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

UCLA

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1

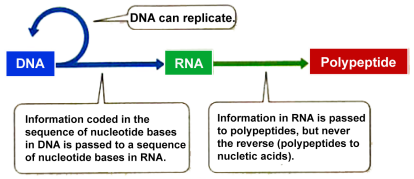
THEMES

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

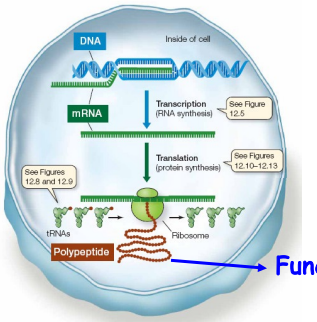
How Do Genes Work?

① Replication

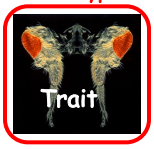


② Gene Activity to Function & Phenotype

Gene Activity → Protein → Function → Phenotype (Trait)



Function → Phenotype



A Gene is NOT Expressed Unless A Functional Protein Produced!

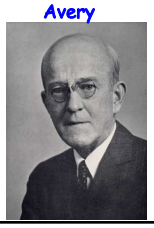
3

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

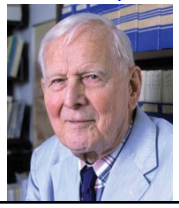
Griffith

Avery



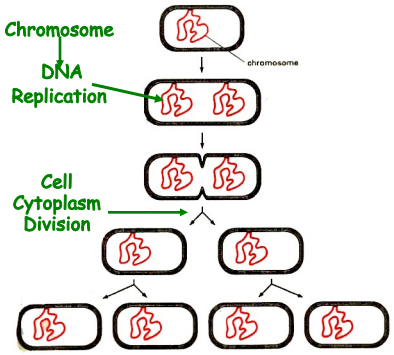
McCleod

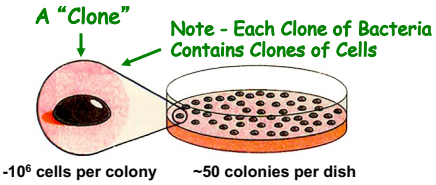
McCarty



4

1 How Are Genes Replicated Each Cell Generation?





Clones

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

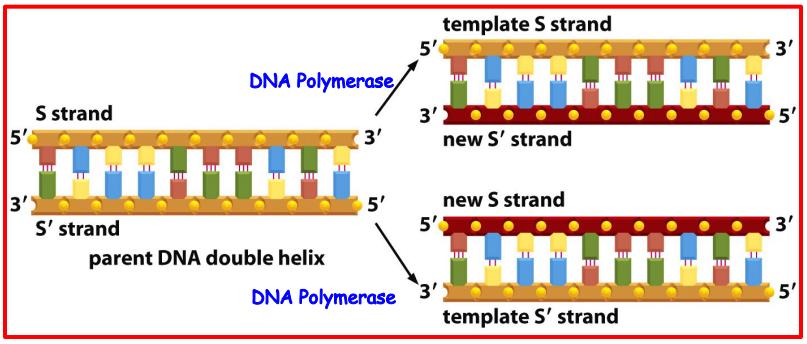
Each Daughter Cell Contains The Same Collection of Genes

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

Clones!

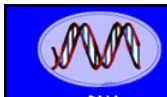
5

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 Using DNA Polymerase
3. New DNA Molecules are Precise Copies of Parental DNA - Each Containing One Newly Synthesized Complementary Strand
4. Predicted by Watson & Crick!!

6


DNA
Genetic Code of Life

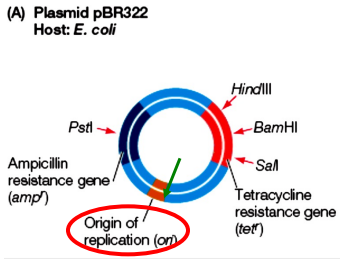

Entire Genetic Code of a Bacteria


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!

It's in the Sequence= Function

∴ Vectors can be Engineered!

Ori's can be cloned/synthesized!

MODULAR!!

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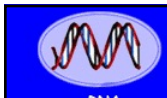
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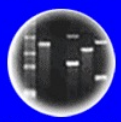

DNA Fingerprinting


Cloning: Ethical Issues and Future Consequences


Plants of Tomorrow

Genetic Engineering Breakthroughs

Classical Breeding – 8,000 BCE

Our Primitive Biotechnology Ancestors

Transgenic Genetic Engineering – 1973

Berg, Cohen, & Boyer

Polymerase Chain Reaction (PCR) – 1985

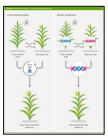
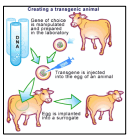
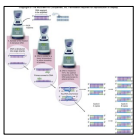
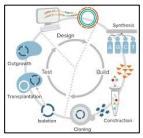
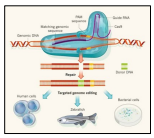
Mullis

Synthetic Genomes – 2000

Venter

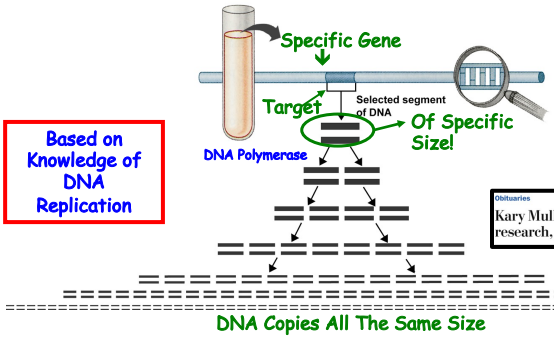
Gene Editing or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) – 2015

Doudna & Carpentier

8

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 1944-2019 RIP

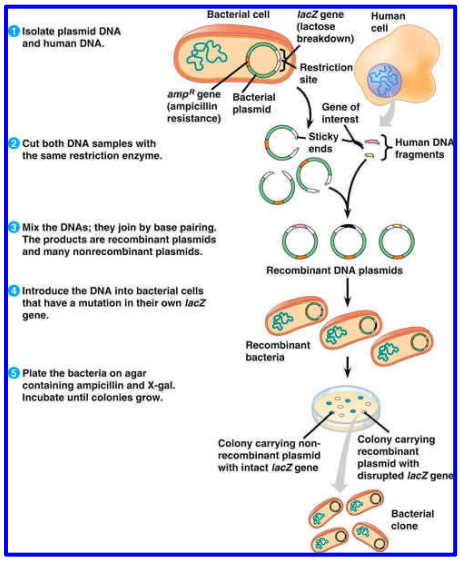
1. PCR Has Revolutionized DNA Analysis!
Specific DNA Sequences/Genes Can Be "Copied" Directly From "Tiny" Amount of DNA!

