

DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

UCLA

TUSKEGEE
UNIVERSITY

UC DAVIS
UNIVERSITY OF CALIFORNIA

HC70A, SAS70A, & PLSS599
Winter 2022
**Genetic Engineering in Medicine,
Agriculture, and Law**

**Professors Bob Goldberg, John Harada, &
Channapatna Prakash**

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

1



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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?

2

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!

DNA Complexes With Proteins in Chromosomes

Chromosomal DNA is compacted ~ 1000 fold to fit within cell

Human Metaphase Chromosomes

13 14 15 16 17 18 19 20 21 22 X Y

3

Recombinant DNA Manipulation Means.....

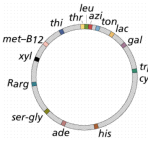
1. Specific DNA/Genes Can Be Isolated From Any Organism
2. DNA Segments of Any Kind From Any Organism Can Be Combined (Genetic Engineering!!!!!!)
3. Isolated Genes Can Be Re-Inserted Into the Chromosomes of Any Organism and Made to Work
4. Genes and Genomes Can Be Synthesized and Made To Work in Any Organism

Paul Berg

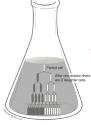
STANLEY COHEN HERBERT BOYER

4

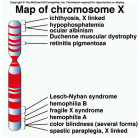
“Why” Clone Genes From An Organism’s Genome? An Essential HC70A Concept!



Cut & Splice →



← Cut & Splice




1. **PURIFY** Individual Genes From the Genome (e.g., One of 25,000 Human Genes) Using Restriction Enzymes & Plasmids (*Cut & Slice*)
2. **AMPLIFY** The Gene Within Plasmids in Bacterial Cells to Obtain Enough DNA For Study
3. **THEN** Use the Cloned Gene To:
 - a) Sequence & Study Gene Structure & Function (THE Major Use!)
 - b) Use to Convert Cells Into Factories To Make Drugs and Pharmaceuticals
 - c) Use to Diagnose Genetic Diseases
 - d) Use to Identify Individuals (e.g., paternity, forensics)
 - e) Use to Correct Genetic Disease
 - f) Use to Engineer New Crops and Farm Animals

5

A Chromosome Contains Many Genes Operating Independently

What is the Evidence?

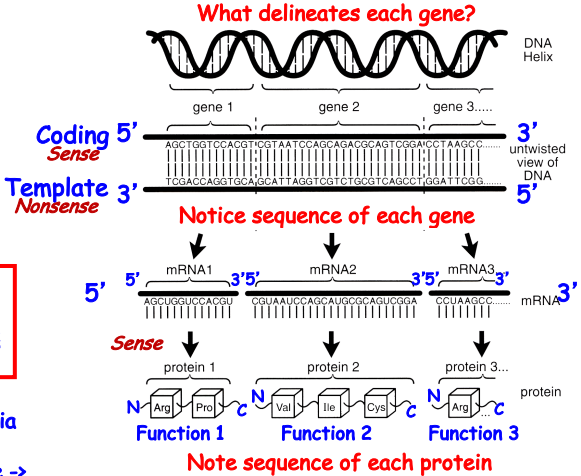


Position of Genes
1, 2, & 3 in
chromosome

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?

Notice sequence of each gene

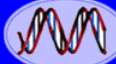
Note sequence of each protein

IMPORTANT HC70A CONCEPT!


COLINEARITY BETWEEN GENE SEQUENCE AND PROTEIN SEQUENCE

6

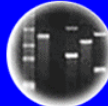
Genes Reside at Specific Locations (Loci) That Can Be Mapped Genetically or By DNA Sequencing




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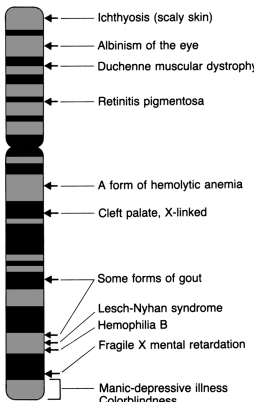
DNA Fingerprinting



Cloning: Ethical Issues and Future Consequences

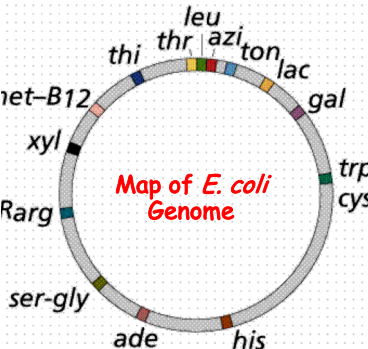


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Human X Chromosome

Linear DNA
How Know?



Map of E. coli Genome

Circular DNA
How Know?

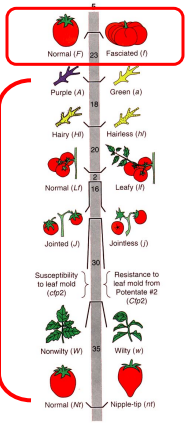
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Alleles Reside at the Same Position on a Chromosome

Allele Phenotypes Specify Markers For Each Gene Location!

Different Genes

Alleles



mutations result in genetic diversity!!!

