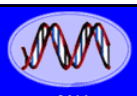


DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

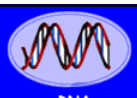
Plants of Tomorrow

HC70A
Spring 2021
Genetic Engineering in Medicine, Agriculture, and Law

Professor Bob Goldberg

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

1



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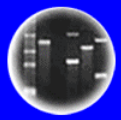

DNA Fingerprinting

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Plants of Tomorrow

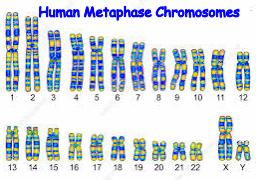
THEMES

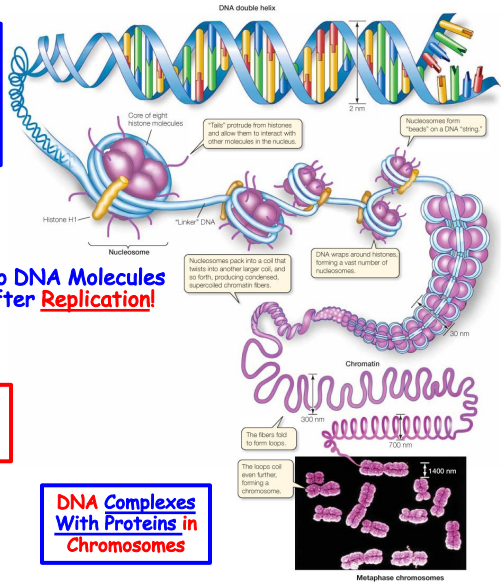
1. How Are Genes & DNA Organized Into Chromosomes?
2. How Do Switches Regulate Genes in Space & Time?
3. Restriction Maps Enable Switches to be Isolated.
4. How Does DNA Replication Occur?
5. What is the Polymerase Chain Reaction (PCR) and How is PCR Used in Society?
6. How Do Mutations Occur?
7. How 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)

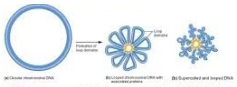
DNA in Human & Eukaryotic Chromosomes is Linear!





Two DNA Molecules After Replication!

DNA in Most Bacteria is Circular!



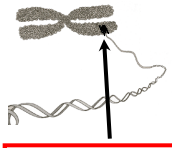
- Chromosomal DNA is compacted ~ 1000 fold to fit within cell

DNA Complexes With Proteins in Chromosomes

3

A Chromosome Contains Many Genes Operating Independently

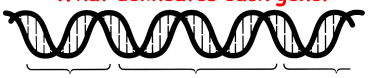
What is the Evidence?



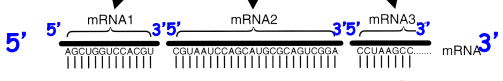
Position of Genes 1, 2, & 3 in chromosome

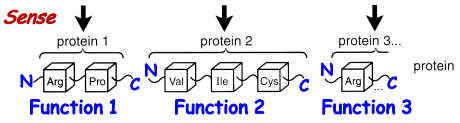
Discrete Units!

What delineates each gene?



Notice sequence of each gene





Note sequence of each protein

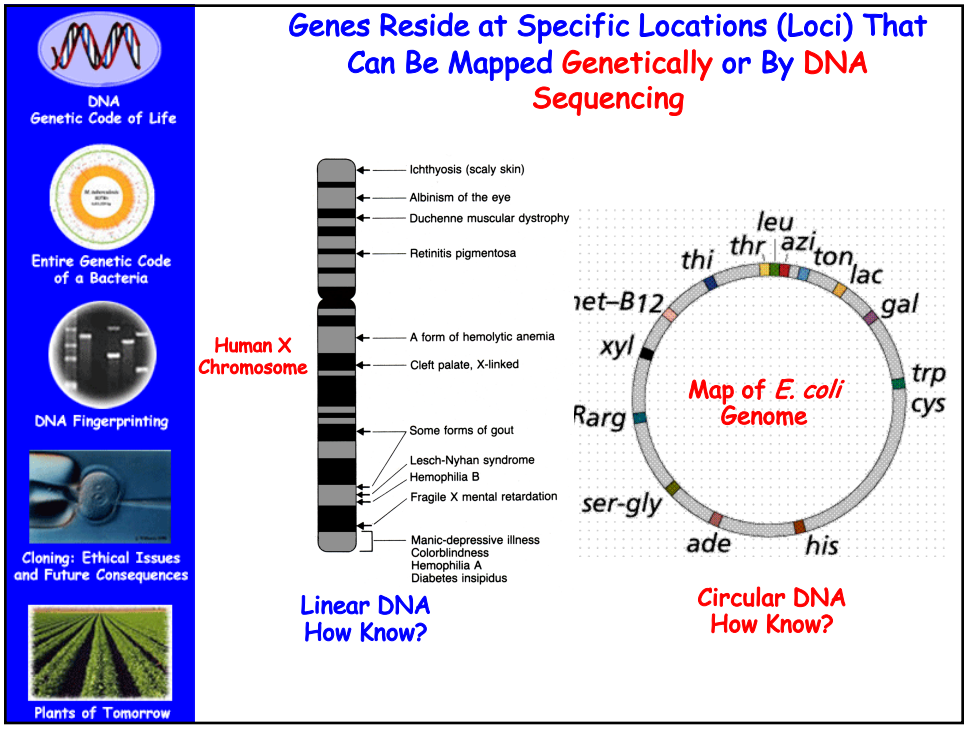
Notice- Each gene, mRNA, & protein has a unique order/sequence of monomeric units

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

IMPORTANT HC70A CONCEPT!

COLINEARITY BETWEEN GENE SEQUENCE AND PROTEIN SEQUENCE

4


DNA
Genetic Code of Life
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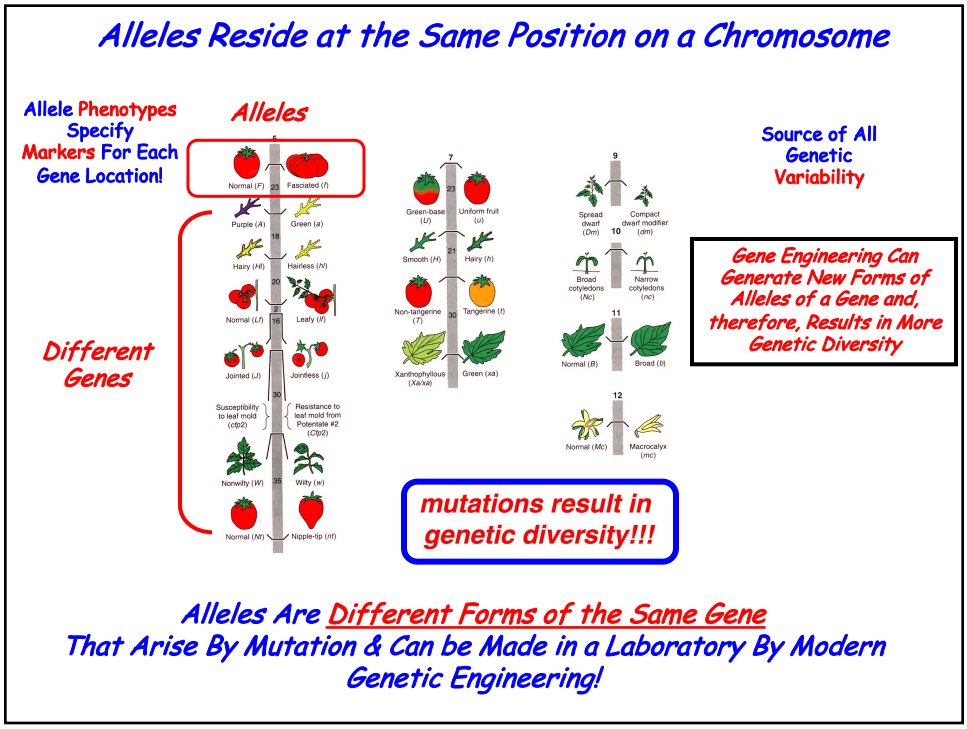
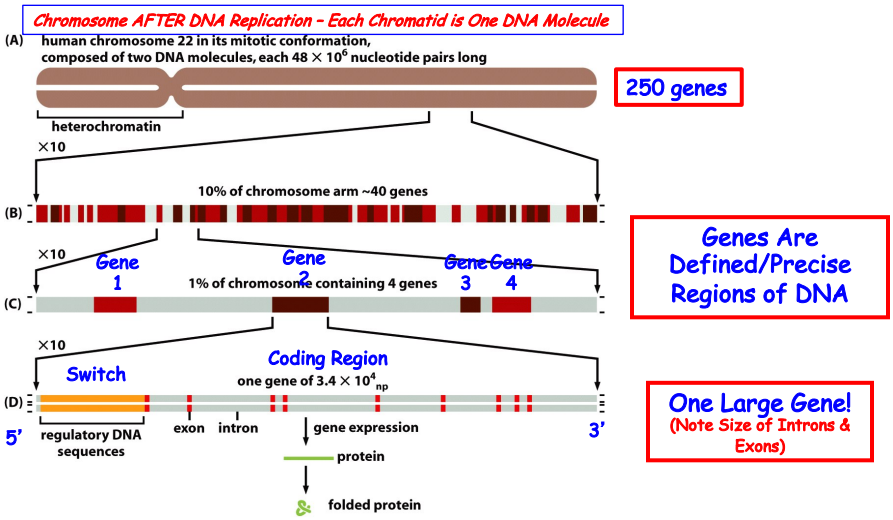
Genes Reside at Specific Locations (Loci) That Can Be Mapped Genetically or By DNA Sequencing
Human X Chromosome
Ichthyosis (scaly skin)
Albinism of the eye
Duchenne muscular dystrophy
Retinitis pigmentosa
A form of hemolytic anemia
Cleft palate, X-linked
Some forms of gout
Lesch-Nyhan syndrome
Hemophilia B
Fragile X mental retardation
Manic-depressive illness
Colorblindness
Hemophilia A
Diabetes insipidus
Linear DNA
How Know?
leu
thr
azi
ton
lac
gal
trp
cys
ade
his
ser-gly
Rarg
xyl
thi
Map of E. coli Genome
Circular DNA
How Know?

