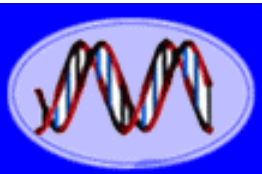
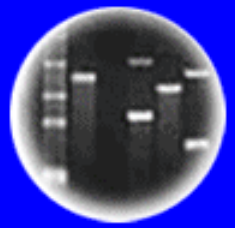


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

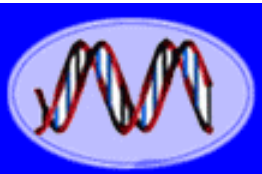
HC70A & SAS70A Winter 2009
Genetic Engineering in Medicine, Agriculture, and Law

Professors John Harada & Bob Goldberg

Lecture 4
What Are Genes & How Do They Work: Part Three

UCLA

UC DAVIS
UNIVERSITY OF CALIFORNIA



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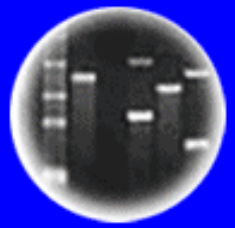

DNA Fingerprinting

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and Future Consequences

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Review of Last Tuesday's Lecture: What Are Genes & How Do They Work - Part Two

1. How Are Genes Regulated - Switched On & Off?
2. How Does DNA Replication Occur?
3. What is the Polymerase Chain Reaction (PCR) and How is PCR used?

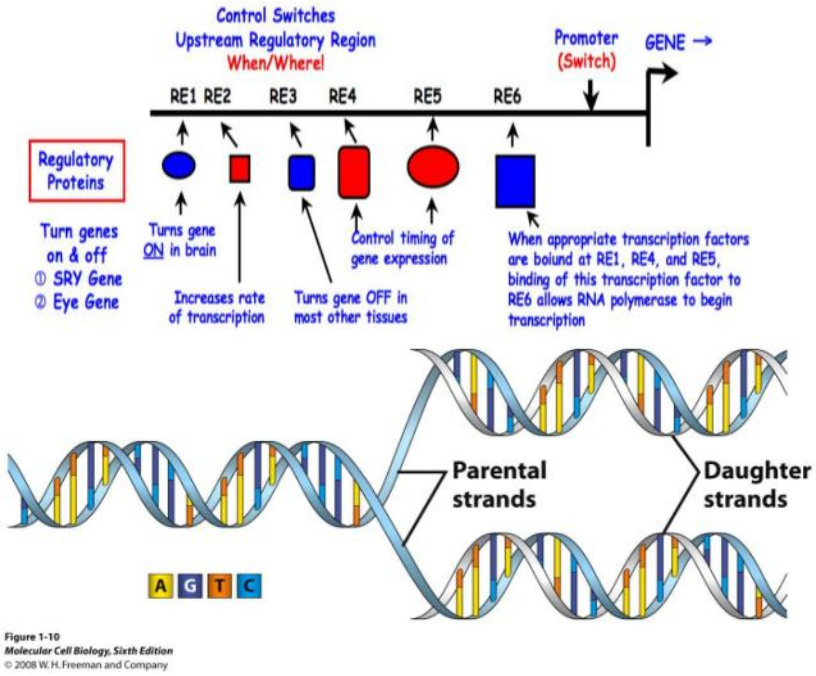

Figure 1-10
Molecular Cell Biology, Sixth Edition
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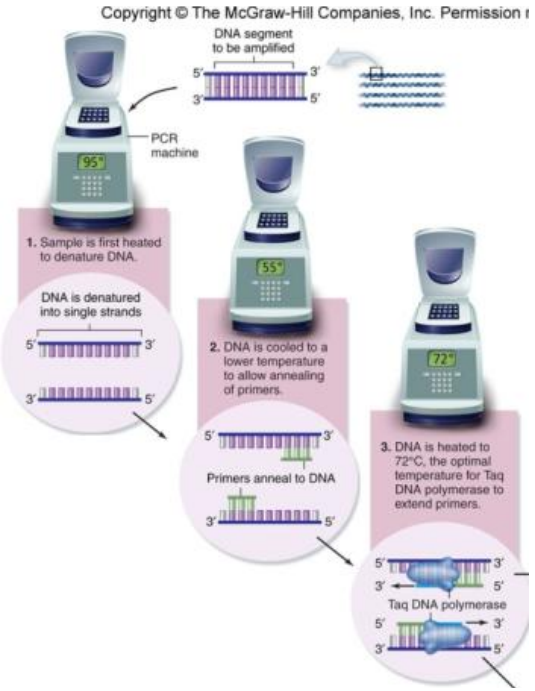
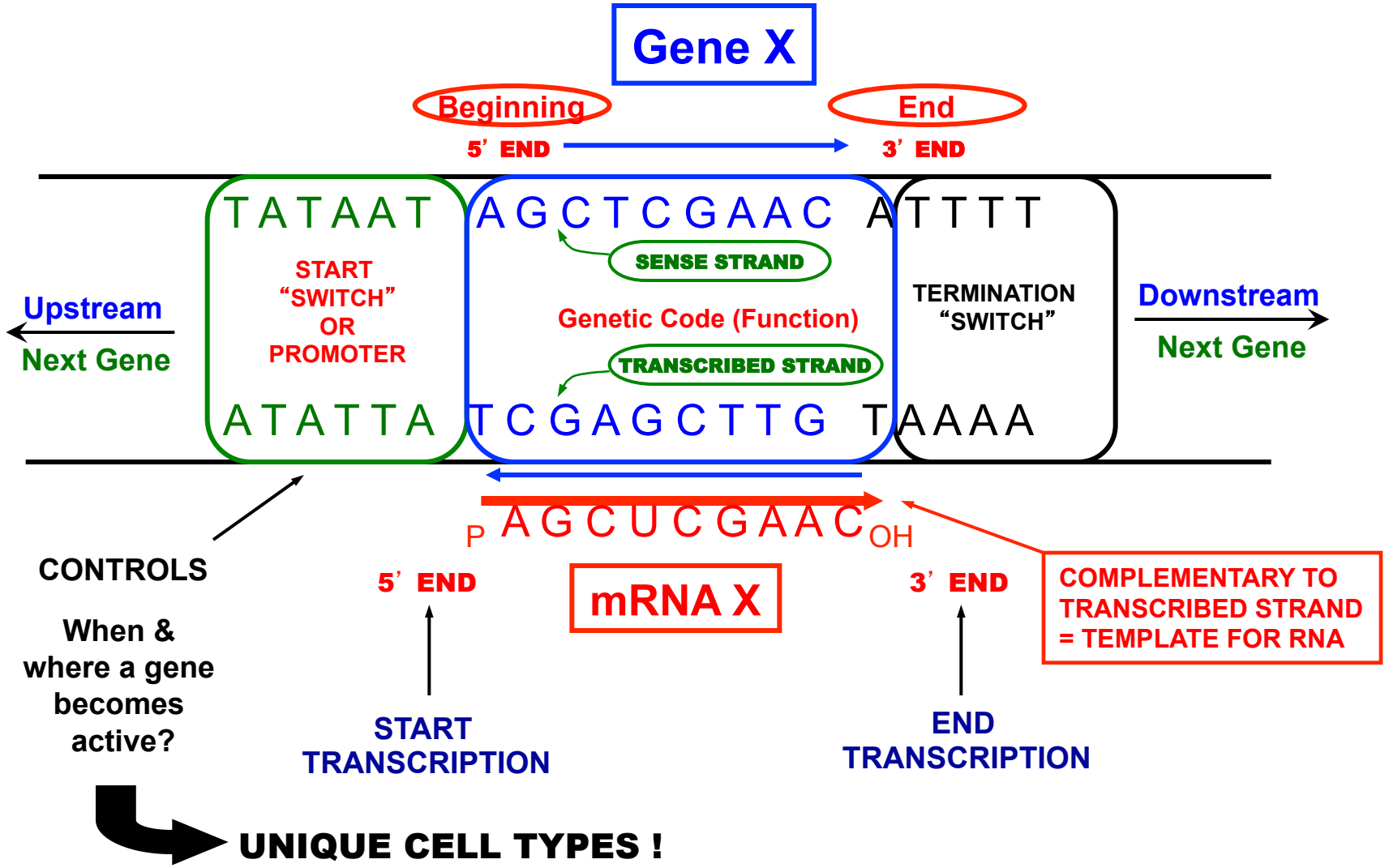


A Gene is a Specific DNA Sequence that Directs the Expression of a Unique Trait

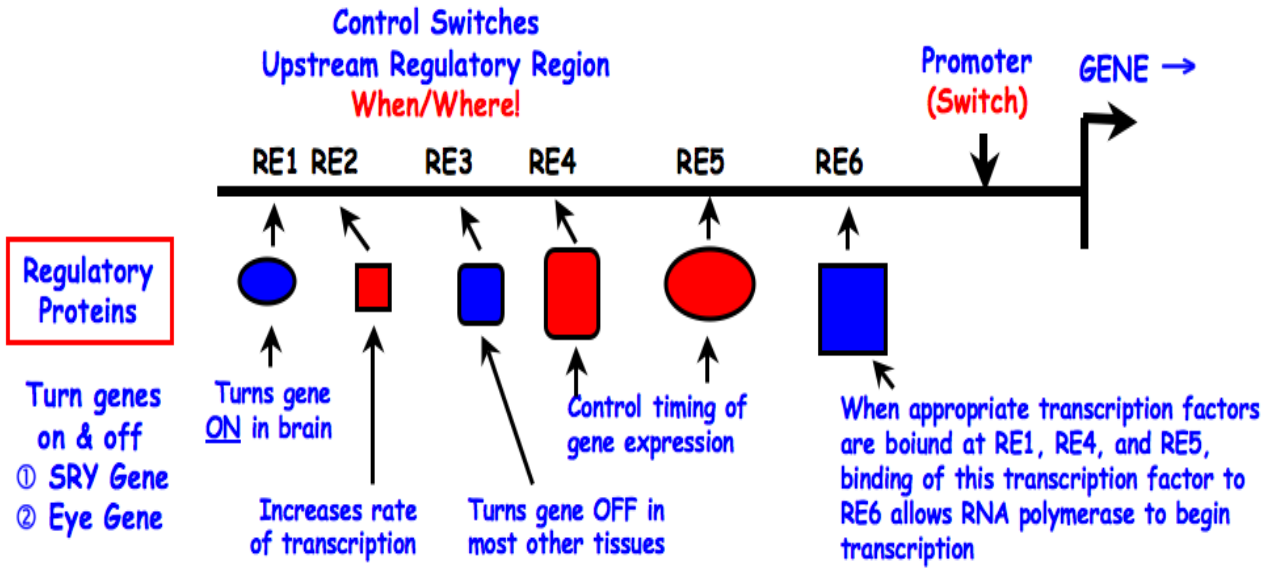


Note: mRNA Sequence = Sense Strand Sequence

Control Switches Are Unique DNA Sequences & Can Be Cloned

AND used to re-engineer organisms!!

Switches act independently of gene!!

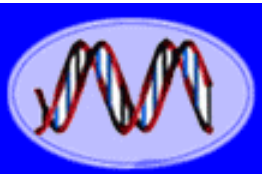


Genome Projects Reveal Both the Genes & the Logic that Controls them!

Each Switch = Unique DNA Sequence

RULE: Sequence → Biology!!

No "Hocus Pocus" Yo! It's in the DNA!



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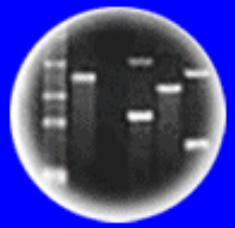

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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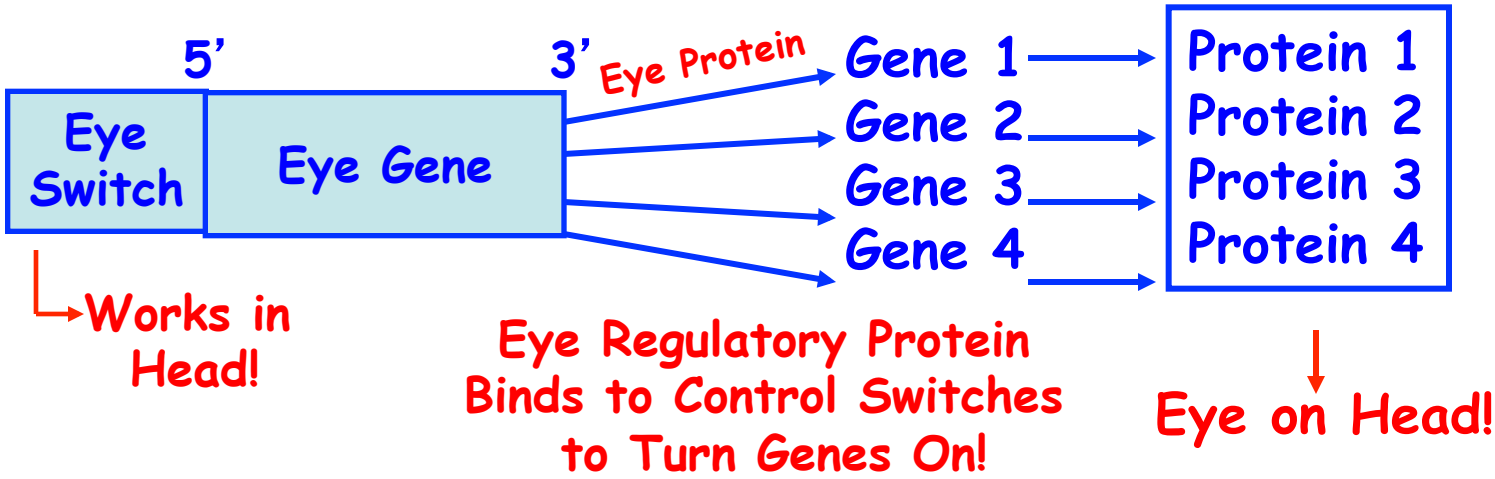
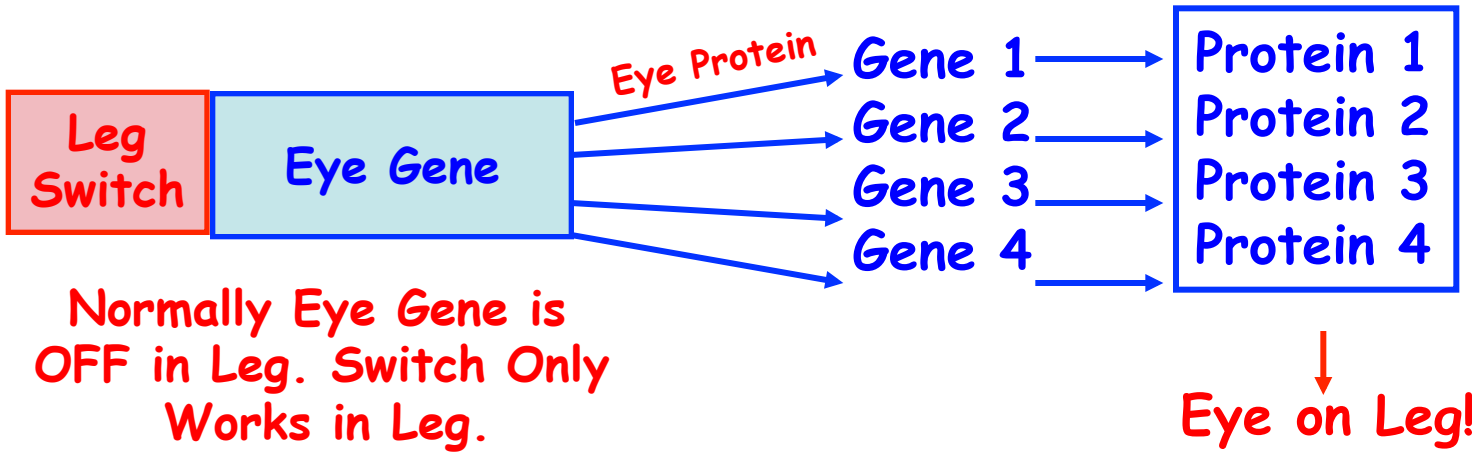
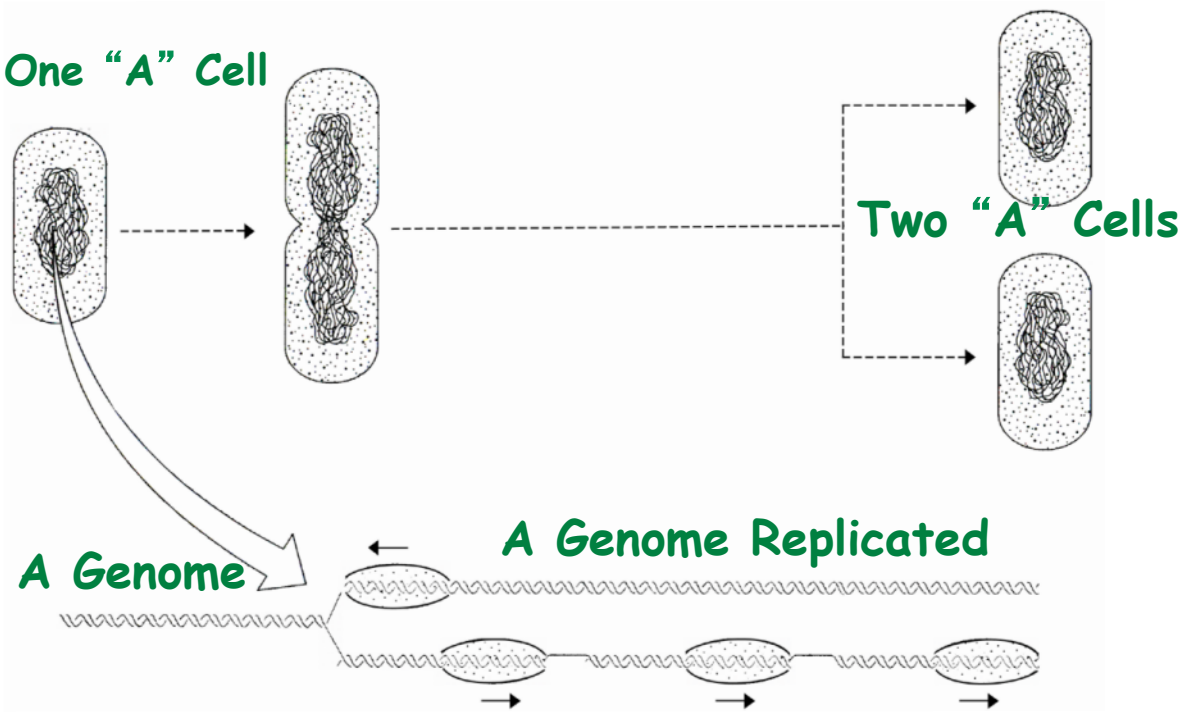
Eye Regulatory Network