2. No Bacterial Cloning Needed!

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

9

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



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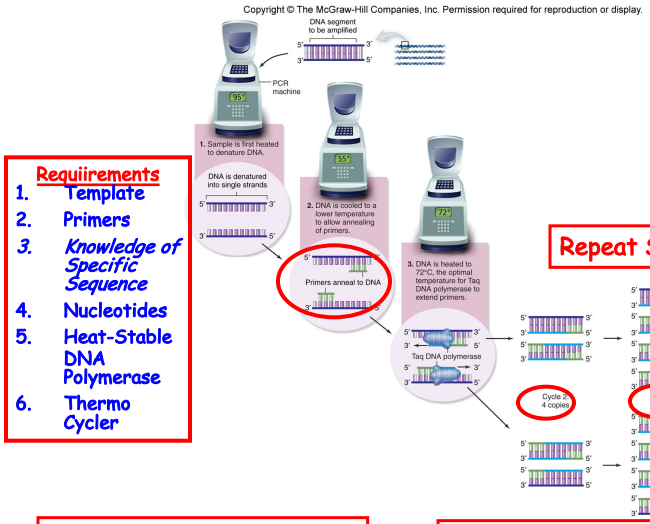
PCR is A Cyclical Process of DNA Replication & Eliminates the Need For Vectors & Bacteria!



Requirements

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

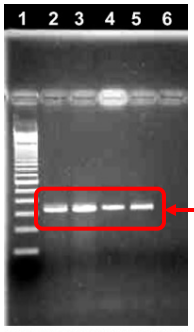
Repeat Steps or Cycle

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

11

Using Gel Electrophoresis to Visualize PCR Products

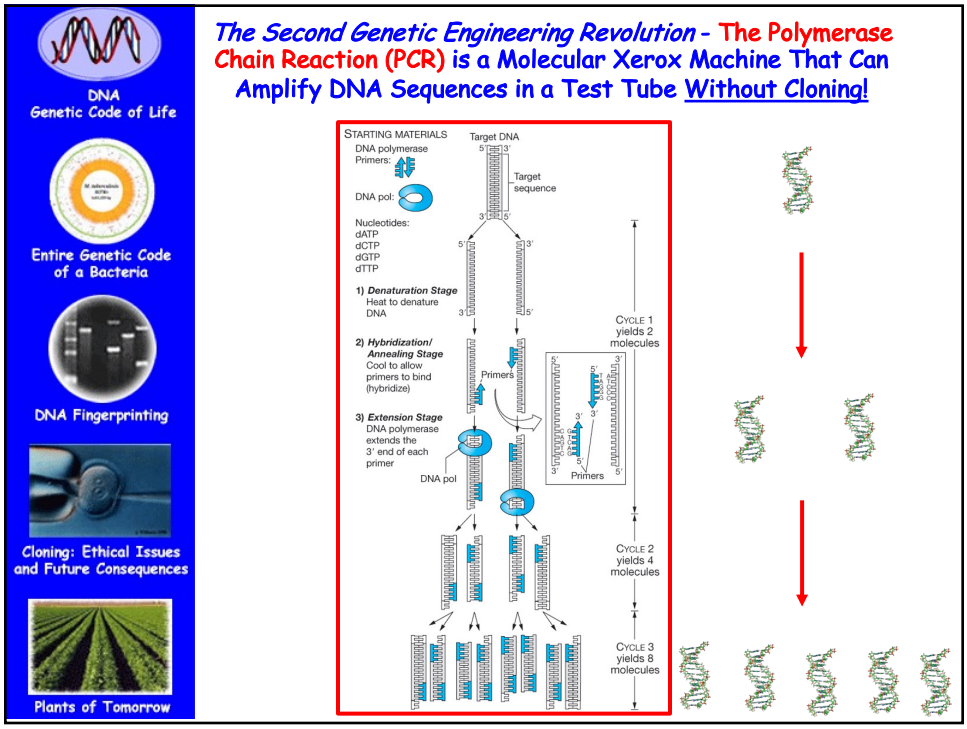


Specific Diagnostic DNA Band Unique to DNA Sequence Being Amplified

- 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*!!!