5


Alleles Reside at the Same Position on a Chromosome
Allele Phenotypes Specify Markers For Each Gene Location!
Alleles
Different Genes
Normal (F)
Fasciated (f)
Purple (A)
Green (a)
Hairy (Hl)
Hairless (hl)
Normal (Lf)
Leafy (lf)
Jointed (J)
Jointless (j)
Susceptibility to leaf mold (ctp2)
Resistance to leaf mold from Potentate #2 (Ctp2)
Nonwilty (W)
Wilty (w)
Normal (Nt)
Nipple-tip (nt)
Green-base (U)
Uniform fruit (u)
Smooth (H)
Hairy (h)
Non-tangerine (T)
Tangerine (t)
Xanthophyllous (Xa/xa)
Green (xa)
Spread dwarf (Dm)
Compact dwarf modifier (dm)
Broad cotyledons (Nc)
Narrow cotyledons (nc)
Normal (B)
Broad (b)
Normal (Mc)
Macrocalyx (mc)
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 Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!

6

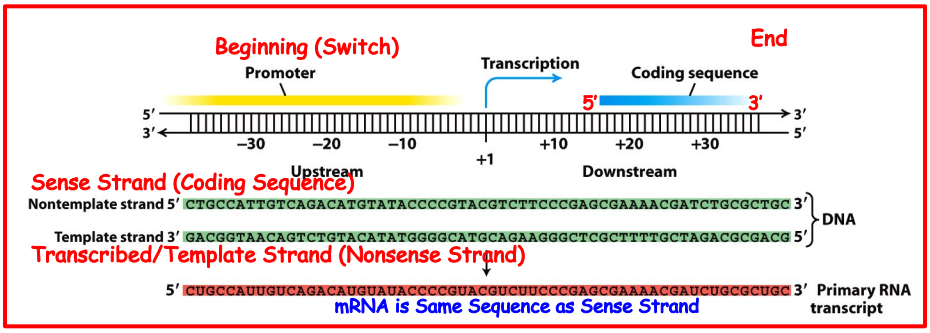
Organization of Genes on Human Chromosome 22

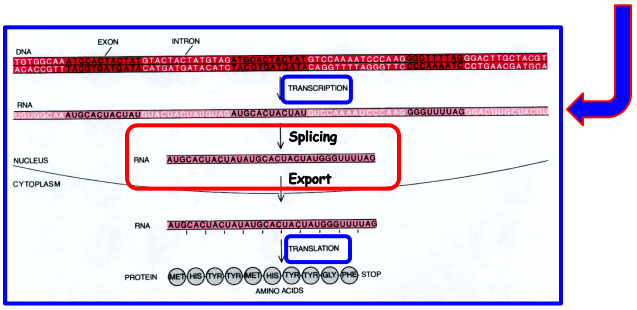


Genes Act As Individual Units!!!
How Know? GloFish Experiment! Genetic Engineering Antibiotic^{R}!

7

A Conceptualized Gene

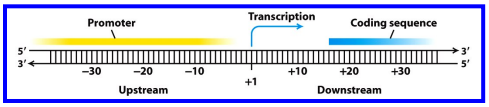


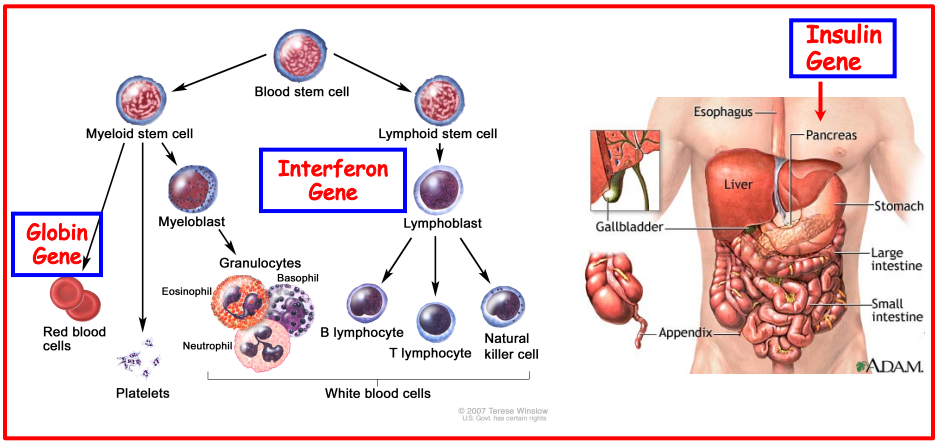


DNA to Protein
No Protein
No Phenotype or Trait

8

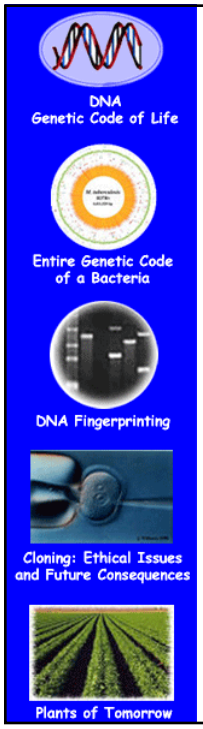
Switches Control Where & When A Gene Is Active
Unique Functions → Unique Cells





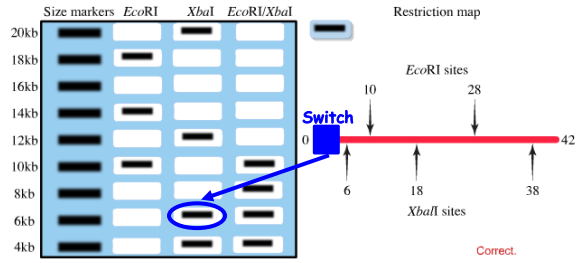
Different Switches!!!!

