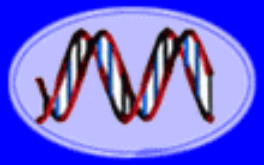
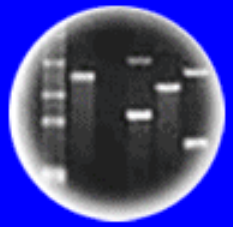


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

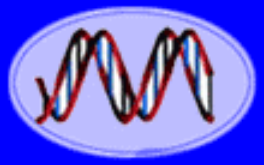
HC70A & PLSS059
Winter 2020
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & Channapatna Prakash

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

UCLA

TUSKEGEE UNIVERSITY



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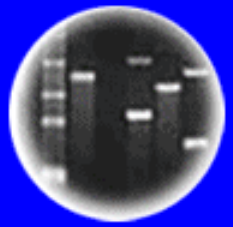

DNA Fingerprinting

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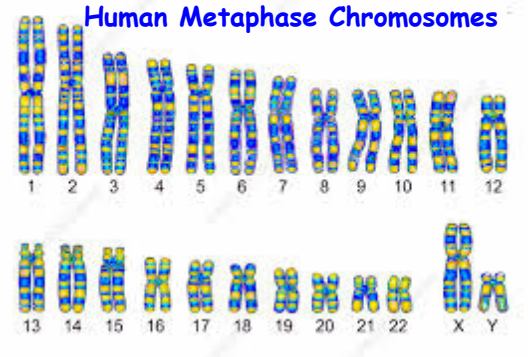
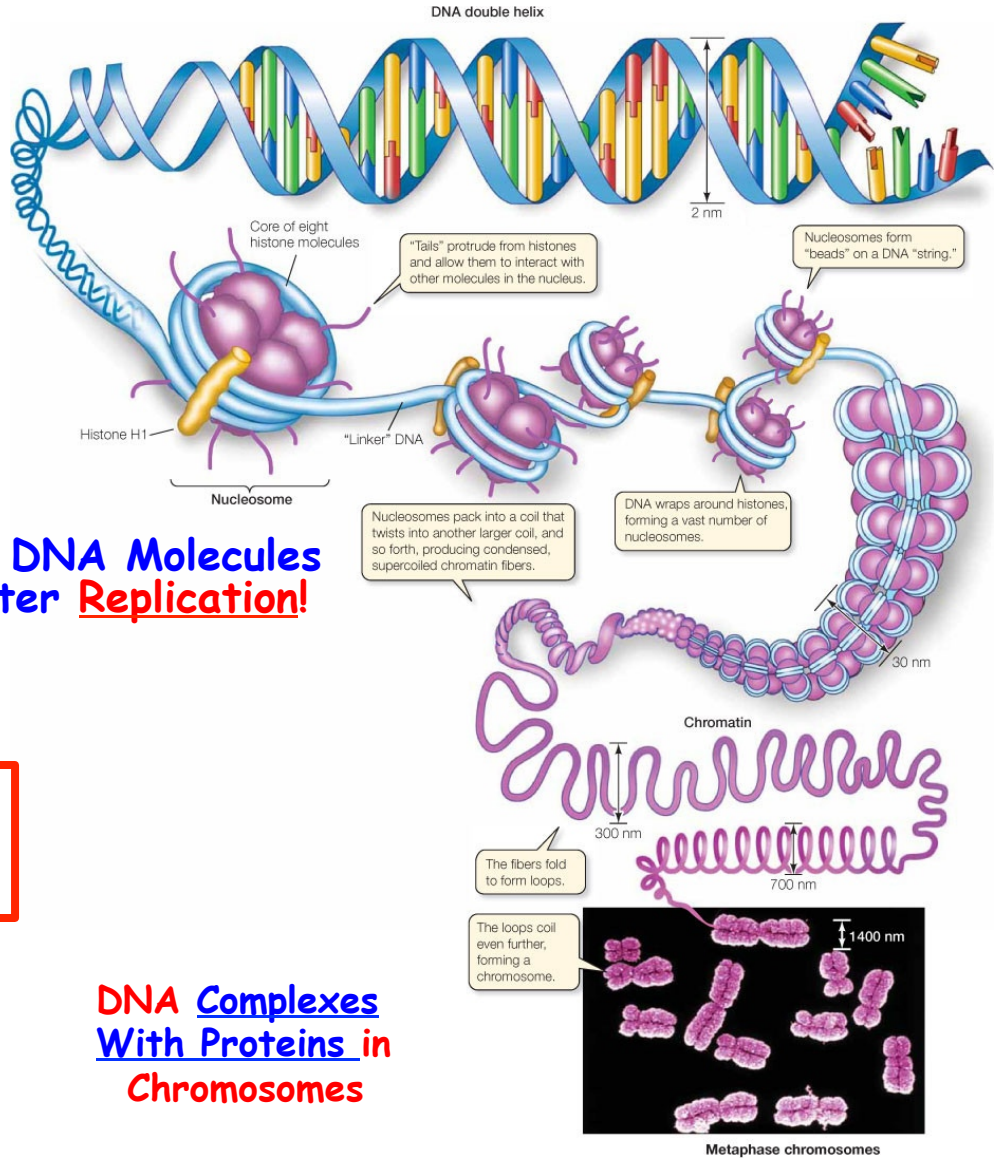
THEMES

1. How Are Genes & DNA Organized Into Chromosomes?
2. How Do Switches Regulate Genes in Space & Time?
3. Restriction Maps Enable Switches to be Isolated.
4. How Does DNA Replication Occur?
5. What is the Polymerase Chain Reaction (PCR) and How is PCR Used in Society?
6. How Do Mutations Occur?
7. How Can Pedigrees Be Used To Follow the Inheritance of Mutant Genes With Phenotypes and RFLPs?
8. How Do Mutations Change Phenotypes?
9. What is the Colinearity Between Genes & Proteins (i.e. how does the DNA sequence specify a protein sequence)?
10. What is the Genetic Code?
11. Yo!-It's in the DNA Sequences- What Are the Implications For Genetic Engineering?

A Chromosome Contains One (or Two!!) Continuous DNA Molecule(s)

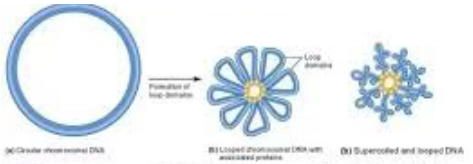
DNA in Human & Eukaryotic Chromosomes is Linear!





Two DNA Molecules After Replication!

DNA in Most Bacteria is Circular!

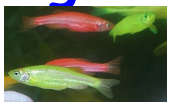


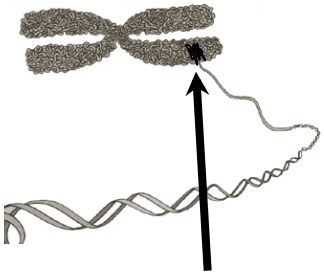
- Chromosomal DNA is compacted ~ 1000 fold to fit within cell

DNA Complexes With Proteins in Chromosomes

A Chromosome Contains Many Genes Operating Independently

What is the Evidence?





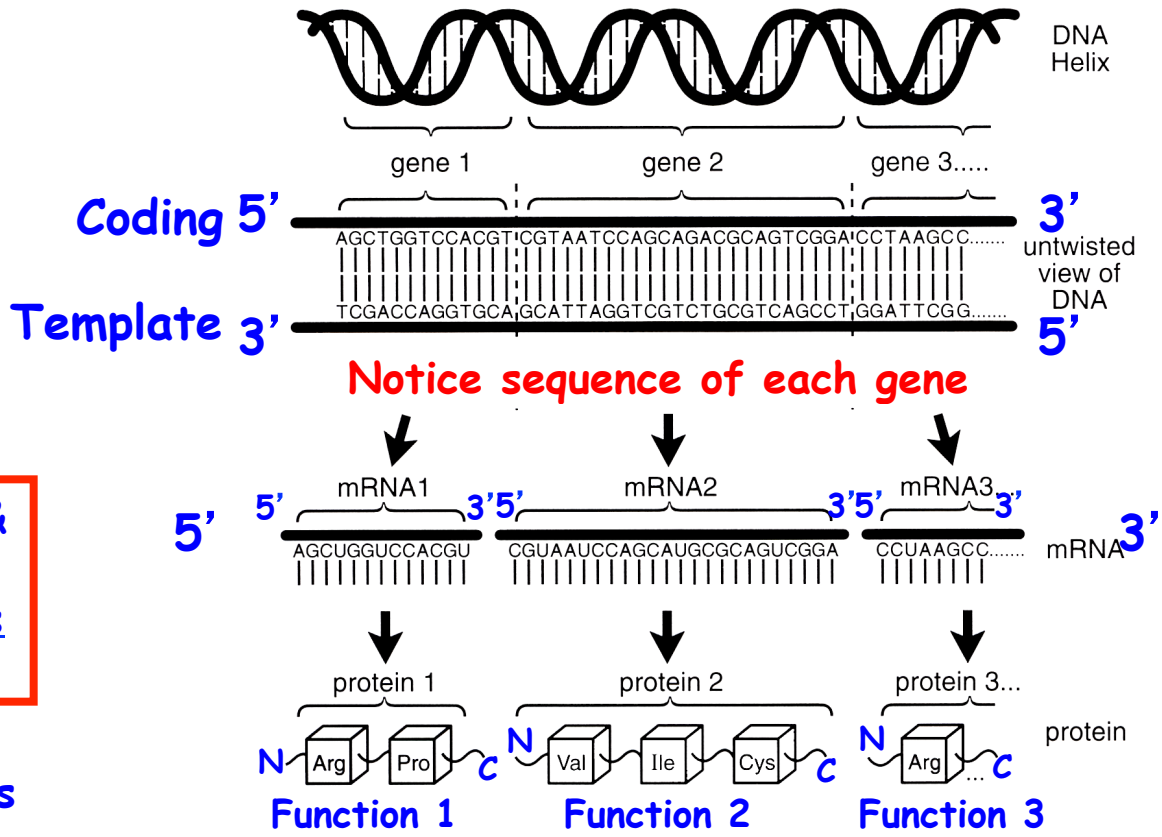
Position of Genes 1, 2, & 3 in chromosome

Discrete Units!

Notice- Each gene, mRNA, & protein has a unique order/ sequence of monomeric units

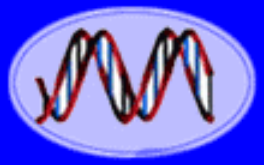
Central Dogma
∴Genes -> Functions in Cells via Proteins
Cells duplicate & stay the same -> DNA replication

What delineates each gene?



IMPORTANT HC70A CONCEPT!

COLINEARITY BETWEEN GENE SEQUENCE AND PROTEIN SEQUENCE



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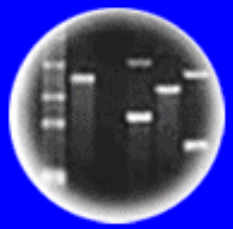


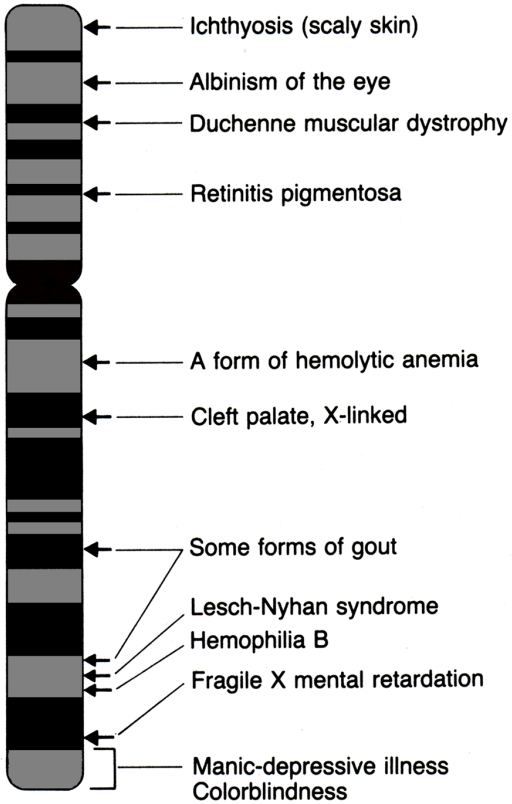
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

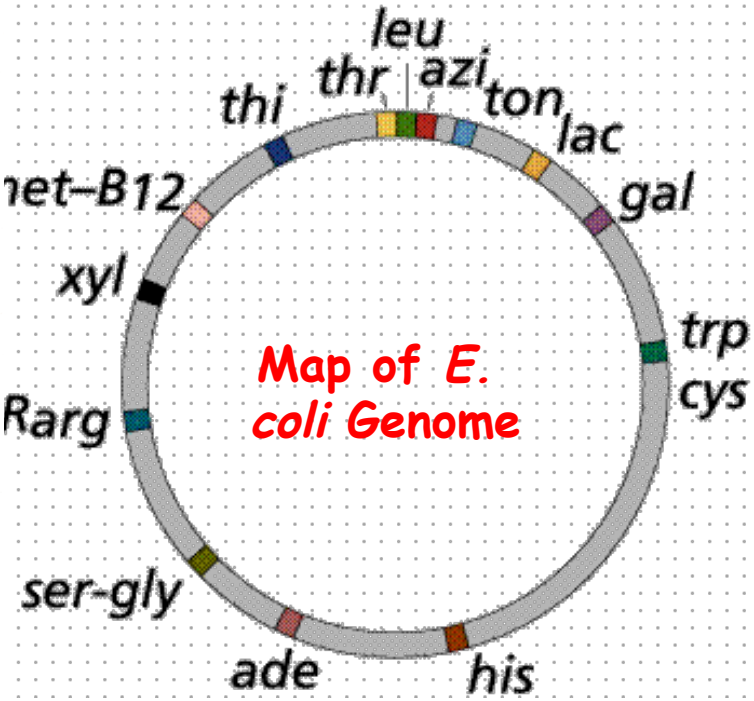
Plants of Tomorrow

Genes Reside at Specific Locations (Loci) That Can Be Mapped Genetically or By DNA Sequencing

Human X Chromosome



Linear DNA
How Know?



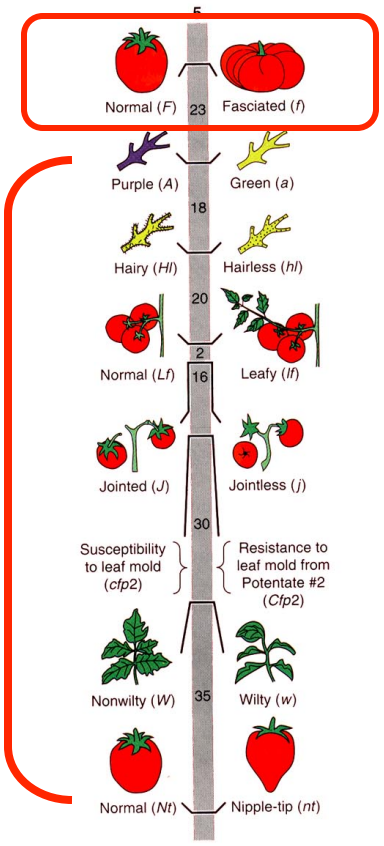
Circular DNA
How Know?

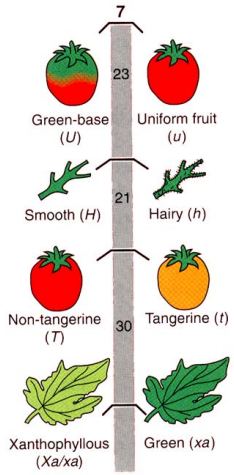
Alleles Reside at the Same Position on a Chromosome

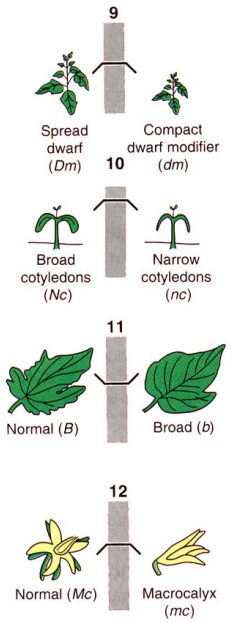
Allele Phenotypes Specify Markers For Each Gene Location!

