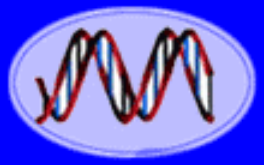
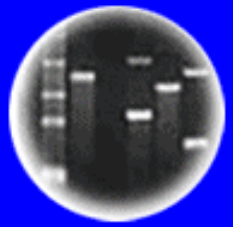


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A, PLS530, & SAS70A
Winter 2012
Genetic Engineering in Medicine, Agriculture, and Law

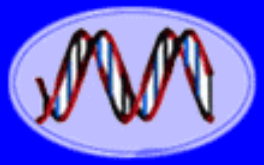
Professors Bob Goldberg, Channapatna Prakash, & John Harada

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

UCLA

TUSKEGEE UNIVERSITY

UCDAVIS
UNIVERSITY OF CALIFORNIA



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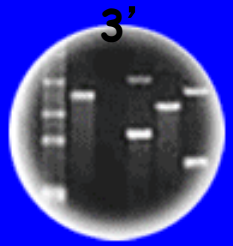

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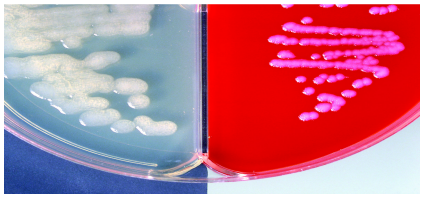
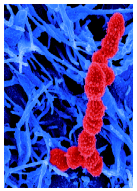
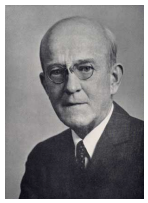
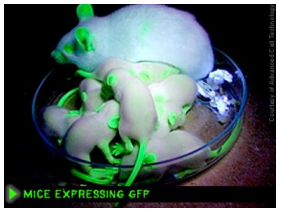
Last Tuesday's Lecture: What Are Genes & How Do They Function - Part One

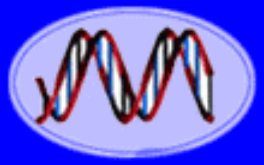
1. What Are the Properties of Genes?
   a) Replication
   b) Direct the Production of Traits
   c) Universality
   d) Stability
2. What is the Evidence For DNA Being the Genetic Material?
   a) Griffith Experiment
   b) Bacterial Cell Structure & Pneumonia
   c) Avery et al. Experment
   d) How Does the Avery Experiment Satisfy the Predictions of DNA as the Genetic Material?
3. Transformation Can Be Done Universally & Is the Foundation of Genetic Engineering
4. Film: Cutting & Splicing of DNA – Gene Origins!
5. Demonstrations
   a) Bacterial "Cloning"
   b) Gel Electrophoresis











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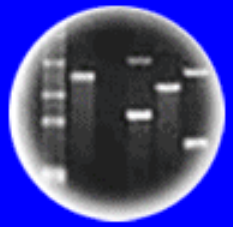

DNA Fingerprinting

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THEMES

1. What is the Function of a Gene-Review?
2. How Are Genes Regulated - Switched On & Off?
3. How Does DNA Replication Occur?
4. What is the Polymerase Chain Reaction (PCR) and How is PCR used?
5. How Do Mutations Occur?
6. How Can Pedigrees Be Used To Follow the Inheritance of Mutant Genes?
7. How Do Mutations Change Phenotypes?
8. What is the Colinearity Between Genes & Proteins (how does DNA→protein)?
9. What Is the Genetic Code?
10. How Do Gene Expression Processes Differ in Eukaryotes & Prokaryotes?
11. How Can Splicing Cause One Gene To Specify Several Different Proteins?
12. Yo!-It's in the DNA Sequences- What Are the Implications For Genetic Engineering?
13. Epigenetics – Modifications of DNA

What is A Gene?

The β-Globin Gene

Blood Protein Carries Oxygen to All Genes From Lungs ⇨ Energy

Begin 5’

Sequence or Order of Nucleotides Coding DNA Strand

```
TGAAAATCCAAAAAAATAGGA
GTTTGGTGTTTGGGTTTTAGG
TAGGAAATAATTTGGGTCTTT
TTTAGGTTTCGGGTTTGGGTT
ATTTGAGTGTTTGACATTTGA
AATTTCGGTGTTTCATCTTCG
TGGGTGTGCCAGTGGCGTGAG
TGTTCCCCGGTTTCGTCAACT
TACGGTTTAGGGTTTACCAAG
TTAGGGTTTAGGGTTTGAGAT
GGCGGCCATTTCTCATGTTTG
AAACAAAGCCTGAAAATCAAA
TGGGTGTGCCGGTGGCGTGAG
CGTTCCCCGGTTCCGTCAACT
ATCAAGTACCCATGTTTGGGA
TGAACGTCAATGAACACGAAA
AAAAAAATAGGAAATCGACCC
AGAAAAGGGAGGGTGGCCATT
ACTATCACGTAACAACAAAAC
ATTTTTTTGCGTGGGTGTGCC
ATAAATAGATTTTTCCCTTGT
CCTTTTCCATGTTCAAGTACC
TTTCTCATGTTTTGAAGTCAA
CCTGAAAAATCCAAAAAAAATAG
CAGTGGCGTGAGACATTGGAG
GATACGTCAACTAACACGTAA
CATGTTTGGGATTTTTTTCCG
AGAACCCAAAAAAAATAGTCT
GAAATCGACCCTTTTCCATGT
GGGCAGCCATTTCTCTTGTTT
AAAACAAAGCCTGAATATCTA
GTGAGTGTGCCAGTGGCGTGA
TCGTTCCCCGGTTCCTTCAAC
GTTCAAGTACCCATGTTTGGG
TTGGACGTCAAAGAAACCAAA
CAAAAAAATAGGAAATCGACC
AGAAAATGGAGGGCGGCCAAT
CTGACACGTAAAAACAAAGCT
TTTTTTCGCGTGGGTGTGCCA
AAAATAGTCCCGTTCCCCGTT
TTTTCCATGTTCAATTACCCA
TCTCATATTTGGACGTCAAAG
```

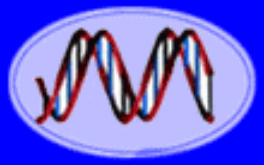
End 3’

A Gene is a <u>Unique Sequence</u> of Nucleotides Specifying a Function

DNA Sequence = Biology!
What If Sequence Changed?

SEQUENCE → FUNCTION

Relative to Coding or Sense Strand of Gene



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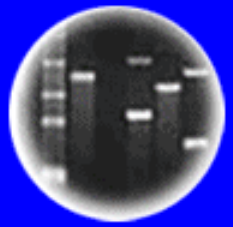

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Genes & Genomes Differ Because the Sequence of DNA Differs

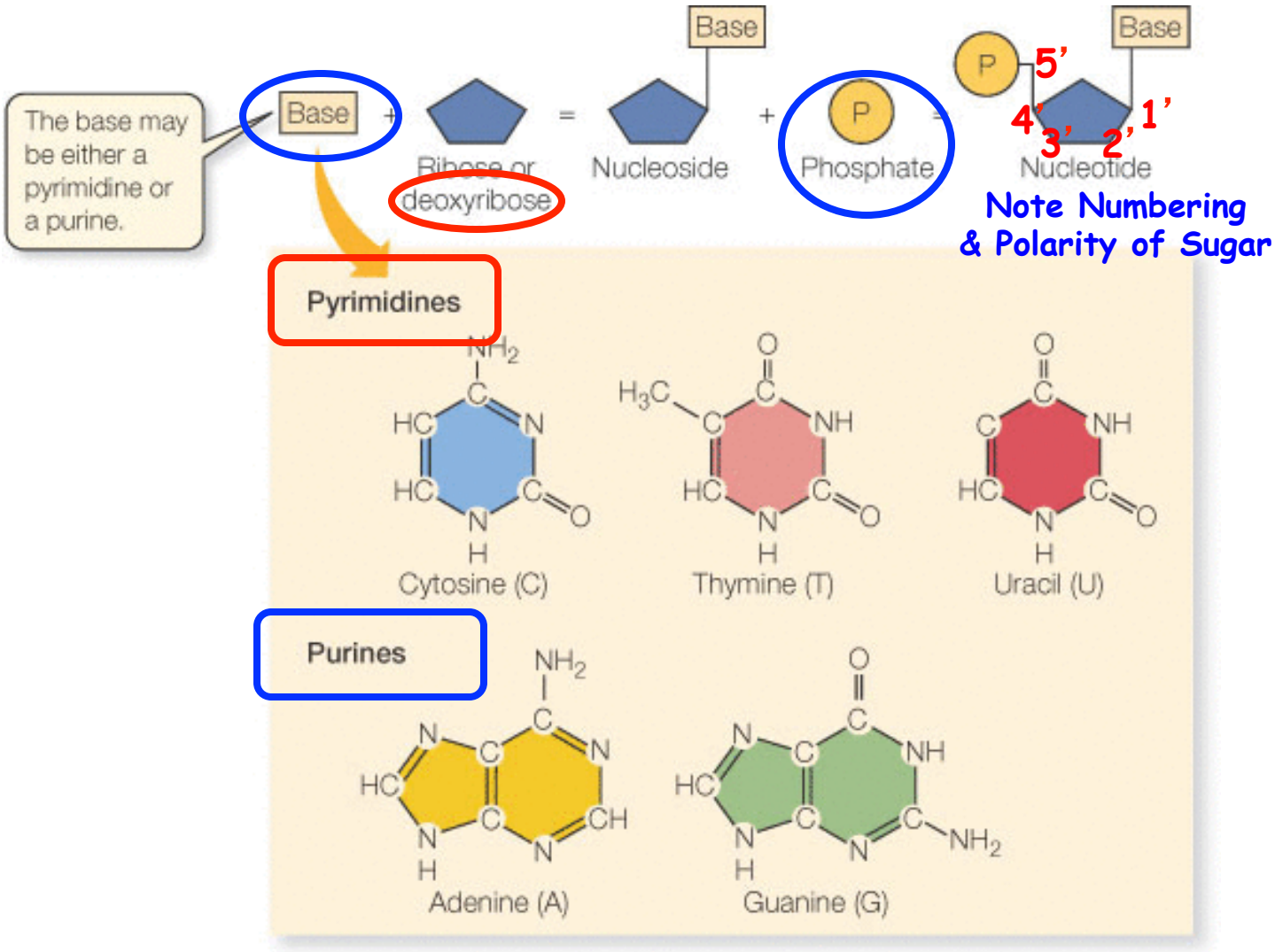
DNA Sequence
Beginning → End
5' 3'

→ Biological Uniqueness

If You Know the DNA Sequence, You Can Engineer Anything! Even Make New Genes & Genome!

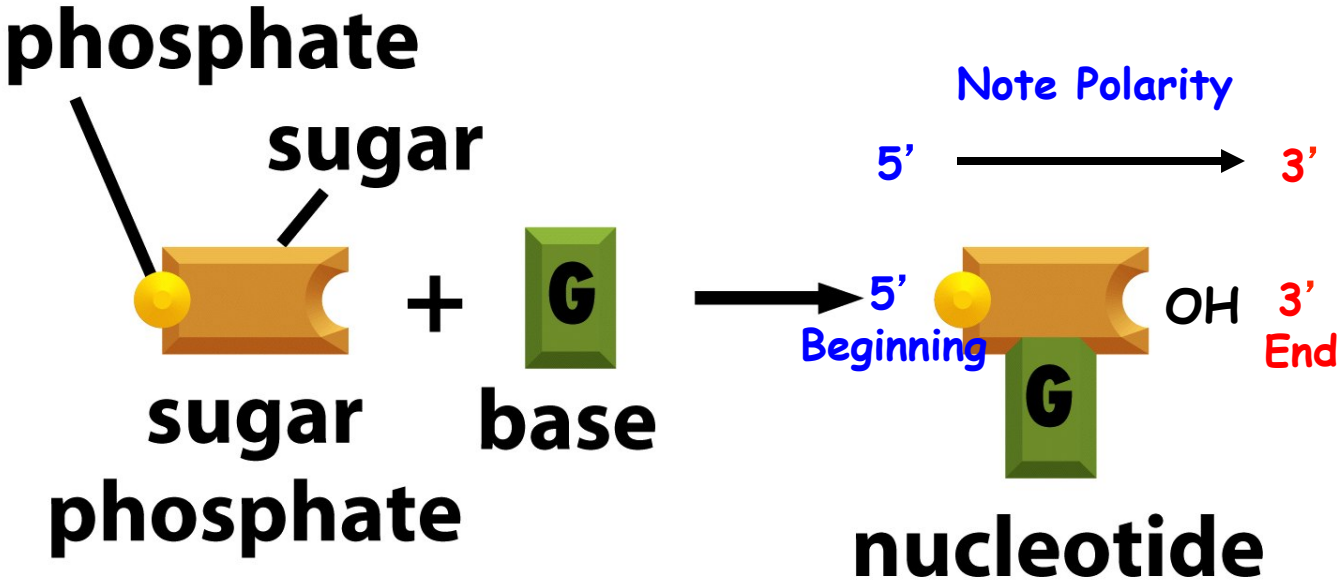
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Genes Have Four Different Nucleotides



Note Chemical Differences in Bases -- Chemistry Leads to Biology!!

Nucleotides Have Polarity
Based on What is Bonded to the Five-Carbon Sugar
Phosphate on 5' Carbon and OH on 3' Carbon



The Sugar is the HUB

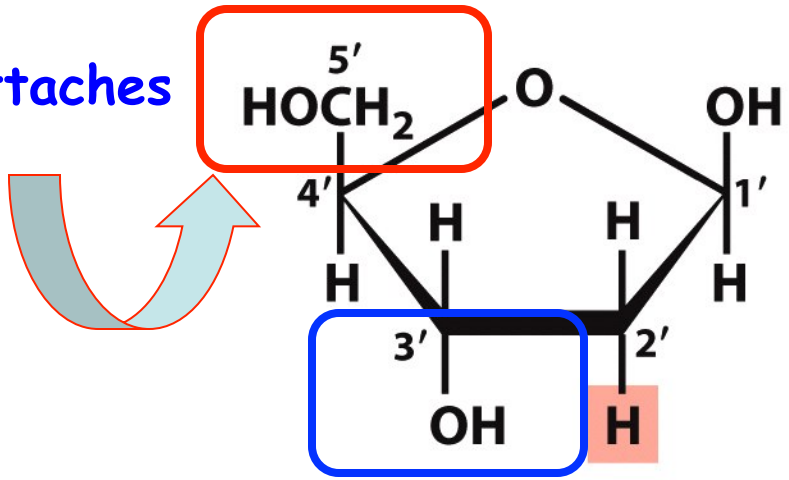
DNA Sequence Defined By Nucleotide Order

DNA Sequence = Functional Uniqueness = Biology

Figure 1-2a *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

Note Structure and Polarity of Deoxyribose Sugar

Phosphate Attaches



2-Deoxyribose

Nucleotides Are Joined By 5' to 3' Phosphodiester Bonds

5'

C A G

ORDER

3'

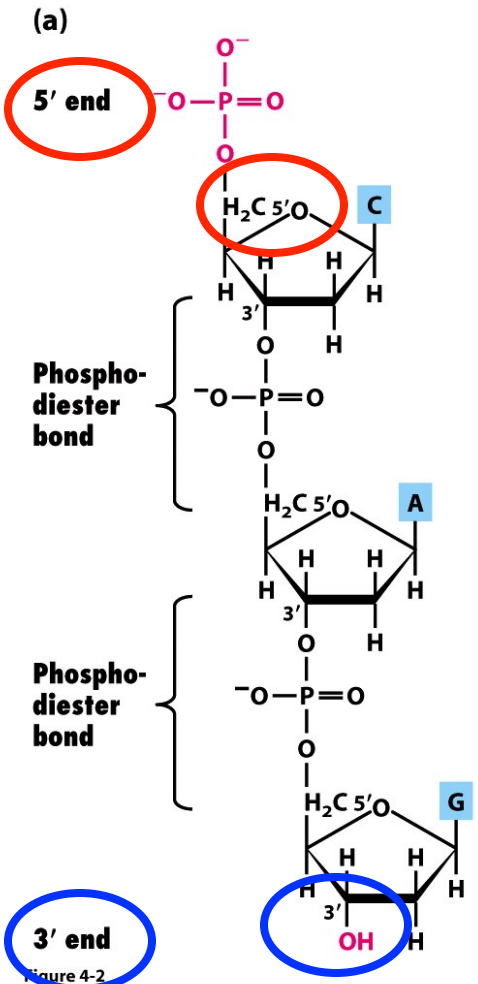
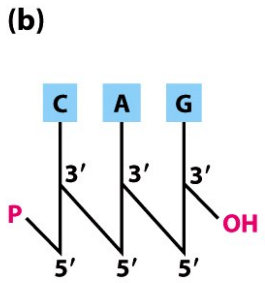


Figure 4-2
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company



Short-Hand Notation

5′ C-A-G 3′

1. The Order is Specified by the Nucleotides That Join 5' to 3'
2. This is the Basis For All of Biology
3. Order is Maintained During DNA Replication
4. Basis of All Genetic Engineering

Polarity Defined By Sugars & Order Specified By Bases

Clues to the Double Helix-Chargaff's Rules
Purines = Pyrimidines

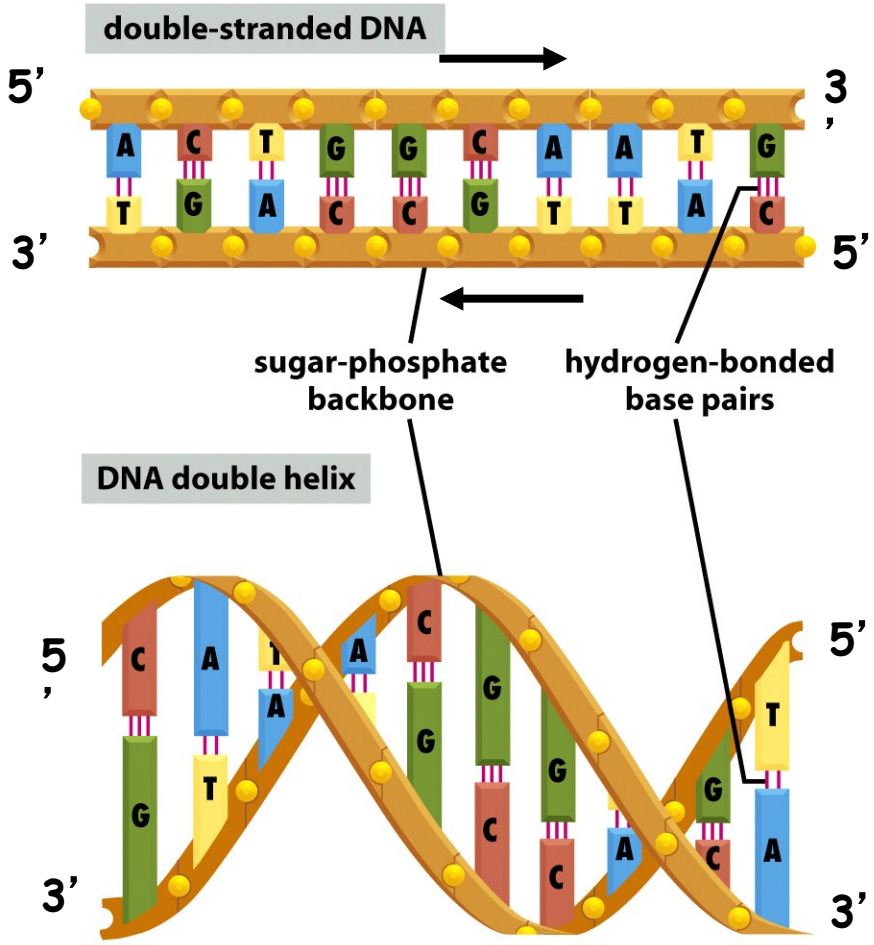
TABLE 6.1 Chargaff's Data on Nucleotide Base Composition in the DNA of Various Organisms

	Percentage of Base in DNA				Ratios	
Organism	*A*	*T*	*G*	*C*	*A:T*	*G:C*
Staphylococcus afermentams	12.8	12.9	36.9	37.5	0.99	0.99
Escherichia coli	26.0	23.9	24.9	25.2	1.09	0.99
Yeast	31.3	32.9	18.7	17.1	0.95	1.09
*Caenorhabditis elegans**	31.2	29.1	19.3	20.5	1.07	0.96
*Arabadopsis thaliana**	29.1	29.7	20.5	20.7	0.98	0.99
Drosophila melanogaster	27.3	27.6	22.5	22.5	0.99	1.00
Honeybee	34.4	33.0	16.2	16.4	1.04	0.99
Mus musculus (mouse)	29.2	29.4	21.7	19.7	0.99	1.10
Human (liver)	30.7	31.2	19.3	18.8	0.98	1.03

*Data for *C. elegans* and *A. thaliana* are based on those for close relative organisms.
Note that even though the level of any one nucleotide is different in different organisms, the amount of A always approximately equals the amount of T, and the level of G is always similar to that of C. Moreover, as you can calculate for yourself, the total amount of purines (A plus G) nearly always equals the total amount of pyrimidines (C plus T).

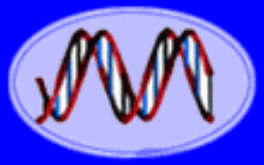
What Would You Predict For a Single-Stranded DNA?

DNA is a Double Helix of Two Complementary Chains of DNA Wound Around Each Other



Watson and Crick, Nature, 1953

1. Complementary Strands
2. A=T and G=C (Four Bases)
3. Sequence of Strands Differ
4. Bases to Interior
5. Phosphate-Sugar Backbone on Exterior
6. DNA Strands in Opposite Direction (Only Way Helix Fits)
7. Sequence of One Chain Automatically Specifies Sequence of Complementary Chain (Basis of Replication!)
8. No Constraint on Sequence (4^n=n # sequences)
9. DNA has dimensions (Know # bp Know Length: 20Å diameter, 3.4Å/bp, 10bp/turn)
10. Sequence = Biology



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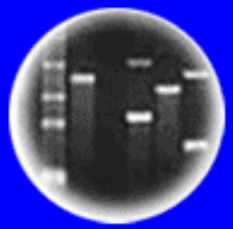

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Sciencexpress Research Article

A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus Science, December 2, 2010

December 2, 2010

Subsisting on Arsenic, a Microbe May Redefine Life

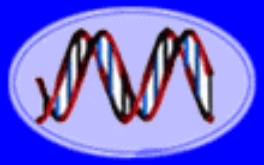
By DENNIS OVERBYE New York Times, December 2, 2010

The newfound bacteria thrives in the arsenic-rich waters of Mono Lake in California.

33
As

15
P

33
As



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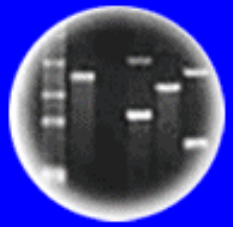

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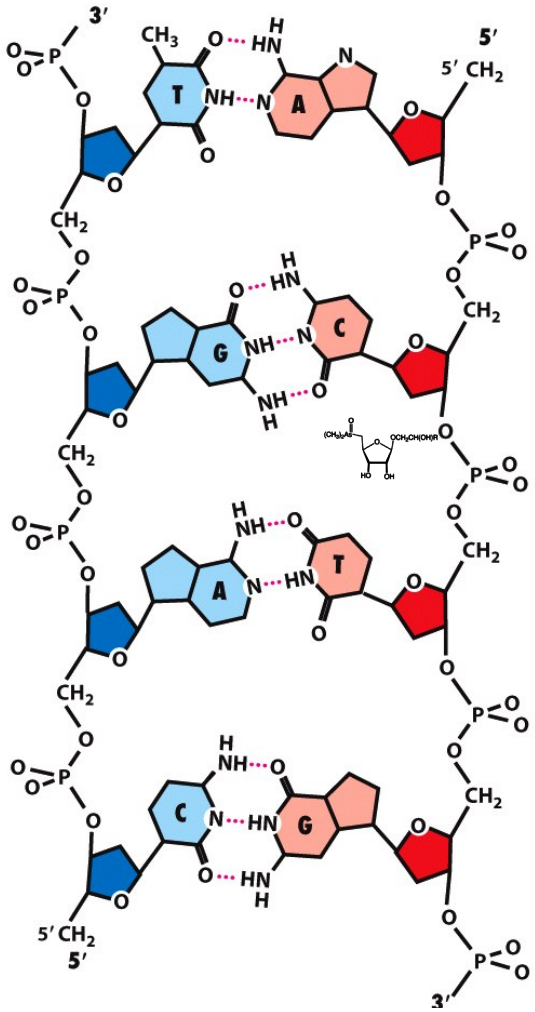
The Mysterious Case of Arsenic DNA

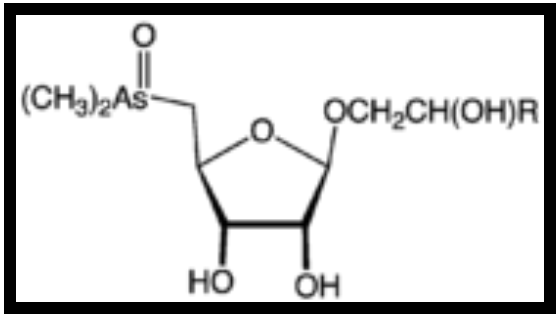


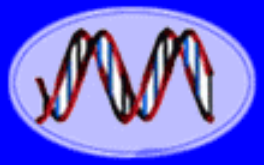
Arsenic

sugar

sugar + G

sugar base





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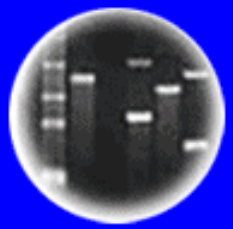

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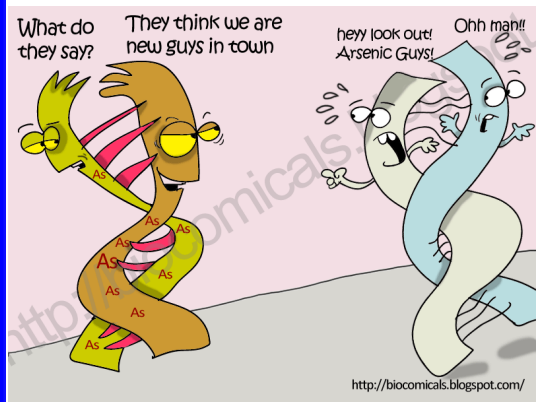
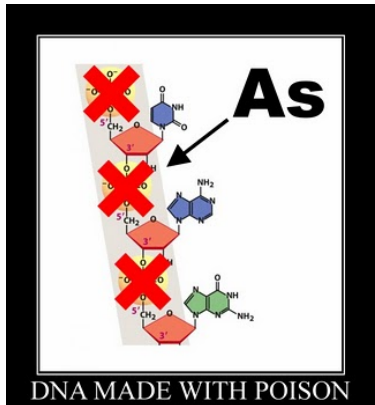
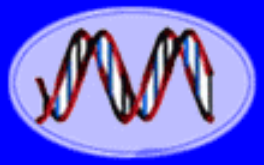
Does Growing Bacteria on Arsenic and Showing that Radioactive Arsenic is Associated With a DNA Fraction of the Cell Demonstrate Unambiguously That the Nucleotides in the Double Helix are Bound Together by Bonds Containing Arsenic?

a. Yes
b. no







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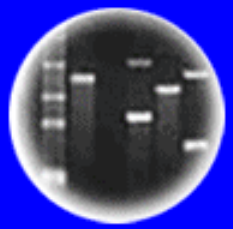

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No Arsenic in Arsenic Bacteria's Genome!!

'Arseniclife' bug lacks arsenic in genome's DNA

By Dan Vergano, USA TODAY

Updated 12/2/2011 5:49 PM

Halamonas sp. 3.5 Mb and 3500 genes, Phung et al., November 30, 2011

Reprints & Permissions

Genome of Controversial Arsenic Bacterium Sequenced

by Elizabeth Pennisi on 5 December 2011, 5:42 PM | 9 Comments

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Our data show evidence for arsenate in macromolecules that normally contain phosphate, most notably nucleic acids and proteins.

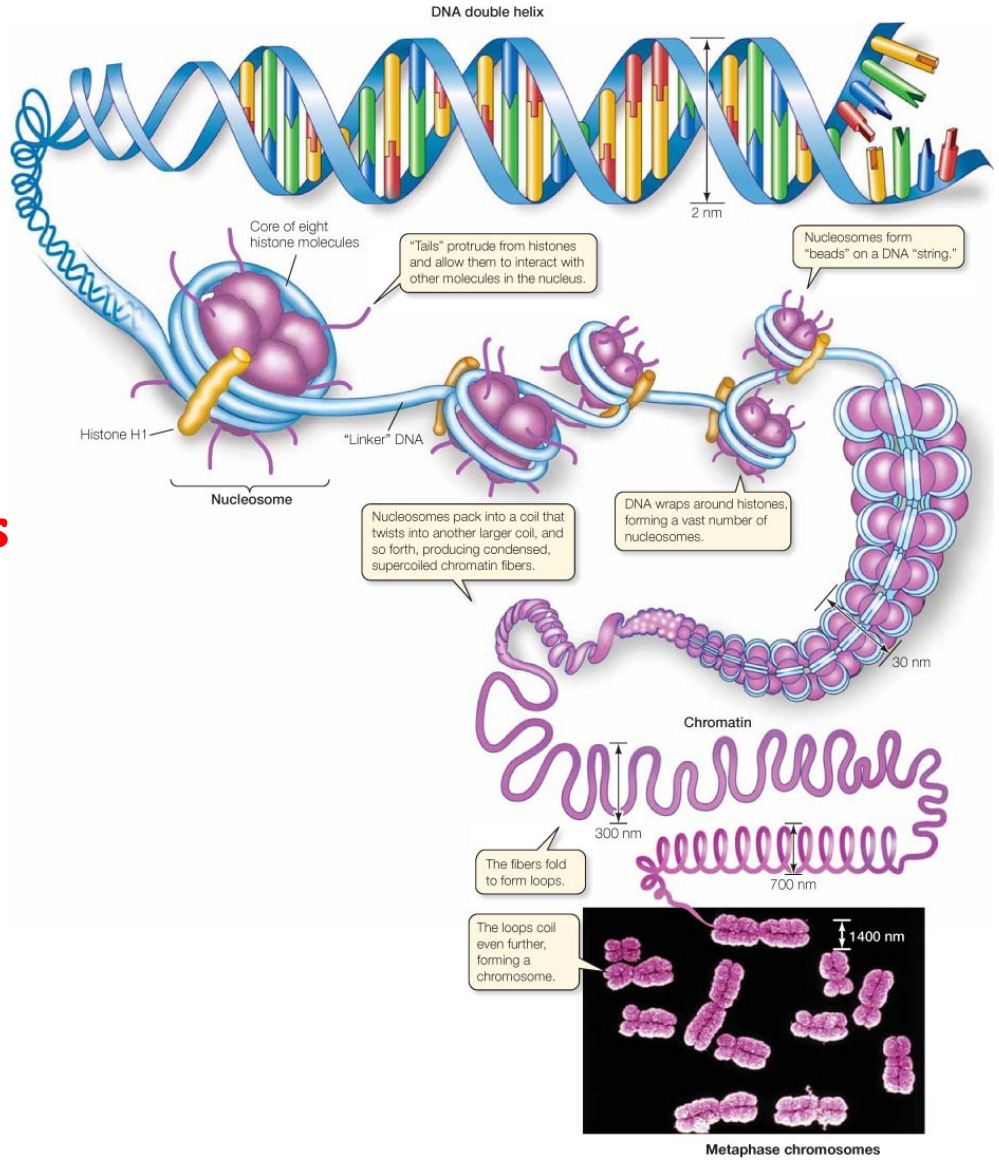
One year ago those 18 words ignited quite a media controversy when Felisa Wolfe-Simon and her colleagues held a press conference to announce the discovery of a bacterium that not only survived high levels of arsenic in its environment but also seemed to use that element in its DNA. Five months later, the debate resurfaced with the publication of critical comments on the original research.

