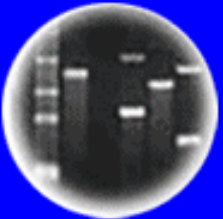


DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

HC70A, PLSS530, & SAS70A Winter 2012 Genetic Engineering in Medicine, Agriculture, and Law

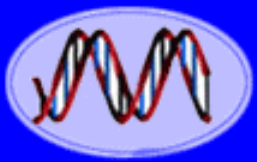
Professors Bob Goldberg,
Channapatna Prakash, & John Harada

Lecture 5 What Are Genes & How Do They Work: Part Three

UCLA



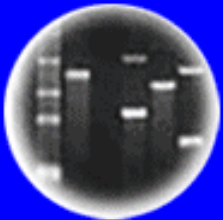
UC DAVIS
UNIVERSITY OF CALIFORNIA



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and Future Consequences

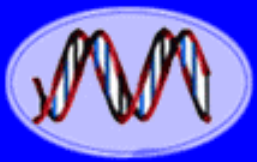


Plants of Tomorrow

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

1. **What is a Gene?**
2. **What are the Structure & Chemical Properties of DNA?**
3. **How is Polarity in DNA Established & Lead to Biological Uniqueness?**
 - a) **What is the Role of Deoxyribose Sugar in Establishing Polarity 5' to 3'? The Hub!**
 - b) **How Does the Formation of Phosphodiester Bonds Lead to 5' to 3' Polarity and Unique Sequences?**
4. **Where Are Genes Located in the Cell?**
 - a) **What are Chromosomes and What Do They Do?**
 - b) **What is the Structure of Eukaryotic Chromosomes? How do they Fit into a "Small" Nucleus**
 - c) **What is the Evidence That Genes Work Independently of One Another? What is Colinearity Between Genes & Proteins?**
5. **What Are Alleles & How Do They Contribute to Genetic Diversity?**
6. **What is the Anatomy of a Gene?**
7. **What Are Control Sequences & How Do They Program Development?**
8. **How Does the Eye Gene Control Eye Development?**
 - a) **How Does the Eye Genetic Regulatory Network Work to Program Eye Formation?**
9. **How Can an Eye Be Produced on a Leg Using Genetic Engineering?**
10. **Demonstration - DNA Fingerprinting**

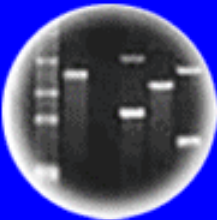
TODAY'S THEMES



DNA
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DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences

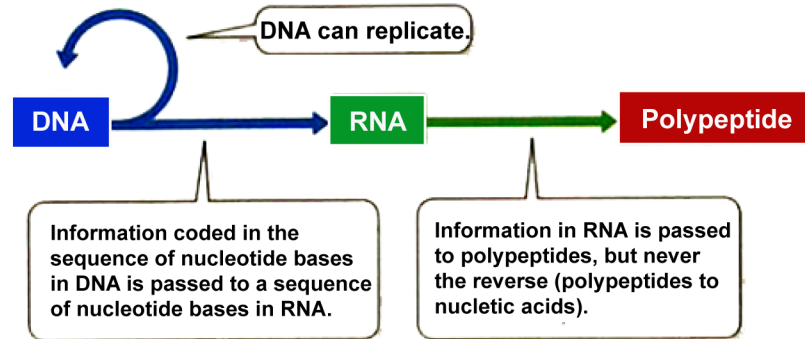


Plants of Tomorrow

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

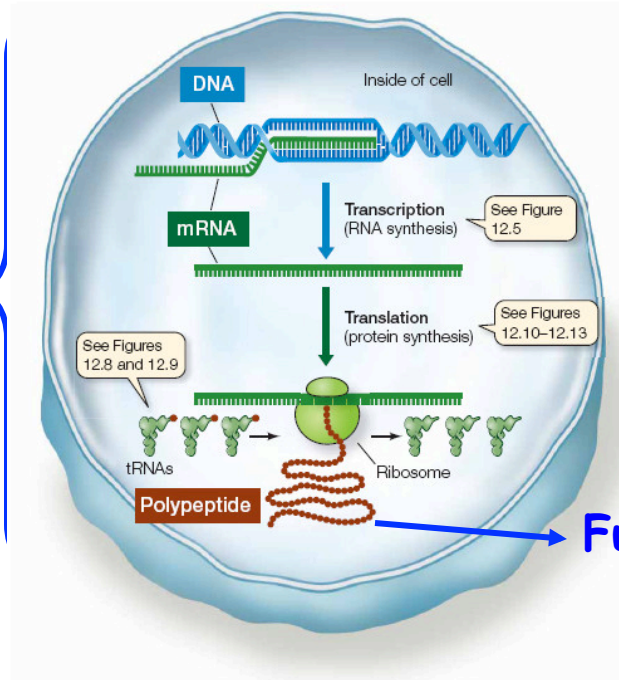
How Do Genes Work-A Review

① Replication

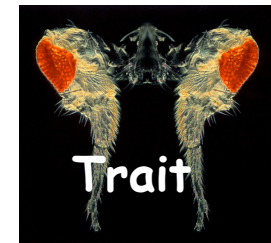


② Gene Activity to Function & Phenotype

Gene Activity
↓
Protein
↓
Function
↓
Phenotype (Trait)



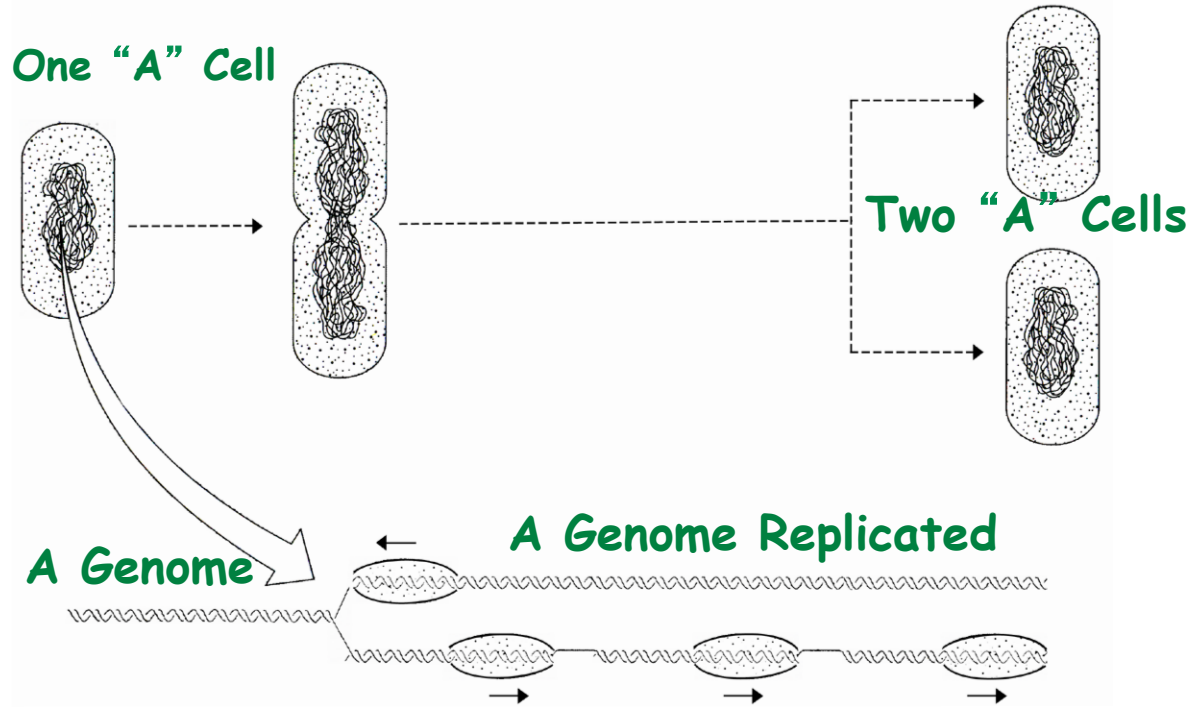
Function →



A Gene is NOT Expressed Unless A Functional Protein Produced!

1

How Are Genes Replicated Each Cell Generation?

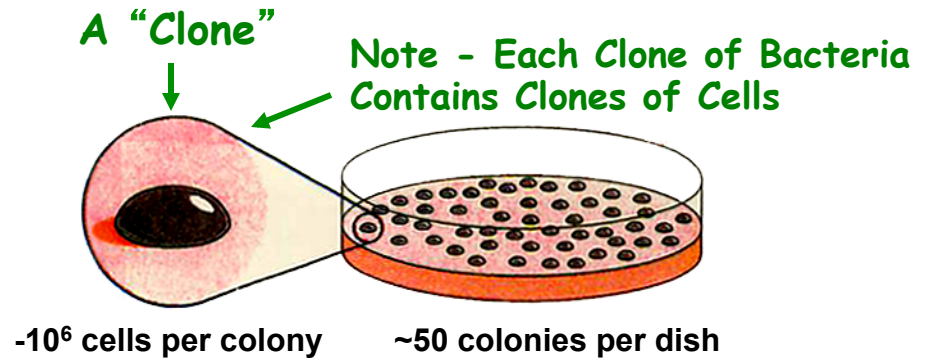
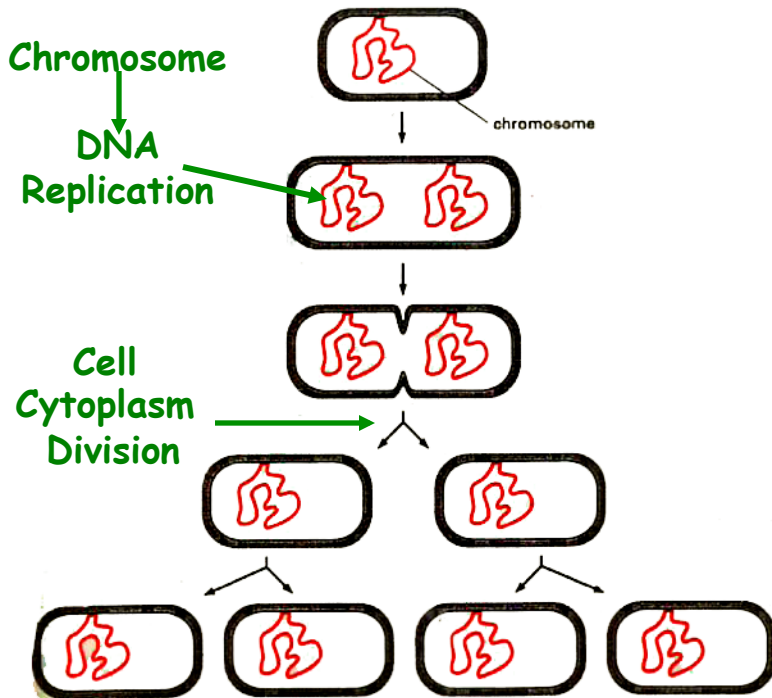


How is the DNA Sequence Copied/
Replicated Each Cell Division?

Pass on Genes to Next Generation Precisely?

BASIS OF LIFE!

Genes Are Replicated During Each Cell Division



Clones

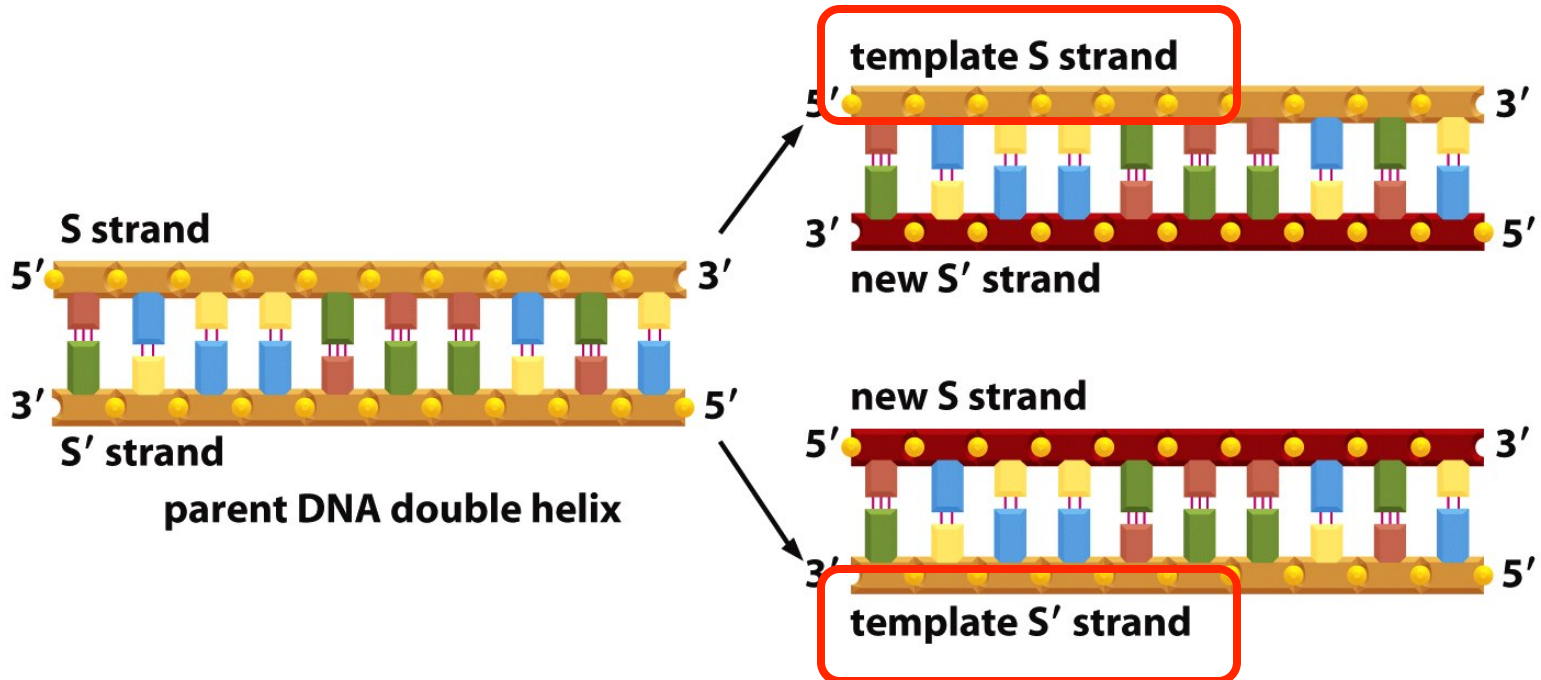
A Bacterial Colony Contains Many Copies of Same Cell, or Clones, Which are Genetically Identical!

Each Daughter Cell Contains The Same Collection of Genes

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

Clones!

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



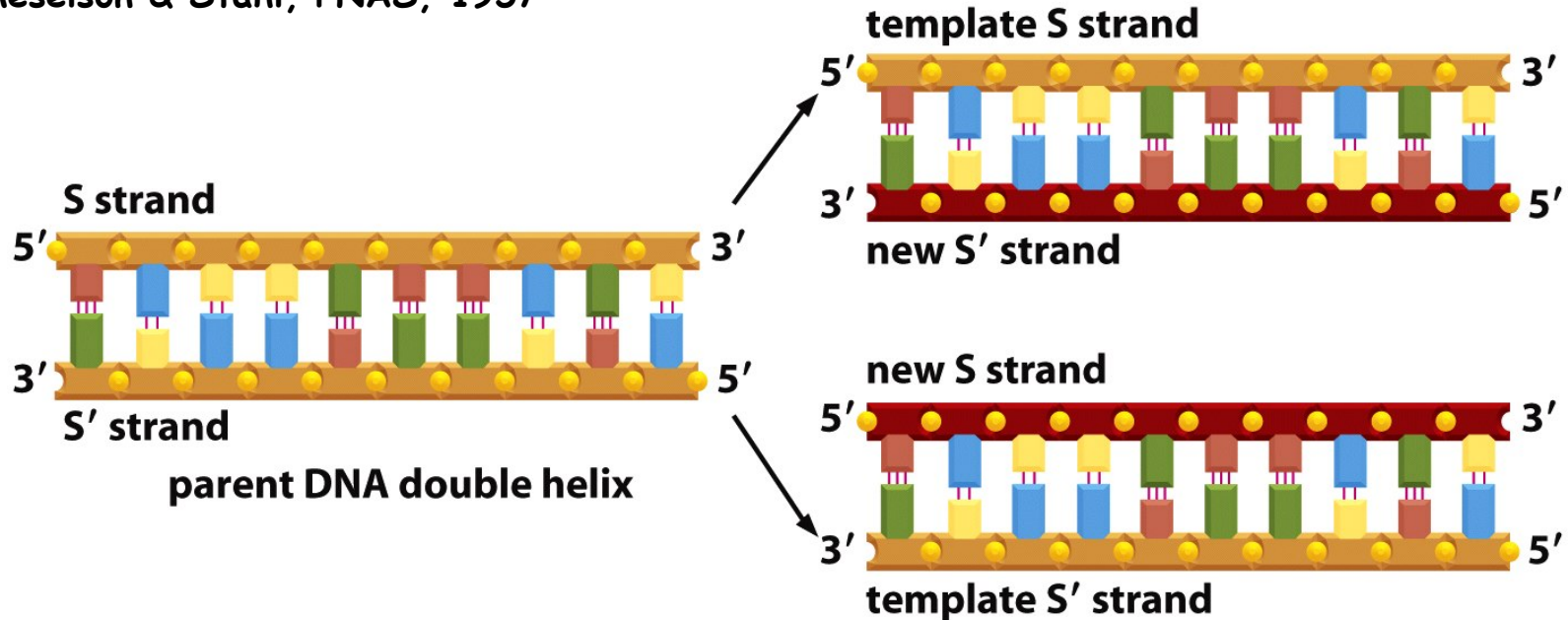
How Does This Occur?
Property of The DNA Molecule