Different Genes

Alleles







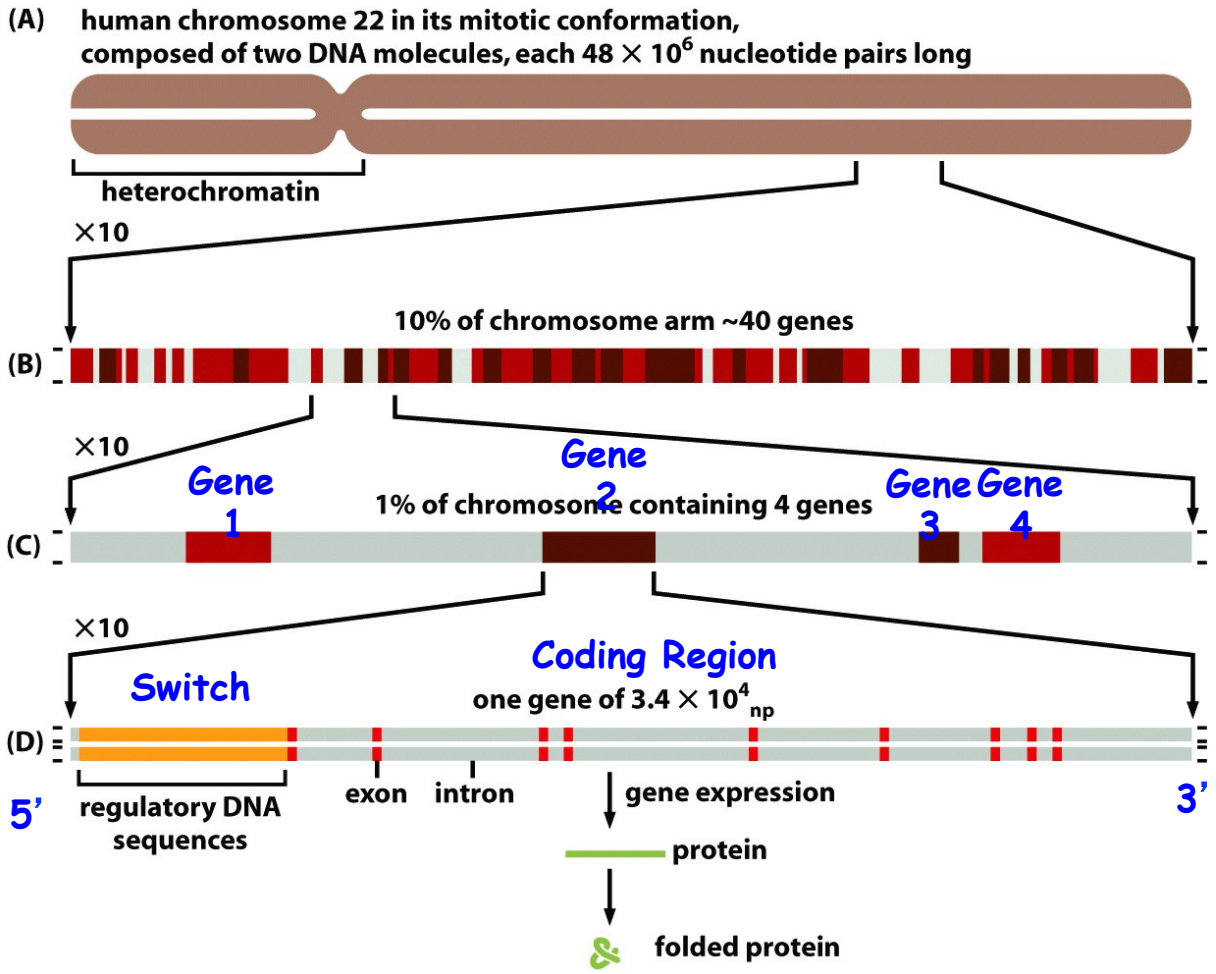
Source of All Genetic Variability

Gene Engineering Can Generate New Forms of Alleles of a Gene and, therefore, Results in More Genetic Diversity

mutations result in genetic diversity!!!

Alleles Are <u>Different Forms of the Same Gene</u>
That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!

Organization of Genes on Human Chromosome 22



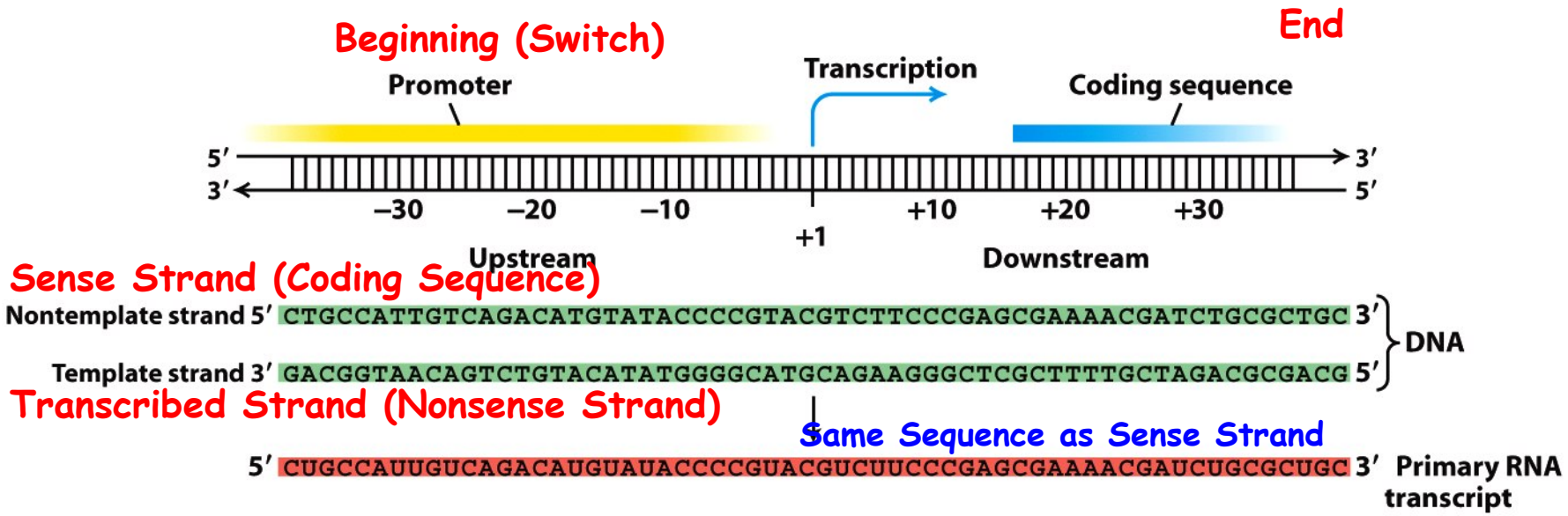
250 genes

Genes Are Defined/ Precise Regions of DNA

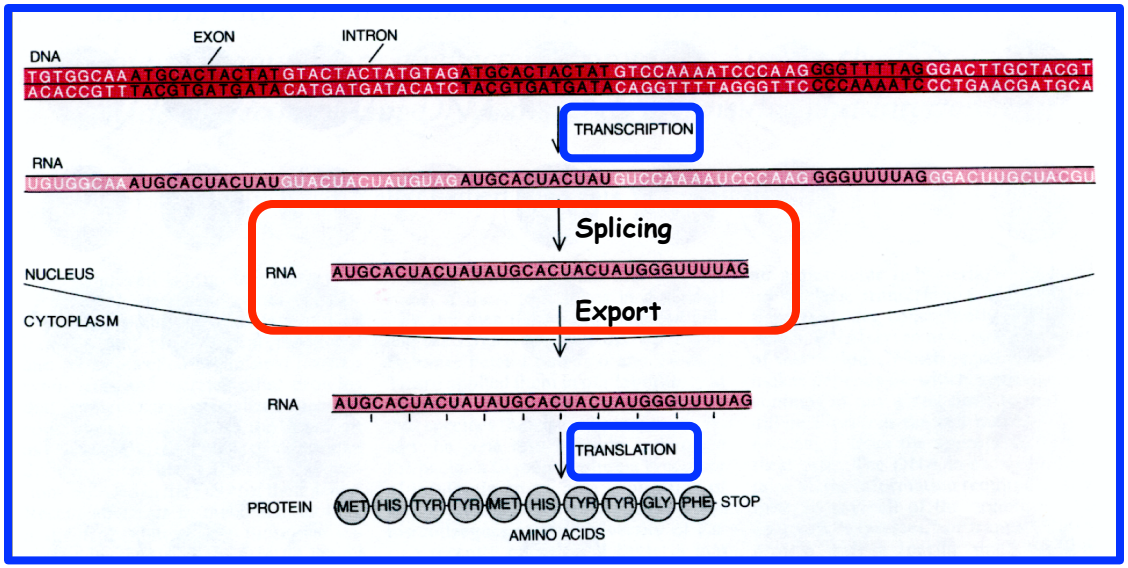
One Large Gene!
(Note Size of Introns & Exons)

Genes Act As Individual Units?
How Know? GloFish Experiment! Genetic Engineering Antibiotic^{R}!

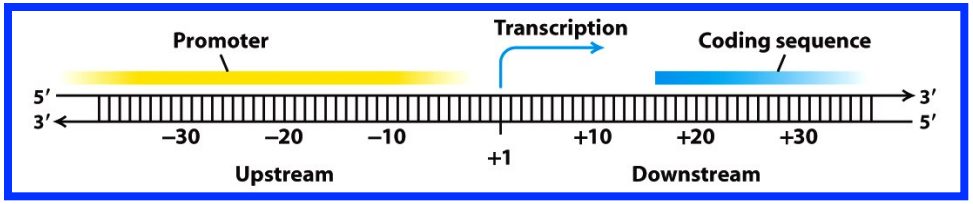
A Conceptualized Gene

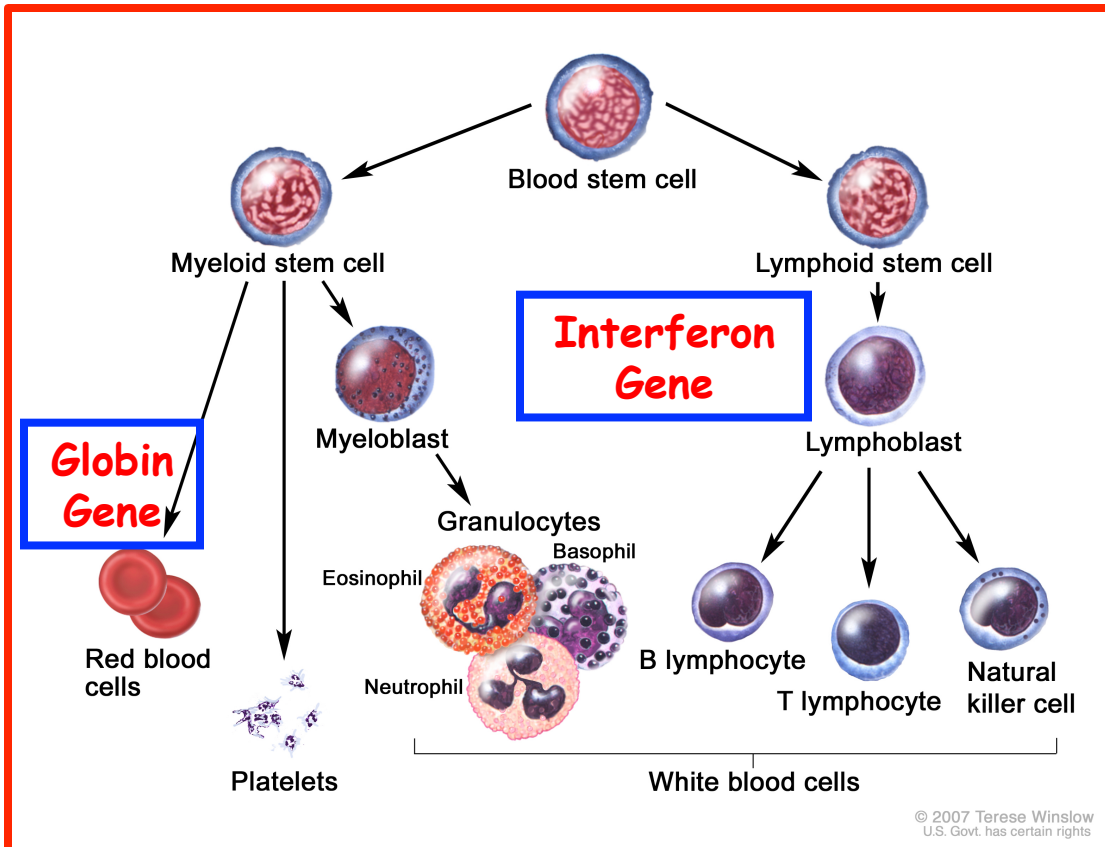


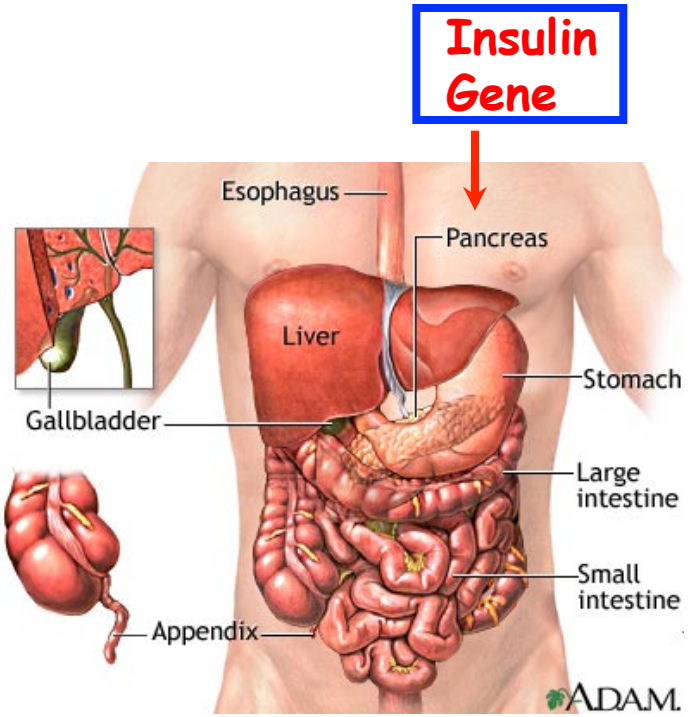
DNA to Protein
No Protein
No Phenotype or Trait



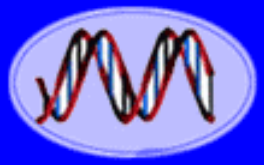
Switches Control Where & When A Gene Is Active
Unique Functions → Unique Cells







Different Switches!!!!