Last week, the genome of the bacterium, known as GFAJ-1, was posted in Genbank, the public repository of DNA sequences for all who care to take a look. But it doesn't settle the debate over whether arsenic is used in DNA.

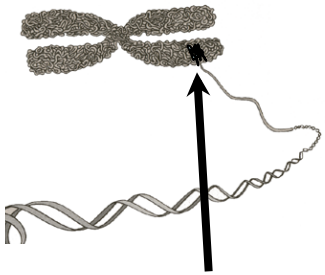
A Chromosome Contains One (or Two!!) Continuous DNA Molecule(s)

DNA in Human & Eukaryotic Chromosomes is Linear and Wrapped Around Proteins Called Histones!

DNA in Most Bacteria is Circular!



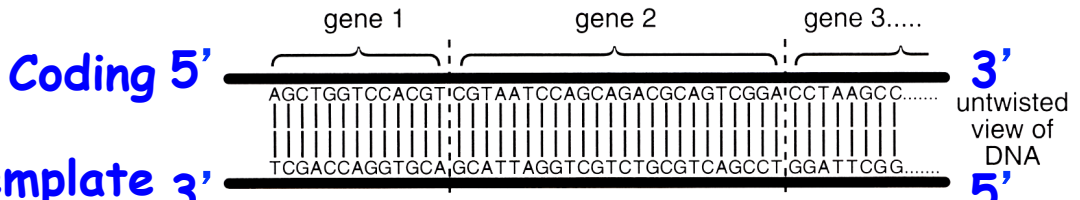
A Chromosome Contains Many Genes That Work As Individual Units (How Know?)



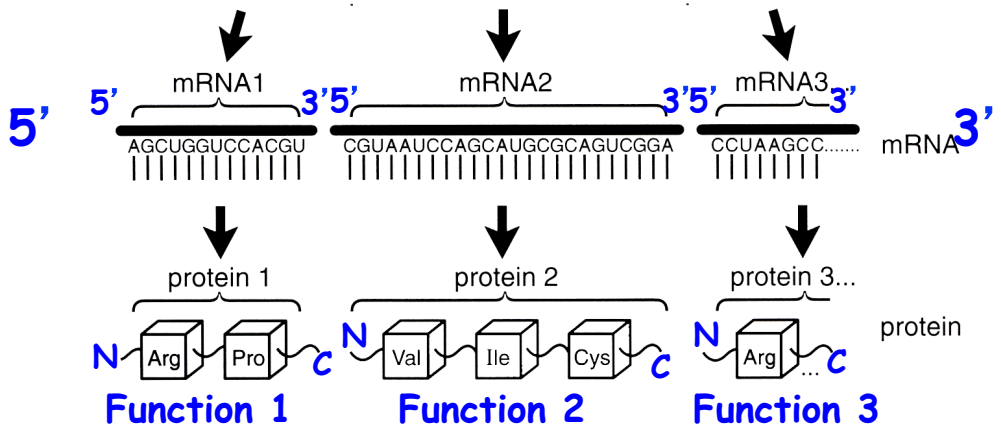
Position of Genes 1, 2, & 3 in chromosome

Discrete Units! Evidence?

What delineates each gene?



Notice sequence of each gene



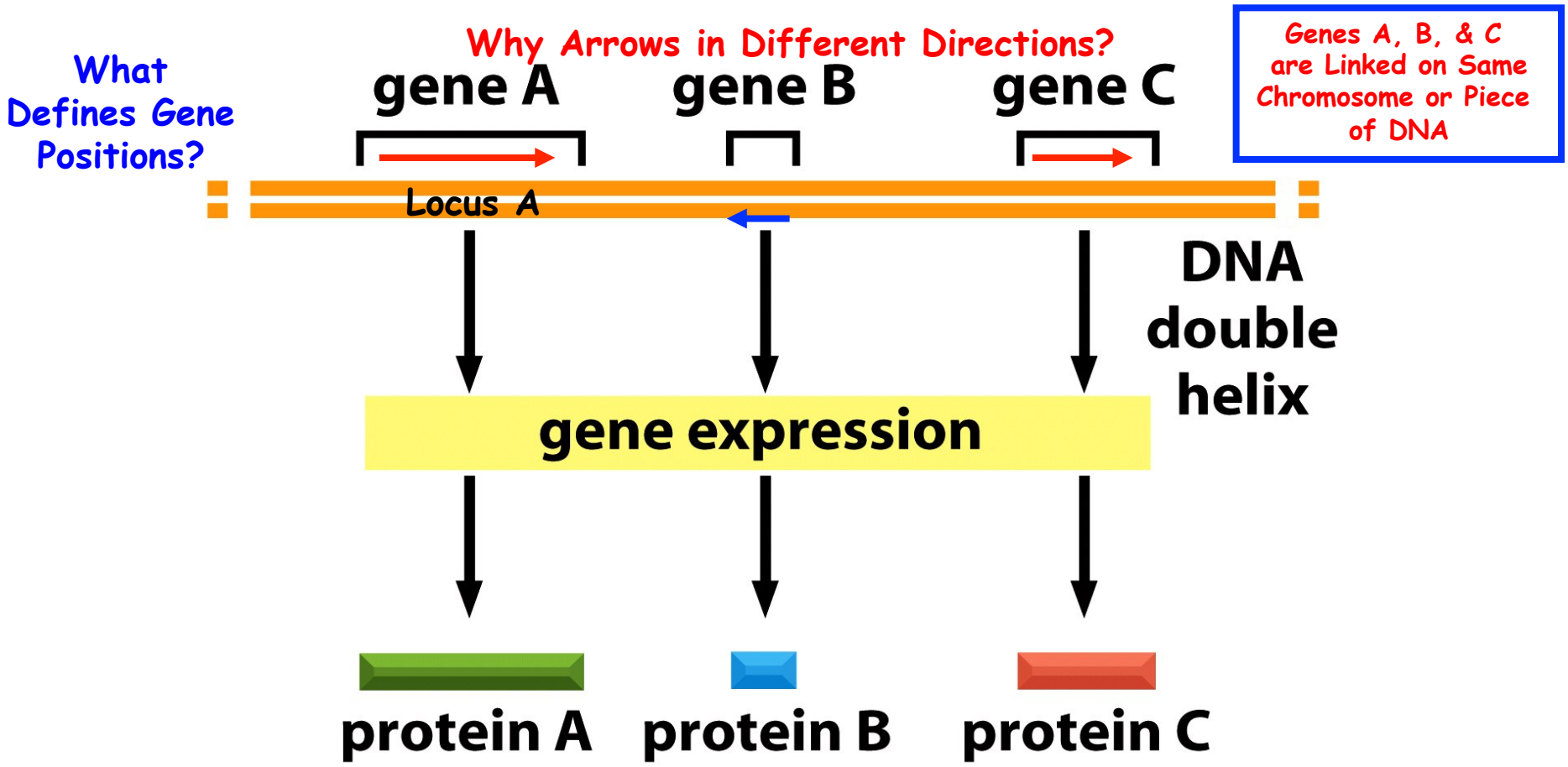
Note sequence of each protein

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

VERY IMPORTANT CONCEPT!
COLINEARITY BETWEEN GENE SEQUENCE AND PROTEIN SEQUENCE

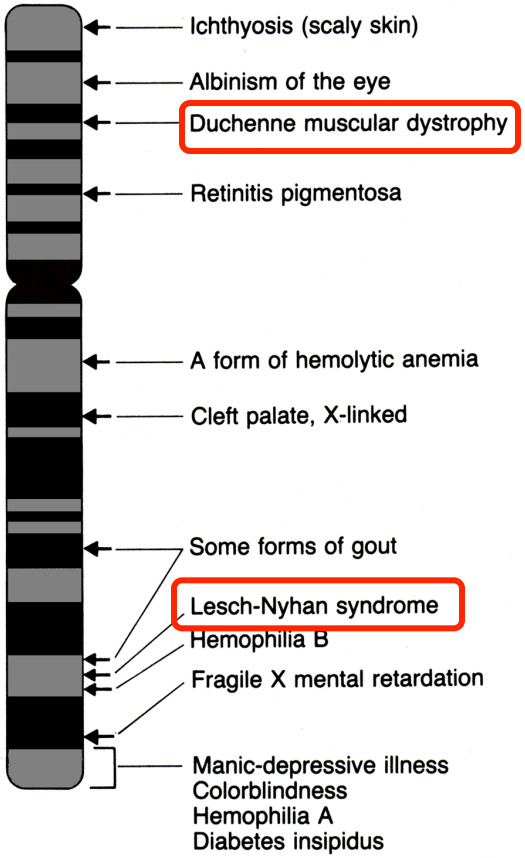
A Chromosome Contains Many Genes That Reside at Specific Positions (Loci) and Have Unique Functions



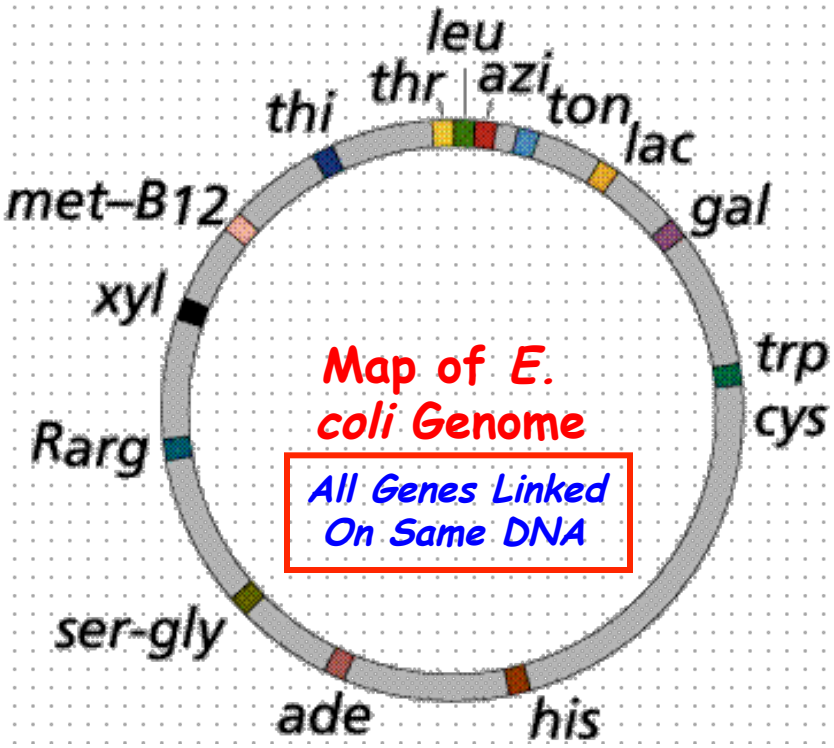
Because DNA Contains Two Strands--Genes Can Be Transcribed From Either Strand--But Only One Per Gene

Genes Reside at Specific Locations

All Genes Linked on Same Chromosome or Piece of DNA



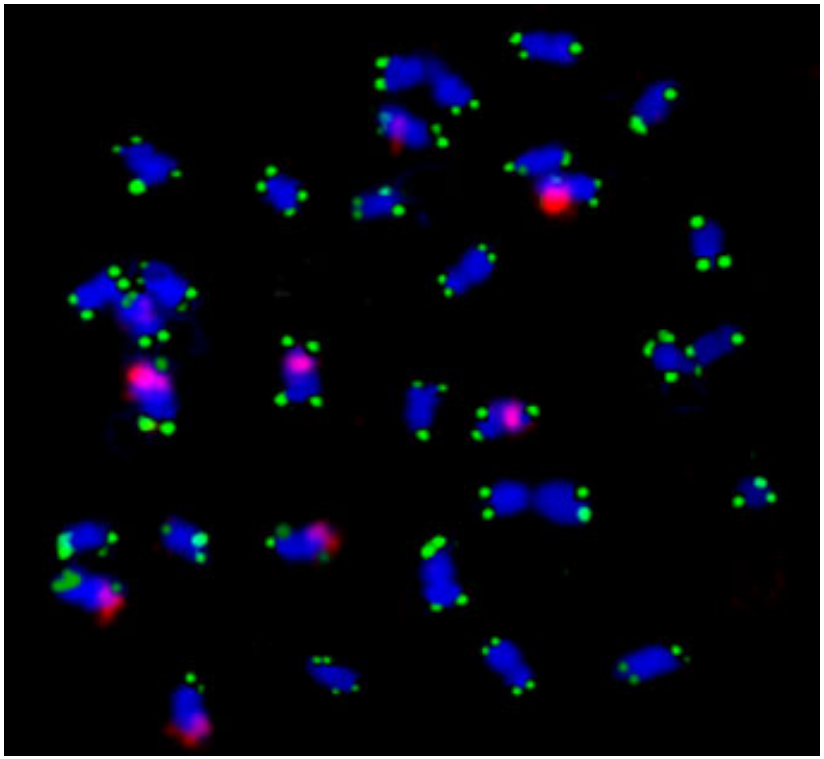
Linear DNA
How Know?



Circular DNA
How Know?

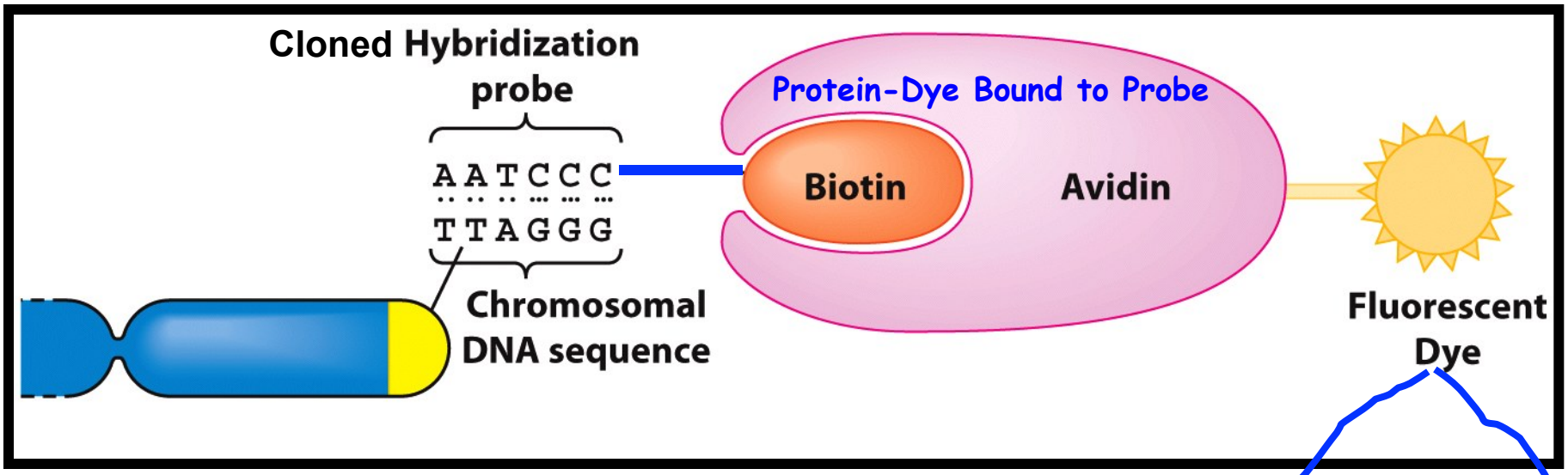
- Note Marker Bands - What are these?
- How Know Gene Positions? Chromosome Number?

Genes Reside at Specific Positions, or Loci, That Can Be Mapped and Visualized

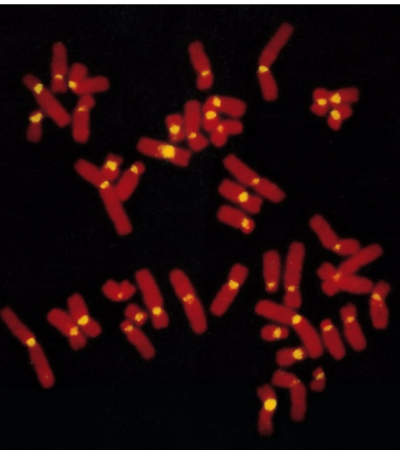


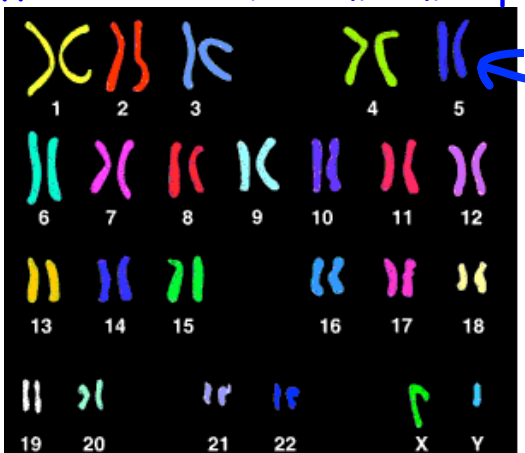
Gene Position = Locus = Unique DNA Sequence

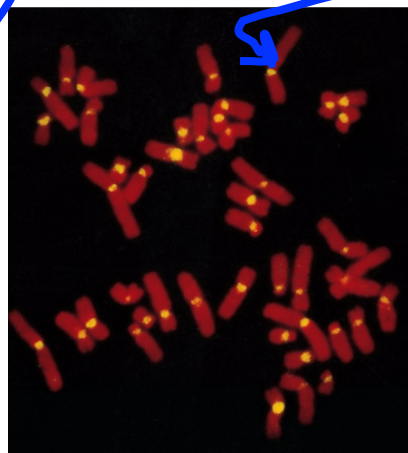
Visualization of Specific Gene Loci Using Fluorescence In Situ Hybridization (FISH)



Dyes of Different Colors & Chromosome-Specific Probes



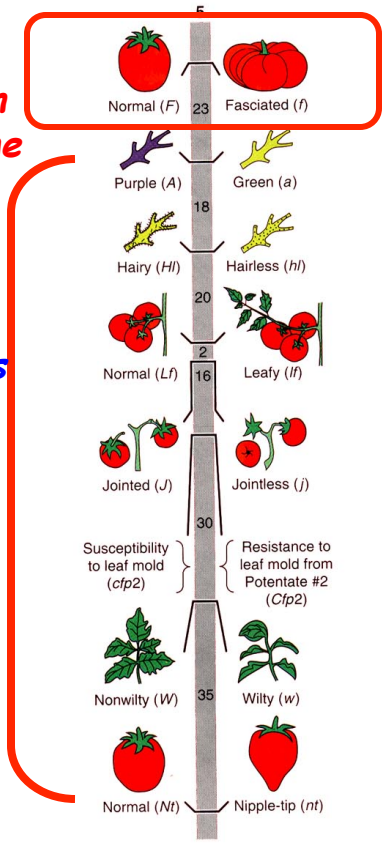


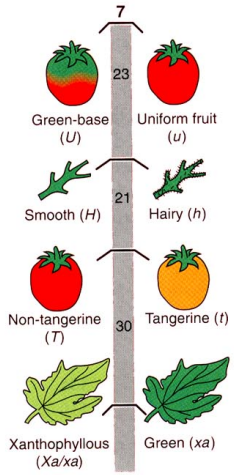


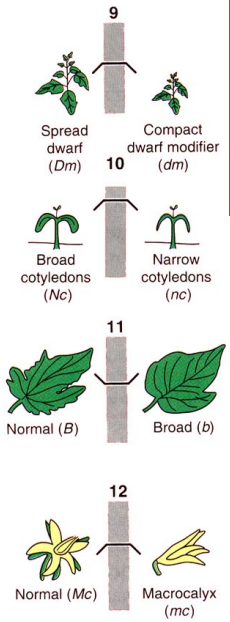
Alleles Reside at the Same Position on a Chromosome

Different Alleles at Same Position on Chromosome

Different Genes All Linked on One Chromosome







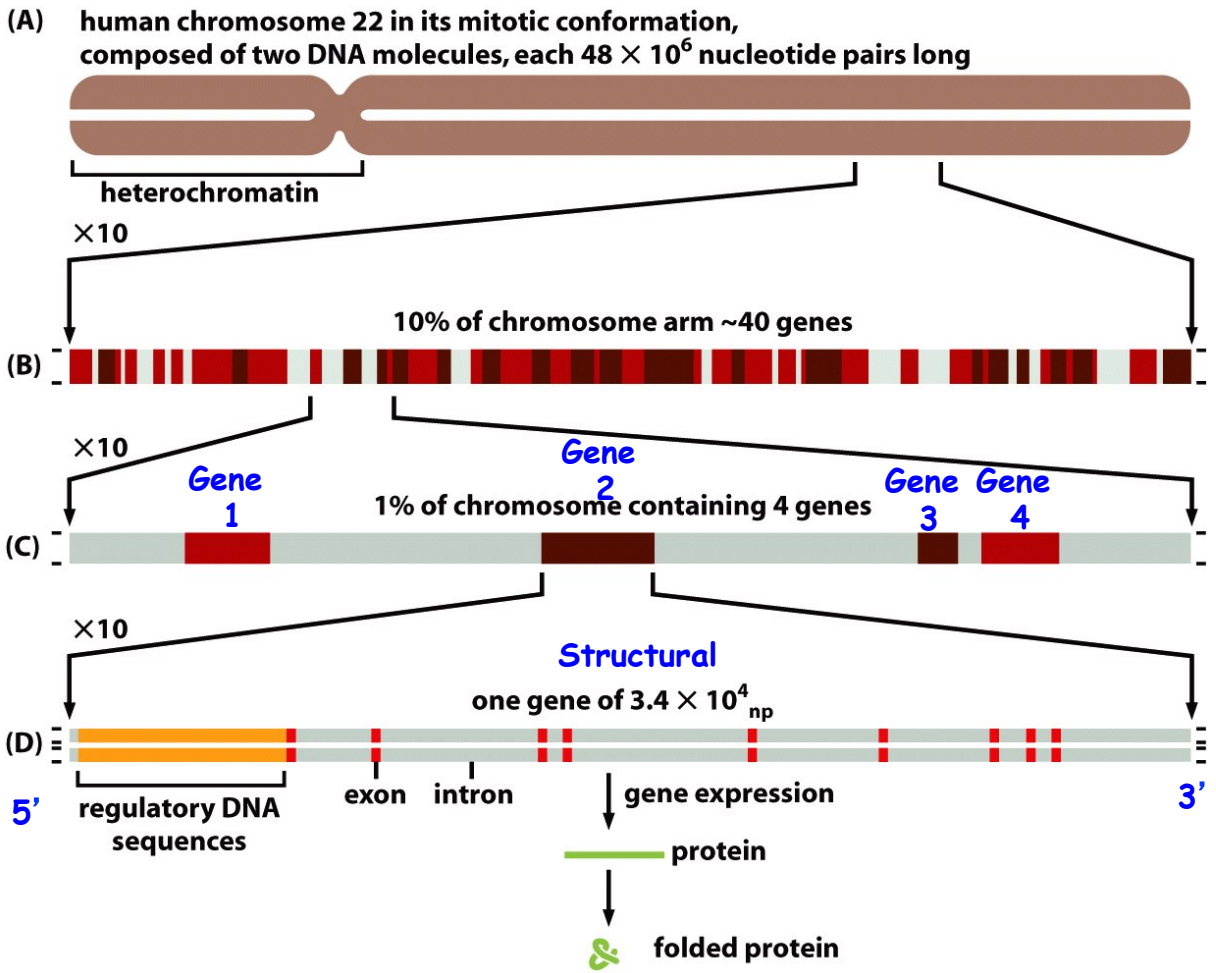
Gene Engineering Can Generate New Forms of Alleles of a Gene and, therefore, Results in More Genetic Diversity

Each Phenotype is a MARKER for the Allele & It's Position on the Chromosome Genetically!

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!

Organization of Genes on Human Chromosome 22



Genes Are Defined/ Precise Regions of DNA

One Large Gene!

Genes Act As Individual Units?
How Know? Design an Experiment!!

Figure 4-15 *Molecular Biology of the Cell* (© Garland Science 2008)

A Conceptualized Gene

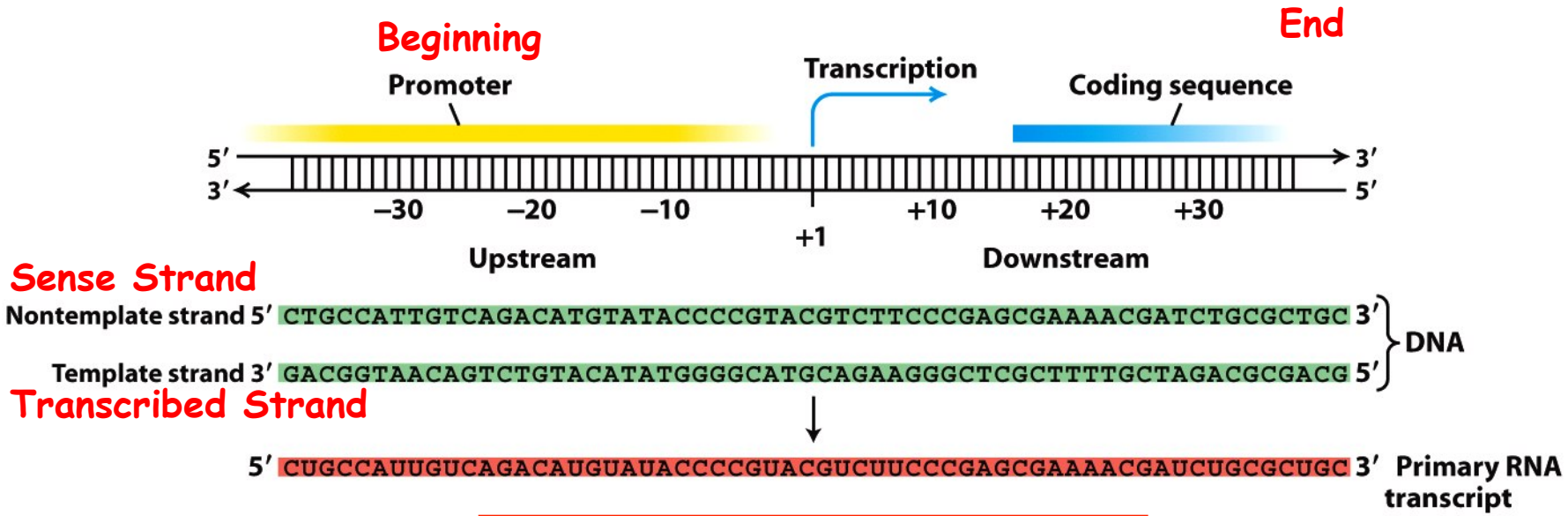
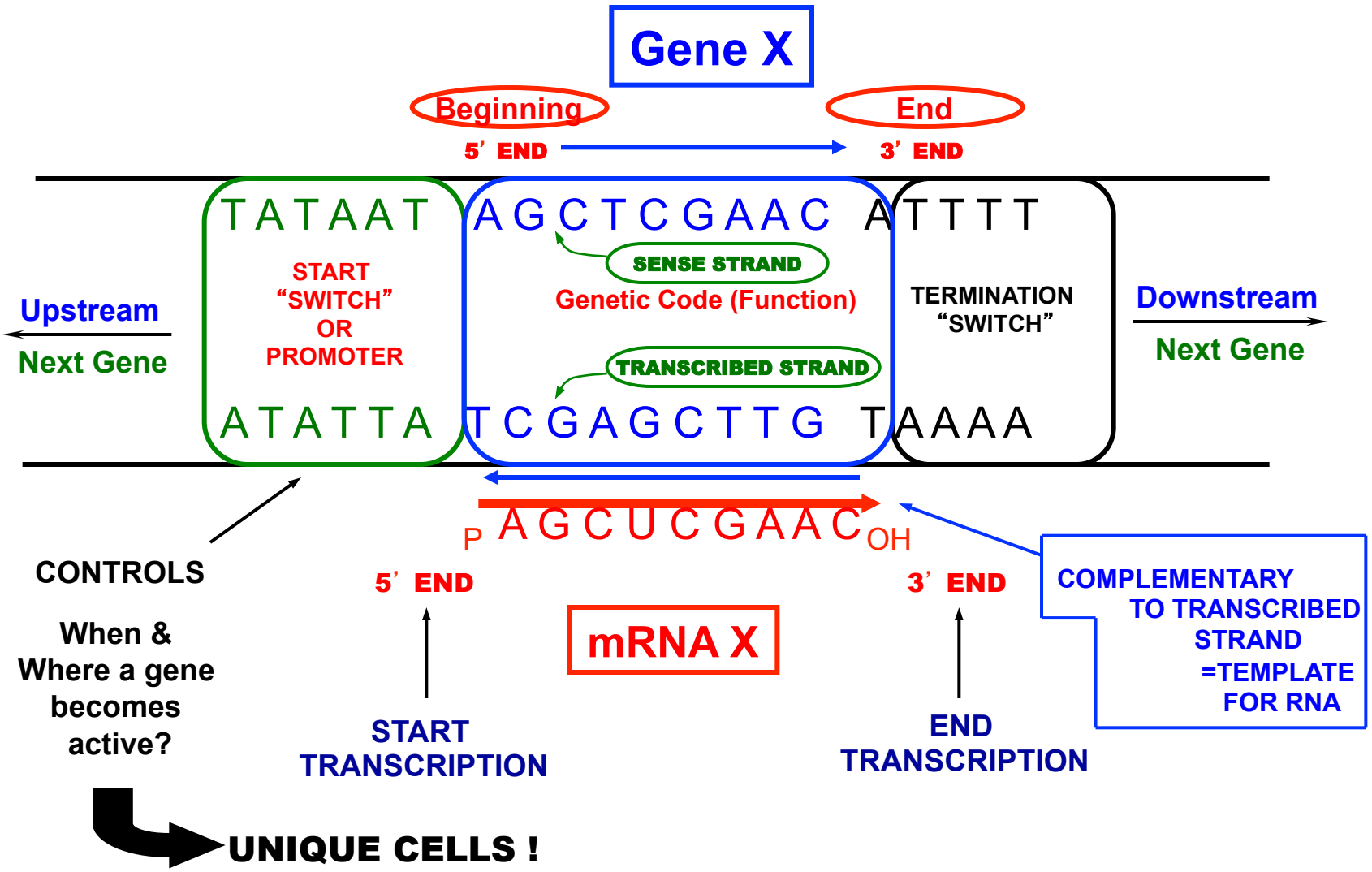


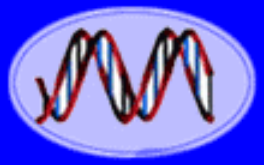
Figure 4-10b
Molecular Cell Biology, Sixth Edition

Complementary to Transcribed Strand
or SAME Sequence
as Sense Strand

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

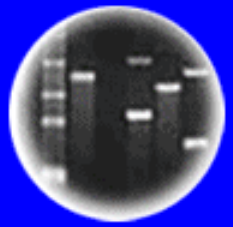


Note: mRNA Sequence = Sense Strand Sequence



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

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A "Simple" Gene Reviewed

1. Sense Strand = Genetic Code
2. Sense Strand = 5' → 3' Direction (all DNA sequences specified 5' → 3')
3. AntiSense Strand = Complement of Sense Strand & is Transcribed Strand
4. mRNA = Same Sequence As Sense Strand & Complementary to Antisense Strand
5. mRNA = 5' → 3'
6. Switch Turns Gene On - Not Transcribed But Upstream of Coding Region

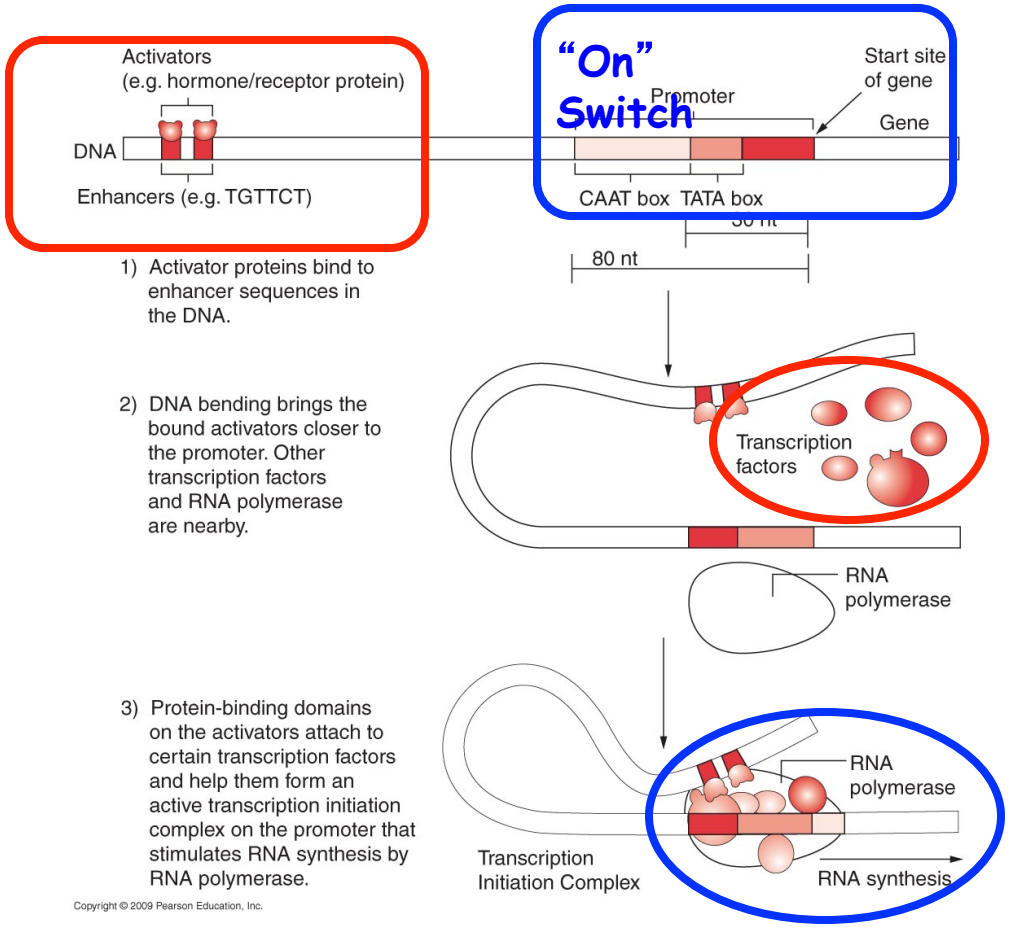
Genes Function As Independent Units -
Design Experiment to Show!

