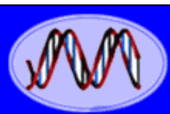
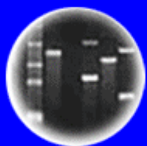


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

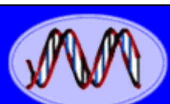
HC70A & SAS70A
Winter 2018
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg, John Harada, & Channapatna Prakash

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

UCLA

UCDAVIS
UNIVERSITY OF CALIFORNIA



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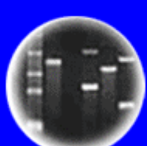


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

THEMES

1. What Are Genes & Their Properties
2. How Do Switches Regulate Genes in Space & Time?
3. How Does DNA Replication Occur?
4. What is the Polymerase Chain Reaction (PCR) and How is PCR Used in Society?
5. How Do Mutations Occur?
6. How Can Pedigrees Be Used To Follow the Inheritance of Mutant Genes With Phenotypes and RFLPs?
7. How Do Mutations Change Phenotypes?
8. What is the Colinearity Between Genes & Proteins (i.e. how does the DNA sequence specify a protein sequence)?
9. What is the Genetic Code?
10. Yo!-It's in the DNA Sequences- What Are the Implications For Genetic Engineering?

What is A Gene?

Begin 5'

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

End 3'

Sequence or Order of Nucleotides Coding DNA Strand
(Coding Strand)

The β-Globin Gene

Blood Protein Carries Oxygen to All Genes From Lungs ⇨ Energy

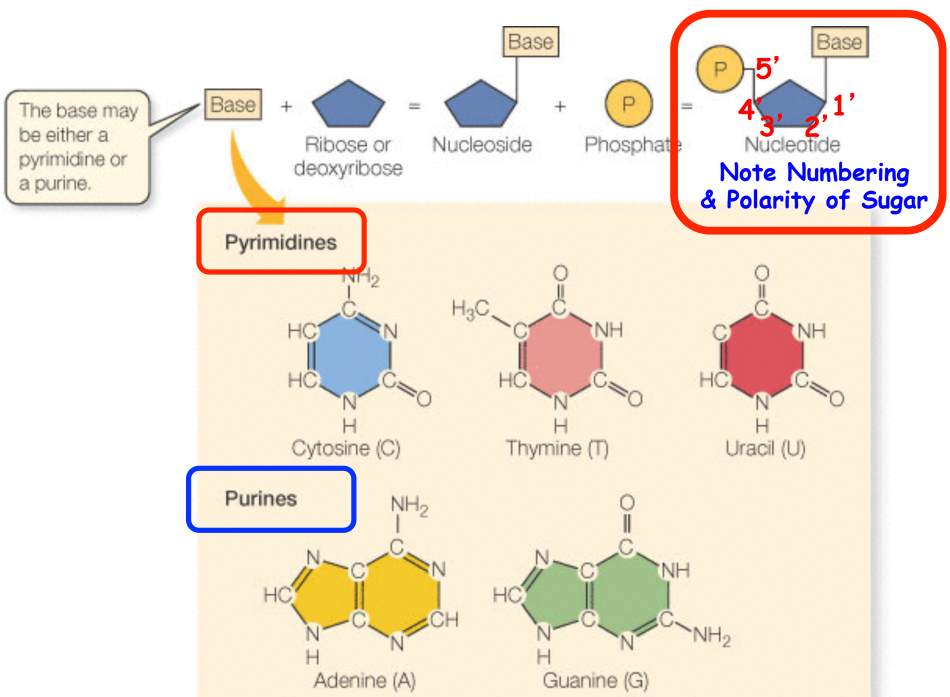
A Gene is a <u>Unique Sequence</u> of Nucleotides Specifying a Function

DNA Sequence = Biology!
What If Sequence Changed?