Note →

SEQUENCE & POLARITY

DNA Replication Occurs Semi-Conservatively

Meselson & Stahl, PNAS, 1957



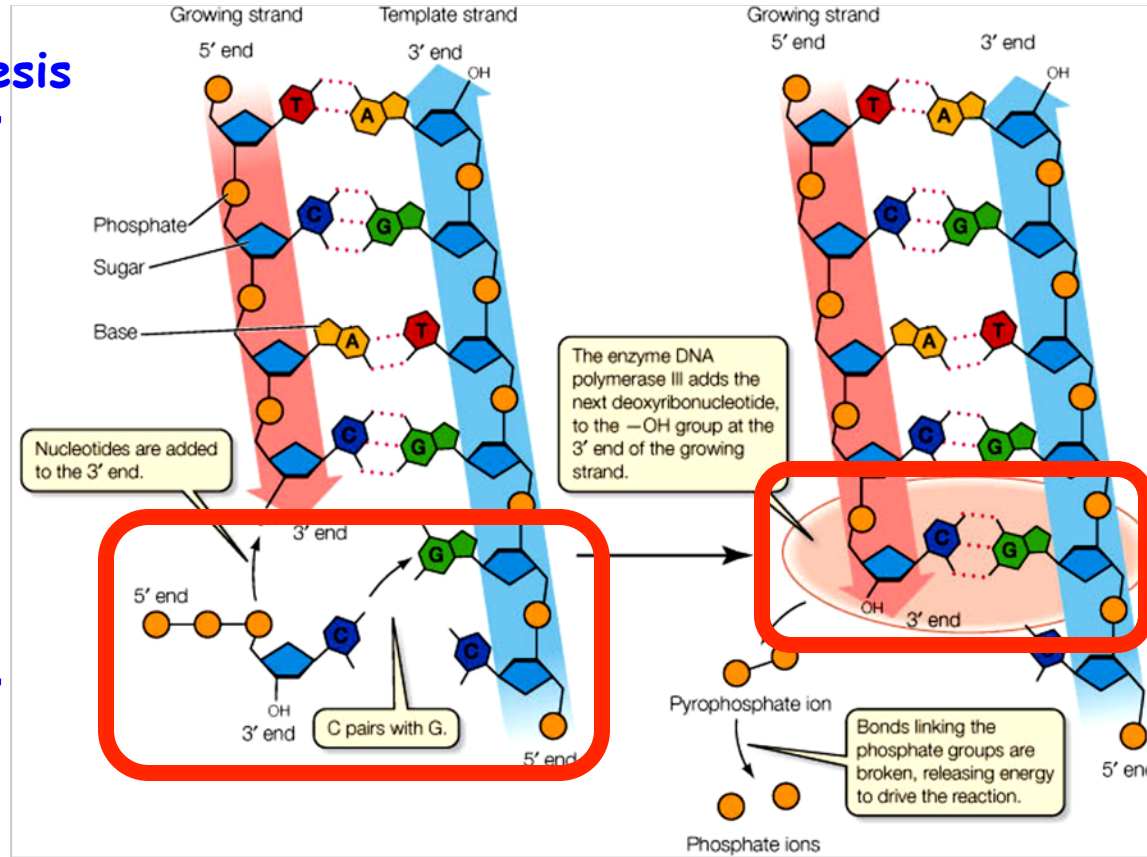
1. DNA Structure Allows DNA Sequence to Be Maintained by Complementary Base Pairing
2. Each Strand Serves as a Template for the Synthesis of a Complementary Strand
3. New DNA Molecules are Precise Copies of Parental DNA - Each Containing One Newly Synthesized Complementary Strand

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

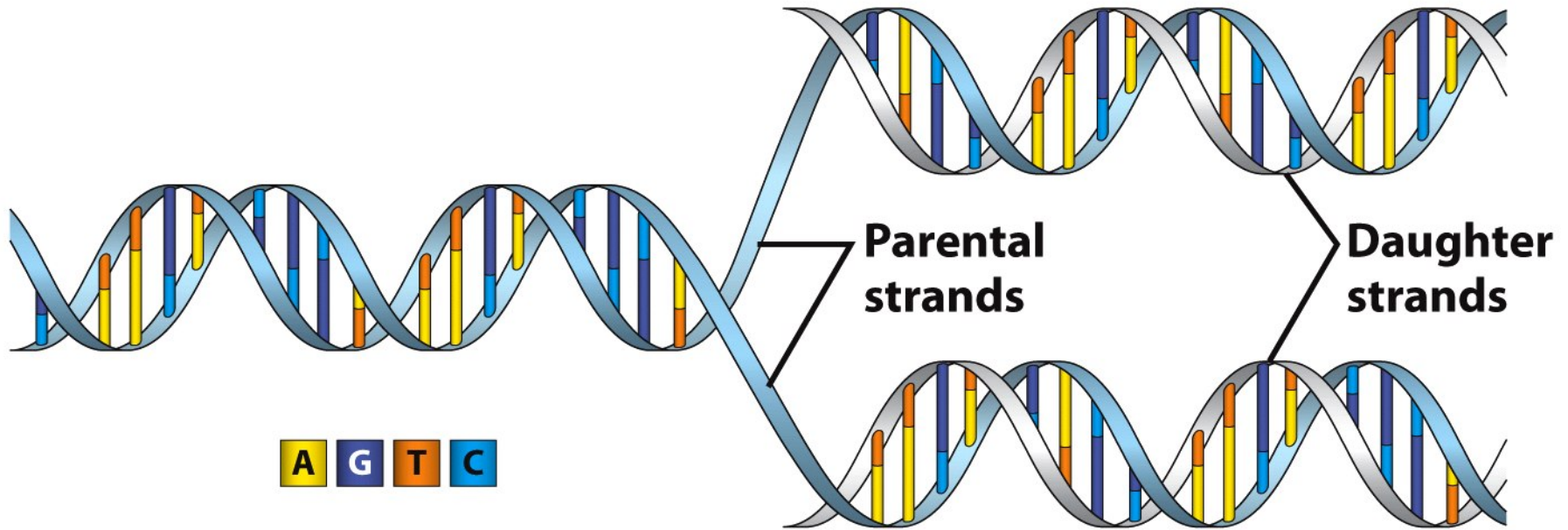


Figure 1-10
Molecular Cell Biology, Sixth Edition
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The DNA Sequence "Lives" Forever!

What is Required For DNA Replication to Occur and What Role Does DNA Replication Play in Genetic Engineering?

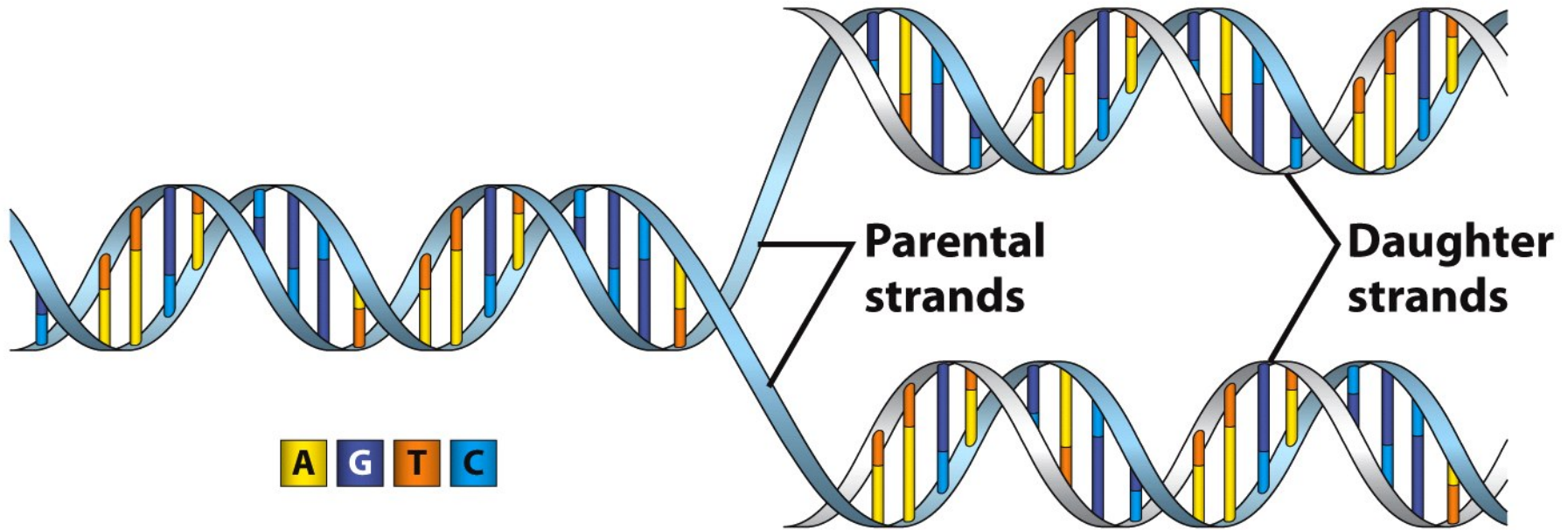
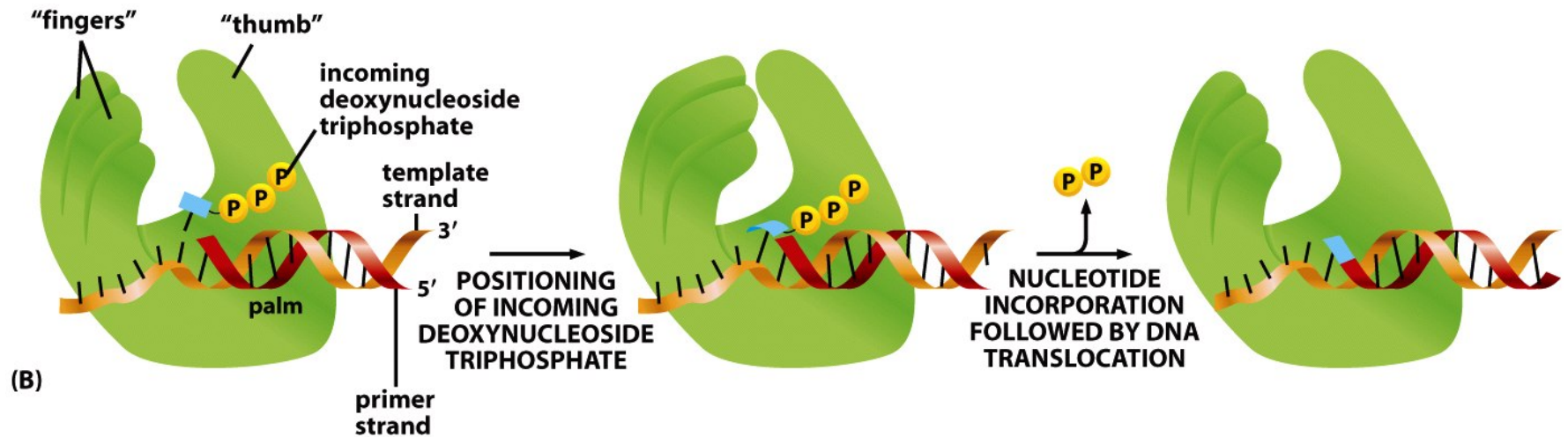
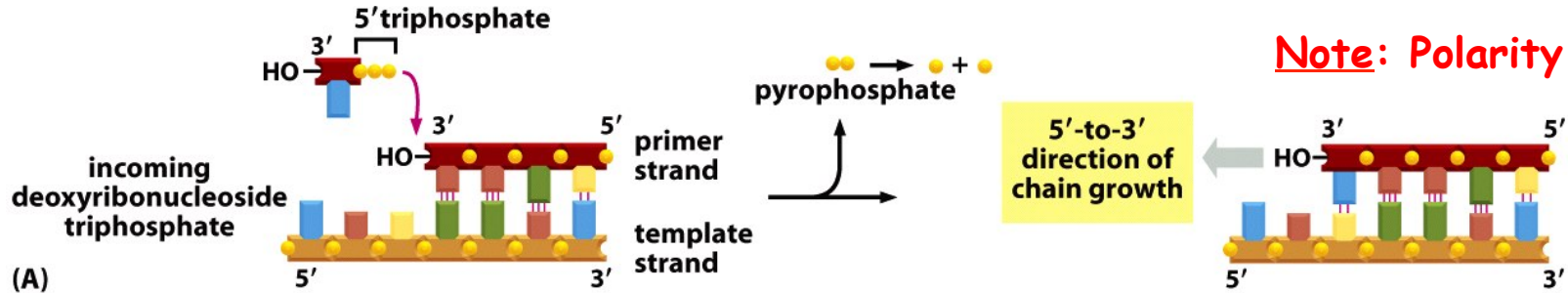


Figure 1-10
Molecular Cell Biology, Sixth Edition
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Does a Vector Have the Ability to Replicate Independently?

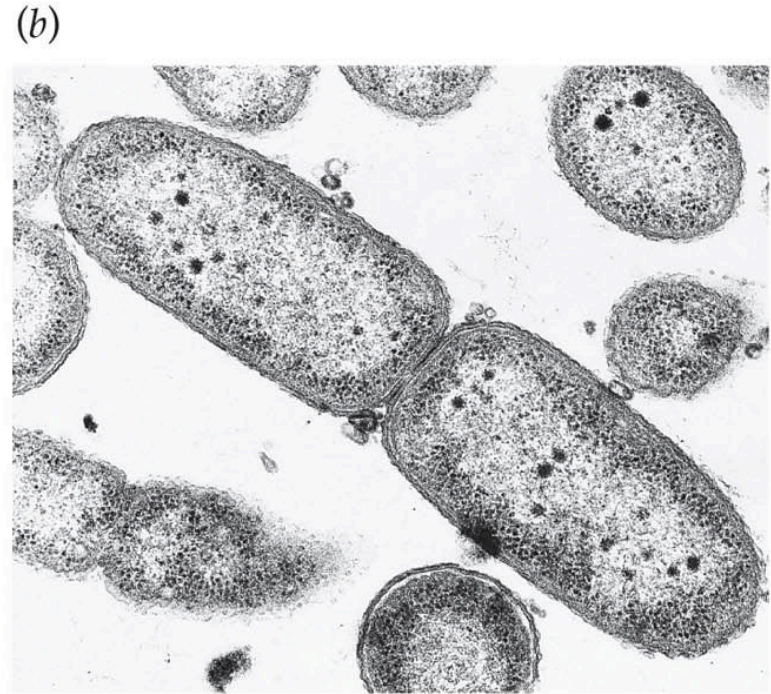
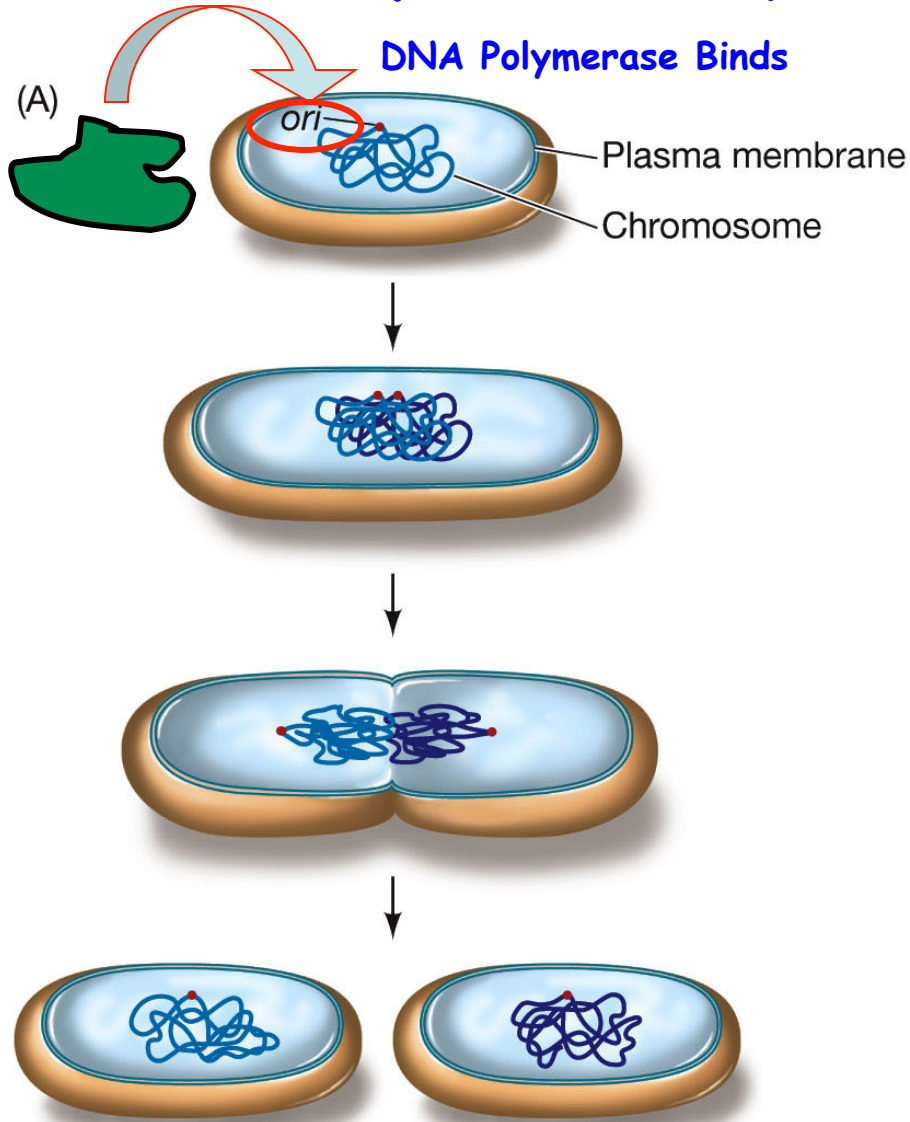
DNA Replication Requires An Enzyme - DNA Polymerase - Which is a Nano Machine!

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



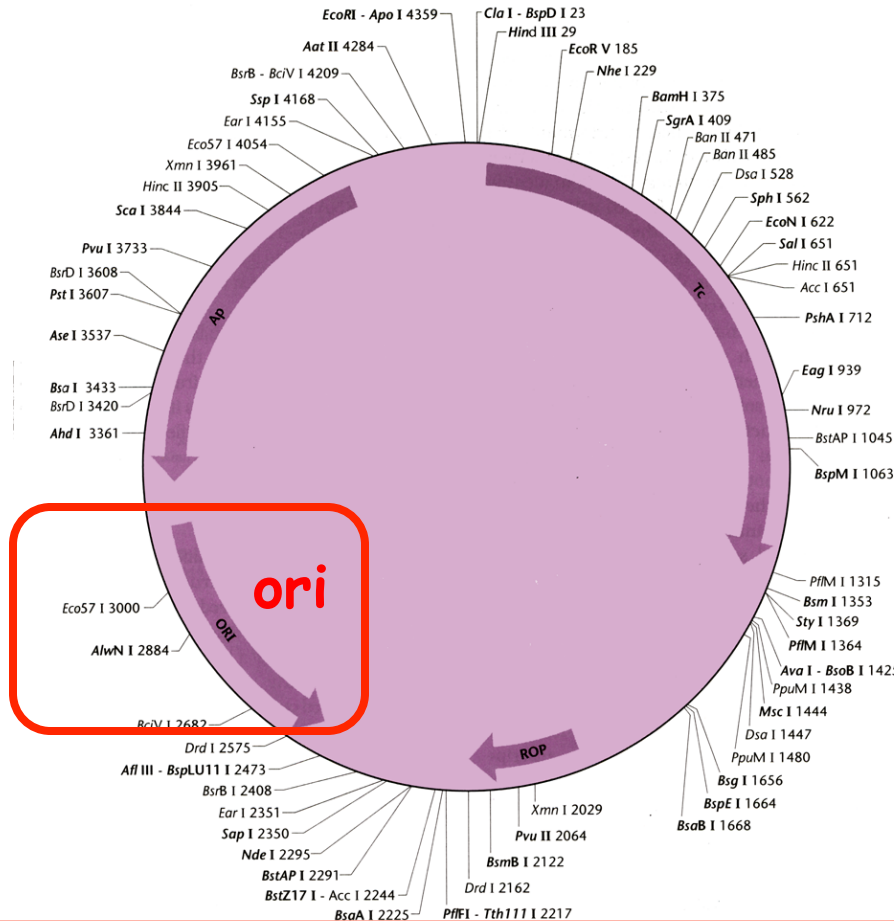
DNA Replication Also Requires:

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

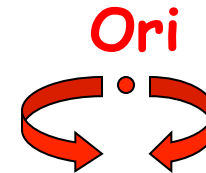
Two IDENTICAL Cells - Phenotypically & Genotypically - From One Cell

Ori

DNA Replication Starts at The Origin of Replication



DNA Replication is
Bidirectional From
the Ori!!!