9



Restriction Maps Are Essential For Isolating Specific Gene Parts

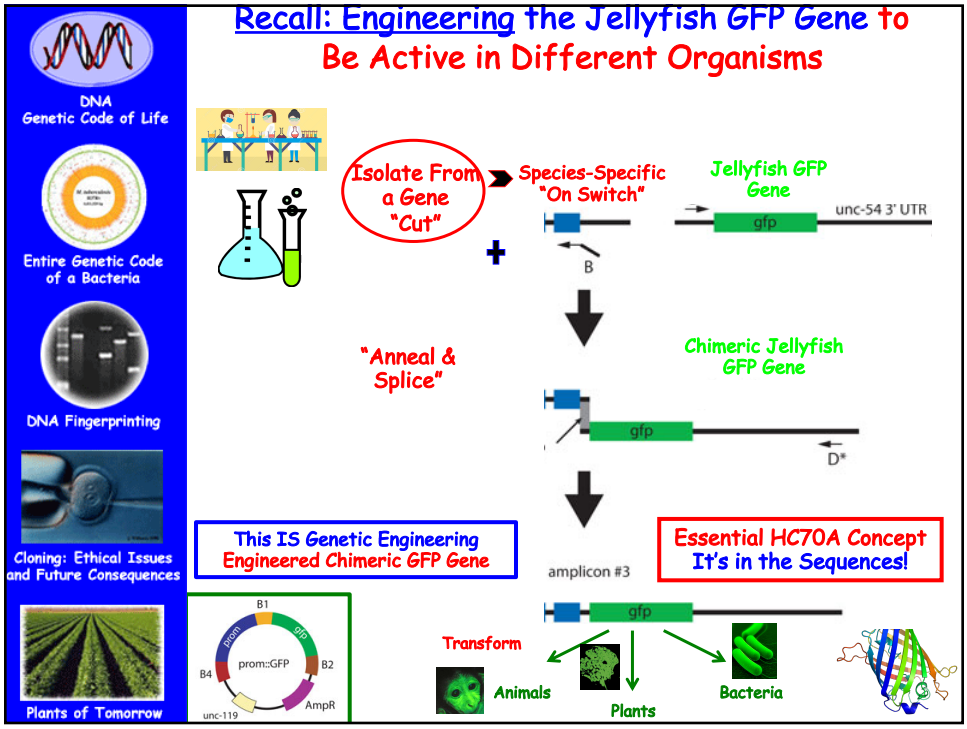
Switches and Other Gene Parts Can Be Cloned & "Shuffled" Creating New Genes That Have No Counterparts in Nature – IF Parts Have Been Localized on Specific Cloned DNA Fragments



To Isolate Switch Use XbaI

And Combining Them With Other Gene Parts Like a Lego Set

10


Recall: Engineering the Jellyfish GFP Gene to Be Active in Different Organisms
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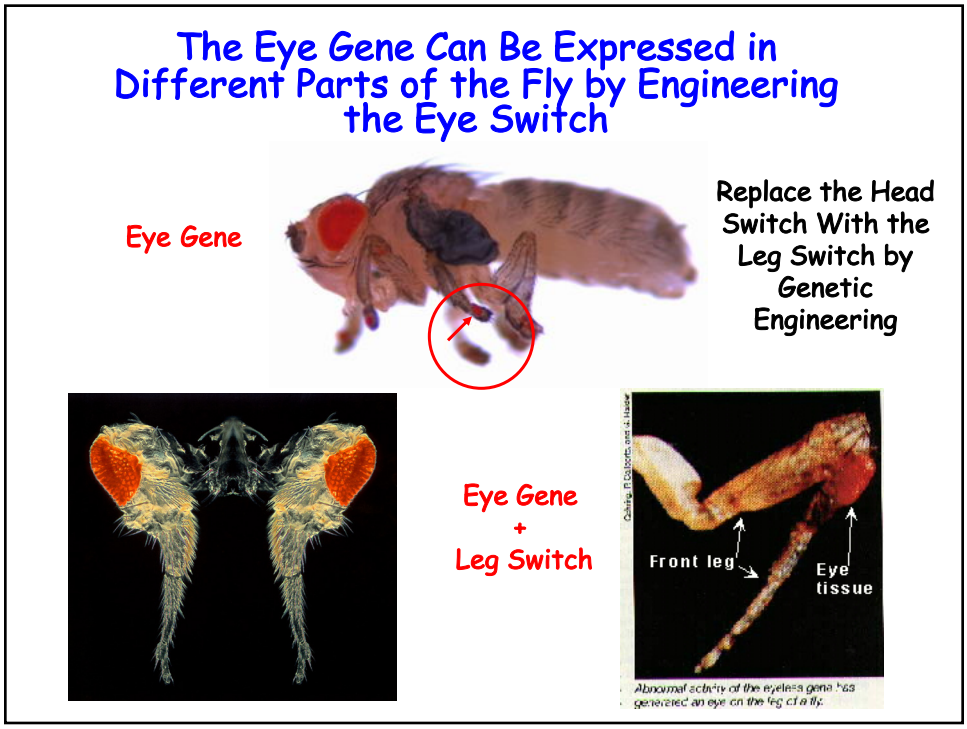
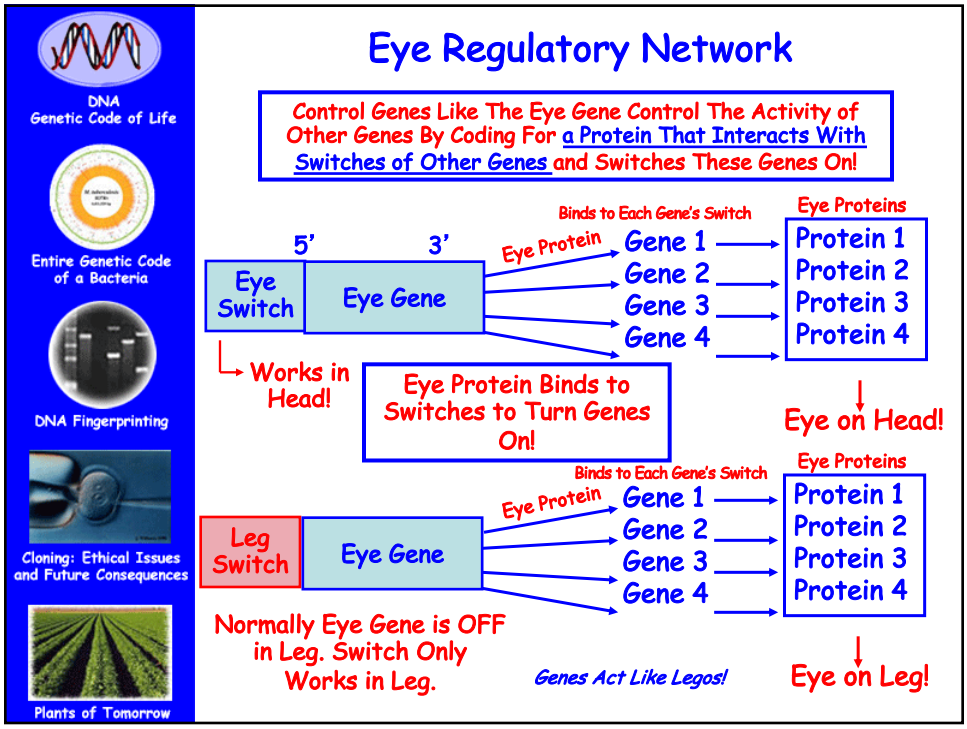
Isolate From a Gene "Cut"
Species-Specific "On Switch"
Jellyfish GFP Gene
gfp
unc-54 3' UTR
B
"Anneal & Splice"
Chimeric Jellyfish GFP Gene
D*
This IS Genetic Engineering
Engineered Chimeric GFP Gene
Essential HC70A Concept
It's in the Sequences!
amplicon #3
prom::GFP
AmpR
unc-119
Transform
Animals
Plants
Bacteria

11


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
Front leg
Eye tissue

12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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!
5'
3'
Eye Switch
Eye Gene
Eye Protein
Binds to Each Gene's Switch
Gene 1
Gene 2
Gene 3
Gene 4
Eye Proteins
Protein 1
Protein 2
Protein 3
Protein 4
Works in Head!
Eye Protein Binds to Switches to Turn Genes On!
Eye on Head!
Leg Switch
Eye Gene
Normally Eye Gene is OFF in Leg. Switch Only Works in Leg.
Genes Act Like Legos!
Eye on Leg!

13


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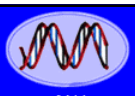
14



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GENES AND SWITCHES ARE UNIQUE DNA SEQUENCES

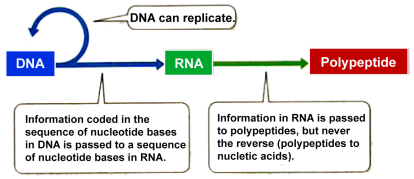
1. They Can Be Cloned & "Shuffled" & Engineered Creating New Genes That Have No Counterparts in Nature
2. These New Genes Can Be Transcribed in New Cell Types (Switch Change) &/or Organisms &/or Both (e.g., Human Genes in Bacteria)
3. All Genes are Regulated & Controlled by Switches Genetic Engineering 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!!