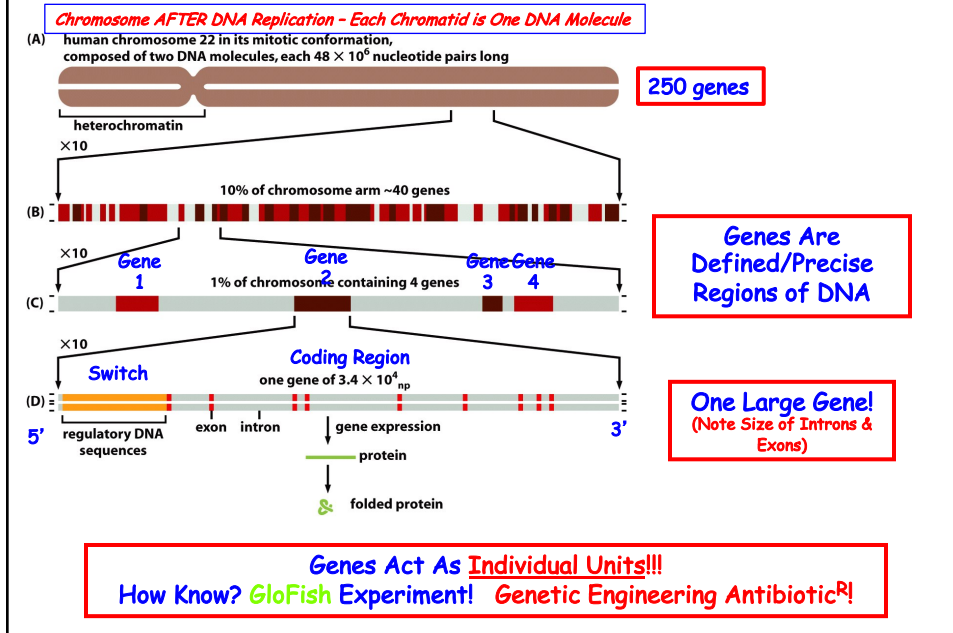
Source of All Genetic Variability

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

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

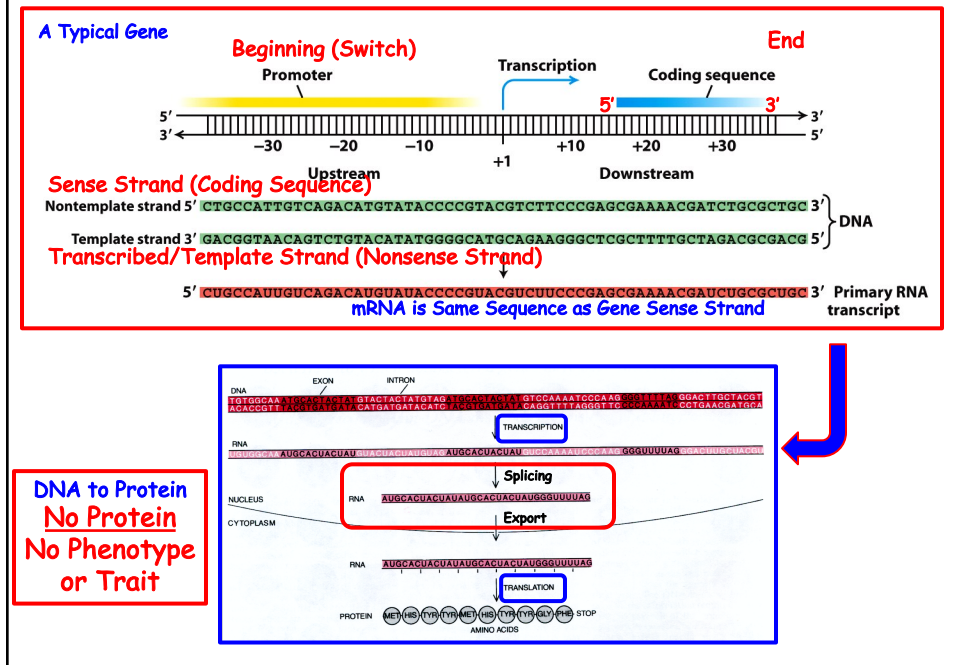
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Organization of Genes on Human Chromosome 22



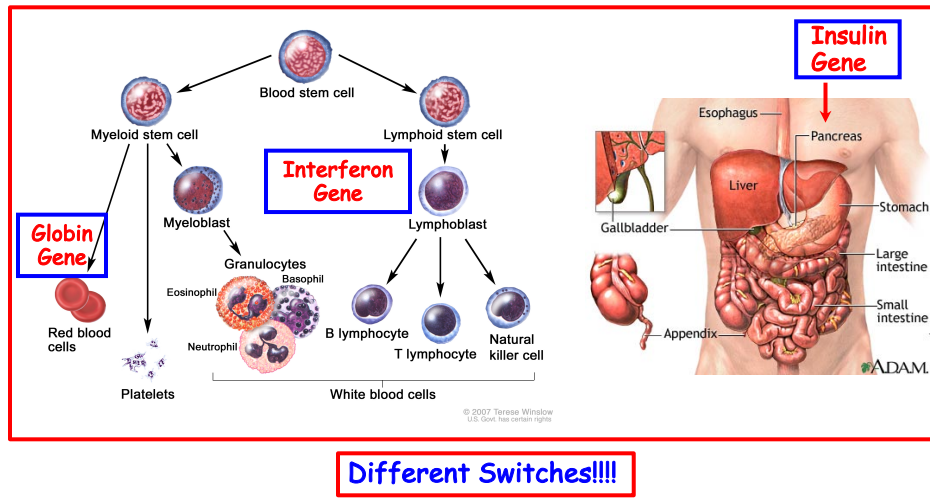
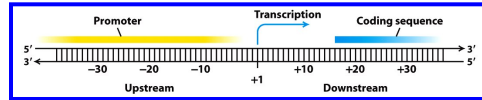
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A Conceptualized Gene



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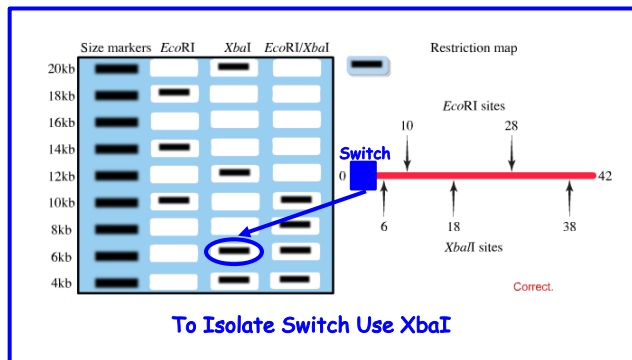
Switches Control Where & When A Gene Is Active Unique Functions → Unique Cells



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Restriction Maps of Cloned Genes 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



