyAa</u>								
	SYA	SYa	SyA	Sya	sYA	sYa	syA	sya
SYA	SSYYAA	SSYYAa	SSYyAA	SSYyAa	SsYYAA	SsYYAa	SsYyAA	SsYyAa
SYa	SSYYaA	SSYYaa	SSYyaA	SSYyaa	SsYYaA	SsYYaa	SsYyaA	SsYyaa
SyA	SSyYAA	SSyYAa	SSyyAA	SSyyAa	SsyYAA	SsyYAa	SsyyAA	SsyyAa
Sya	SSyYaA	SSyYaa	SSyyaA	SSyyaa	SsyYaA	SsyYaa	SsyyaA	Ssyyaa
sYA	sSYYAA	sSYYAa	sSYyAA	sSYyAa	ssYYAA	ssYYAa	ssYyAA	ssYyAa
sYa	sSYYaA	sSYYaa	sSYyaA	sSYyaa	ssYYaA	ssYYaa	ssYyaA	ssYyaa
syA	sSyYAA	sSyYAa	sSyyAA	sSyyAa	ssyYAA	ssyYAa	ssyyAA	ssyyAa
sya	sSyYaA	sSyYaa	sSyyaA	sSyyaa	ssyYaA	ssyYaa	ssyyaA	ssyyaa

- 1/64 AABBCC, 2/64 AABBCc, 1/64 AABBcc, 2/64 AABbCC, 4/64 AABbCc, 2/64 AABbcc, 1/64 AAbbCC, 2/64 AAbbCc, 1/64 AAbbcc, 2/64 AaBBCC, 4/64 AaBBCc, 2/64 AaBBcc, 4/64 AaBbCC, 8/64 AaBbCc, 4/64 AaBbcc, 2/64 AabbCC, 4/64 AabbCc, 2/64 Aabbcc, 1/64 aaBBCC, 2/64 aaBBCc, 1/64 aaBBcc, 2/64 aaBbCC, 4/64 aaBbCc, 2/64 aaBbcc, 1/64 aabbCC, 2/64 aabbCc, 1/64 aabbcc.
- 1/64 abc, 3/64 Abc, 3/64 aBc, 9/64 ABc, 3/64 abC, 9/64 AbC, 9/64 aBC, 27/64 ABC.

# Mitosis and Meiosis

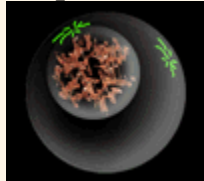
(Taken from [http://www.biology.arizona.edu/cell\\_bio/tutorials/cell\\_cycle/cells3.html](http://www.biology.arizona.edu/cell_bio/tutorials/cell_cycle/cells3.html))

## Interphase



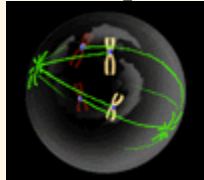
The cell is engaged in metabolic activity and performing its prepare for mitosis (the next four phases that lead up to and include nuclear division). Chromosomes are not clearly discerned in the nucleus, although a dark spot called the nucleolus may be visible. The cell may contain a pair of centrioles (or microtubule organizing centers in plants) both of which are organizational sites for microtubules.

## Prophase



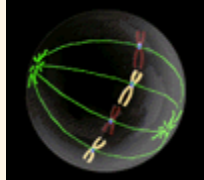
Chromatin in the nucleus begins to condense and becomes visible in the light microscope as chromosomes. The nucleolus disappears. Centrioles begin moving to opposite ends of the cell and fibers extend from the centromeres. Some fibers cross the cell to form the mitotic spindle.

## Prometaphase



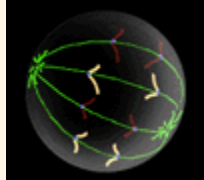
The nuclear membrane dissolves, marking the beginning of prometaphase. Proteins attach to the centromeres creating the kinetochores. Microtubules attach at the kinetochores and the chromosomes begin moving.