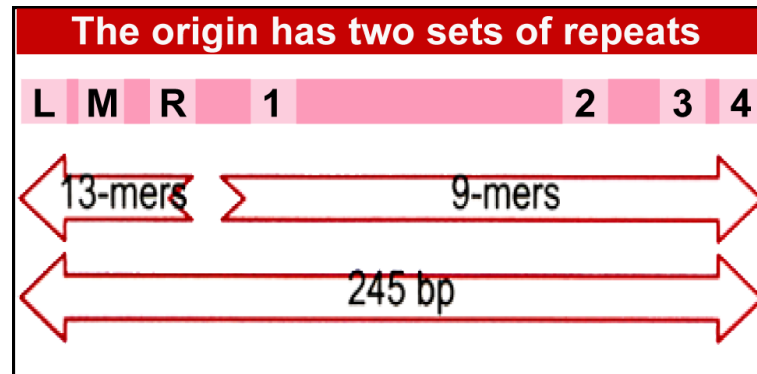
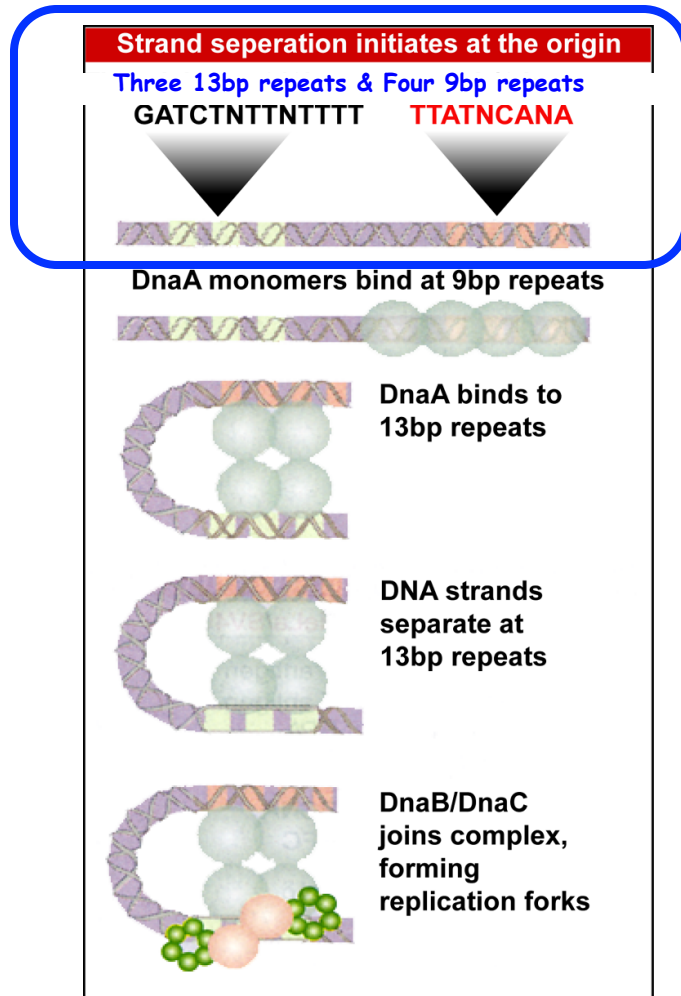


Hypothesis For
Two Direction
Synthesis?

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

How Control Division?

The Origin of Replication is a Specific Sequence



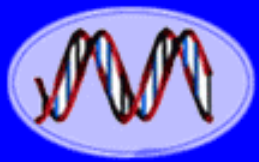
1. How Clone An Origin of Replication?

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

3. What is The Significance For Genetic Engineering?

4. Can Replicating "Chromosomes" Be Made?

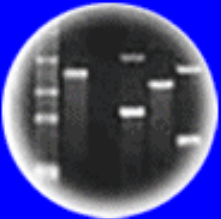
Vectors Are Needed To Replicate Genes In Transformed Cells



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Entire Genetic Code
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DNA Fingerprinting

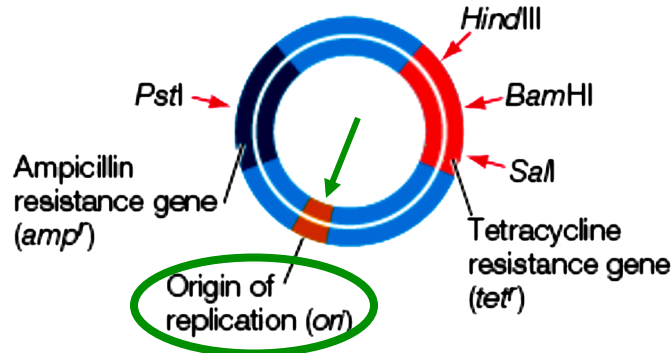


Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

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



↓ Recognition Site for Restriction Enzymes

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!

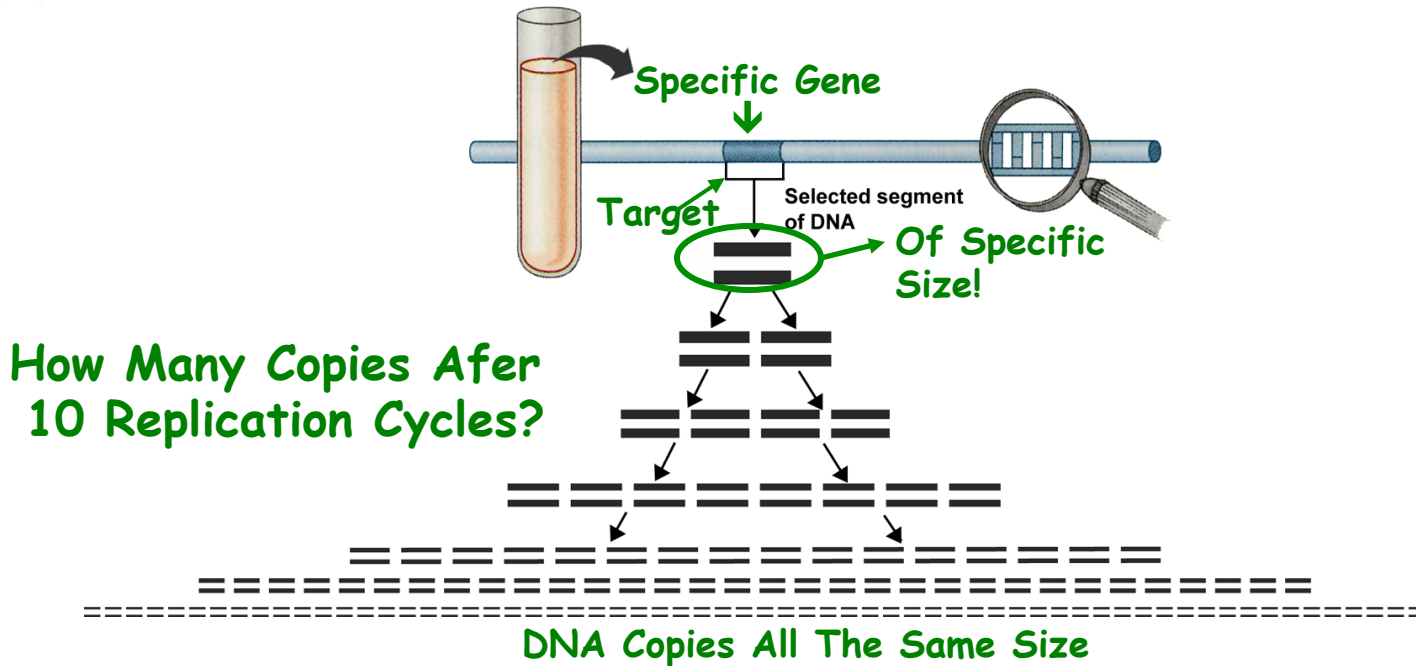
Ori's can be cloned/synthesized!

MODULAR!!

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!



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!

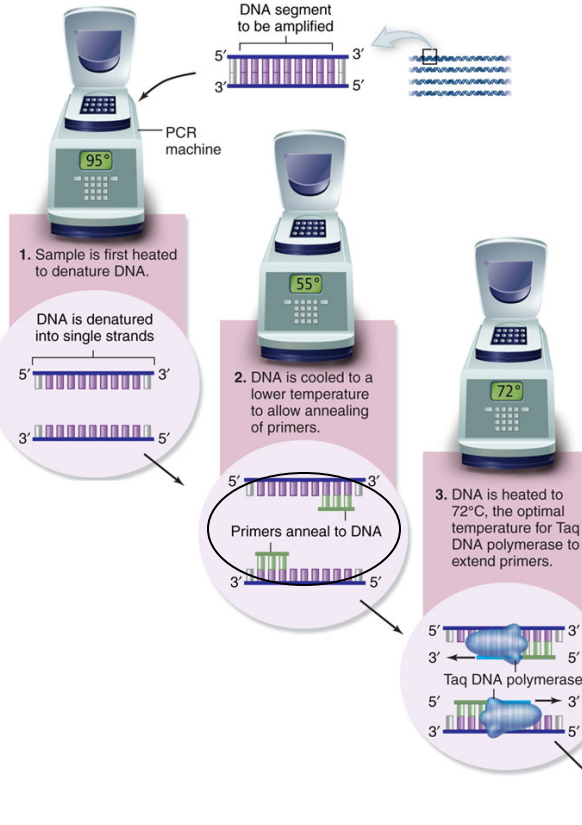
2. No Cloning Needed!

3. But Need Sequence! Therefore - Have to Clone “Gene” First

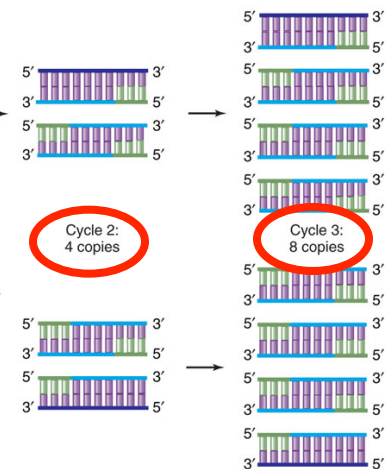
PCR is A Cyclical Process of DNA Replication

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



Repeat Steps or Cycle



2ⁿ 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

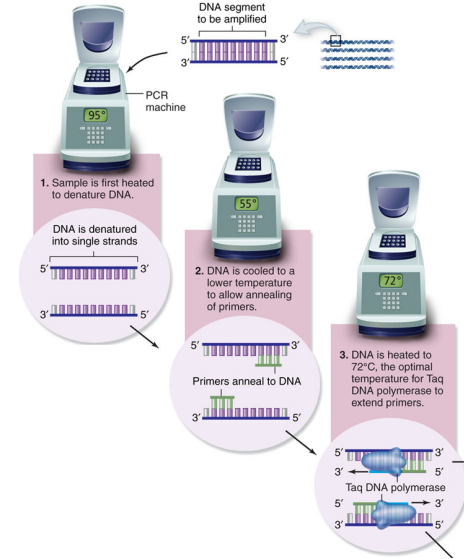
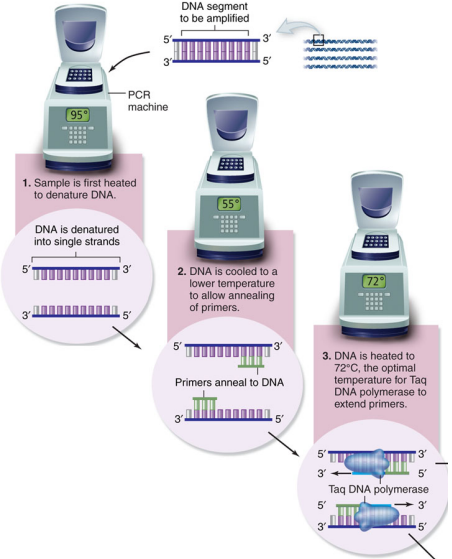
- Target-Specific Band
- Diagnostic For Specific DNA Sequence
- Band Size Unique For Specific Sequence
- Primers "Surround" the Target Sequence

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

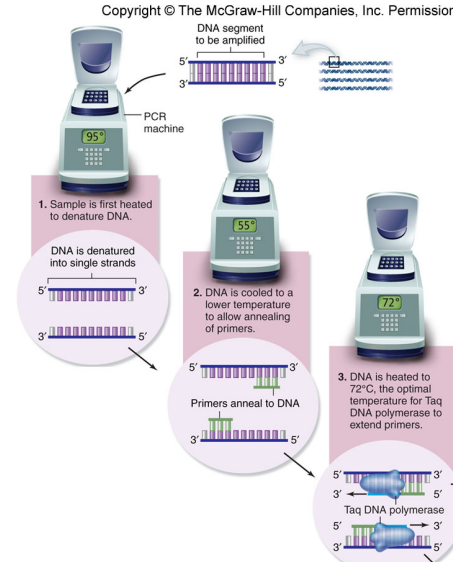
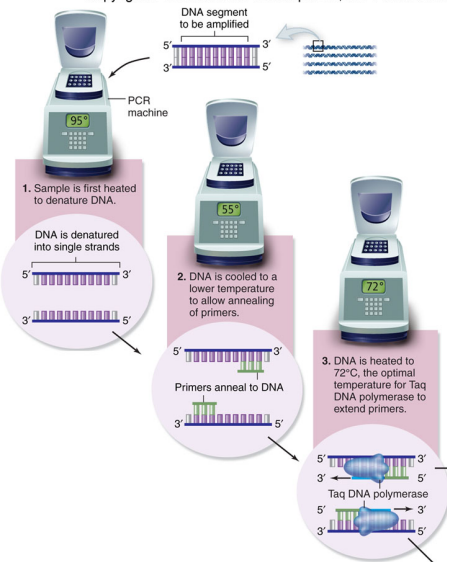
Requirements For PCR

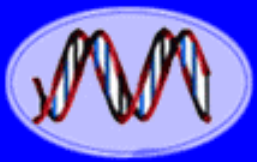
1. Knowledge of a Specific Sequence to Amplify (e.g., insulin gene)
 - a) Must Have First Cloned & Sequenced DNA of Interest the “Old-fashioned Way”
2. Primers That Recognize Specific DNA Sequences & Initiate DNA Synthesis & DNA Polymerase Binding To Template
3. Template (e.g., DNA From Human Cheek Cell)
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), & Permitting New dsDNA Molecules to Form

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



Examples of PCR Applications

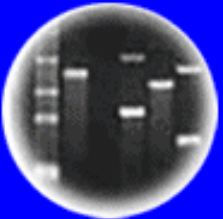




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DNA Fingerprinting



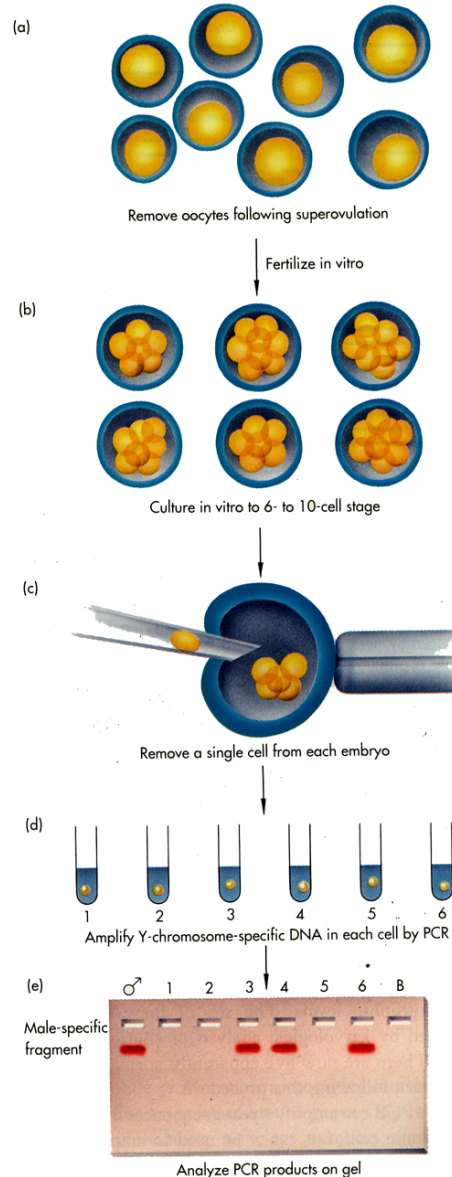
Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

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

PGD Pre- Implantation Genetic Diagnosis



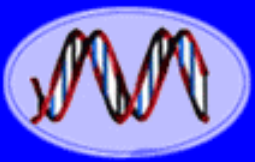
**What is The
Implication of
This Procedure
Considering That
The Human
Genome Has Been
Sequenced?**

**Sex Determination
in 8-cell Embryo!**

Determining the Genetic Identity of a Human Embryo Before Implantation!



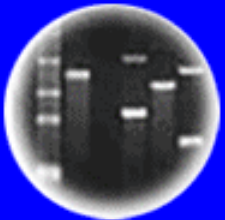
Prenatal Genetic Diagnosis (PGD)



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of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences

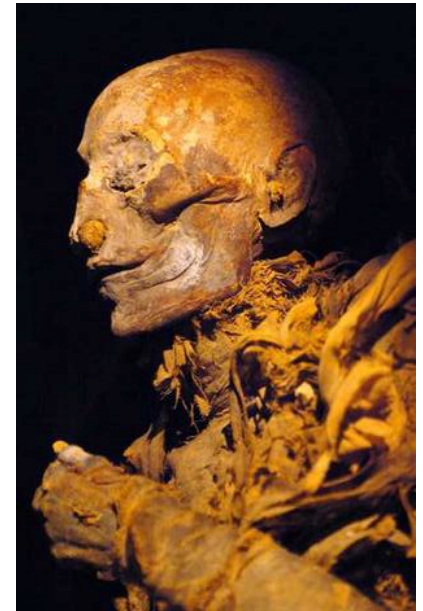
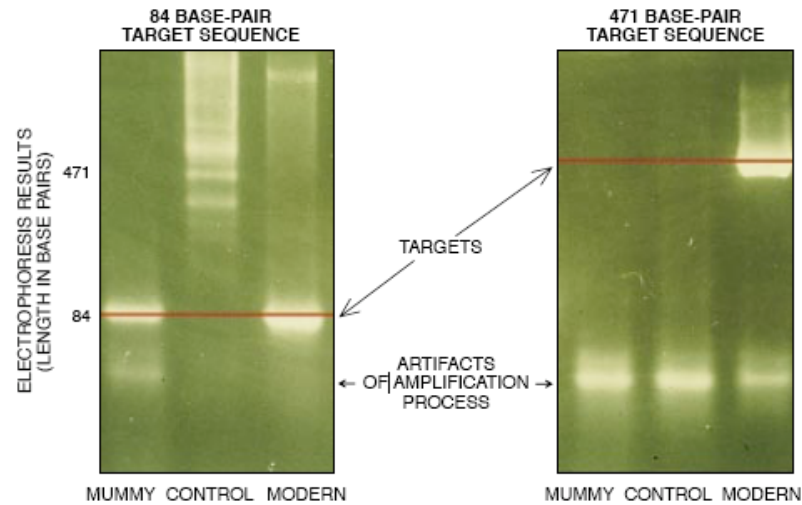


Plants of Tomorrow

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
For Example - King Tut

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 Miller¹, Daniela I. Drautz¹, Aakrosh Ratan¹, Barbara Pusey¹, Ji Qi¹, Arthur M. Lesk¹, Lynn P. Tomsho¹, Michael D. Packard¹, Fangqing Zhao¹, Andrei Sher^{2,†}, Alexei Tikhonov³, Brian Raney⁴, Nick Patterson⁵, Kerstin Lindblad-Toh⁵, Eric S. Lander⁵, James R. Knight⁶, Gerard P. Irzyk⁶, Karin M. Fredrikson⁷, Timothy T. Harkins⁷, Sharon Sheridan⁷, Tom Pringle⁸ & Stephan C. Schuster¹