And Combining Them With Other Gene Parts Like a Lego Set

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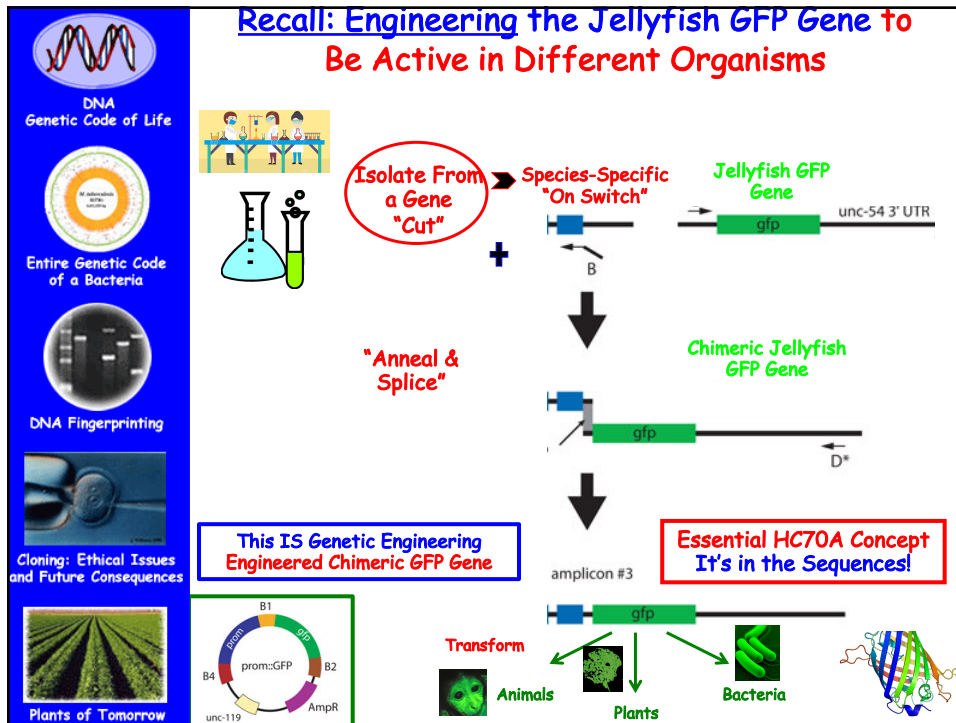
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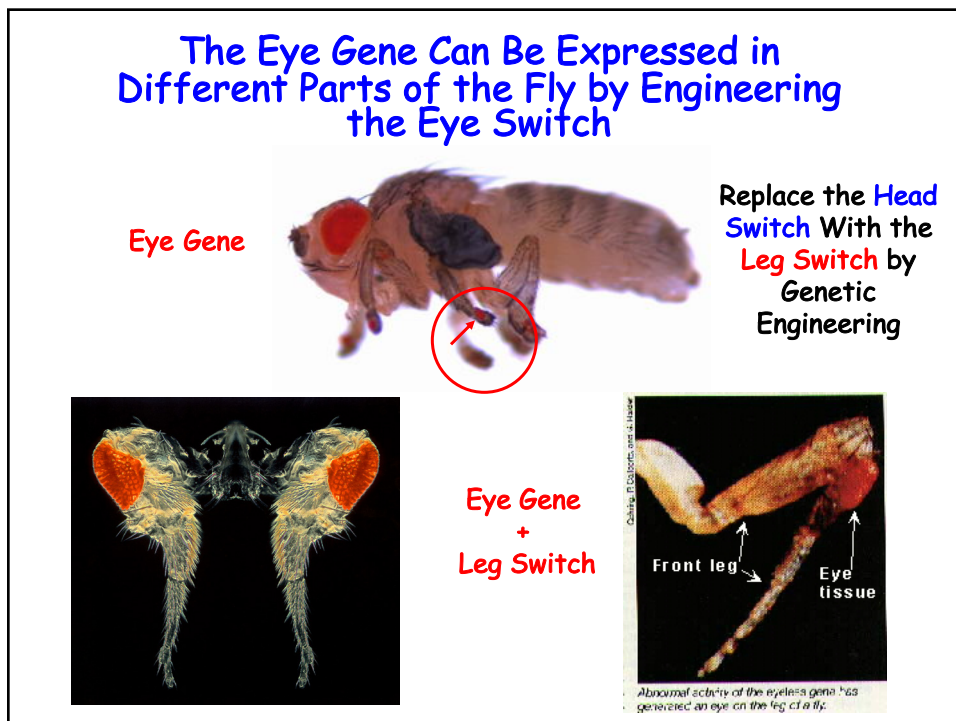
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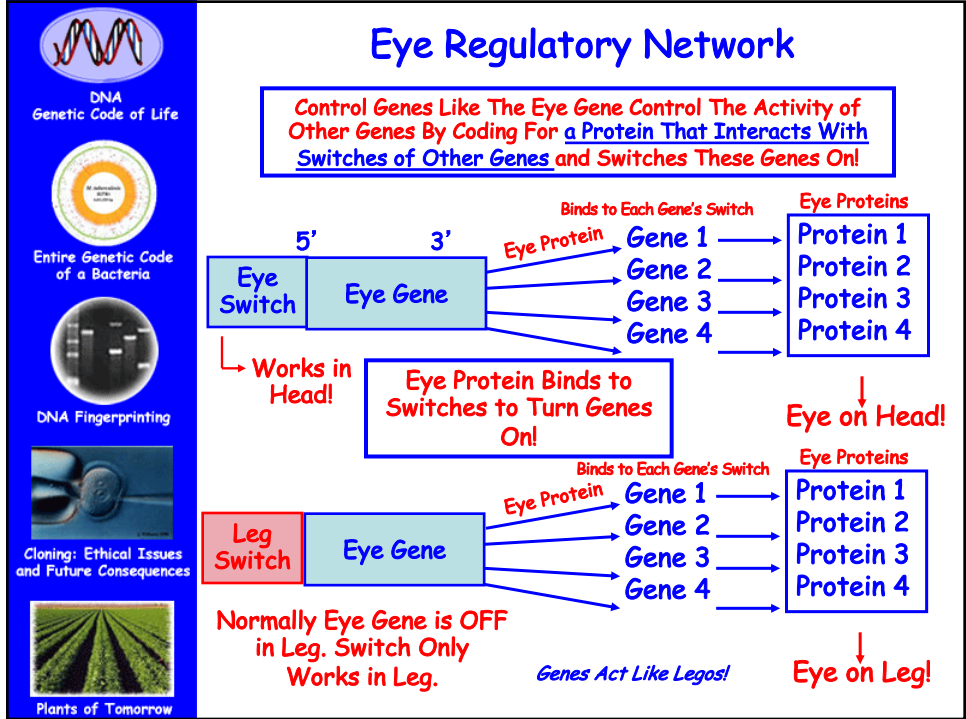
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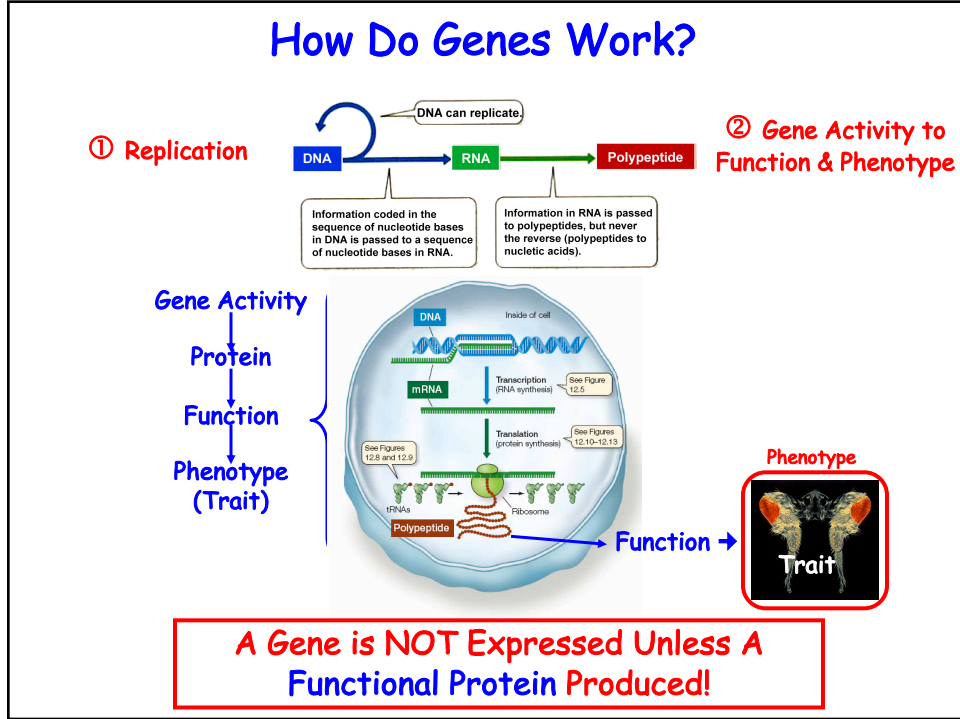
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GENES AND SWITCHES ARE UNIQUE DNA SEQUENCES

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!!

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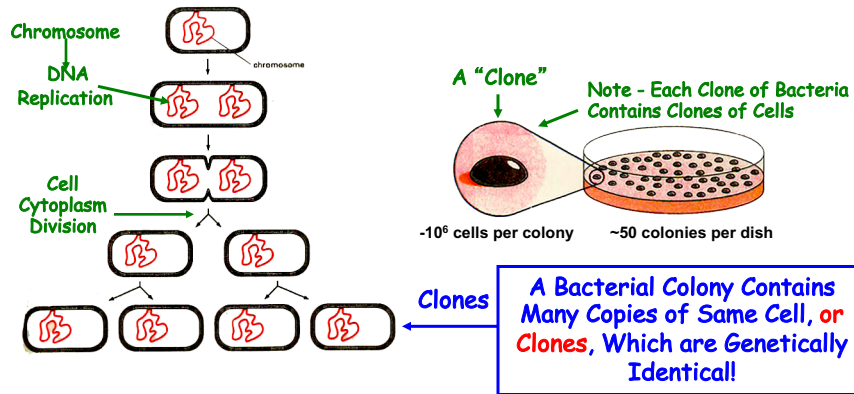
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WHAT ARE THE PROPERTIES OF A GENE?