Regulatory Genes Like the Eye Gene Control the Activity of Other Genes!





How Are Genes Replicated During Each Cell Generation?

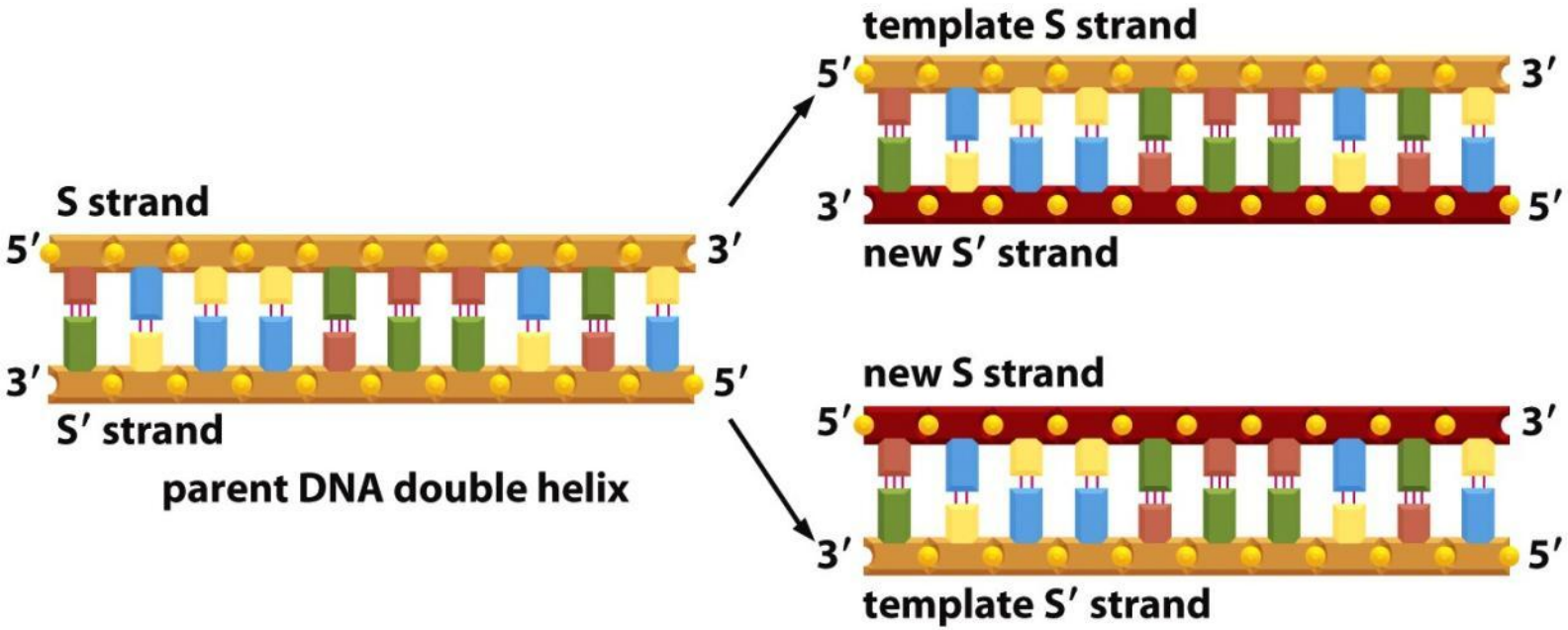


How is The DNA Sequence Copied/ Replicated Each Cell Division?

Pass on Genes to Next Generation Precisely?

BASIC OF LIFE!

DNA Replication



1. DNA replication is semi-conservative - new DNA molecules are precise copies of parental DNA – each containing one newly synthesized complementary strand
2. DNA sequence is maintained by complementary base pairing
3. DNA synthesis is polar - occurs 5' to 3' and requires a primer.
4. DNA replication is continuous and discontinuous synthesis.

DNA Replication Begins at an Origin of Replication, a Specific DNA Sequence, and Proceeds Bidirectionally

Genetic Engineering Concept

Foreign DNA Segments Use the ORI of Chromosomes/DNA into Which They Are Inserted

e.g., bacteria insect resistance gene uses plant ori

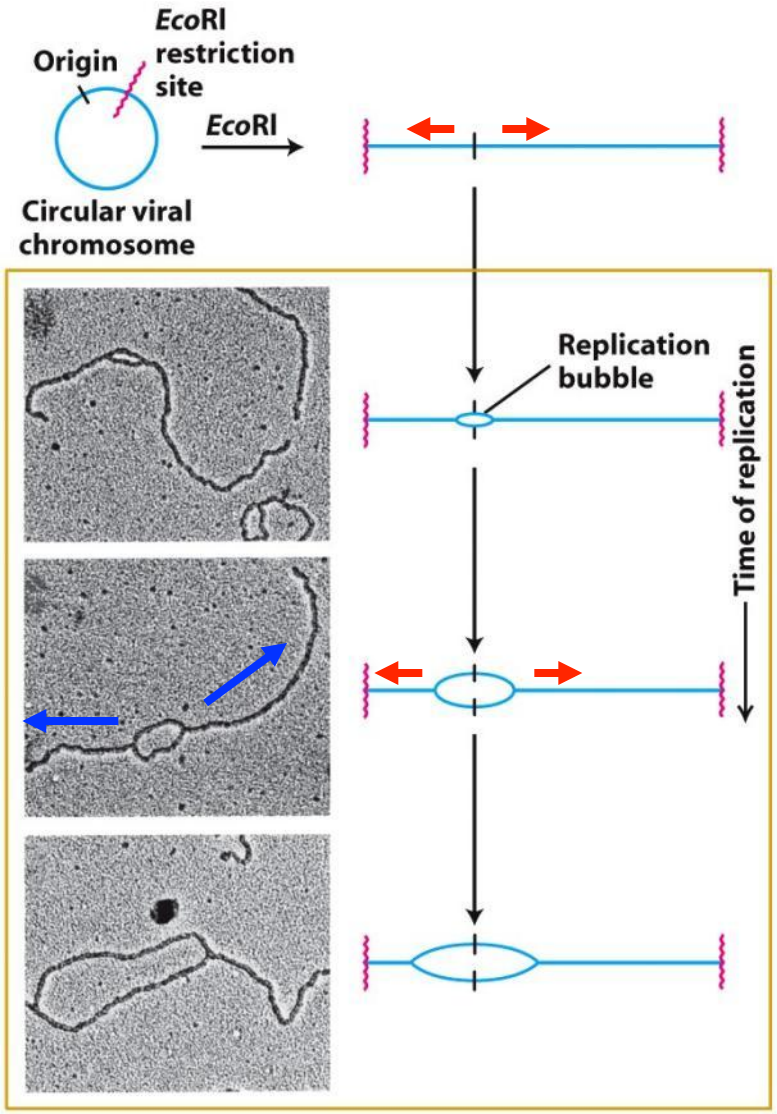
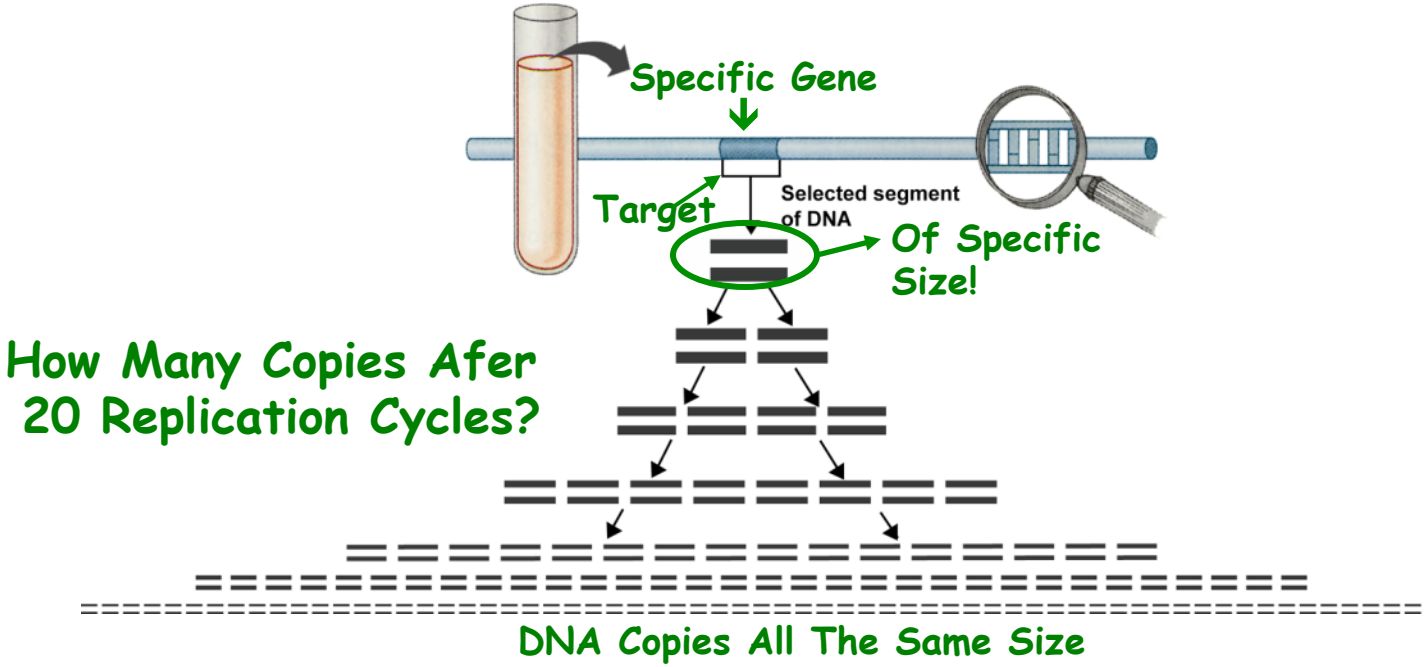


Figure 4-32
Molecular Cell Biology, Sixth Edition

The Polymerase Chain Reaction or PCR
is a Molecular Xerox Machine



1. PCR Has Revolutionized DNA Analysis!
Specific DNA Sequences/Genes Can Be "Copied" Directly From "Tiny" Amount of DNA!

2. No Cloning Needed!

3. But Need Sequence!

Requirements For PCR

1. Knowledge of a Specific Sequence to Amplify (e.g.,insulin gene)
   a) Must Have First Cloned & Sequenced DNA of Interest the "Old-fashioned Way"
2. Primers That Recognize Specific DNA Sequences & Initiate DNA Synthesis & DNA Polymerase Binding To Template
3. Template (e.g., DNA From Human Cheek Cell)
4. Heat-Stable DNA Polymerase
5. Nucleotides
6. Thermoprogrammer/Cycler To Heat & Cool DNA in Cycles- Separating DNA Strands, Allowing Primers To Bind Complementary Sequences (Anneal), & Permiting New dsDNA Molecules to Form

It's All in the DNA Sequences -- Know Sequence & Can Synthesize an Infinite Amount of Specific DNA Sequences.
It know Takes One Hour To Do What Used to Take YEARS!

PCR Has Many Uses, Has Changed Many Fields, and Led To New Ones That Have Had a Big Impact On Our Lives

1. Amplify Any DNA Sequence, or Gene, From "Tiny" Amounts of DNA or Biological Materials IF ORIGINAL SEQUENCE KNOWN
2. Study DNA From Limited and/or Degraded Sources Such As:
    1. A Single Human Hair or Cheek Cell
    2. An Ancient Fossil (e.g., Neanderthal Bone or Mammoth Hair)
    3. An Ancient Insect Trapped in Amber
    4. Human Remains (e.g., 9/11 Victims)
    5. A Single Human Embryo Cell
    6. Contaminated Meat To Determine the Causal Organism
3. Used In:
    1. DNA Fingerprinting-Individual Identification-Genetic Disease Screening
    2. Forensics (Crime Scenes, Mass Graves, Criminal Suspects, Wrongfully Convicted)
    3. Paternity & Family Relationships (e.g., Immigration, Tracing Lost Children)
    4. Disease Diagnosis & Pathogen Identification (Humans, Animals, & Plants)
    5. Human Origins & Migrations
    6. Ancient Genome Sequences & Evolutionary Studies
    7. Specific mRNA Detection
    8. "Cloning" Specific DNA Sequences
    9. Tracing Plant & Animal Sources (e.g., Stolen Cattle, Cactus)
4. Need as Little as One Molecule of DNA & Can Replicate an ∞ Amount of Specific Sequences

Revolutionized How To Study & Manipulate DNA

The New York Times

Carp DNA Is Found in Lake Michigan

Spencer Green/Associated Press

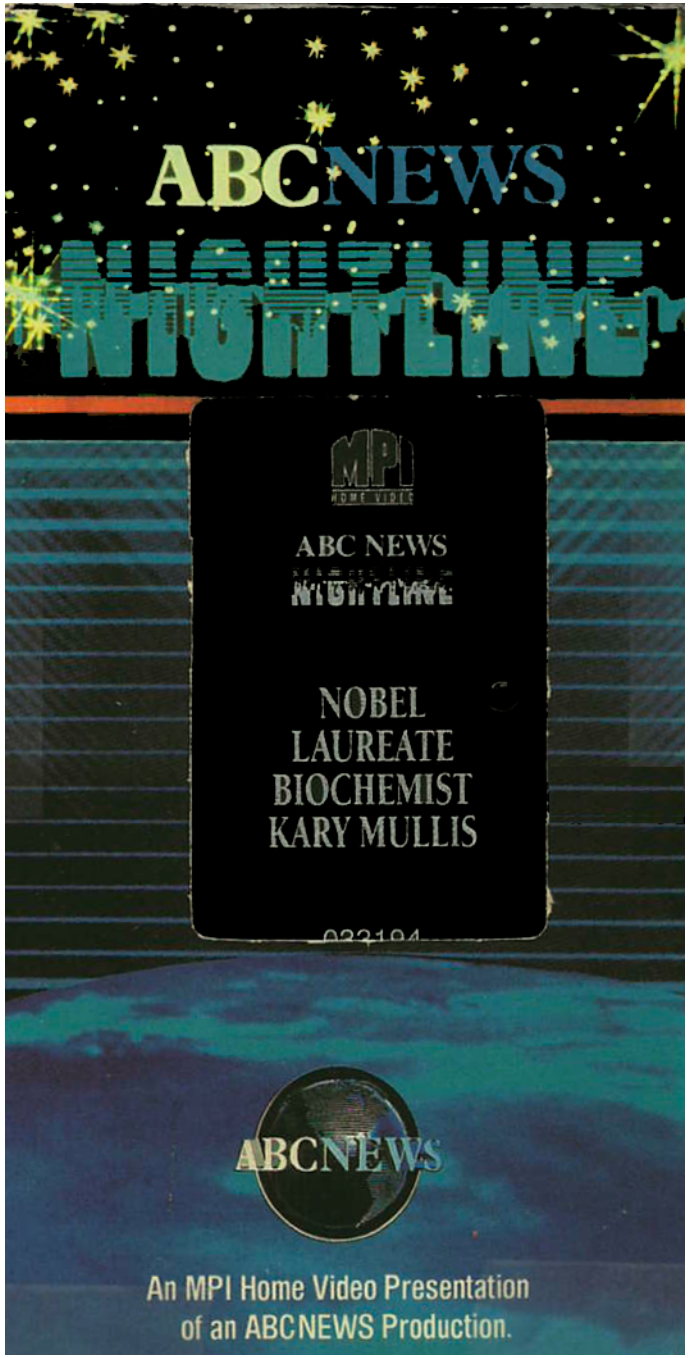
Crews searched an Illinois shipping canal last month for Asian carp, a voracious invasive species.