"Everything" Follows the Double Helix & Its Rules -
Anti-parallel Chains & Complementary Base Pairing!

Control Switches Are Unique DNA Sequences & Can Be Cloned

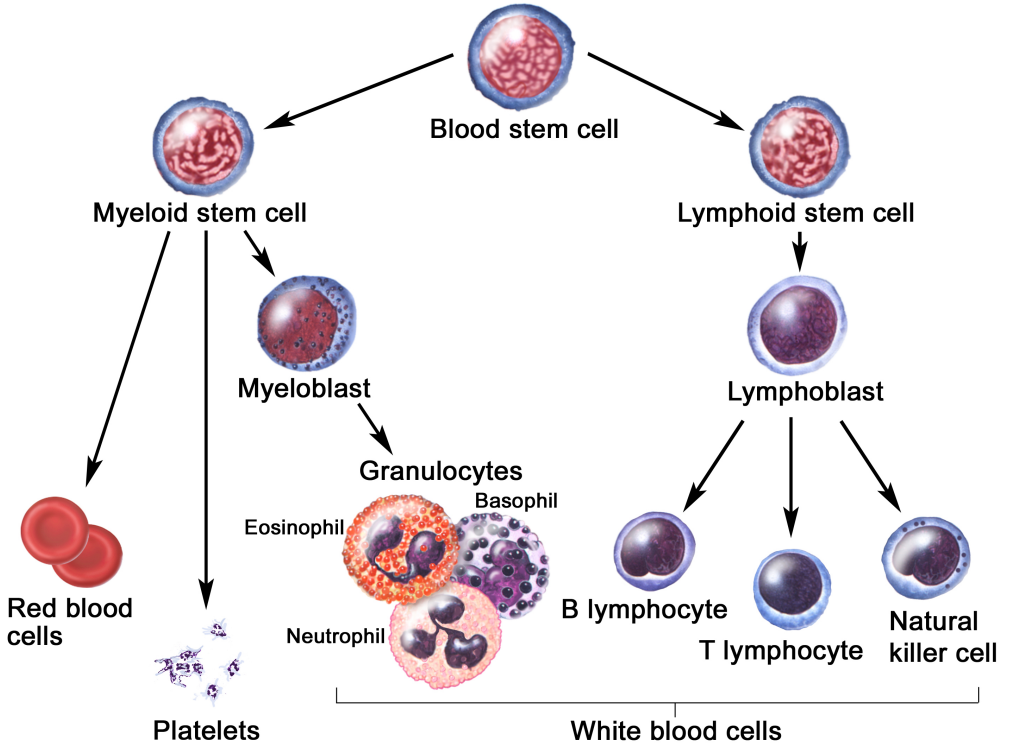
AND used to Re-Engineer Organisms!! Switches Act Independently of Gene!!

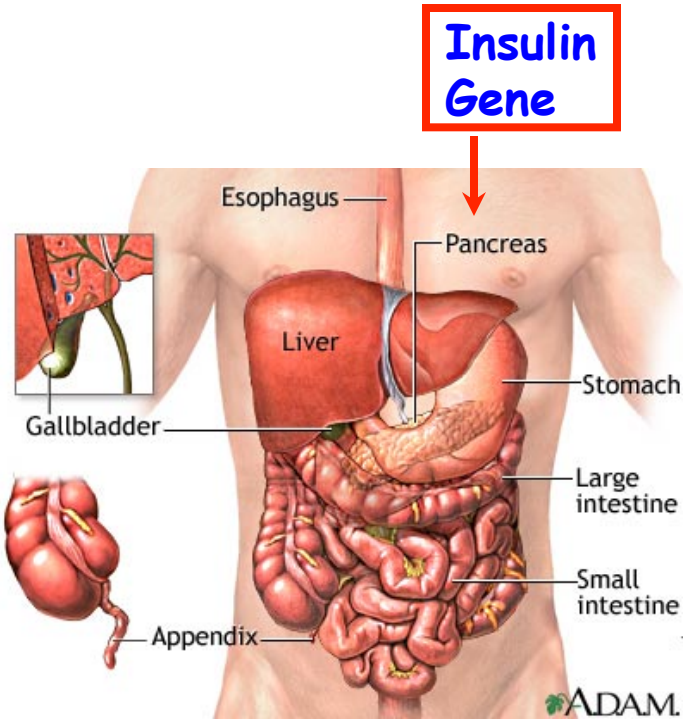
"Control" Switch

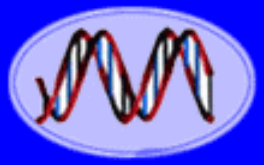


1. Each Switch Has a Unique DNA Sequence
2. Genome Projects Reveal Genes & Logic Controlled by the Switches
3. Sequence = Biology
4. No Hocus Pocus
5. Yo! It's in the DNA!!

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

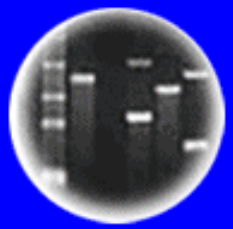






DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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THE GENE AND SWITCHES ARE UNIQUE DNA SEQUENCES

1. They Can Be Cloned & "Shuffled" & Engineered Creating New Genes That Have No Counterparts in Nature. ⇨ **Genetic Engineering**
2. These New Genes Can Be Transcribed in New Cell Types (Switch Change) &/or Organisms &/or Both.

   Human Genes + Plant Leaf Switch
   Bacterial Switch + Human Insulin cDNA
3. All Genes are Regulated & Controlled by Switches. Genome Projects Reveal Both The Genes & The Switches & Wiring Together of All Switches in Gene. ⇨ Program of Life From Birth to Death

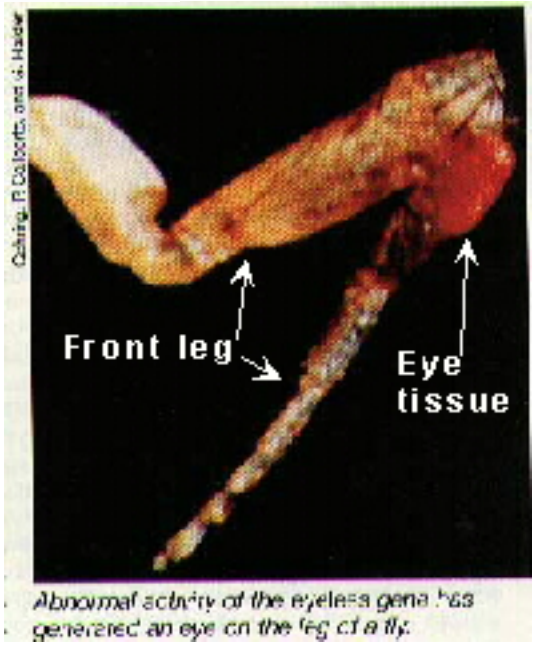
Yo! It's in the Sequences!!

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

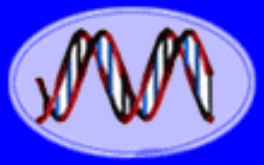
Eye Gene

Replace the Head Switch With the Leg Switch by Genetic Engineering

Eye Gene
+
Leg Switch



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



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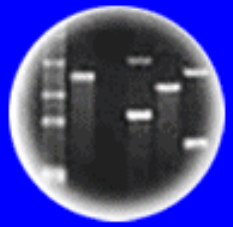

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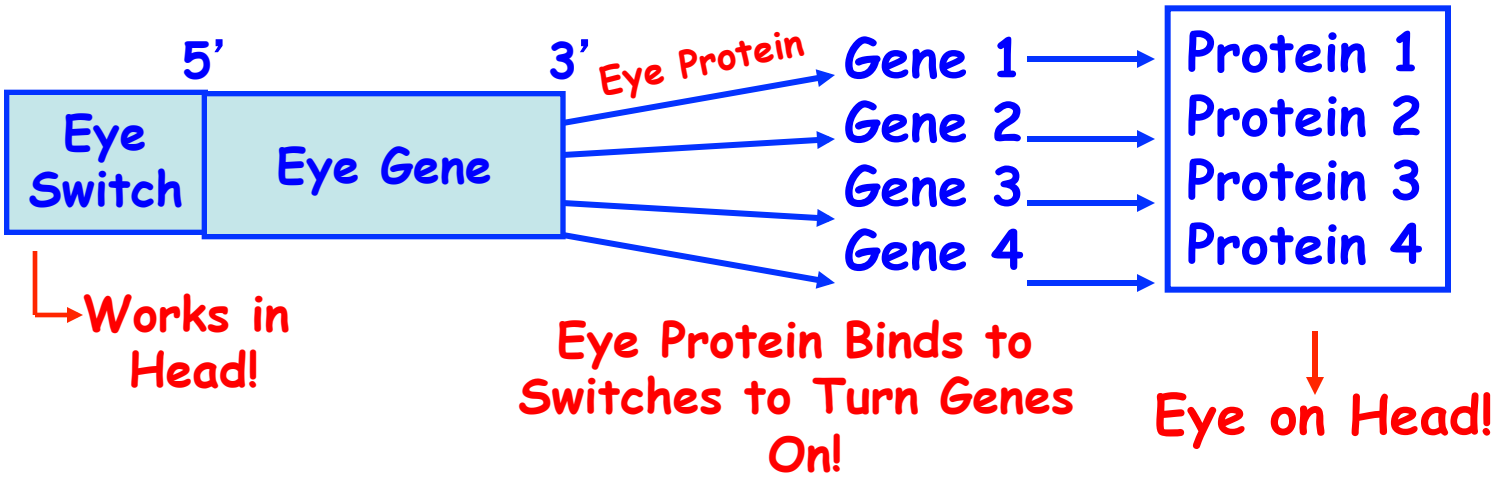
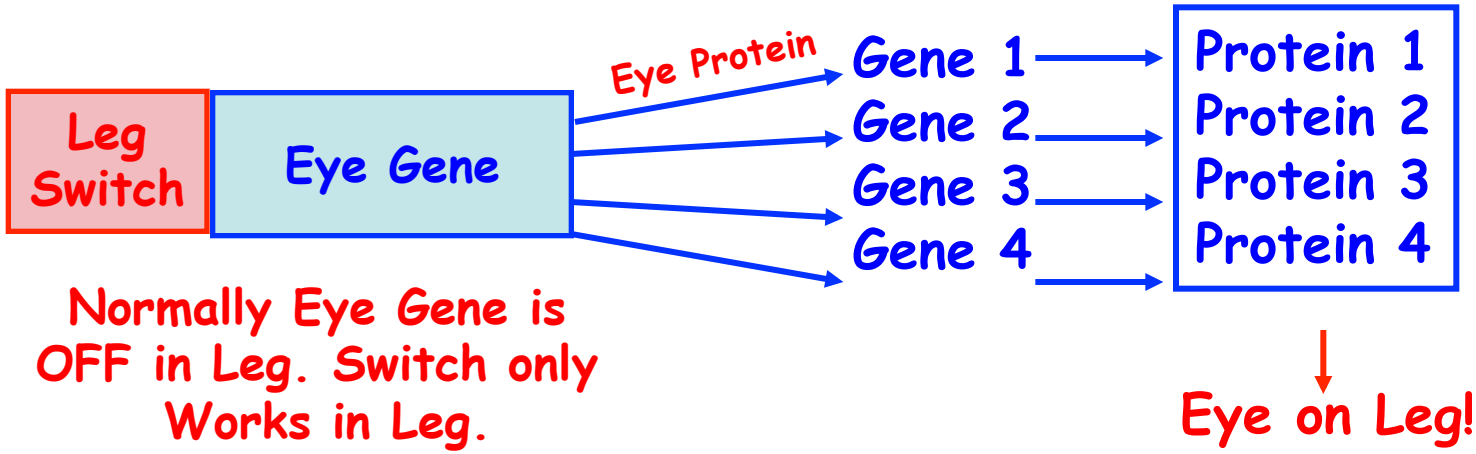
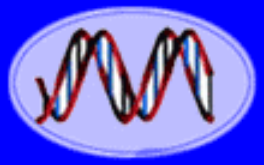
Eye Genetic Regulatory Network (GRN)

Control Genes Like The Eye Gene Control The Activity of Other Genes!







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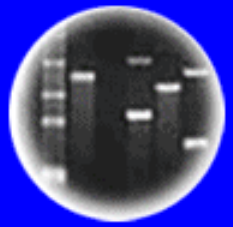

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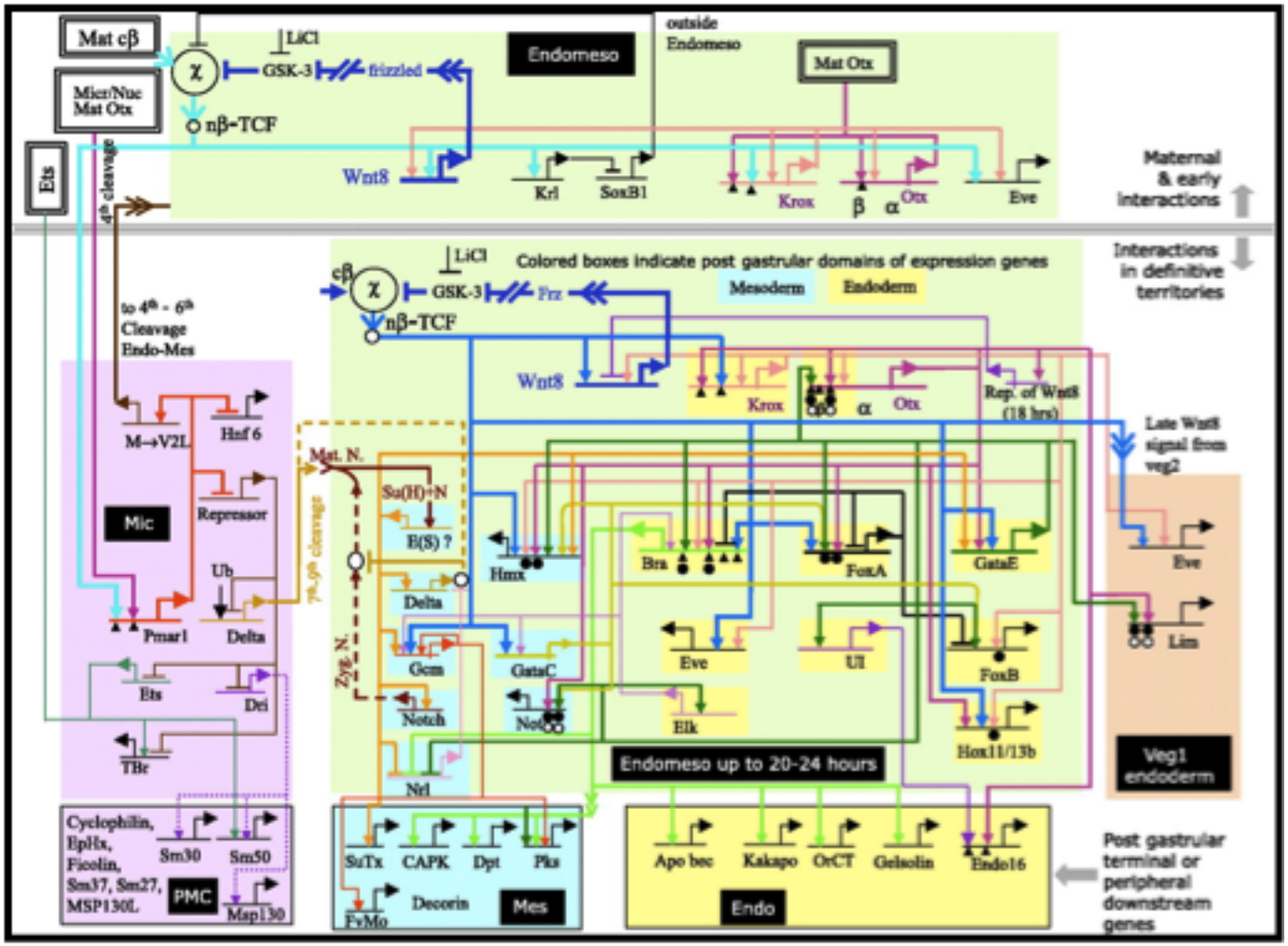
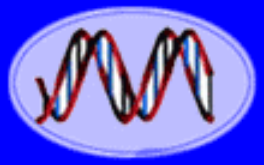
Ultimate Goal: To Dissect Genetic Regulatory Networks Programming Human Development From Birth to Death!



Genetic Networks Programming Early Sea Urchin Development



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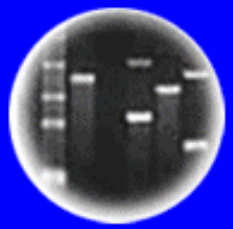


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

100 Years Into The Future

1. If the Entire Human Genome is Sequenced?
2. If the Function/Protein of All Genes Are Known?
3. If All the Switches Are Identified & How They Go On & Off From Birth to Death?
4. If We Understand How Genes Are Choreographed & All the Sequences That Program them

What Does the Future Hold?

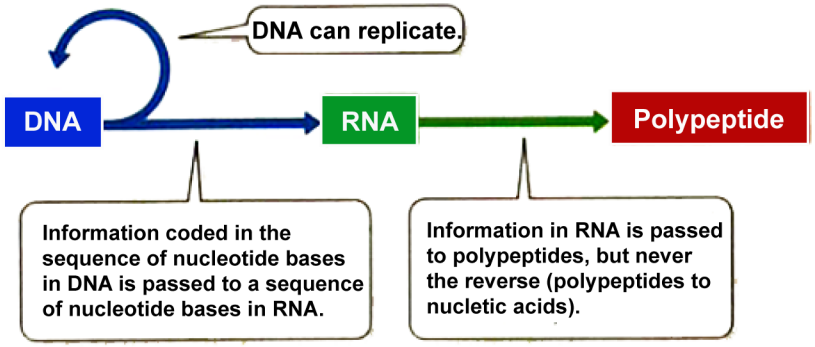
We Will Know at the DNA Level What Biological Information Programs Life to Death!

What Does This Mean For The Future of Humanity?

Remember - Mendel's Law Were Only Rediscovered 100 Years Ago & Look What We Can Do & Now!

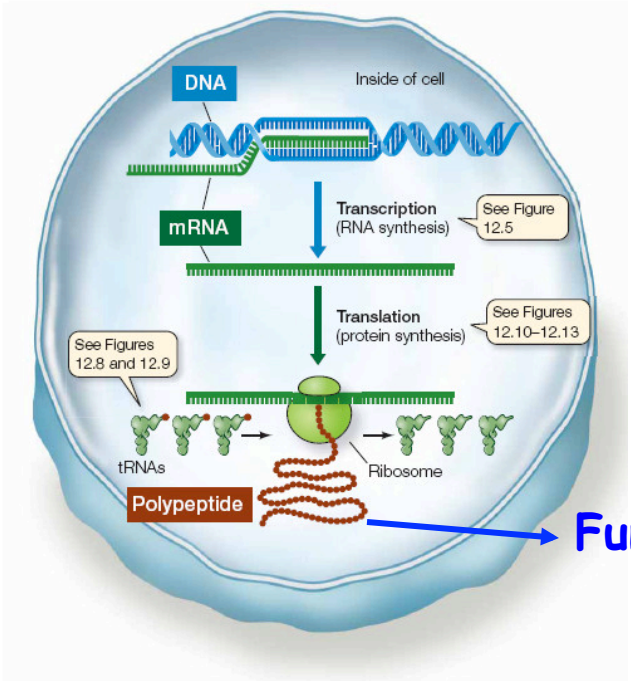
How Do Genes Work-A Review

① Replication



② Gene Activity to Function & Phenotype

Gene Activity → Protein → Function → Phenotype (Trait)

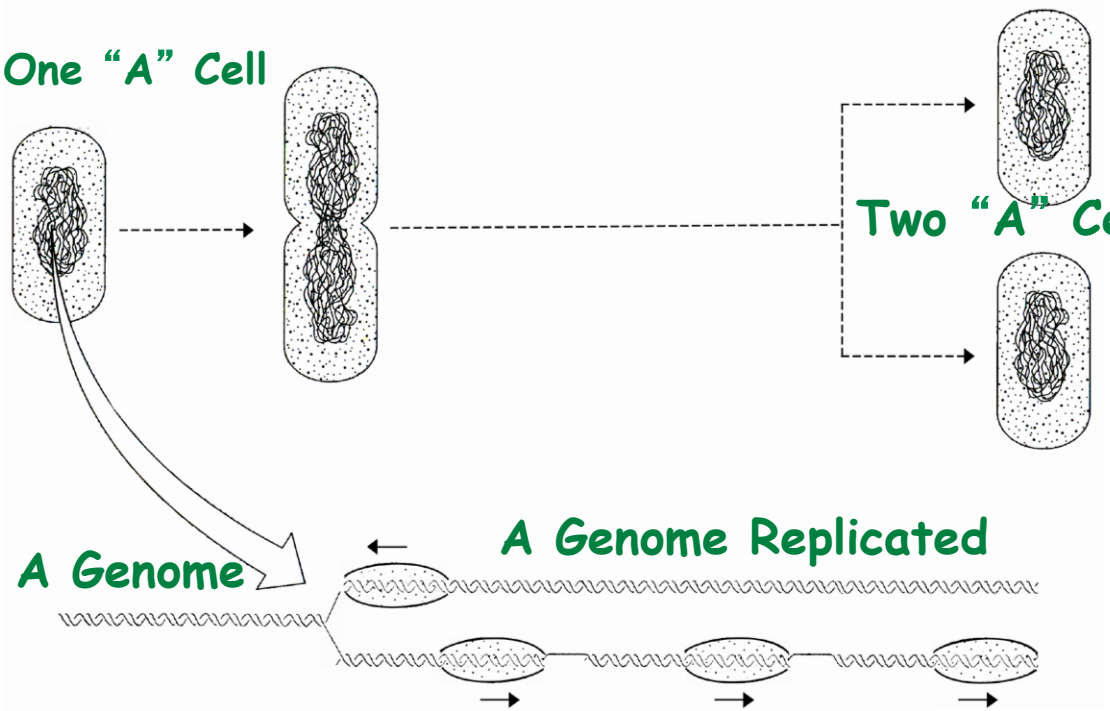


Function →

A Gene is NOT Expressed Unless A Functional Protein Produced!

1

How Are Genes Replicated Each Cell Generation?

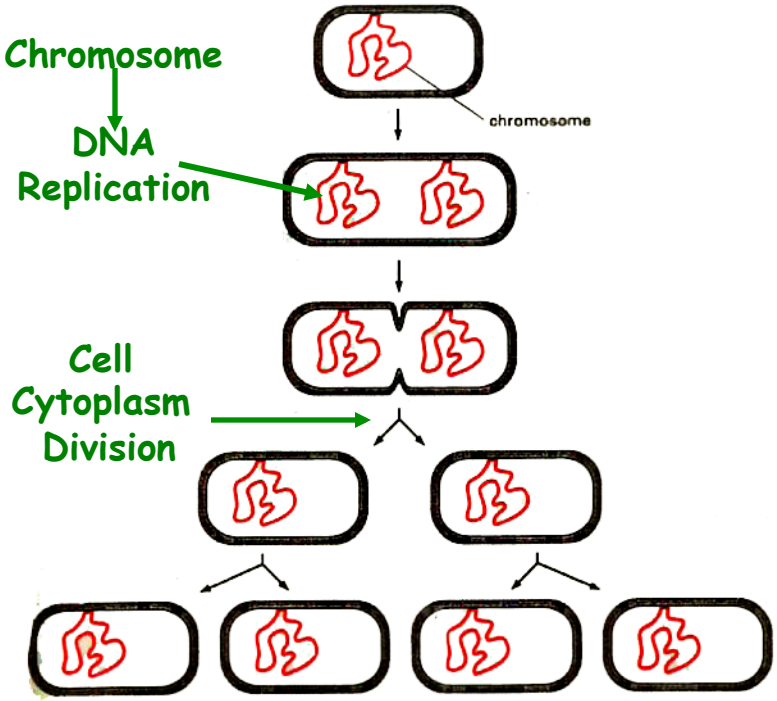


How is The DNA Sequence Copied/ Replicated Each Cell Division?

Pass on Genes to Next Generation Precisely?

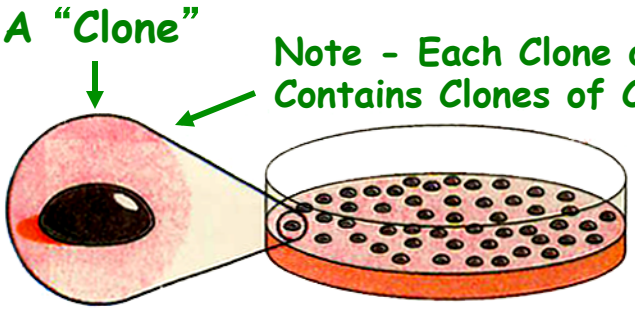
BASIS OF LIFE!

Genes Are Replicated During Each Cell Division



Clones

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



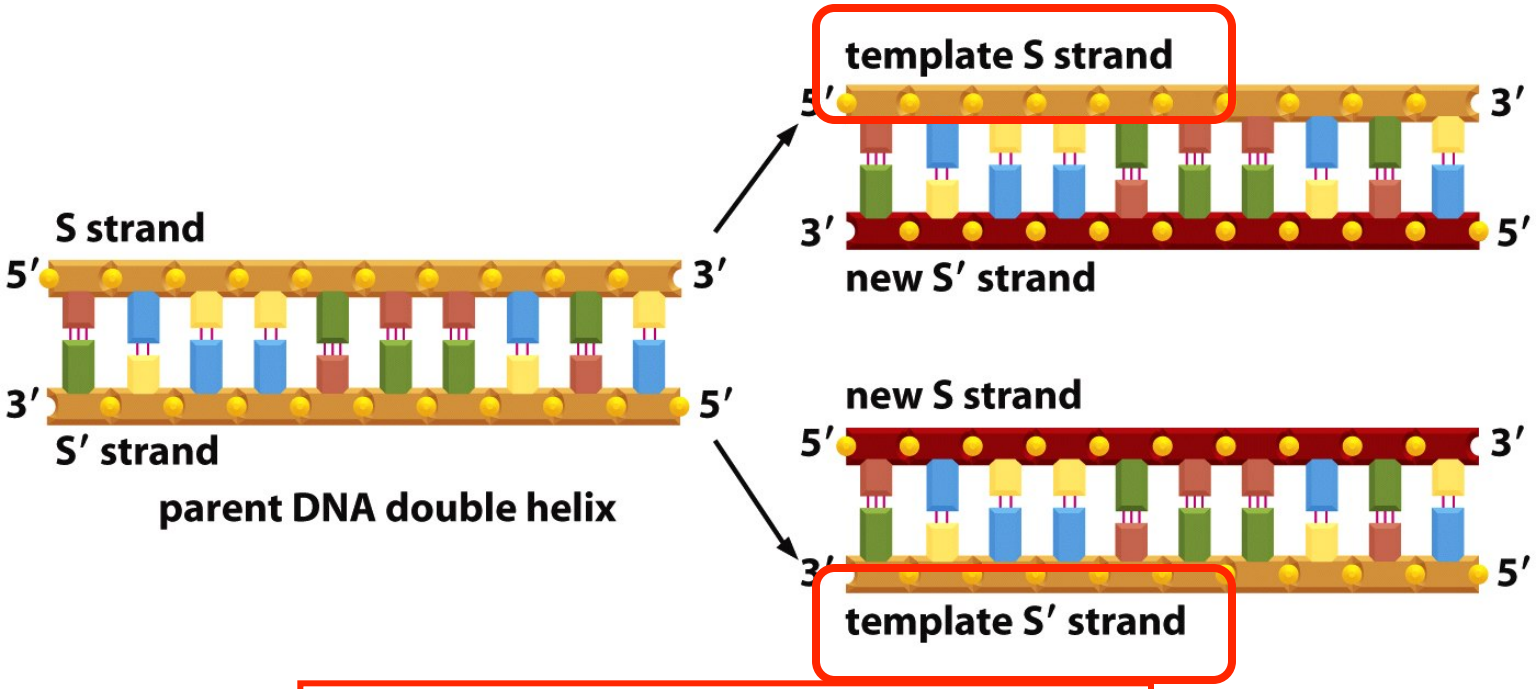
-10^6 cells per colony ~50 colonies per dish

Each Daughter Cell Contains The Same Collection of Genes

Clones!

Major Properties of Genetic Material
Replication, Stability, & All Cells!!