1. Replication
2. Stability (Mutations)
3. Universality
 - a) All Cells
 - b) All Organisms
4. Direct Cell
Function/Phenotype

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1 How Are Genes Replicated Each Cell Generation?



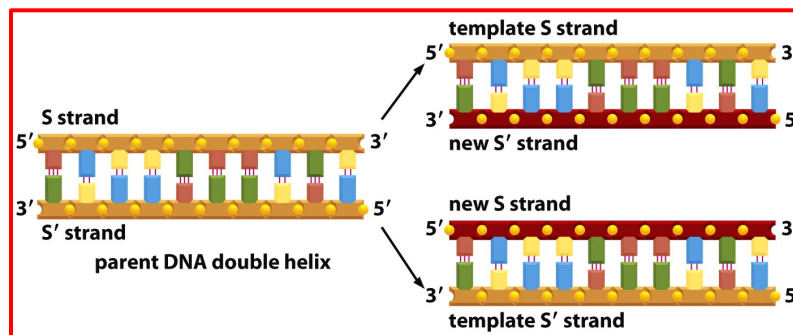
Each Daughter Cell Contains The Same Collection of Genes

Each Daughter Cell Will Also Replicate a Foreign Gene in a Plasmid - Increasing its Amount For Study or Expression

Clones!

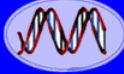
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DNA Replication Occurs Semi-Conservatively




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!!


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DNA Fingerprinting



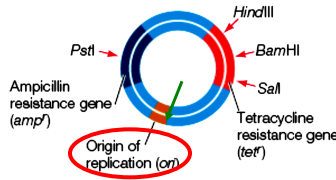
Cloning: Ethical Issues
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Vectors Are Needed To Replicate Genes In Transformed Cells

(A) Plasmid pBR322
Host: *E. coli*



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!

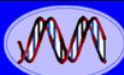
Note → Need Bacterial Ori to Clone Human Gene in Bacteria.
Need Human Ori to Replicate a Bacterial Gene in Human Cells.

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


It's in the Sequence= Function
∴ Vectors can be Engineered!
Ori's can be cloned/synthesized!

MODULAR!!


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DNA Fingerprinting



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Genetic Engineering Breakthroughs

Classical Breeding - 8,000 BCE

Our Primitive Biotechnology Ancestors

Transgenic Genetic Engineering - 1973

Berg, Cohen, & Boyer

Polymerase Chain Reaction (PCR) - 1985

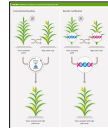
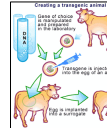
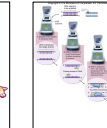
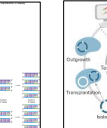

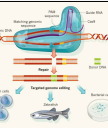
Mullis

Synthetic Genomes - 2000

Venter

Gene Editing or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) - 2015

Doudna & Carpentier

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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

DNA Copies All The Same Size

Kary Mullis
1993

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

Kary Mullis, unconventional Nobel laureate who unlocked DNA research, dies at 74. 1944–2019 RIP

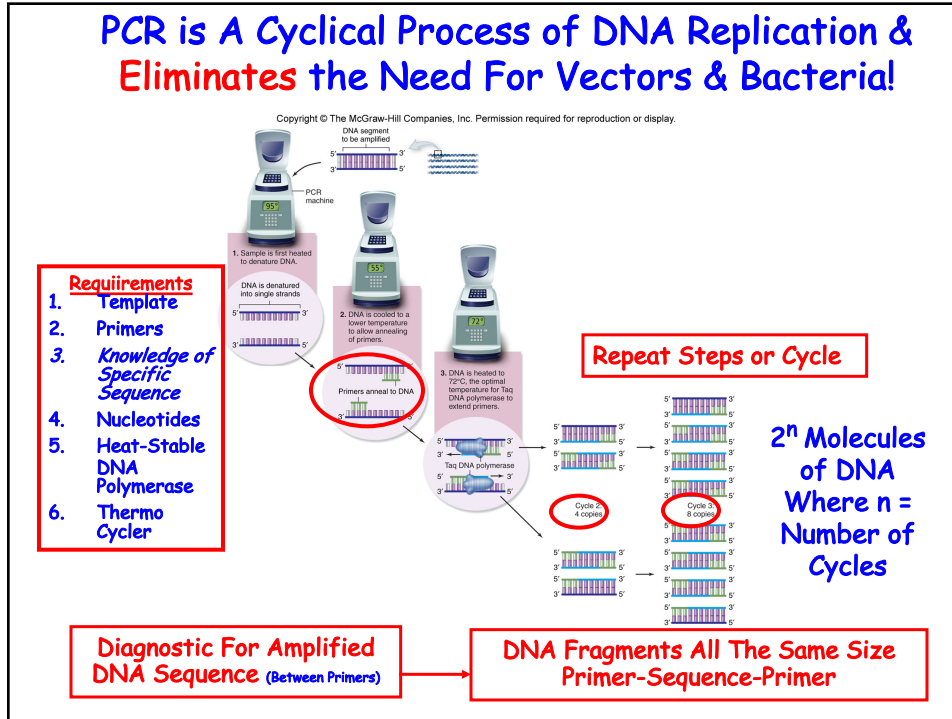
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DNA Cloning the “Old Fashioned” Way is a Lot of Work!

- 1 Isolate plasmid DNA and human DNA.
- 2 Cut both DNA samples with the same restriction enzyme.
- 3 Mix the DNAs; they join by base pairing. The products are recombinant plasmids and many nonrecombinant plasmids.
- 4 Introduce the DNA into bacterial cells that have a mutation in their own *lacZ* gene.
- 5 Plate the bacteria on agar containing ampicillin and X-gal. Incubate until colonies grow.

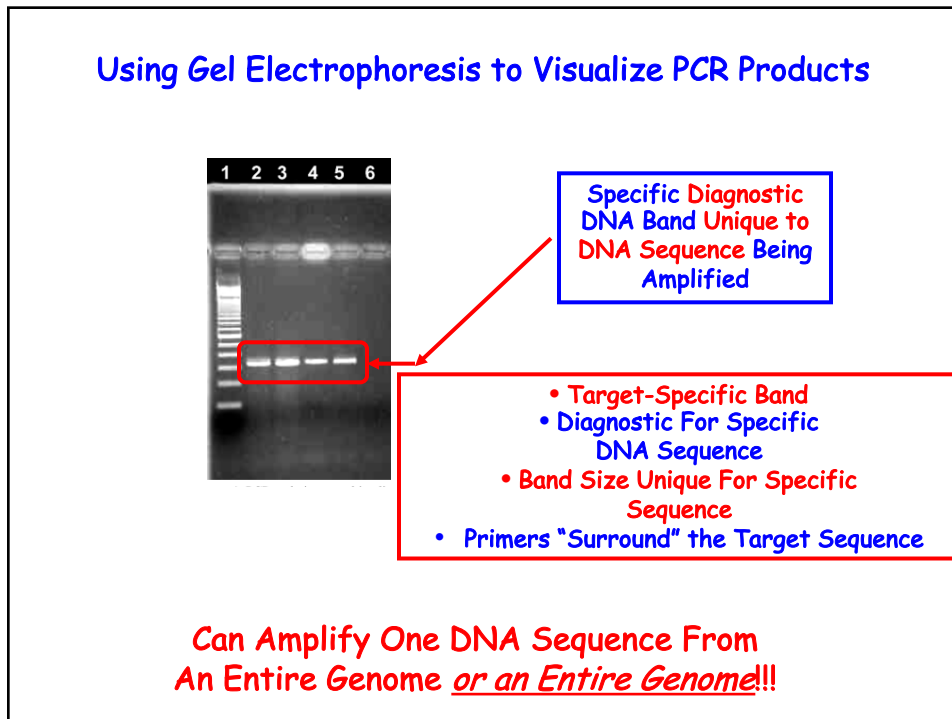
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PCR is A Cyclical Process of DNA Replication & Eliminates the Need For Vectors & Bacteria!