## Metaphase



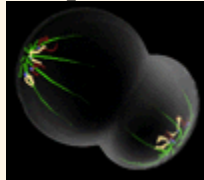
Spindle fibers align the chromosomes along the middle of the cell nucleus. This line is referred to as the metaphase plate. This organization helps to ensure that in the next phase, when the chromosomes are separated, each new nucleus will receive one copy of each chromosome.

## Anaphase



The paired chromosomes separate at the kinetochores and move to opposite sides of the cell. Motion results from a combination of kinetochore movement along the spindle microtubules and through the physical interaction of polar microtubules.

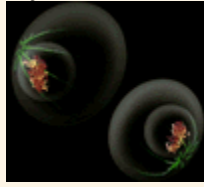
## Telophase



Chromatids arrive at opposite poles of cell, and new membranes form around the daughter nuclei. The chromosomes disperse and are no longer visible under the light microscope. The spindle fibers disperse, and cytokinesis or the partitioning of the cell may also begin

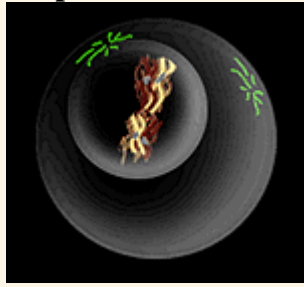
during this stage.

### **Cytokinesis**



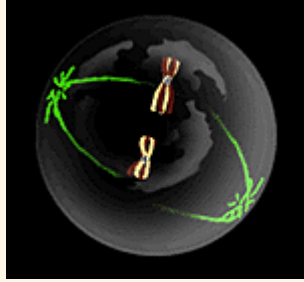
In animal cells, cytokinesis results when a fiber ring composed of a protein called actin around the center of the cell contracts pinching the cell into two daughter cells, each with one nucleus. In plant cells, the rigid wall requires that a cell plate be synthesized between the two daughter cells.

### Prophase I



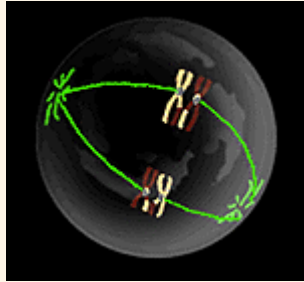
DNA replication precedes the start of meiosis I. During prophase I, homologous chromosomes pair and form synapses, a step unique to meiosis. The paired chromosomes are called bivalents, and the formation of chiasmata caused by genetic recombination becomes apparent. This genetic recombination is also called **crossing over**. Chromosomal condensation allows these to be viewed in the microscope. Note that the bivalent has two chromosomes and four chromatids, with one chromosome coming from each parent.

### Prometaphase I



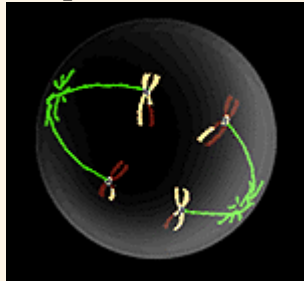
The nuclear membrane disappears. One kinetochore forms per chromosome rather than one per chromatid, and the chromosomes attached to spindle fibers begin to move.

### Metaphase I



Bivalents, each composed of two chromosomes (four chromatids) align at the metaphase plate. The orientation is random, with either parental homologue on a side. This means that there is a 50-50 chance for the daughter cells to get either the mother's or father's homologue for each chromosome.

### Anaphase I



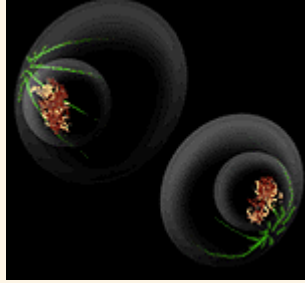
Chiasmata separate. Chromosomes, each with two chromatids, move to separate poles. Each of the daughter cells is now haploid (23 chromosomes), but each chromosome has two chromatids.