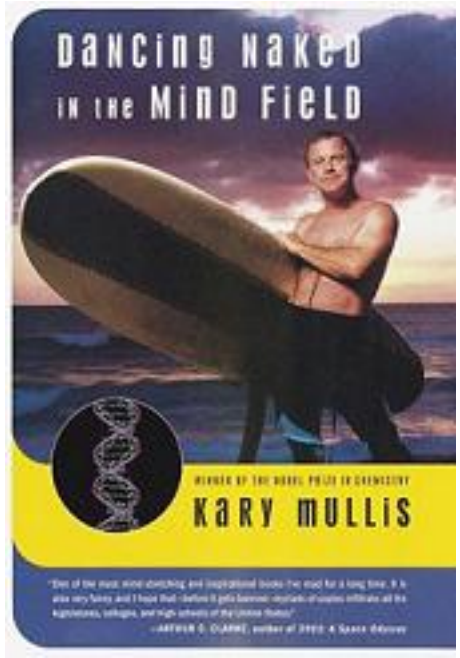
By SUSAN SAULNY
Published: January 19, 2010

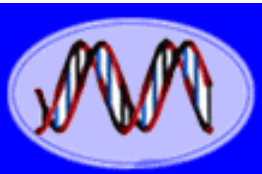
CHICAGO — Genetic material from the Asian carp, a voracious invasive species long feared to be nearing the Great Lakes, has been identified for the first time at a harbor within Lake Michigan, near the Illinois-Indiana border, ecologists and federal officials said Tuesday.

Asian Carp DNA was identified using the environmental DNA method. Water from the lake was analyzed for DNA sequences from Asian Carp using PCR.

March 31, 1994







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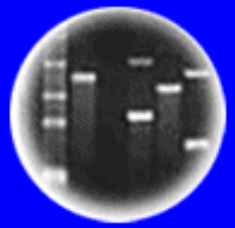


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

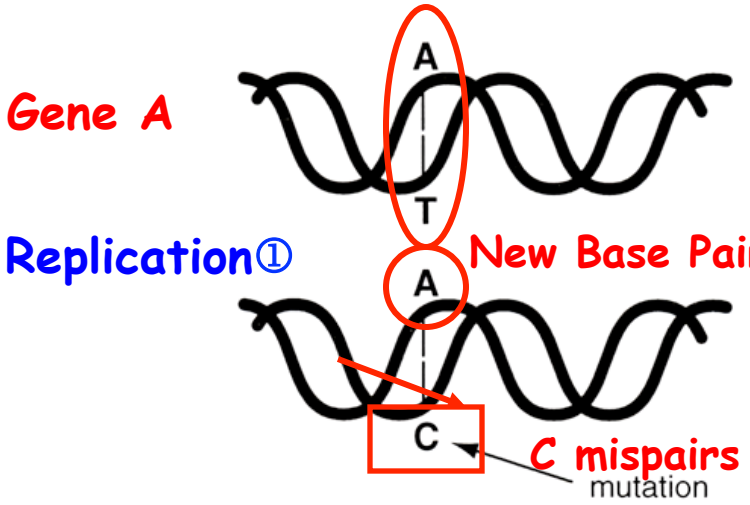
THEMES

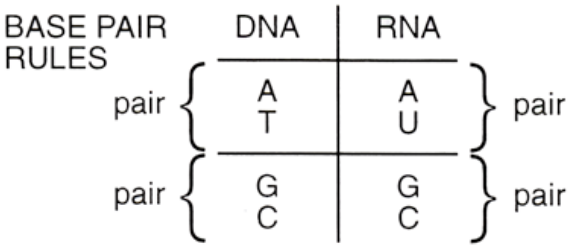
1. How Do Mutations Occur?
2. How Can Pedigrees Be Used To Follow the Inheritance of Mutant Genes?
3. How Do Mutations Change Phenotypes?
4. What is the Colinearity Between Genes & Proteins (how does DNA→protein)?
5. What Is the Genetic Code?
6. How Do Gene Expression Processes Differ in Eukaryotes & Prokaryotes?
7. How Can Splicing Cause One Gene To Specify Several Different Proteins?
8. Yo!-It's in the DNA Sequences- What Are the Implications For Genetic Engineering?

DEMONSTRATION

1. DNA Fingerprinting

DNA Replication is Precise:
But Mistakes or Mutations Can Occur!



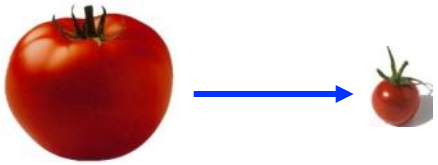


MUTATION DURING REPLICATION ← **Rare Base Mismatch**

Mutation Seen As Change in Phenotype

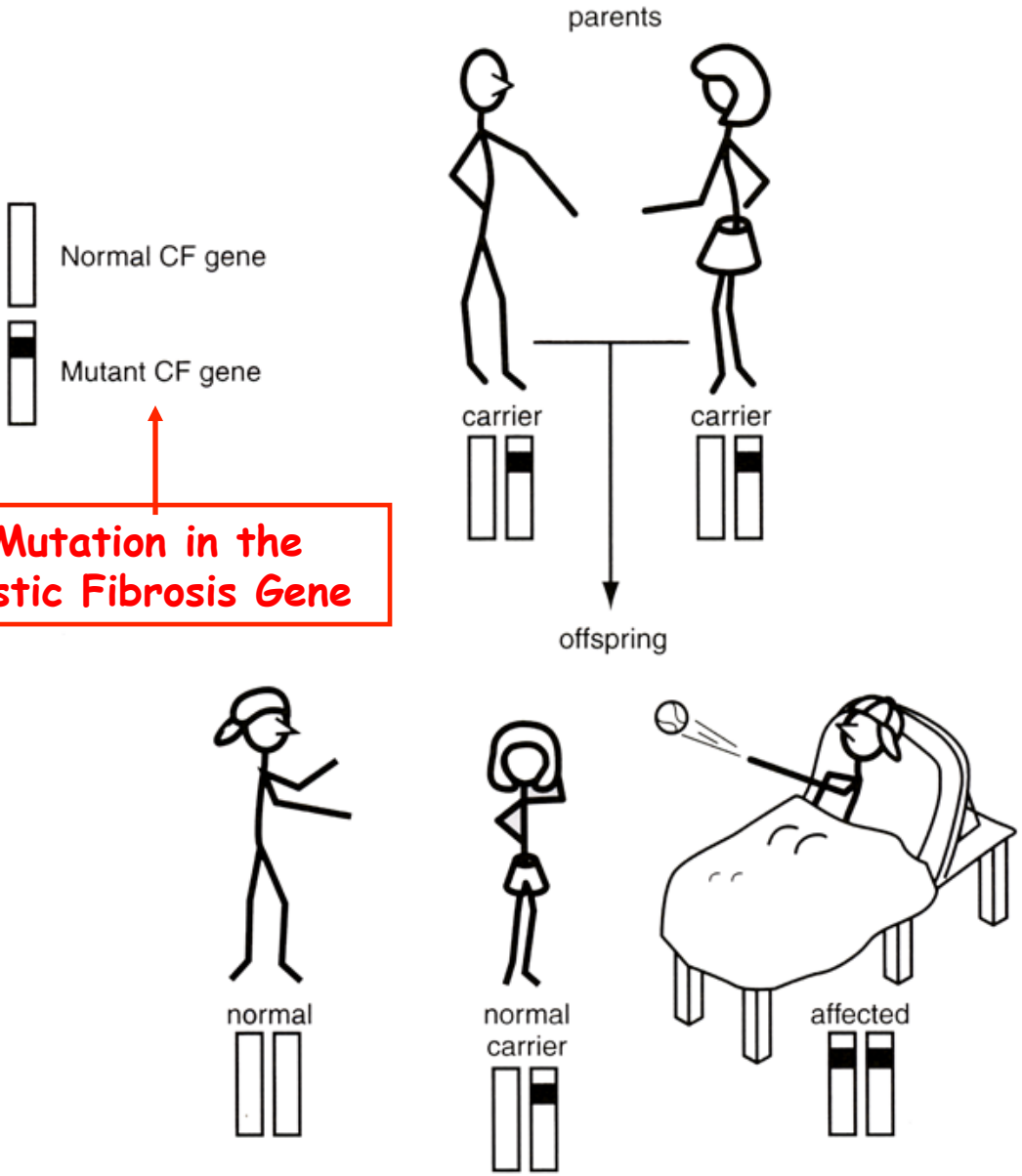
Change DNA Sequence From A = T to G ≡ C

∴ Change Protein Amino Acid Sequence »» Alter Function!



Big Tomato to Small Tomato

Mutation in Genes Are Rare But Are Inherited

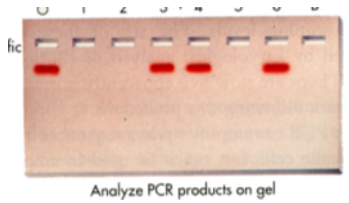


One Gene per Gamete

♀ + ♂

Two Genes per Somatic Cells

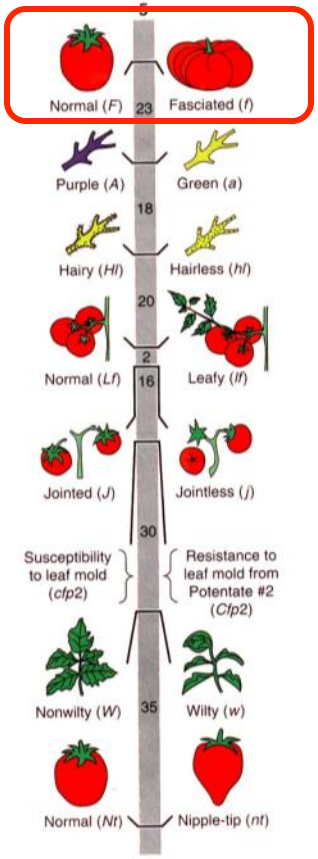
How Follow Inheritance?
What Allows Disease To Be Followed?

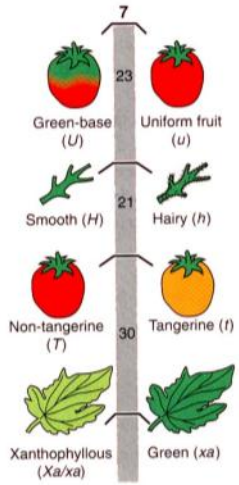


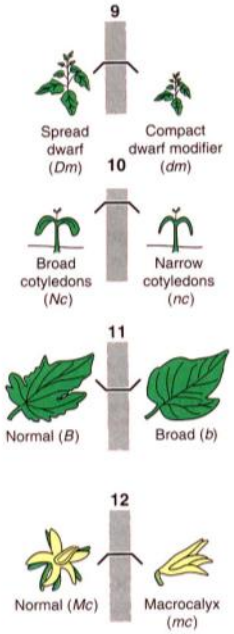
DNA Marker or Fingerprint!

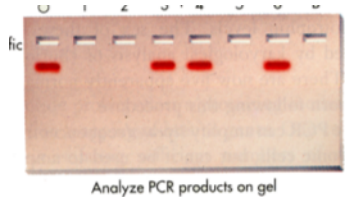
Alternative Forms of the Same Gene – Alleles – Lead to Genetic Diversity

Alleles









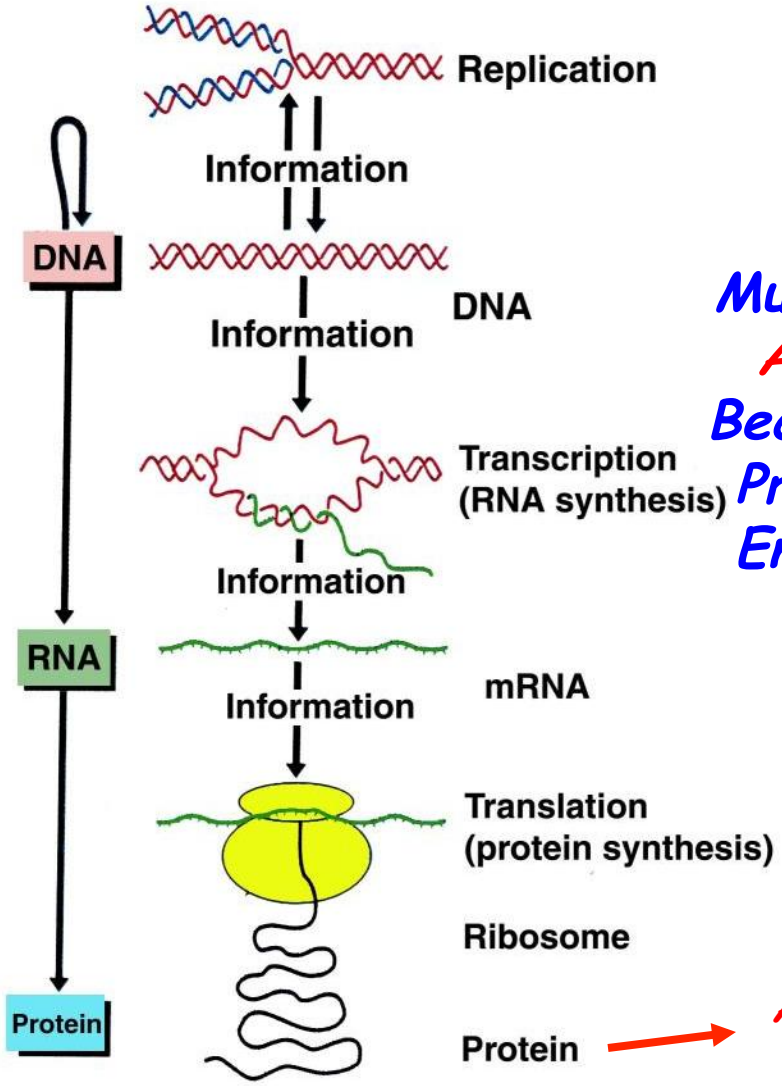
Can Follow These Traits With DNA Markers As Well

mutations result in genetic diversity!!!