The Sequence of Each DNA Strand Must Be Maintained Division After Division

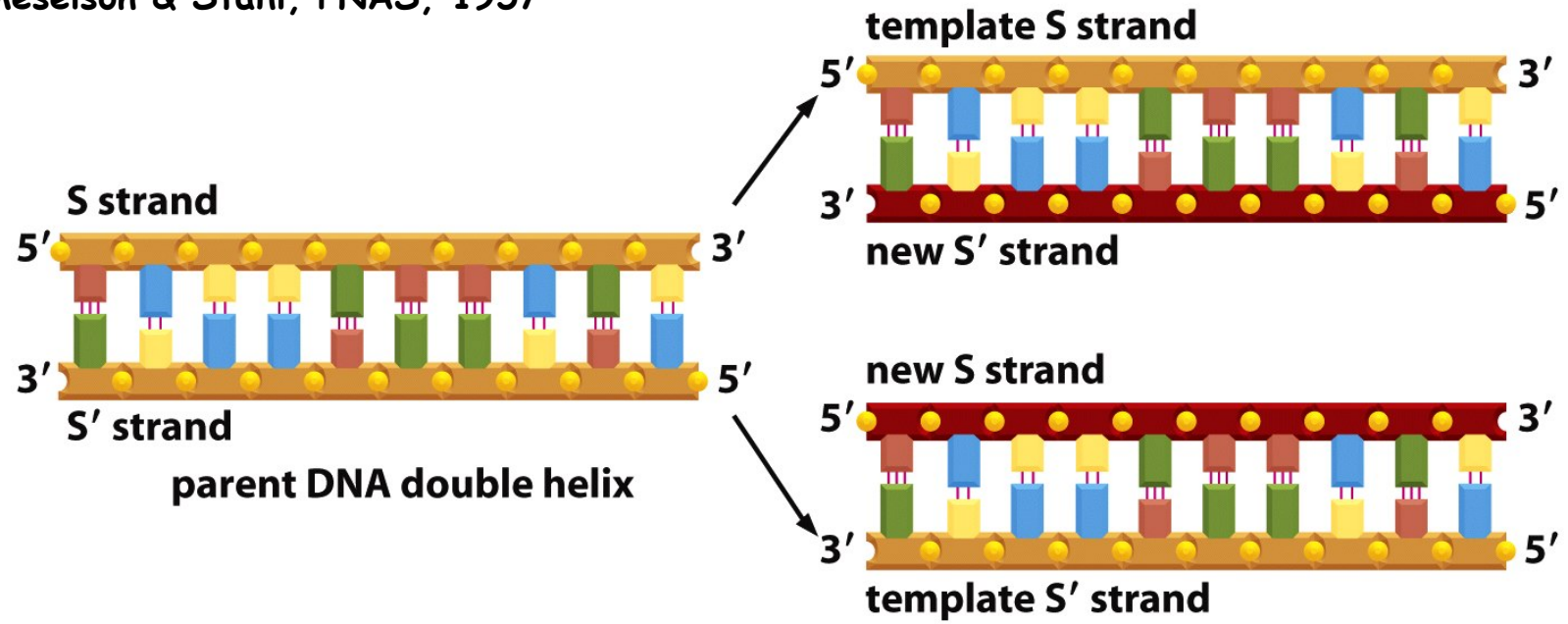


How Does This Occur?
Property of The DNA Molecule

Note → SEQUENCE & POLARITY

DNA Replication Occurs Semi-Conservatively

Meselson & Stahl, PNAS, 1957



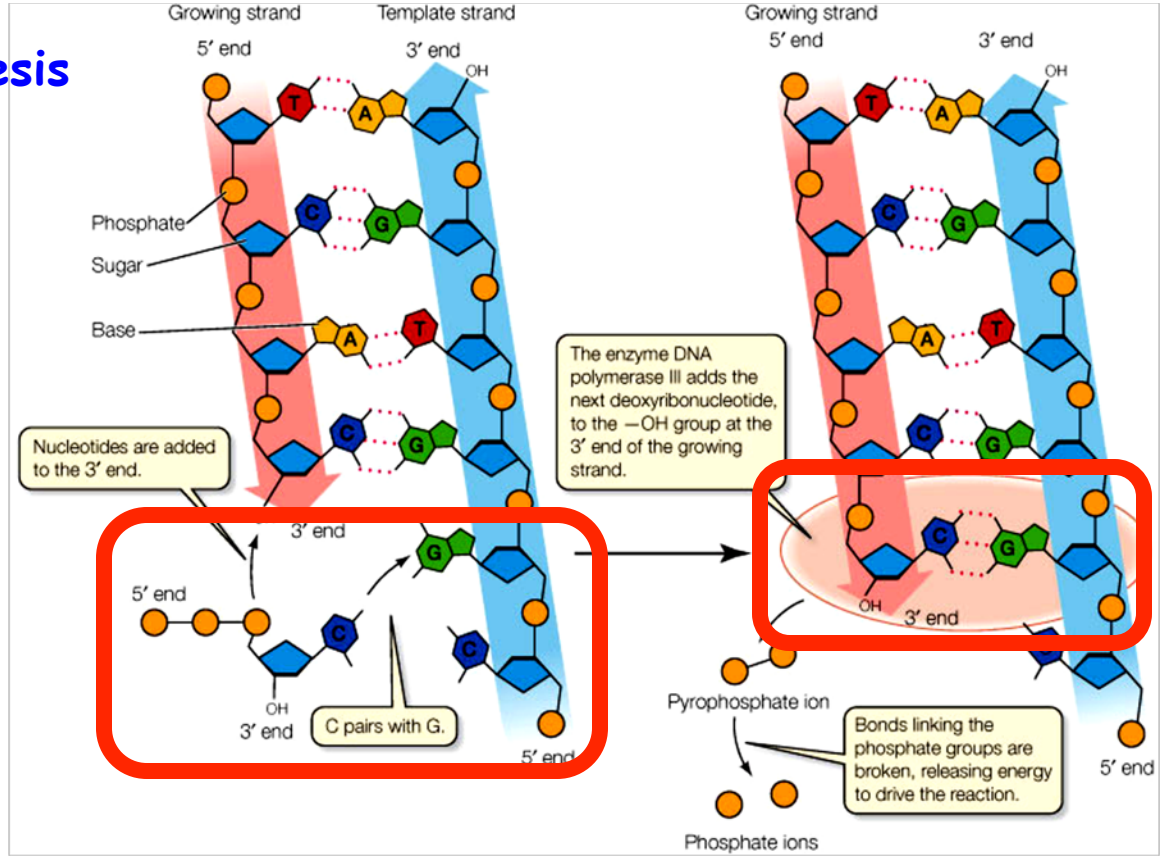
1. DNA Structure Allows DNA Sequence to Be Maintained by Complementary Base Pairing
2. Each Strand Serves as a Template for the Synthesis of a Complementary Strand
3. New DNA Molecules are Precise Copies of Parental DNA - Each Containing One Newly Synthesized Complementary Strand

DNA Sequence of One Strand is A Template For The New Strand

Synthesis

5'

3'



Sequence is Specified by Complementary Bases

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

5' to 3' Polarity Specifies Sequence

The DNA Sequence is Maintained Generation To Generation

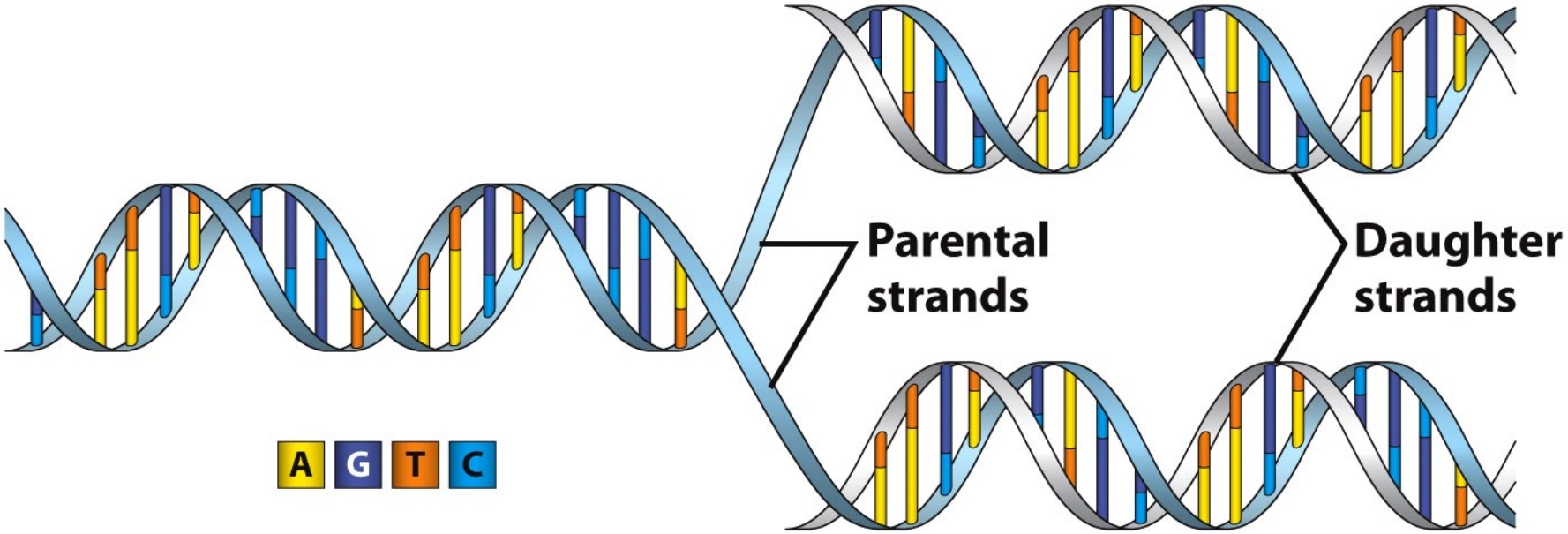
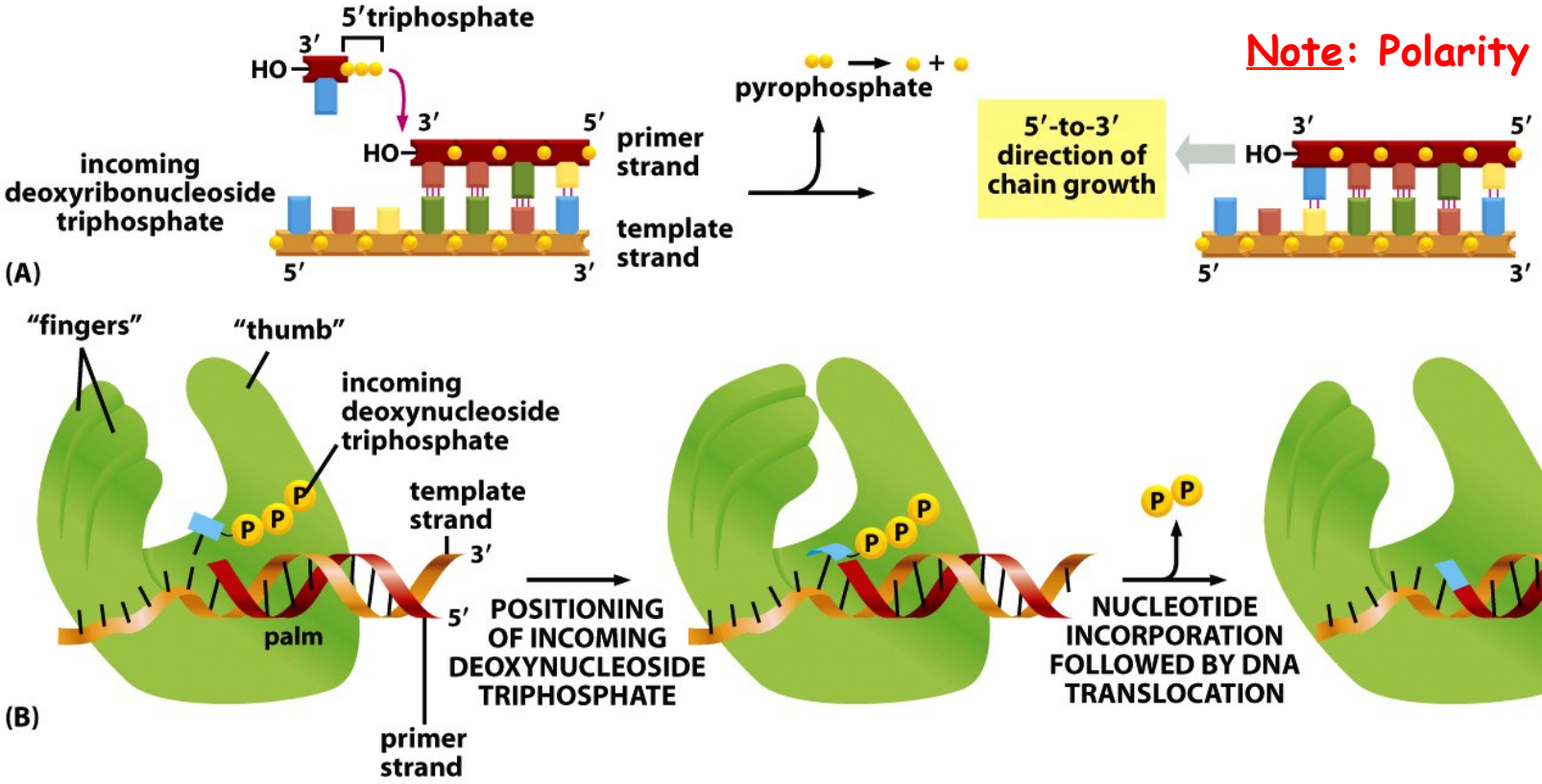


Figure 1-10
Molecular Cell Biology, Sixth Edition

The DNA Sequence "Lives" Forever!

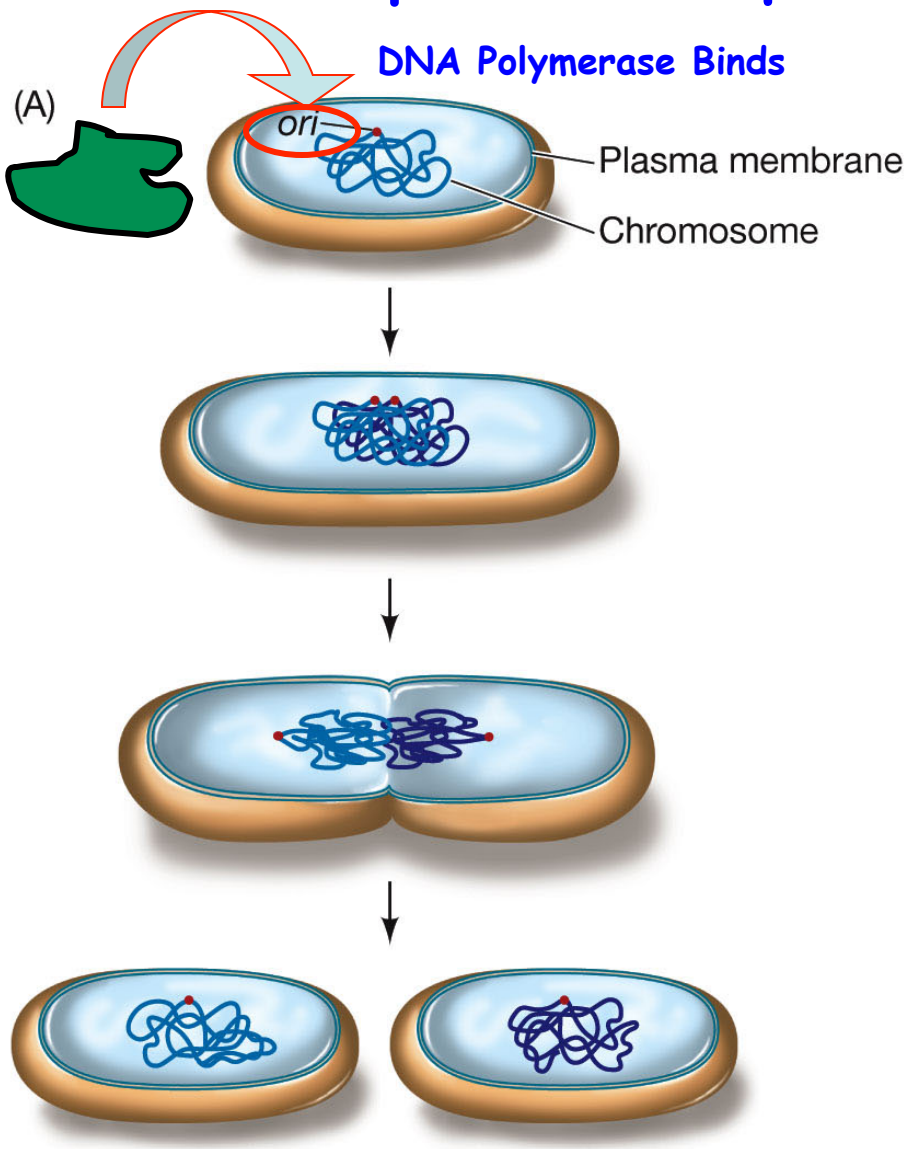
DNA Replication Requires An Enzyme - DNA Polymerase

Note: Nucleotide, Primer, & Template



1. DNA Polymerase Catalyzes 3'-5' Phosphodiester Bonds & Copies the Template
2. DNA Replication Needs a Primer, Template, DNA Polymerase,& Nucleotides

DNA Replication Requires An Origin of Replication



Two IDENTICAL Cells - Phenotypically & Genotypically - From One

(b)

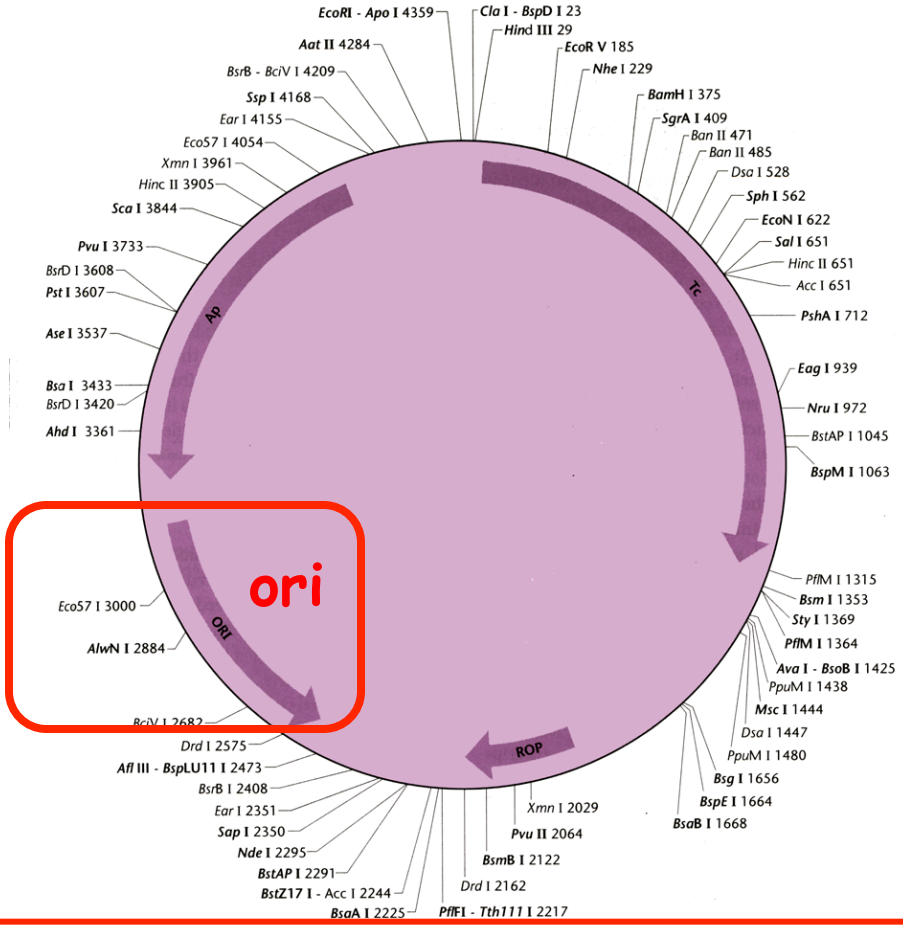
DNA Replication Also Requires:

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

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

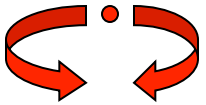
Ori

DNA Replication Starts at The Origin of Replication



DNA Replication is <u>Bidirectional</u> From the Ori!!!

Ori

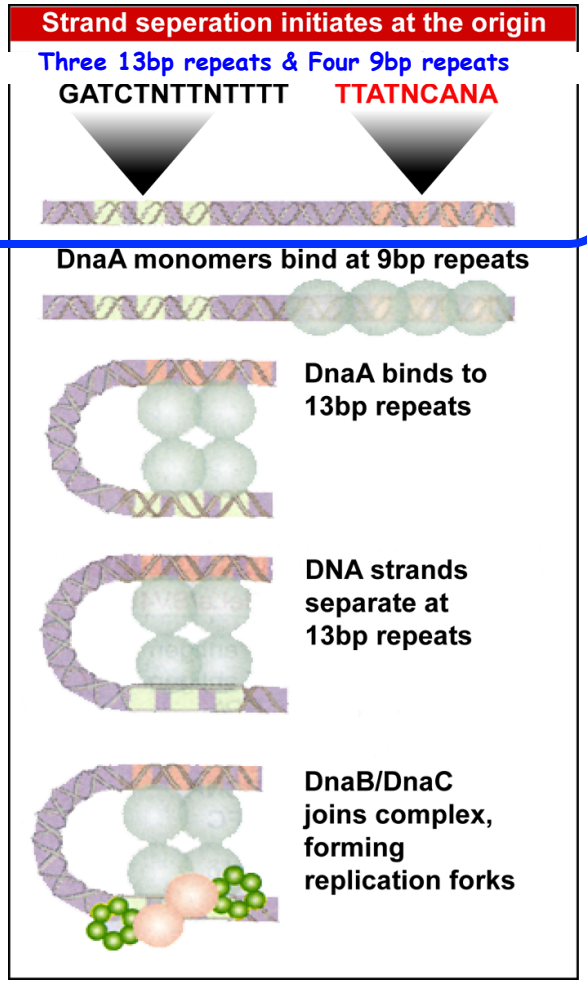


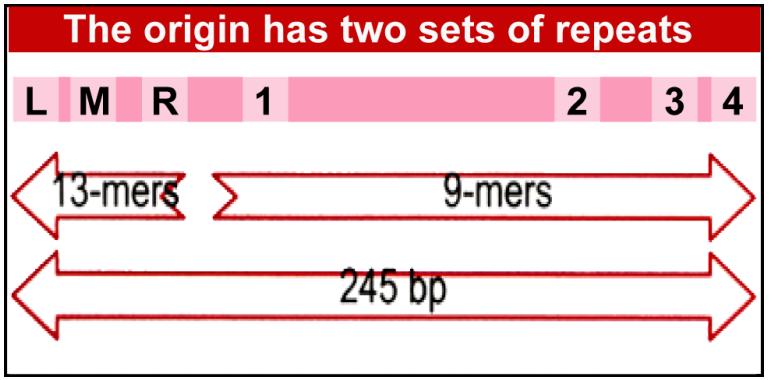
Hypothesis For Two Direction Synthesis?

DNA Polymerase Binds to The Origin of Replication (Ori) to Begin DNA Synthesis

How Control Division?

The Origin of Replication is a Specific Sequence



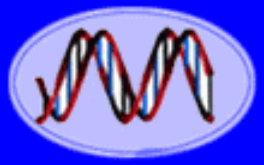


1. How Clone An Origin of Replication?

2. Specific Sequence - What Does This Mean For Genetic Engineering?

3. What is The Significance For Genetic Engineering?

4. Can Replicating "Chromosomes" Be Made?



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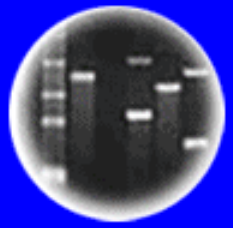

DNA Fingerprinting

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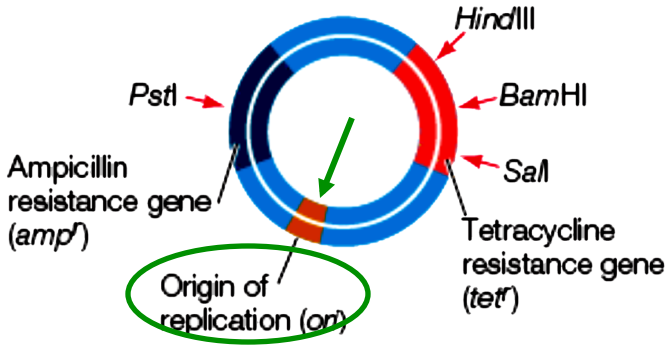
Vectors Are Needed To Replicate Genes In Transformed Cells

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



Recognition Site for Restriction Enzymes

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.

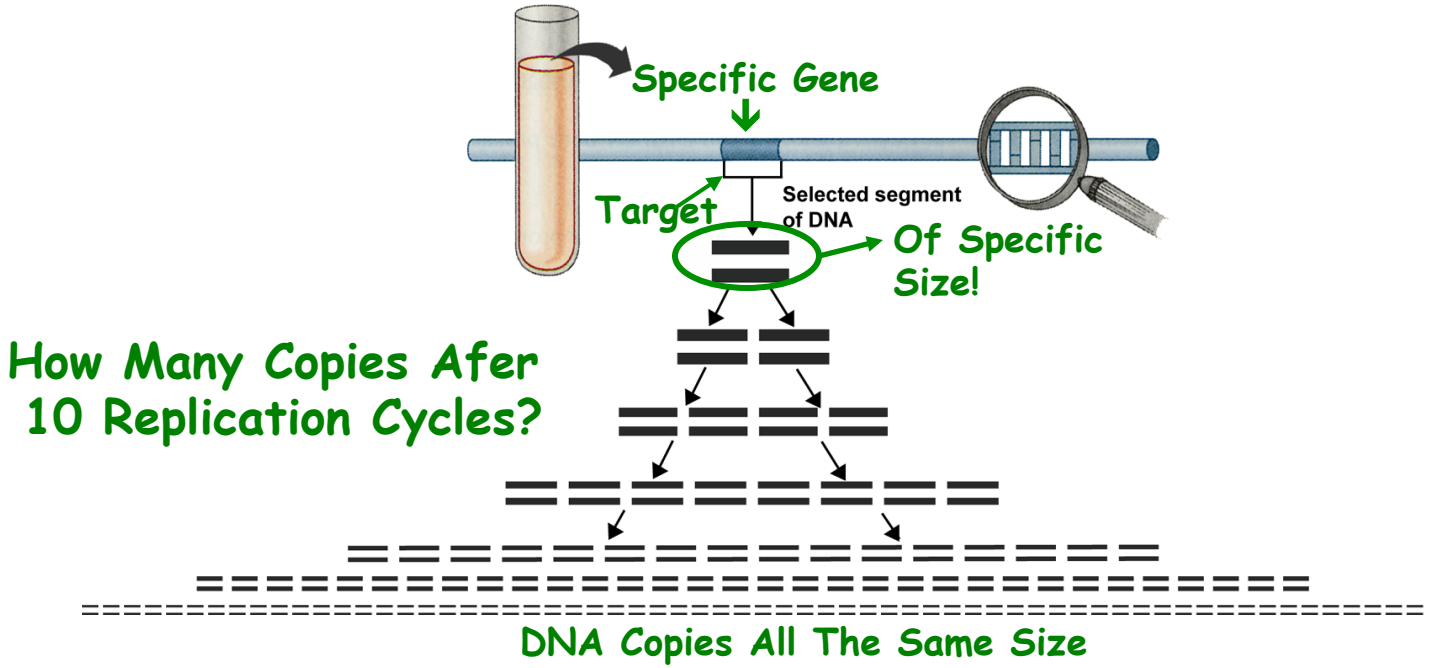
Yo! It's in the Sequence= Function

∴ Vectors can be Engineered!

Ori's can be cloned/synthesized!

MODULAR!!

The Polymerase Chain Reaction or PCR is A Molecular Xerox Machine



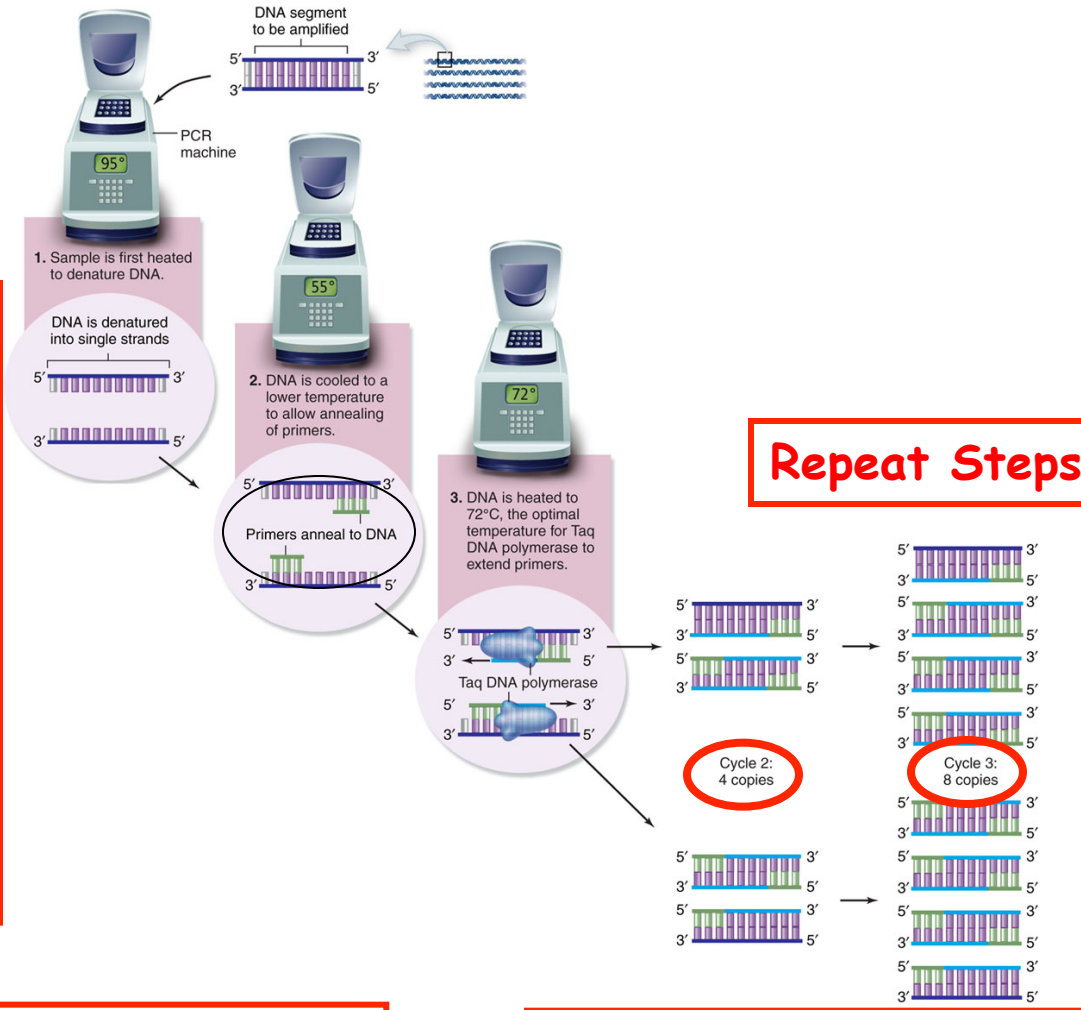
1. PCR Has Revolutionized DNA Analysis!

Specific DNA Sequences/Genes Can Be "Copied" Directly From "Tiny" Amount of DNA!

2. No Cloning Needed!

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

PCR is A Cyclical Process of DNA Replication



Requires

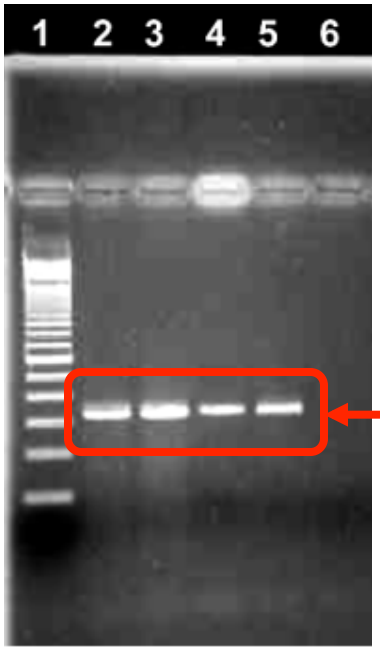
1. Template
2. Primers
3. Knowledge of Specific Sequence
4. Nucleotides
5. Heat-Stable DNA Polymerase
6. Cycler

Repeat Steps or Cycle

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

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

Using Gel Electrophoresis to Visualize PCR Products



Specific Diagnostic DNA Band Unique to DNA Sequence Being Amplified

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

Can Amplify One DNA Sequence From An Entire Genome!!!