12



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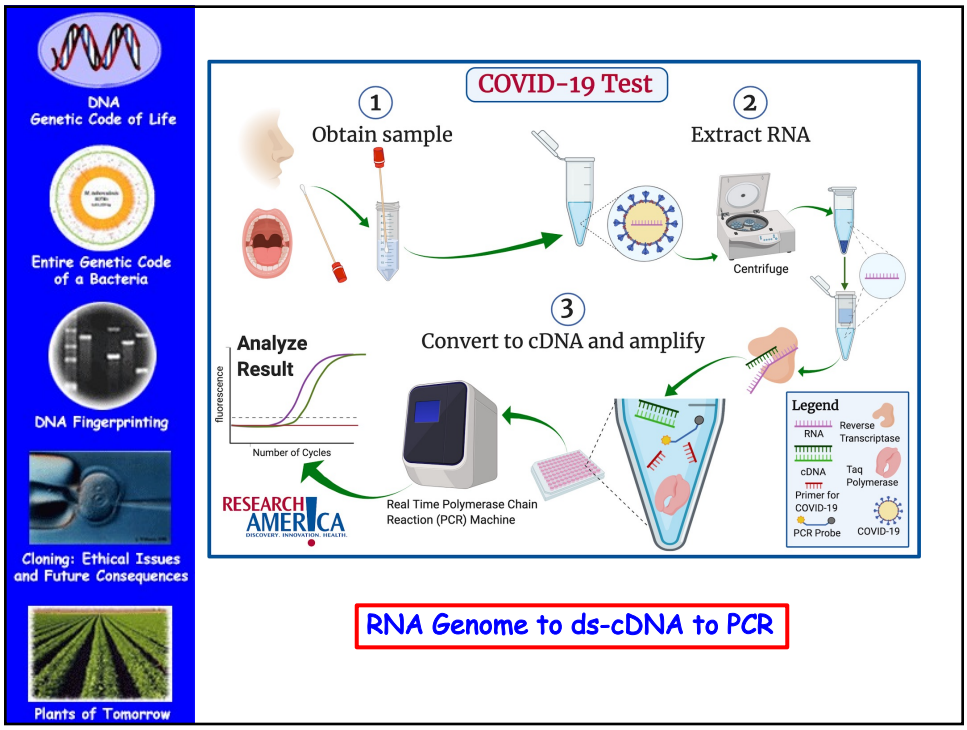
PCR Revolutionized Genetic Engineering & Working With DNA

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

14


DNA
Genetic Code of Life
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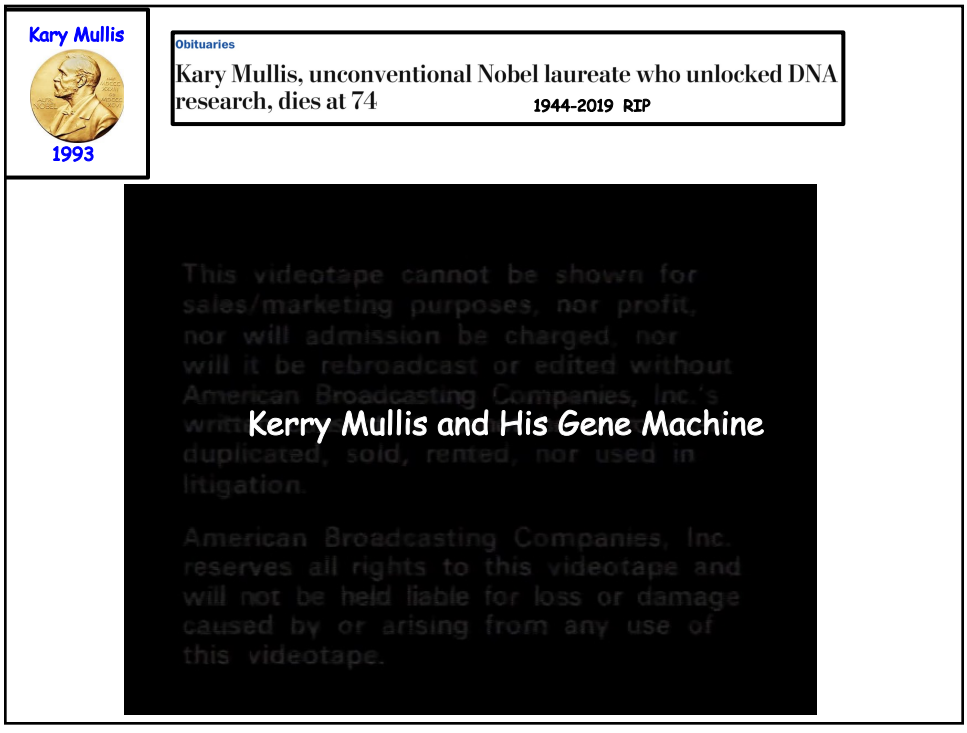
Plants of Tomorrow
COVID-19 Test
1
Obtain sample
2
Extract RNA
Centrifuge
3
Convert to cDNA and amplify
Analyze Result
fluorescence
Number of Cycles
RESEARCH AMERICA
DISCOVERY. INNOVATION. HEALTH.
Real Time Polymerase Chain Reaction (PCR) Machine
Legend
RNA
Reverse Transcriptase
cDNA
Taq Polymerase
Primer for COVID-19
PCR Probe
COVID-19
RNA Genome to ds-cDNA to PCR

15


Kary Mullis
1993
Obituaries
Kary Mullis, unconventional Nobel laureate who unlocked DNA research, dies at 74
1944-2019 RIP
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Kerry Mullis and His Gene Machine

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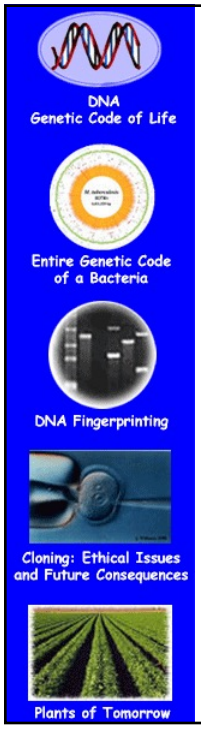

WHAT ARE THE PROPERTIES OF A GENE?