SEQUENCE → FUNCTION

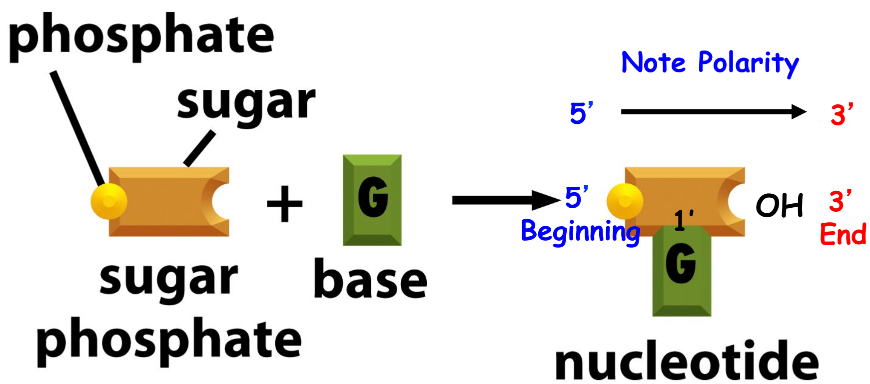
Relative to Coding or Sense Strand of Gene

There Are Four Different Nucleotides in DNA Joined Together in a Chain By Phosphodiester Bonds



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



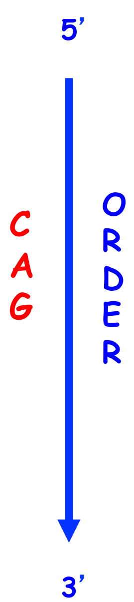
The Sugar is the HUB

DNA Sequence Defined By Nucleotide Order

DNA Sequence = Functional Uniqueness = Biology

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

Nucleotides Are Joined Together in a DNA Chain By 5' to 3' Phosphodiester Bonds



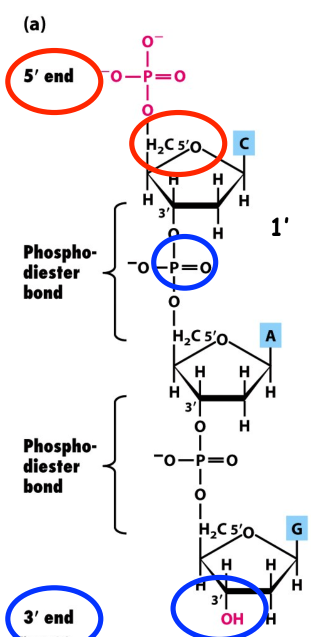
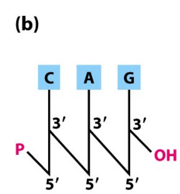


Figure 4-2 *Molecular Cell Biology, Sixth Edition*
© 2008 W. H. Freeman and Company