### Telophase I



Nuclear envelopes may reform, or the cell may quickly start meiosis II.

## Cytokinesis



Analogous to mitosis where two complete daughter cells form.

Meiosis II is similar to mitosis. However, there is no "S" phase. The chromatids of each chromosome are no longer identical because of recombination. Meiosis II separates the chromatids producing two daughter cells each with 23 chromosomes (haploid), and each chromosome has only one chromatid.

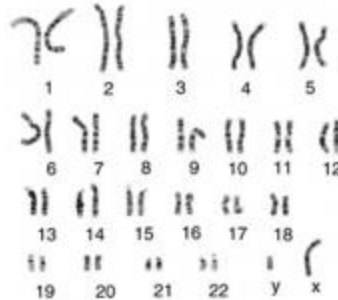
## Human Sex Determination

- Each gamete contains one set of 23 chromosomes.
- The X and Y Chromosome is referred to as the Sex Chromosome and is instrumental in determining the human gender.
  - XX is female.
  - XY is male; the presence of Y chromosome provides the masculine characteristics.
- The egg will always have 22 autosomal chromosomes and an X chromosome. X chromosome, in fact, is necessary for survival. (Makes for a good feminist joke)
- The sperm will have 22 autosomal chromosomes and either X or Y chromosome.
  - Thus, the sperm determines the gender of the offspring.

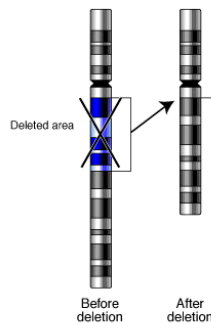
## Karyotypes

- Chart that displays all chromosomes.
- An ordinary Karyotype of a human autosomal cell should display two sets of 23 chromosomes.
  - There are 22 autosomal chromosomes, labeled with numbers, for bodily functions plus the sex chromosomes.
- Any lack of, or presence of extra chromosome may cause genetic disorder and may even be lethal to a certain degree.
  - This is caused by **nondisjunction**-During the separation of anaphase in formation of gametes, the chromosomes may not separate correctly, resulting in a gamete with two of certain chromosome
  - Notable examples of trisomy are:
    - Trisomy 21: Three chromosome no.21, also known as Down Syndrome.
    - Trisomy 18: Three chromosome no.18, also known as Edwards Syndrome.
    - Trisomy 13: Three chromosome no.13, also known as Patau Syndrome.

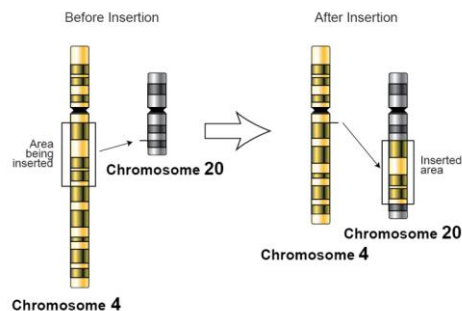
- Triple X Syndrome: Three X Chromosomes.
- Klinefelter's Syndrome: XXY Chromosomes.
- XYY is also possible; however, the condition appears to be phenotypically normal.



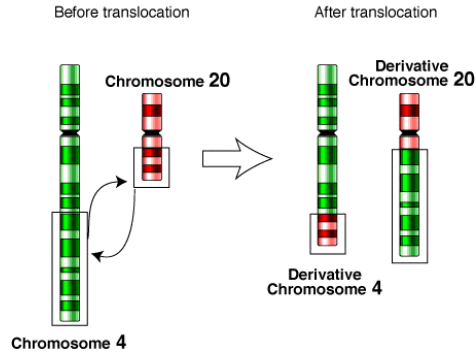
- Analysis of karyotypes may reveal these disorders caused by nondisjunction.
- There are also other errors in chromosomes detectable through karyotypes:
  - Deletion: Certain segment of the chromosome is deleted. This may result from errors in crossing over in prophase I of meiosis.
    - Deletion Syndromes: Deletion of Chromosome 5 results in Cri Du Chat Syndrome. Large deletion can be fatal.
    - Deletion will be signified by a chromosome that is shorter than the other in a karyotype.