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

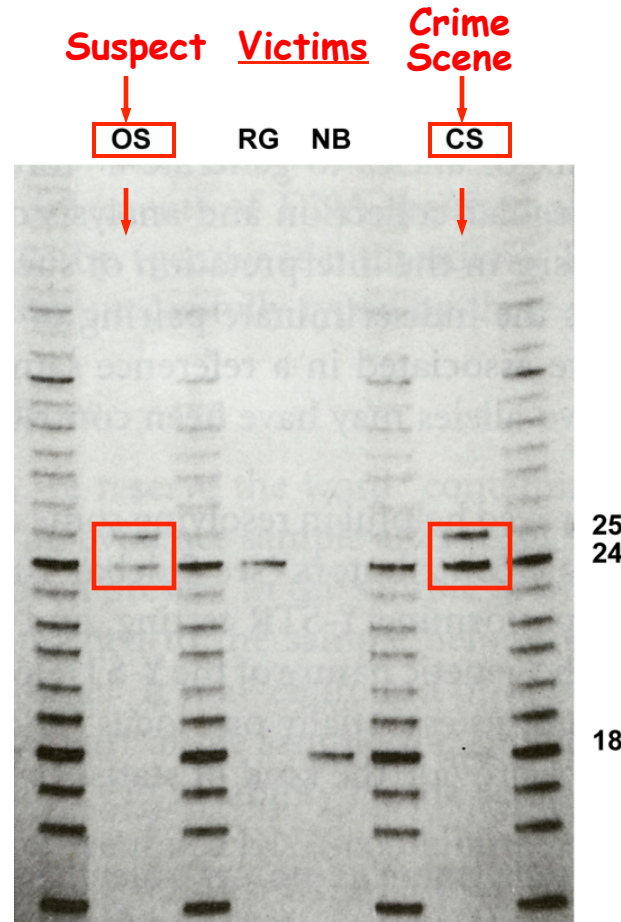
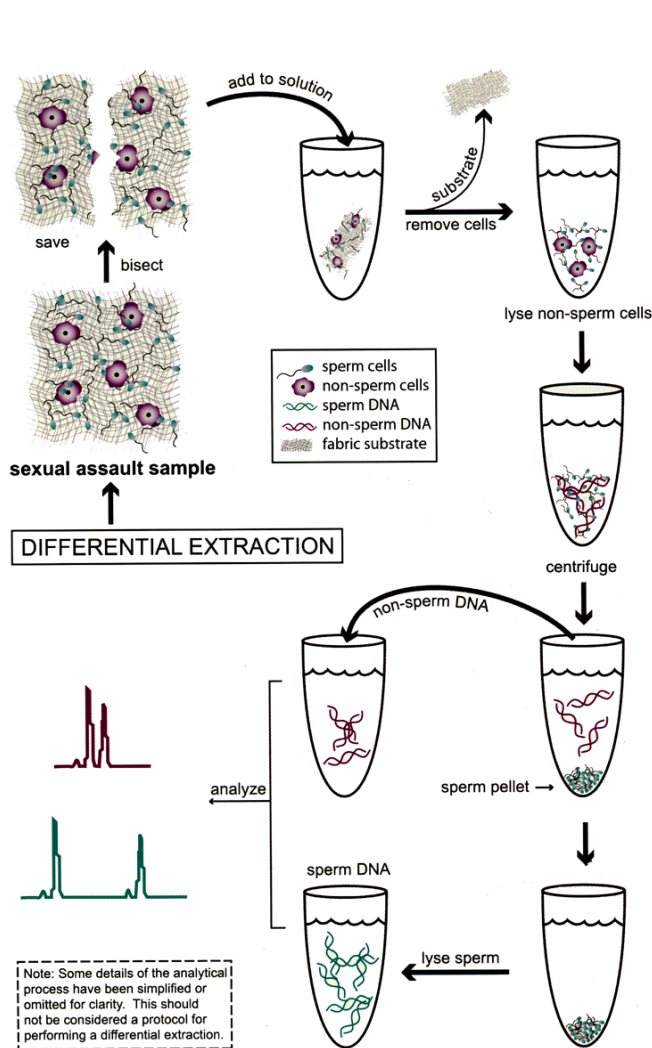
Richard E. Green¹, Johannes Krause¹, Susan E. Ptak¹, Adrian W. Briggs¹, Michael T. Ronan², Jan F. Simons², Lei Du², Michael Egholm², Jonathan M. Rothberg², Maja Paunovic³† & Svante Pääbo¹



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



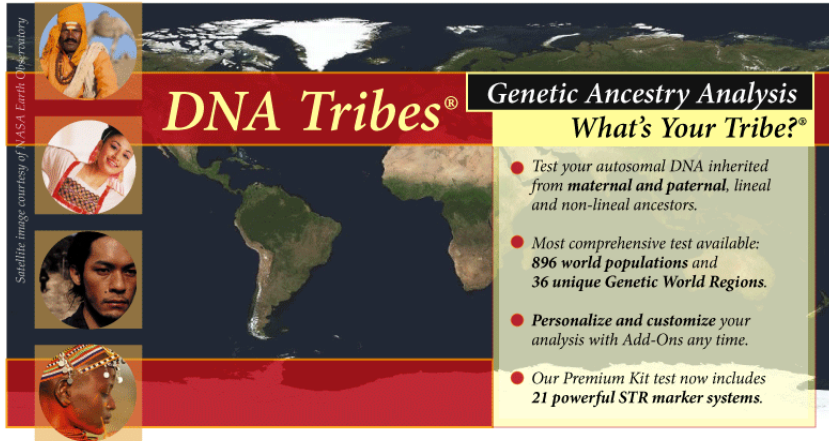
Figure 19-31

Genetics: A Conceptual Approach, Third Edition

© 2009 W. H. Freeman and Company

Newsweek, January 12, 2009

Using PCR To Determine an Individual's Ancestry



DNA Tribes® Genetic Ancestry Analysis
What's Your Tribe?®

- Test your autosomal DNA inherited from **maternal and paternal**, lineal and non-lineal ancestors.
- Most comprehensive test available: **896 world populations and 36 unique Genetic World Regions.**
- **Personalize and customize** your analysis with Add-Ons any time.
- Our Premium Kit test now includes **21 powerful STR marker systems.**

Satellite image courtesy of NASA Earth Observatory



Discover Your Past!

- ✓ Determine if two people are related
- ✓ Determine if two people descend from the same ancestor
- ✓ Find out if you are related to others with the same surname
- ✓ Prove or disprove your family tree research
- ✓ Provide clues about your ethnic origin

ORDER YOUR TEST NOW!

PCR Started a New Industry



Adopted?

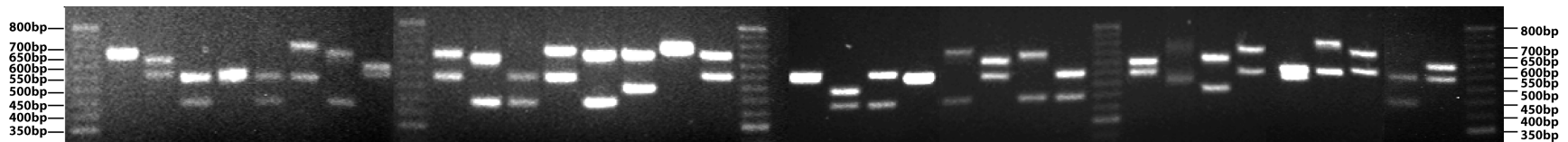
Find out about your ancestry...

JOIN THE ADOPTEE PROJECT



Maternal & Paternal Testing

ORDER YOUR TEST NOW!



Using PCR to Verify Remains of Russian Royal Family

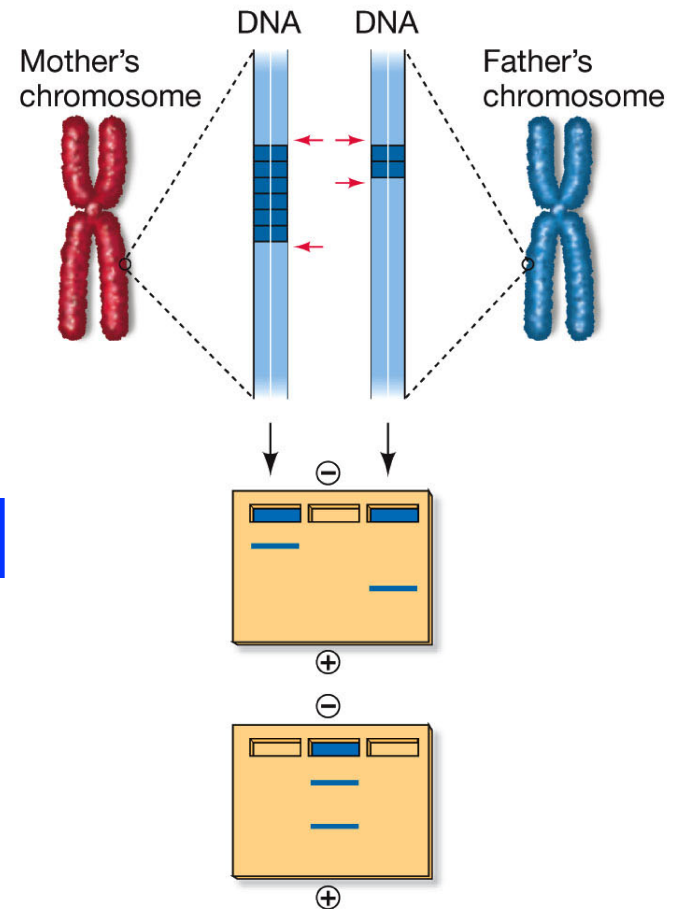


	Number of repeats		
STR-1	15,16	15,16	
STR-2	8,8	7,10	
STR-3	3,5	7,7	
STR-4	12,13	12,12	
STR-5	32,36	11,32	

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!



Genomic identification in the historical case of the Nicholas II royal family **PNAS, March, 2009**

Evgeny I. Rogayev^{a,b,c,d,1}, Anastasia P. Grigorenko^{b,d}, Yuri K. Mollaka^a, Gulnaz Fashkudimova^a, Andrey Goltsov^d, Arlene Lahti^a, Curtis Hildebrandt^a, Ellen L. W. Kittler^e, and Irina Morozova^a

^aDepartment of Genomics and Laboratory of Evolutionary Genomics, Vavilov Institute of General Genetics, Russian Academy of Science, Gubkina Street, 3, Moscow, 119991, Russian Federation; ^bBrudnick Neuropsychiatric Research Institute, University of Massachusetts Medical School, 303 Belmont Street, Worcester, MA 01604; ^cFaculty of Bioinformatics and Bioengineering, Lomonosov Moscow State University, Moscow, 119991, Russian Federation; ^dResearch Center of Mental Health, Russian Academy of Medical Science, Zagorodnoe Shosse 2/2, Moscow, 113152, Russia; ^eMolecular World, Inc., Thunder Bay, ON, Canada P7B 2T1; and ^fUniversity 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

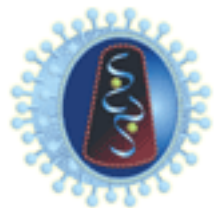
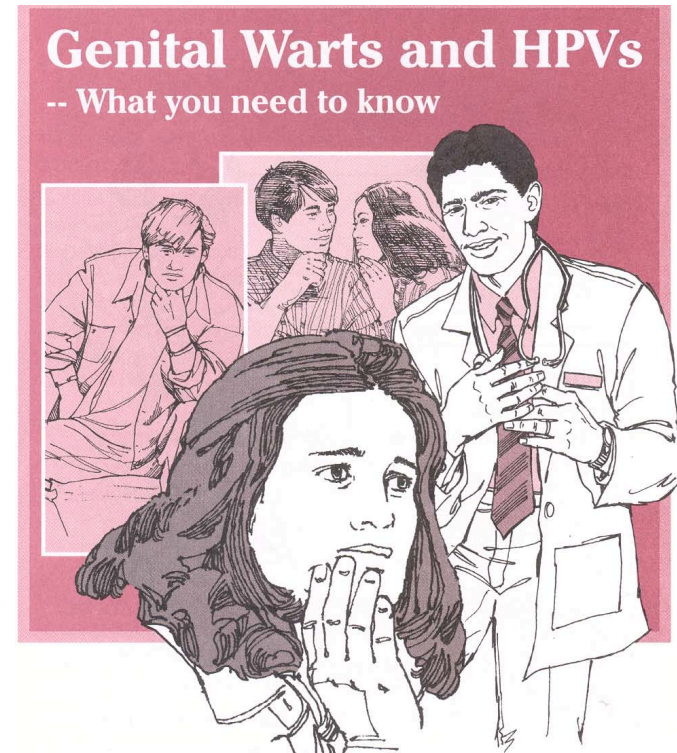
Michael D. Coble^{1,2,3,4,5}, Odile M. Loreille^{1,2,3}, Mark J. Wadhams¹, Suni M. Edson³, Kerry Maynard¹, Carna E. Meyer¹, Harald Niederstätter², Cordula Berger², Burkhard Berger², Anthony B. Falsetti³, Peter Gill^{4,5}, Walther Parson², Louis N. Finelli¹

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)



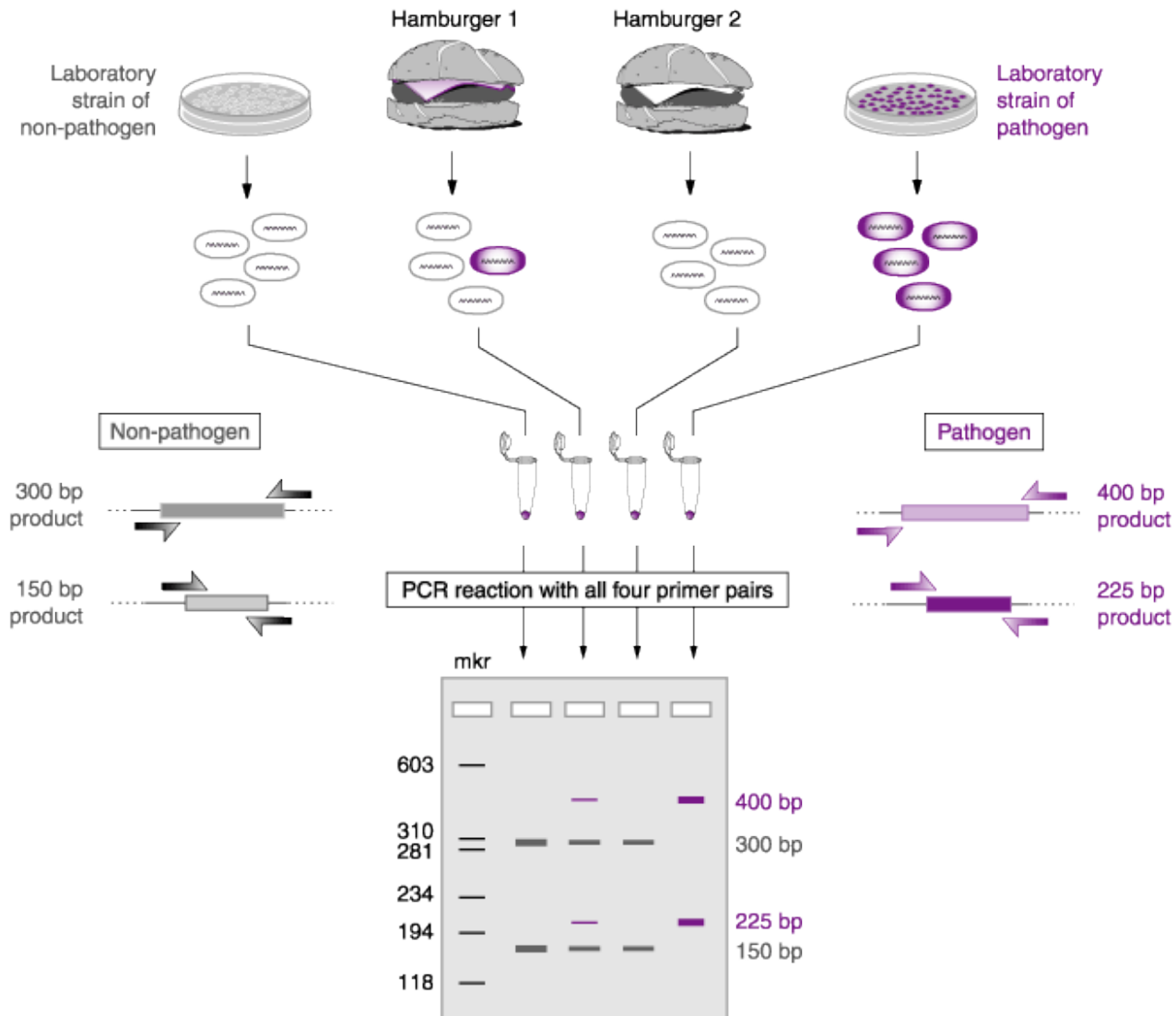
ViroSeq™
HIV-1 Genotyping System

DIVISION OF HIV/STD
VDH VIRGINIA
DEPARTMENT
OF HEALTH

"This booklet has been reviewed and approved by a state panel for use in general settings."

**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

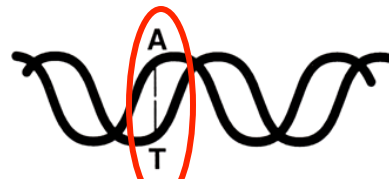


DNA Replication is Precise But Mistakes or Mutations Can Occur!

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

BASE PAIR RULES

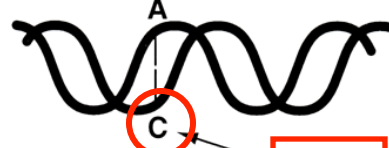
Gene A



ORIGINAL BASE PAIR

Rare Base Mismatch

Replication ①



MUTATION DURING REPLICATION

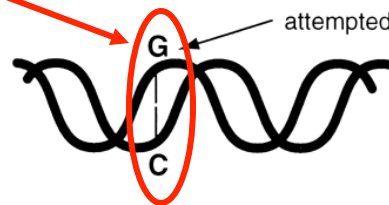
New Base Pair

mutation

C mispairs with A

Replication ②

Gene A'
Allelic Variant



attempted repair

RESULTING DEFECT

See Mutation As Change in Phenotype

Change DNA Sequence From A = T to G = C

∴ Change Protein Amino Acid Sequence ⇨ Alter Function!