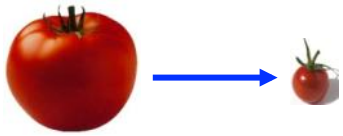
Spontaneous Mutations Give Rise to Alleles, or Different Forms of the Same Gene, as a Result of Small DNA Sequence Changes (e.g., SNPs or Single Nucleotide Polymorphisms)

Translating the Genetic Code Into Proteins is a Conserved Process

Mutations Are Inherited Because Altered Gene Replicates

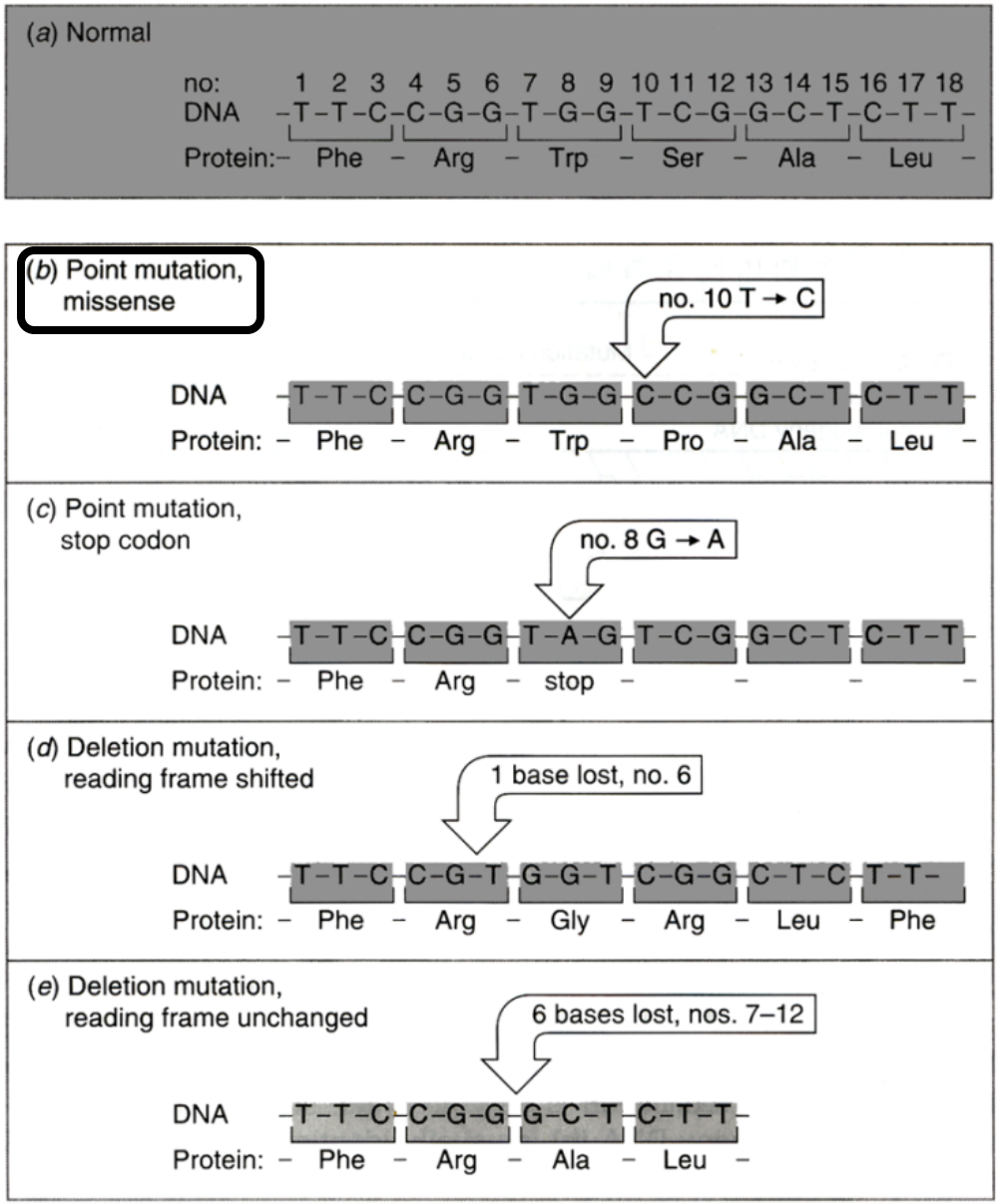


Mutations Lead To Altered Protein Because mRNA and Protein Sequence Encoded By Gene Changes



Mutations Lead to Altered Traits/Phenotype Because Protein Structure Changed

Mutations Occur in Many Different Ways



1. Base-Pair Change
2. Insert or Delete a Base (Indel)
3. Move Gene, or Part of Gene, to New Location (Switches Change)!

Function of Protein Lost and/or Changed

∴Phenotype Changes

Human Genetic Disorders Occur As a Result of Mutations

TABLE 13.2 Some Important Genetic Disorders

Disorder	Symptom	Defect	Dominant/ Recessive	Frequency Among Human Births
Hemophilia	Blood fails to clot	Defective blood-clotting factor VIII	X-linked recessive	1/10,000 (Caucasian males)
Huntington disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24,000
Muscular dystrophy (Duchenne)	Muscles waste away	Degradation of myelin coating of nerves stimulating muscles	X-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood lead to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

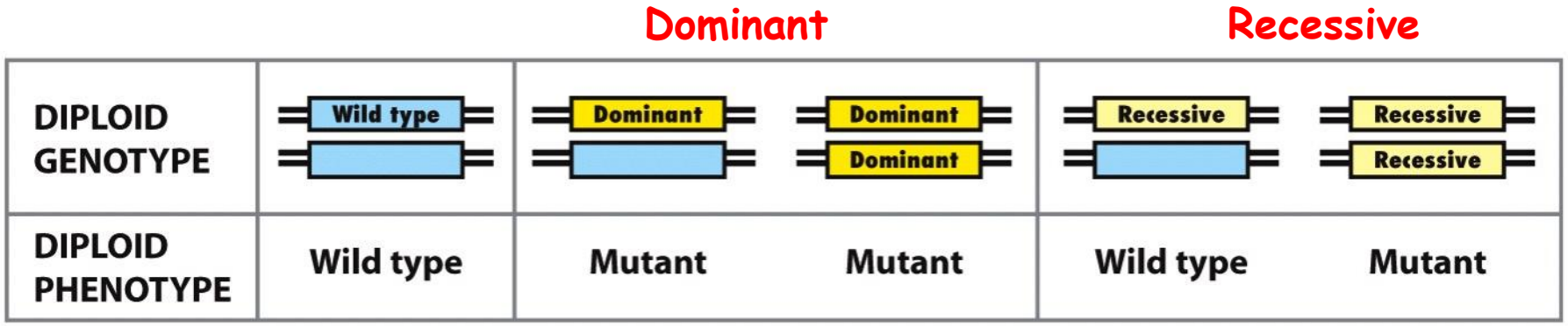
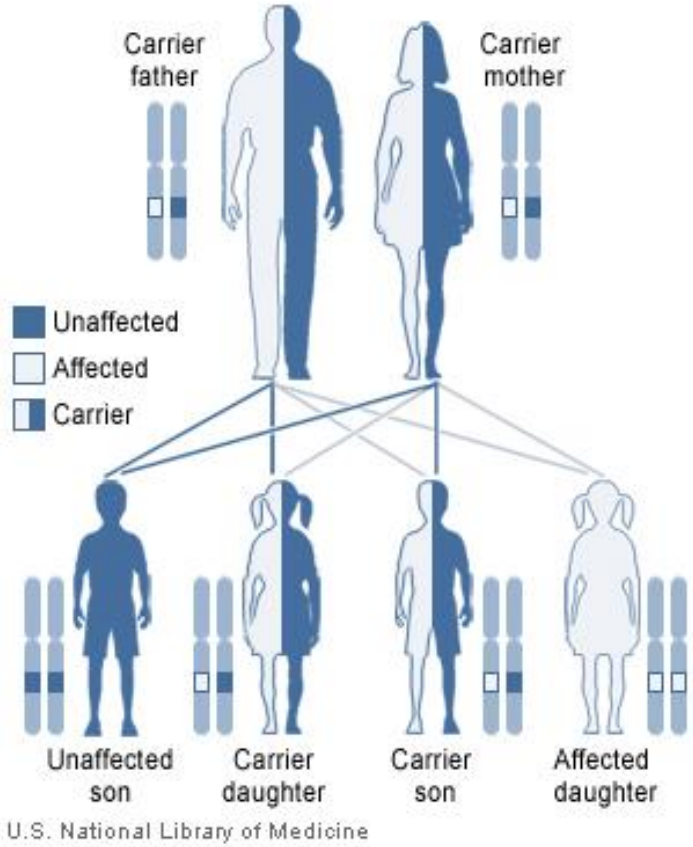


Figure 5-2
Molecular Cell Biology, Sixth Edition

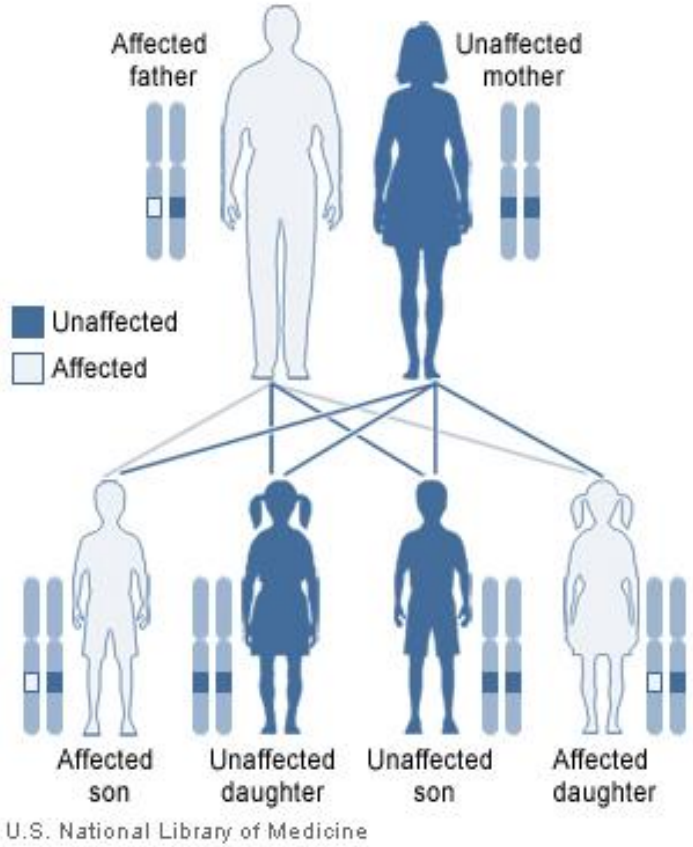
Dominant & Recessive Mutations

A = Normal Allele
a = Mutant Allele

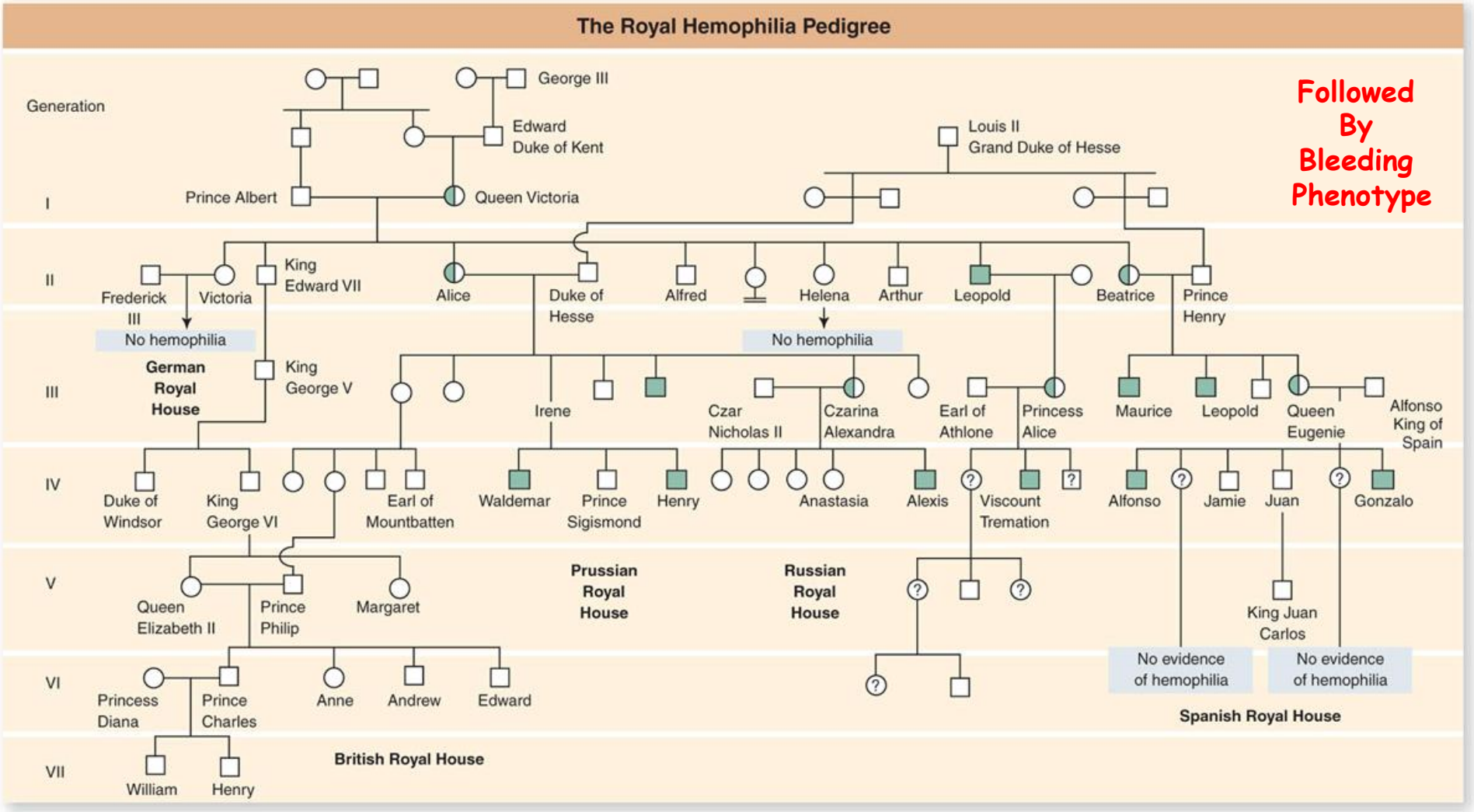
Recessive Mutation
AA, Aa, aA »» no disease
aa »» disease



Dominant Mutation
AA »» no disease
, Aa, aA, aa »» disease



Pedigrees Can Be Used To Follow Disease Genes in Human Families

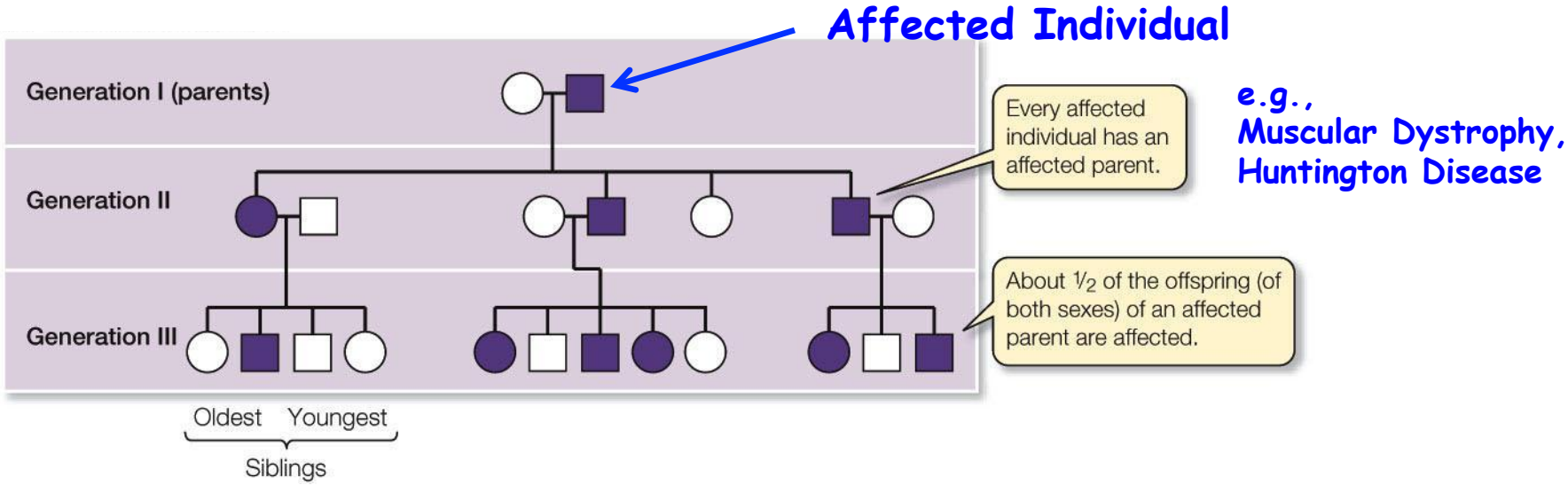


Followed By Bleeding Phenotype

Hemophilia = any of several hereditary blood-coagulation disorders in which the blood fails to clot normally because of a deficiency or an abnormality of one of the clotting factors.