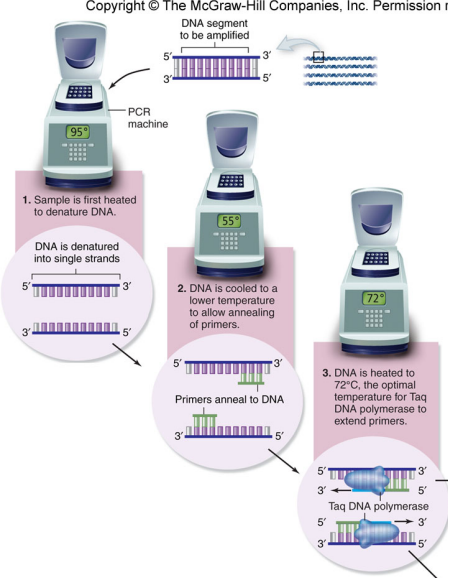
Requirements For PCR

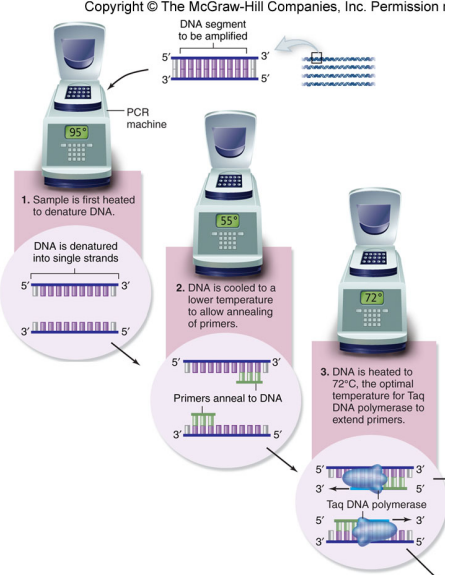
1. Knowledge of a Specific Sequence to Amplify (e.g.,insulin gene)
   a) Must Have First Cloned & Sequenced DNA of Interest the "Old-fashioned Way"
2. Primers That Recognize Specific DNA Sequences & Initiate DNA Synthesis & DNA Polymerase Binding To Template
3. Template (e.g., DNA From Human Cheek Cell)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

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

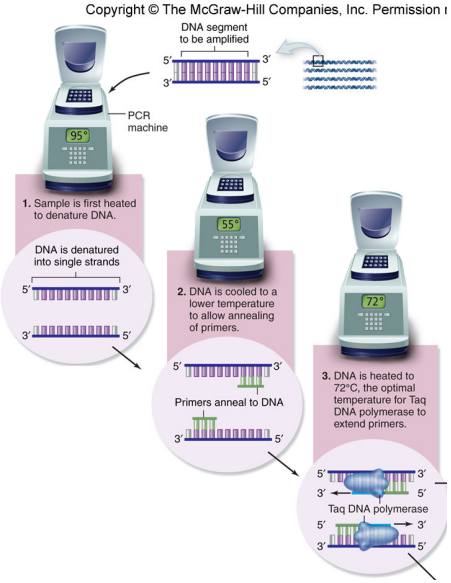
PCR Has Made DNA Cloning and Recombinant DNA Technology Obsolete?

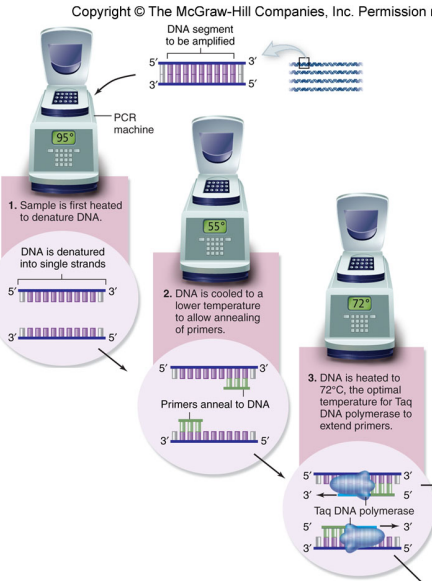
a. Yes
b. No

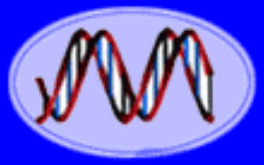




Examples of PCR Applications

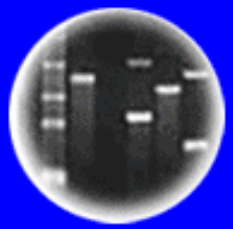






DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



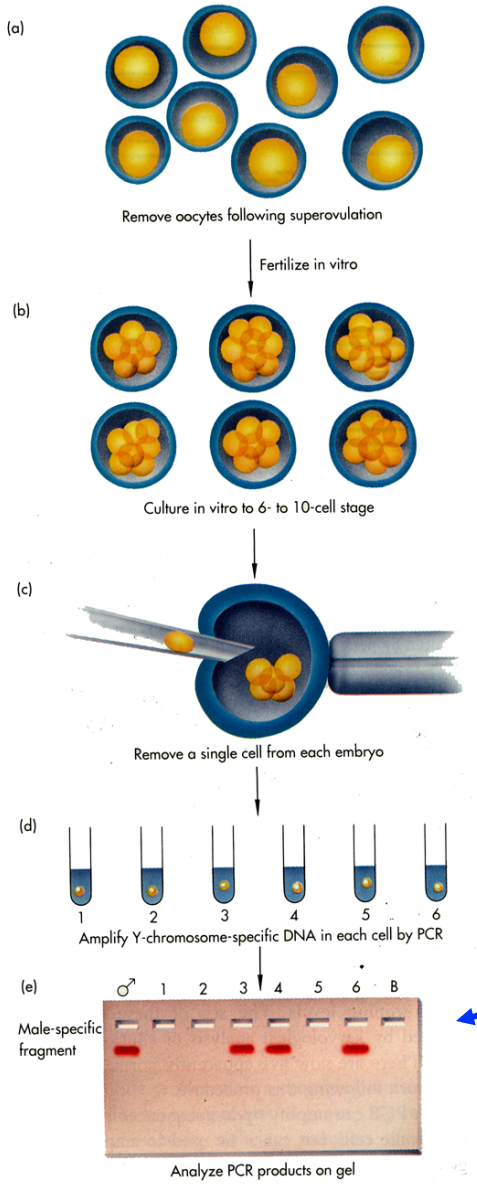
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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

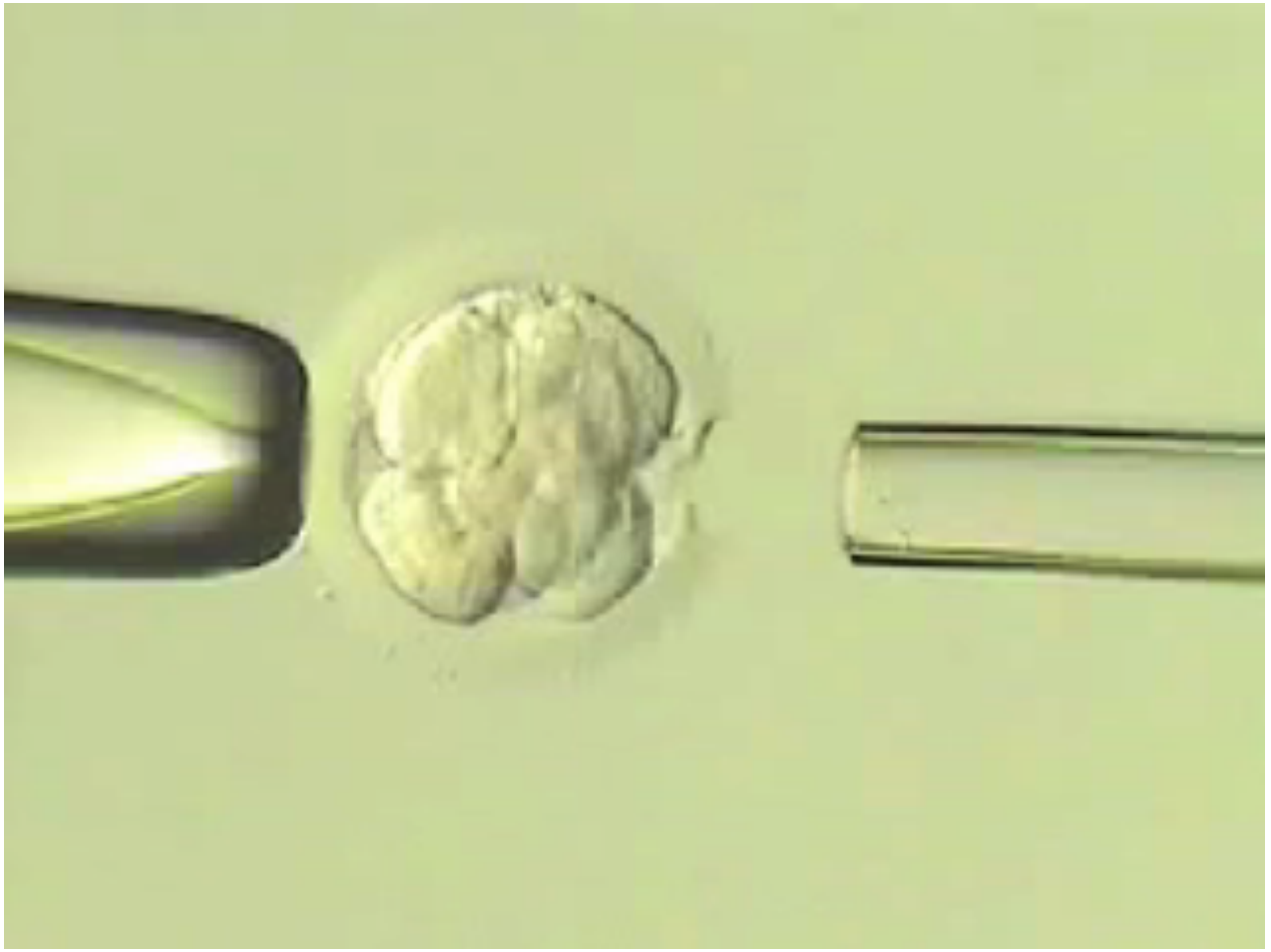
PGD
Pre-Implantation Genetic Diagnosis



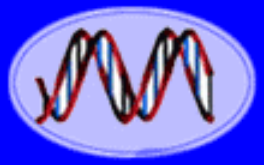
What is The Implication of This Procedure Considering That The Human Genome Has Been Sequenced?

Sex Determination in 8-cell Embryo!

Determining the Genetic Identity of a Human Embryo Before Implantation!

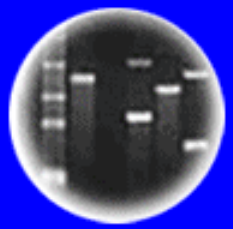


Prenatal Genetic Diagnosis (PGD)



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

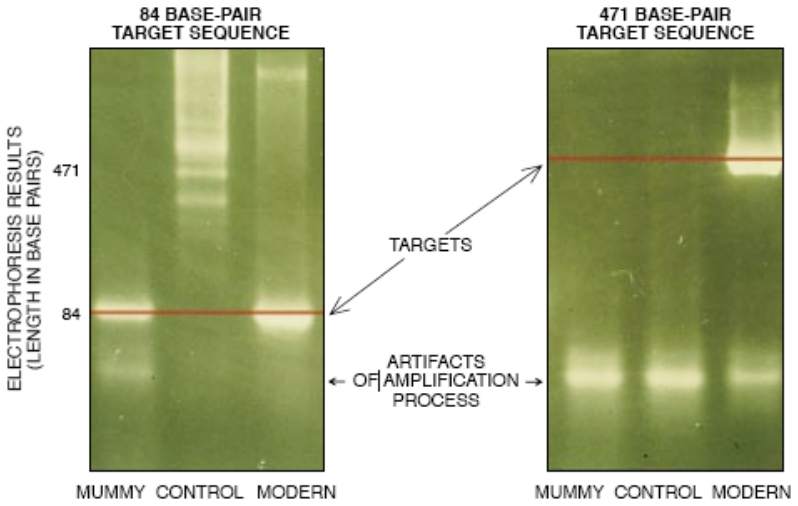
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Parents Should Be Allowed To Use PGD To Test Their Embryos For Gender and Select the Sex of Their Child?

a. Yes
b. No

Using PCR To Detect Genes in Mummy DNA



Sequence to Determine Relationships

Using PCR to Amplify Mammoth DNA From Fossilized Hair & Sequence The Entire Genome!

Nature, November 2008

Sequencing the nuclear genome of the extinct woolly mammoth

Webb Miller[1], Daniela I. Drautz[1], Aakrosh Ratan[1], Barbara Pusey[1], Ji Qi[1], Arthur M. Lesk[1], Lynn P. Tomsho[1], Michael D. Packard[1], Fangqing Zhao[1], Andrei Sher[2]‡, Alexei Tikhonov[3], Brian Raney[4], Nick Patterson[5], Kerstin Lindblad-Toh[5], Eric S. Lander[5], James R. Knight[6], Gerard P. Irzyk[6], Karin M. Fredrikson[7], Timothy T. Harkins[7], Sharon Sheridan[7], Tom Pringle[8] & Stephan C. Schuster[1]

Using PCR to Amplify Neanderthal Bone DNA & Sequence The Entire Genome!

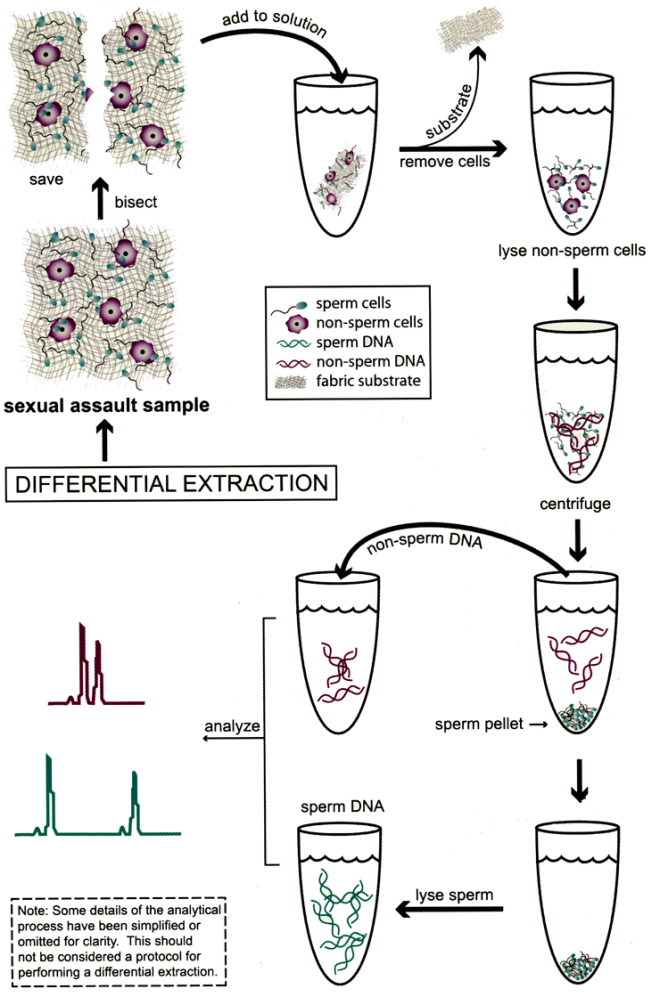
Analysis of one million base pairs of Neanderthal DNA

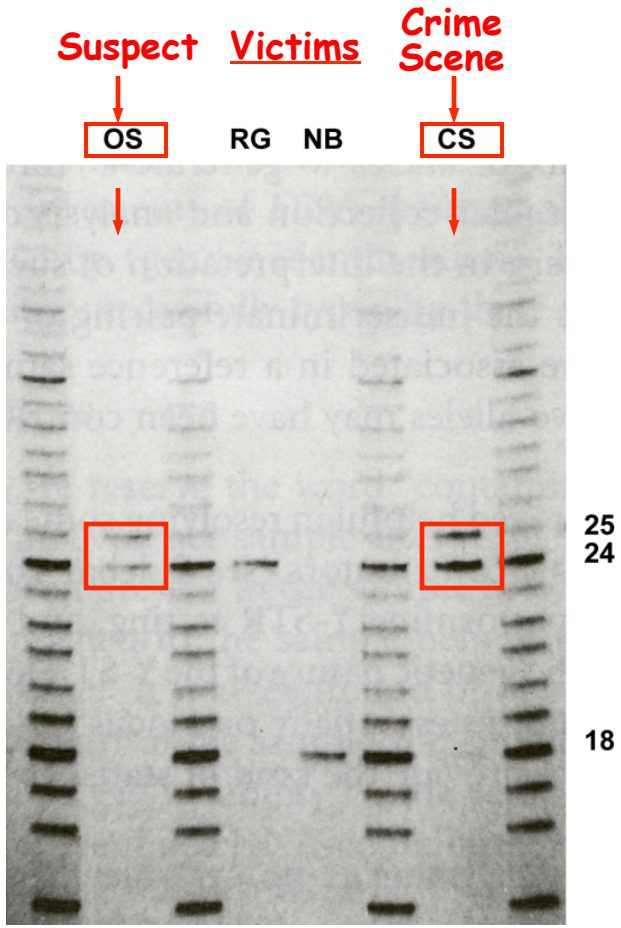
From a 45,000 Year-Old Bone

Richard E. Green[1], Johannes Krause[1], Susan E. Ptak[1], Adrian W. Briggs[1], Michael T. Ronan[2], Jan F. Simons[2], Lei Du[2], Michael Egholm[2], Jonathan M. Rothberg[2], Maja Paunovic[3]‡ & Svante Pääbo[1]

Nature, November, 2006

Using PCR in Crime Scenes





OS = Suspect
CS = Crime Scene
RG & NB = Victims

"Match" What is Probability That This Will Occur by Chance?

DNA Doesn't "Lie" !!

Identifying Victims of 9/11 Using PCR and DNA Fingertinting

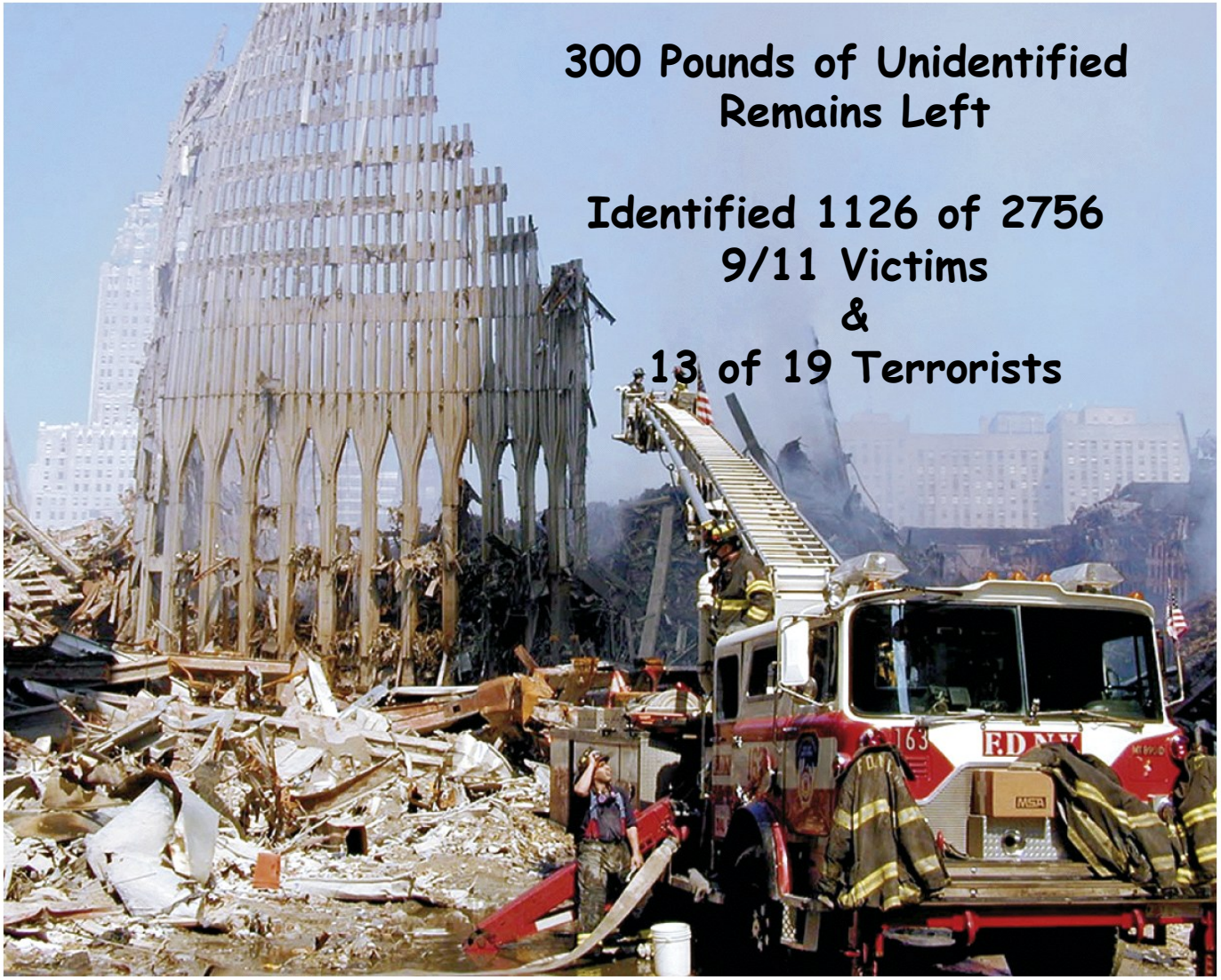
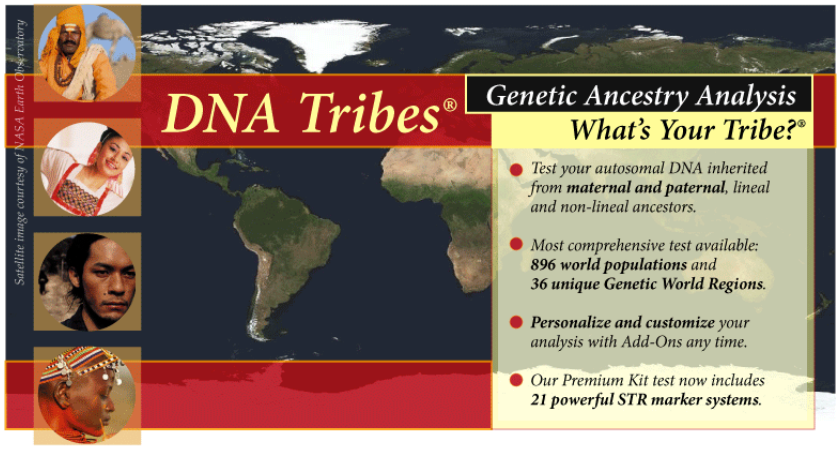


Figure 19-31
Genetics: A Conceptual Approach, Third Edition

Newsweek, January 12, 2009

Using PCR To Determine an Individual's Ancestry



PCR Started a New Industry

DNA can reveal ancestors' lies and secrets

LA Times, January 18, 2009

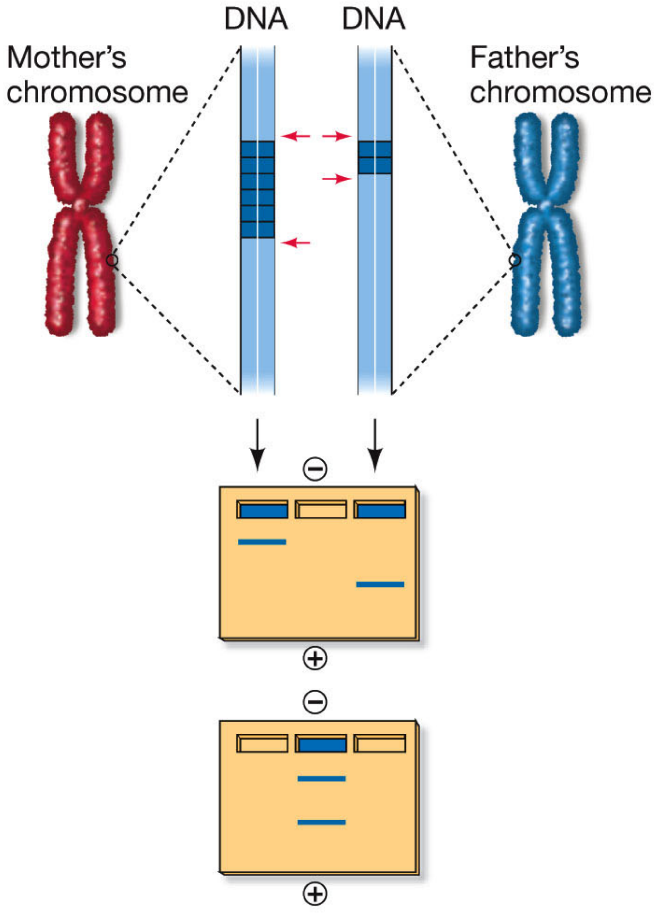
Using PCR to Verify Remains of Russian Royal Family

	Number of repeats	
STR-1	15,16	15,16
STR-2	8,8	7,10
STR-3	3,5	7,7
STR-4	12,13	12,12
STR-5	32,36	11,32

Tsarina Alexandra ○ — □ Tsar Nicholas II

STR-1	15,16	15,16	15,16
STR-2	8,10	7,8	8,10
STR-3	5,7	5,7	3,7
STR-4	12,13	12,13	12,13
STR-5	11,32	11,36	32,36

VNTRs!



PNAS

Genomic identification in the historical case of the Nicholas II royal family

PNAS, March, 2009

Evgeny I. Rogaev[a,b,c,d,1], Anastasia P. Grigorenko[b,d], Yuri K. Moliaka[b], Guinaz Faskhutdinova[b], Andrey Goltsov[d], Arlene Lahti[e], Curtis Hildebrandt[e], Ellen L. W. Kittler[f], and Irina Morozova[a]

[a]Department of Genomics and Laboratory of Evolutionary Genomics, Vavilov Institute of General Genetics, Russian Academy of Science, Gubkina Street, 3, Moscow, 119991, Russian Federation; [b]Brudnick Neuropsychiatric Research Institute, University of Massachusetts Medical School, 303 Belmont Street, Worcester, MA 01604; [c]Faculty of Bioinformatics and Bioengineering, Lomonosov Moscow State University, Moscow, 119991, Russian Federation; [d]Research Center of Mental Health, Russian Academy of Medical Science, Zagorodnoe Shosse 2/2, Moscow, 113152, Russia; [e]Molecular World, Inc., Thunder Bay, ON, Canada P7B 2T1; and [f]University of Massachusetts Medical School, Center for AIDS Research, Worcester, MA 01605

Communicated by James D. Watson, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, November 14, 2008 (received for review October 8, 2008)

RESEARCH ARTICLE OPEN ACCESS

Mystery Solved: The Identification of the Two Missing Romanov Children Using DNA Analysis

Michael D. Coble[1#*], Odile M. Loreille[1#], Mark J. Wadhams[1], Suni M. Edson[1], Kerry Maynard[1], Carna E. Meyer[1], Harald Niederstätter[2], Cordula Berger[2], Burkhard Berger[2], Anthony B. Falsetti[3], Peter Gill[4,5], Walther Parson[2], Louis N. Finelli[1]

1 Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, Rockville, Maryland, United States of America, **2** Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria, **3** University of Florida, Gainesville, Florida, United States of America, **4** Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom, **5** Institute of Forensic Medicine, University of Oslo, Oslo, Norway

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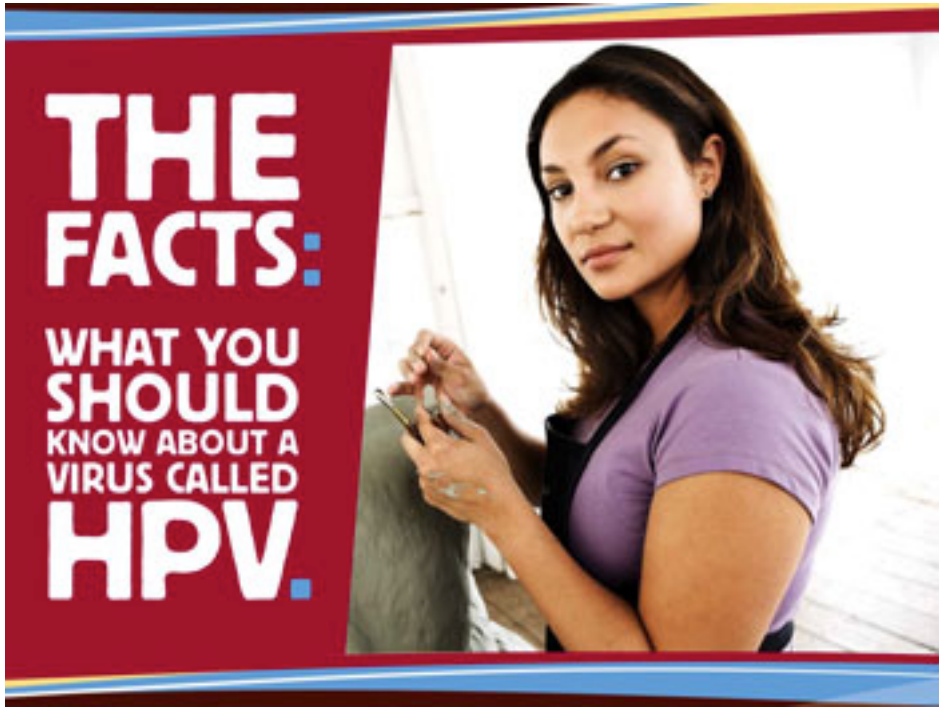
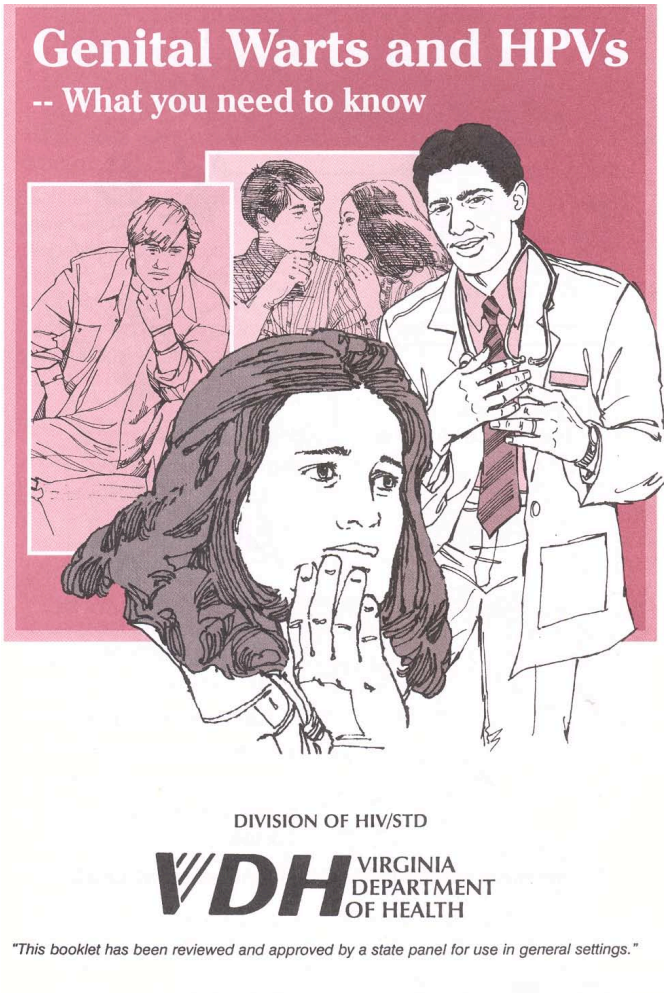
PLOS, March, 2009

Using PCR To Detect Human Pathogens
(Viruses, Fungi, Bacteria)

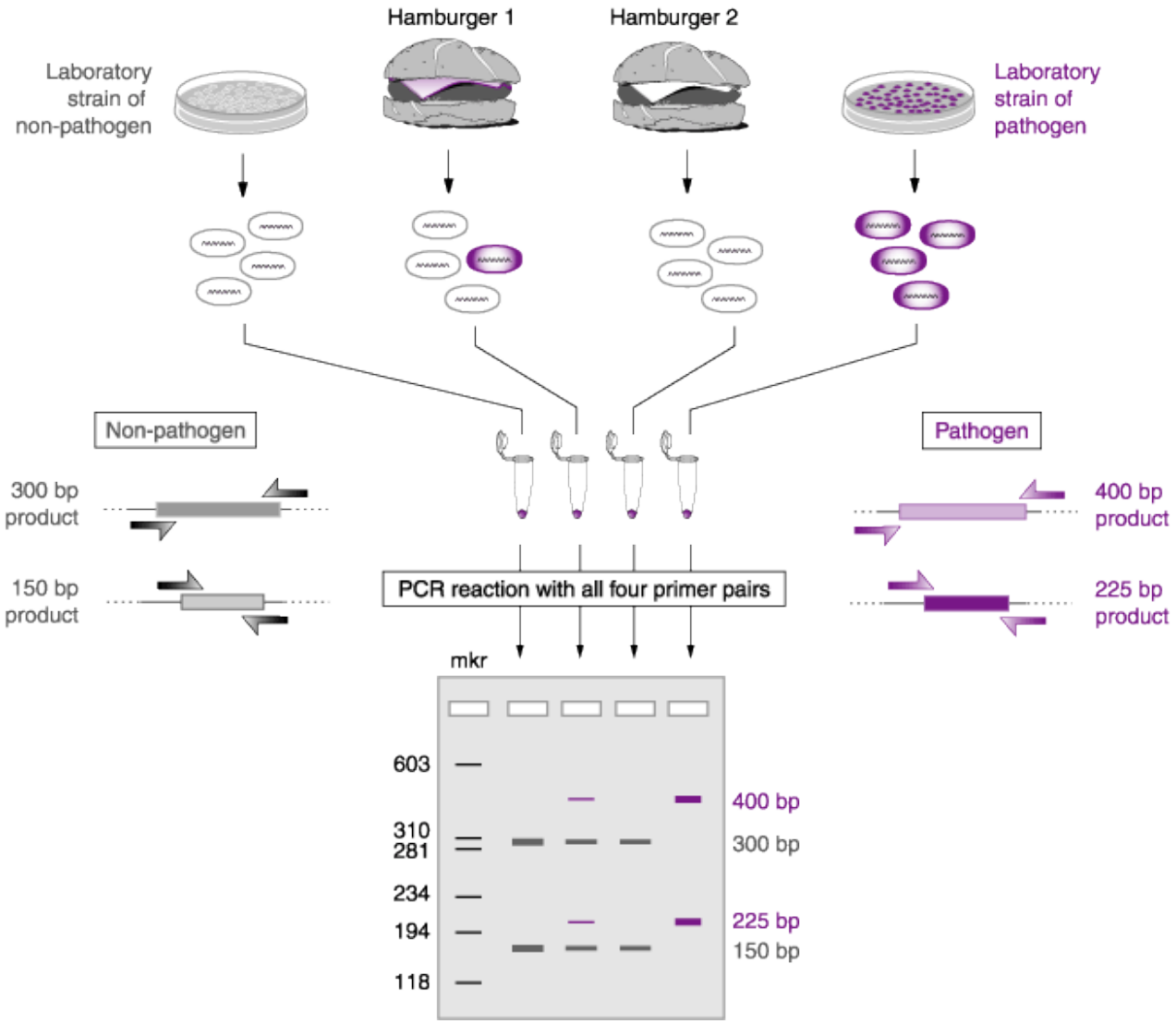




ViroSeq™
HIV-1 Genotyping System

Each Genome Has Specific DNA Sequences That Can Be Used For Screening And Diagnosis Using PCR

Using PCR To Detect Food Pathogens



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

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

Revolutionized How To Study & Manipulate DNA

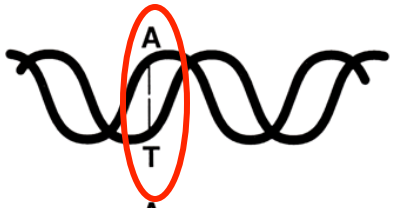

ABCNEWS WASHINGTON
Kerry Mullis and PCR
Nightline March, 1994

DNA Replication is Precise
But Mistakes or Mutations Can Occur!