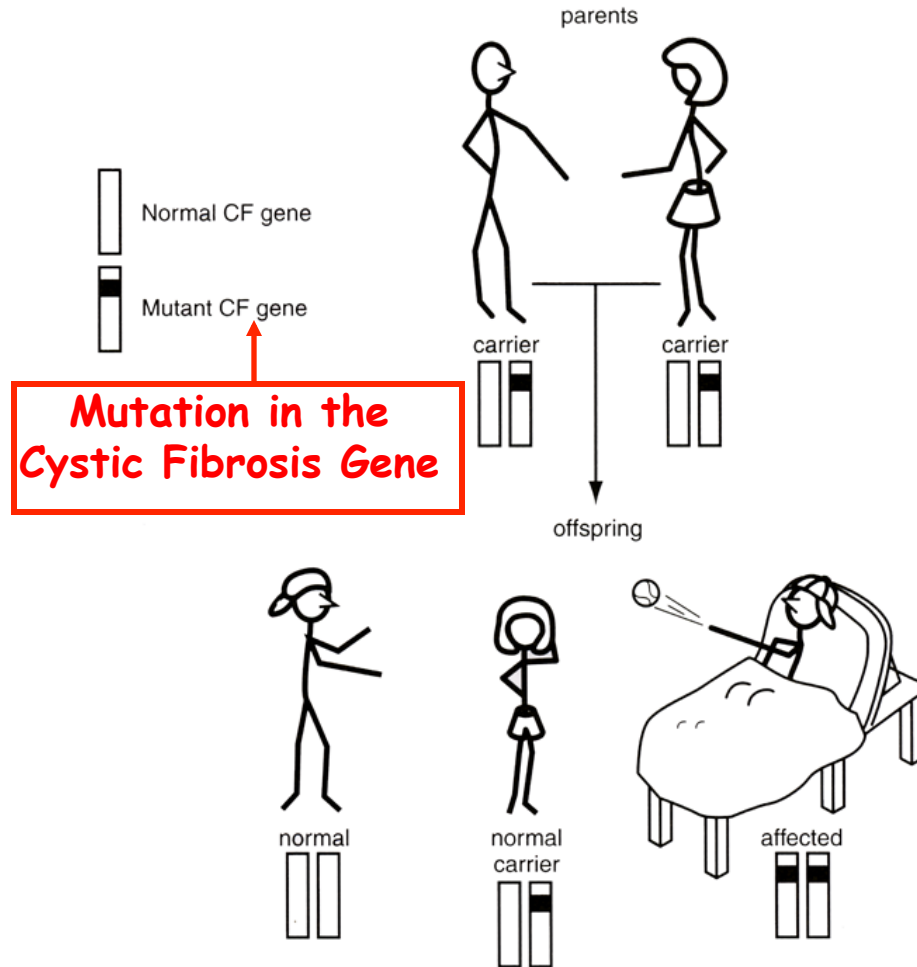
Big Tomato to Small Tomato

Mutation in Genes Are Rare But Are Inherited

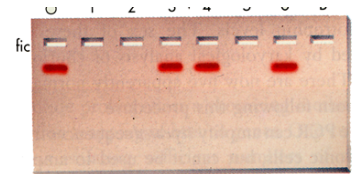
One Gene Per Gamete

♀ + ♂

Two Genes per Somatic Cells



**How Follow Inheritance?
What Allows Disease To Be Followed?**

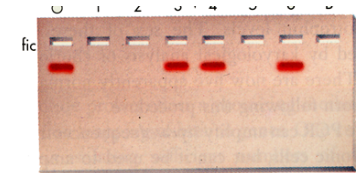
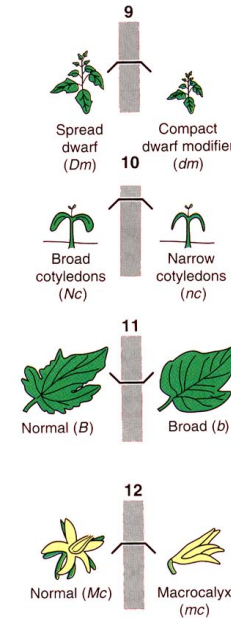
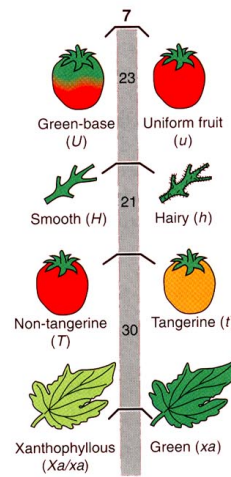
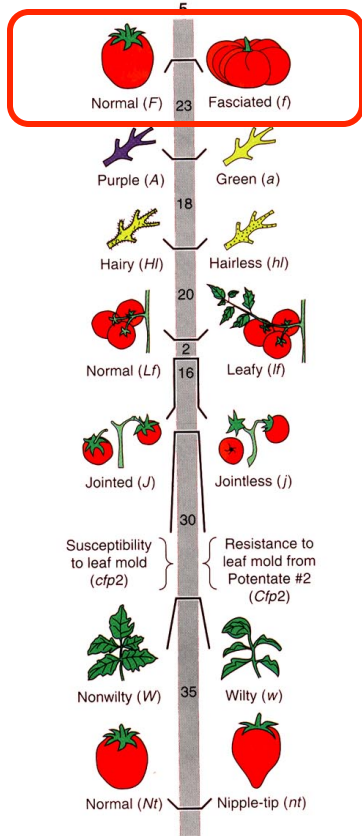


Analyze PCR products on gel

DNA Marker or Fingerprint!

Alternative Forms of the Same Gene Lead to Genetic Diversity

Alleles



Analyze PCR products on gel

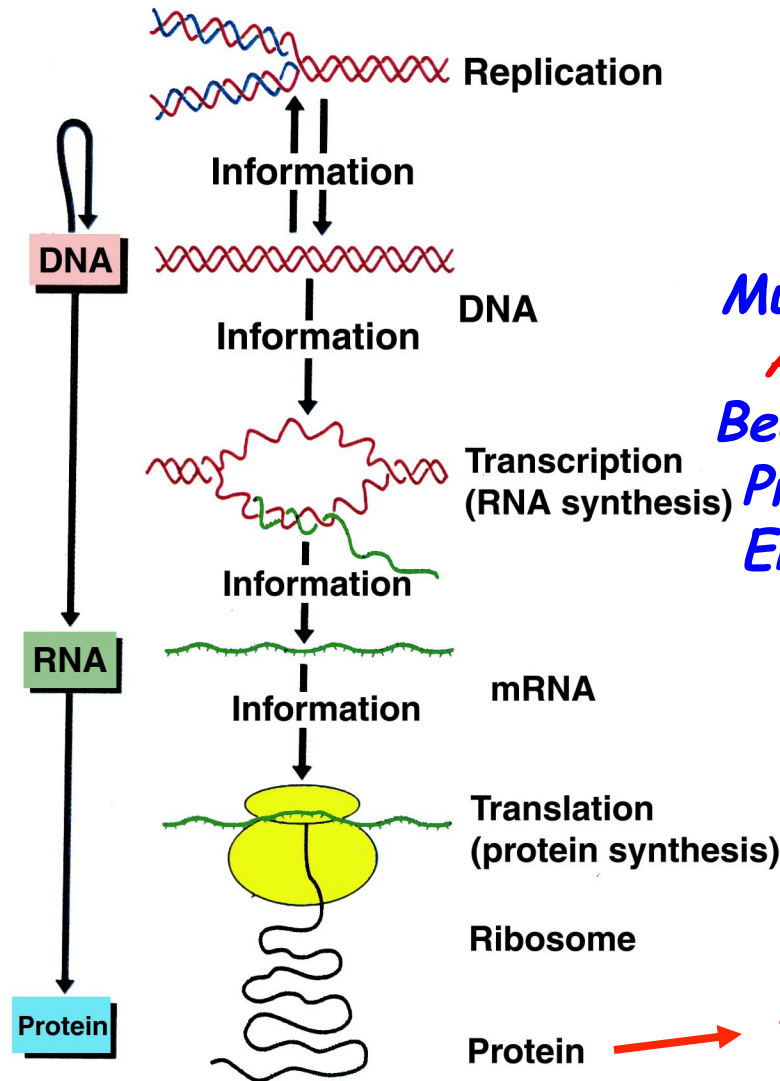
Can Follow These Traits With DNA Markers As Well

mutations result in genetic diversity!!!

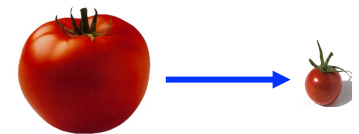
Spontaneous Mutations Give Rise To Alleles, or Different Forms of the Same Gene, And result in Small DNA Sequence Changes (e.g., SNPs or Single Nucleotide Polymorphisms)

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

Human Genetic Disorders Occur As a Result of Mutations

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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

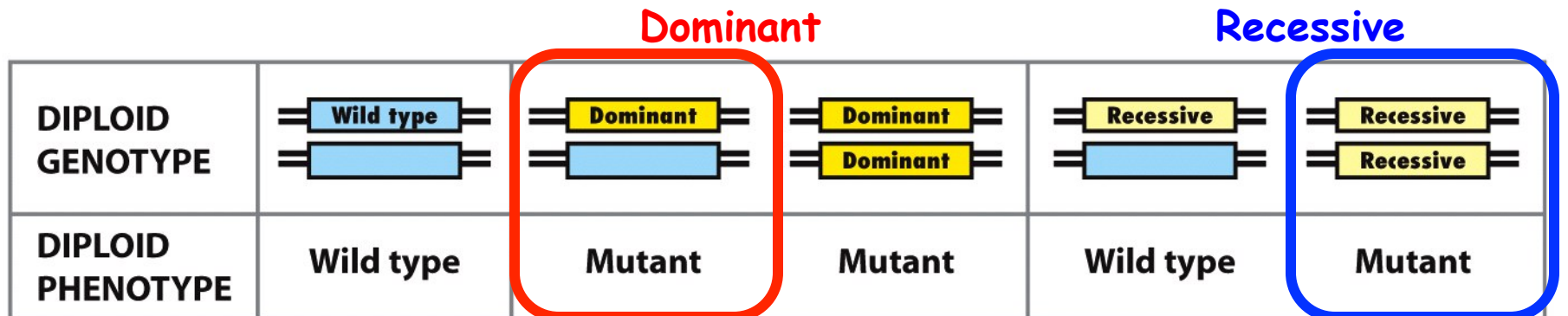
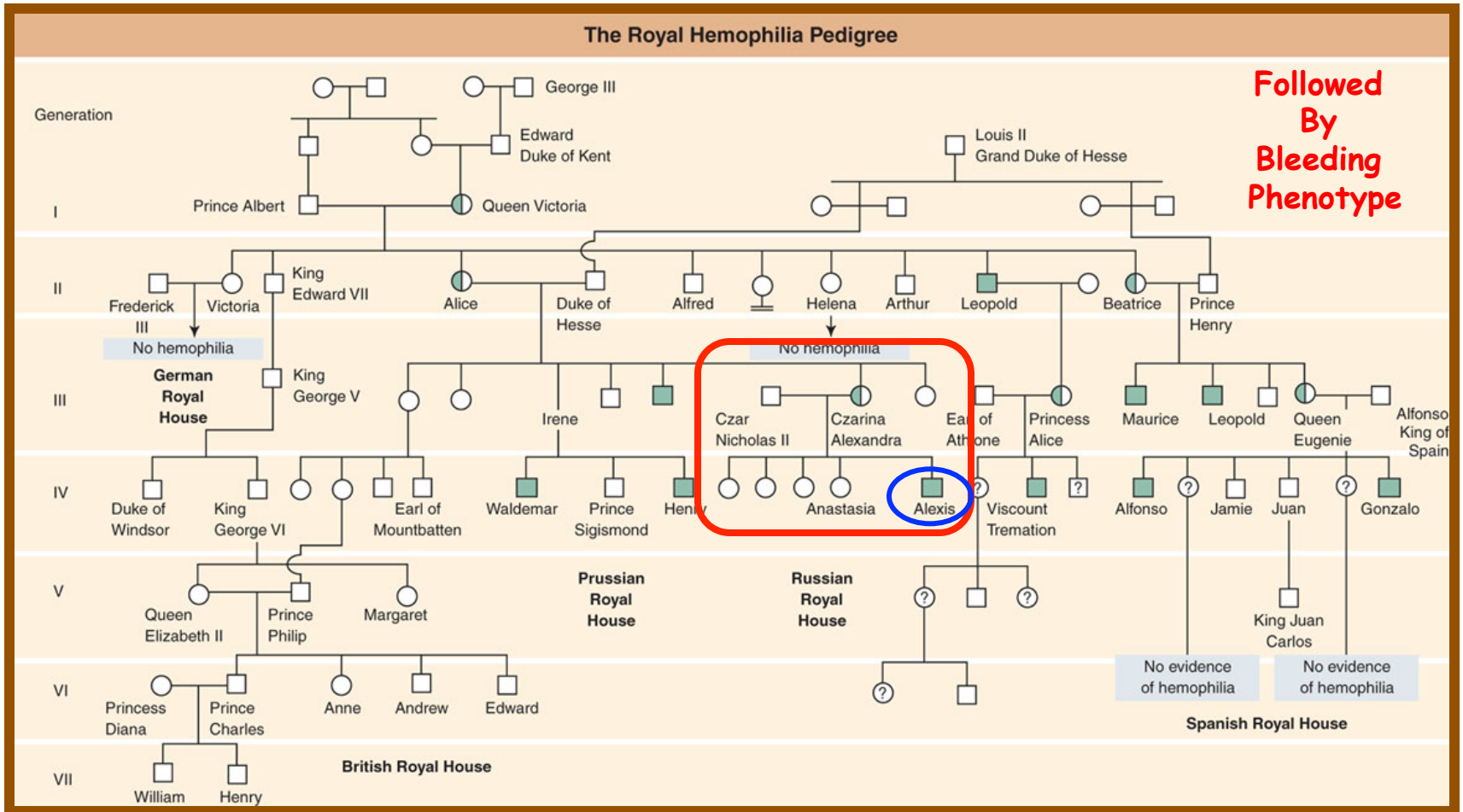


Figure 5-2
Molecular Cell Biology, Sixth Edition
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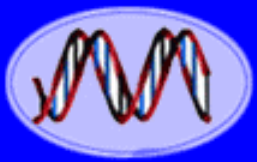
Need One Allele

Need Two Alleles

Pedigrees Can Be Used To Follow Disease Genes in Human Families



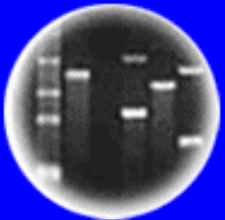
Recessive Sex Linked



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



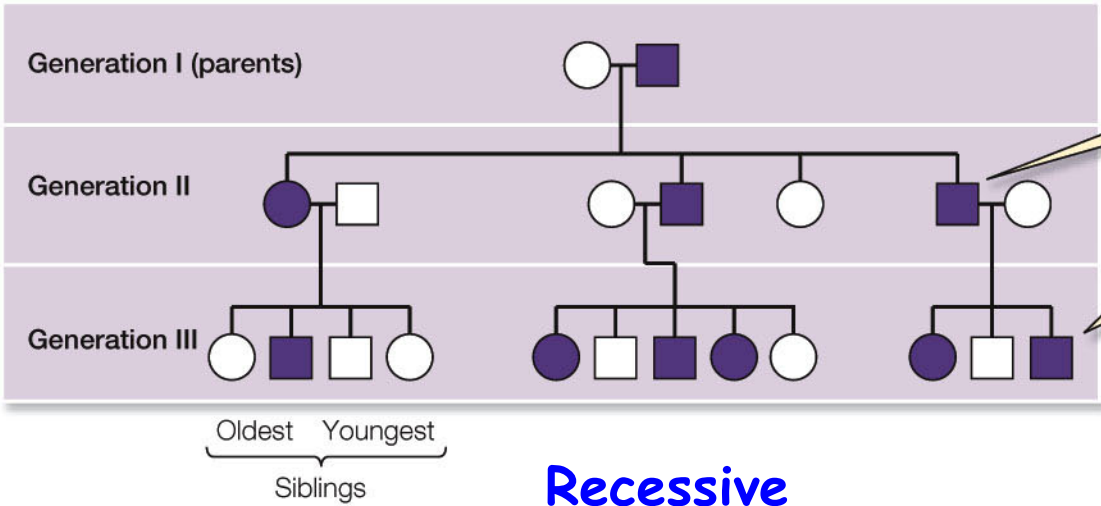
Plants of Tomorrow

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

**Each Type of Inheritance Predicts
Specific Results in Each Generation**

Dominant

(A) Dominant inheritance



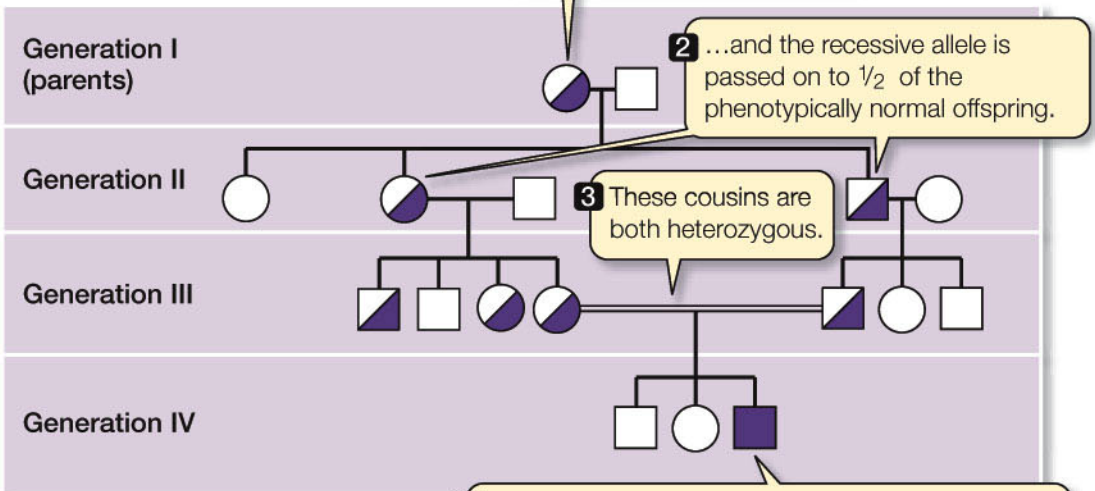
Every affected individual has an affected parent.

**Muscular Dystrophy
Huntington Disease**

About 1/2 of the offspring (of both sexes) of an affected parent are affected.

Recessive

(B) Recessive inheritance

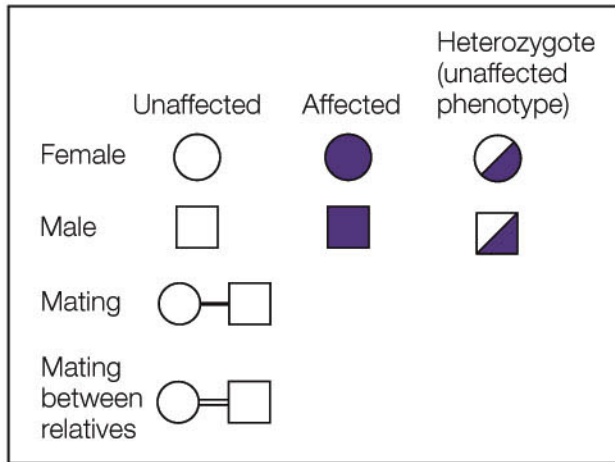


1 One parent is heterozygous...

2 ...and the recessive allele is passed on to 1/2 of the phenotypically normal offspring.