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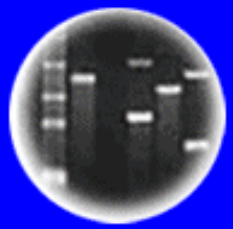


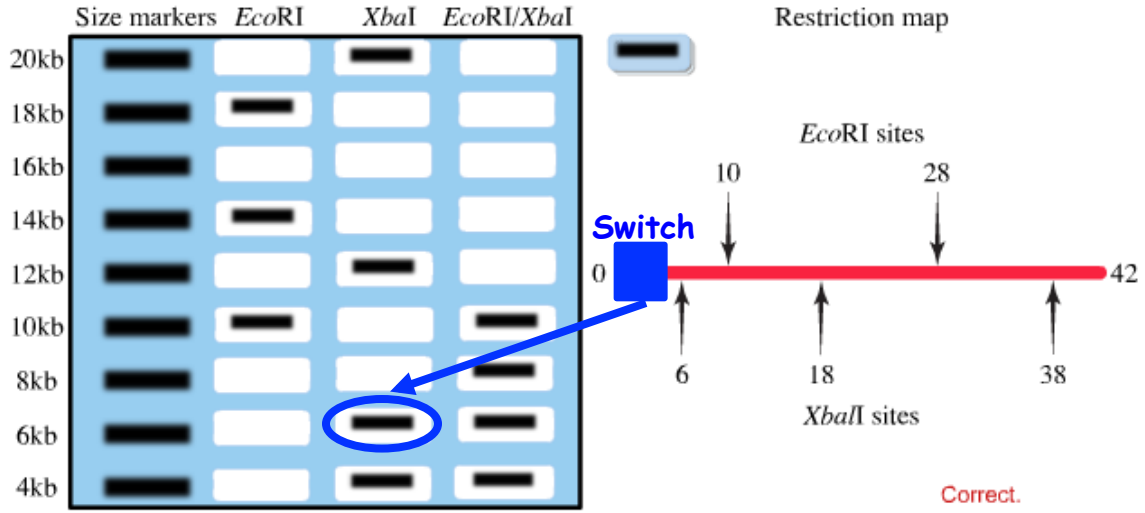
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

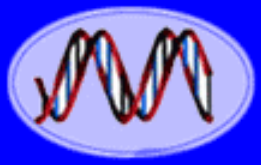
Restriction Maps Are Essential For Isolating Specific Gene Parts

Switches and Other Gene Parts Can Be Cloned & "Shuffled" Creating New Genes That Have No Counterparts in Nature – IF Parts Have Been Localized on Specific Cloned DNA Fragments



To Isolate Switch Use XbaI

And Combining Them With Other Gene Parts Like a Lego Set



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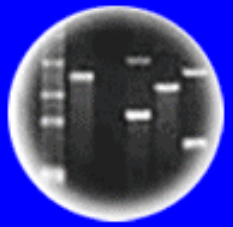

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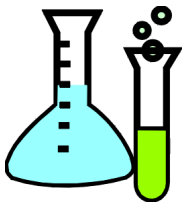
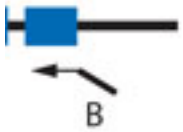
Recall: Engineering the Jellyfish GFP Gene to Be Active in Different Organisms



Isolate From a Gene "Cut"

Species-Specific "On Switch"



Jellyfish GFP Gene

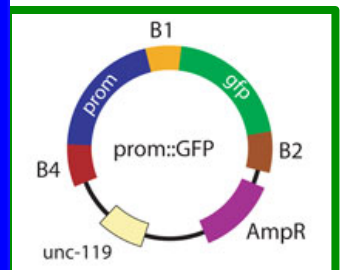
"Anneal & Splice"

Chimeric Jellyfish GFP Gene

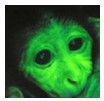
amplicon #3

This IS Genetic Engineering
Engineered Chimeric GFP Gene

Essential HC70A Concept
It's in the Sequences!



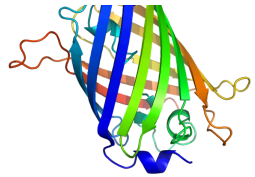
Transform



Animals

Plants

Bacteria

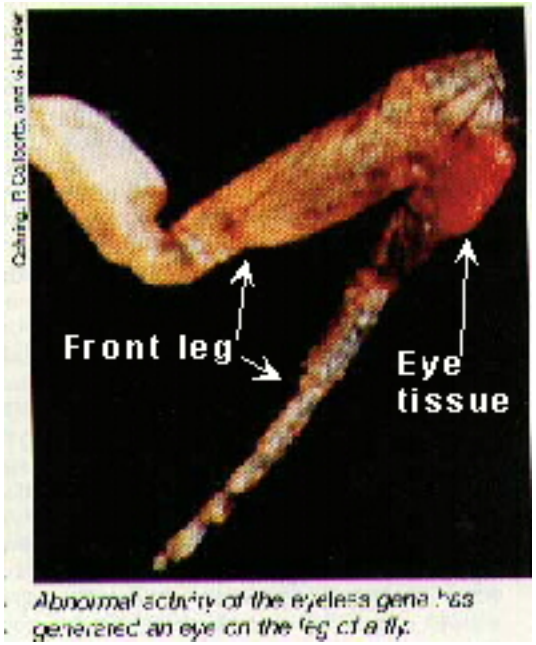


The Eye Gene Can Be Expressed in Different Parts of the Fly by Engineering the Eye Switch

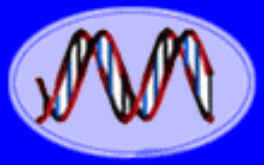
Eye Gene

Replace the Head Switch With the Leg Switch by Genetic Engineering

Eye Gene
+
Leg Switch



Abnormal activity of the eyeless gene has generated an eye on the leg of a fly.



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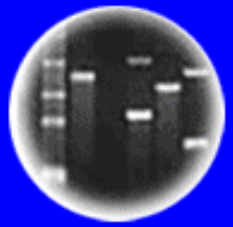

DNA Fingerprinting

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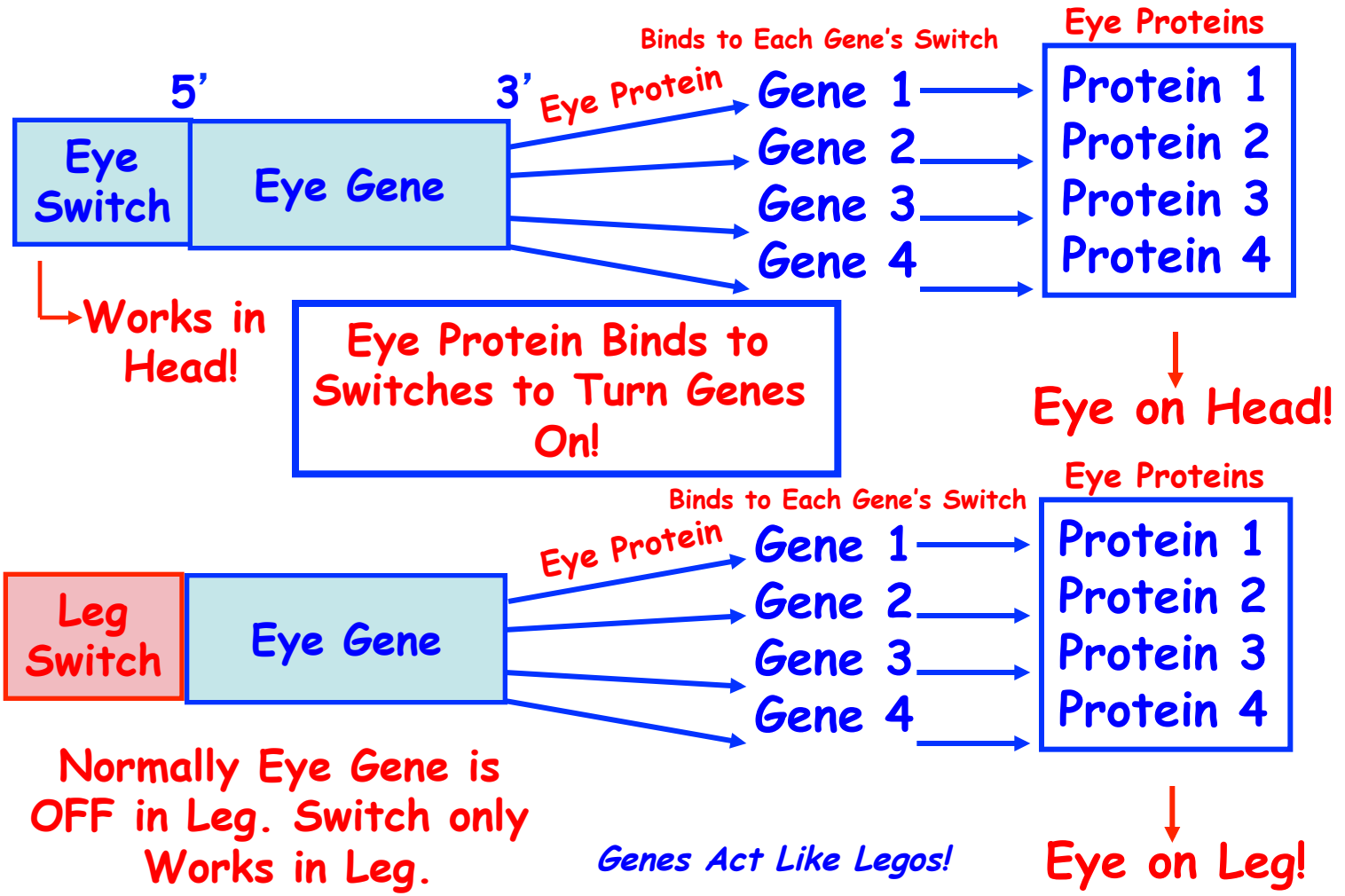
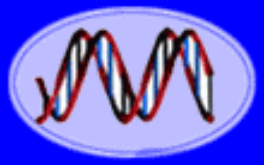
Plants of Tomorrow

Eye Regulatory Network

Control Genes Like The Eye Gene Control The Activity of Other Genes By Coding For a Protein That Interacts With Switches of Other Genes and Switches These Genes On!





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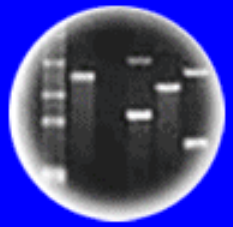

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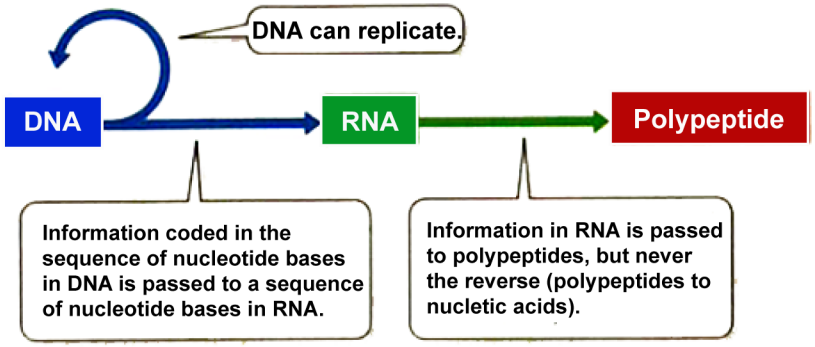
GENES AND SWITCHES ARE UNIQUE DNA SEQUENCES

1. They Can Be Cloned & "Shuffled" & Engineered Creating New Genes That Have No Counterparts in Nature
2. These New Genes Can Be Transcribed in New Cell Types (Switch Change) &/or Organisms &/or Both (e.g., Human Genes in Bacteria)
3. All Genes are Regulated & Controlled by Switches Genetic Engineering Can Uncover Genes & their Switches & the Wiring Together of All Switches in All Genes ⇨ Program of Life From Birth to Death

Yo! It's in the Sequences!!

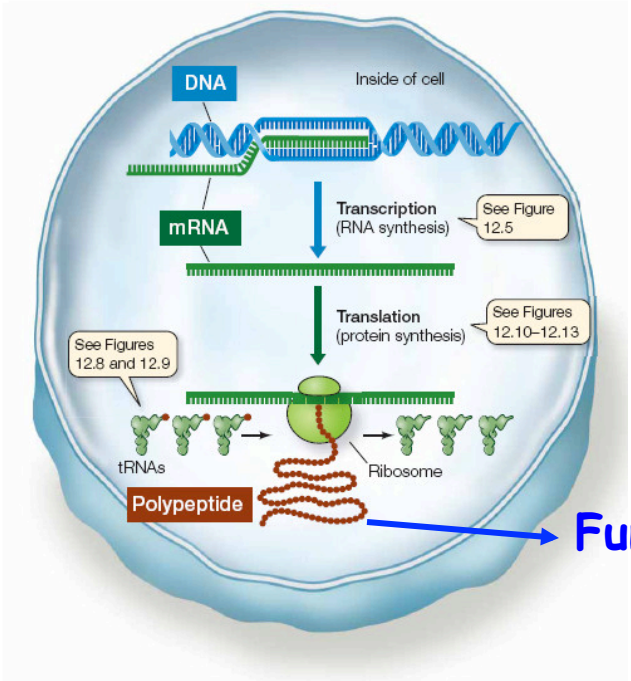
How Do Genes Work?

① Replication



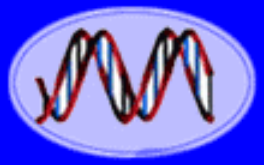
② Gene Activity to Function & Phenotype

Gene Activity → Protein → Function → Phenotype (Trait)



Function →

A Gene is NOT Expressed Unless A Functional Protein Produced!



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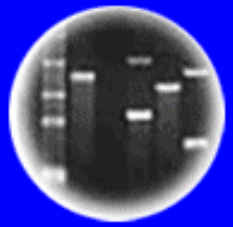

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WHAT ARE THE PROPERTIES OF A GENE?

1. Replication

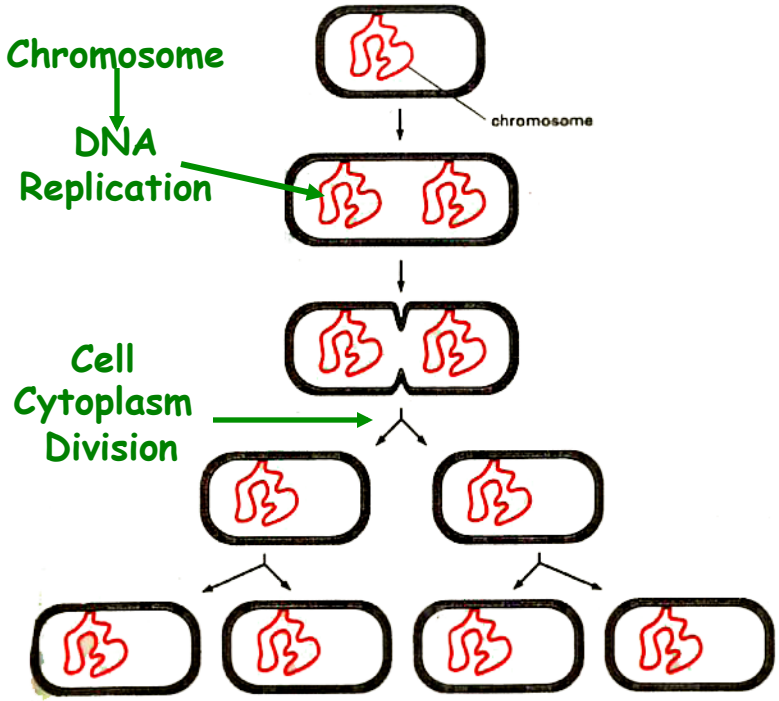
2. Stability (Mutations)

3. Universality
   a) All Cells
   b) All Organisms

4. Direct Cell Function/
   Phenotype

1

How Are Genes Replicated Each Cell Generation?

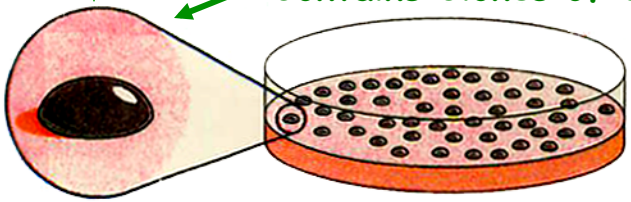


Clones

A Bacterial Colony Contains Many Copies of Same Cell, or Clones, Which are Genetically Identical!