15

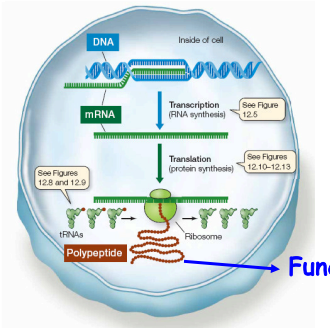
How Do Genes Work?

① Replication



② Gene Activity to Function & Phenotype

Gene Activity
Protein
Function
Phenotype (Trait)

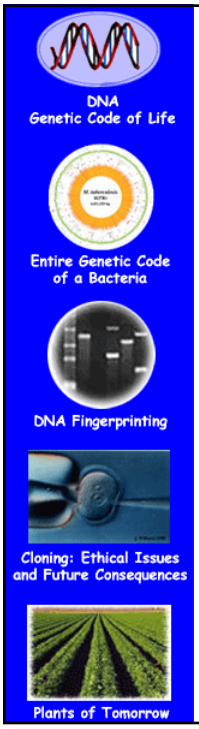


Function →

Phenotype

A Gene is NOT Expressed Unless A Functional Protein Produced!

16



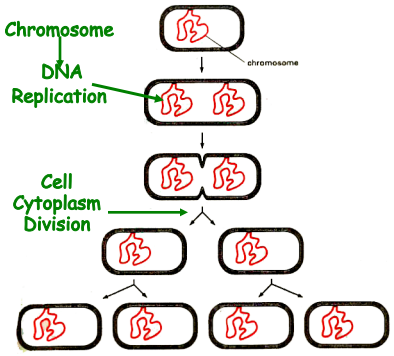
WHAT ARE THE PROPERTIES OF A GENE?

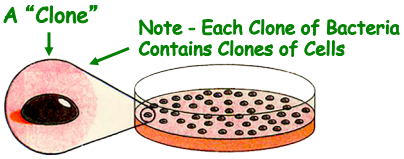
1. Replication
2. Stability (Mutations)
3. Universality
   a) All Cells
   b) All Organisms
4. Direct Cell Function/Phenotype

17

How Are Genes Replicated Each Cell Generation?

1





Clones

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

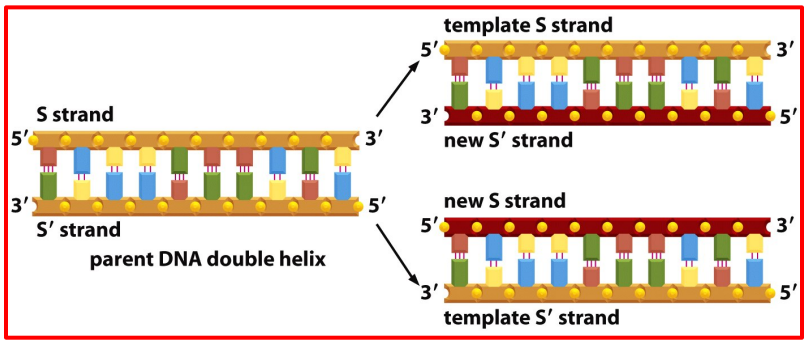
Each Daughter Cell Contains The Same Collection of Genes

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

Clones!

18

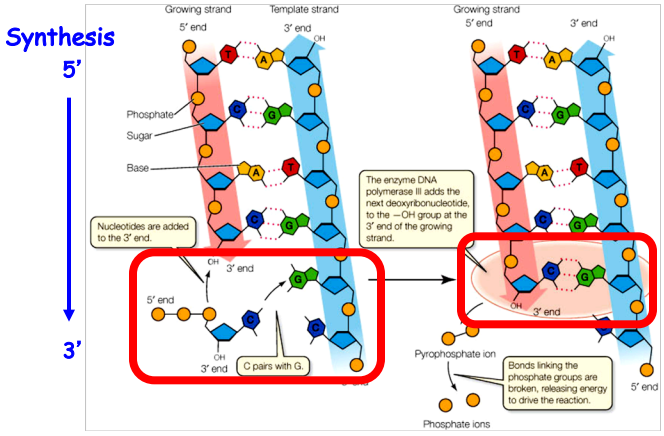
DNA Replication Occurs Semi-Conservatively



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!!

19

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

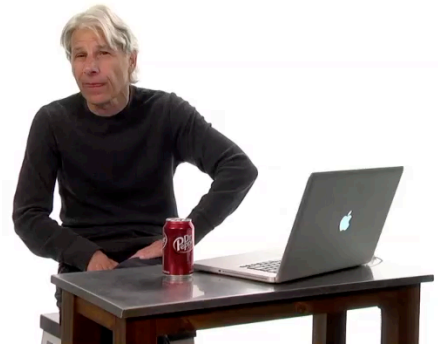


Sequence is Specified by Complementary Bases

Note: 5′ (P) & 3′ (OH)

5′ to 3′ Polarity Specifies Sequence

20



DNA Replication

Polarity is maintained

Sequence is maintained

Each strand serves as a template for new daughter strands (semi-conservative)

Daughter strands are precise copies

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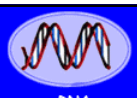
21



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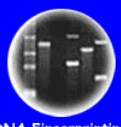

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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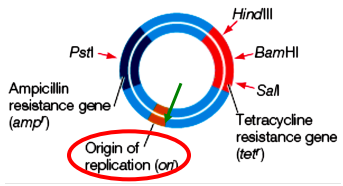
Vectors Are Needed To Replicate Genes In Transformed Cells

(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!

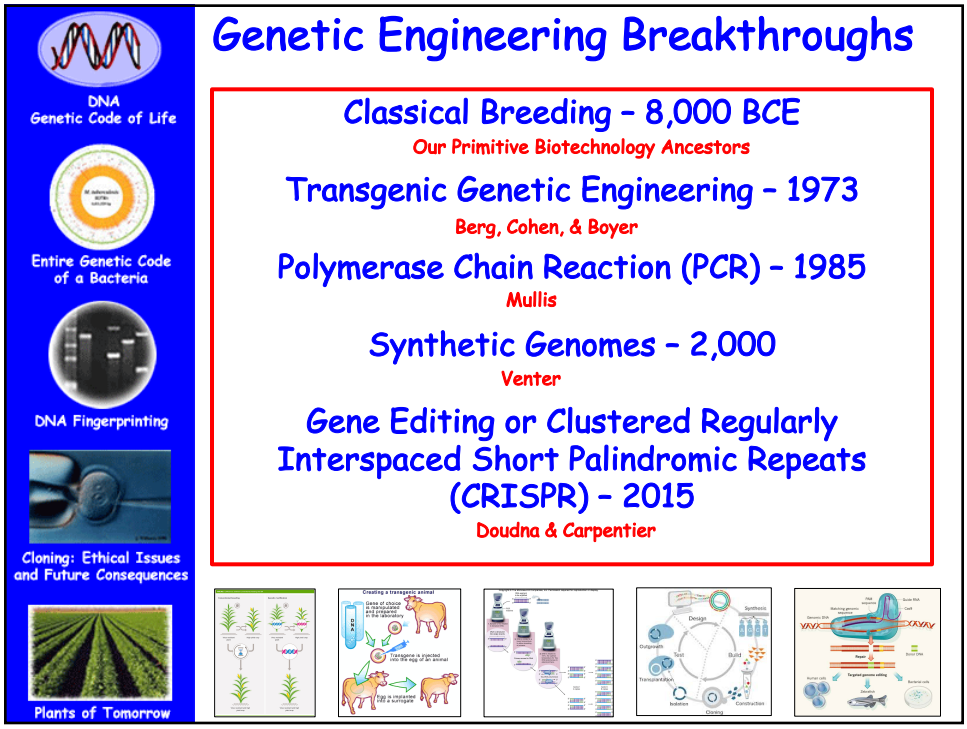
It's in the Sequence= Function

∴ Vectors can be Engineered!

Ori's can be cloned/synthesized!

MODULAR!!