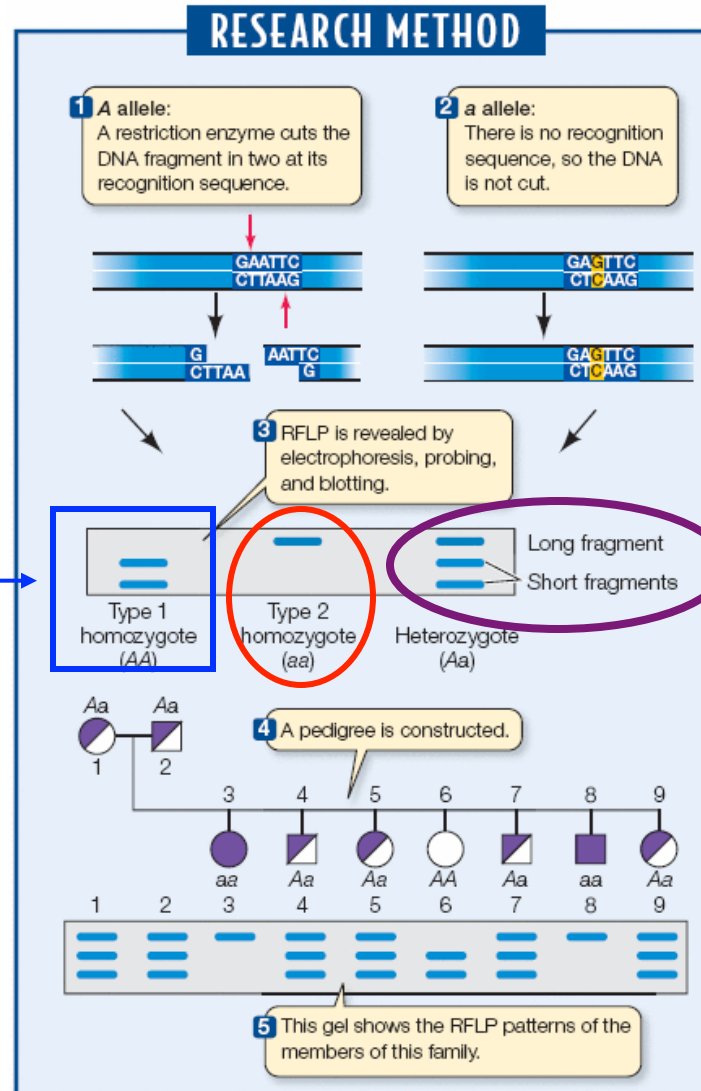
3 These cousins are both heterozygous.

4 Mating of heterozygous recessive parents may produce homozygous recessive (affected) offspring.



**Sickle Cell Anemia
Cystic Fibrosis
Tay-Sachs Disease**

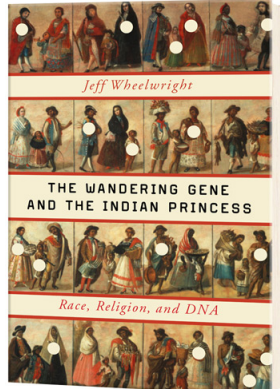
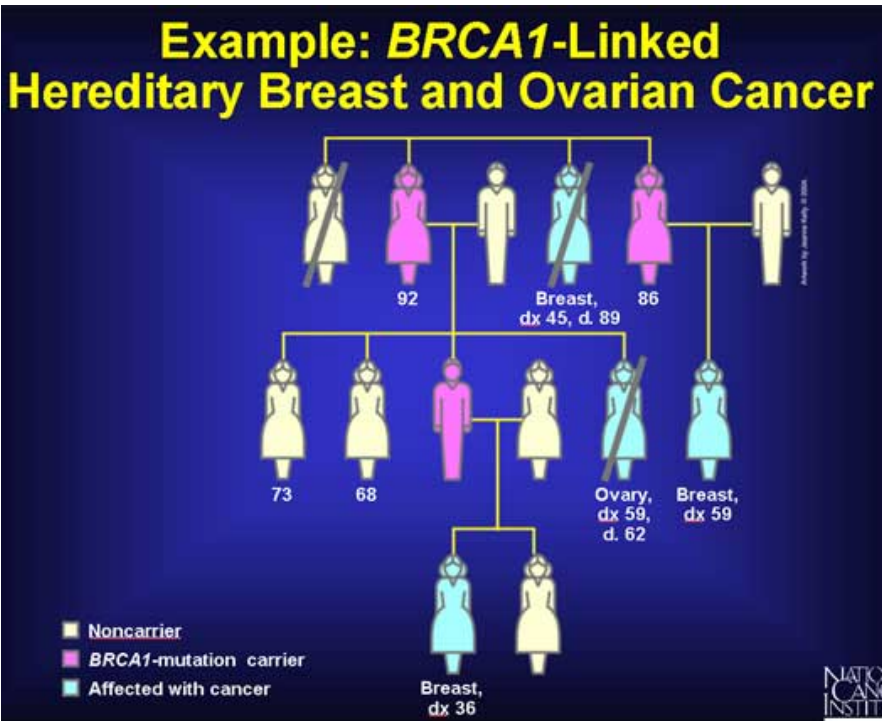
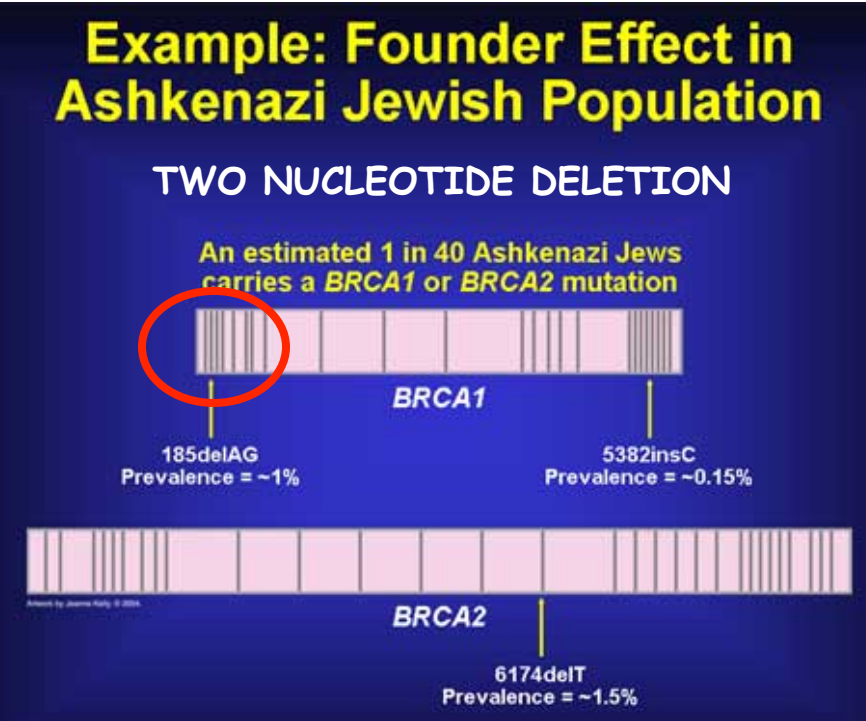
Genetic Diseases Can Be Followed in Families Using Molecular Methods (e.g., DNA Blots or PCR)



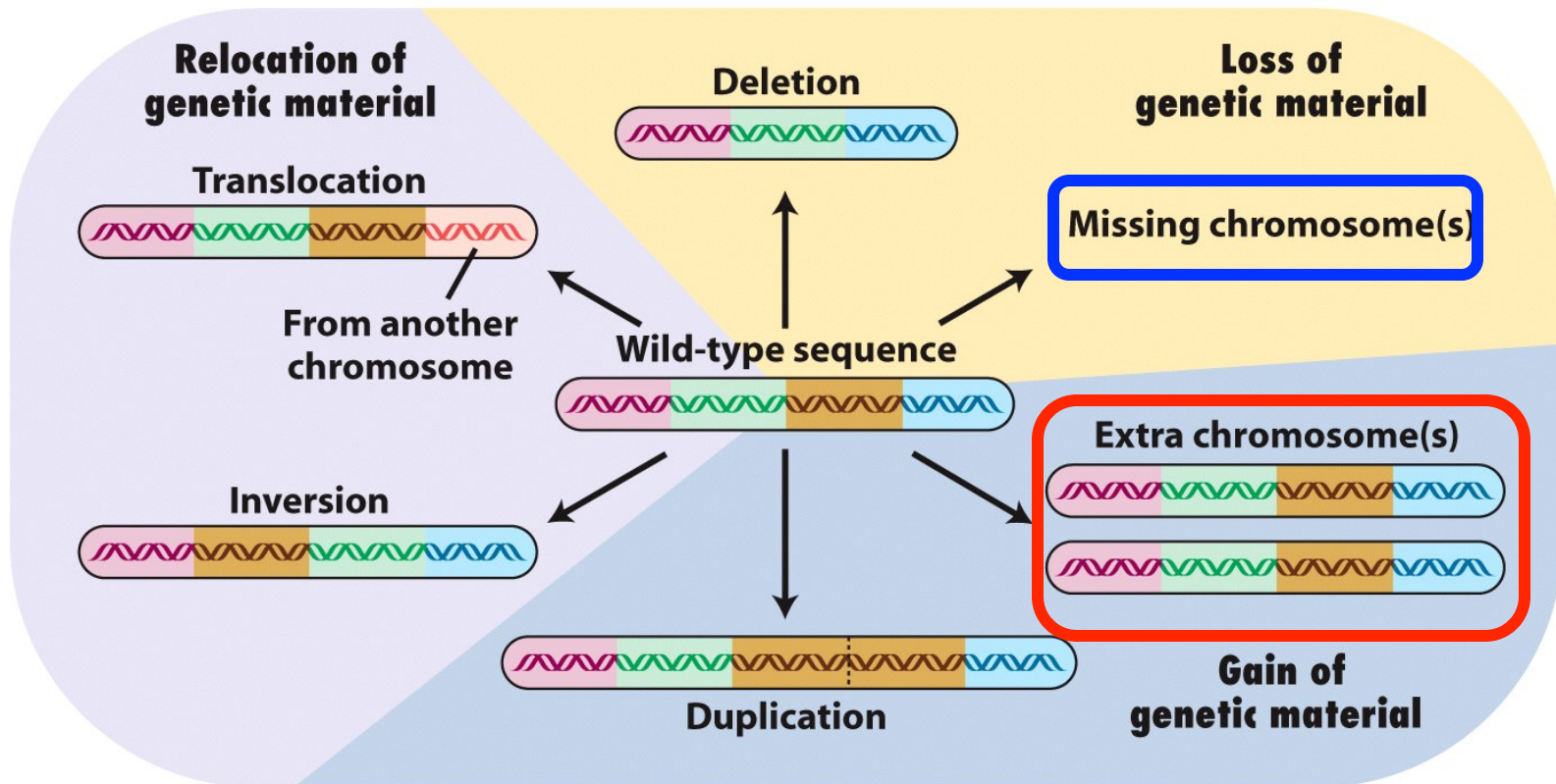
DNA Fingerprints →

← RFLP -Restriction
Fragment
Length Polymorphism

Disease Mutations Can Originate in Single Populations and Be Maintained in Population Ancestors



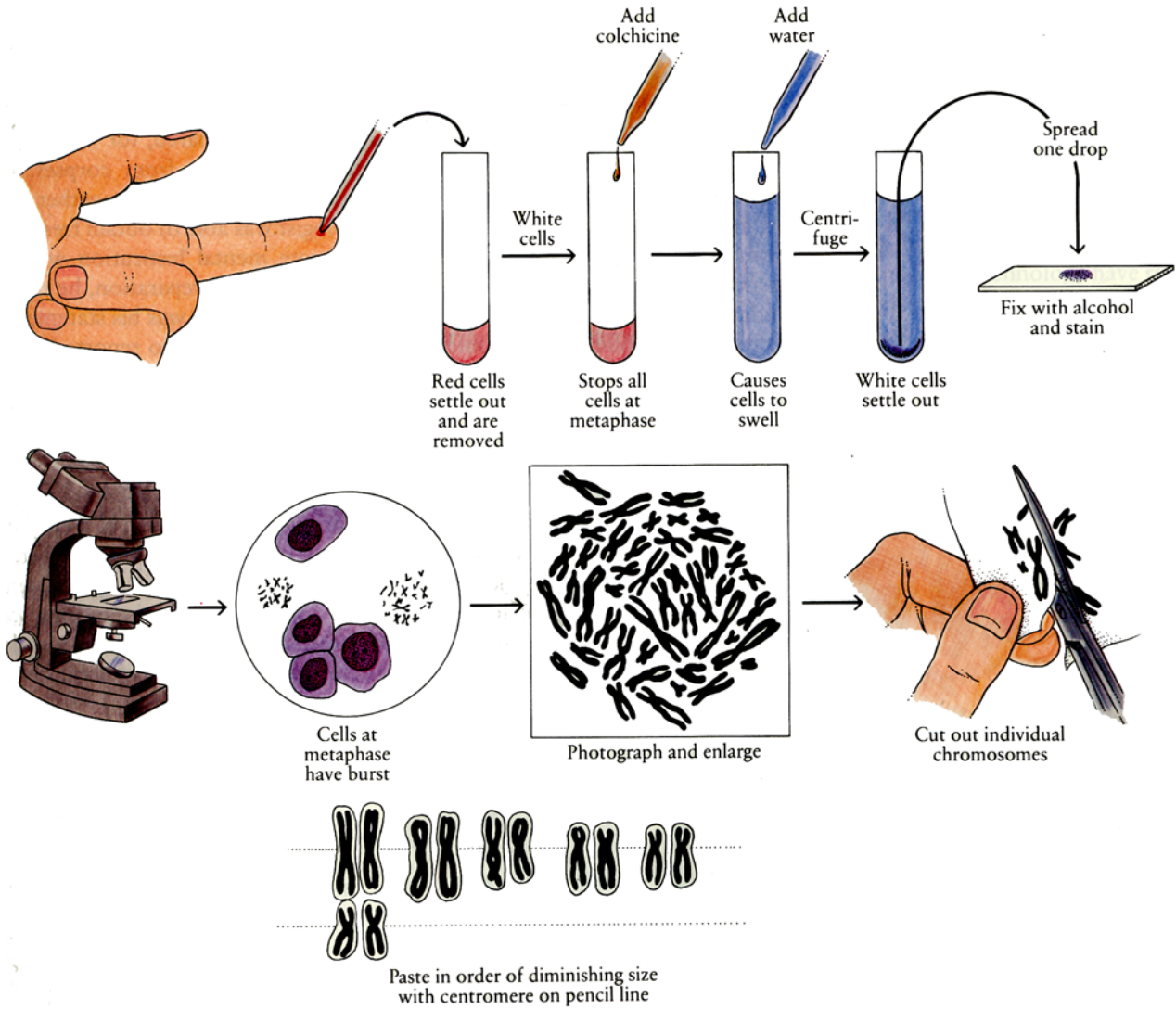
“Mutations” Can Also Occur By Large Chromosomal Changes



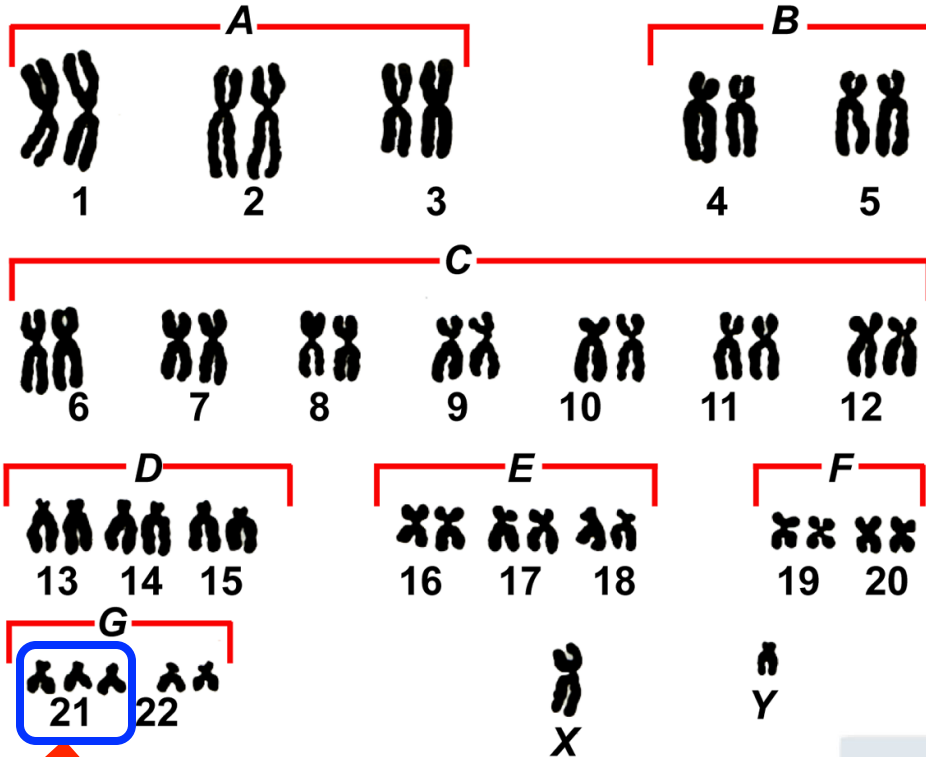
These changes affect many genes!

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

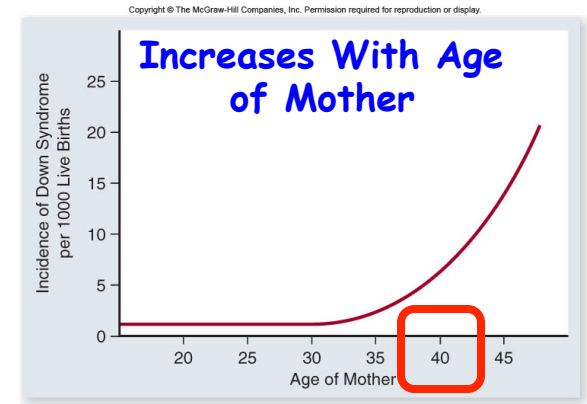
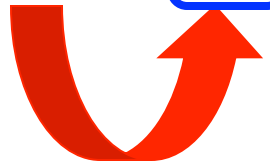
Karyotypes Can Be Used To Detect Changes in Chromosome Structure and Number



A Down's Syndrome Karyotype

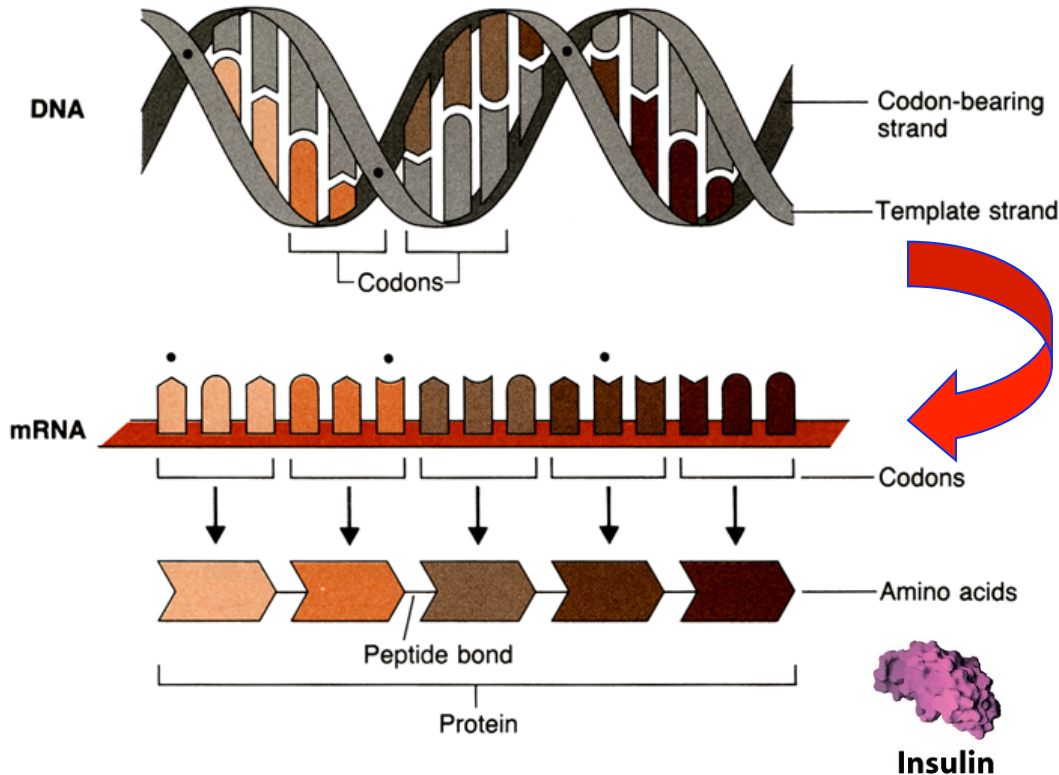


Three Chromosome
21s



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② How Does A Gene Lead To A Phenotype?