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

BASE PAIR RULES

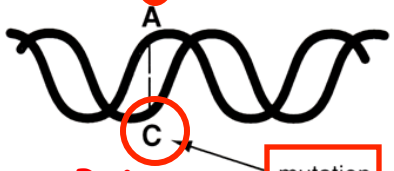
Gene A



ORIGINAL BASE PAIR

Rare Base Mismatch

Replication①



MUTATION DURING REPLICATION

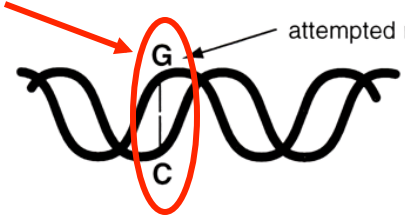
mutation

C mispairs with A

New Base Pair

See Mutation As Change in Phenotype

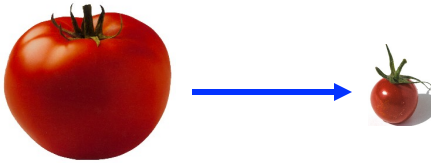
Replication②
Gene A'
Allelic Variant



RESULTING DEFECT

Change DNA Sequence From A = T to G ≡ C

∴ Change Protein Amino Acid Sequence ⇨ Alter Function!



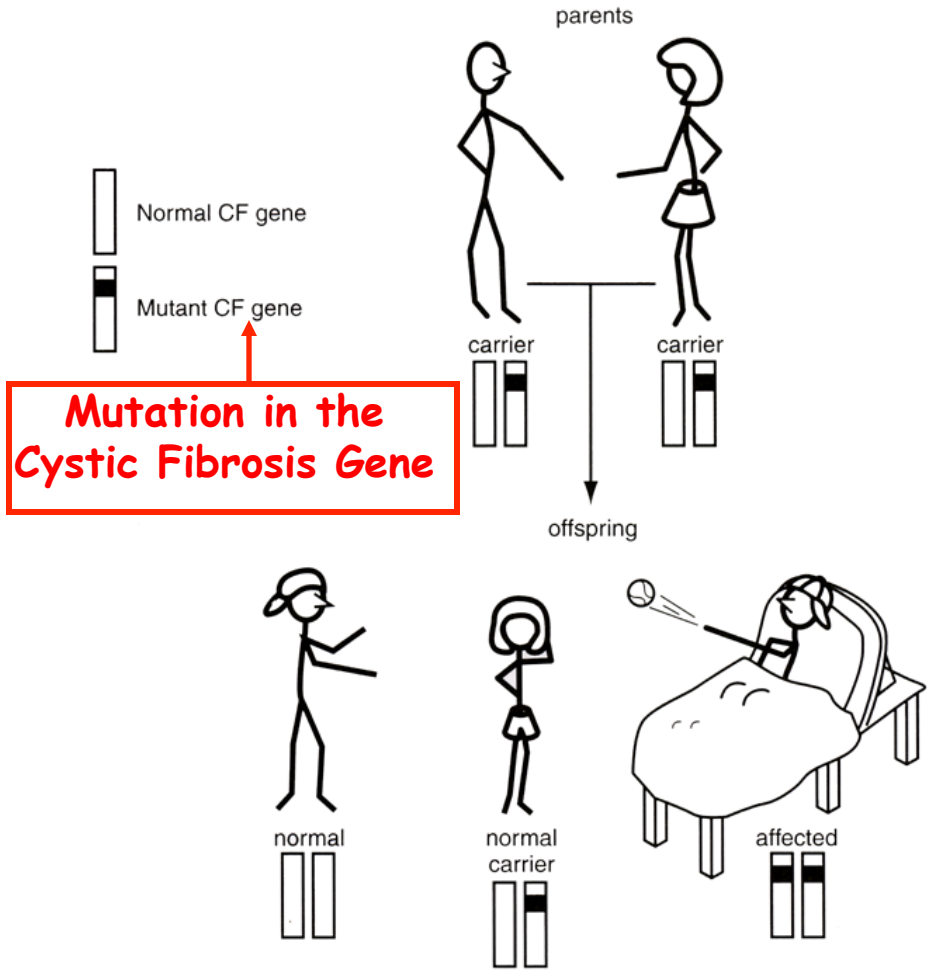
Big Tomato to Small Tomato

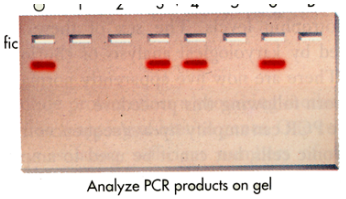
Mutation in Genes Are Rare But Are Inherited

One Gene Per Gamete

♀ + ♂

Two Genes per Somatic Cells



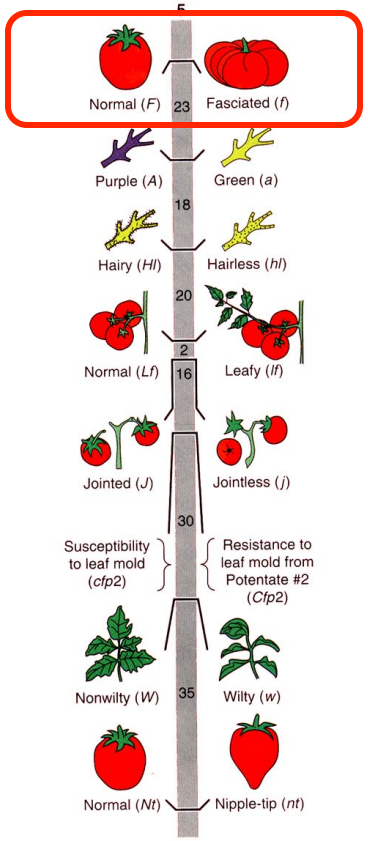


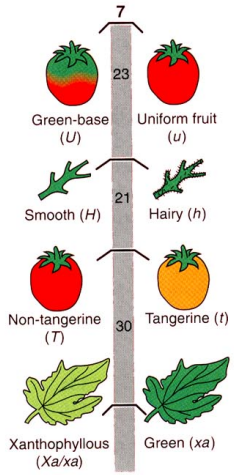
How Follow Inheritance?
What Allows Disease To Be Followed?

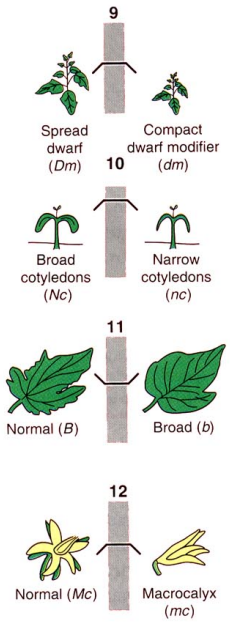
DNA Marker or Fingerprint!

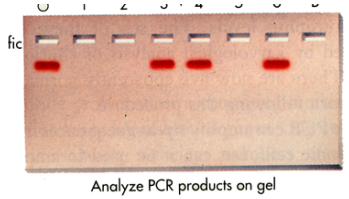
Alternative Forms of the Same Gene Lead to Genetic Diversity

Alleles









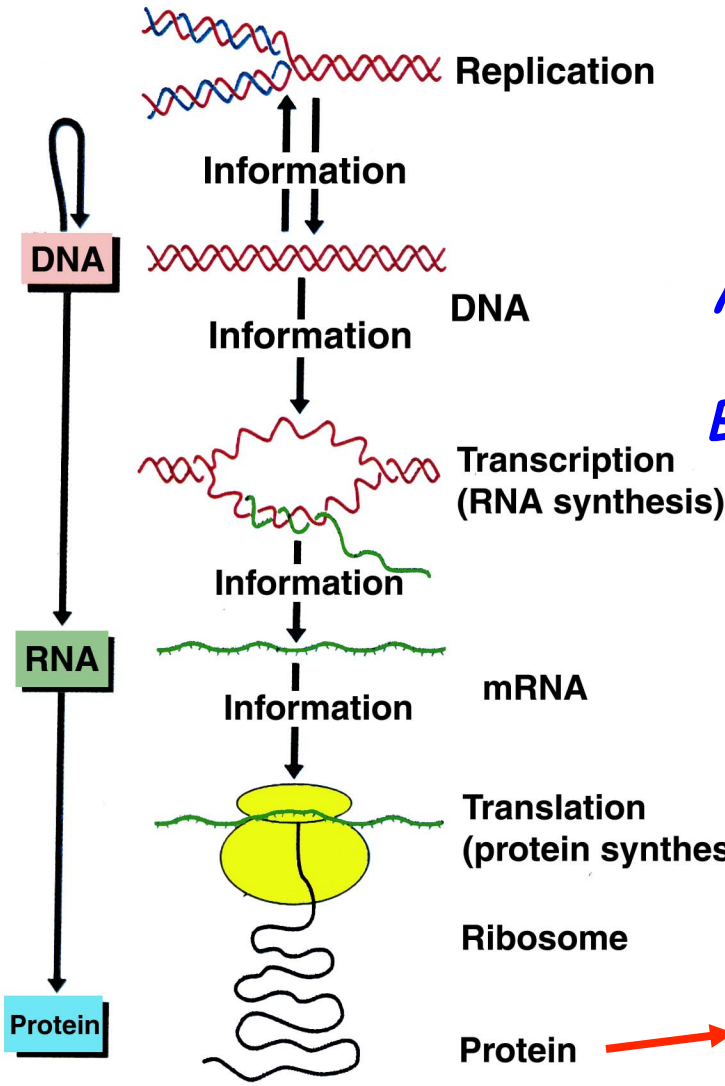
Can Follow These Traits With DNA Markers As Well

mutations result in genetic diversity!!!

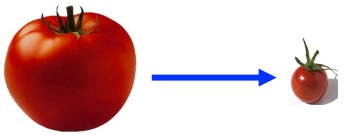
Spontaneous Mutations Give Rise To Alleles, or Different Forms of the Same Gene, And result in Small DNA Sequence Changes (e.g., SNPs or Single Nucleotide Polymorphisms)

Translating The Genetic Code Into Proteins is a Conserved Process

Mutations Are Inherited Because Altered Gene Replicates

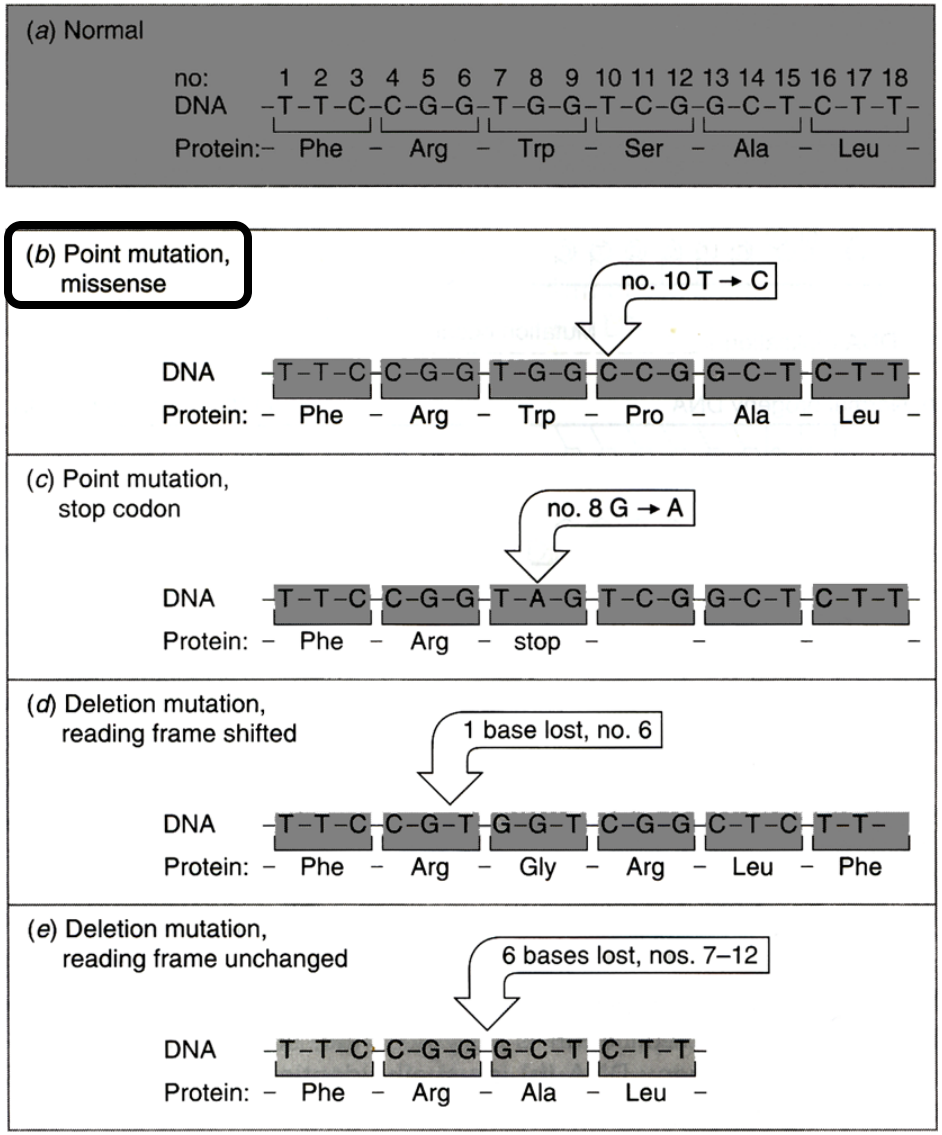


Mutations Lead To Altered Protein Because mRNA and Protein Sequence Encoded By Gene Changes



Mutations Lead to Altered Traits/Phenotype Because Protein Structure Changed

Mutations Can Occur Different Ways



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

Function of Protein Lost and/or Changed

∴

Phenotype Changes

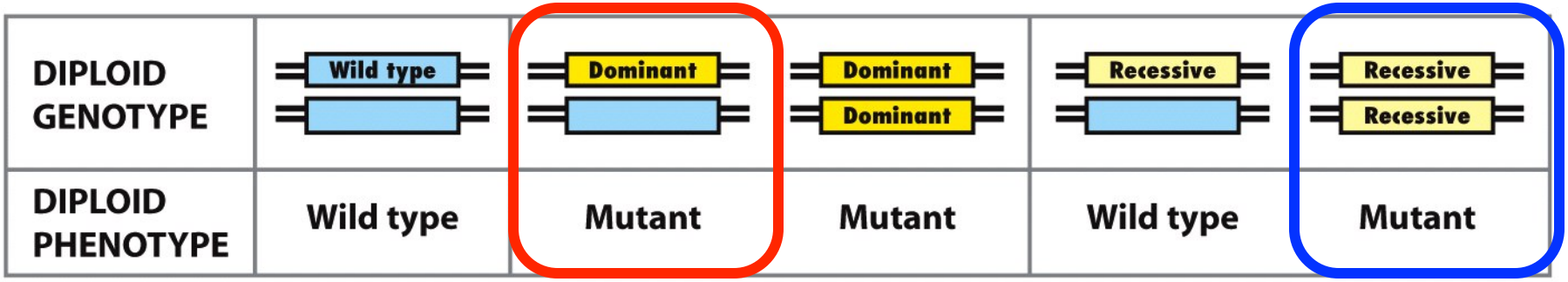
Human Genetic Disorders Occur As a Result of Mutations

TABLE 13.2 Some Important Genetic Disorders

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

Dominant

Recessive

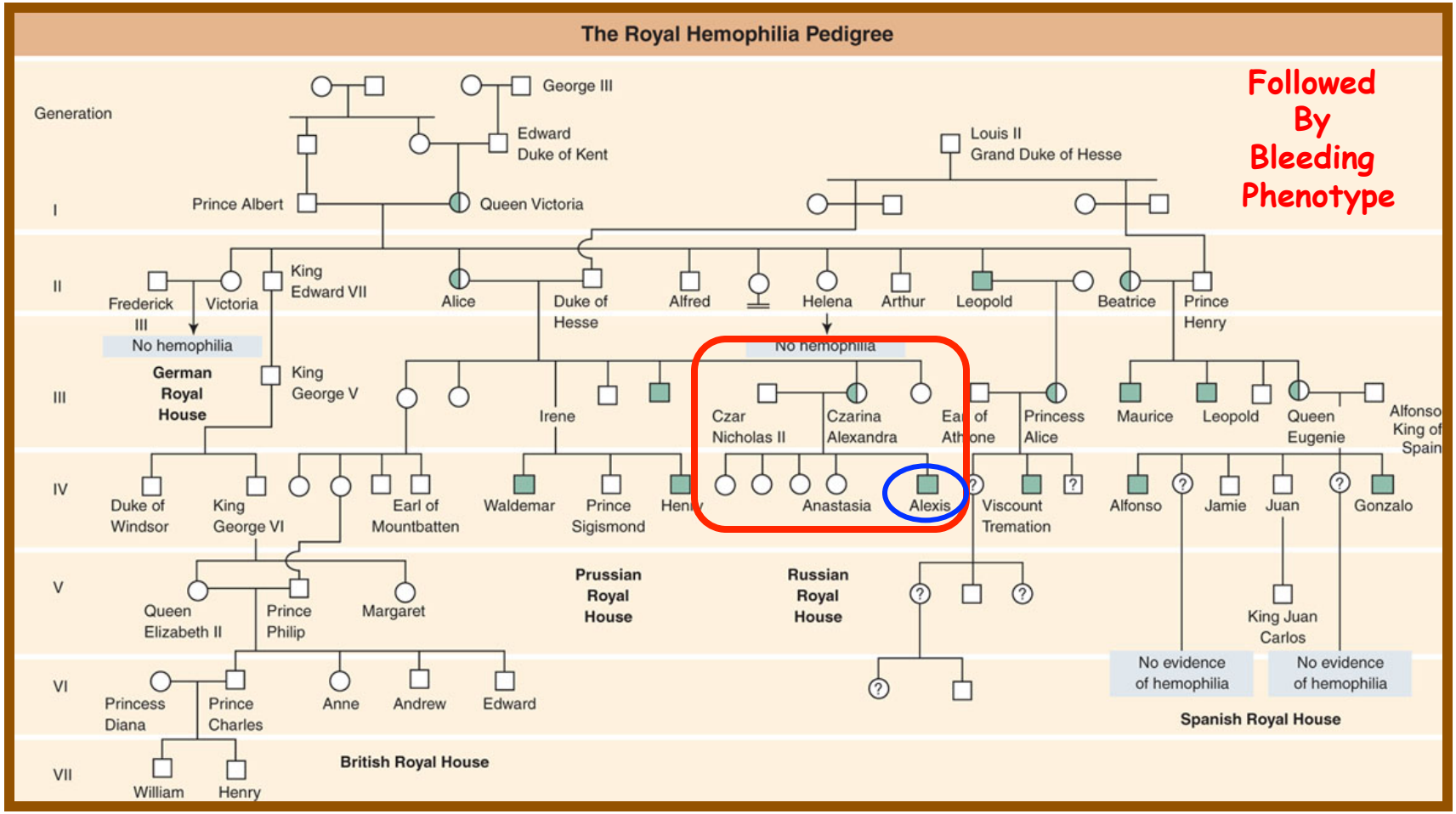


Need One Allele

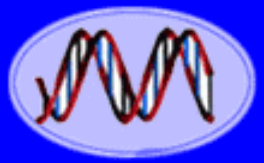
Need Two Alleles

Figure 5-2
Molecular Cell Biology, Sixth Edition

Pedigrees Can Be Used To Follow Disease Genes in Human Families



Recessive Sex Linked



DNA
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Entire Genetic Code
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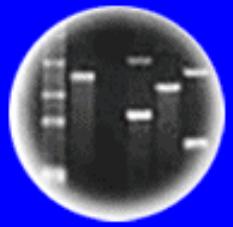

DNA Fingerprinting

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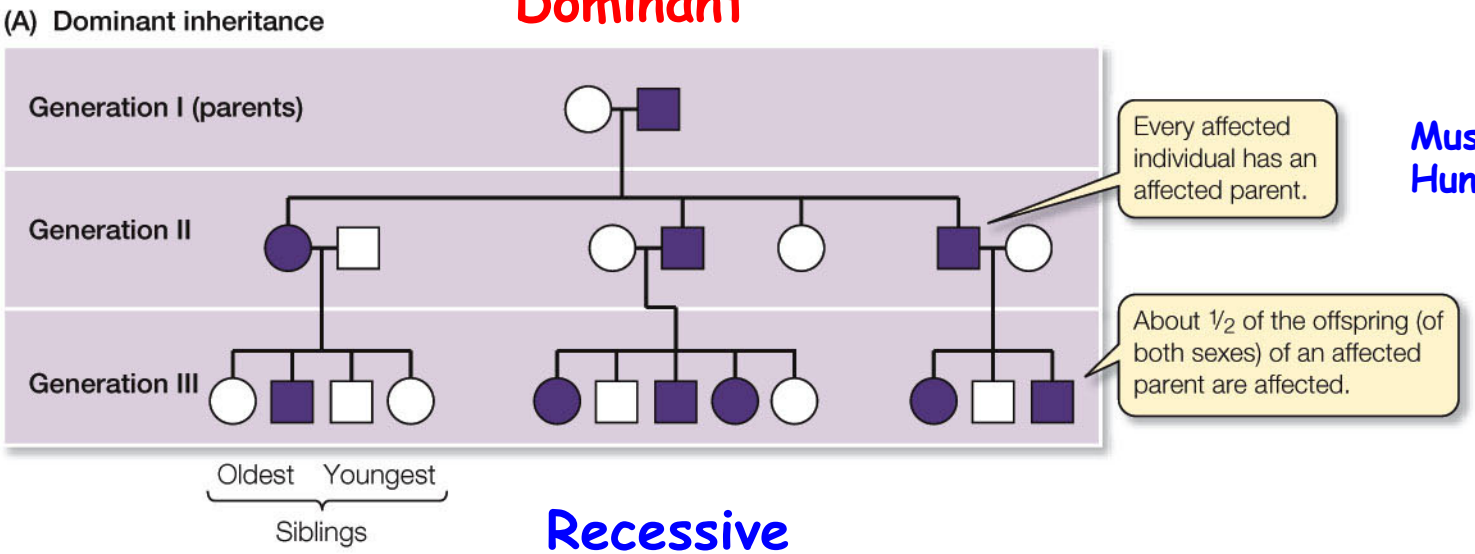
Pedigrees Can Be Used To Determine If a Trait is Dominant or Recessive

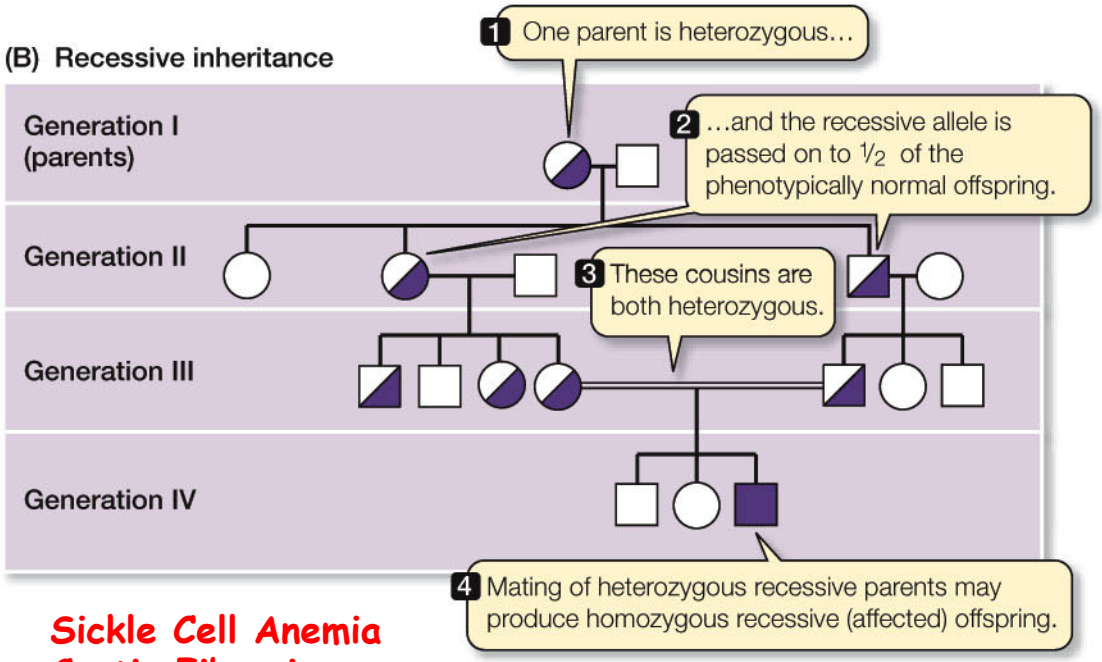
Each Type of Inheritance Predicts Specific Results in Each Generation

Dominant

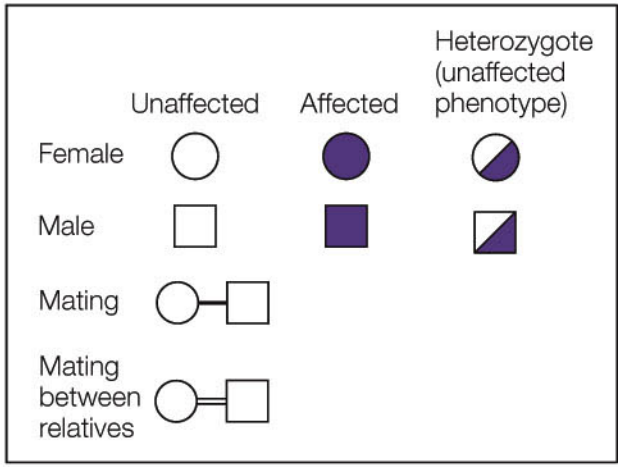


Muscular Dystrophy
Huntington Disease

Recessive

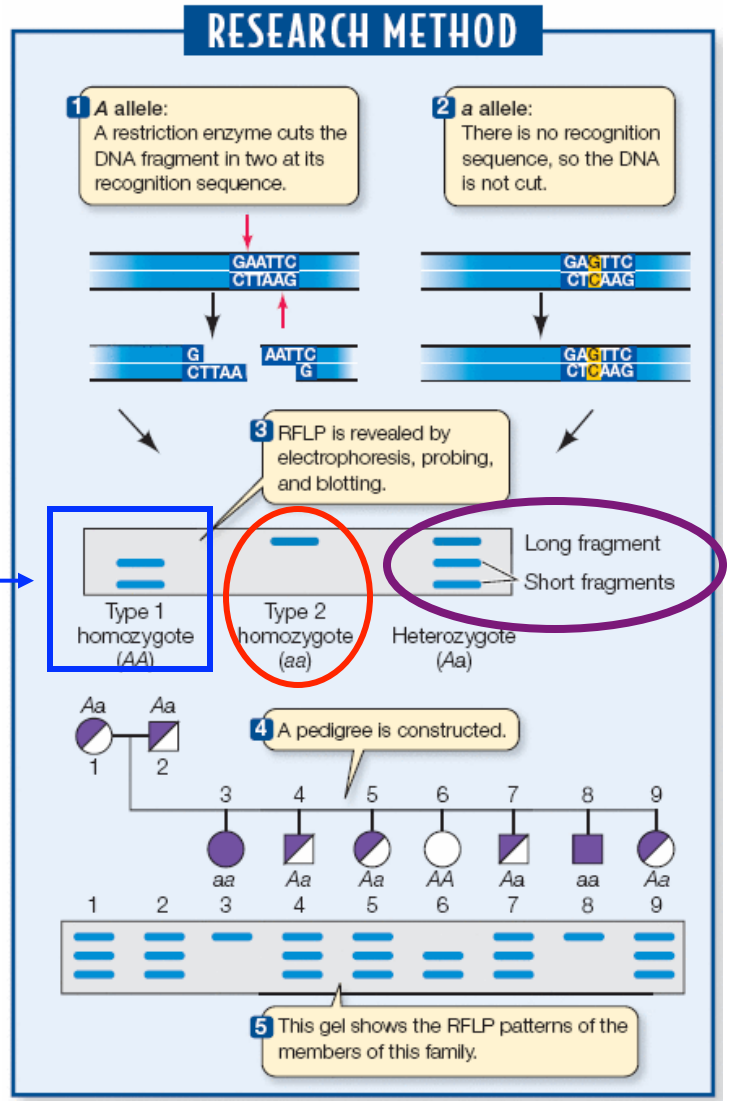


Sickle Cell Anemia
Cystic Fibrosis
Tay-Sachs Disease



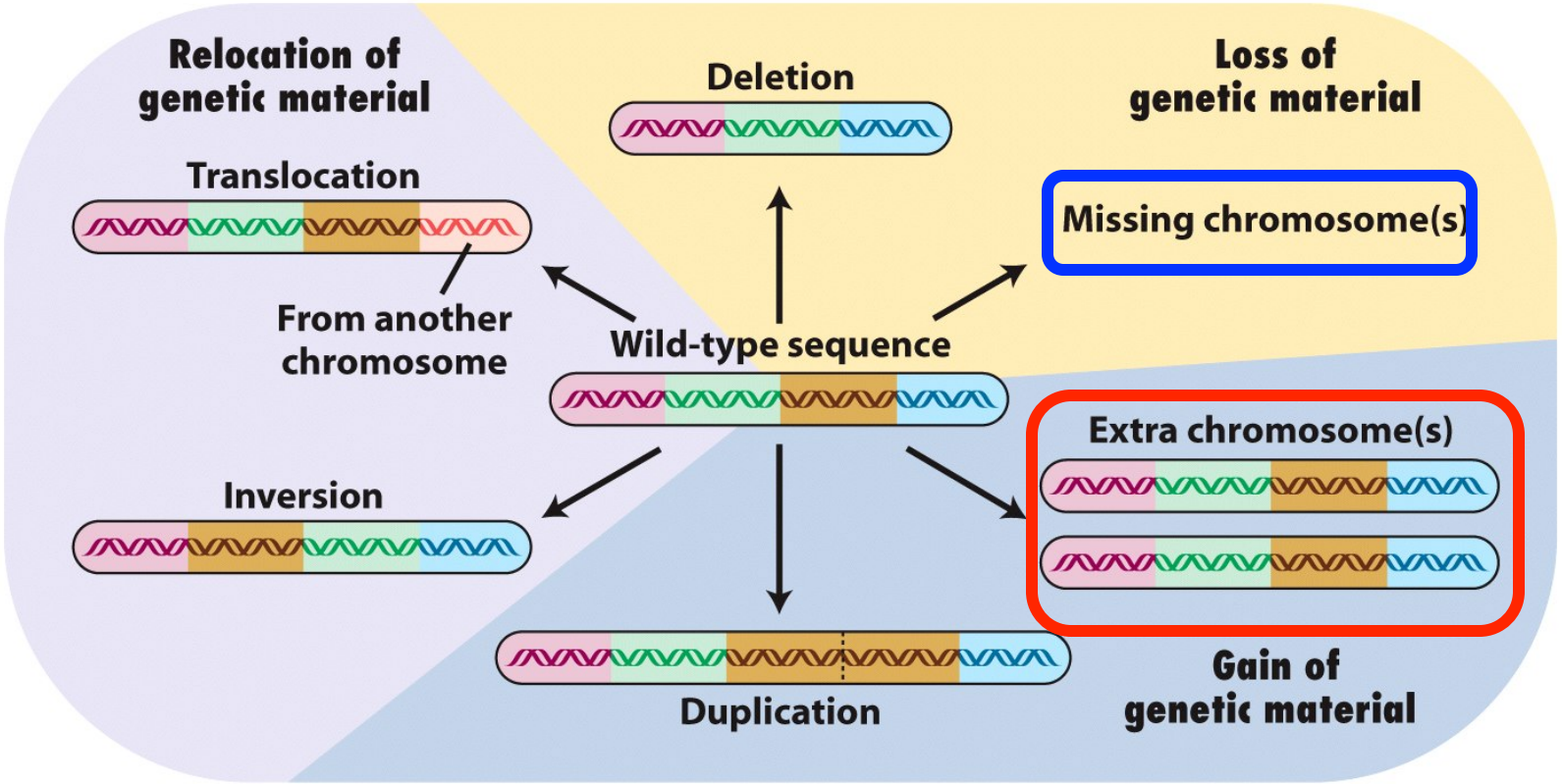
Genetic Diseases Can Be Followed in Families Using Molecular Methods (e.g., DNA Blots or PCR)