22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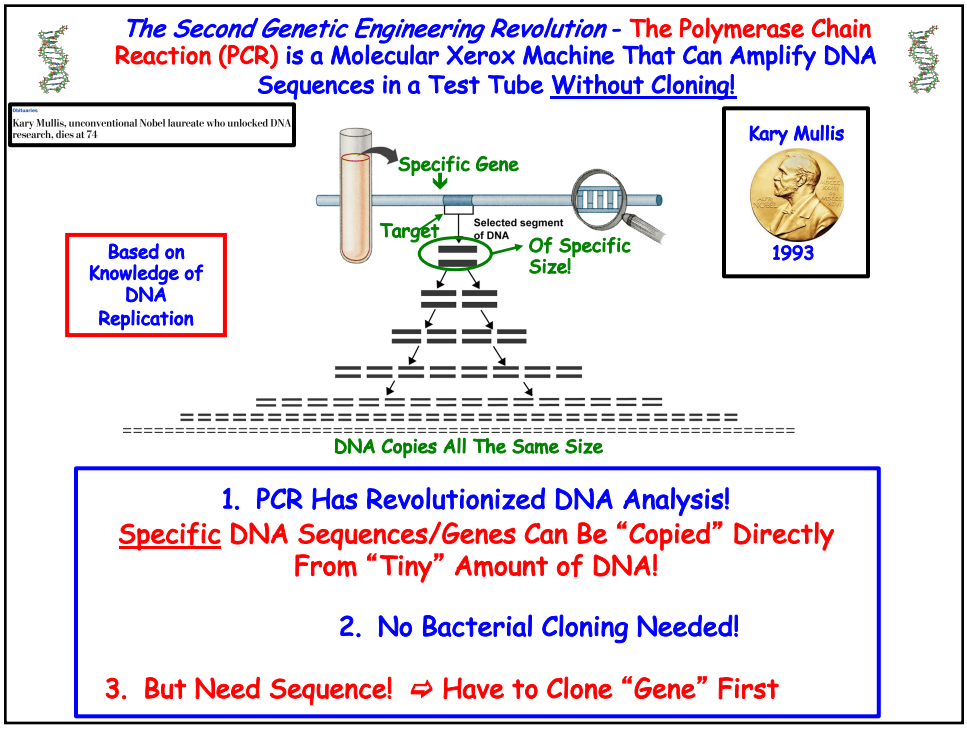
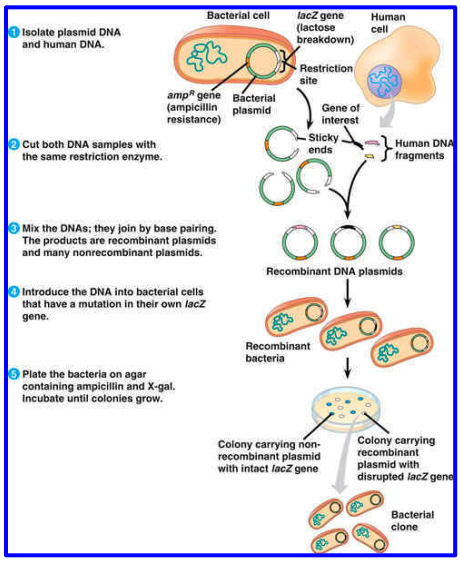
Genetic Engineering Breakthroughs
Classical Breeding – 8,000 BCE
Our Primitive Biotechnology Ancestors
Transgenic Genetic Engineering – 1973
Berg, Cohen, & Boyer
Polymerase Chain Reaction (PCR) – 1985
Mullis
Synthetic Genomes – 2,000
Venter
Gene Editing or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) – 2015
Doudna & Carpentier

23


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!
Kary Mullis, unconventional Nobel laureate who unlocked DNA research, dies at 74
Specific Gene
Target
Selected segment of DNA
Of Specific Size!
Based on Knowledge of DNA Replication
Kary Mullis
1993
DNA Copies All The Same Size
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

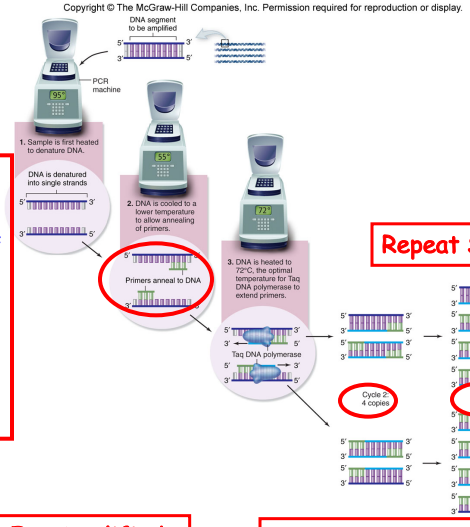
24

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



25

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

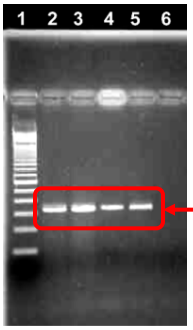
Repeat Steps or Cycle

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

26

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*!!!

27

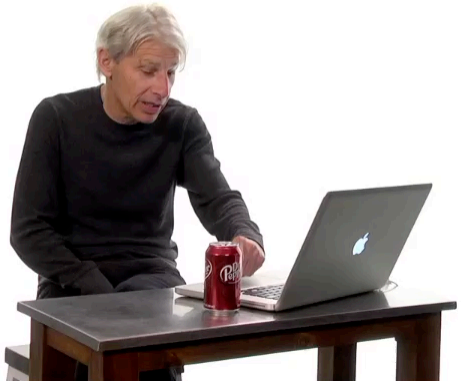
PCR Revolutionized Genetic Engineering & Working With DNA

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

28

UCLA

29



UCLA

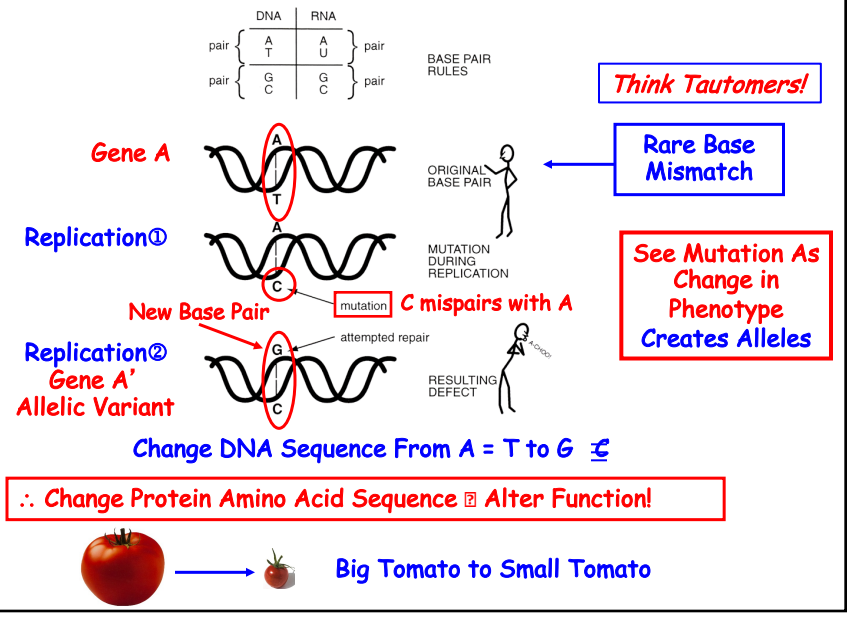
30

WHAT ARE THE PROPERTIES OF A GENE?

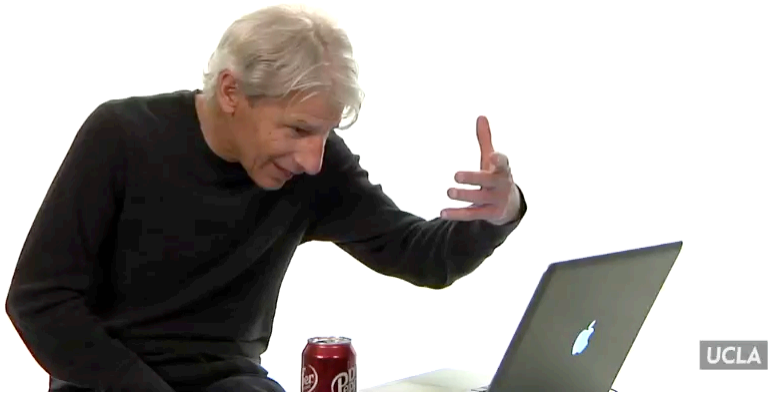
1. Replication
2. Stability (Mutations)
3. Universality
   a) All Cells
   b) All Organisms
4. Direct Cell Function/Phenotype

31

DNA Replication is Precise But Mistakes or Mutations Can Occur!