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Using Gel Electrophoresis to Visualize PCR Products

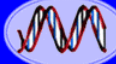


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PCR Revolutionized Genetic Engineering & Working With DNA

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**

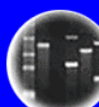
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DNA Fingerprinting

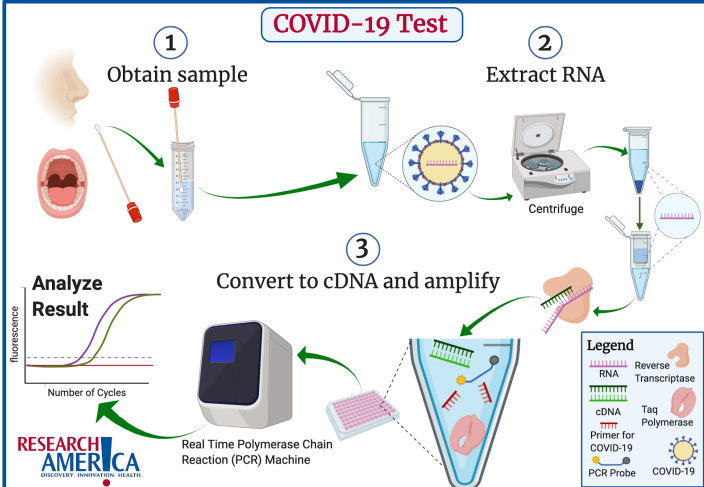


Cloning: Ethical Issues and Future Consequences



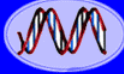
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COVID-19 Test




RNA Genome to ds-cDNA to PCR


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DNA Fingerprinting



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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


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

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


BASE PAIR RULES

Gene A



ORIGINAL BASE PAIR


Replication



MUTATION DURING REPLICATION

New Base Pair mutation **C** mispairs with **A**

Replication



attempted repair

Gene A'
Allelic Variant

RESULTING DEFECT



Think Tautomers!

Rare Base Mismatch

See Mutation As Change in Phenotype Creates Alleles

Change DNA Sequence From A = T to G ≡ C

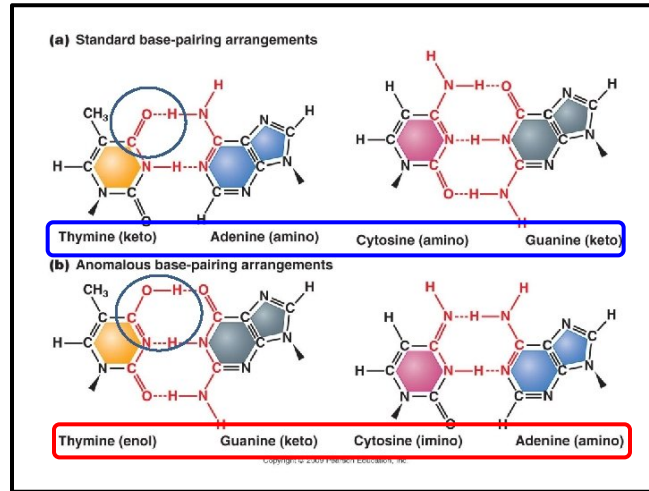
∴ Change Protein Amino Acid Sequence ☒ Alter Function!


→


Big Tomato to Small Tomato

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TAUTOMERS CHANGE BASE PAIRING RULES

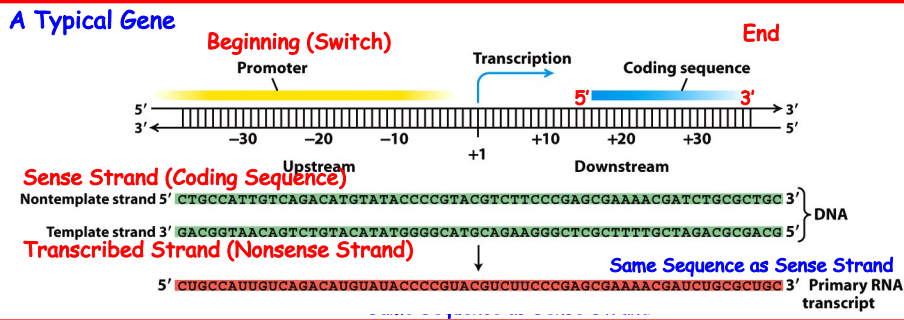


And Lead To Mistakes in DNA Replication & Mutations ➤ Genetic Diversity
Chemistry Leads to Biology!!

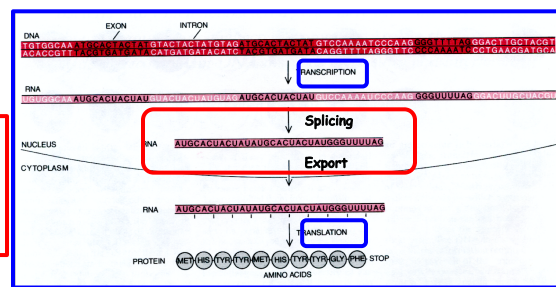


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Mutations Can Occur At Any Nucleotide or Gene Region

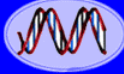


Mutation
No Functional Protein
No Phenotype or Trait




- Switch
- Coding Sequence
- Splice Junctions


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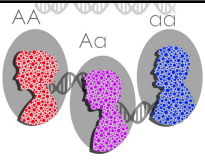

DNA Fingerprinting






Cloning: Ethical Issues and Future Consequences



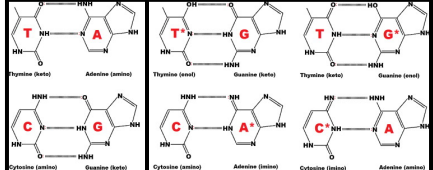
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Populations of All Organisms Contain Genetic Variability Derived From Random Mutations







Base Pairing Mistakes During DNA Replication

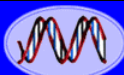


Chemistry → Biology
Change DNA Sequence
Change Phenotype!!!!