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

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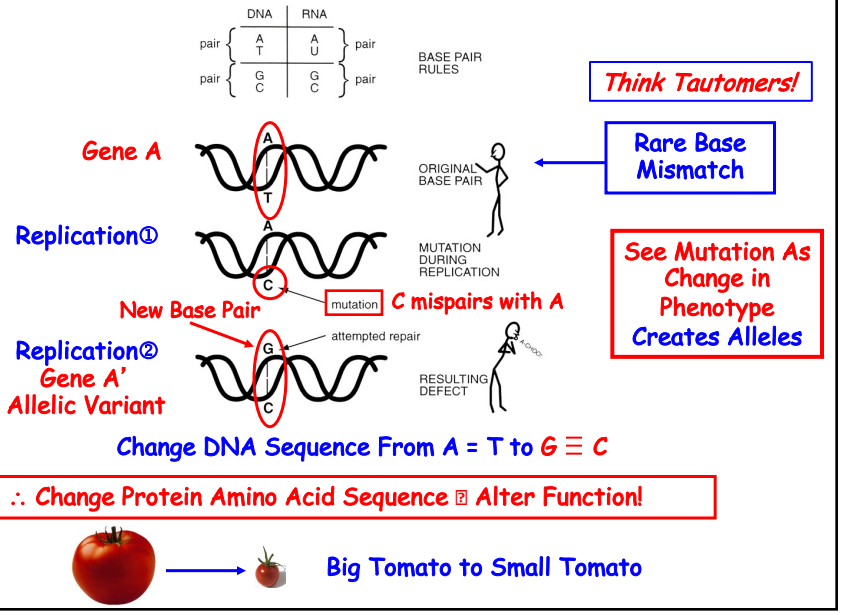
DNA Replication is Precise But Mistakes or Mutations Can Occur!



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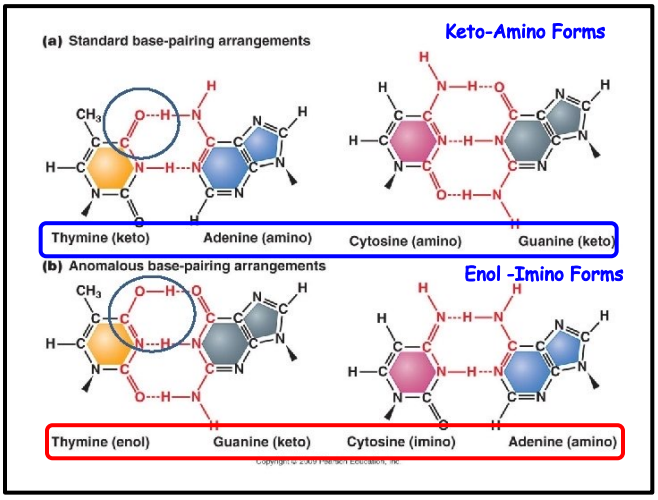
TAUTOMERS CHANGE BASE PAIRING RULES



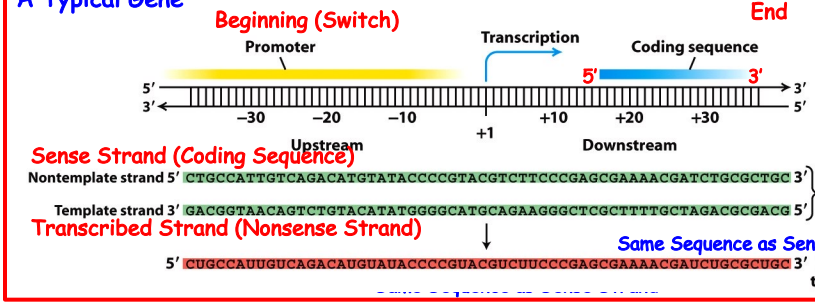
And Lead To Mistakes in DNA Replication & Mutations ➤ Genetic Diversity

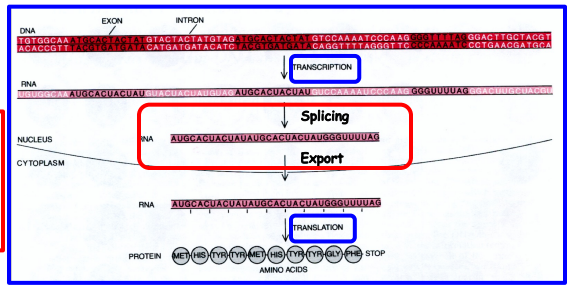
Chemistry Leads to Biology!!

19

Mutations Can Occur At Any Nucleotide or Gene Region

A Typical Gene





Mutation
No Functional Protein
No Phenotype or Trait

- Switch
- Coding Sequence
- Splice Junctions

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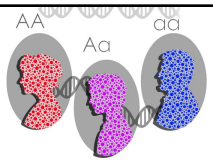
DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

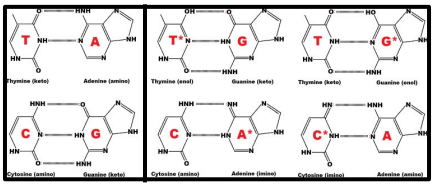
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow



Populations of All Organisms Contain Genetic Variability
Derived From Random Mutations

Base Pairing Mistakes During DNA Replication



Spontaneous Tautomeric Base Shifts

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

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ARTICLE

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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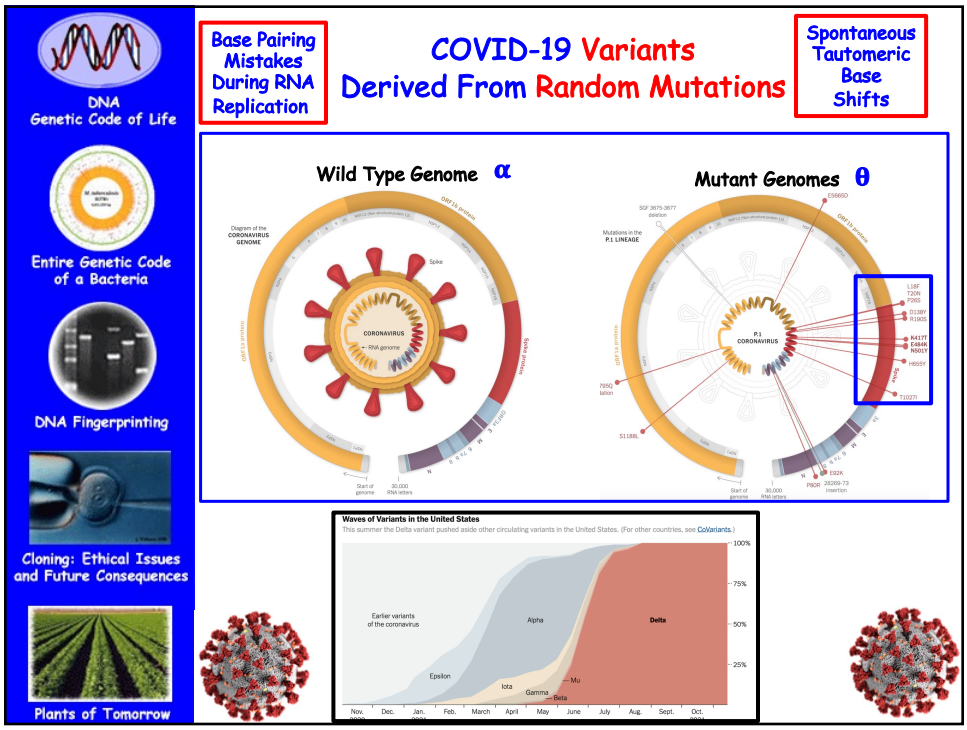