32

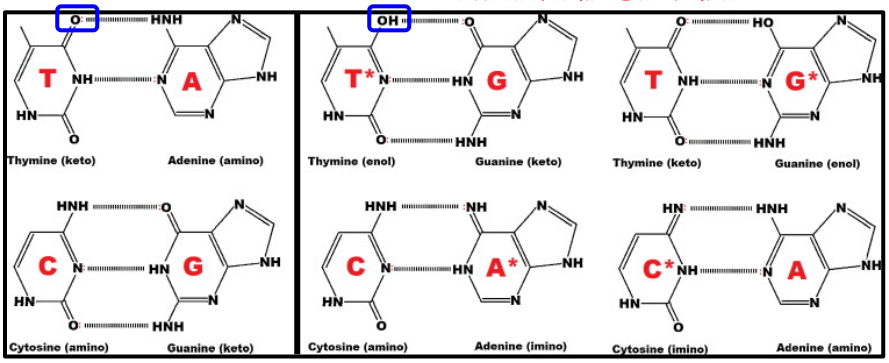


33

TAUTOMERS CHANGE BASE PAIRING RULES

Normal Forms – Keto & Amino

"Mutant" Forms – Enol & Imino

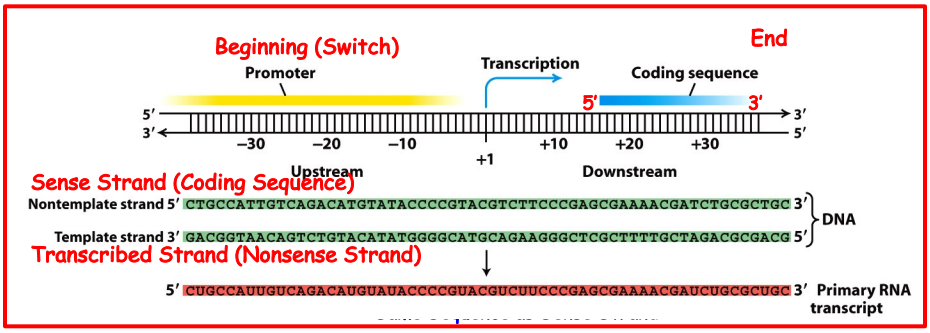


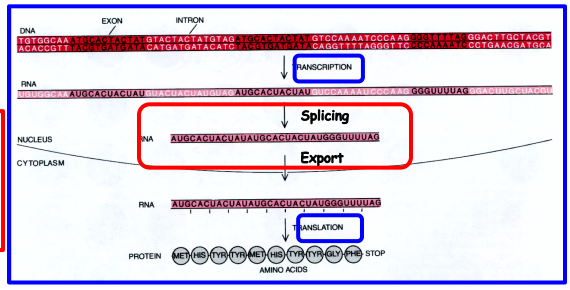
And Lead To Mistakes in DNA Replication & Mutations ➡ Genetic Diversity

Chemistry Leads to Biology!!

34

Mutations Can Occur At Any Nucleotide or Gene Region

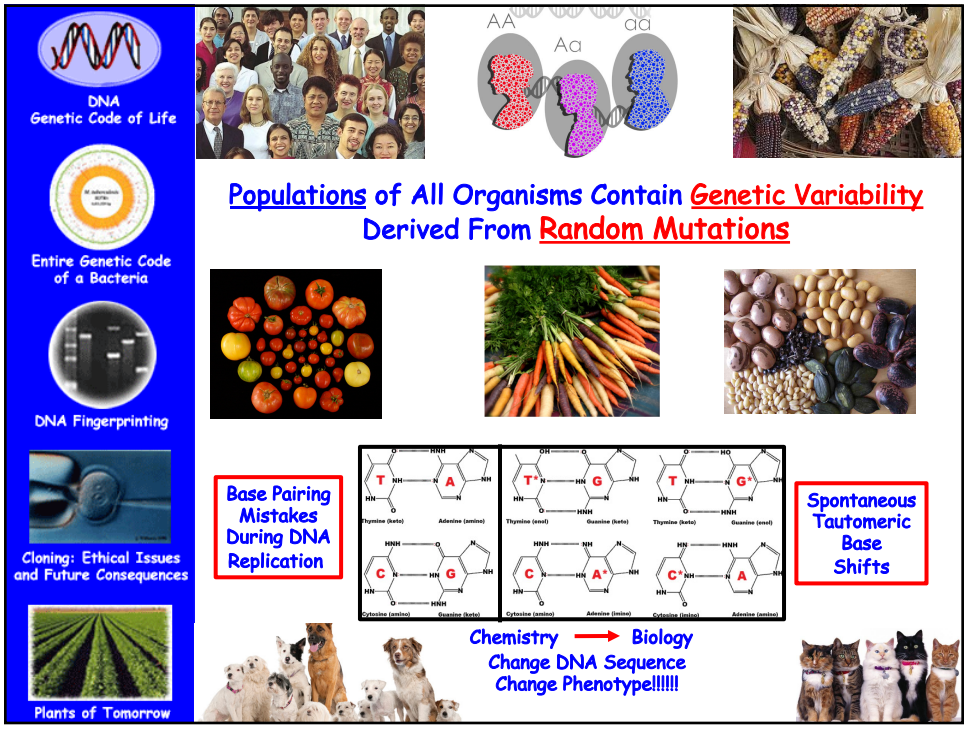




Mutation
No Functional Protein
No Phenotype or Trait

- Switch
- Coding Sequence
- Splice Junctions

35



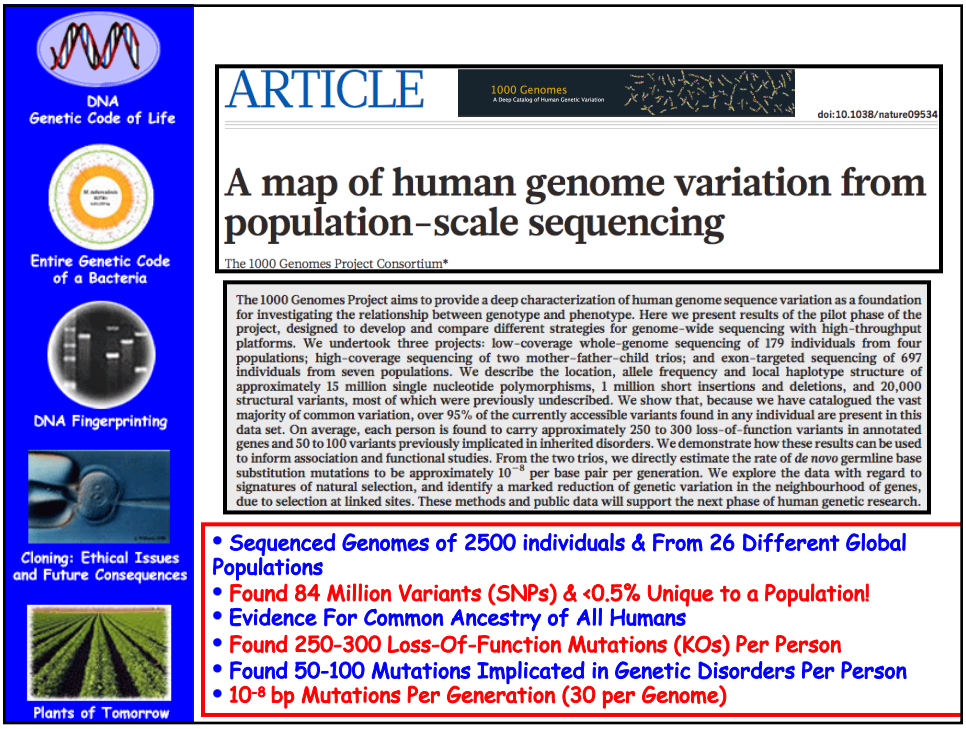
Populations of All Organisms Contain Genetic Variability
Derived From Random Mutations