A "Clone"

Note - Each Clone of Bacteria Contains Clones of Cells



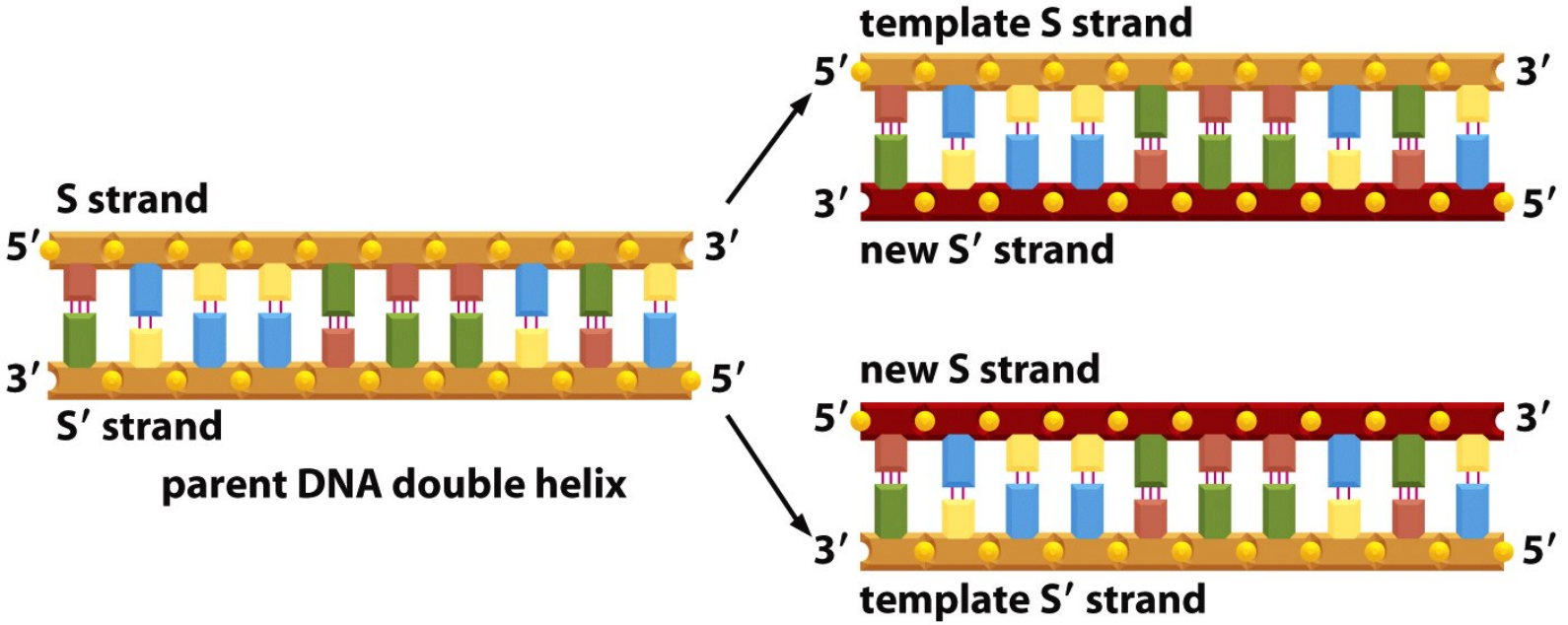
-10^6 cells per colony

~50 colonies per dish

Each Daughter Cell Contains The Same Collection of Genes

Clones!

DNA Replication Occurs Semi-Conservatively



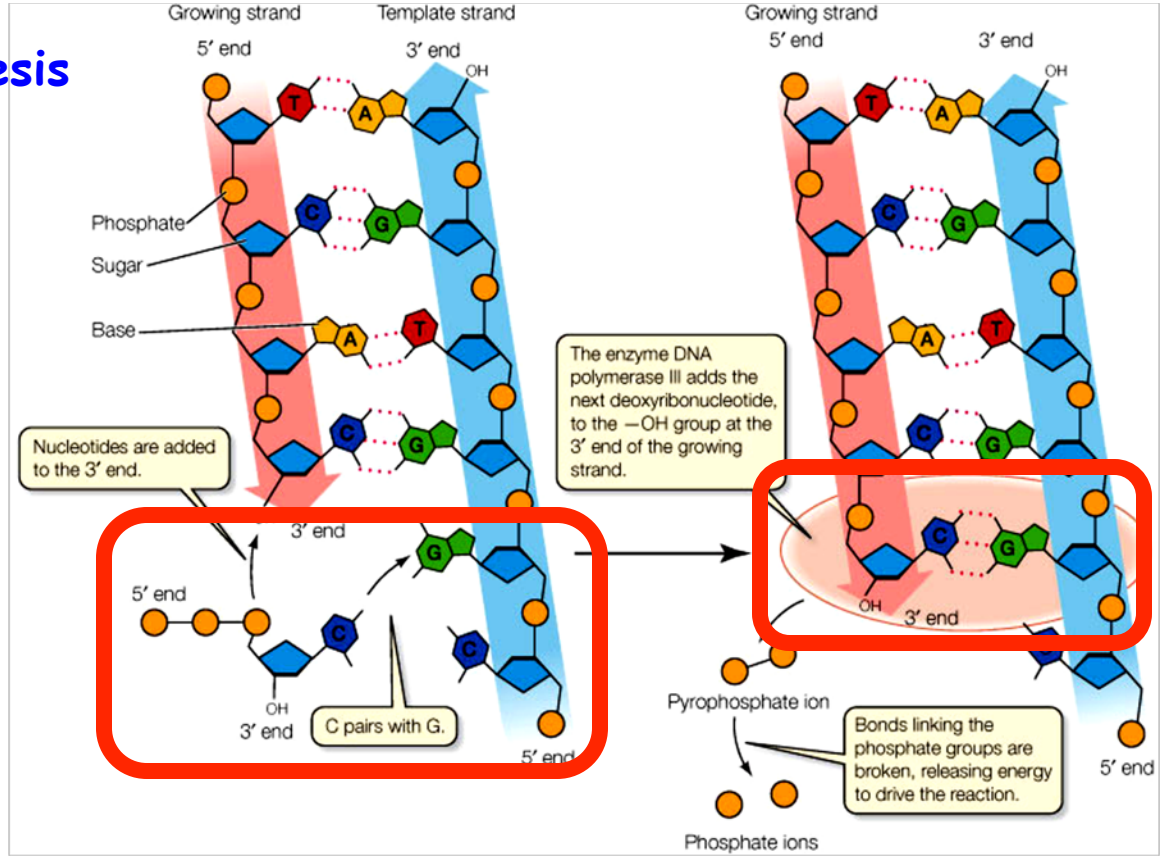
1. DNA Structure Allows DNA Sequence to Be Maintained by Complementary Base Pairing
2. Each Strand Serves as a Template for the Synthesis of a Complementary Strand
3. New DNA Molecules are Precise Copies of Parental DNA - Each Containing One Newly Synthesized Complementary Strand
4. Predicted by Watson & Crick!!

DNA Sequence of One Strand is a Template For the New Strand

Synthesis

5'

3'

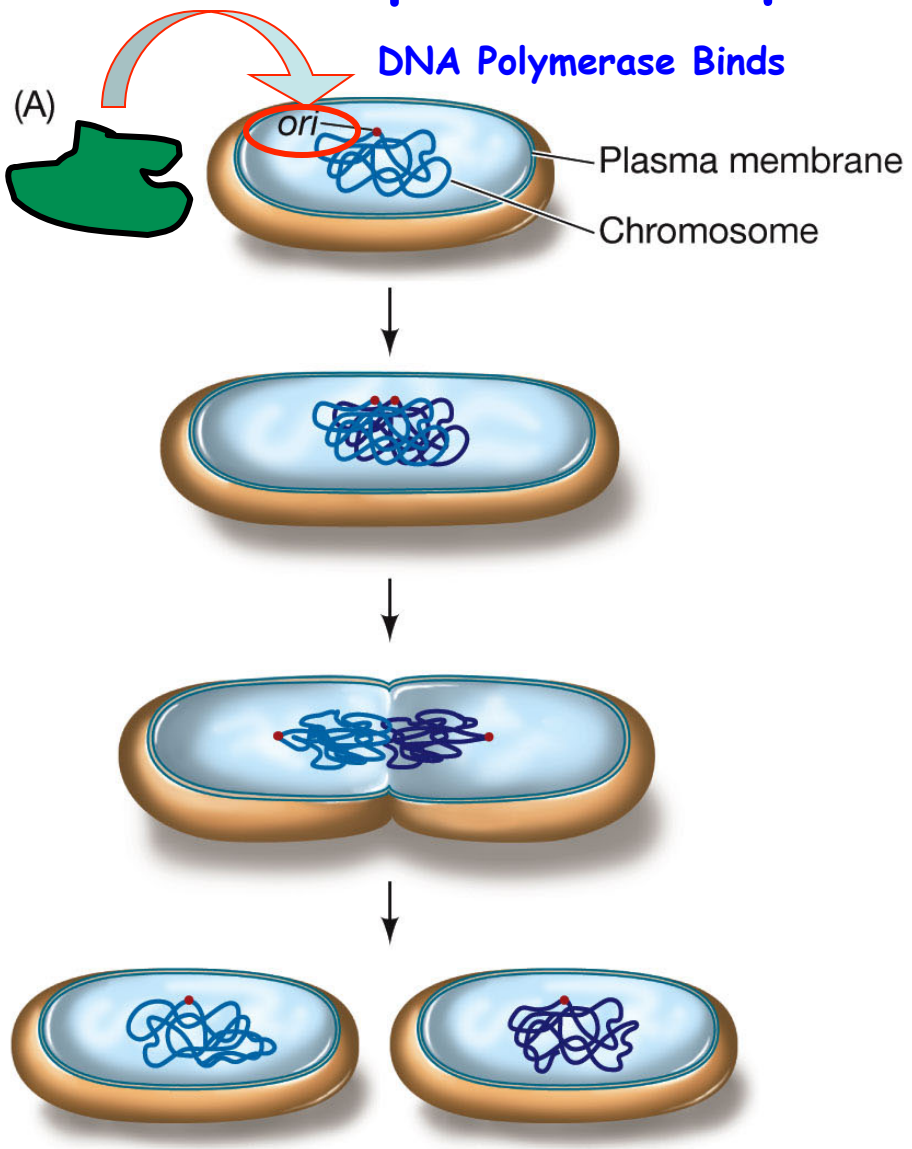


Sequence is Specified by Complementary Bases

Note: 5' (P) & 3' (OH)

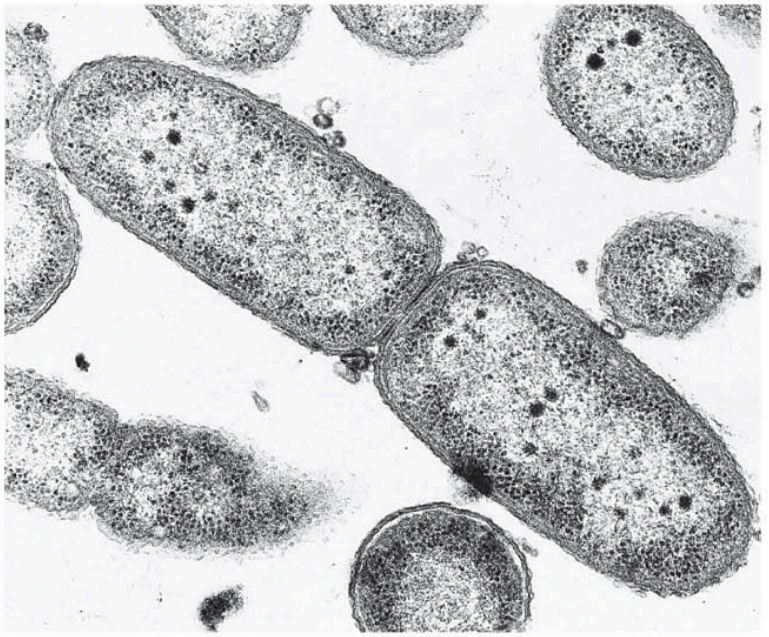
5' to 3' Polarity Specifies Sequence

DNA Replication Requires An Origin of Replication



Two IDENTICAL Cells - Phenotypically & Genotypically - From One

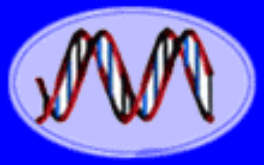
(b)



DNA Replication Also Requires:

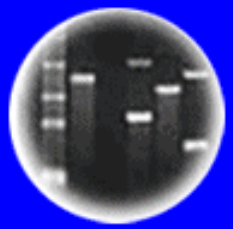
1. Template DNA Strand
2. Nucleotides
3. DNA Polymerase (Machine)
4. "Primer" to Start Replication

LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.2 Prokaryotic Cell Division



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

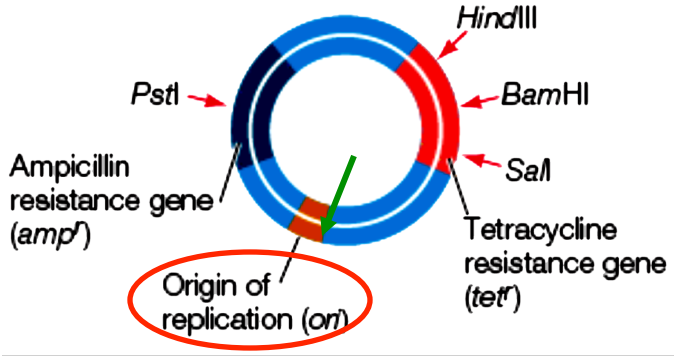


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Vectors Are Needed To Replicate Genes In Transformed Cells



1. Ori is a Specific Sequence
2. Ori is Genome & Organism Specific
3. DNA Polymerases are Specific For Each Organism Therefore, Need Correct Ori to Replicate Gene in a Specific Organism!

Note → Need Bacterial Ori to clone human gene in bacteria. Need human Ori to replicate a bacterial gene in human cells.

Ori Along Chromosomes Allows Gfp Gene to be Replicated. Uses Endogenous Ori!

Yo! It's in the Sequence= Function

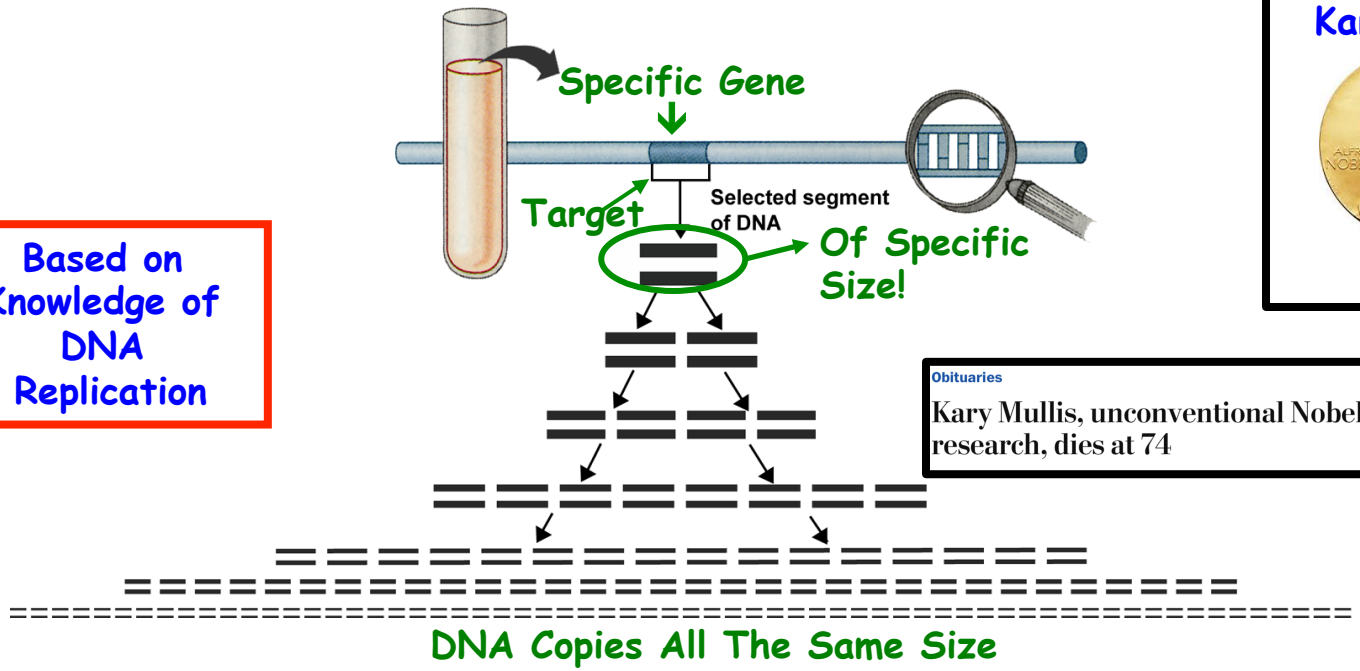
∴ Vectors can be Engineered!

Ori's can be cloned/synthesized!

MODULAR!!