DNA Fingerprints →



← RFLP –Restriction Fragment Length Polymorphism

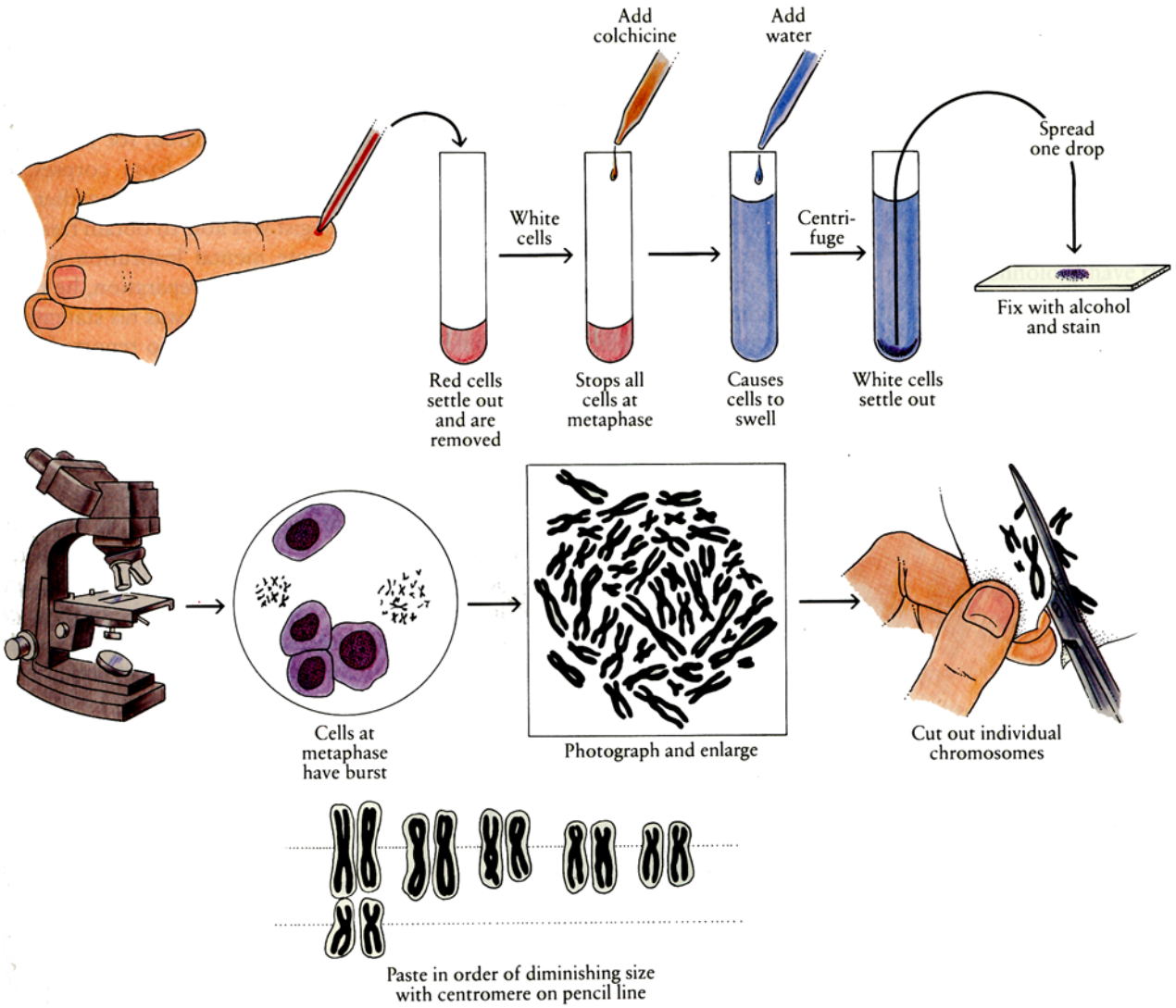
"Mutations" Can Also Occur By Large Chromosomal Changes



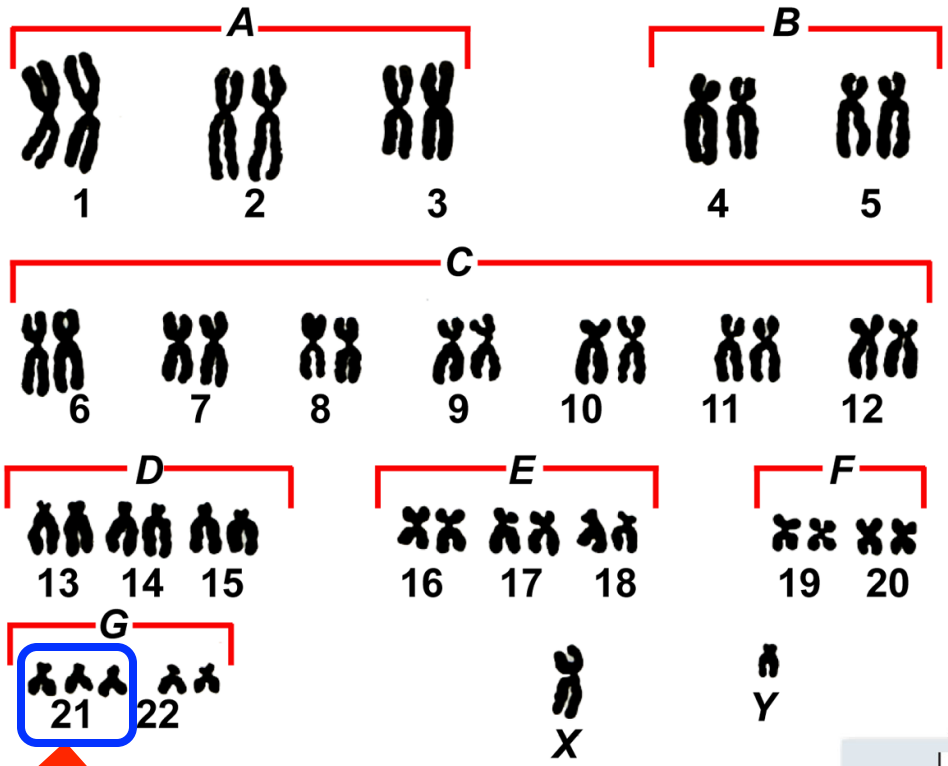
These changes affect many genes!

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

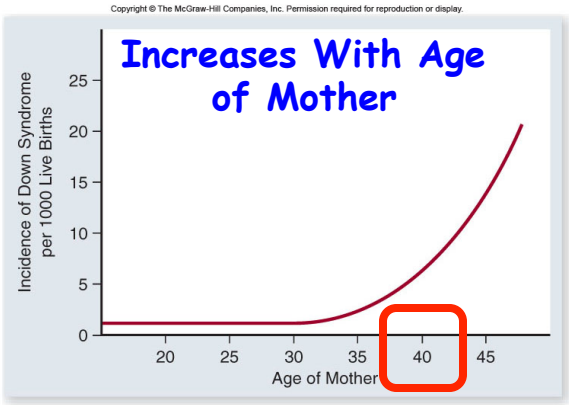
Karyotypes Can Be Used To Detect Changes in Chromosome Structure and Number



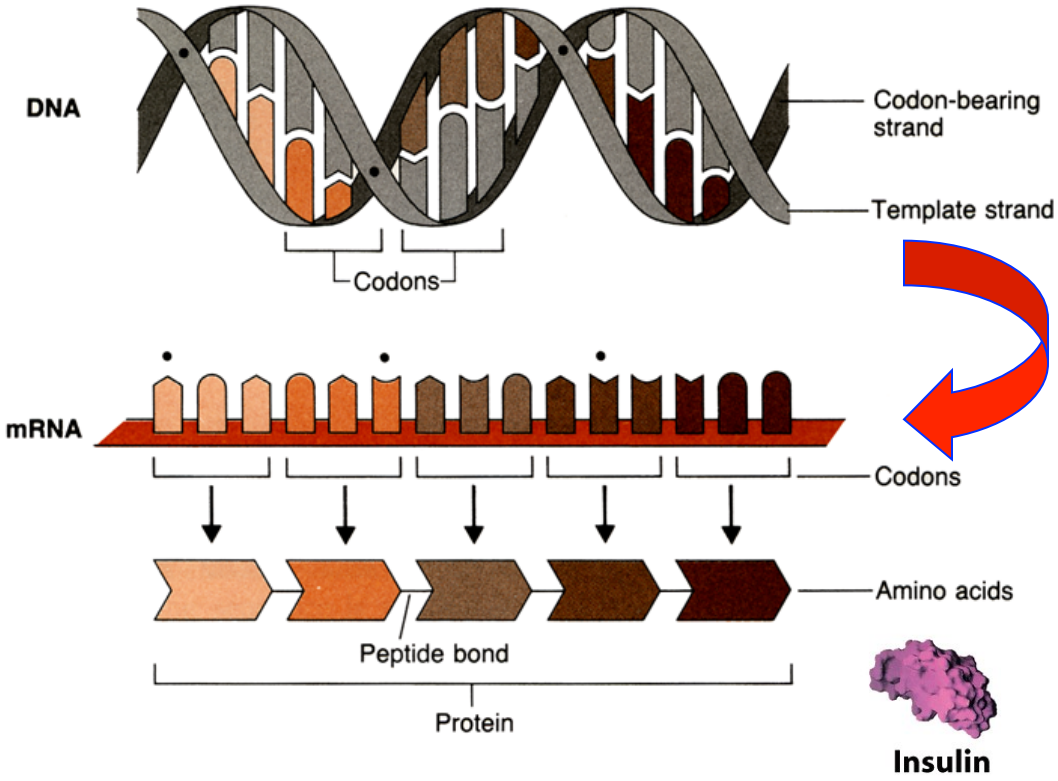
A Down's Syndrome Karyotype



Three Chromosome # 21s



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

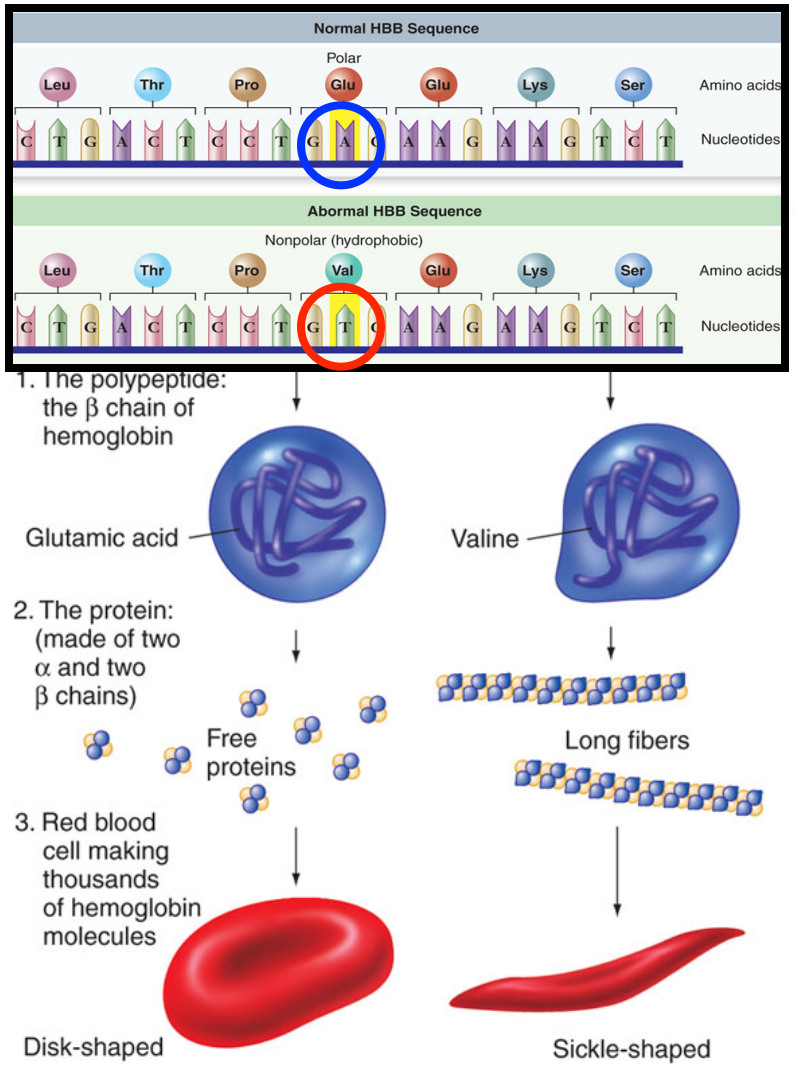
∴ Sequence of Gene
Sequence of mRNA
Sequence of Protein

Colinearity of Sequences!

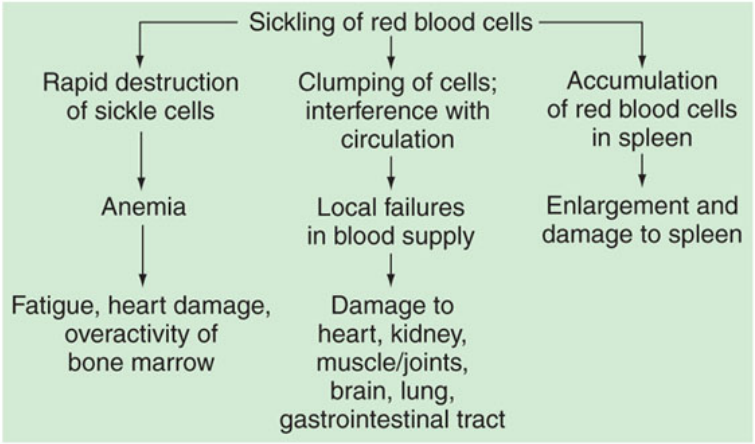
Know Sequence
Know Protein → Engineer New Protein

Human Genetic Disorders Occur As A Result of Mutations

Chromosome 11



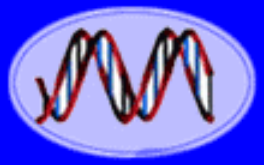
(b) Sickle-cell anemia is pleiotrophic



(c) β-chain substitutions/variants

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

Sickle-Cell Anemia



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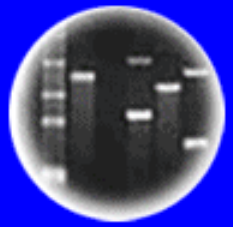

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and Future Consequences

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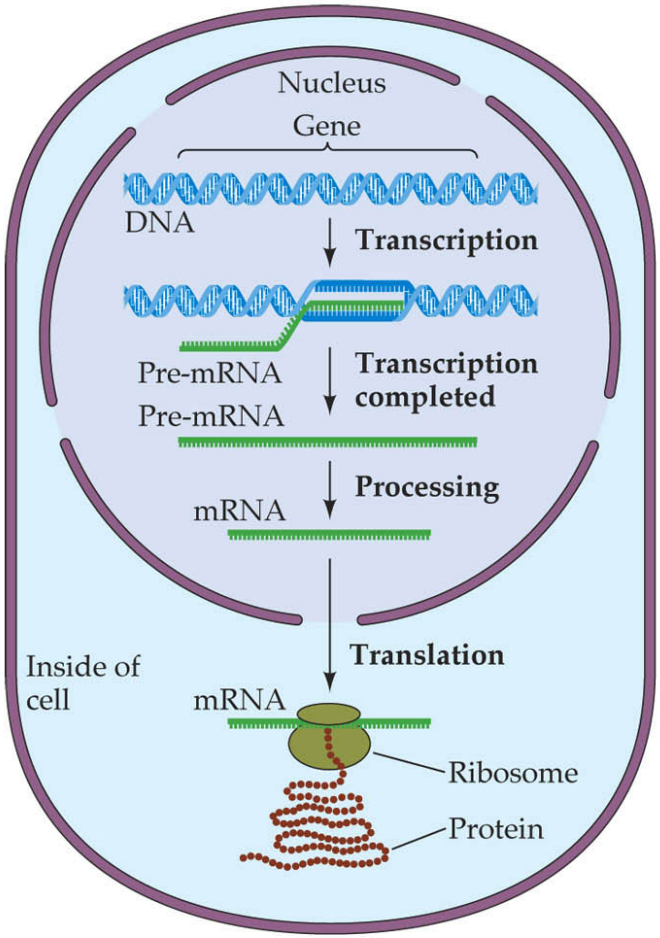
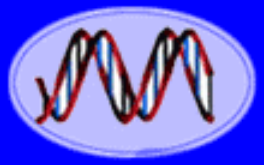
An Elaborate Cellular Machinery Requiring Thousands Of Genes is Required To Produce Proteins Encoded By Specific Genes!!



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

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



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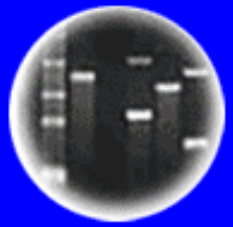

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Unique Proteins Have A Unique Composition & Order of Amino Acids & Have Unique Sizes, Shapes, & Functions

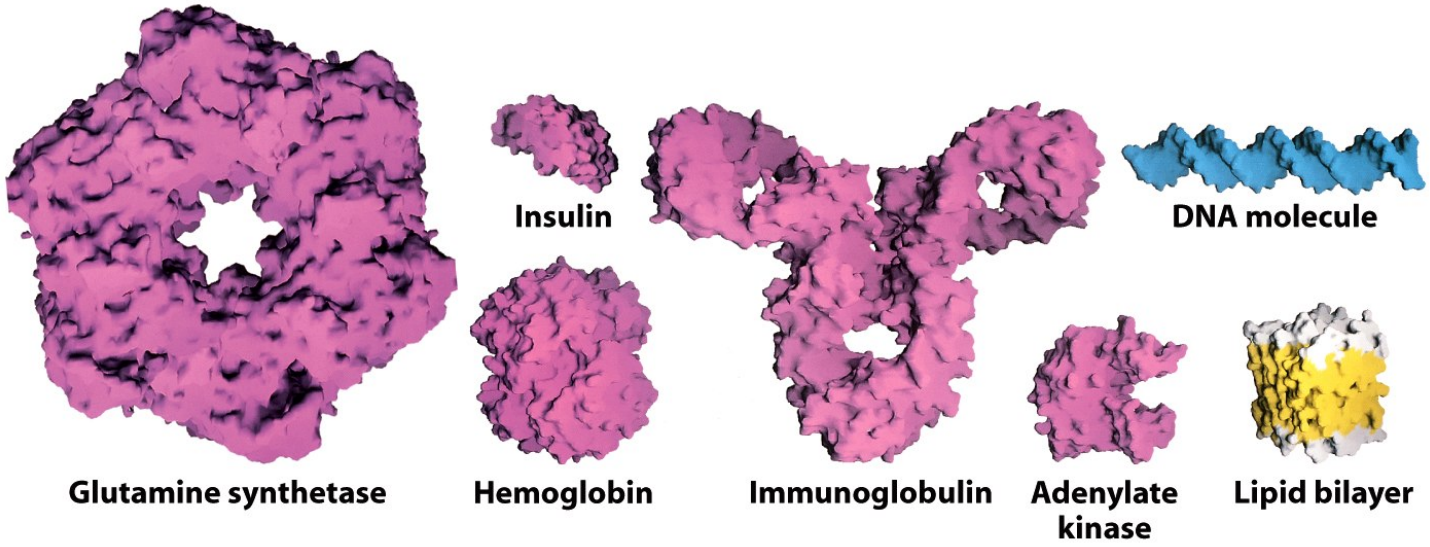
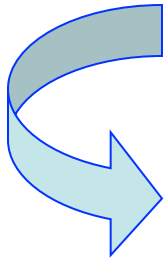
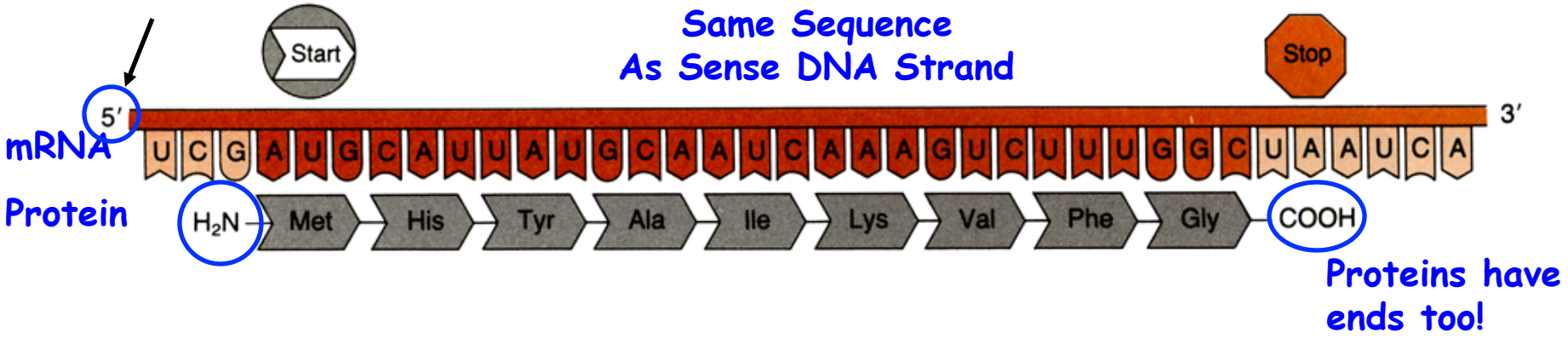


Figure 1-9
Molecular Cell Biology, Sixth Edition



Novel Cell Functions & Phenotypes

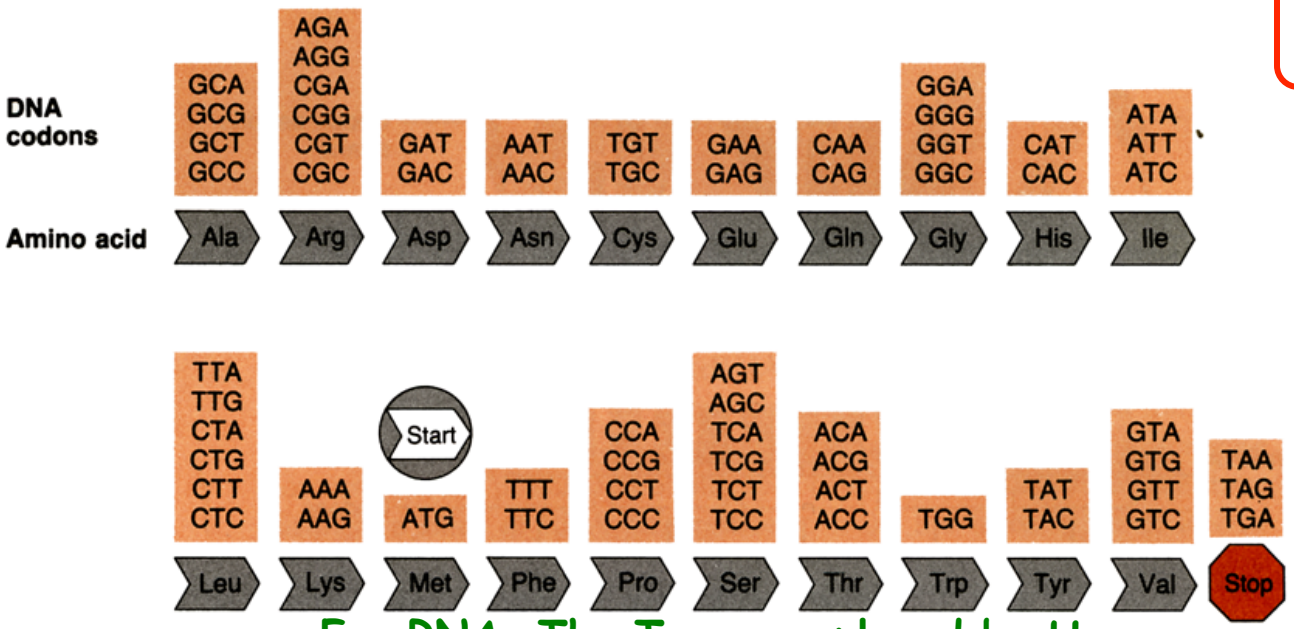
Genetic Code Allows The Sequence of Nucleotides in mRNA/ sense strand of Gene to be Translated into Sequence of Amino Acids in Proteins



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 Nts in gene = order amino acid in protein!

The Genetic Code is Universal!

How Know?



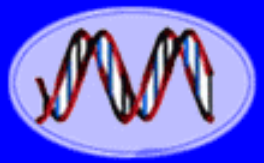
For RNA, The Ts are replaced by Us.

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

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

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

Design An Experiment to Show Code is Universal!



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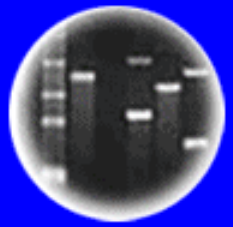

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Expression of Jellyfish Green Fluorescence Protein (GFP) in Pigs Shows That Genetic Code is Universal!!

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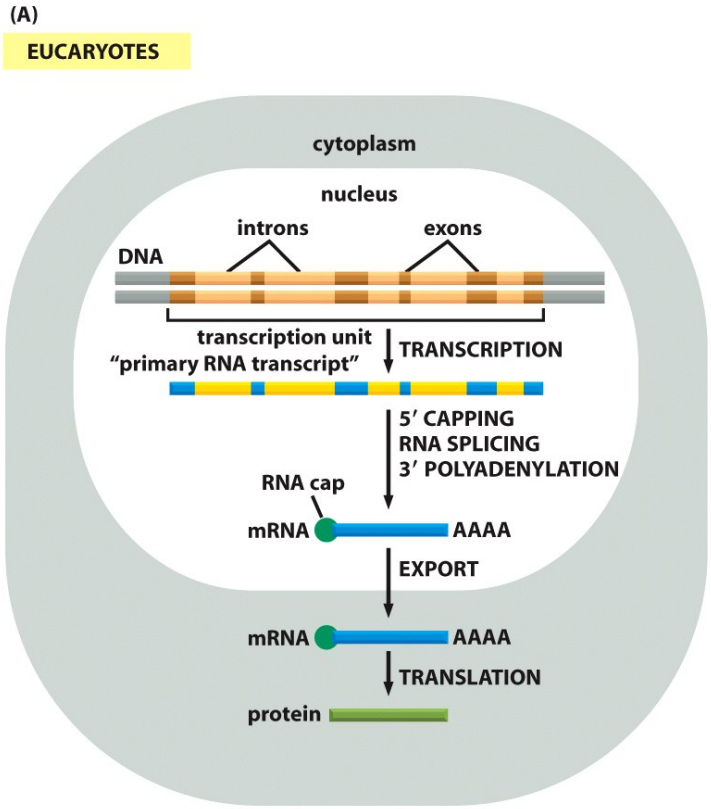
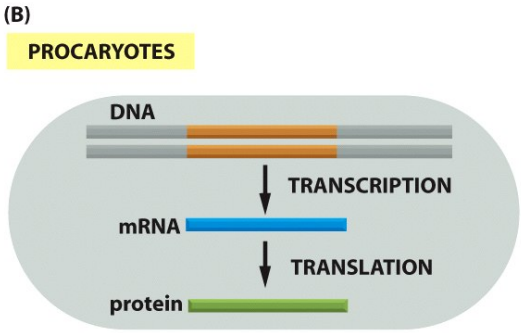
Eukaryotic and Prokaryotic Gene Expression Processes Differ Slightly



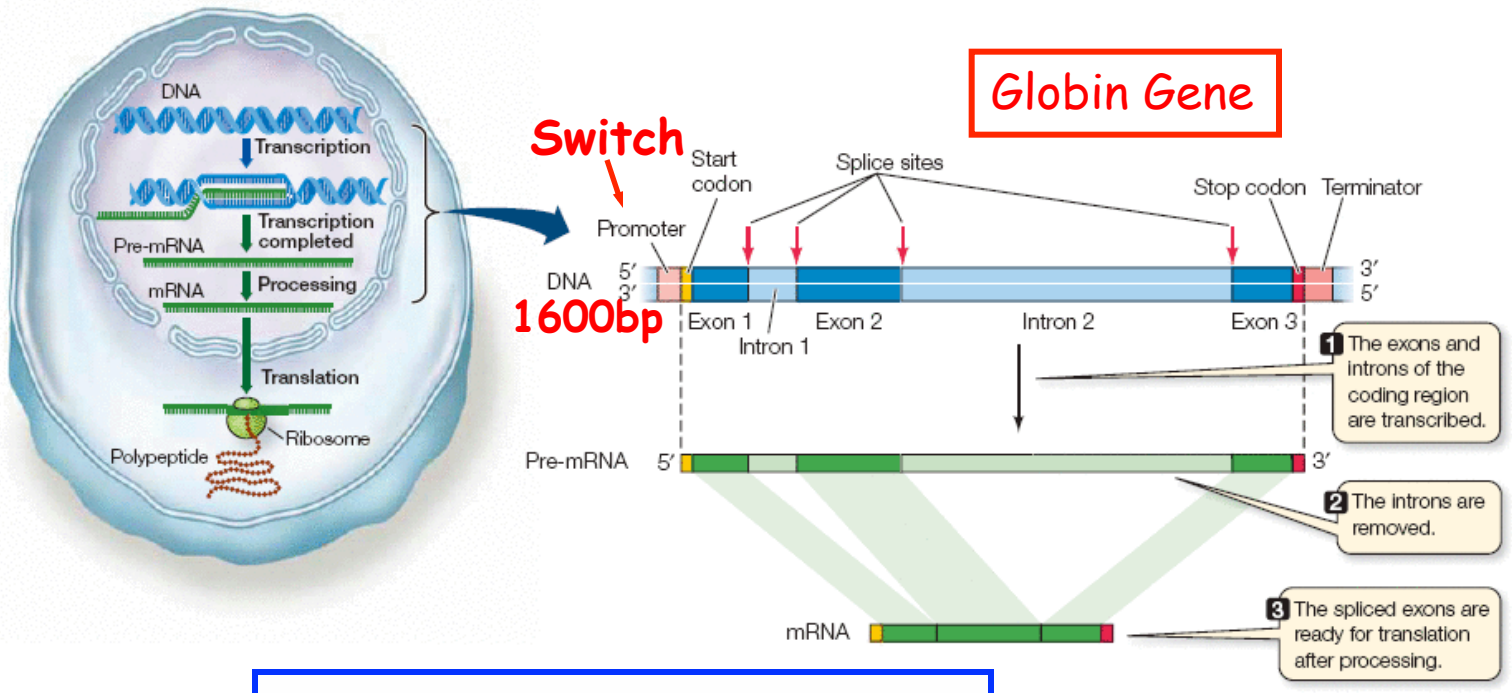


Genes Differ
Switches Differ
Genetic Code the Same
General Processes Same
Eukaryotic Gene Have Introns & Non-Coding Region in Gene!