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Human Genetic Disorders Occur As a Result of Rare Spontaneous Mutations in Gametes

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

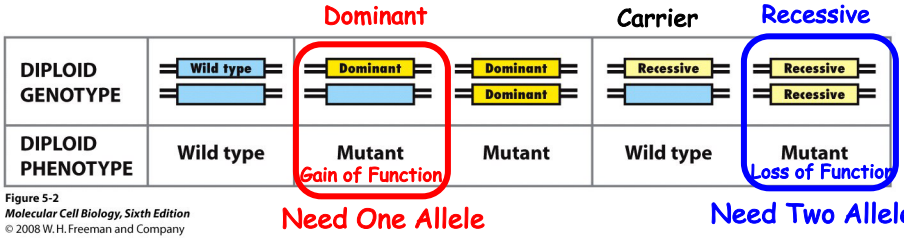

Figure 5-2
Molecular Cell Biology, Sixth Edition

Need One Allele

Need Two Alleles

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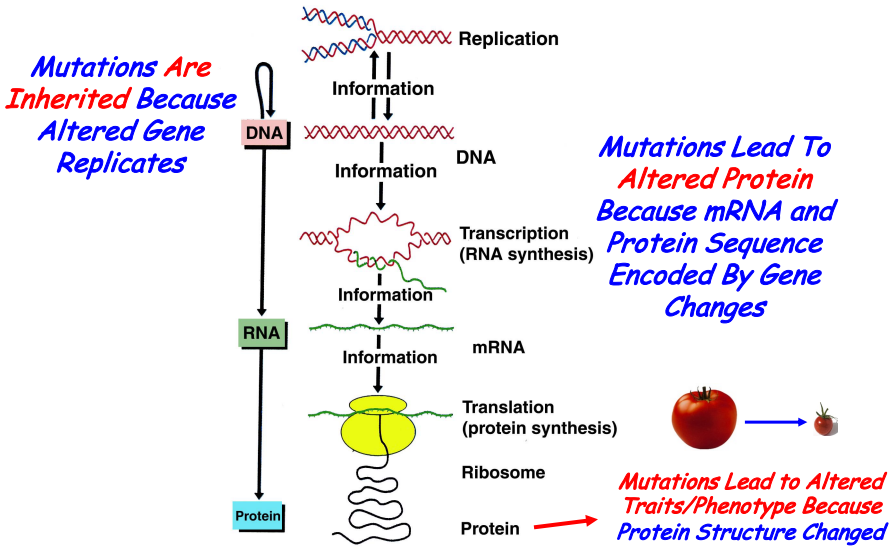
Translating The Genetic Code Into Proteins is a Conserved Process



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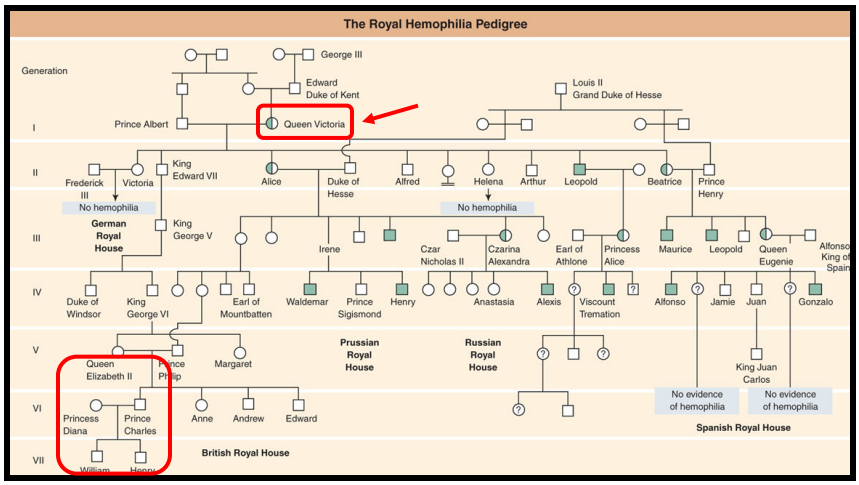
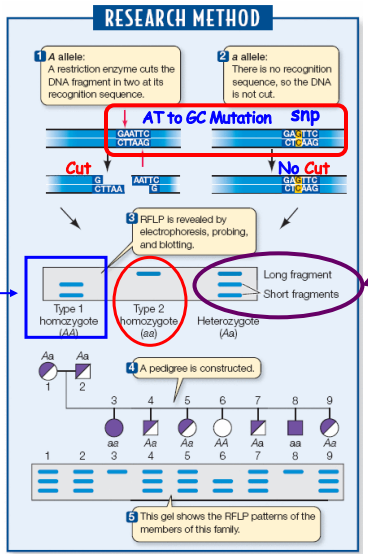
Pedigrees Can Be Used To Follow Disease Genes in Human Families



Recessive Sex Linked

26

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



Essential HC70A Concept!