Pedigrees Can Be Used To Determine If a Trait is Dominant or Recessive

Each Type of Inheritance Predicts Specific Results in Each Generation

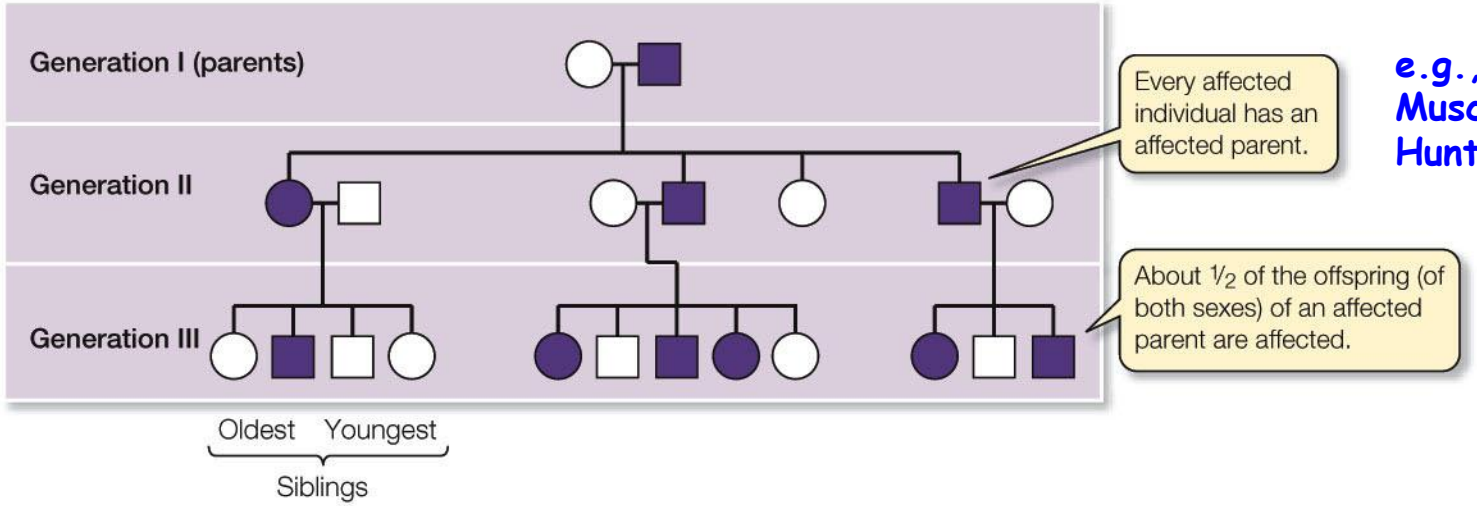


e.g.,
Muscular Dystrophy,
Huntington Disease

Does this pedigree show that the disease is caused by a recessive or dominant mutation?

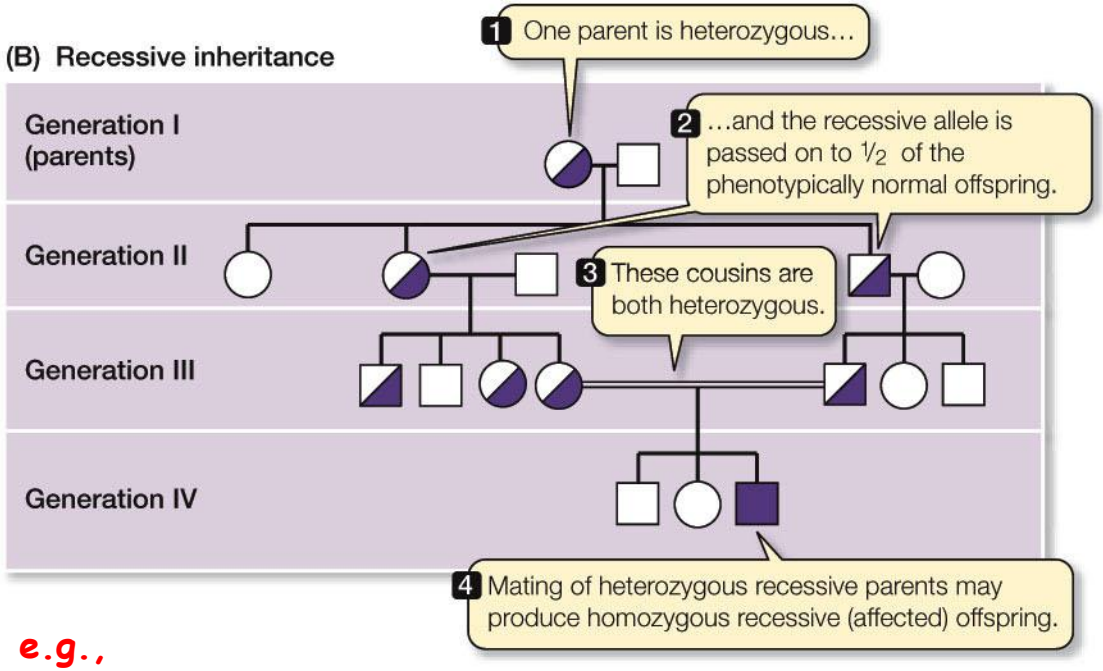
a. Recessive
b. Dominant

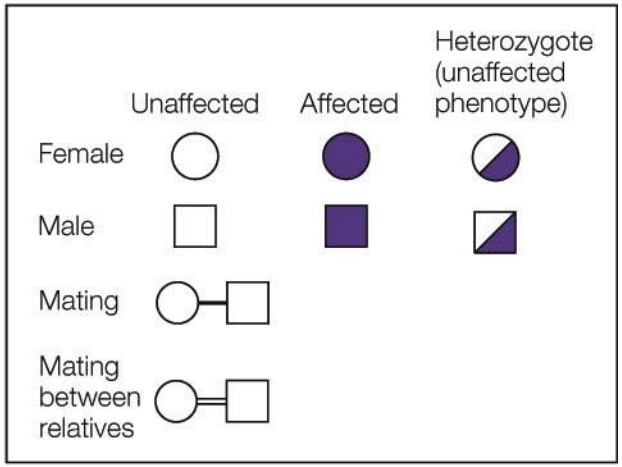
(A) Dominant inheritance



e.g.,
Muscular Dystrophy,
Huntington Disease

(B) Recessive inheritance

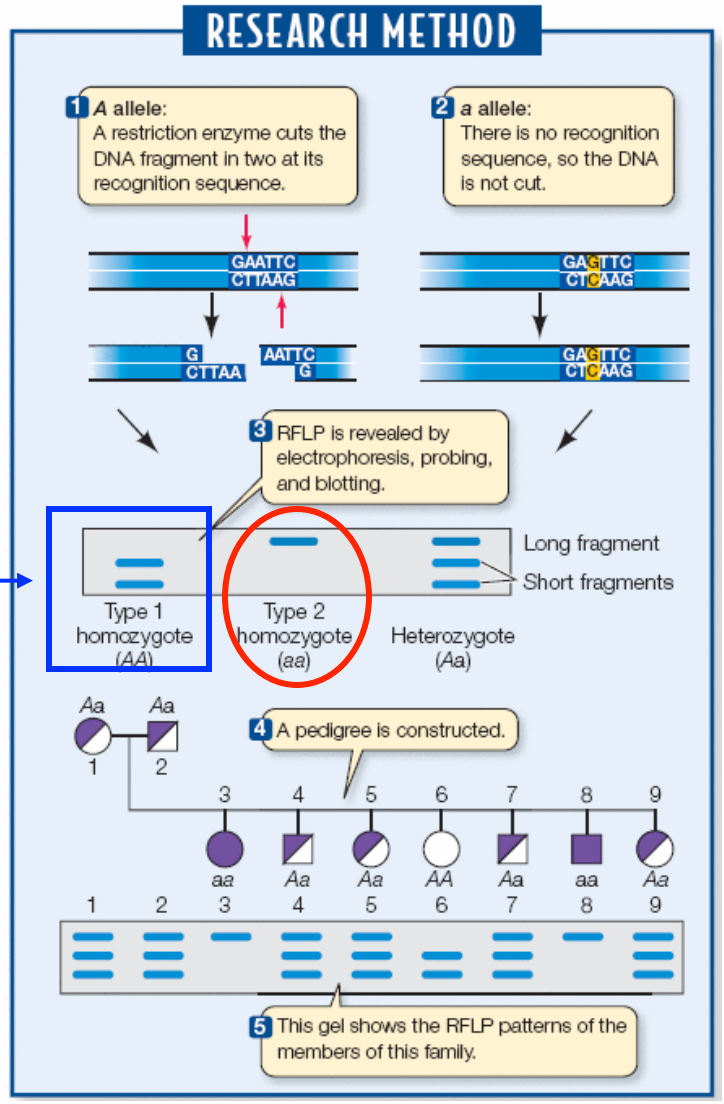




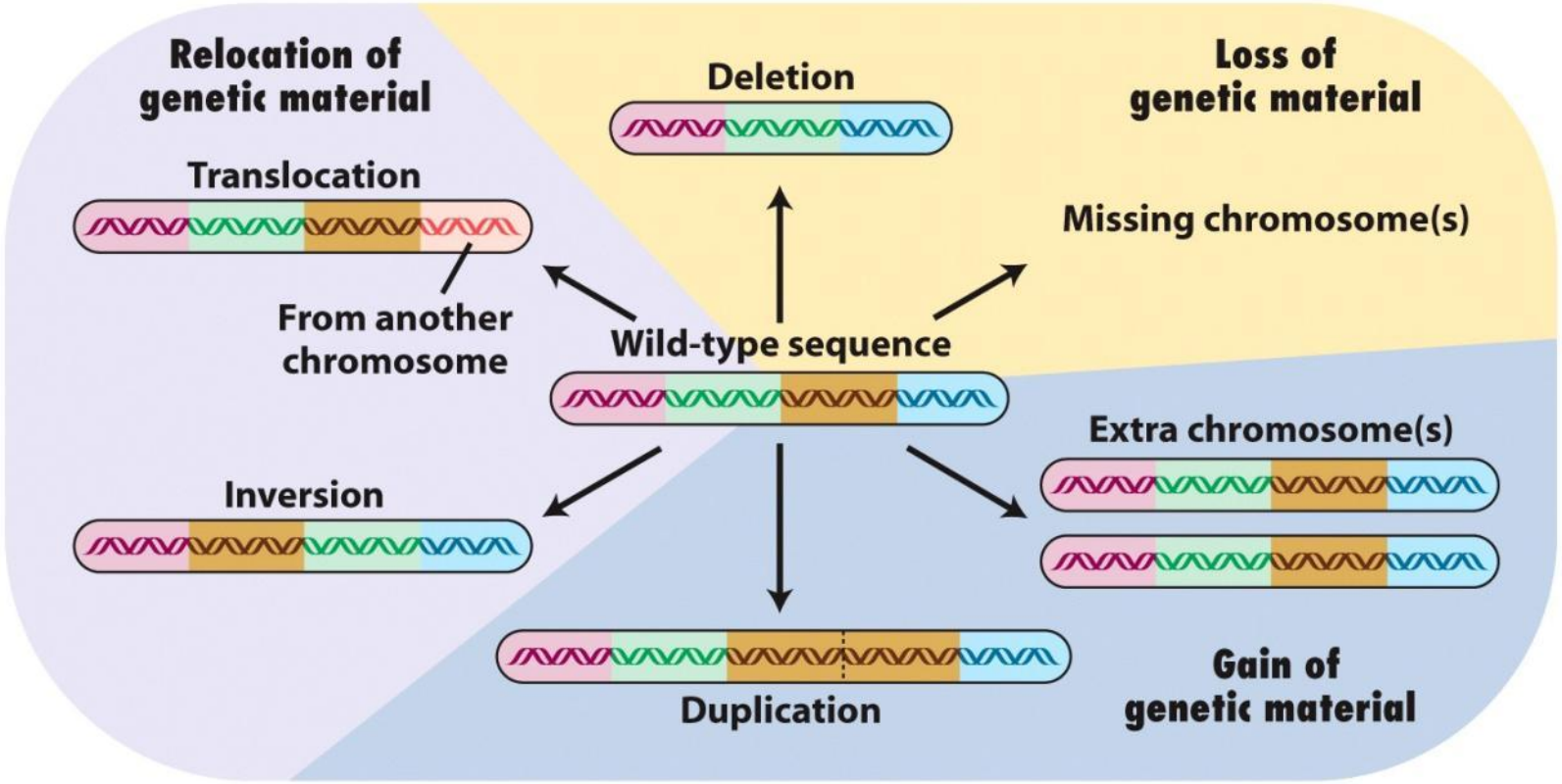
e.g.,
Sickle Cell Anemia
Cystic Fibrosis,
Tay-Sachs Disease

Genetic Diseases Can Be Followed in Families Using Molecular Methods (e.g., PCR)

DNA Fingerprints →



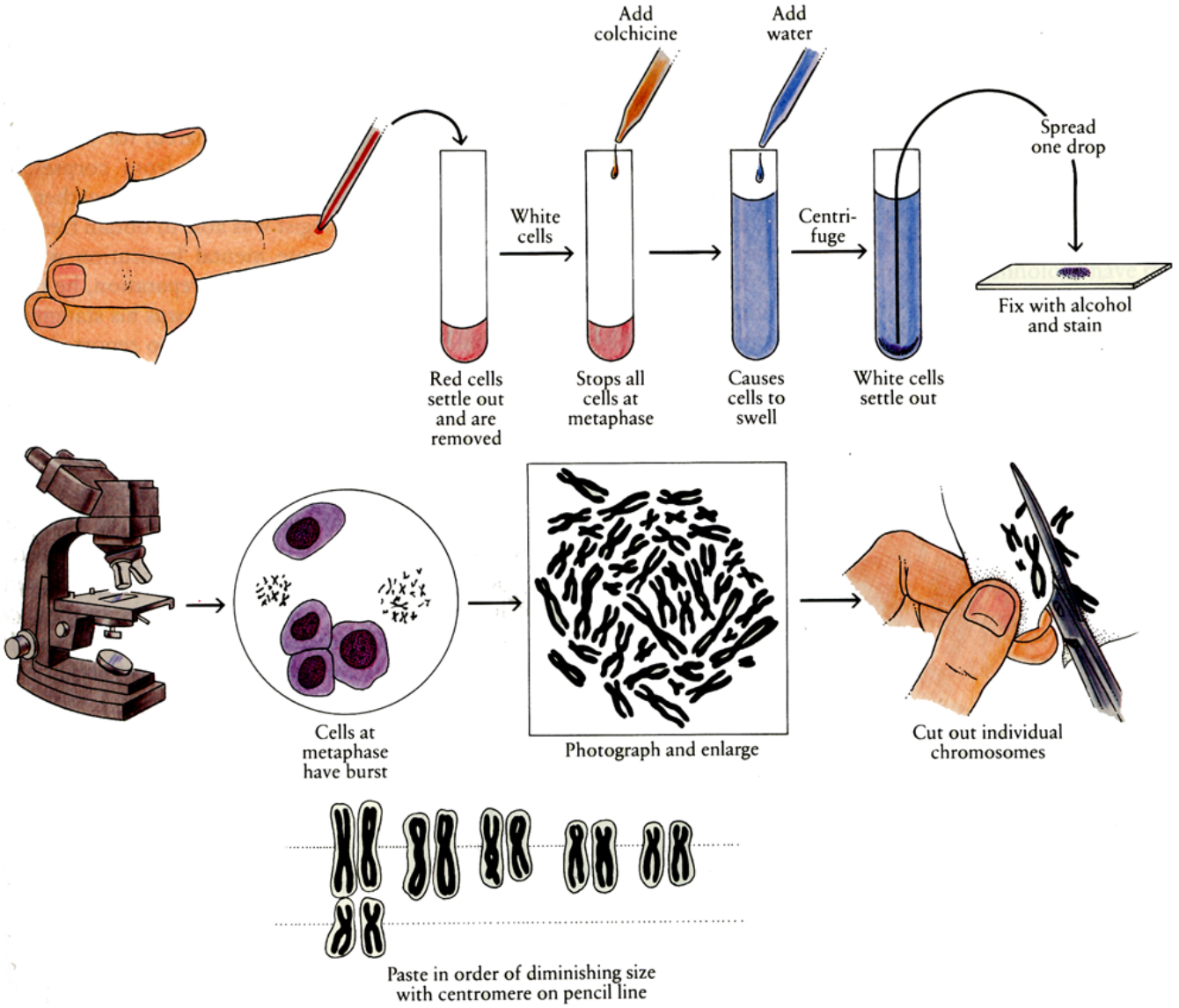
"Mutations" Can Also Occur By Large Chromosomal Changes