Spontaneous Tautomeric Base Shifts

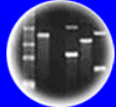
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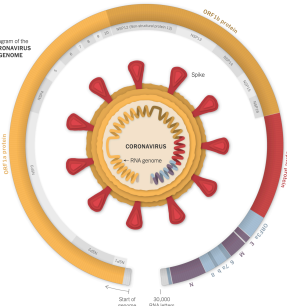
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Base Pairing Mistakes During RNA Replication

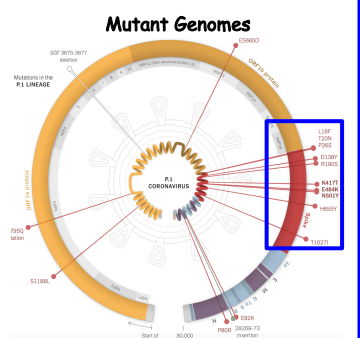
COVID-19 Genetic Variability Derived From Random Mutations

Spontaneous Tautomeric Base Shifts

Wild Type Genome

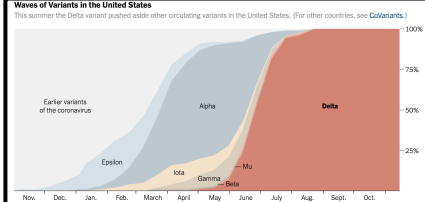


Mutant Genomes

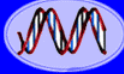


Waves of Variants in the United States


This summer the Delta variant pushed aside other circulating variants in the United States. (For other countries, see CoVariants.)




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ARTICLE

1000 Genomes
A New Catalog of Human Genetic Variation

doi:10.1038/nature09534

A map of human genome variation from population-scale sequencing

The 1000 Genomes Project Consortium*

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)

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Human Genetic Disorders Occur As a Result of Rare Spontaneous Mutations in Gametes

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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

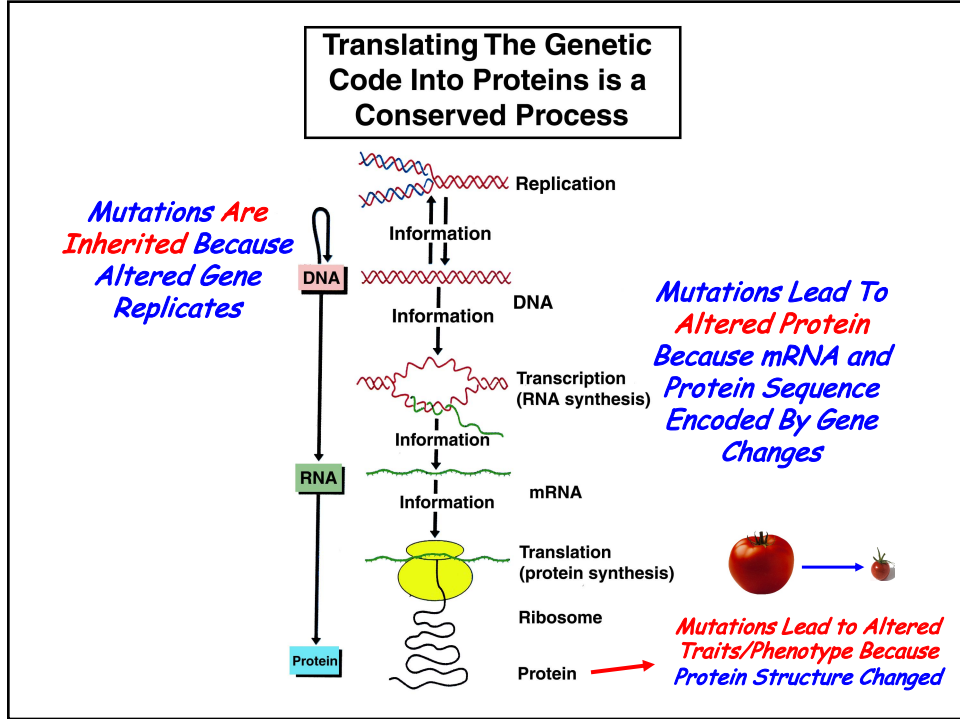
	Dominant		Carrier	Recessive
DIPLOID GENOTYPE	= Wild type =	= Dominant =	= Recessive =	= Recessive =
DIPLOID PHENOTYPE	Wild type	Mutant	Wild type	Mutant

Need One Allele

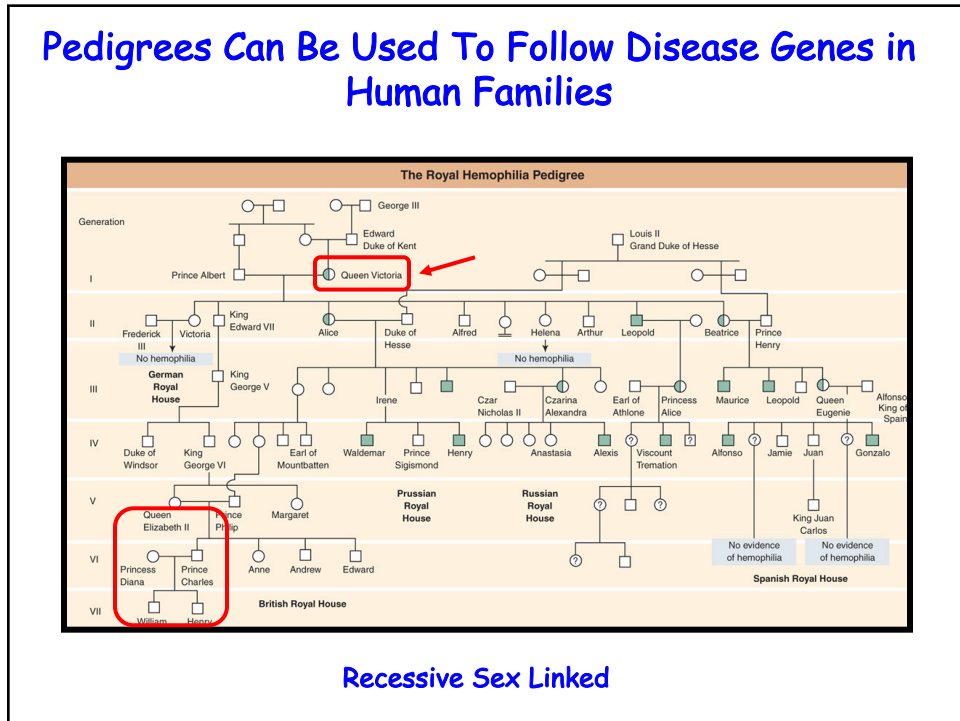
Need Two Alleles

Figure 5-2
Molecular Cell Biology, Sixth Edition
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Genetic Diseases Can Also Be Followed in Families Using DNA Methods (e.g., PCR) & Pedigrees - With DNA Markers Linked to the Disease Phenotype

RESEARCH METHOD

1 A allele: A restriction enzyme cuts the DNA fragment in two at its recognition sequence.

2 a allele: There is no recognition sequence, so the DNA is not cut.

↓ **AT to GC Mutation**

Cut → **No Cut**

3 RFLP is revealed by electrophoresis, probing, and blotting.

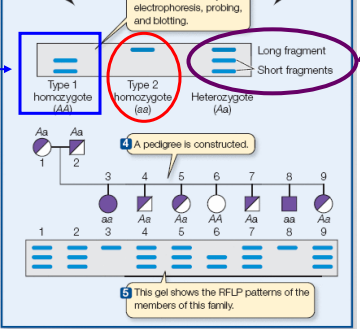
4 A pedigree is constructed.

5 This gel shows the RFLP patterns of the members of this family.



Essential HC70A Concept!