DNA Fingerprints

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

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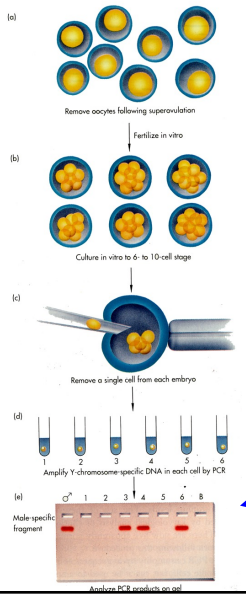
PCR & RFLPs Can Be Used To Analyze Human Genes in A Single Embryo Cell

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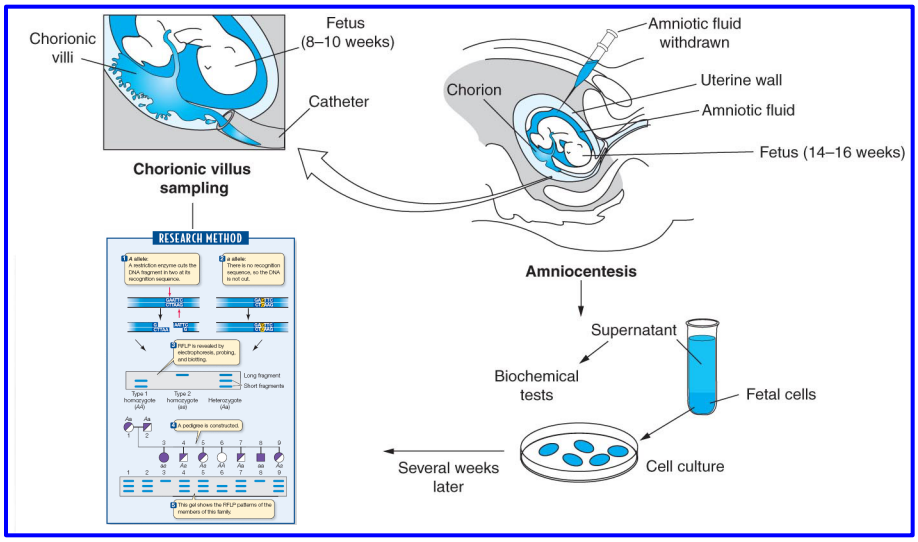
Also……DNA Testing Can Be Carried Out Before Child Birth During Pregnancy



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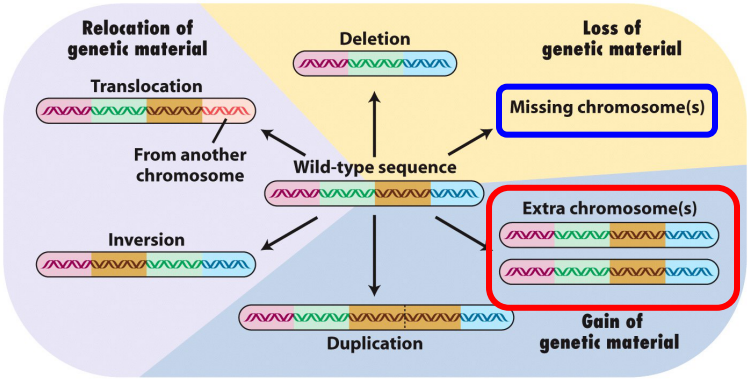
"Mutations" Can Occur By Large Chromosomal Changes – Altering Blocks of Genes at a Time



These Changes Affect Thousands of Genes and Cause Major Developmental Abnormalities

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

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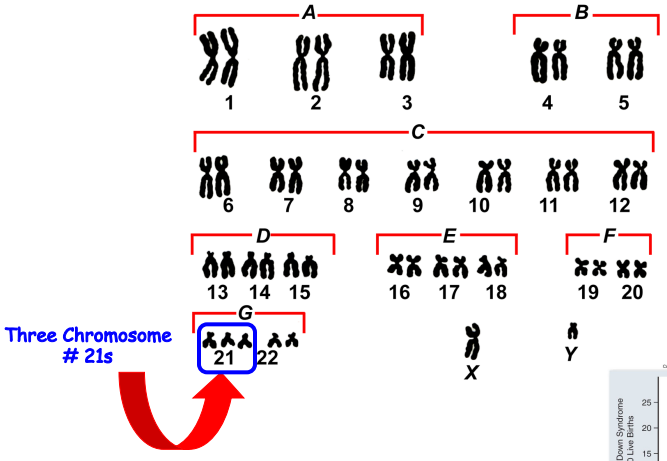
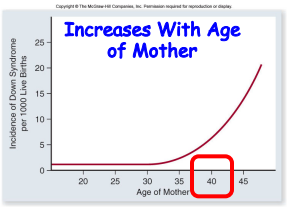
A Down's Syndrome Karyotype





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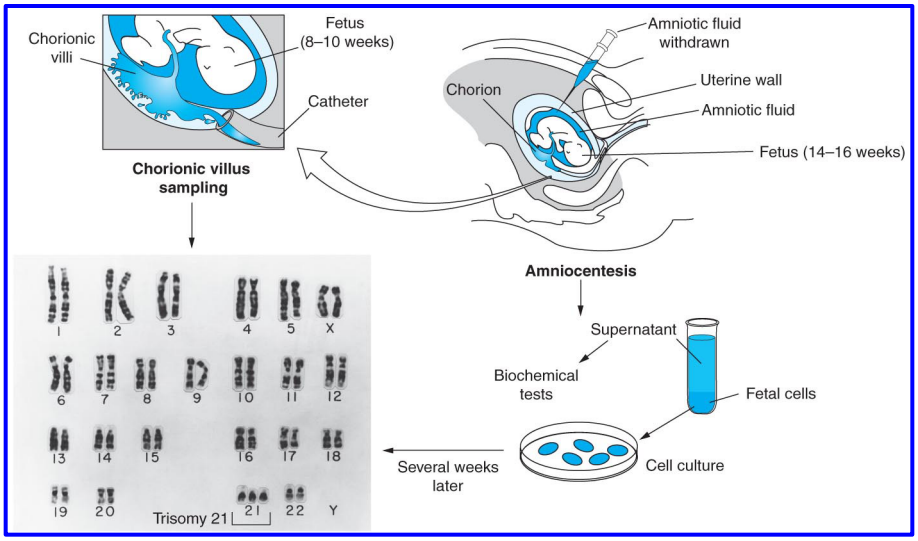
Chromosome Testing Can Be Also Be Carried Out During Pregnancy or Before (New DNA Tests)