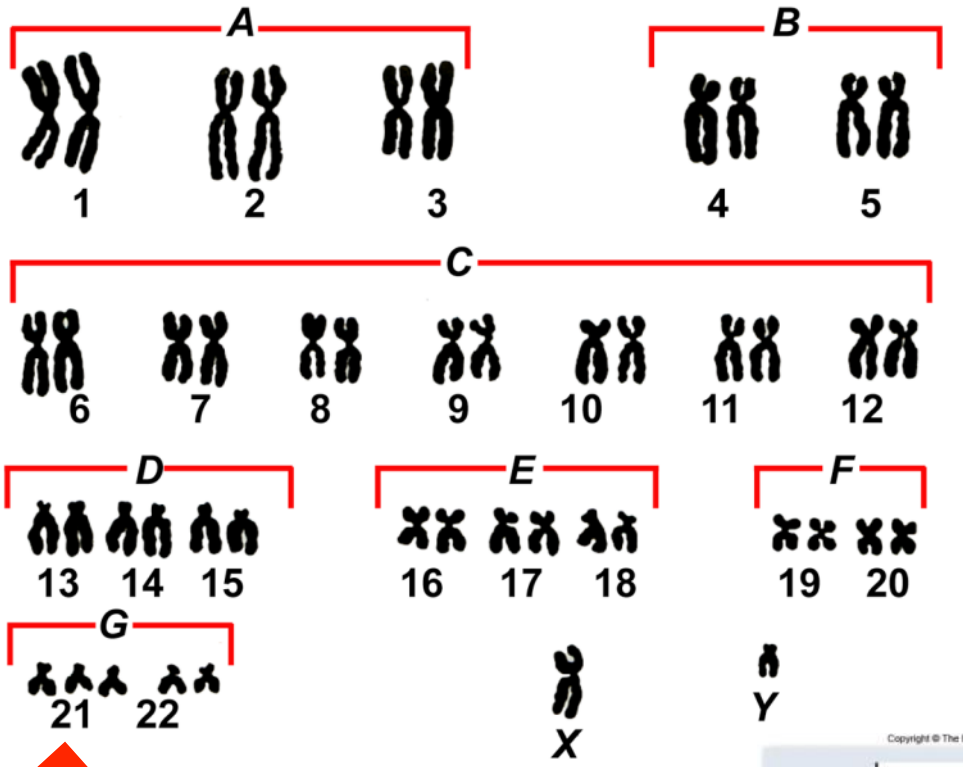
These changes affect many genes!

e.g. Down's Syndrome (3 Chromosome #21s)

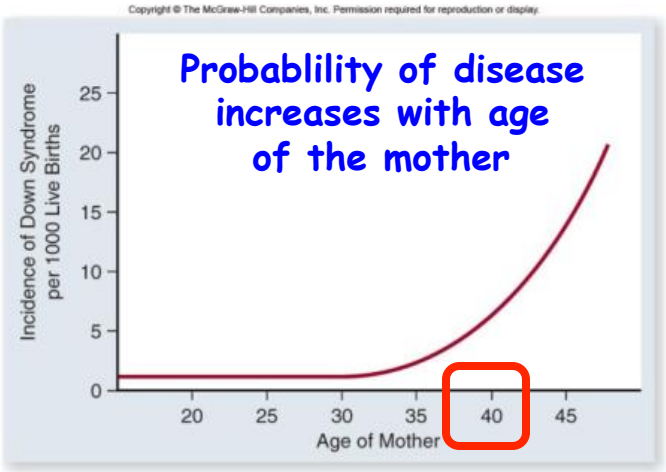
Karyotypes Can Be Used To Detect Changes in Chromosome Structure and Number



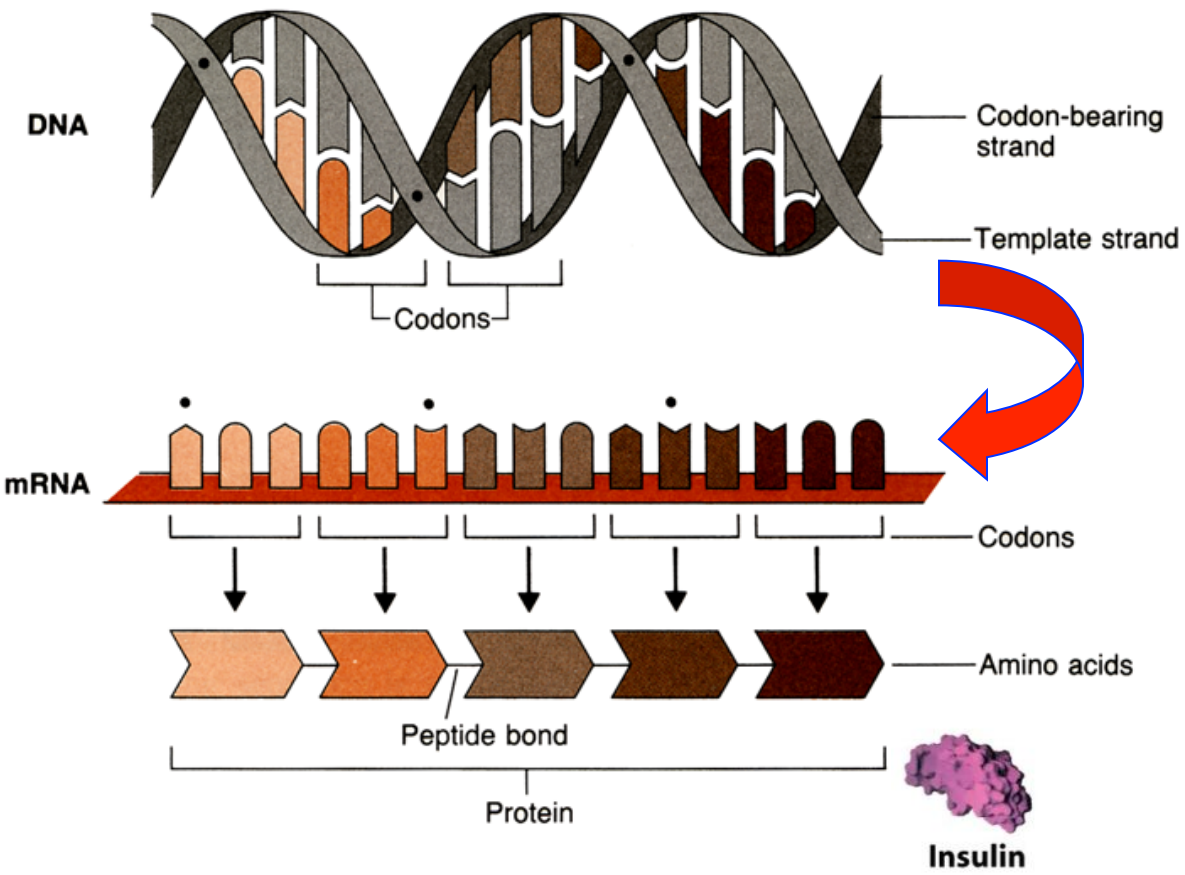
A Down Syndrome Karyotype



Three Chromosome # 21s



How Does A Gene Lead To A Phenotype?



① mRNA Synthesized by Transcription

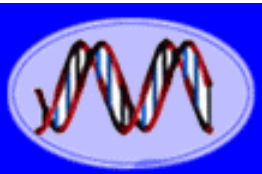
- Complementary to Transcribed, Non-Sense Strand
- Same Sequence As Sense Strand

② mRNA Translated into Protein by Translation of The Genetic Code

Genetic Code on mRNA Translated to Protein Sequence

∴ Sequence of Gene
↓
Sequence of mRNA
↓
Sequence of Protein

Know DNA Sequence
Know Protein
→
Engineer New Protein



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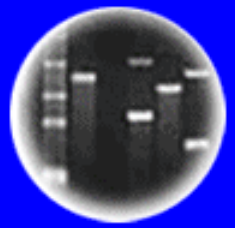

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Each Protein has a Unique Composition and Order of Amino Acids, and a Unique Size, Shape, and Function

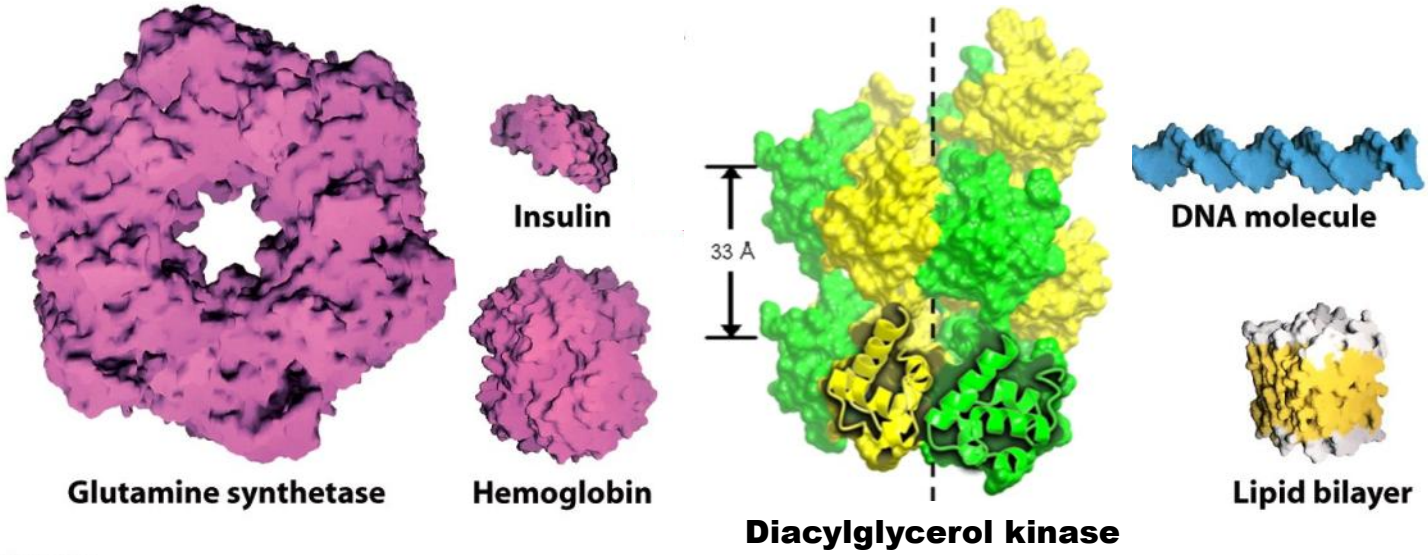
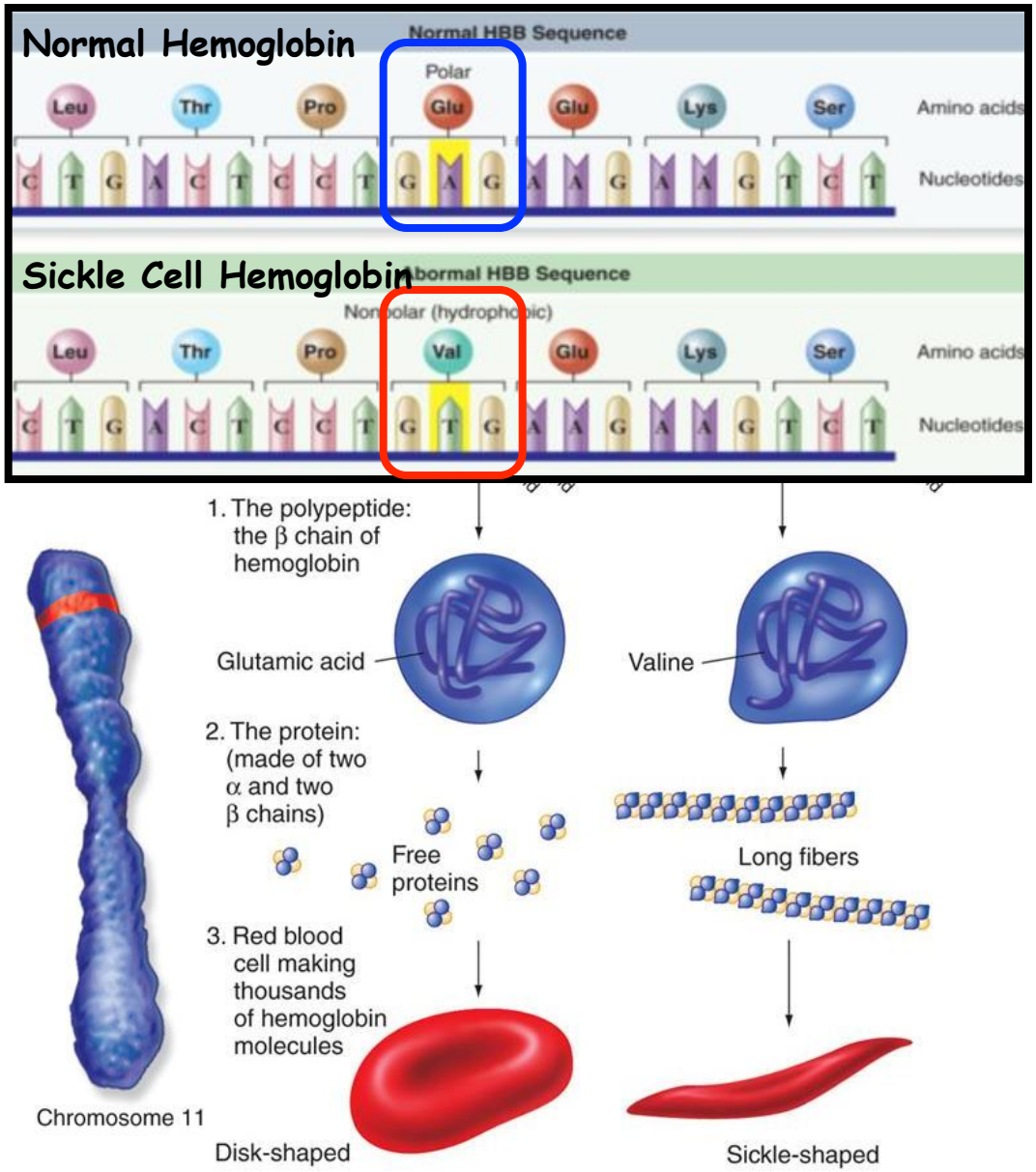