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

What Are the Implications For Genetic Engineering?

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



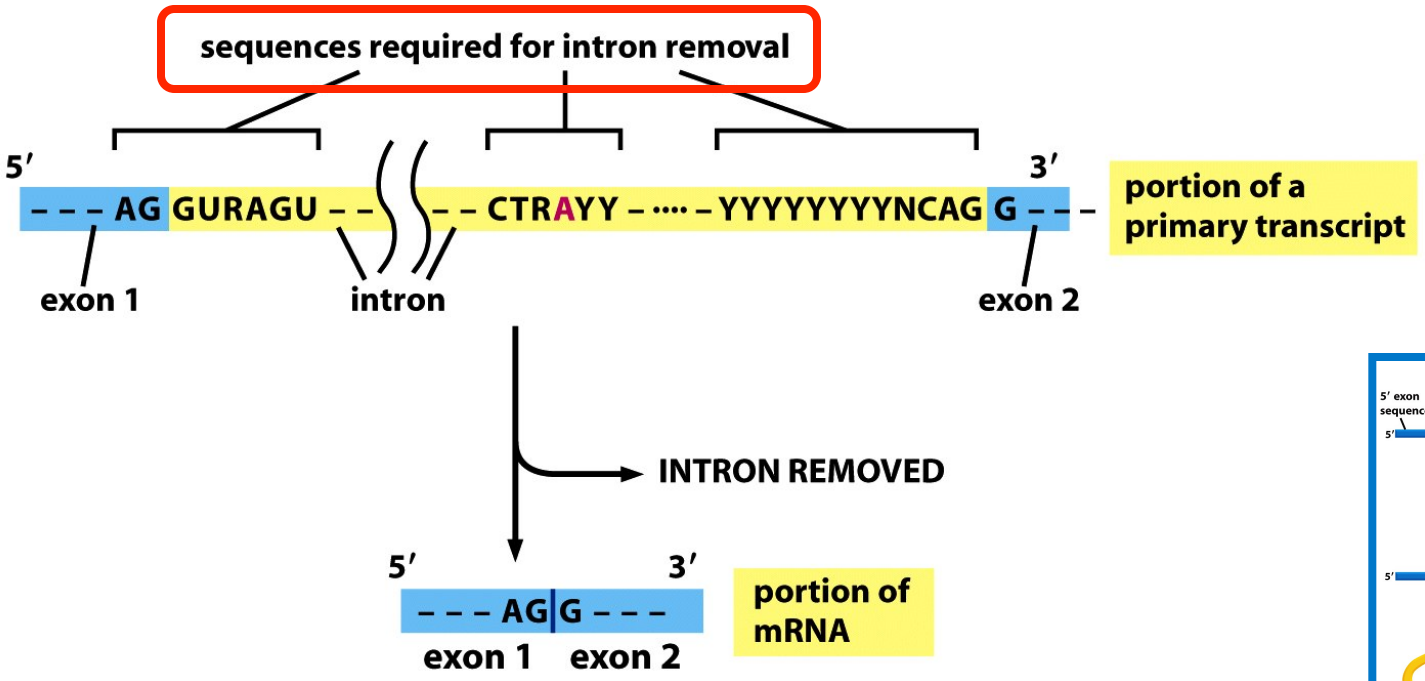
Mutations→ Blood Disorders
Where can these occur?

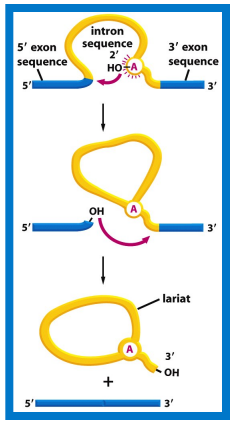
Mutations Can Occur in Coding Region, Switch, & RNA Splice Sites

→ Mutant Phenotype

Implications For Engineering Eukaryotic Gene in Bacterial Cell For Expression?

Yo! It's In The Sequences!

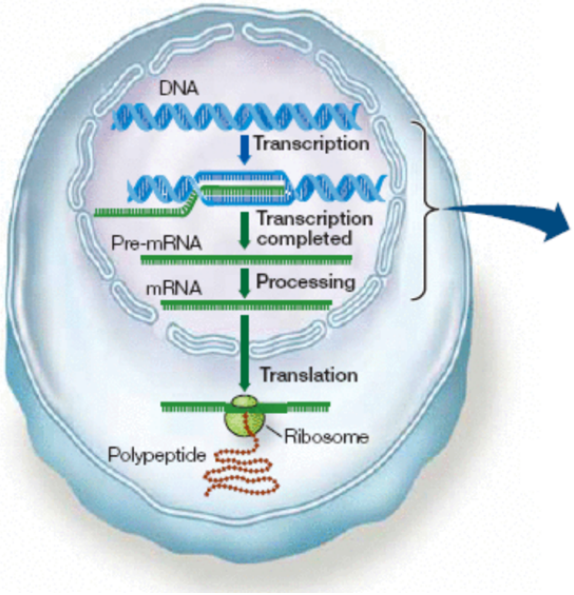




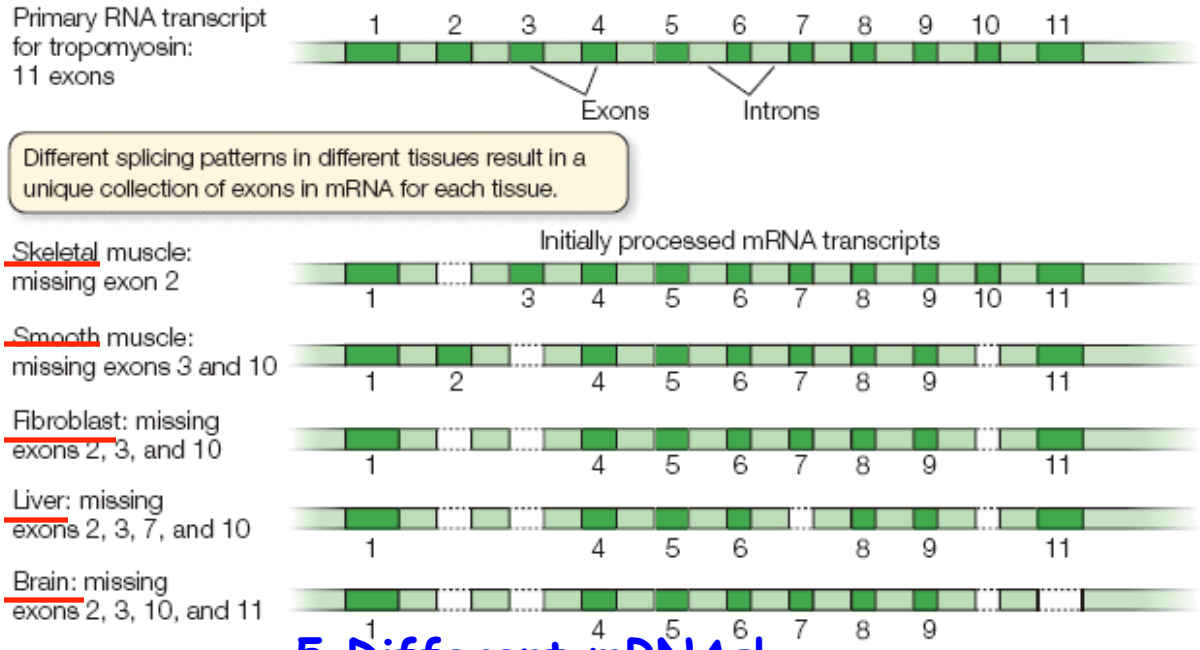
Specific Sequences Required For RNA Splicing!

What Happens If These Sequences Are Mutated in a Gene?

Alternative Splicing- One Gene → Several mRNAs & Proteins



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



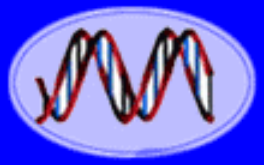
5 Different mRNAs!

Different mRNA = Different Proteins = Different Functions!

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

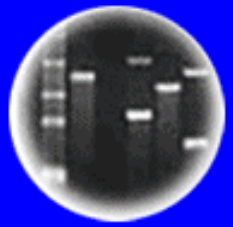
Reason Why Human Genome Can Contain Same Number of Genes as Fly and Plant Genomes!!

Implications for Genetic Engineering? Use Specific cDNA!



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



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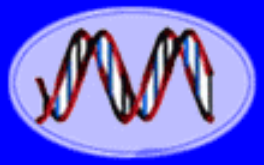
Implications For "Yo - Its in The DNA!!"

Modular Organization of Sequences

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

Modules → Anything You Want To Do Using Genetic Engineering!



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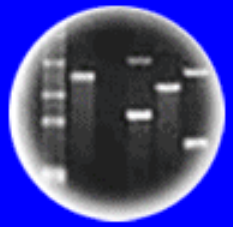

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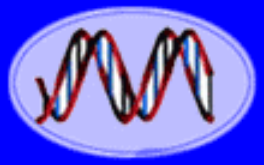
Summary: Engineering Genes Requires:

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 part)
5. Translation Start And Stop Switches
6. Kingdom-Specific Switches/ Signals

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

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

→ Human Insulin in Bacteria!!



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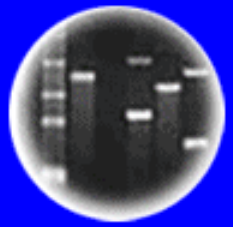

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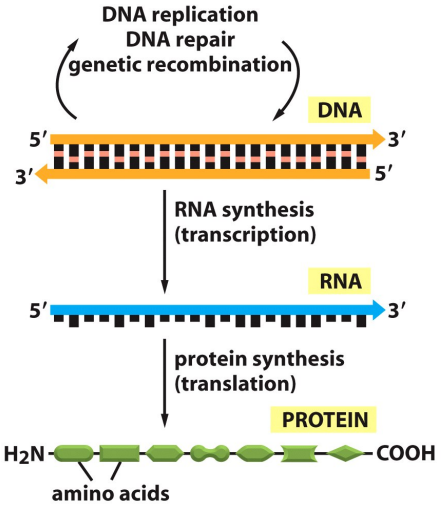
How Do Genes Work & What are Genes in Context of…

Thinking About The Consequences of GMOs



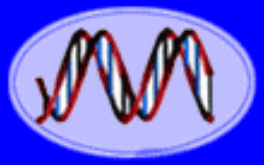
1. What is a Gene?
2. What is the Anatomy of a gene?
3. How Does the Gene Replicate?
4. How Does the Gene Direct Synthesis of a Protein?
5. Does the Gene Work Independently of other Genes?
6. What is the Sequence & Structure of the Protein?
7. How does it work in cell?
8. Does the Protein Structure imply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

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

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

"Behind" All Traits!

Same Processes!



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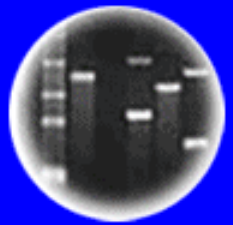

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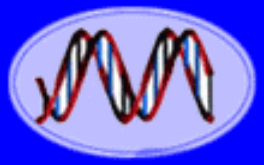
Can Identical Twins Be Different?

"Things Written in Pen You Cannot Change. That's DNA!
Things Written in Pencil You Can. That's Epigenetics"
Geneticist Danielle Reed



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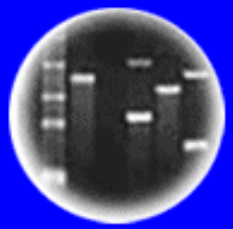

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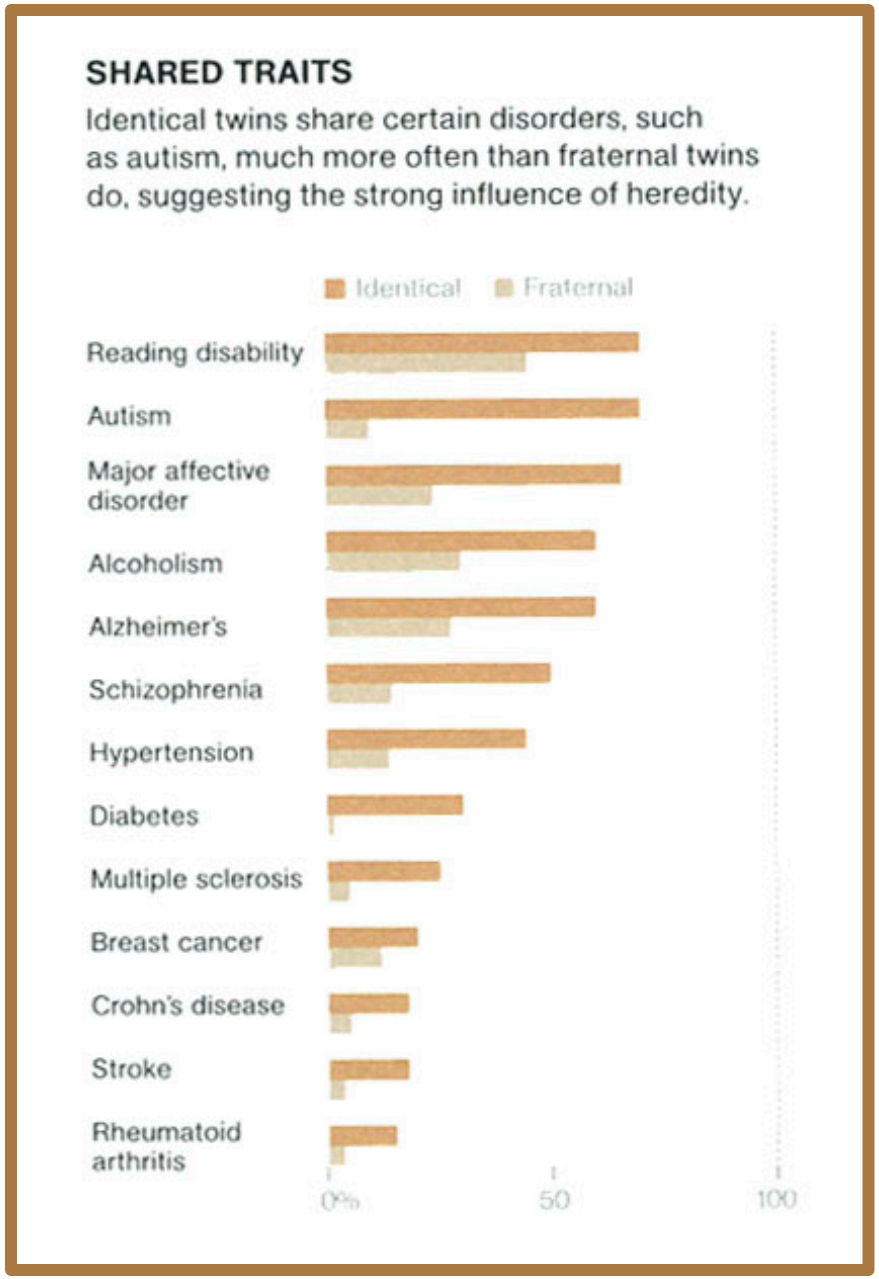
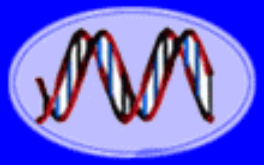
Nature vs. Nurture?



Because Genes Replicate Generation to Generation!

But Environment Can Play a Role

We Are Beginning to Learn Why!



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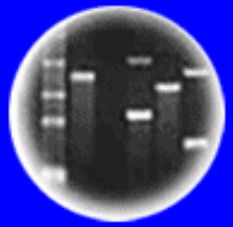
Entire Genetic Code of a Bacteria

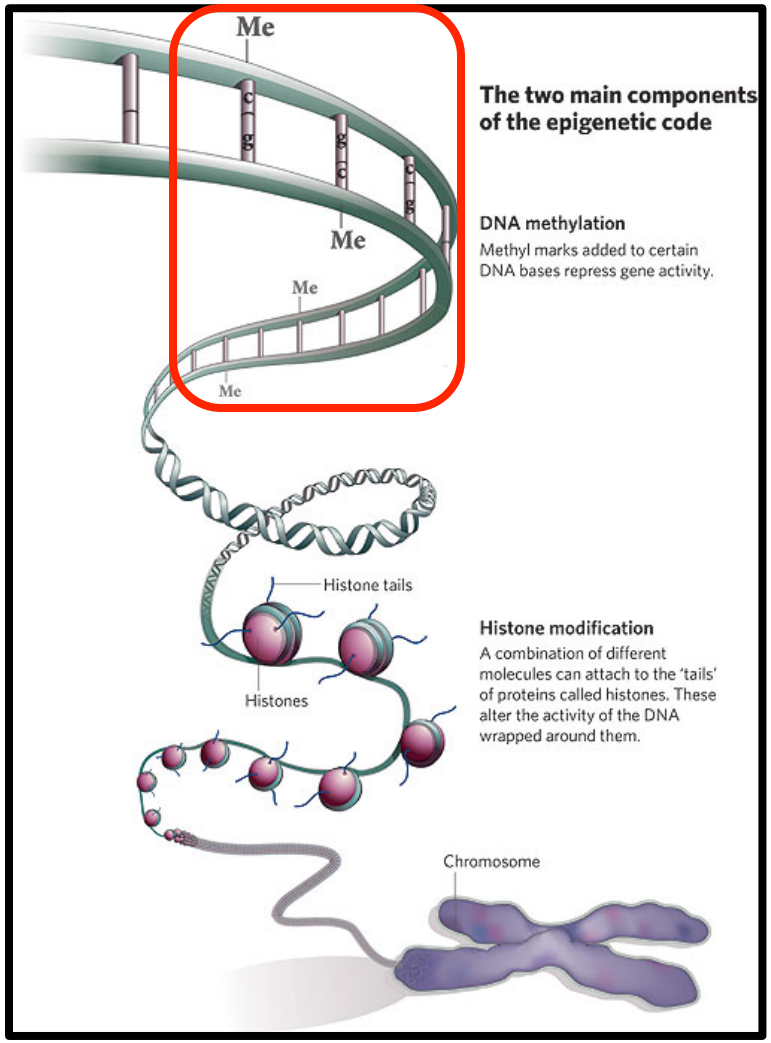


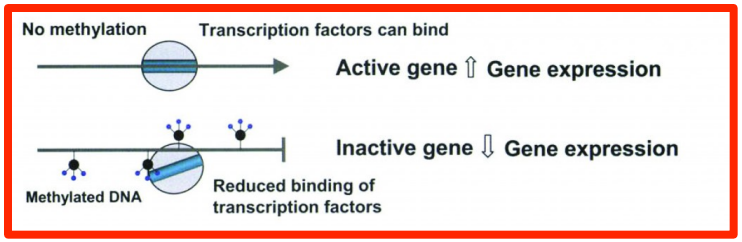
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

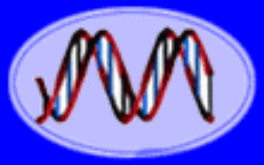
Plants of Tomorrow

Epigenetic Changes, or Chemical Modifications of Switches and Genes, Can Affect Gene Activity!





These Changes Are Re-Set Each Generation, but Environmental Factors Can Influence Modification of DNA



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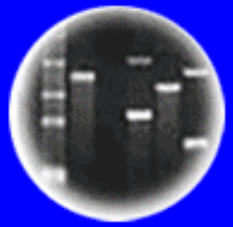

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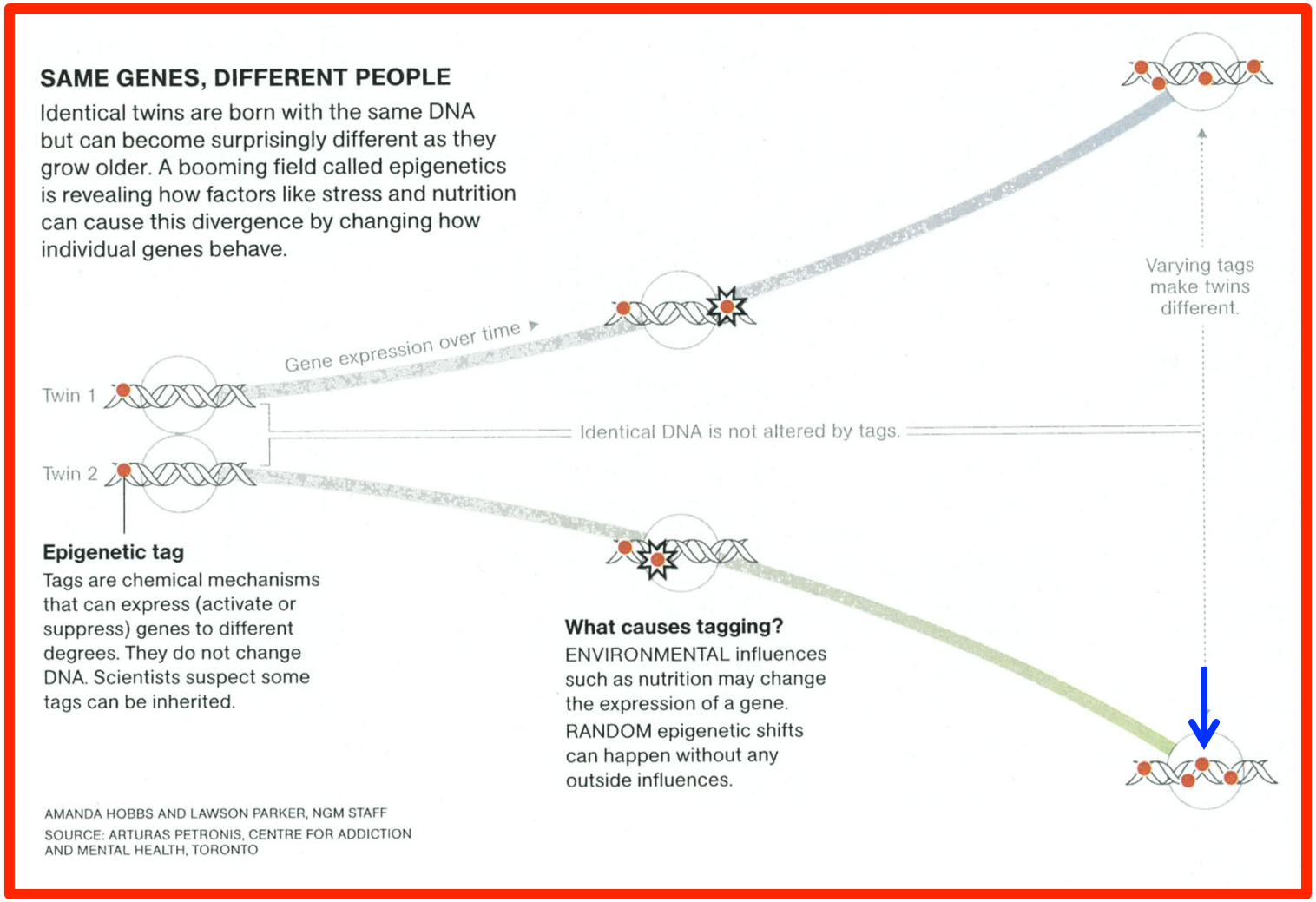
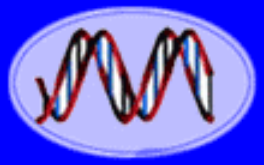
Nature vs. Nurture?



Rare Epigenetic Events Can Affect Individuals Differently!



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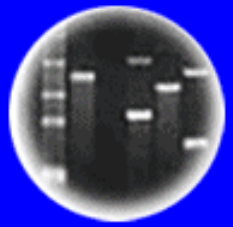

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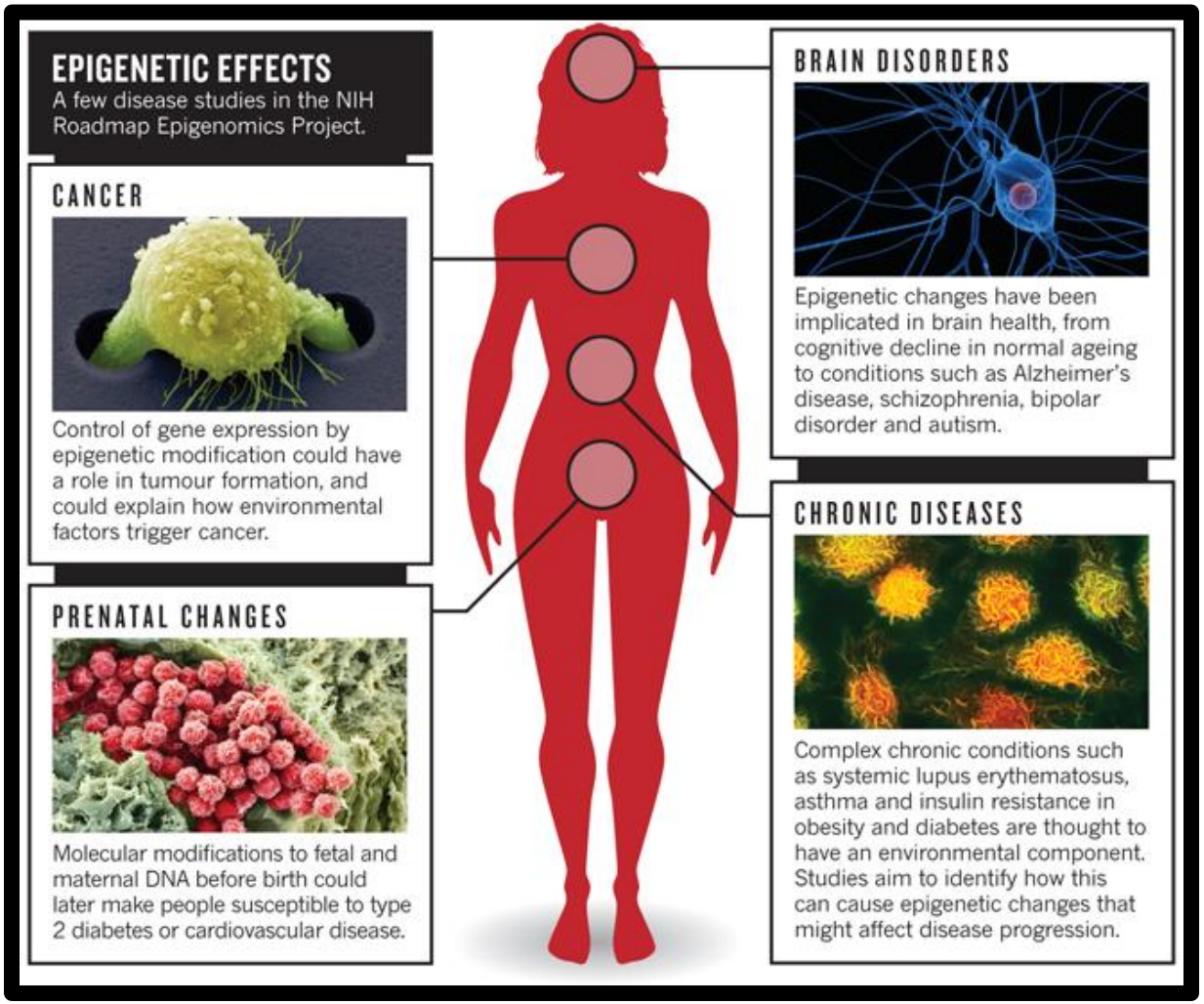
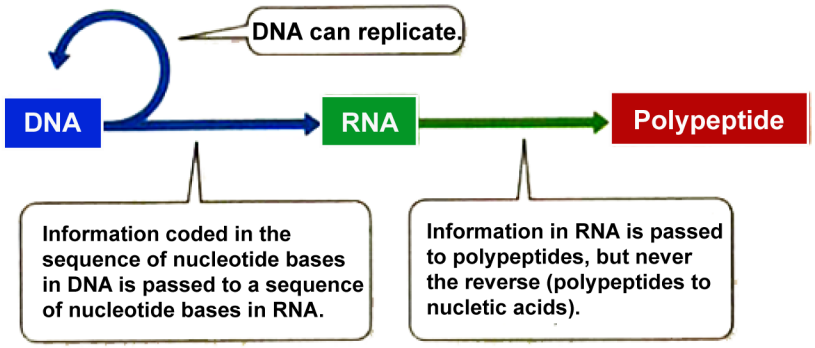
Epigenetic Effects in Disease?



Rare Epigenetic Events Affect Individuals Differently!

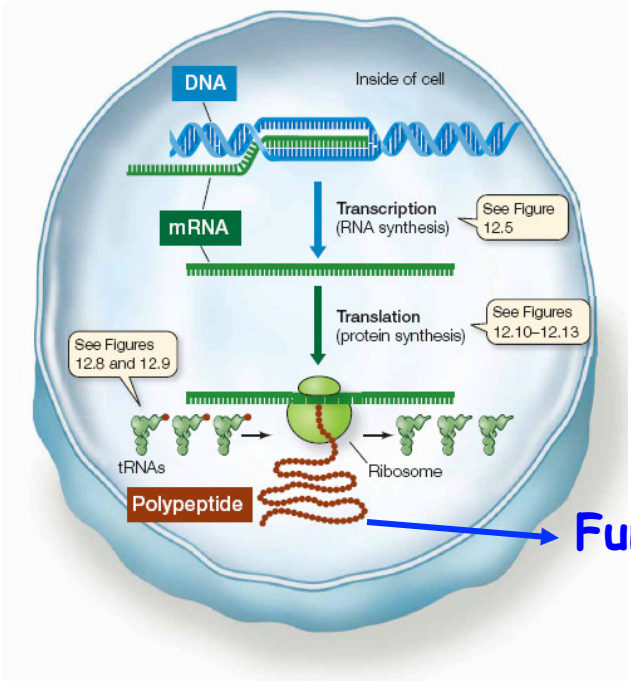
How Do Genes Work-Not As Simple As We Think!

① Replication



② Gene Activity to Function & Phenotype

Gene Activity → Protein → Function → Phenotype (Trait)



Function →

But Precise Cellular Rules Are Followed That We Can Use For Genetic Engineering!