The Second Genetic Engineering Revolution - The Polymerase Chain Reaction (PCR) is a Molecular Xerox Machine That Can Amplify DNA Sequences in a Test Tube Without Cloning!

Based on Knowledge of DNA Replication



Kary Mullis

1993

Obituaries

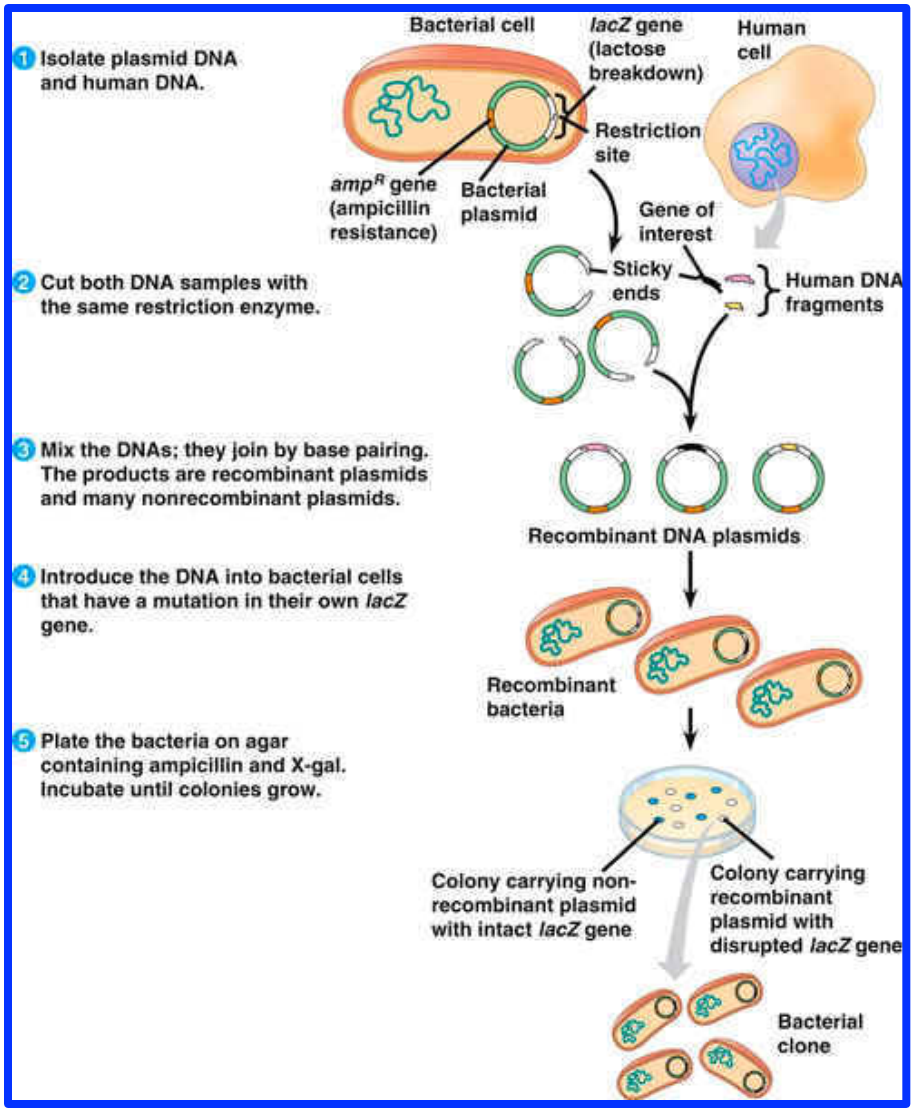
Kary Mullis, unconventional Nobel laureate who unlocked DNA research, dies at 74

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

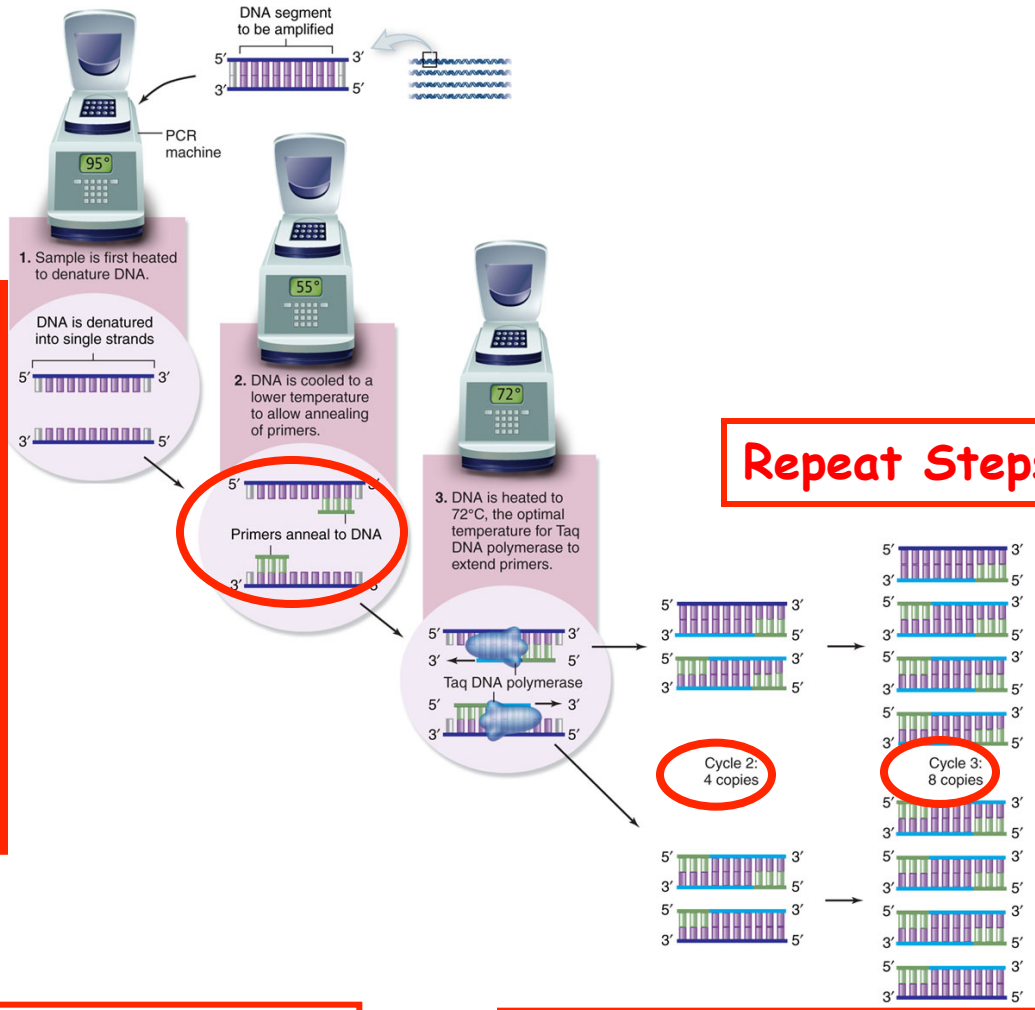
2. No Bacterial Cloning Needed!

3. But Need Sequence! ⇨ Have to Clone "Gene" First

DNA Cloning the "Old Fashioned" Way is a Lot of Work!



PCR is A Cyclical Process of DNA Replication & Eliminates the Need For Vectors & Bacteria!



Requires

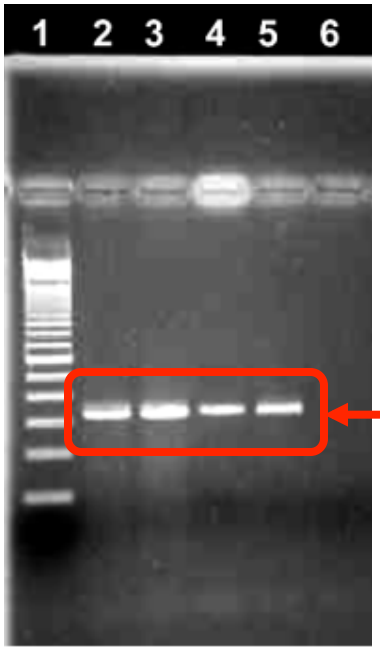
1. Template
2. Primers
3. Knowledge of Specific Sequence
4. Nucleotides
5. Heat-Stable DNA Polymerase
6. Thermo Cycler

Repeat Steps or Cycle

2^n Molecules of DNA Where n = Number of Cycles

Diagnostic For Amplified DNA Sequence (Between Primers) → **DNA Fragments All The Same Size Primer-Sequence-Primer**

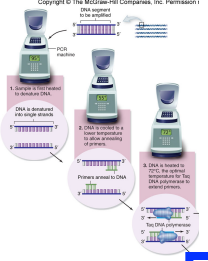
Using Gel Electrophoresis to Visualize PCR Products



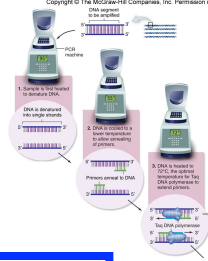
Specific Diagnostic DNA Band Unique to DNA Sequence Being Amplified

- Target-Specific Band
- Diagnostic For Specific DNA Sequence
- Band Size Unique For Specific Sequence
- Primers "Surround" the Target Sequence

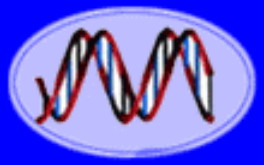
Can Amplify One DNA Sequence From An Entire Genome *or an Entire Genome*!!!



PCR Revolutionized Genetic Engineering & Working With DNA



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:
   a. A Single Human Hair or Cheek Cell
   b. An Ancient Fossil (e.g., Neanderthal Bone or Mammoth Hair)
   c. An Ancient Insect Trapped in Amber
   d. Human Remains (e.g., 9/11 Victims)
   e. A Single Human Embryo Cell
   f. Contaminated Meat To Determine the Causal Organism
3. Used In:
   a. DNA Fingerprinting-Individual Identification-Genetic Disease Screening
   b. Forensics (Crime Scenes, Mass Graves, Criminal Suspects, Wrongfully Convicted)
   c. Paternity & Family Relationships (e.g., Immigration, Tracing Lost Children)
   d. Disease Diagnosis & Pathogen Identification (Humans, Animals, & Plants)
   e. Human Origins & Migrations
   f. Ancient Genome Sequences & Evolutionary Studies
   g. Specific mRNA Detection
   h. "Cloning" Specific DNA Sequences
   i. Tracing Plant & Animal Sources (e.g., Poaching Stolen Cattle, Cactus)
4. Need as Little as One Molecule of DNA & Can Replicate an ∞ Amount of Specific Sequences



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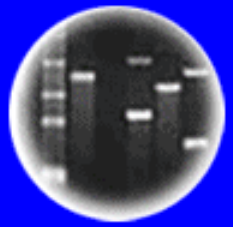


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

WHAT ARE THE PROPERTIES OF A GENE?

1. Replication

2. Stability (Mutations)

3. Universality
   a) All Cells
   b) All Organisms

4. Direct Cell Function/
   Phenotype

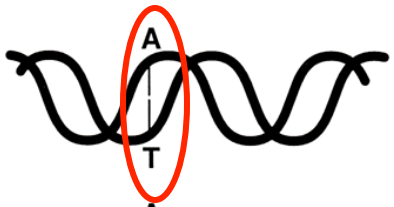
DNA Replication is Precise
But Mistakes or Mutations Can Occur!

	DNA	RNA	
pair	A T	A U	pair
pair	G C	G C	pair

BASE PAIR RULES

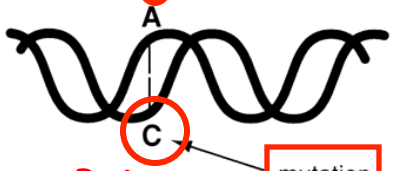
Think Tautomers!

Gene A



Rare Base Mismatch

Replication①



MUTATION DURING REPLICATION

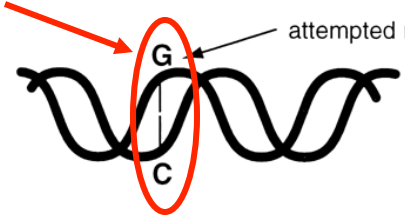
mutation

C mispairs with A

See Mutation As Change in Phenotype Creates Alleles

New Base Pair

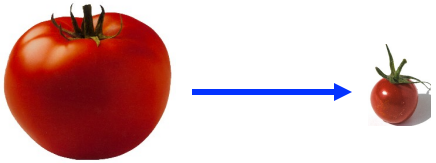
Replication②
Gene A'
Allelic Variant



RESULTING DEFECT

Change DNA Sequence From A = T to G ≡ C

∴ Change Protein Amino Acid Sequence ⇨ Alter Function!

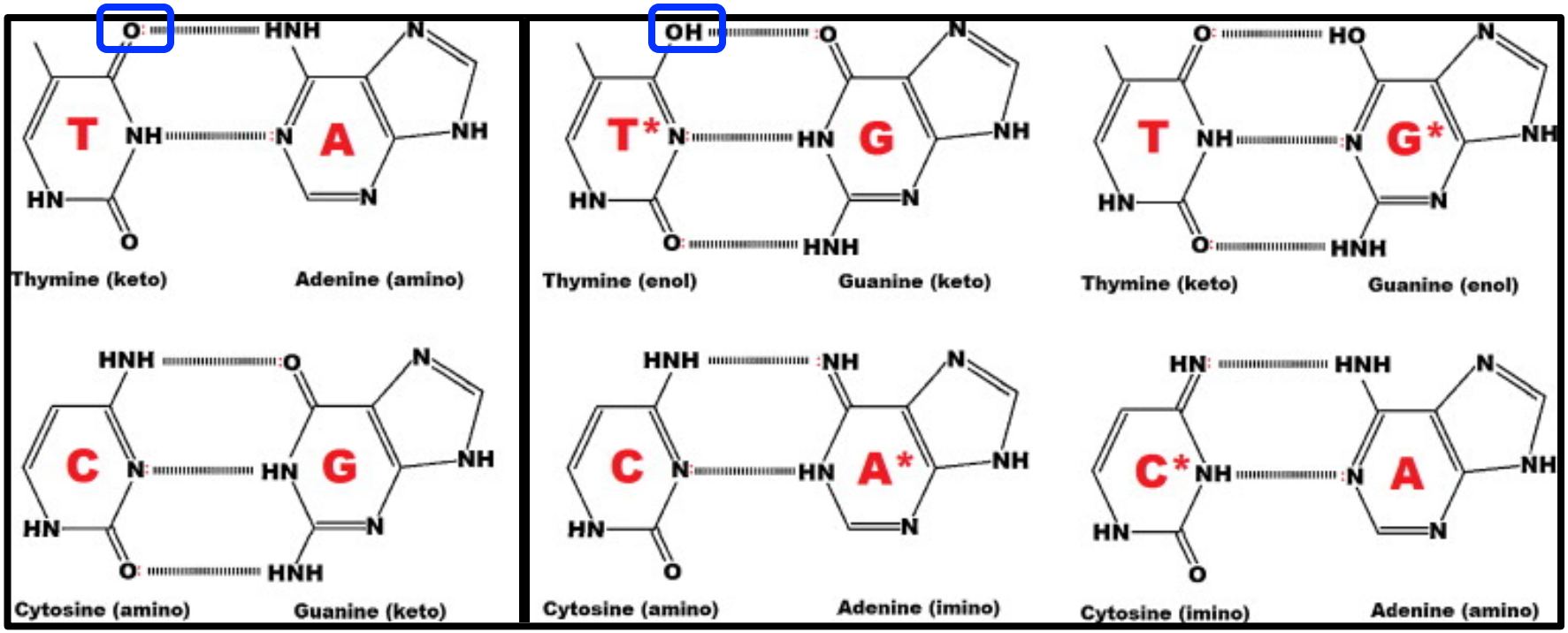


Big Tomato to Small Tomato

TAUTOMERS CHANGE BASE PAIRING RULES

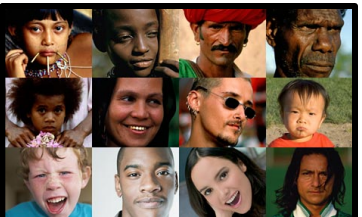
Normal Forms – Keto & Amino

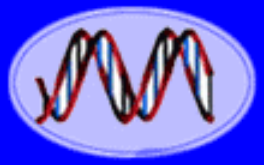
"Mutant" Forms – Enol & Imino



And Lead To Mistakes in DNA Replication & Mutations ➤ Genetic Diversity

Chemistry Leads to Biology!!