DNA Fingerprints →

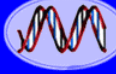
Mutation in Restriction Enzyme Site = RFLP or Restriction Fragment Length Polymorphism




Long fragment
Short fragments

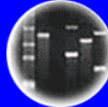
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DNA
Genetic Code of Life




Entire Genetic Code of a Bacteria



DNA Fingerprinting



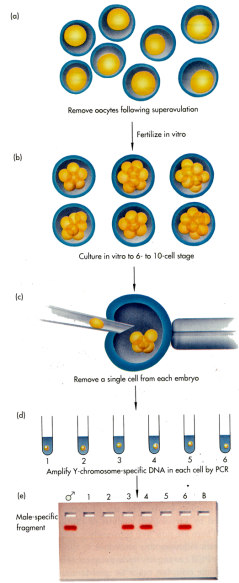
Cloning: Ethical Issues and Future Consequences



Plants of Tomorrow

PCR & RFLPs Can Be Used To Analyze Human Genes in A Single Embryo Cell

PGD
Pre-Implantation Genetic Diagnosis

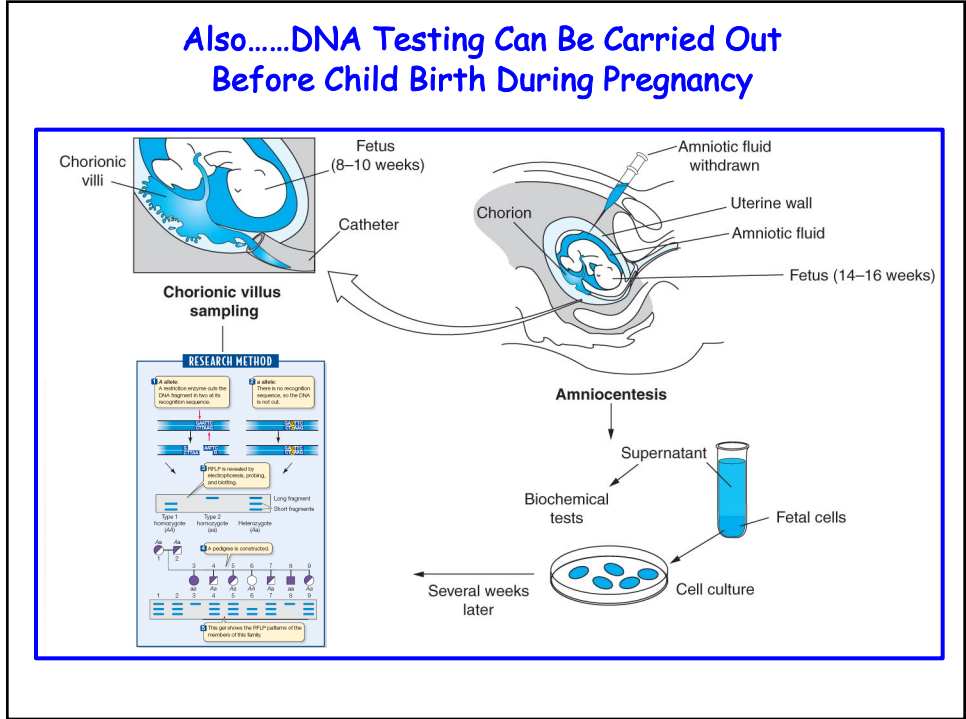


What is The Implication of This Procedure Considering That The Human Genome Has Been Sequenced?

Sex Determination in 8-cell Embryo!

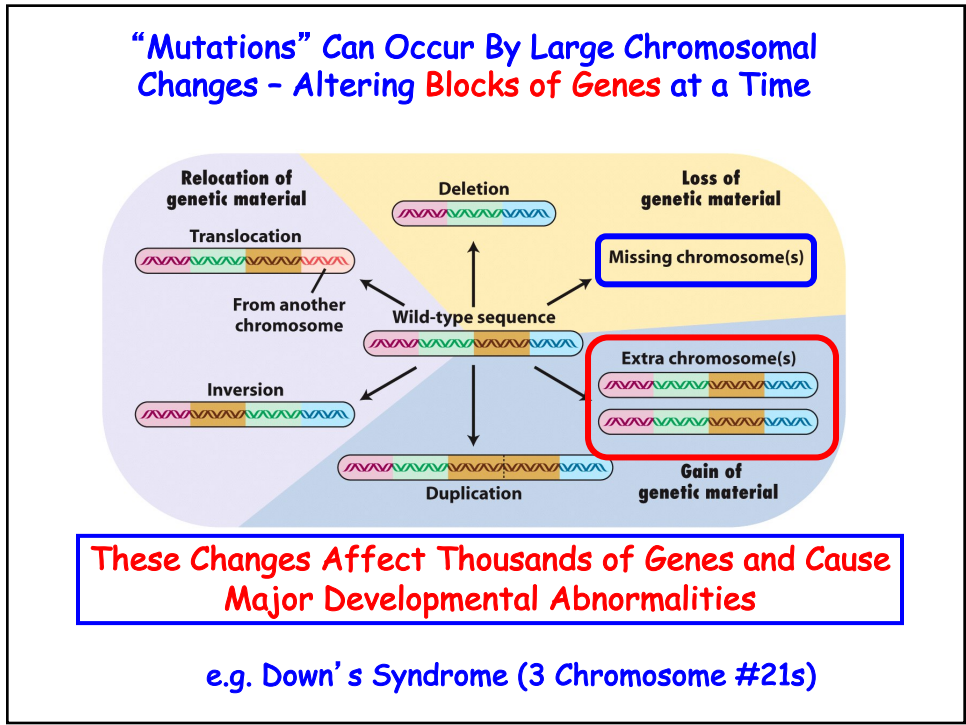
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Also.....DNA Testing Can Be Carried Out Before Child Birth During Pregnancy