Figure 1-9
Molecular Cell Biology, Sixth Edition

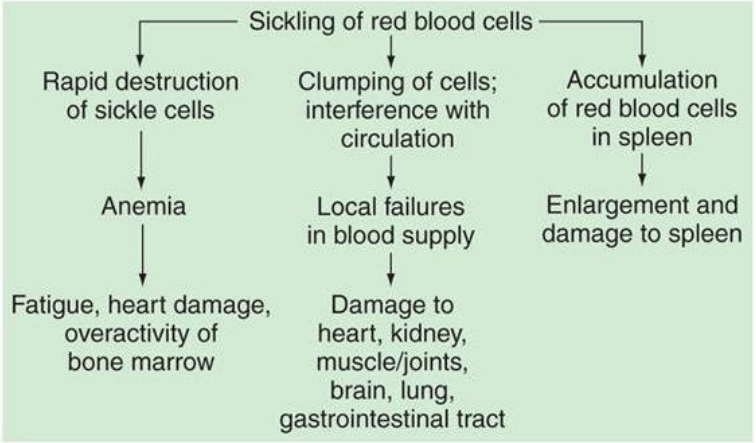
Novel Cell Functions & Phenotypes

Human Genetic Disorders Occur As a Result of Mutations



Sickle-Cell Anemia

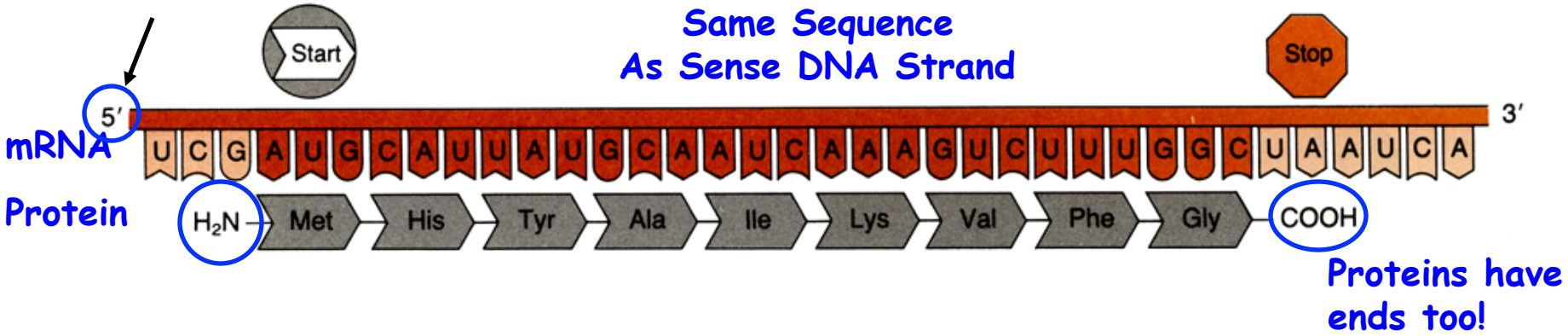
(b) Sickle-cell anemia is pleiotrophic



(c) β-chain substitutions/variants

	Amino-acid position									
	1	2	3 ··· 6		7 ··· 26 ··· 63 ··· 67···125···146					
Normal (HbA)	Val	His	Leu	Glu	Glu	Glu	His	Val	Glu	His
HbS	Val	His	Leu	Val	Glu	Glu	His	Val	Glu	His
HbC	Val	His	Leu	Lys	Glu	Glu	His	Val	Glu	His
HbG San Jose	Val	His	Leu	Glu	Gly	Glu	His	Val	Glu	His
HbE	Val	His	Leu	Glu	Glu	Lys	His	Val	Glu	His
HbM Saskatoon	Val	His	Leu	Glu	Glu	Glu	Tyr	Val	Glu	His
Hb Zurich	Val	His	Leu	Glu	Glu	Glu	Arg	Val	Glu	His
HbM Milwaukee 1	Val	His	Leu	Glu	Glu	Glu	His	Glu	Glu	His
HbDβ Punjab	Val	His	Leu	Glu	Glu	Glu	His	Val	Gln	His

Genetic Code Allows the Sequence of Nucleotides in mRNA/sense Strand of Gene to be Translated into the Sequence of Amino Acids in Proteins

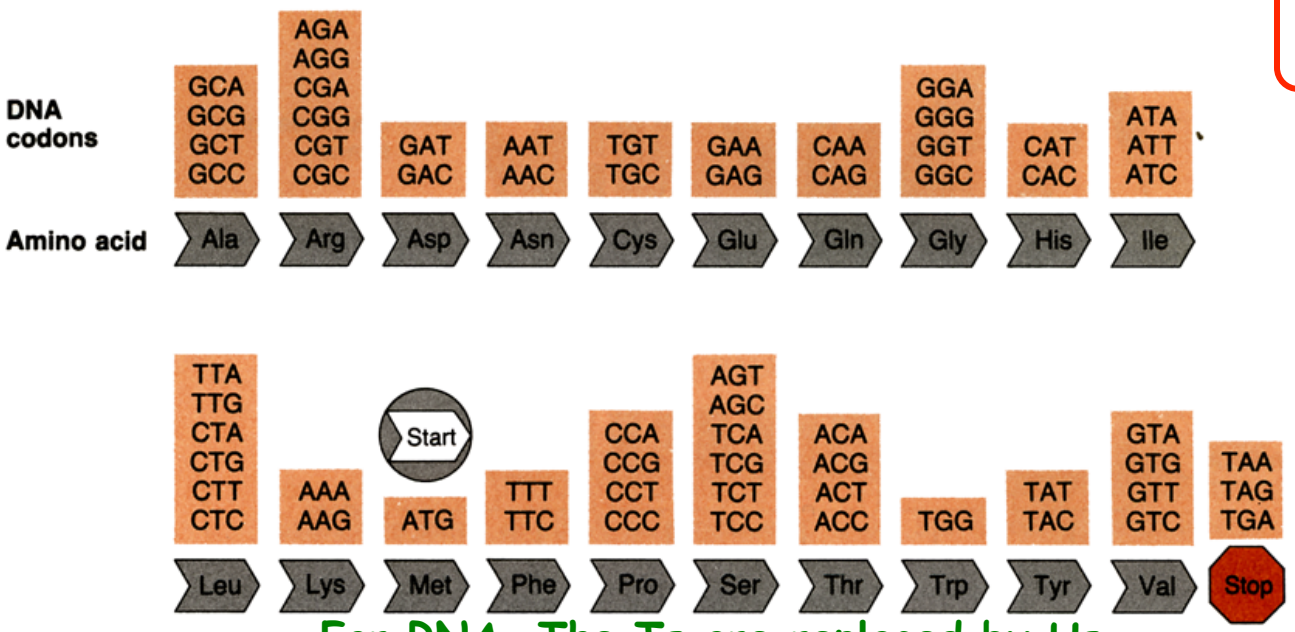


Notes: There are 20 amino acids

Sequence in mRNA (= Sense Gene Strand) is translated 5'→3' (= beginning of sense strand to end) & Protein made in N→C direction therefore order Nts in gene = order amino acid in protein!

The Genetic Code is Universal!

How Know?



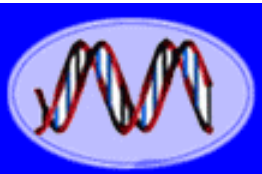
For RNA, The Ts are replaced by Us.

1. Universal
2. Triplet
3. Punctuation
4. Degenerate

Know Sequence of Gene-Know Sequence of Protein Using Genetic Code

Big Implication For Genetic Engineering! Can Make Genes, Genomes & Specify Proteins Wanted! Can Express Genes From One Organism in Another!

Design An Experiment to Show Code is Universal!



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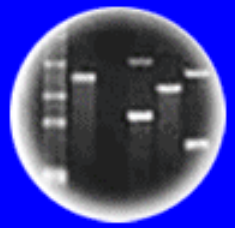
Entire Genetic Code
of a Bacteria



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Expression of Jellyfish Green Fluorescence Protein (GFP) in Pigs Shows That Genetic Code is Universal!!

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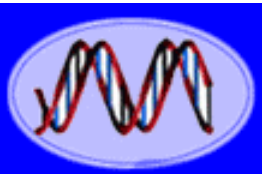

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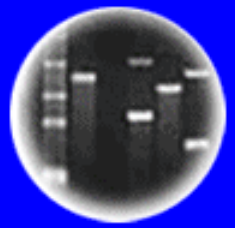

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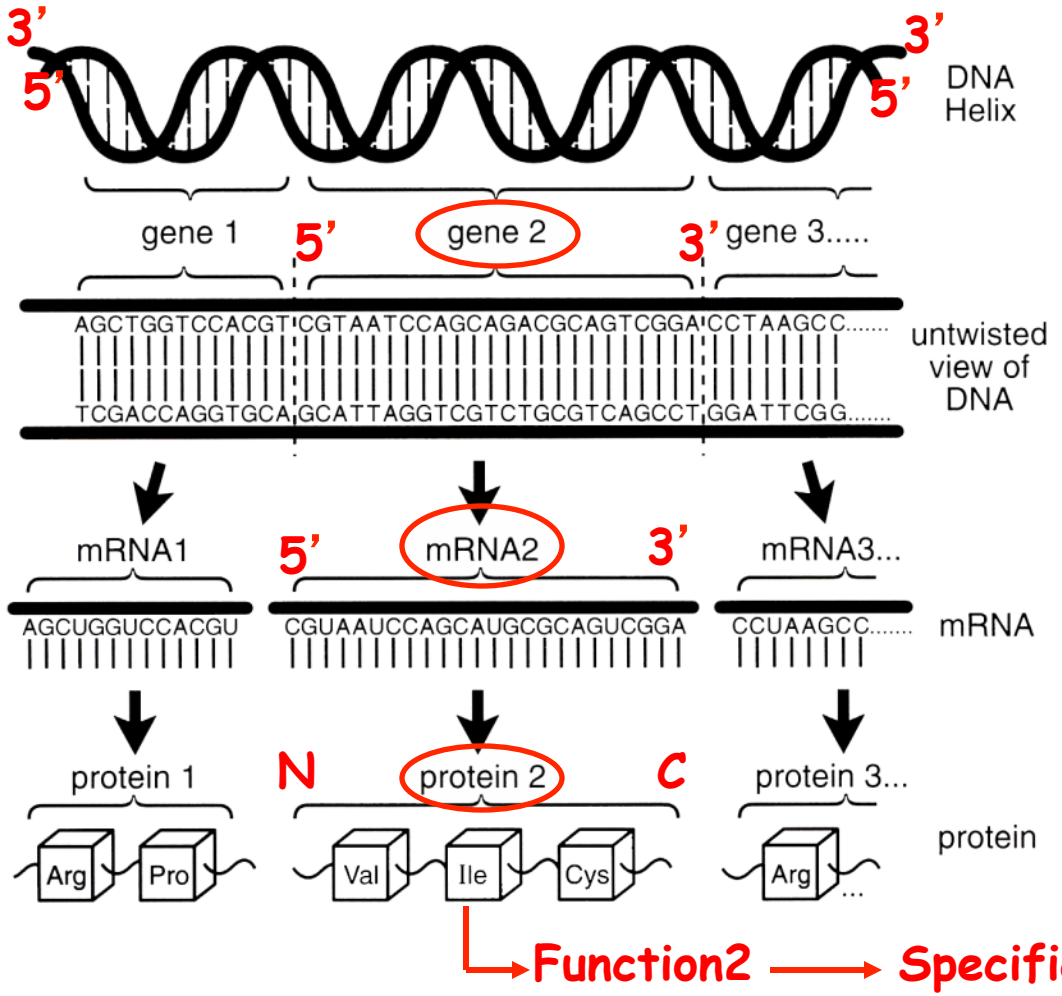
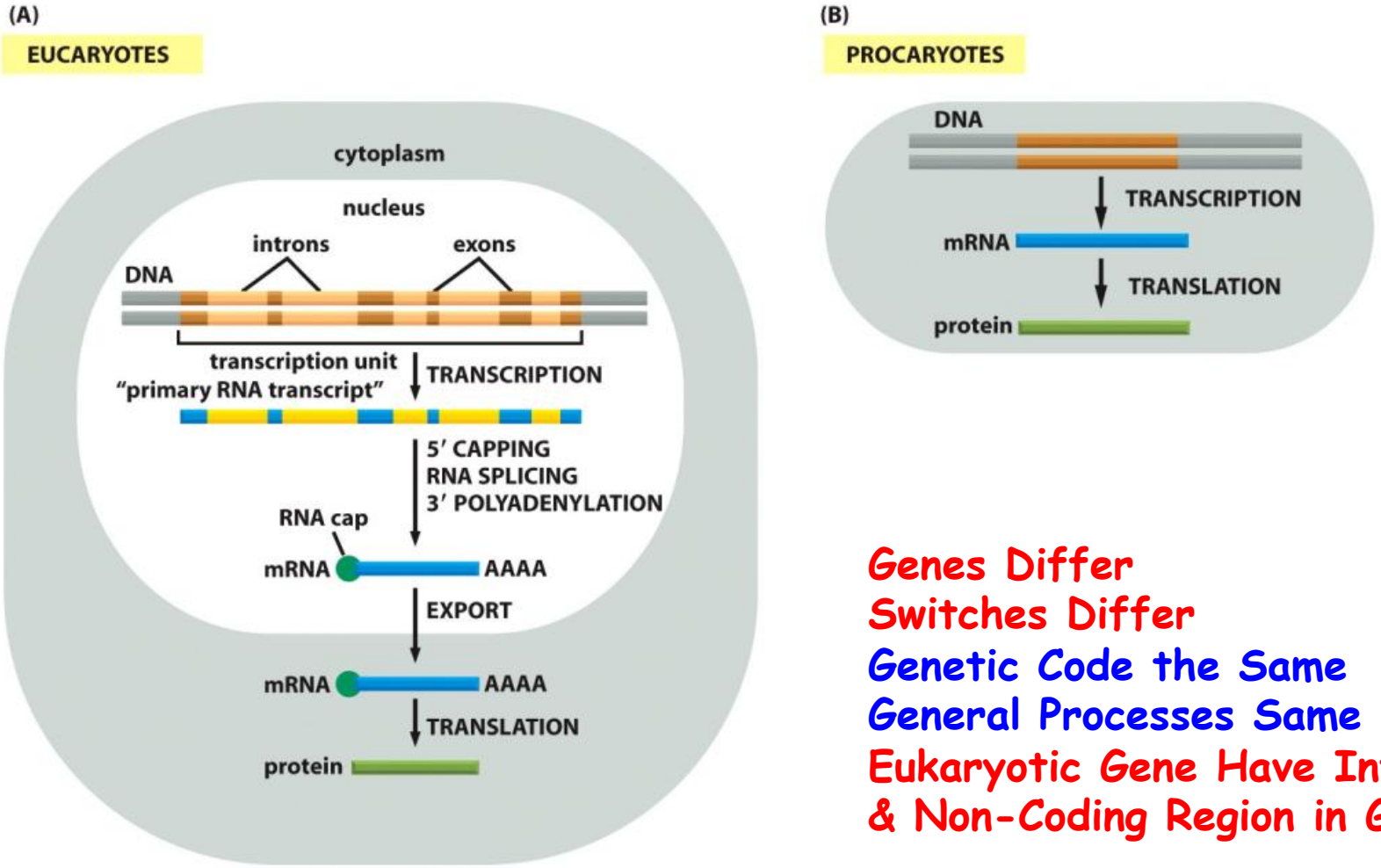
There is Colinearity Between the DNA Sequence of a Gene & the Amino Acid Sequence of a Protein



Genes Function As Individual Units!

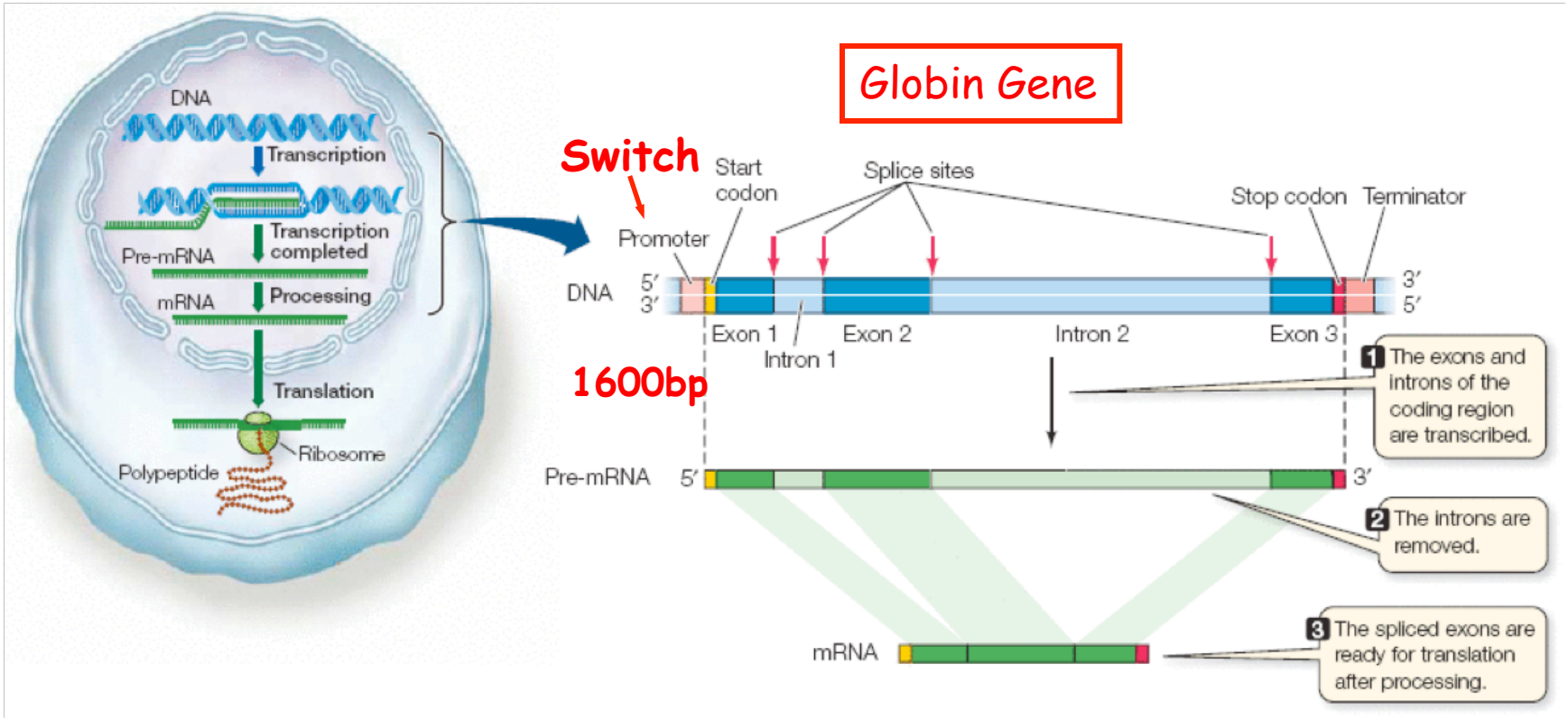
Eukaryotic and Prokaryotic Gene Expression Processes Differ Slightly



Genes Differ
Switches Differ
Genetic Code the Same
General Processes Same
Eukaryotic Gene Have Introns & Non-Coding Region in Gene!