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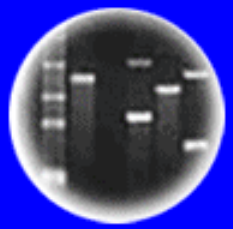

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Plants of Tomorrow

ARTICLE

doi:10.1038/nature09534

A map of human genome variation from population-scale sequencing

The 1000 Genomes Project Consortium*

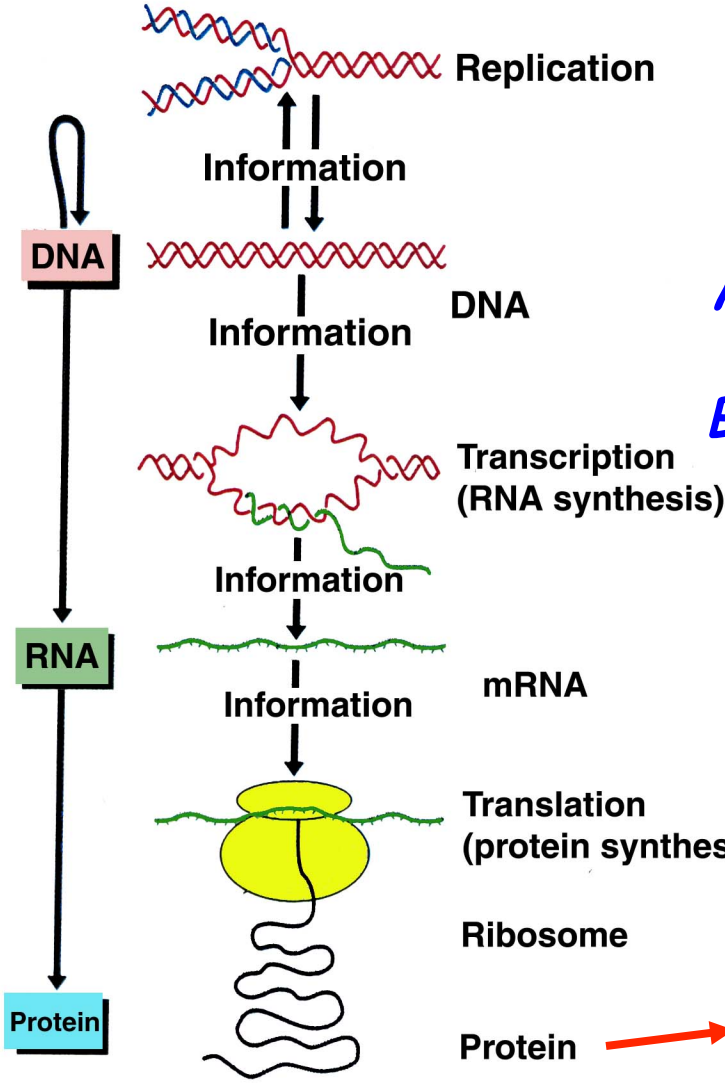
Nature, October 10, 2010

The 1000 Genomes Project aims to provide a deep characterization of human genome sequence variation as a foundation for investigating the relationship between genotype and phenotype. Here we present results of the pilot phase of the project, designed to develop and compare different strategies for genome-wide sequencing with high-throughput platforms. We undertook three projects: low-coverage whole-genome sequencing of 179 individuals from four populations; high-coverage sequencing of two mother–father–child trios; and exon-targeted sequencing of 697 individuals from seven populations. We describe the location, allele frequency and local haplotype structure of approximately 15 million single nucleotide polymorphisms, 1 million short insertions and deletions, and 20,000 structural variants, most of which were previously undescribed. We show that, because we have catalogued the vast majority of common variation, over 95% of the currently accessible variants found in any individual are present in this data set. On average, each person is found to carry approximately 250 to 300 loss-of-function variants in annotated genes and 50 to 100 variants previously implicated in inherited disorders. We demonstrate how these results can be used to inform association and functional studies. From the two trios, we directly estimate the rate of *de novo* germline base substitution mutations to be approximately 10^{-8} per base pair per generation. We explore the data with regard to signatures of natural selection, and identify a marked reduction of genetic variation in the neighbourhood of genes, due to selection at linked sites. These methods and public data will support the next phase of human genetic research.

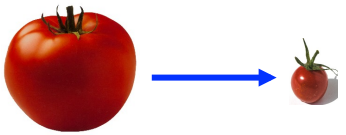
- Sequenced Genomes of 2500 individuals & From 26 Different Global Populations
- Found 84 Million Variants (SNPs) & <0.5% Unique to a Population!
- Evidence For Common Ancestry of All Humans
- Found 250-300 Loss-Of-Function Mutations (KOs) Per Person
- Found 50-100 Mutations Implicated in Genetic Disorders Per Person
- 10^{-8} bp Mutations Per Generation (30 per Genome)

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

Human Genetic Disorders Occur As a Result of Rare 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

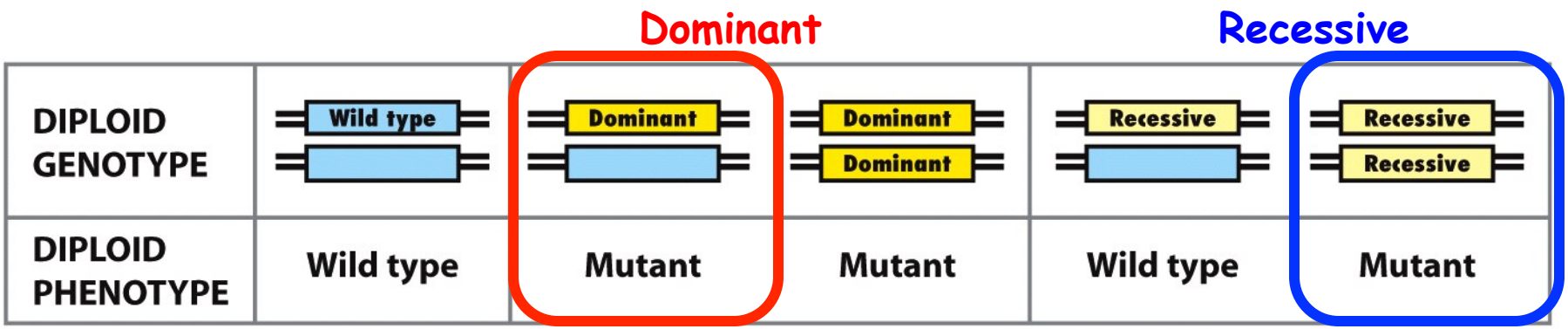
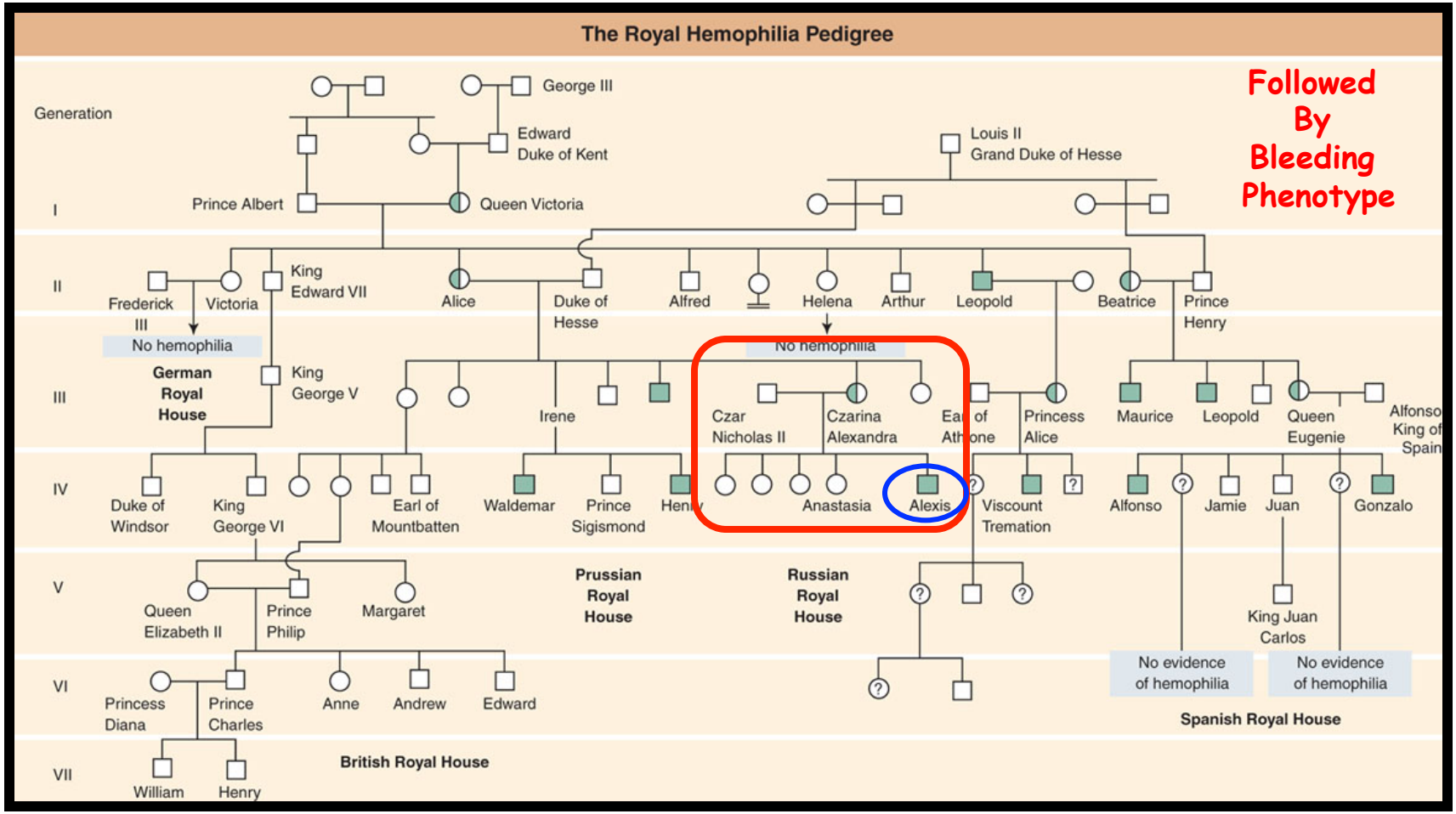


Figure 5-2
Molecular Cell Biology, Sixth Edition

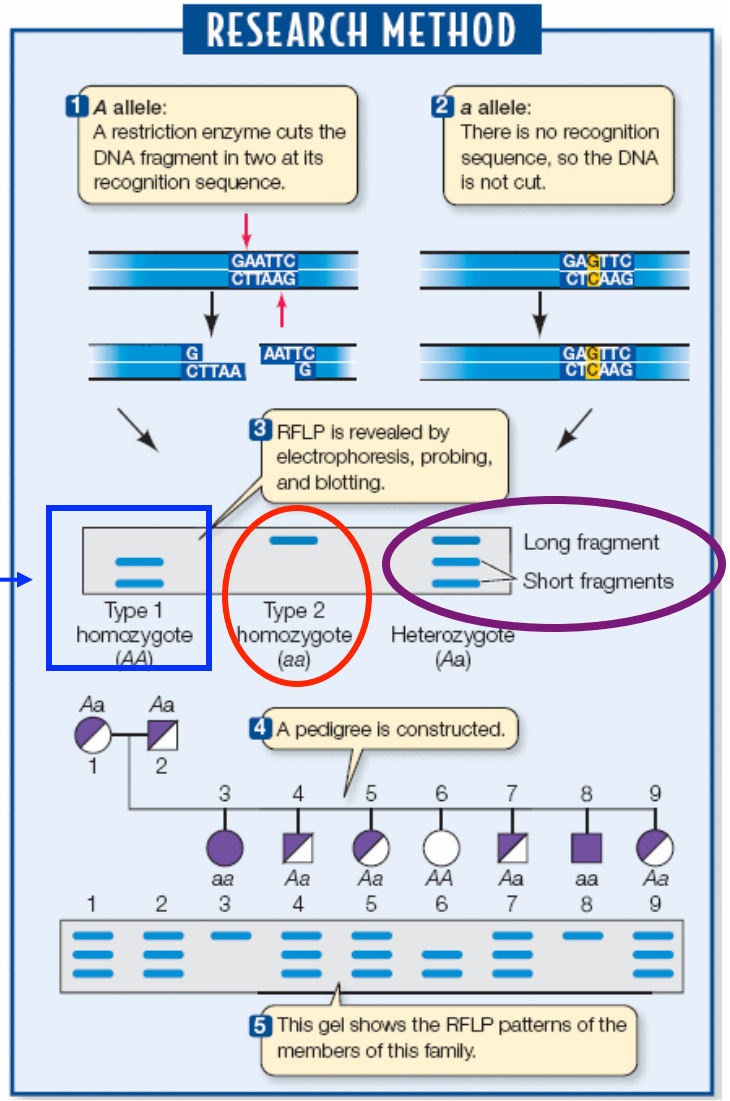
Pedigrees Can Be Used To Follow Disease Genes in Human Families



Recessive Sex Linked

Genetic Diseases Can Also Be Followed in Families Using DNA Methods (e.g., PCR) & Pedigrees – With DNA Markers Linked to the Disease Phenotype

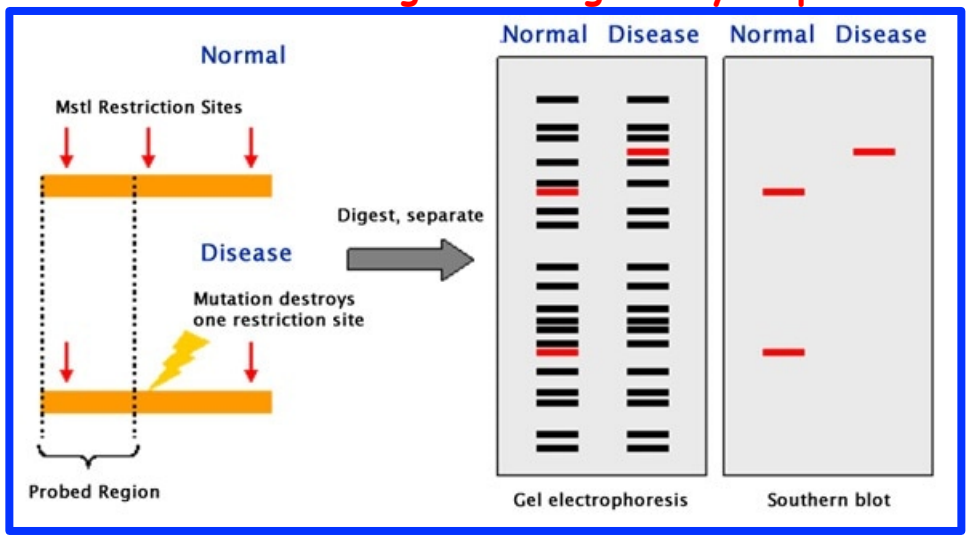
DNA Fingerprints →

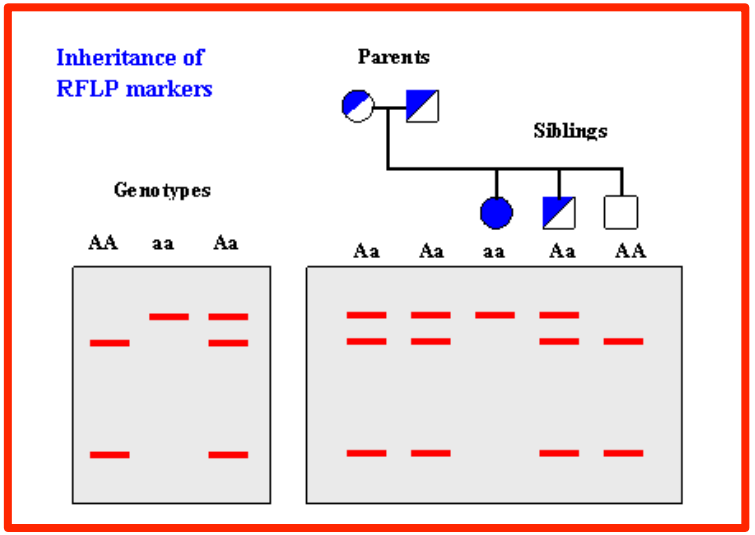


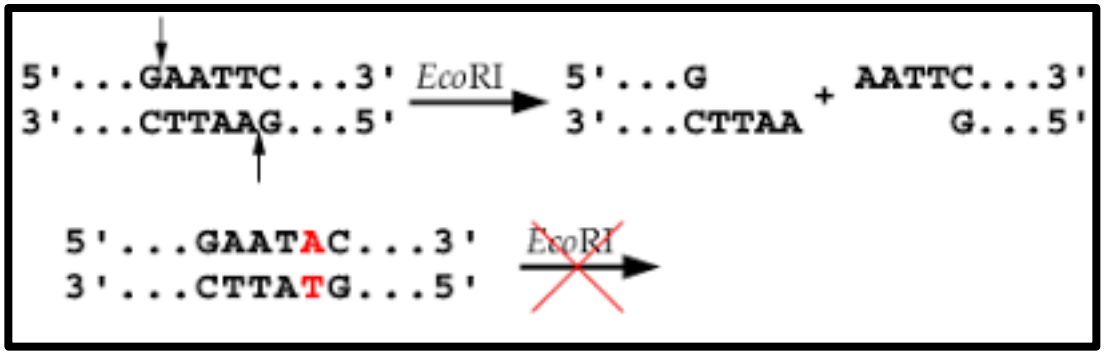
← RFLP – Restriction Fragment Length Polymorphism