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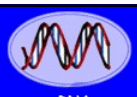

DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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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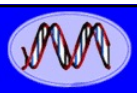

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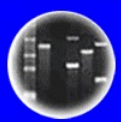

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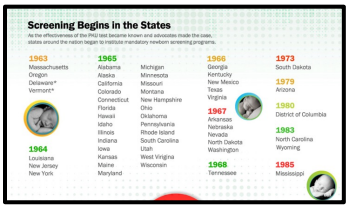
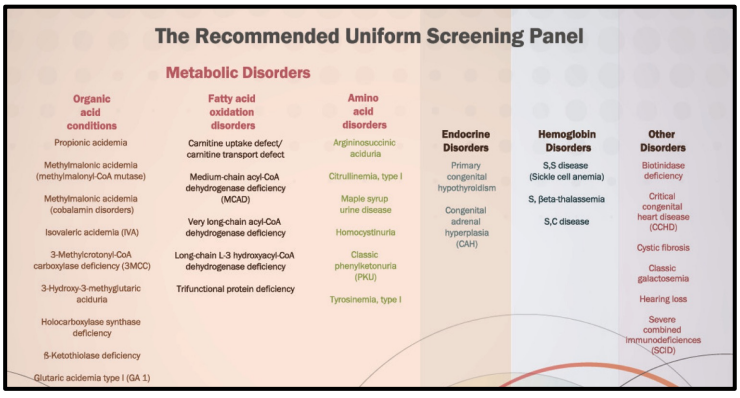
Mandatory Genetic Screening





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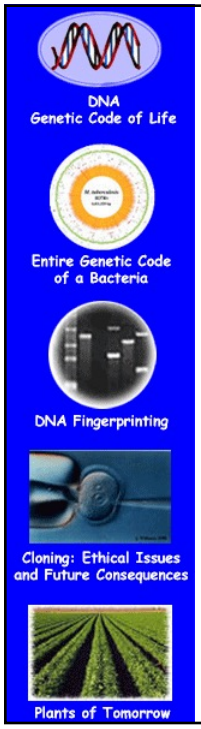

WHAT ARE THE PROPERTIES OF A GENE?

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

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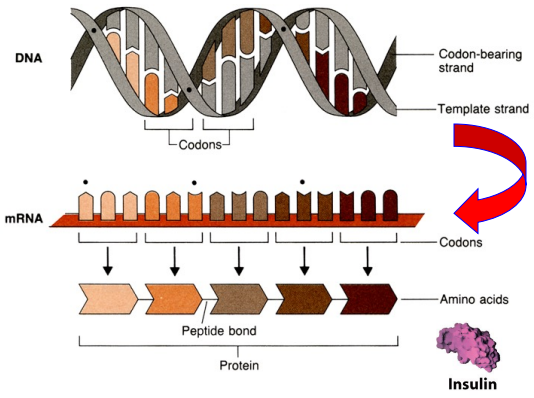
② How Does A Gene Lead To A Phenotype?



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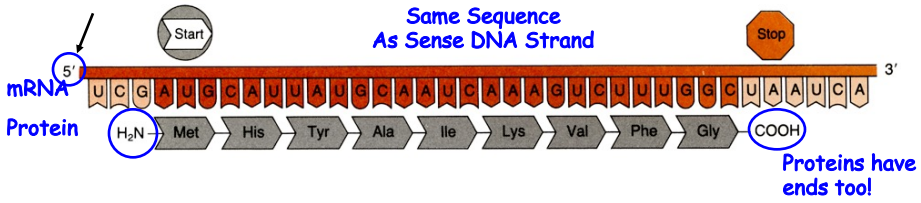
Genetic Code Allows The Sequence of Nucleotides in mRNA/Sense strand of Gene to be Translated into Sequence of Amino Acids in Proteins



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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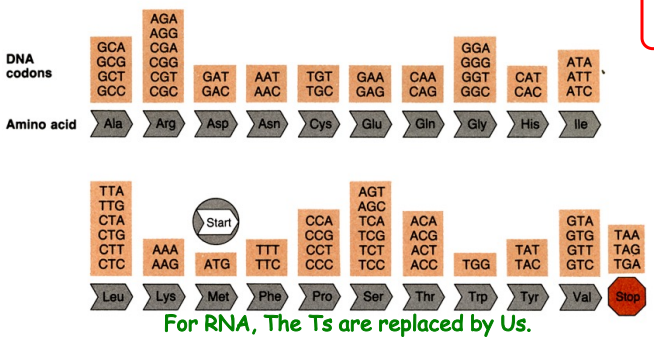
The Genetic Code is Universal!

How Know?



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

For RNA, The Ts are replaced by Us.

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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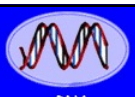

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Expression of Jellyfish Green Fluorescence Protein (GFP) in Pigs Shows That Genetic Code is Universal!!

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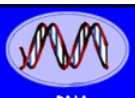

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Implications For Genetic Engineering - "Its in The DNA!!"