- Addition: Certain segment of the chromosome is added and becomes longer. This may result in slipping of DNA polymerase in the STR (repeats) area in a smaller scale, or an error in crossing over in prophase I of meiosis for a larger scale.
  - Addition will be signified by a chromosome that is longer than the other in a karyotype.



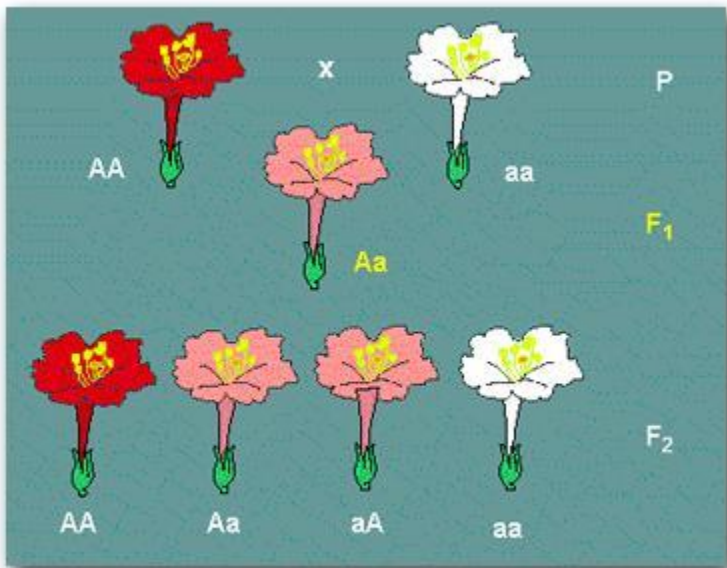
- Translocation: Crossing over occurs between nonhomologous, that is, not the same, chromosome.
  - Cancer may result from translocation
  - Translocation will be signified by a swapped portion of chromosomes in a karyotype.



## Variations to Traditional Mendelian Genetics

- **Incomplete Dominance**

- In some unusual cases, there may be no clear dominant and recessive allele.
- The heterozygous form may display the intermediate phenotype between the homozygous dominant and the homozygous recessive.
- Examples include 4 o'clock flowers and Snapdragon flowers which have red homozygous dominant, pink heterozygous, and white homozygous recessive.
- Number of genotypes equals the number of phenotypes.



- **Codominance**

- For some cases, both alleles may act as being dominant.

- Both alleles are expressed for codominance in the case of a heterozygote as opposed to the usual dominant phenotype.
- There are three phenotypes for codominance: Dominant, recessive, and both dominant and recessive.
- A good example of codominance is roan cattle: A cow can be completely red with dominant RR genotype or completely white with recessive rr genotype; however, heterozygous cow will have both red and white (roan) fur.
- Another example of codominance is AB blood type; Blood type is also an example of multiple alleles.
- **Multiple Alleles**
  - For multiple alleles, there are two or more alleles for a gene in a population.
  - Most famous example is the blood type: ABO. There are three alleles for blood types.
  - The dominant alleles are represented by capital I and have superscript A and B to designate the types. ( $I^A$  and  $I^B$ ) Blood types A and B are always dominant over the recessive i, standing for the blood type O.
  - Thus, there are three alleles  $I^A$ ,  $I^B$ , and i; four genotypes  $I^A i$ ,  $I^B i$ ,  $I^A I^B$ , and ii. Their phenotypes are A, B, AB, and O, respectively. Note, however, that  $I^A I^B$  is an example of codominance as well.