Eukaryotic Cells Must Remove Non-Coding Region of RNA Before Genetic Code Can Be Translated Continuously!

RNA Splicing- Removing Non-Coding Sequences From Primary Transcripts & Generating Functional mRNAs



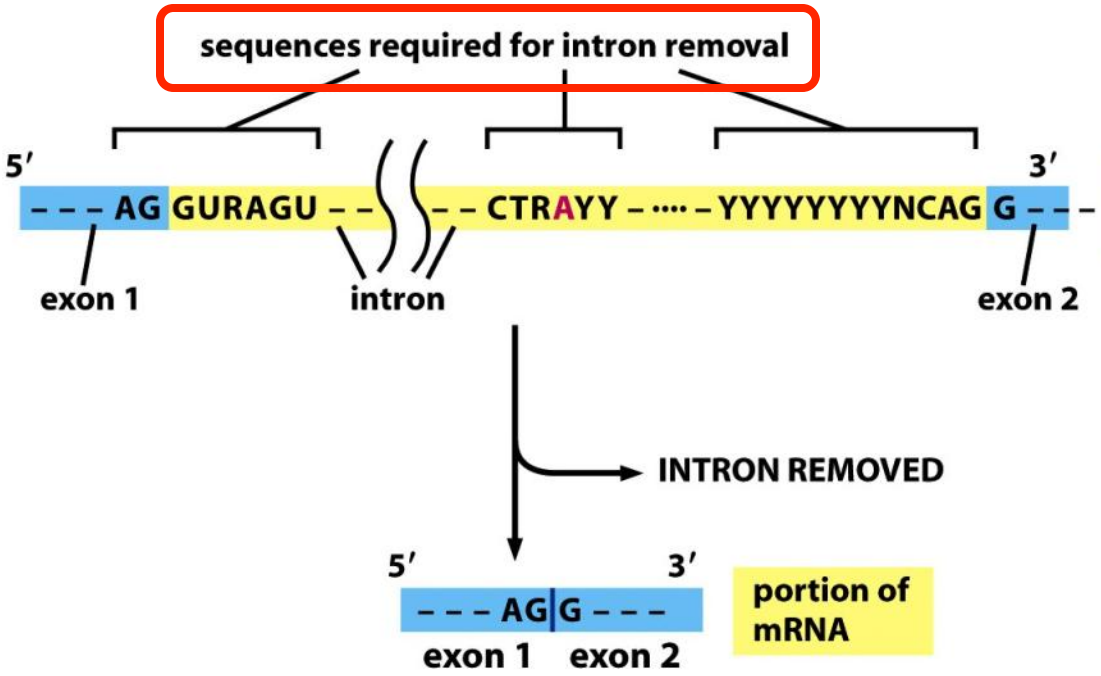
Mutations→ Blood Disorders
Where can these occur?

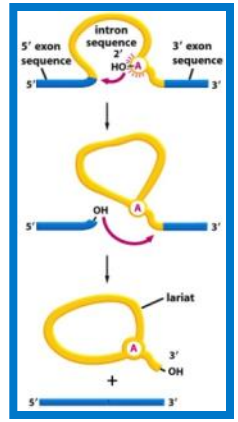
Mutations Can Occur in Coding Region, Switch, & RNA Splice Sites

→ **Mutant Phenotype**

Implications For Engineering Eukaryotic Gene in Bacterial Cell For Expression?

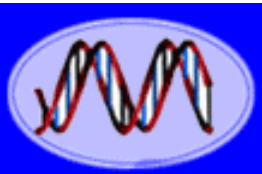
Yo! It's In The Sequences!





Specific Sequences Required For RNA Splicing!

What Happens If These Sequences Are Mutated in A Gene?



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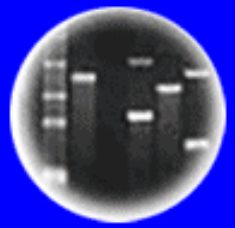

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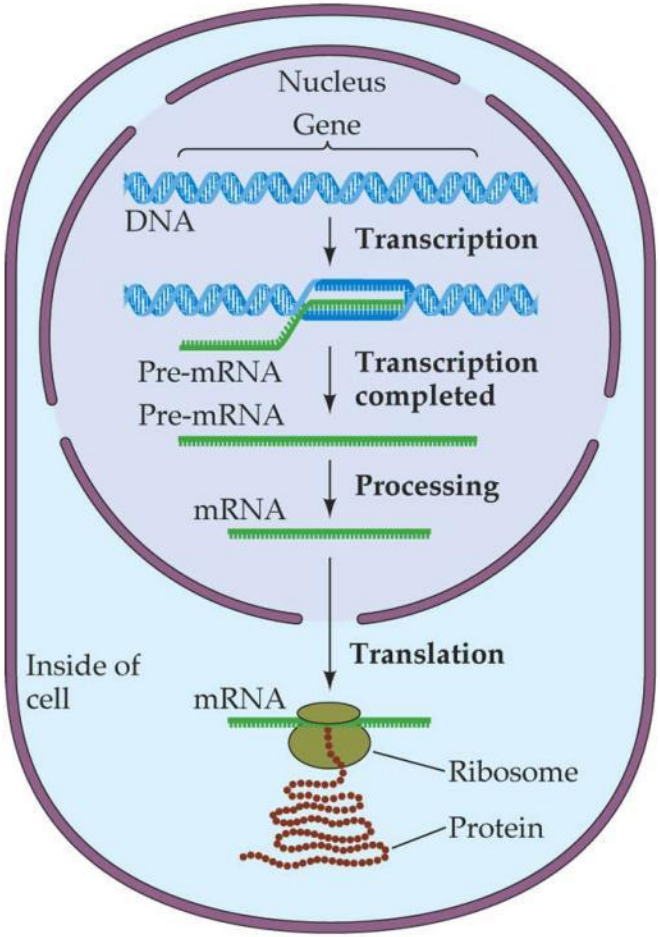
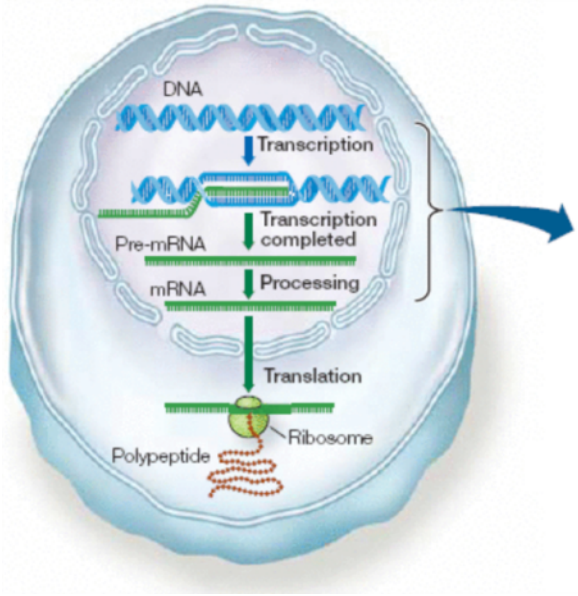
An Elaborate Cellular Machinery Requiring Thousands Of Genes is Required To Produce Proteins Encoded By Specific Genes!!



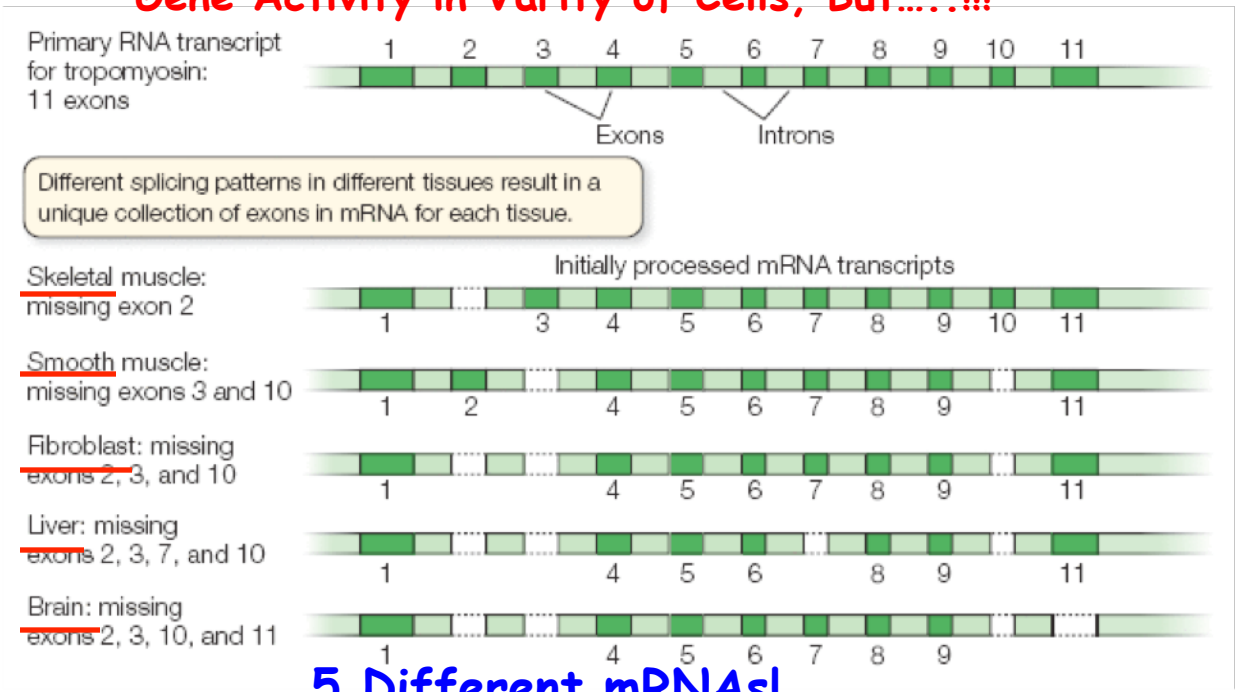
LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 14.1 Eukaryotic mRNA Is Transcribed in the Nucleus but Translated in the Cytoplasm

It takes Genes
to Express
(and Replicate)
A GENE!!!

Alternative Splicing- One Gene → Several mRNAs & Proteins



Gene Activity in Varity of Cells, But…..!!!



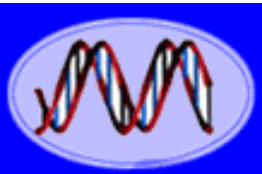
5 Different mRNAs!

Different mRNA = Different Proteins = Different Functions!

Implication- Human Genome Has Only 25,000 Genes But Can Give Rise to Many More Proteins which Are Responsible For Producing the Phenotype

Reason Why Human Genome Can Contain Same Number of Genes as Fly and Plant Genomes!!

Implications for Genetic Engineering? Use Specific cDNA!



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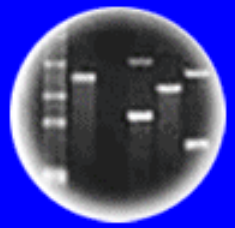

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Implications For "Yo - Its in The DNA!!"

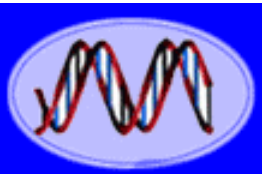
Modular Organization of Sequences

1. DNA Replication
   - Ori
2. Transcription
   - Switch/Regulator
   - Terminator
3. Processing of RNA (Eukaryotes)
   - Splicing Sites
4. Translation
   - Start
   - Stop
   - Genetic Code/Codons
5. Coding Sequence
   - Genetic Code

Modules → Anything You Want To Do Using Genetic Engineering!

The Modular Organization of Genes and Gene Function Implies That There Are No Limits to How Genes Can Be Functionally Changed and Rearranged Using Genetic Engineering?

a. True
b. False



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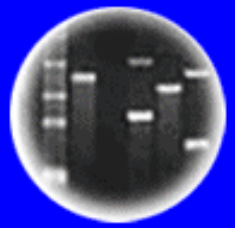

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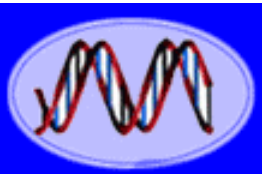
Engineering Genes Requires:

1. The Gene & Its DNA Sequences
2. A Roadmap of Where Coding Sequence & All Switches Located (Sequence, Restriction Site Map)
3. Transcription Start And Stop Switches
4. Coding Region of Gene (genetic code part)
5. Translation Start And Stop Switches
6. Kingdom-Specific Switches/ Signals

Note: The General Process of Gene→Protein is the same in ALL organisms, but the Specific Switches & Enzymes (e.g., RNA Polymerase) are Kingdom Specific

Bacteria Transcription On Switch + Human Insulin Coding Sequence + Bacteria Transcription Off Switch

→ Human Insulin in Bacteria!!



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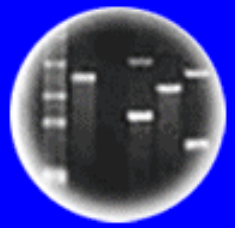

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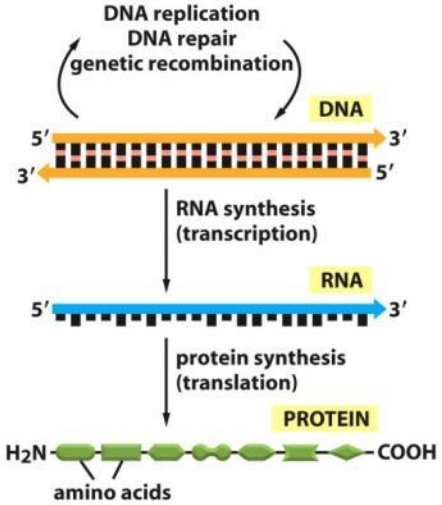
How Do Genes Work & What Are Genes In Context of…

Thinking About The Consequences of GMOs



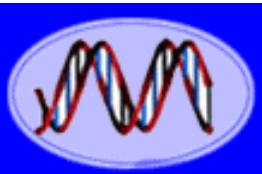
1. What is a Gene?
2. What is the Anatomy of a gene?
3. How Does the Gene Replicate?
4. How Does the Gene Direct Synthesis of a Protein?
5. Does the Gene Work Independently of other Genes?
6. What is the Sequence & Structure of the Protein?
7. How does it work in cell?
8. Does the Protein Structure imply any Potential "Harm"?
9. Does the Gene Change the organism? Fitness?

Need Science-Based Questions & Science-Based Solutions-NOT OPINIONS!

There's NO HOCUS POCUS all hypothesis are testable!!

"Behind" All Traits!

Same Processes!



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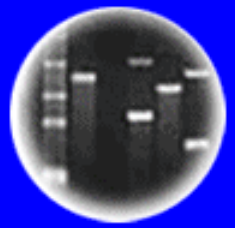

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DNA Fingerprinting Experiment