Base Pairing Mistakes During DNA Replication

Spontaneous Tautomeric Base Shifts

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

36



37

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

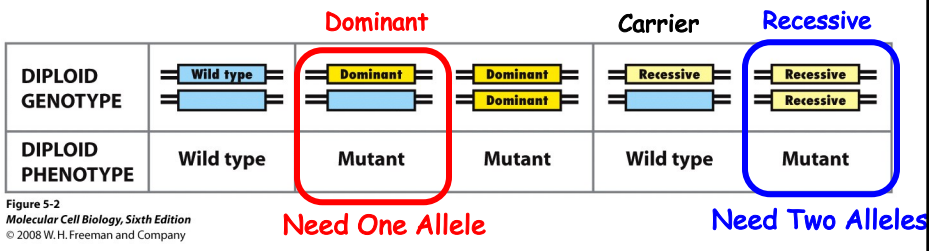


Figure 5-2
Molecular Cell Biology, Sixth Edition
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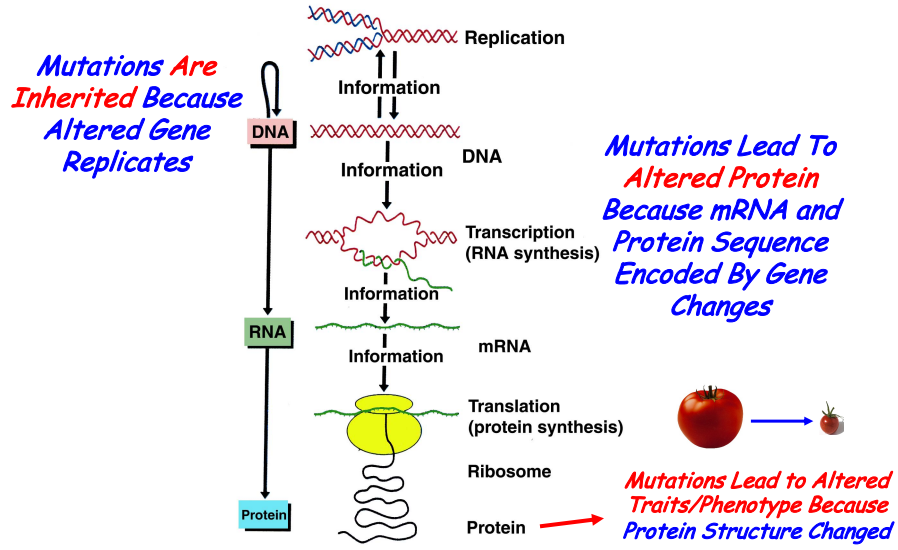
Need One Allele

Need Two Alleles

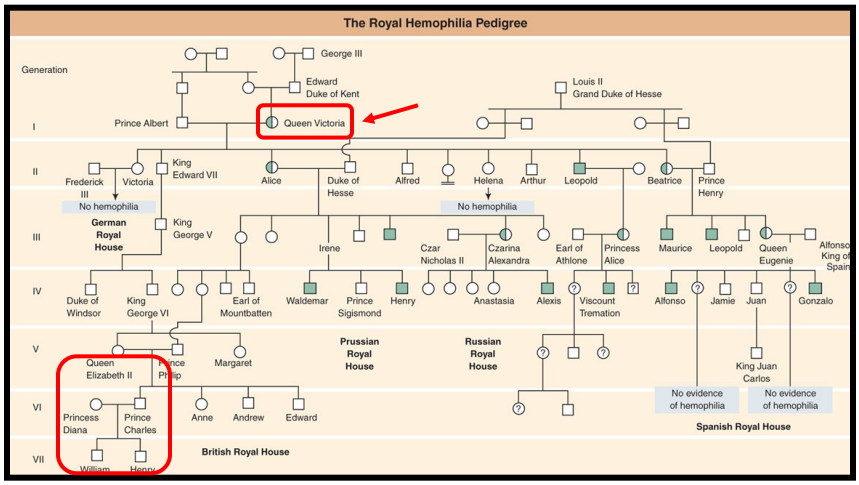
38

Translating The Genetic Code Into Proteins is a Conserved Process



39

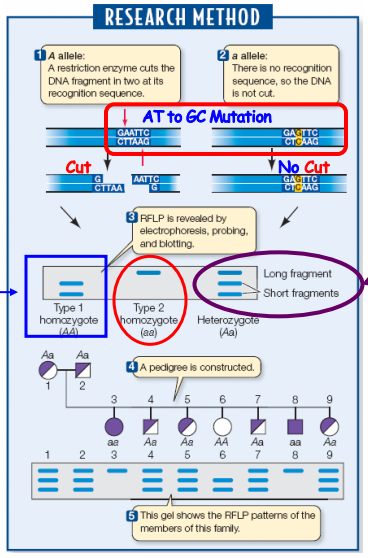
Pedigrees Can Be Used To Follow Disease Genes in Human Families



Recessive Sex Linked

40

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!

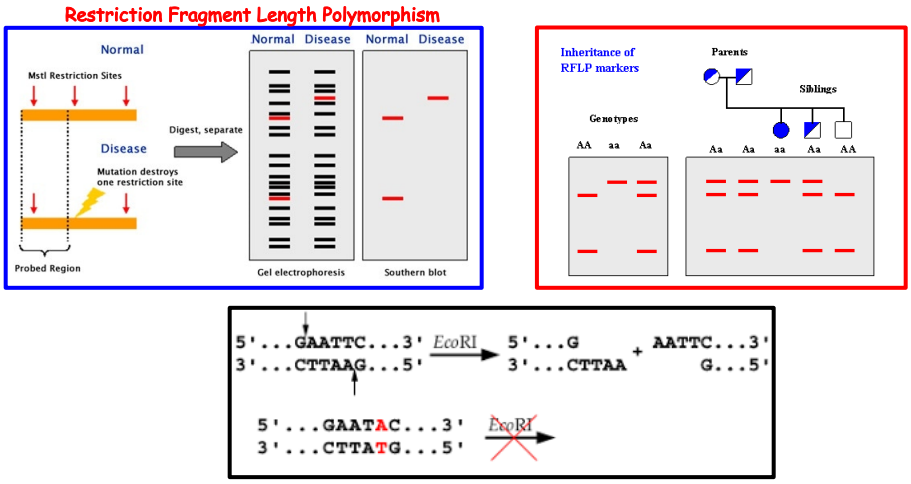
DNA Fingerprints

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

41

42

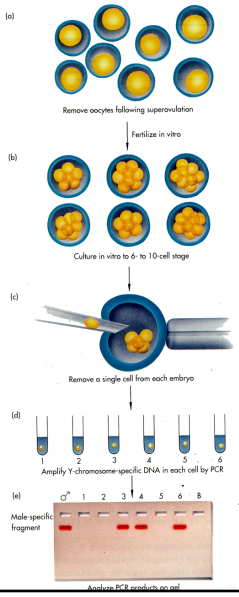
Genetic Diseases Can Be Followed in Families Using DNA Methods (e.g., PCR) & Pedigrees – With DNA Markers (RFLPs) Linked to the Disease Phenotype



43

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

PGD
Pre-Implantation Genetic Diagnosis

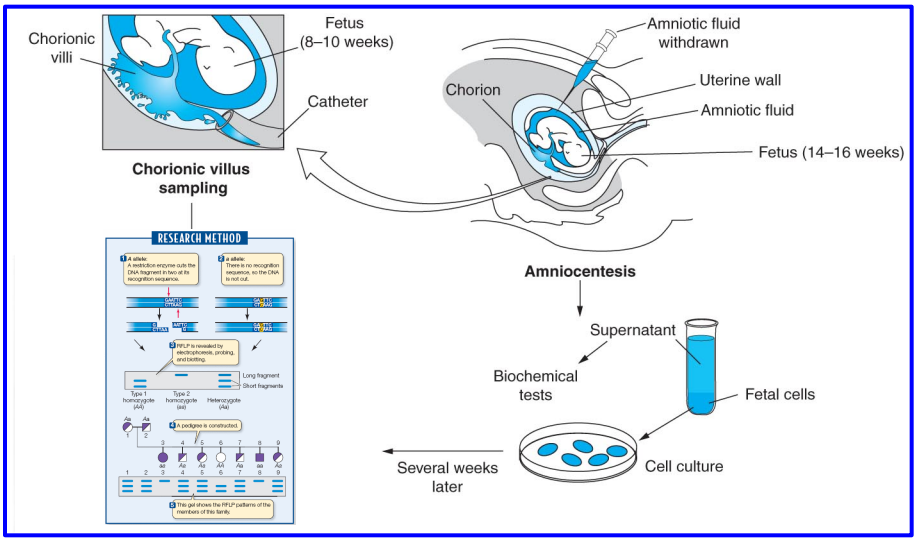


What is The Implication of This Procedure Considering That The Human Genome Has Been Sequenced?