## Epistasis

- More than one gene affects a characteristic.
  - Do not confuse with multiple alleles or codominance; instead of alleles, multiple **genes** interact with each other to produce a certain characteristic.
  - A set of genes may aid the actions of another genes and be **synergistic**.
  - A set of genes may hinder the actions of another genes and be antagonistic.
- Epistasis genes can be either recessive or dominant.
- Albinism is a good example.
  - Alleles for gene regarding albinism is P (normal pigmentation) and p (no pigment.) P is dominant.
  - However, the Enzyme S is also needed for skin coloration; dominant S allele produces the Enzyme S while recessive s allele does not.
  - Thus, without the presence of S enzyme, even if the gene for albinism and skin coloration is Heterozygous or homozygous dominant (PP or Pp), the person is albino. PPss or Ppss would be albino.
- Epistasis results if the enzyme or the protein produced by a certain gene is an intermediary or necessary for the production of another gene.
  - For ex, Enzyme S is necessary for the expression of P; thus, absence of S disregards the entire P gene.
- Dangerous thought: flower color in peas, the organism studied by the famed Gregor Mendel, has epistasis. His monohybrid cross theory with law of segregation would have been believed to be flawed by him had he studied the flower color extensively.



## Sex-linked traits

- Sex-linked traits are associated with genes on the sex chromosomes X and Y.
- The sex-linked traits can be x-linked or y-linked (usually X-linked due to the small size of the y chromosome), dominant or recessive.
- X-linked recessive are most commonly seen; hemophilia and color-blindness are both X-linked recessive.
  - Because hemophilia is x-linked recessive, women cannot have it unless they possess the affected alleles in both X genes; they are carriers otherwise. However, since man only has one X chromosome and one Y chromosome, they cannot be carriers, only affected or not affected. See pedigree section.

## Lethal alleles

- Lethal alleles do not affect the setup of the punnet square regarding the homozygous and the heterozygous.
  - However, the phenotypic ratio would be different due to the death of the affected individuals.
  - The affected individuals' death remove the expected progeny class after the cross.
- Color of the mouse's coat is a good example: the dominant allele Y represents yellow coat while recessive allele y represents white coat. Though the genotypic ratio is as expected 1:2:1 YY, Yy, and yy, the phenotypic ratio of yellow mouse to white mouse was 2:1. This was because the homozygous recessive YY proved to be lethal.

## Hardy-Weinberg Equilibrium (Excellently explained by the scioly wiki)

### Conditions

The *Hardy-Weinberg Law* states that a population will maintain the exact allele and genotype frequencies over each generation unless five specific influences are introduced into the population. These are:

1. Mutations
2. Gene flow (migration in/out of the population)
3. Small population
4. Natural selection
5. Non-random mating

For a population to be in Hardy-Weinberg equilibrium, it must **not** have any of the 5 conditions listed above. Here are the explanations for each condition:

1. **Mutations:** Mutations introduce new alleles into the population.
2. **Gene flow:** Like mutations, migration can introduce new alleles (or diminish another allele)
3. **Small population:** Genetic drift is likely to occur in a smaller population.
4. **Natural selection:** If some traits are discriminated for/against, the genotype frequencies will not be in equilibrium over the generations.
5. **Non-random mating:** Like natural selection, non-random mating could discriminate for/against traits.

An example of Hardy Weinberg: Let's say we were in a world where everyone had either purple or blue skin. S is purple skin, and s is blue skin. The probability of either one of these genes occurring is constant, and both probabilities have to add to 1. Given the probabilities of both blue and purple skin, lets say p for purple and b for blue, the probability of having two purple skin alleles (SS) would be pp, and having a blue and a purple (Ss) would be pb, and so on.

## Equation

There are two equations used in the Hardy-Weinberg Law:

1.  $p^2 + 2pq + q^2 = 1$
2.  $p + q = 1$

where

p is the frequency of the (homozygous) dominant **allele** in the population

q is the frequency of the (homozygous) recessive **allele** in the population

$p^2$  is the **percentage** of the homozygous dominant individuals

$2pq$  is the **percentage** of the heterozygous individuals

and

$q^2$  is the **percentage** of the homozygous recessive individuals.