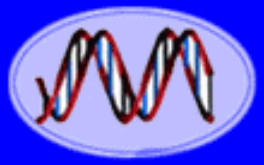
Genetic Diseases Can Also Be Followed in Families Using DNA Methods (e.g., PCR) & Pedigrees – With DNA Markers (RFLPs) Linked to the Disease Phenotype

Restriction Fragment Length Polymorphism









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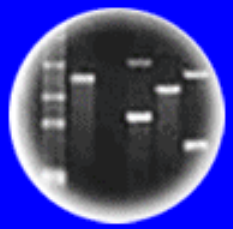

DNA Fingerprinting

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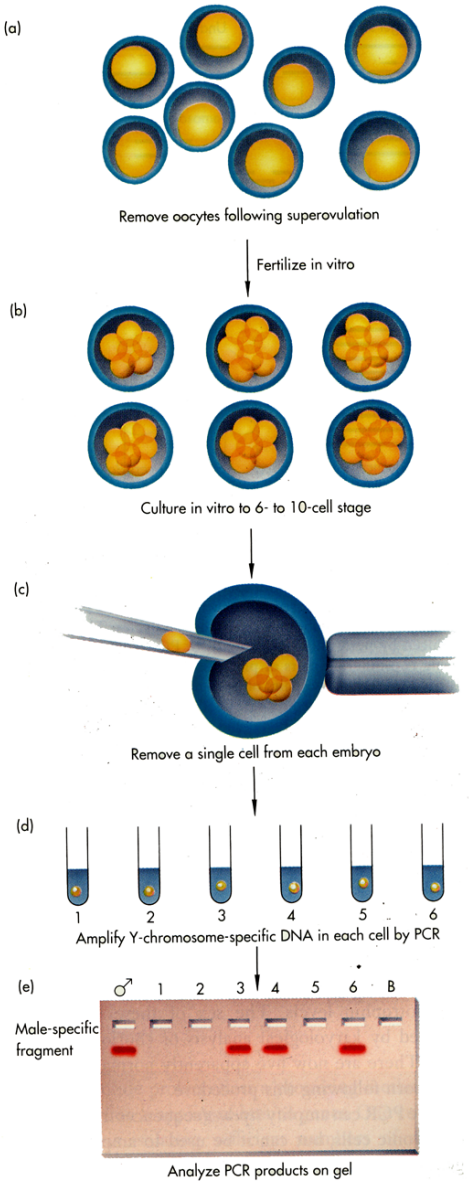
Plants of Tomorrow

PCR Can Be Used To Analyze Gene in A Single Embryo Cell

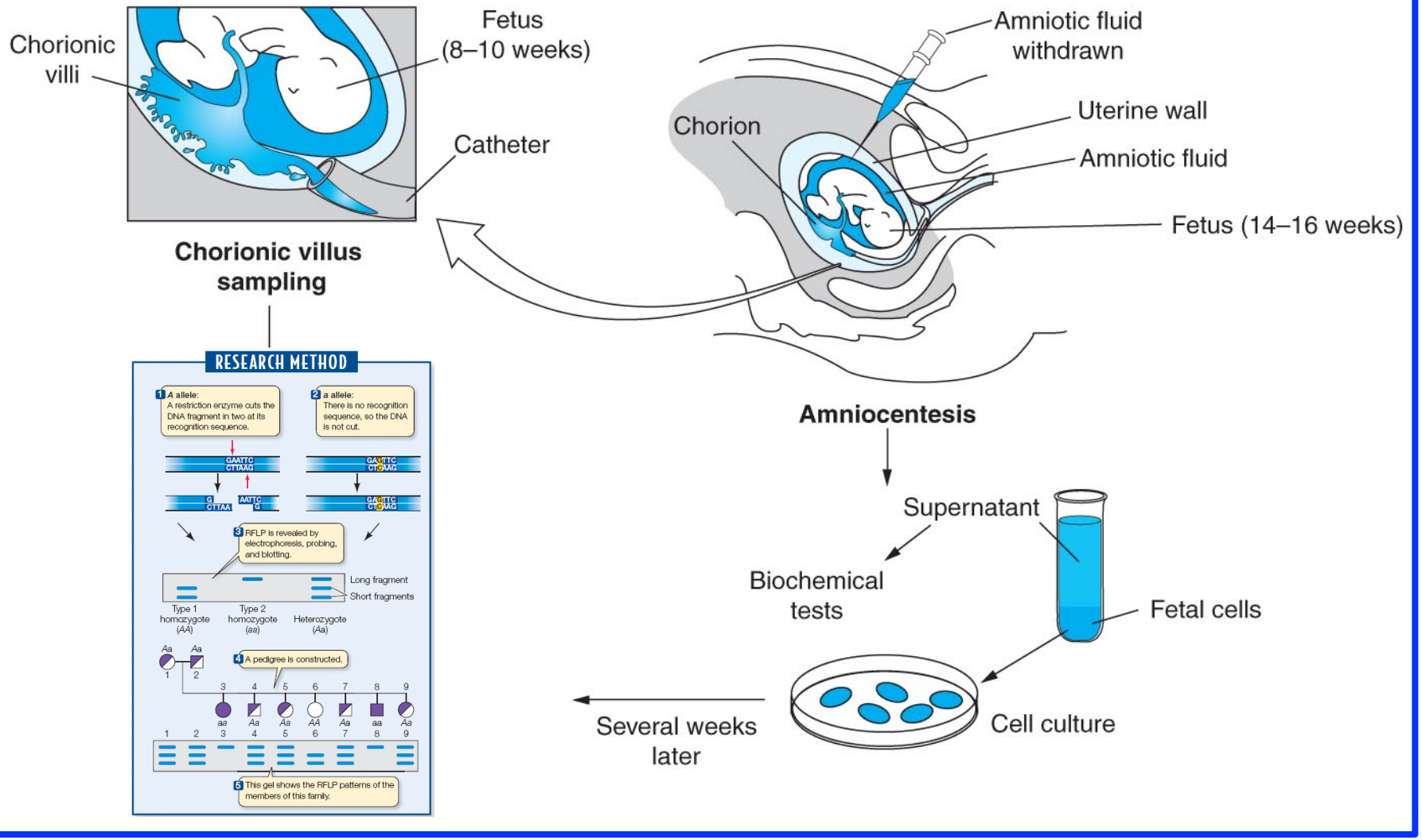
PGD
Pre-Implantation Genetic Diagnosis



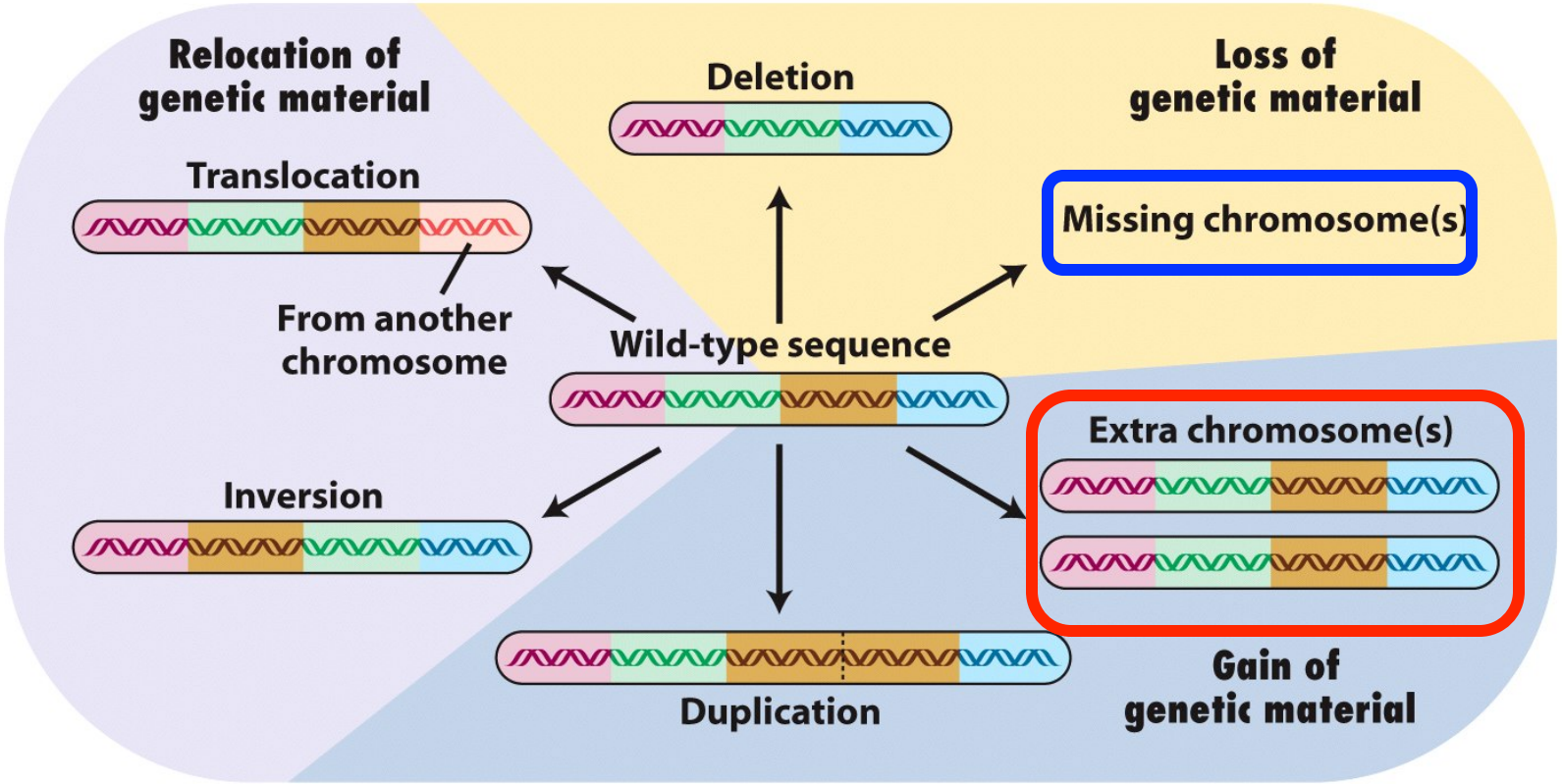
What is The Implication of This Procedure Considering That The Human Genome Has Been Sequenced?

Sex Determination in 8-cell Embryo!

DNA Testing Can Be Carried Out Before Child Birth During Pregnancy



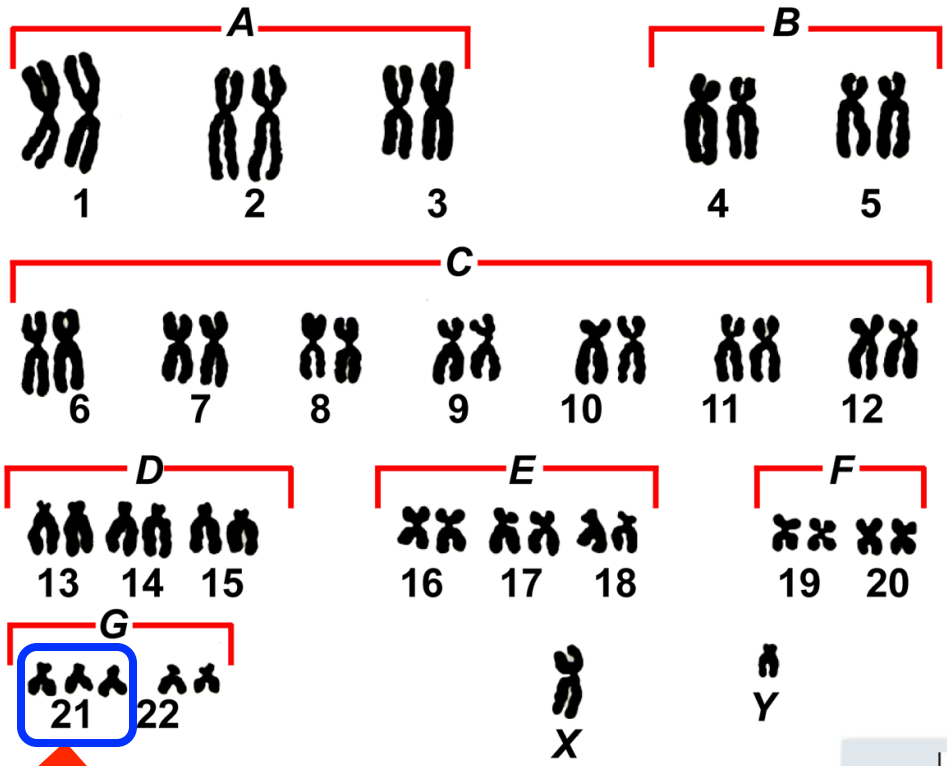
"Mutations" Can Also Occur By Large Chromosomal Changes



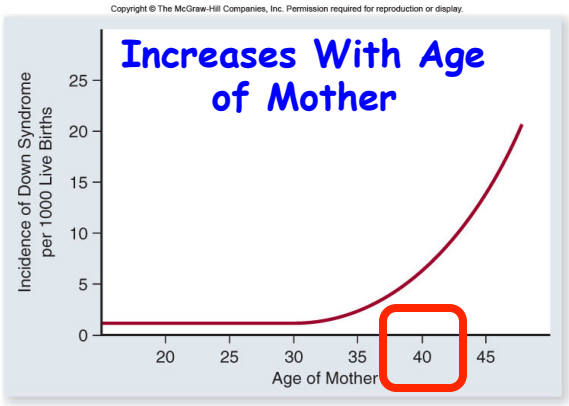
These changes affect many genes!