Sex Determination in 8-cell Embryo!

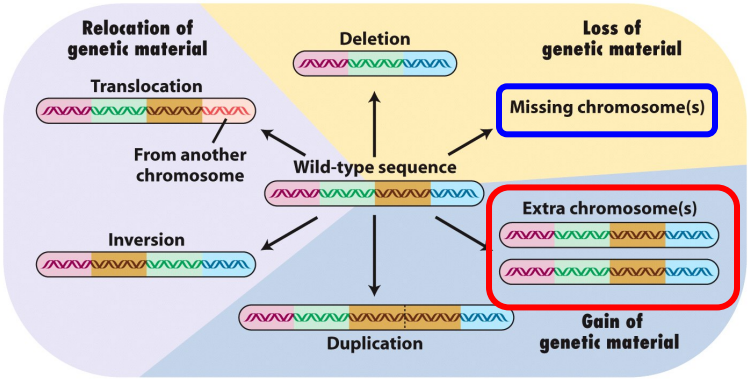
44

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



45

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

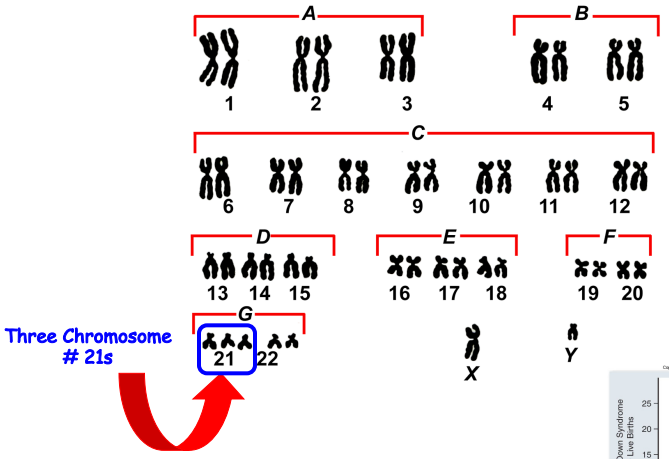


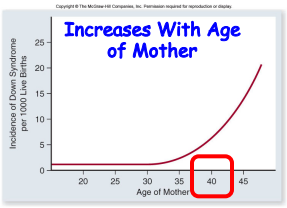
These Changes Affect Thousands of Genes and Cause Major Developmental Abnormalities

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

46

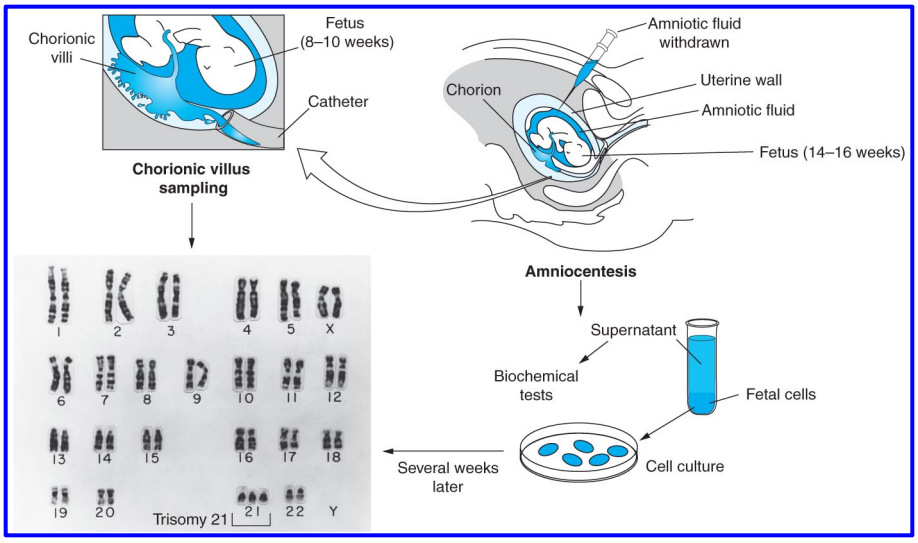
A Down's Syndrome Karyotype



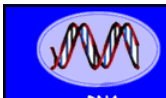


47

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



48



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

DNA Fingerprinting

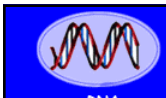
Cloning: Ethical Issues and Future Consequences

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?

49



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WHAT ARE THE PROPERTIES OF A GENE?

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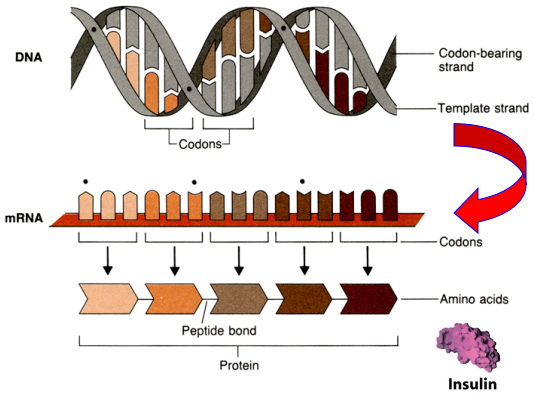
3. Universality
   a) All Cells
   b) All Organisms
4. Direct Cell Function/Phenotype

50

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

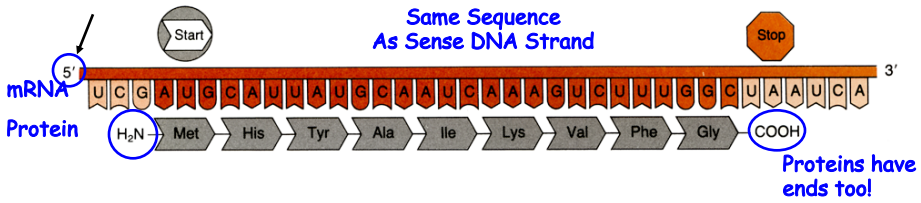
∴ Sequence of Gene
↓
Sequence of mRNA
↓
Sequence of Protein

Colinearity of Sequences!

Know Sequence
Know Protein → Engineer New Protein

51

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

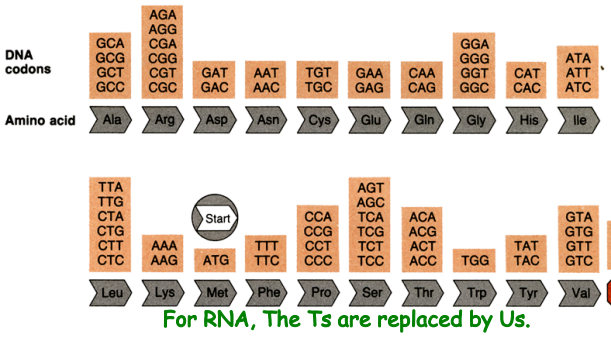


Note: Sequence in mRNA (= Sense Gene Strand) is translated 5'→3' (= beginning of sense strand to end) & protein made in N→C direction – therefore: order nucleotides in gene specifies order of amino acids in protein!

52

The Genetic Code is Universal!

How Know?



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

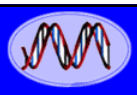
For RNA, The Ts are replaced by Us.

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!

53



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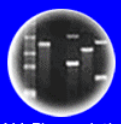


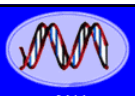
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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

54



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Plants of Tomorrow

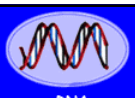
Implications For Genetic Engineering - "Its in The DNA!!"

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

Modules → Anything You Want To Do Using Genetic Engineering!

55



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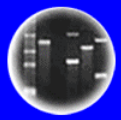

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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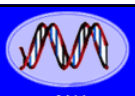
Summary: Engineering Genes Requires:

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!!*

56



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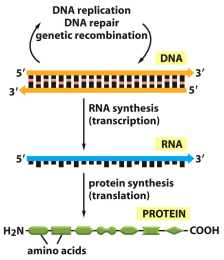
Plants of Tomorrow

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!

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

"Behind" All Traits!

Same Processes!

57