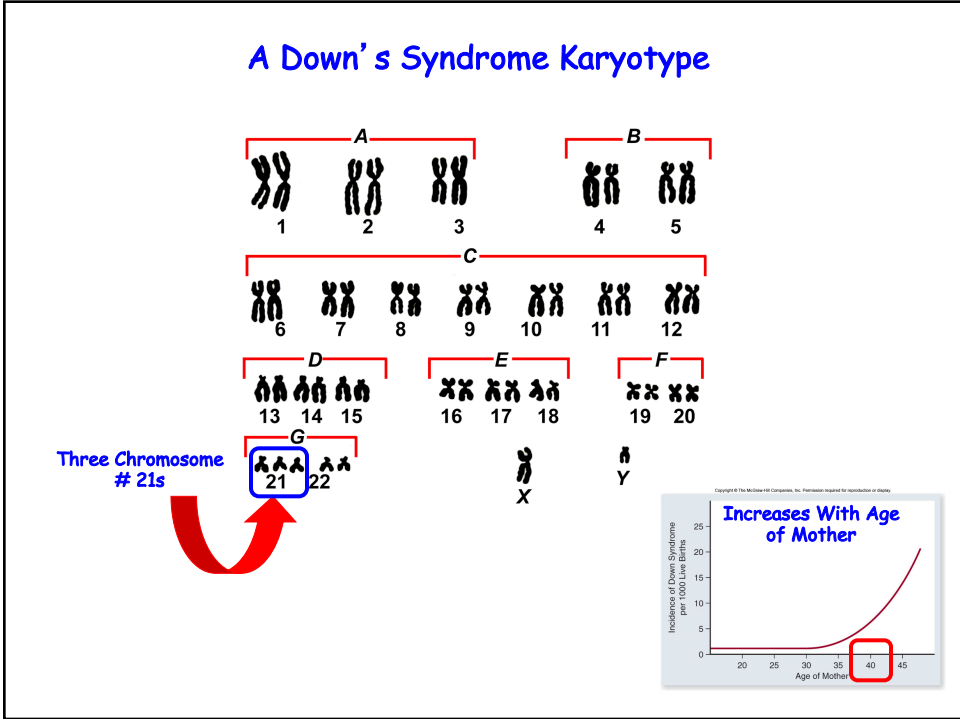


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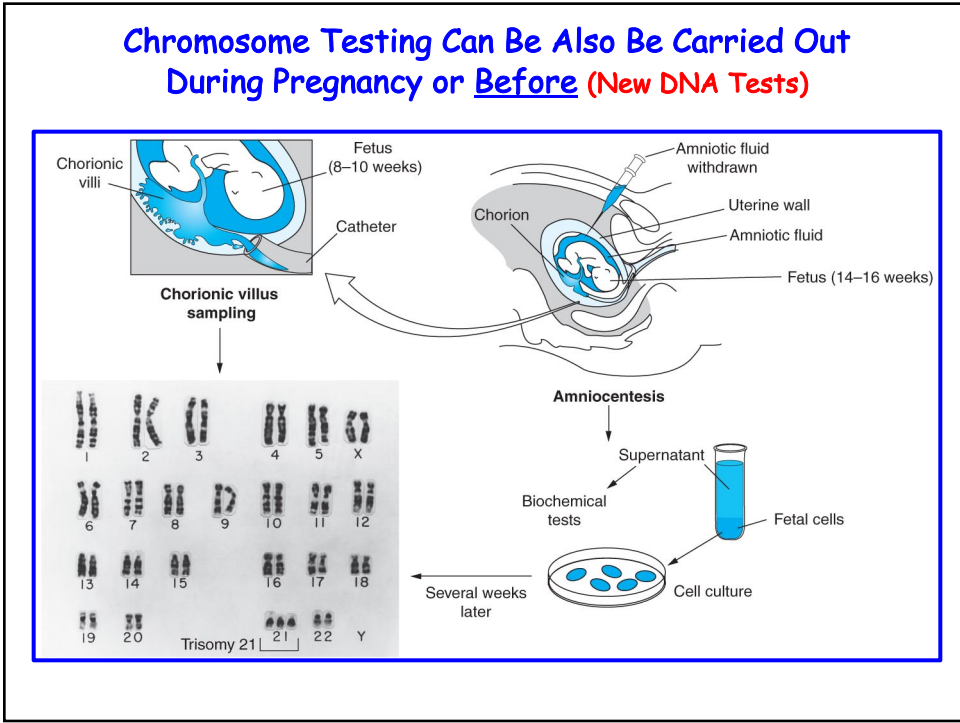
“Mutations” Can Occur By Large Chromosomal Changes - Altering Blocks of Genes at a Time



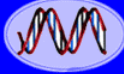
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DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences

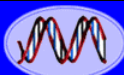


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Genetic Screening Issues

- 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?

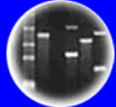
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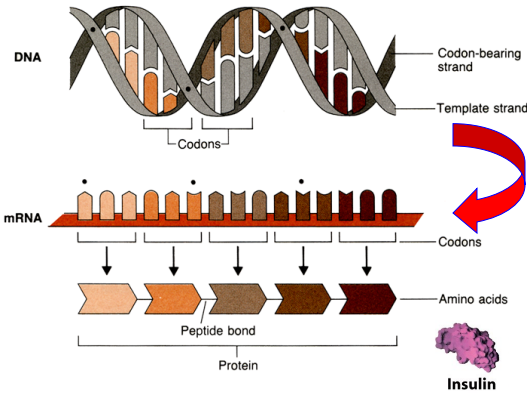
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WHAT ARE THE PROPERTIES OF A GENE?

1. Replication
2. Stability (Mutations)
3. Universality
 - a) All Cells
 - b) All Organisms
4. Direct Cell
Function/Phenotype

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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

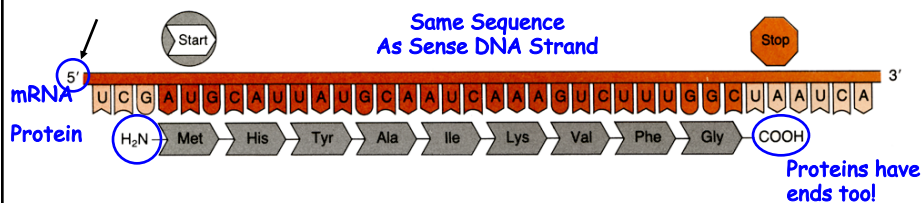
∴ Sequence of Gene
 ↓
 Sequence of mRNA
 ↓
 Sequence of Protein
Colinearity of Sequences!

Know Sequence
 Know Protein

Engineer New Protein

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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!

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The Genetic Code is Universal!

DNA codons	GCA	AGA	GAT	AAT	TGT	GAA	CAA	GGA	CAT	ATA
	GCG	AGG	GAC	AAC	TGC	GAG	CAG	GGG	CAC	ATT
	GCT	CGA	GAC	AAC	TGC	GAG	CAG	GGT	CAC	ATC
	GCC	CGC	CGC	CGC	CGC	CGC	CGC	CGC	CGC	CGC

Amino acid → Ala → Arg → Asp → Asn → Cys → Glu → Gln → Gly → His → Ile

DNA codons	TTA	AAA	ATG	TTT	CCA	AGT	ACA	TGG	TAT	GTA	TAA
	TTG	AAG	ATG	TTC	CCG	AGC	ACG	TGG	TAC	GTG	TAG
	CTA	AAG	ATG	TTC	CCT	TCA	ACT	TGG	TAC	GTT	TGA
	CTT	AAG	ATG	TTC	CCC	TCT	ACC	TGG	TAC	GTC	TGA
	CTC	AAG	ATG	TTC	CCC	TCC	ACC	TGG	TAC	GTC	TGA

Amino acid → Leu → Lys → Met → Phe → Pro → Ser → Thr → Trp → Tyr → Val → Stop

For RNA, The Ts are replaced by Us.

How Know?

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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DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

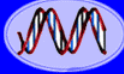
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Expression of Jellyfish Green Fluorescence Protein (GFP) in Pigs Shows That Genetic Code is Universal!!


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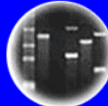
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Plants of Tomorrow

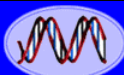
Implications For Genetic Engineering - "Its in The DNA!!"

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

"Legos"/Modules → Sequences to Do Anything You Want
Using Genetic Engineering!

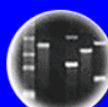
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Summary: Engineering Genes Requires:

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

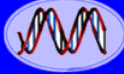
+

Bacteria
Transcription
Off Switch


→

Human Insulin in Bacteria!!


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DNA
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Entire Genetic Code of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues and Future Consequences



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

↓

Thinking About The Consequences of GMOs

DNA replication
DNA repair
genetic recombination
DNA
5' → 3'
3' → 5'
RNA synthesis (transcription)
RNA
5' → 3'
protein synthesis (translation)
PROTEIN
H₂N — amino acids — COOH

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

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?**

"Behind" All Traits!

Same Processes!

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