Remember, the equations only apply if the population is in Hardy-Weinberg equilibrium.

## Solving a Hardy-Weinberg Problem

A typical Hardy-Weinberg problem will resemble the sample problem below:

In a certain population, the percentage of the homozygous recessive genotype (aa) is 36%. Using only that information, find:

1. The frequency of the recessive genotype.
2. The frequency of the recessive allele.
3. The frequency of the dominant allele.
4. The percent of the heterozygous individuals.

**IMPORTANT:** Before attempting to solve the problem, it is critical to analyze all of the given information and approach it in the correct manner. *Make sure to check your work after finishing!* One mistake will throw off the entire problem. When solving a problem, make sure to work in the order as follows:

*Step 1: Determine q.* Since a dominant phenotype can have either a homozygous or heterozygous genotype, it is easier to find the recessive allele first (unless an exact homozygous/heterozygous dominant value is given).

*Step 2: Determine p.* Using the second equation, p can be found once q has been determined.

*Step 3: Determine  $p^2$  and  $q^2$ .* Steps 3 and 4 are interchangeable, but finding  $p^2$  and  $q^2$  first is generally the common practice.

*Step 4: Determine  $2pq$ .*

# Pedigree

- Pedigrees are essentially family trees showing the carriers, affected, and normal people in the family.
- Carriers: Heterozygous for a recessive condition. Affected: Those with the condition. Normal: Those without the condition.
  - Pedigree can give clue to the nature of the condition (ex. homozygous recessive, X-linked recessive, etc.)
  - Some clues: X-linked recessive will occur more frequently in males than females.
  - Autosomal recessive traits will occasionally skip a generation and occurs in equal frequency between male and female.
  - Some traits may even disappear for some cases.
- Following is the legend to aid the production of a pedigree; (taken from [http://www.genomedical.com/documents/Standard\\_Pedigree\\_Symbols.pdf](http://www.genomedical.com/documents/Standard_Pedigree_Symbols.pdf))

## Standard Pedigree Symbols

	Male	Female	Sex Unknown	Comments
Individual				Assign gender by phenotype.
Affected individual				Keylegend used to define shading or other fill (e.g., hatches, dots, etc.).
Multiple individuals, number known				Number of siblings written inside symbol. (Affected individuals should not be grouped.)
Multiple individuals, number unknown				"n" used in place of "?" mark.
Deceased individual				If known, write "d." with age at death beside symbol.
Stillbirth (SB)				Birth of a dead child with gestational age noted.
Pregnancy (P)				Gestational age and karyotype (if known) below symbol. Light shading can be used for affected and defined in keylegend.
Proband				First affected family member coming to medical attention.
Consultand				Individual(s) seeking genetic counseling/testing.
Miscarriage				
Elective AB				

<p><b>Definitions</b></p>	<p><b>Comments</b></p> <p>If possible, male partner should be to left of female partner on relationship line.</p> <p>Siblings should be listed from left to right in birth order (oldest to youngest)</p> <p>For pregnancies not carried to term (SABs and TOPs), the individual's line is shortened.</p>
<p><b>Relationships</b></p> <p>A break in a relationship line indicates the relationship no longer exists.</p>	<p>A break in a relationship line indicates the relationship no longer exists. Multiple previous partners do not need to be shown if they do not affect genetic assessment.</p>
<p><b>Consanguinity</b></p> <p>If degree of relationship is not obvious from pedigree, it should be stated (e.g., third cousins) above relationship line.</p>	

Adapted from Bennet RL, Steinhaus KA, Ulrich SB, et al. "Recommendations for standardized human pedigree nomenclature" Am J Hum Genet 56(5):745-752, 1995.

All Information gathered from *Genomics* and *Biology* textbook.