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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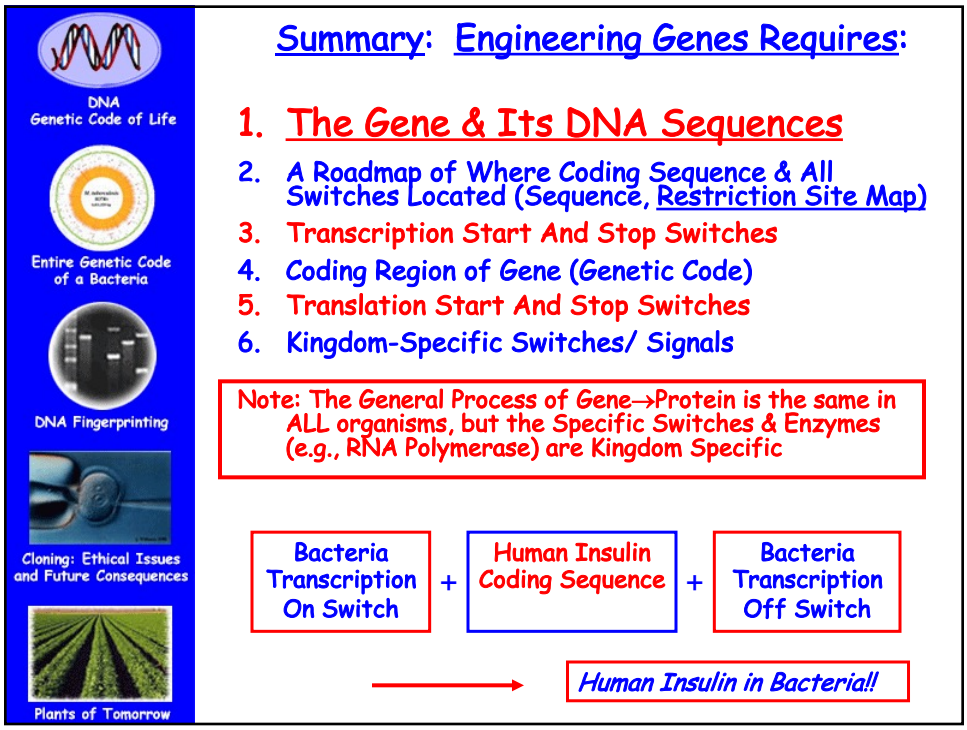
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Summary: Engineering Genes Requires:
1. The Gene & Its DNA Sequences
2. A Roadmap of Where Coding Sequence & All Switches Located (Sequence, Restriction Site Map)
3. Transcription Start And Stop Switches
4. Coding Region of Gene (Genetic Code)
5. Translation 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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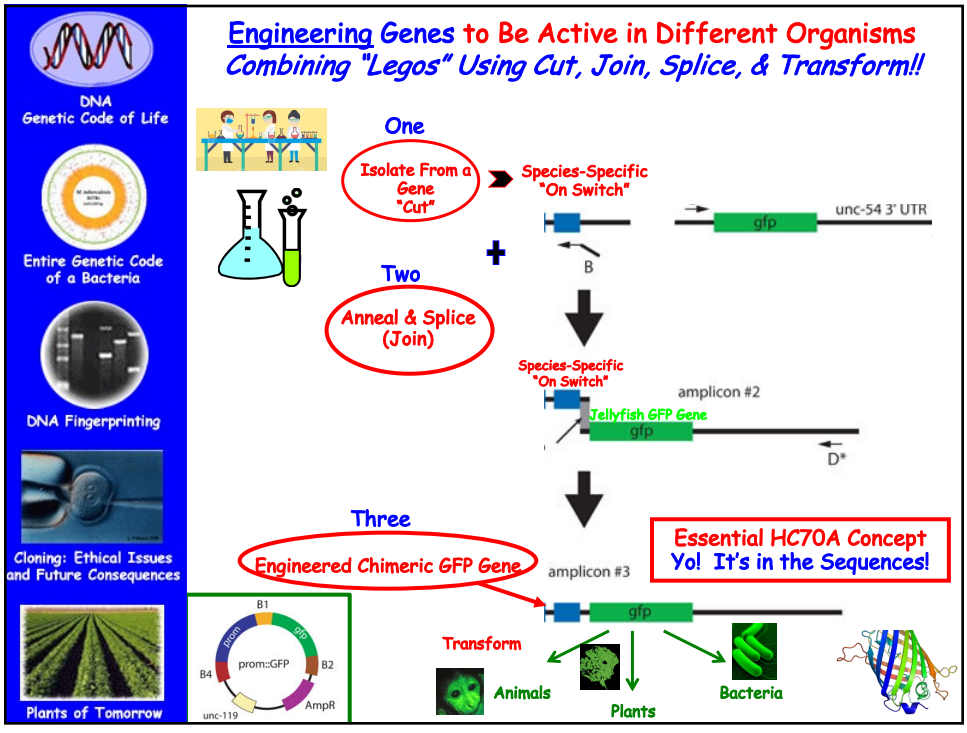
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Engineering Genes to Be Active in Different Organisms
Combining "Legos" Using Cut, Join, Splice, & Transform!!
One
Isolate From a Gene "Cut"
Species-Specific "On Switch"
gfp
unc-54 3' UTR
B
Two
Anneal & Splice (Join)
Species-Specific "On Switch"
amplicon #2
Jellyfish GFP Gene
gfp
D*
Three
Engineered Chimeric GFP Gene
amplicon #3
gfp
Essential HC70A Concept
Yo! It's in the Sequences!
B1
prom::GFP
B2
B4
AmpR
unc-119
Transform
Animals
Plants
Bacteria

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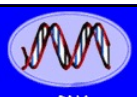

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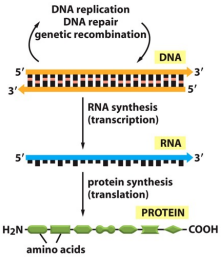
How Do Genes Work & What are Genes in Context of…

Thinking About The Consequences of GMOs



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

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

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!

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