① mRNA Synthesized by Transcription

- Complementary to Transcribed, Non-Sense Strand
- Same Sequence As Sense Strand

② mRNA Translated into Protein by Translation of The Genetic Code

Genetic Code on mRNA Translated to Protein Sequence

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

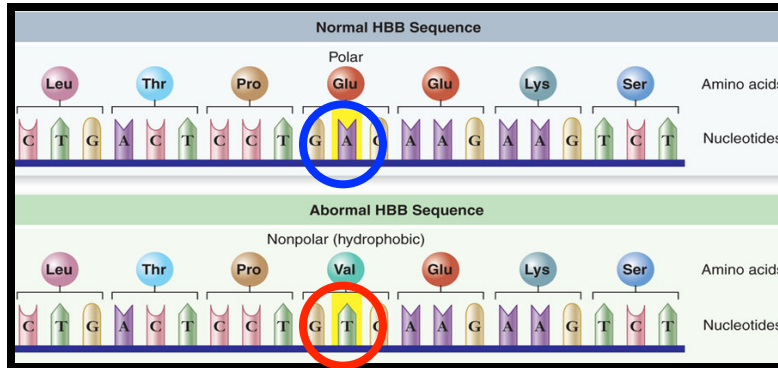
Know Sequence
Know Protein

Engineer New Protein

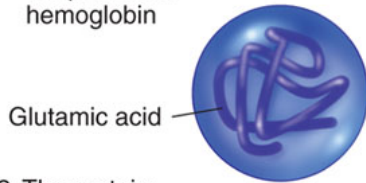
Human Genetic Disorders Occur As A Result of Mutations



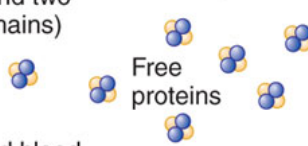
Chromosome 11



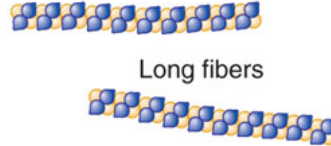
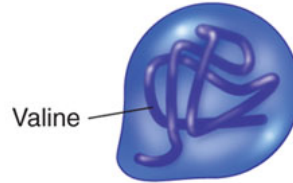
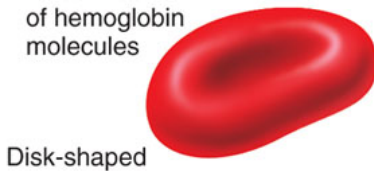
1. The polypeptide: the β chain of hemoglobin



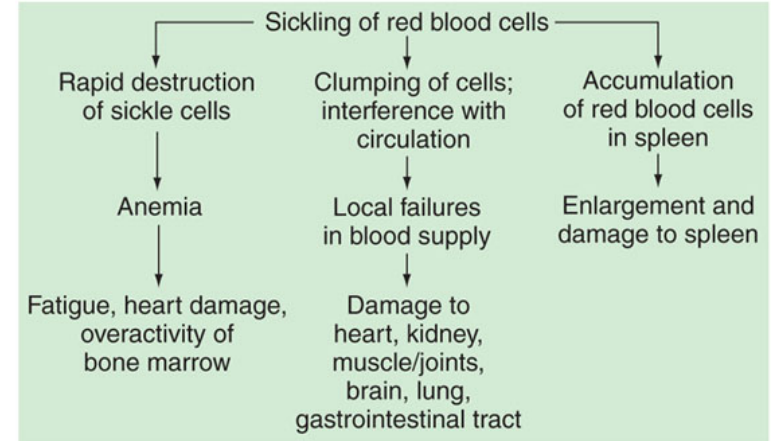
2. The protein: (made of two α and two β chains)



3. Red blood cell making thousands of hemoglobin molecules



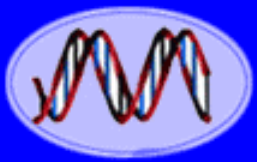
(b) Sickle-cell anemia is pleiotropic



(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							
HbD β Punjab	Val	His	Leu	Glu	Glu	Glu	His	Val	Gln	His							

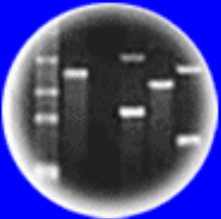
Sickle-Cell Anemia



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting

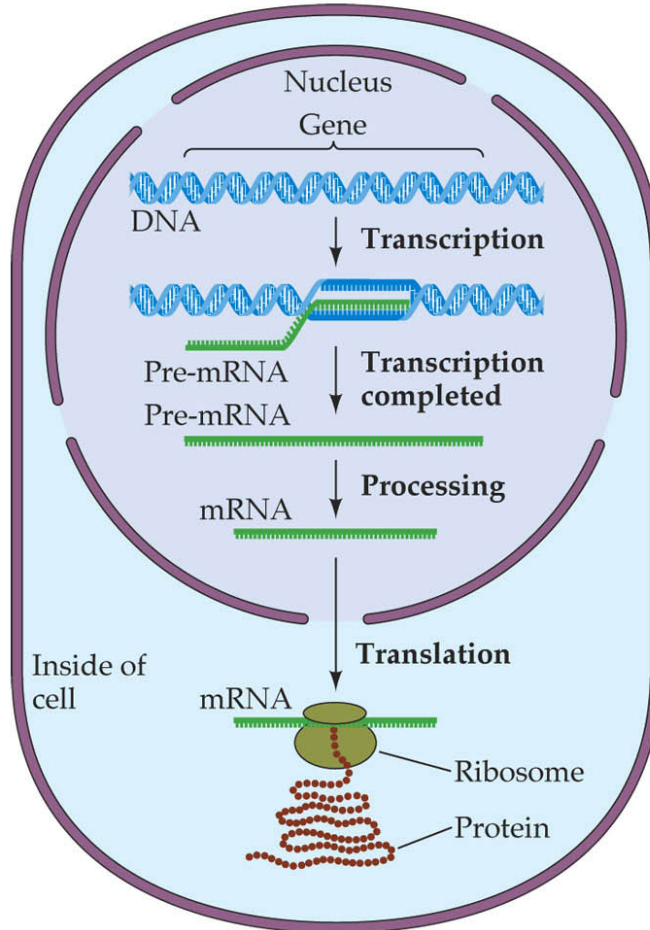


Cloning: Ethical Issues
and Future Consequences

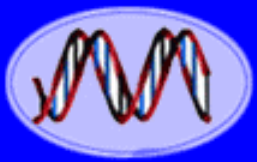


Plants of Tomorrow

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



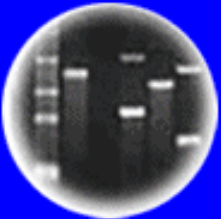
**It takes Genes
to Express
(and Replicate)
A GENE!!!**



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

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

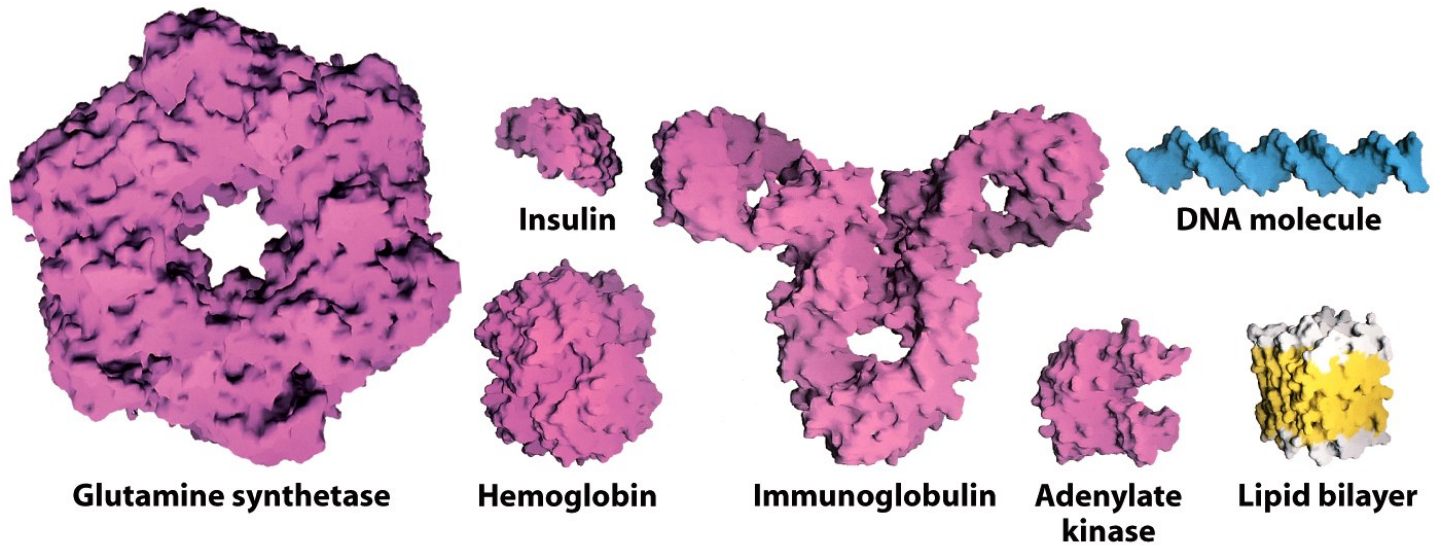
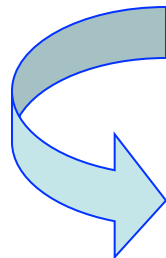
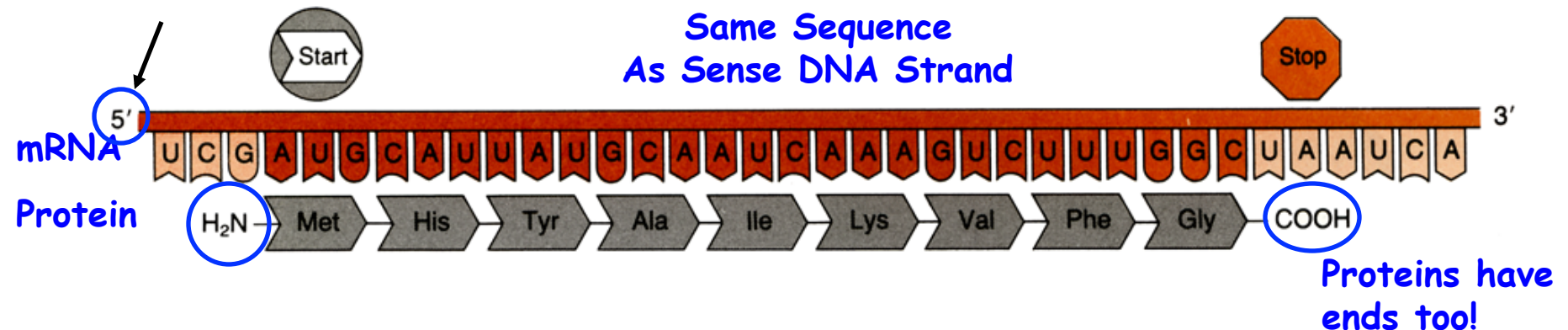


Figure 1-9
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Novel Cell Functions & Phenotypes

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!



DNA codons	Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile
GCA GCG GCT GCC	AGA AGG CGA CGG CGT CGC	GAT GAC	AAT AAC	TGT TGC	GAA GAG	CAA CAG	GGA GGG GGT GGC	CAT CAC	ATA ATT ATC	
TTA TTG CTA CTG CTT CTC	AAA AAG	ATG	TTT TTC	CCA CCG CCT CCC	AGT AGC TCA TCG TCT TCC	ACA ACG ACT ACC	TGG	TAT TAC	GTA GTG GTT GTC	TAA TAG TGA
Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Stop

For RNA, The Ts are replaced by Us.

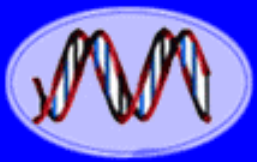
How Know?

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

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

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

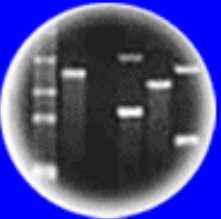
Design An Experiment to Show Code is Universal!



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



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!!

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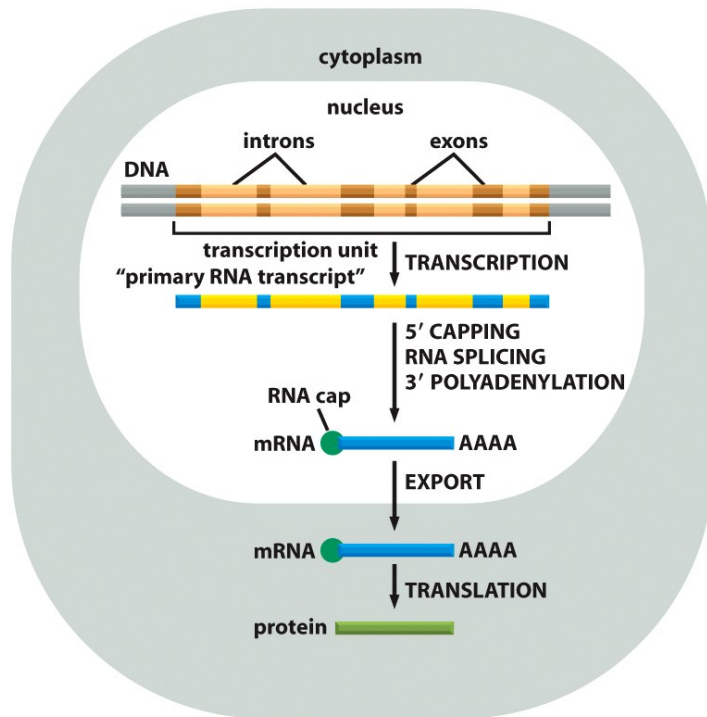


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Eukaryotic and Prokaryotic Gene Expression Processes Differ Slightly

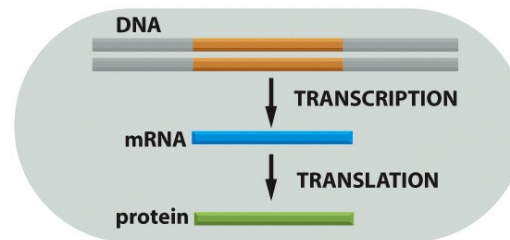
(A)

EUCARYOTES



(B)

PROCARYOTES

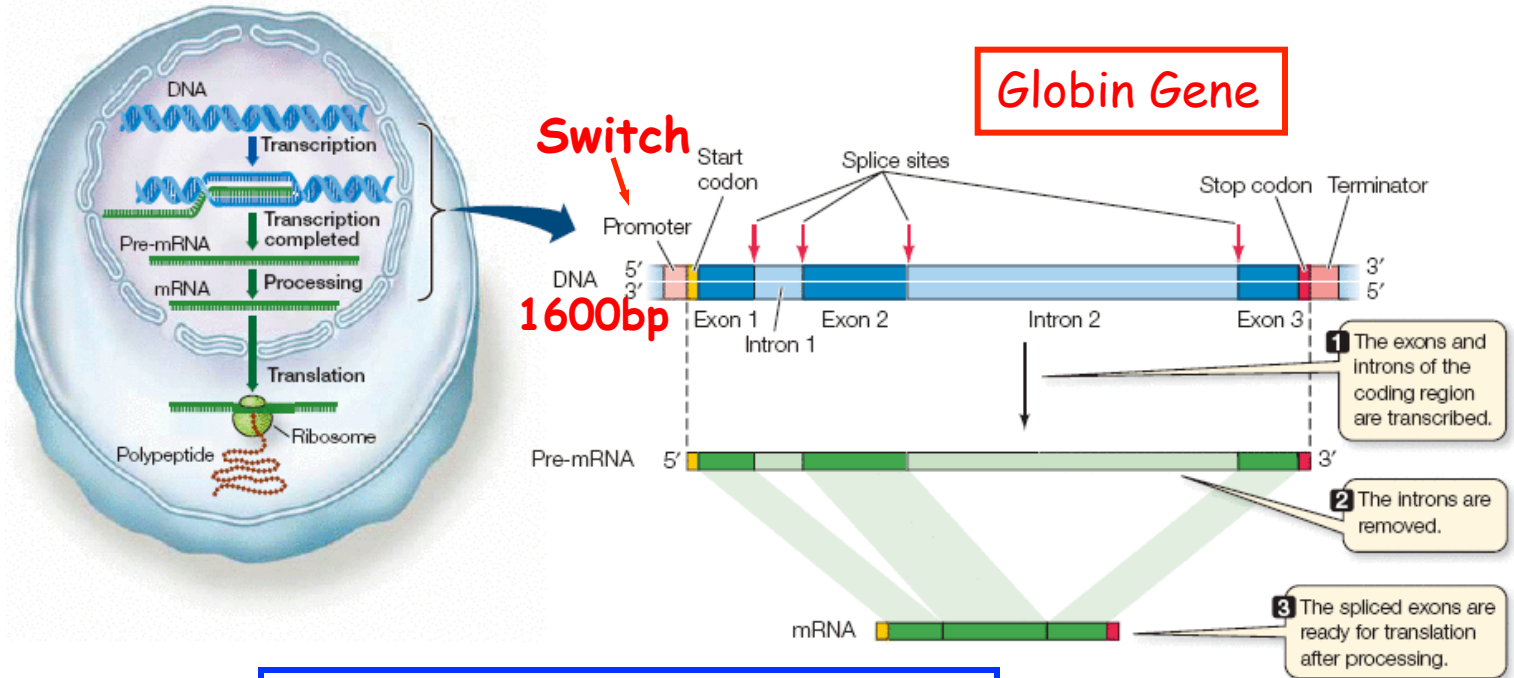


Genes Differ
Switches Differ
Genetic Code the Same
General Processes Same
Eukaryotic Gene Have Introns & Non-Coding Region in Gene!