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

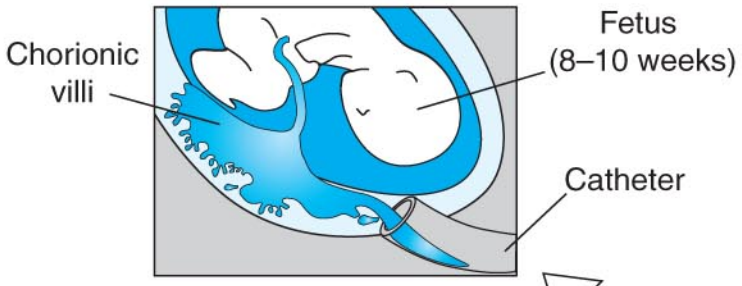
A Down's Syndrome Karyotype



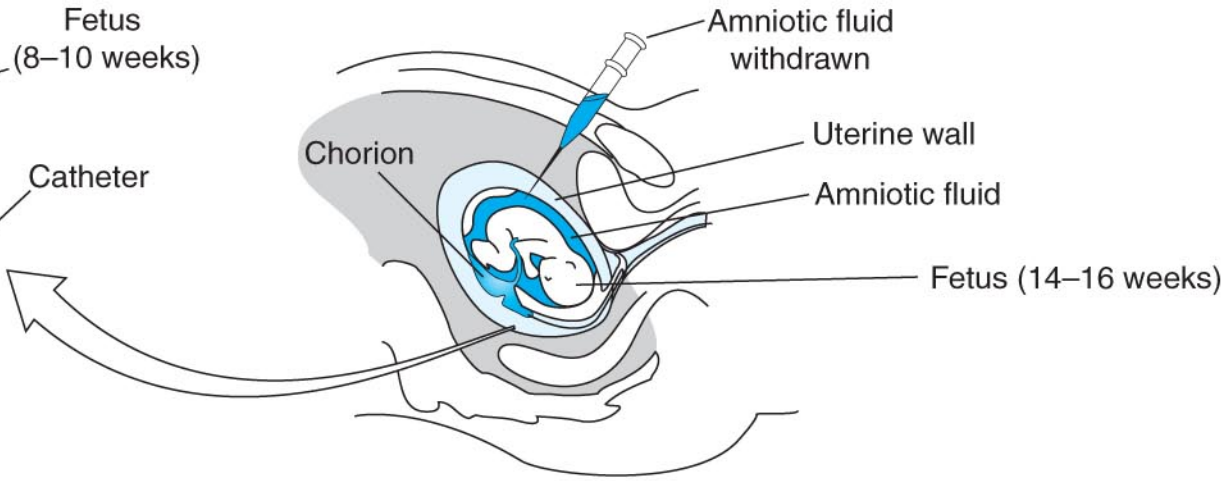
Three Chromosome # 21s



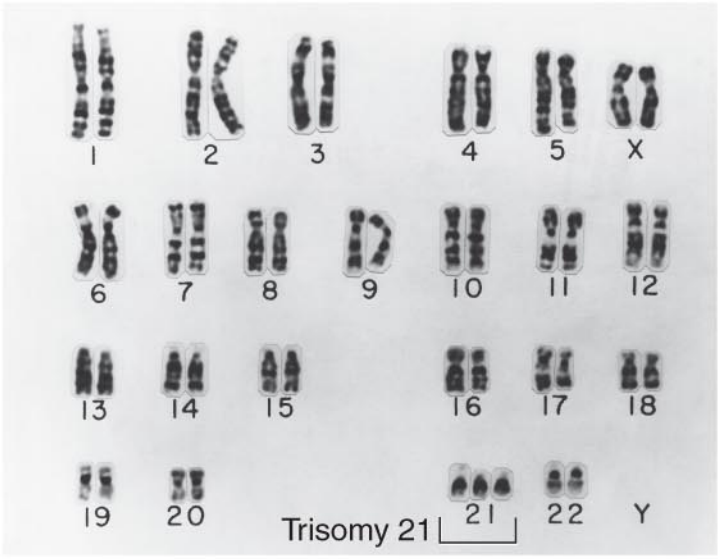
Chromosome Testing Can Be Carried Out During Pregnancy or Before (New DNA Tests)

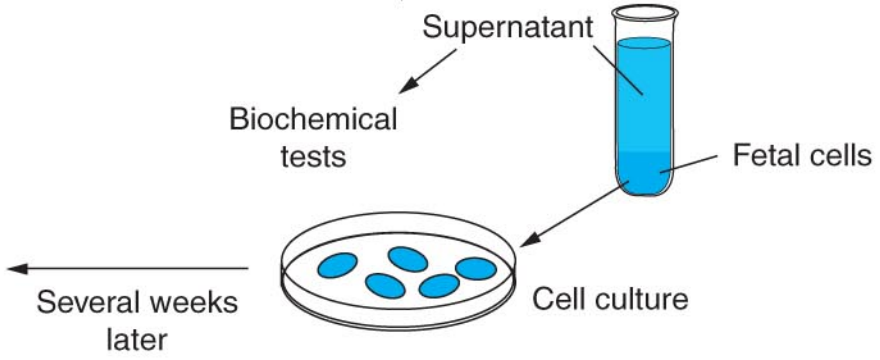


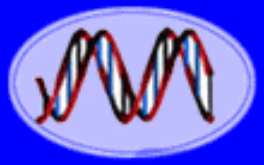
Chorionic villus sampling



Amniocentesis







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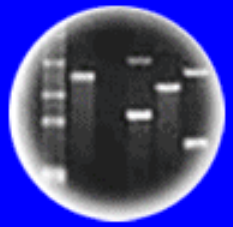


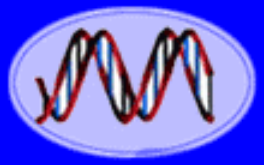
DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Genetic Screening Issues

- Why Screen For Genes?
- When is a Test Accurate Enough?
- Mandatory or Voluntary Screening?
- Who Should Be Tested?
- Employer & Insurance Company Testing?
- Protection From Genotype Discrimination?
- Testing for Genetic Diseases With No Cures?
- How Ensure Privacy & Confidentiality?
- Obligations to Inform Others (Spouse/Sibling) of Genetic Disorder Knowledge?
- Genetic Databases??
- Patents on Tests?



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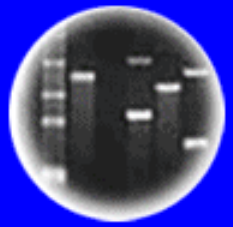

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WHAT ARE THE PROPERTIES OF A GENE?

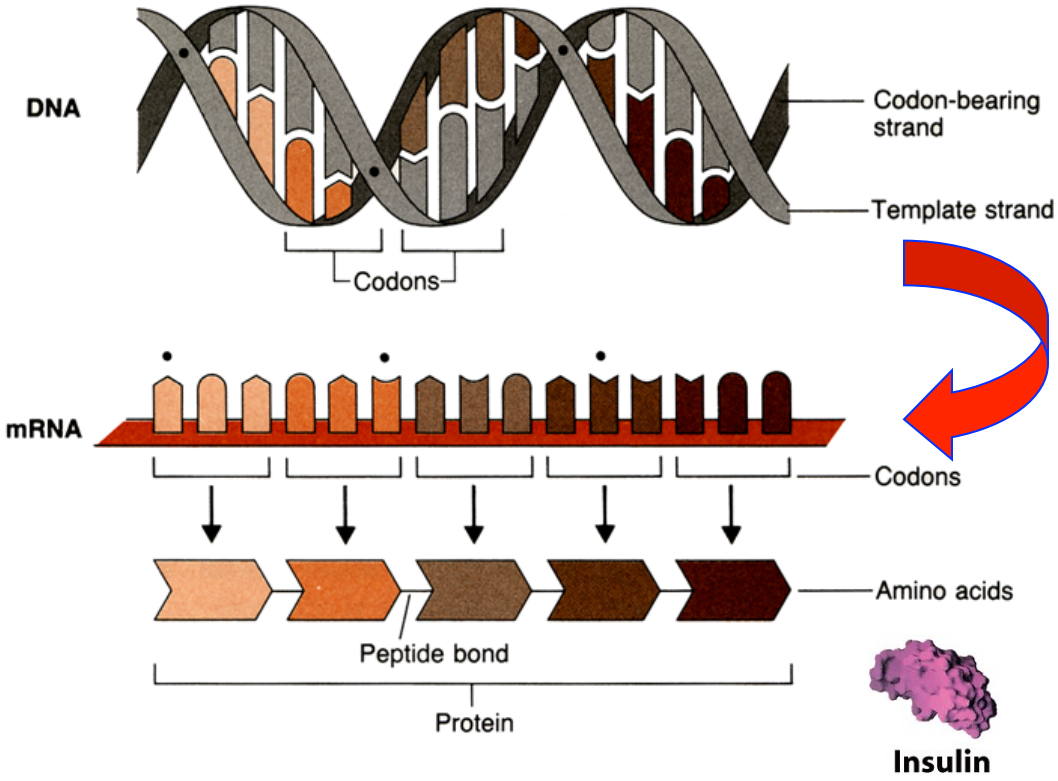
1. Replication

2. Stability (Mutations)

3. Universality
   a) All Cells
   b) All Organisms

4. Direct Cell Function/
   Phenotype

② 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

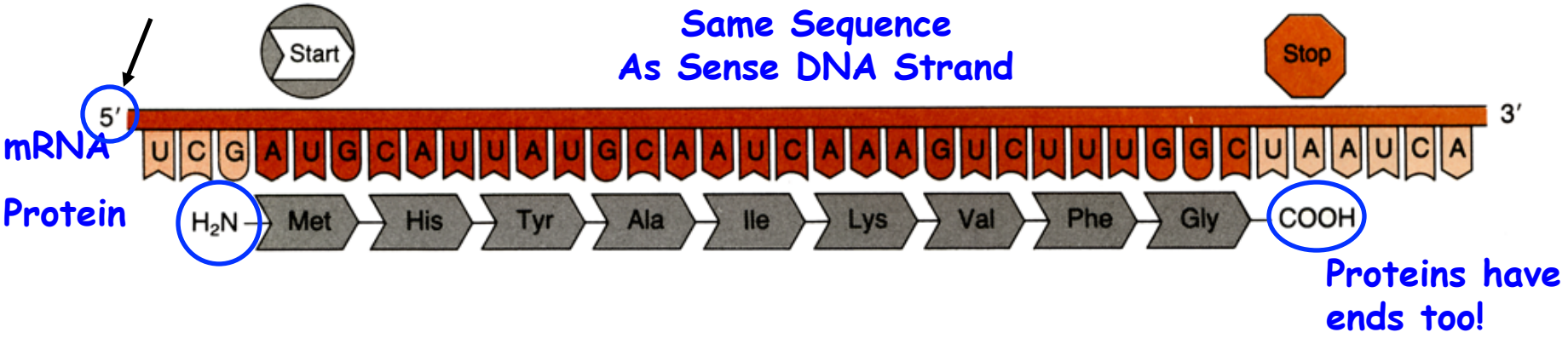
Genetic Code on mRNA Translated to Protein Sequence

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

Colinearity of Sequences!

Know Sequence
Know Protein
→ Engineer New Protein

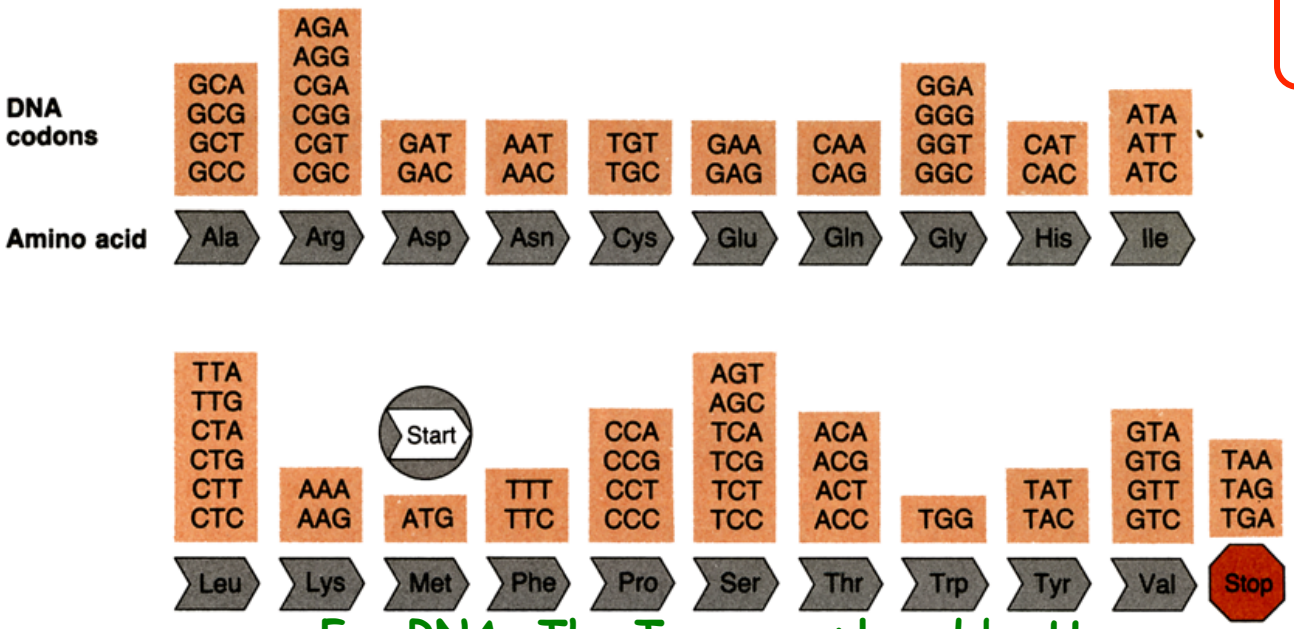
Genetic Code Allows The Sequence of Nucleotides in mRNA/Sense strand of Gene to be Translated into Sequence of Amino Acids in Proteins



Note: Sequence in mRNA (= Sense Gene Strand) is translated 5' →3' (= beginning of sense strand to end) & protein made in N→C direction – therefore: order nucleotides in gene specifies order of amino acids in protein!

The Genetic Code is Universal!

How Know?



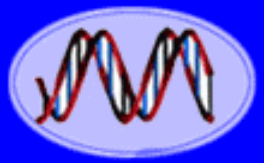
For RNA, The Ts are replaced by Us.

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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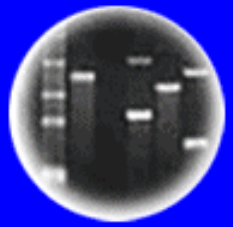

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Expression of Jellyfish Green Fluorescence Protein (GFP) in Pigs Shows That Genetic Code is Universal!!

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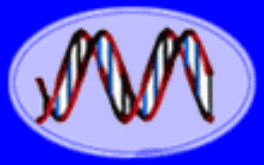

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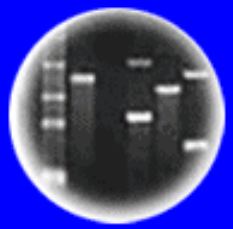

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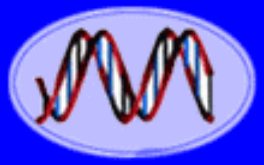
Implications For Genetic Engineering - "Yo - Its in The DNA!!"

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!



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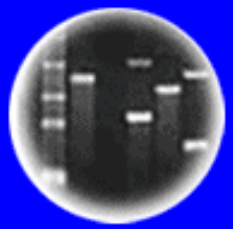

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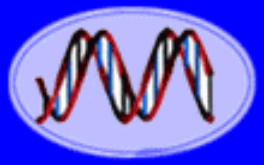
Summary: Engineering Genes Requires:

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)
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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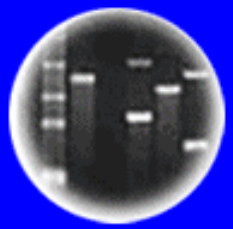

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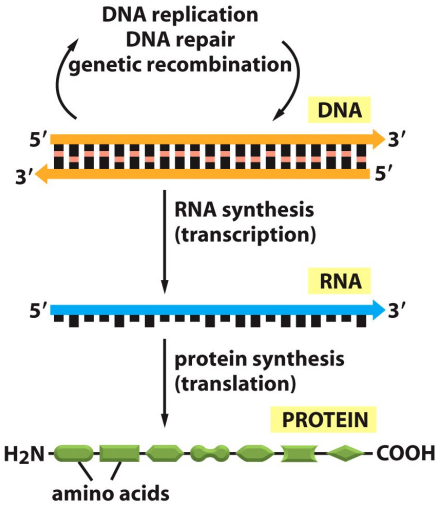
How Do Genes Work & What are Genes in Context of…

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 Gene 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!

"Behind" All Traits!

Same Processes!

There's NO HOCUS POCUS All Hypothesis Are Testable!!