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



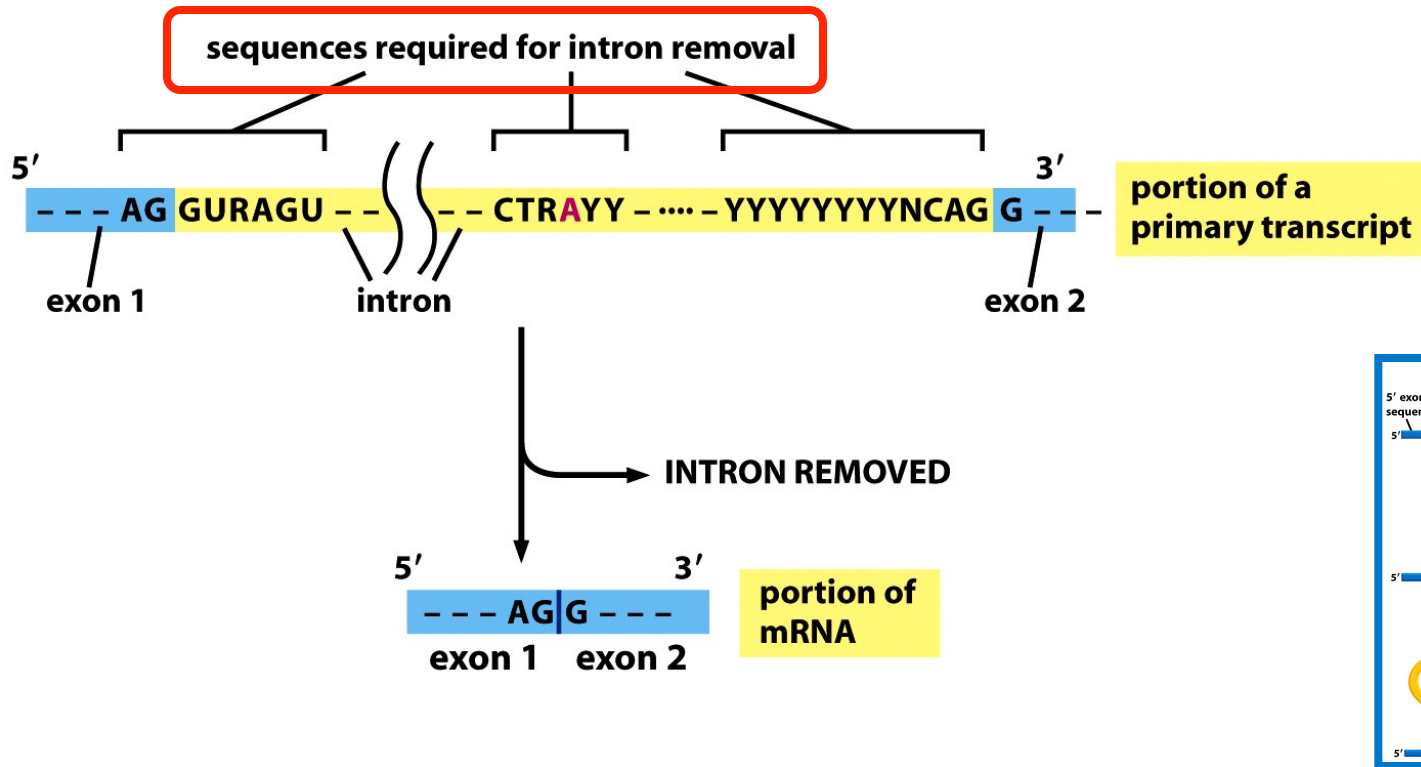
**Mutations → Blood Disorders
Where can these occur?**

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!

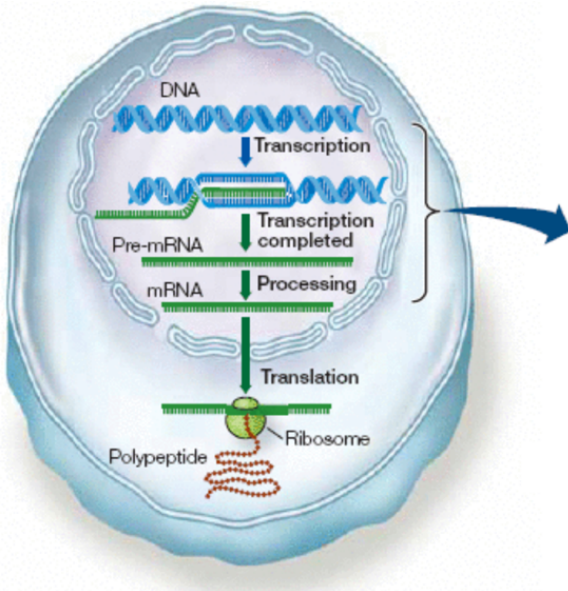


Specific Sequences Required For RNA Splicing!

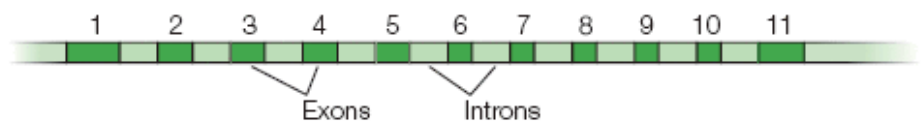
What Happens If These Sequences Are Mutated in a Gene?

Alternative Splicing- One Gene ↳ Several mRNAs & Proteins

Gene Activity in Variety of Cells, But....!!!

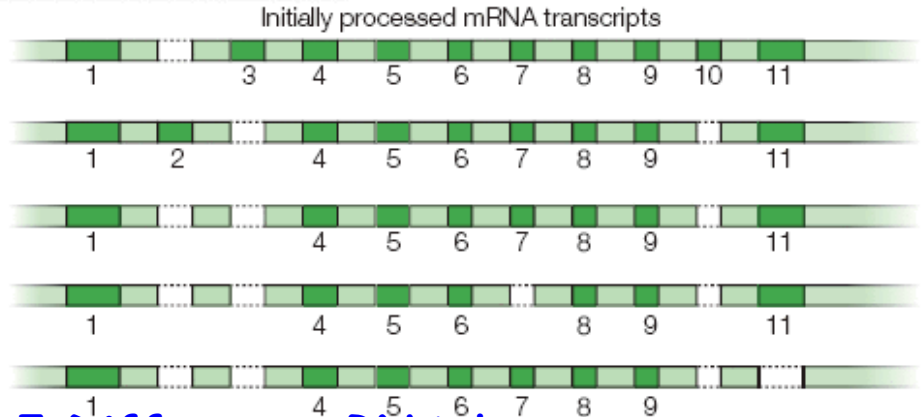


Primary RNA transcript for tropomyosin: 11 exons



Different splicing patterns in different tissues result in a unique collection of exons in mRNA for each tissue.

- Skeletal muscle: missing exon 2
- Smooth muscle: missing exons 3 and 10
- Fibroblast: missing exons 2, 3, and 10
- Liver: missing exons 2, 3, 7, and 10
- Brain: missing exons 2, 3, 10, and 11



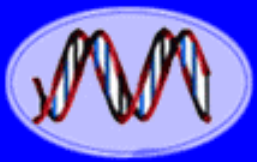
5 Different mRNAs!

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!!

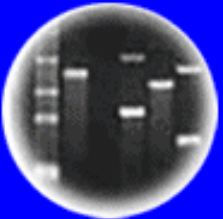
Implications for Genetic Engineering? Use Specific cDNA!



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



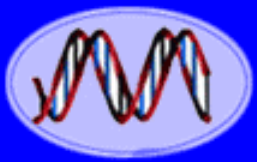
Plants of Tomorrow

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

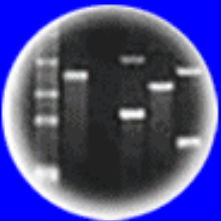
Modules → Anything You Want To Do Using
Genetic Engineering!



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

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

+

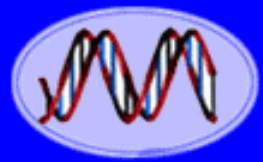
Human Insulin
Coding
Sequence

+

Bacteria
Transcription
Off Switch



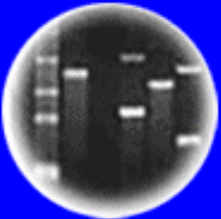
Human Insulin in Bacteria!!



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences

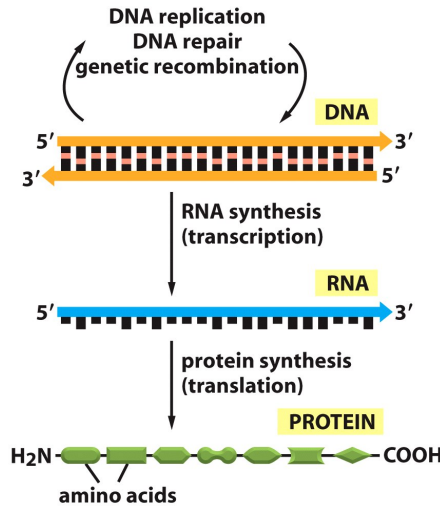


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How Do Genes Work & What are Genes in Context of...



Thinking About The Consequences of GMOs



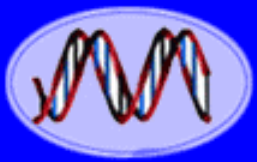
1. What is a Gene?
2. What is the Anatomy of a gene?
3. How Does the Gene Replicate?
4. How Does the Gene Direct Synthesis of a Protein?
5. Does the Gene Work Independently of other Genes?
6. What is the Sequence & Structure of the Protein?
7. How does it work in cell?
8. Does the Protein Structure imply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

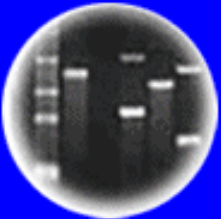
Same Processes!



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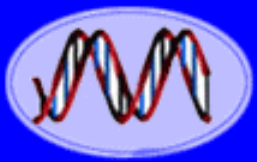
Can Identical Twins Be Different?



“Things Written in Pen You Cannot Change. That's DNA!
Things Written in Pencil You Can. That's Epigenetics”

Geneticist Danielle Reed

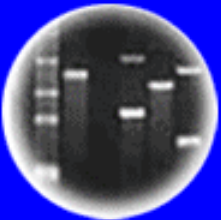
Nature vs. Nurture?



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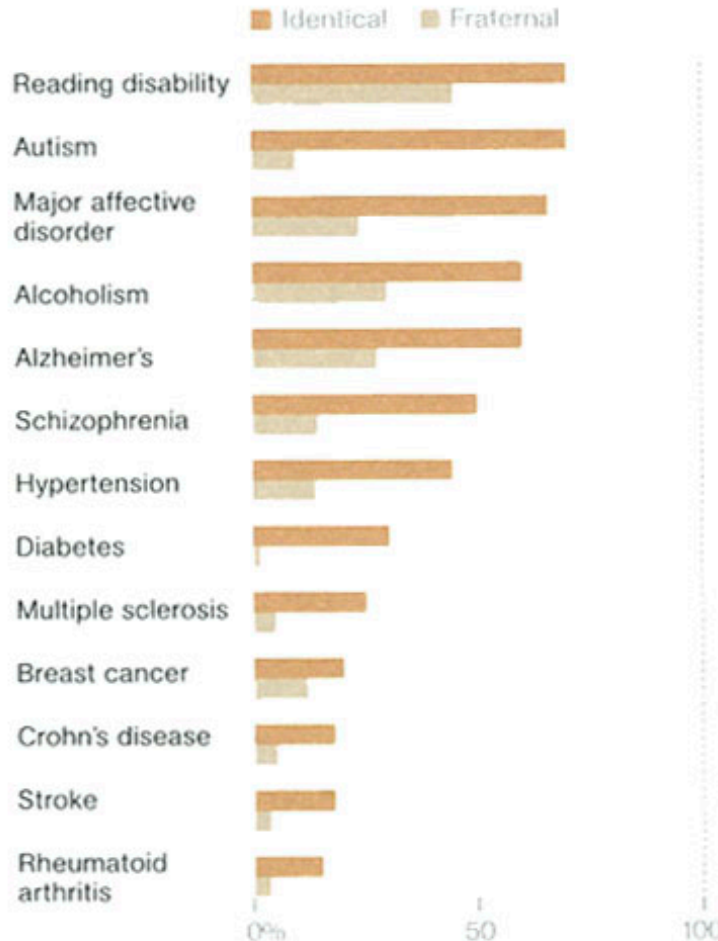
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SHARED TRAITS

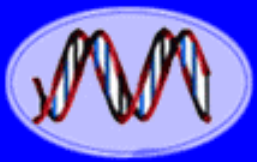
Identical twins share certain disorders, such as autism, much more often than fraternal twins do, suggesting the strong influence of heredity.



Because Genes
Replicate
Generation to
Generation!

But Environment
Can Play a Role

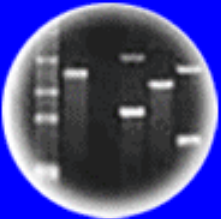
We Are
Beginning to
Learn Why!



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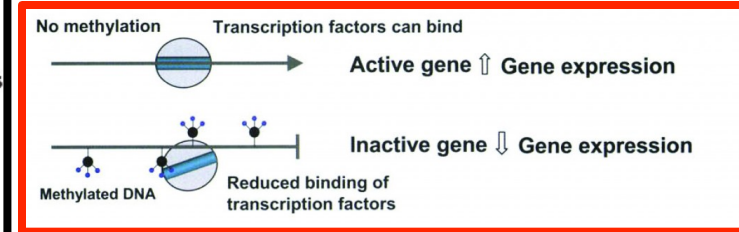
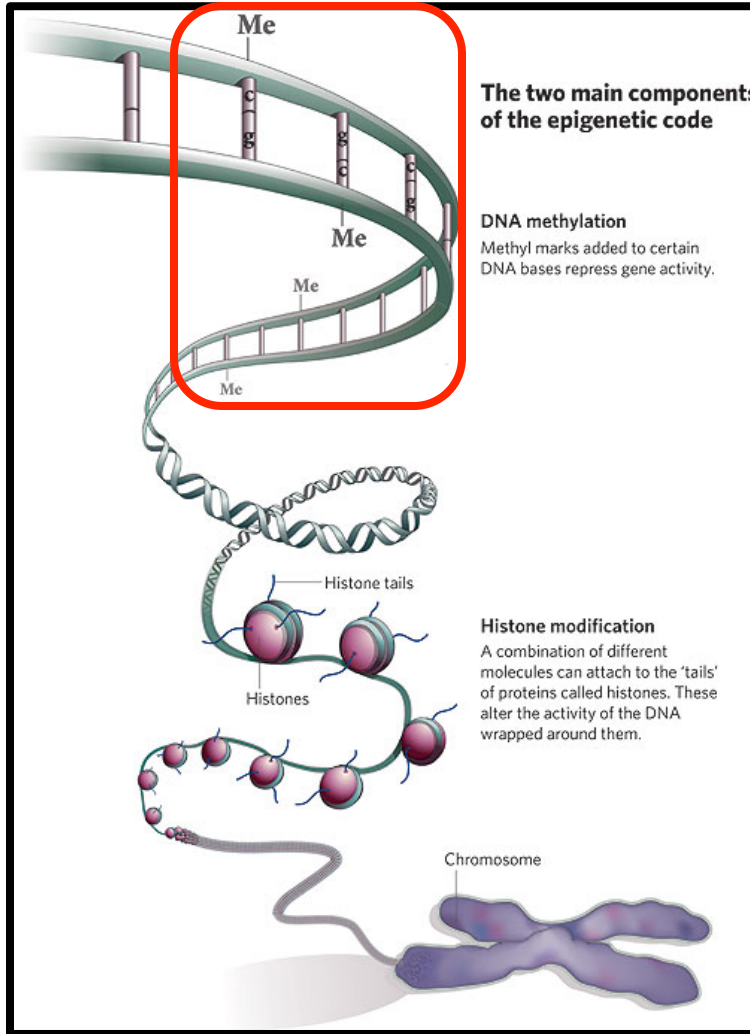


Cloning: Ethical Issues
and Future Consequences



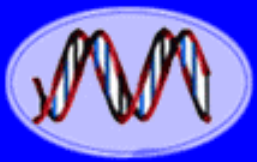
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Epigenetic Changes, or Chemical Modifications of Switches and Genes, Can Affect Gene Activity!



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

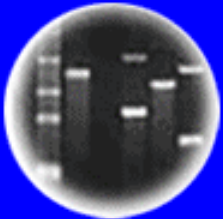
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SAME GENES, DIFFERENT PEOPLE

Identical twins are born with the same DNA but can become surprisingly different as they grow older. A booming field called epigenetics is revealing how factors like stress and nutrition can cause this divergence by changing how individual genes behave.



Epigenetic tag

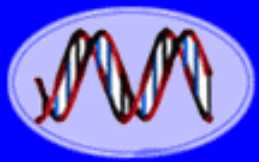
Tags are chemical mechanisms that can express (activate or suppress) genes to different degrees. They do not change DNA. Scientists suspect some tags can be inherited.

What causes tagging?

ENVIRONMENTAL influences such as nutrition may change the expression of a gene.
RANDOM epigenetic shifts can happen without any outside influences.

AMANDA HOBBS AND LAWSON PARKER, NGM STAFF
SOURCE: ARTURAS PETRONIS, CENTRE FOR ADDICTION
AND MENTAL HEALTH, TORONTO

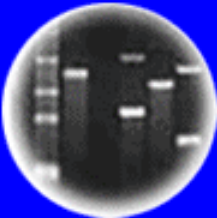
Rare Epigenetic Events Can Affect Individuals Differently!



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Epigenetic Effects in Disease?


EPIGENETIC EFFECTS
A few disease studies in the NIH Roadmap Epigenomics Project.

CANCER




Control of gene expression by epigenetic modification could have a role in tumour formation, and could explain how environmental factors trigger cancer.

PRENATAL CHANGES



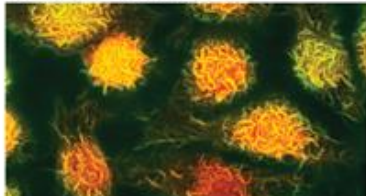
Molecular modifications to fetal and maternal DNA before birth could later make people susceptible to type 2 diabetes or cardiovascular disease.

BRAIN DISORDERS



Epigenetic changes have been implicated in brain health, from cognitive decline in normal ageing to conditions such as Alzheimer's disease, schizophrenia, bipolar disorder and autism.

CHRONIC DISEASES

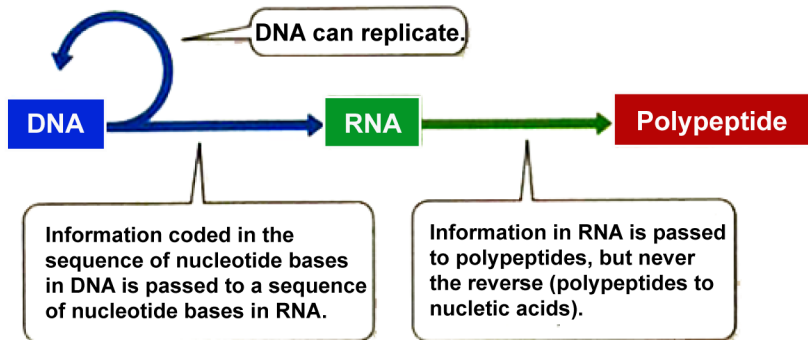


Complex chronic conditions such as systemic lupus erythematosus, asthma and insulin resistance in obesity and diabetes are thought to have an environmental component. Studies aim to identify how this can cause epigenetic changes that might affect disease progression.

Rare Epigenetic Events Affect Individuals Differently!

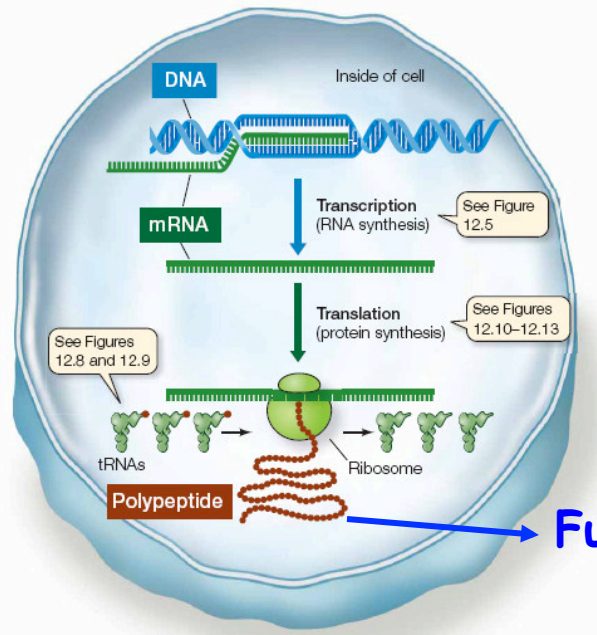
How Do Genes Work-Not As Simple As We Think!

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!