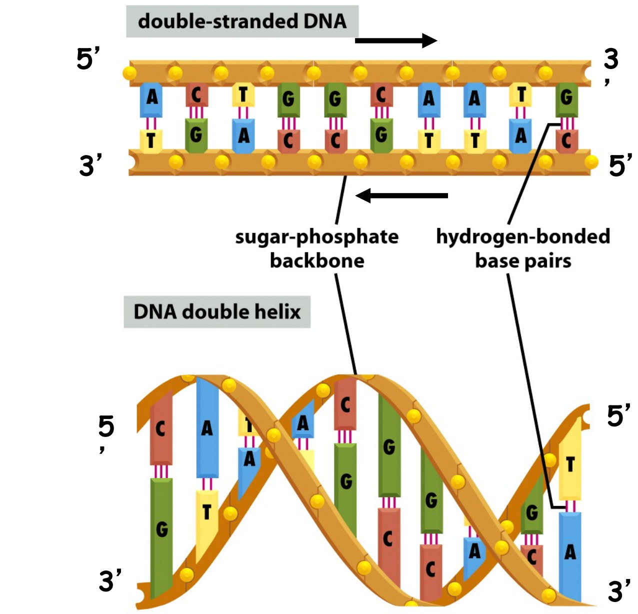
Short-Hand Notation

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

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

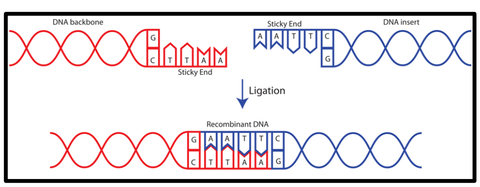


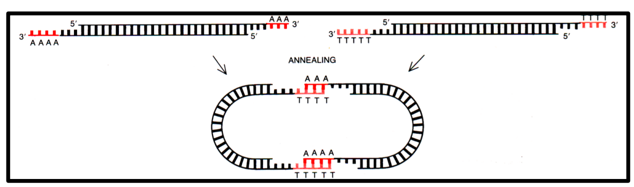
Watson and Crick, Nature, 1953

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

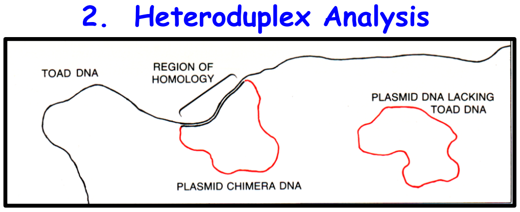
Complementary Base Pairs Are Essential For Genetic Engineering Engineering, Analysis of Recombinant Plasmids, and Polymerase Chain Reaction (PCR)

1. Annealing Two Two Molecules Together (“Cut & Splice”)

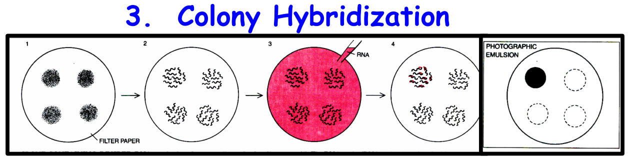




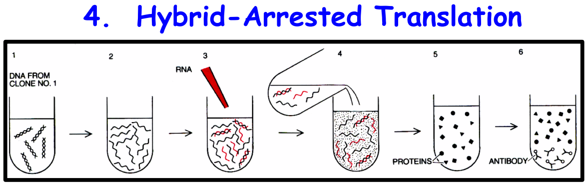
2. Heteroduplex Analysis



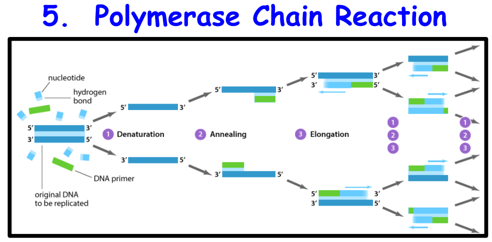
3. Colony Hybridization



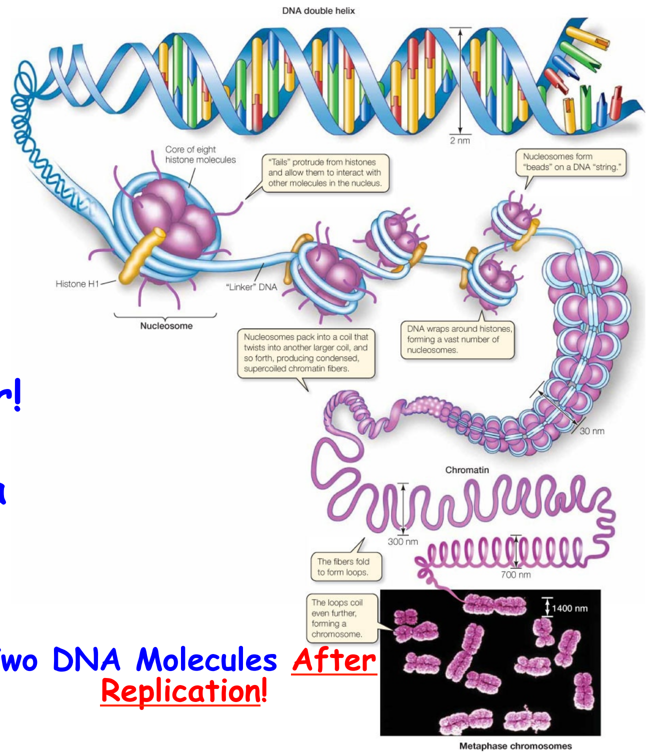
4. Hybrid-Arrested Translation



5. Polymerase Chain Reaction

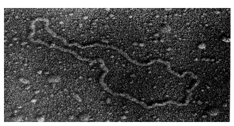


A Human Chromosome Contains One (or Two!!) Continuous DNA Molecule(s)



DNA in Human & Eukaryotic Chromosomes is Linear!
(Has Two Ends)

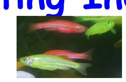
DNA in Most Bacteria is Circular!

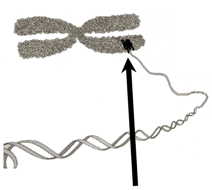


Two DNA Molecules After Replication!

A Chromosome Contains Many Genes Operating Independently

What is the Evidence?



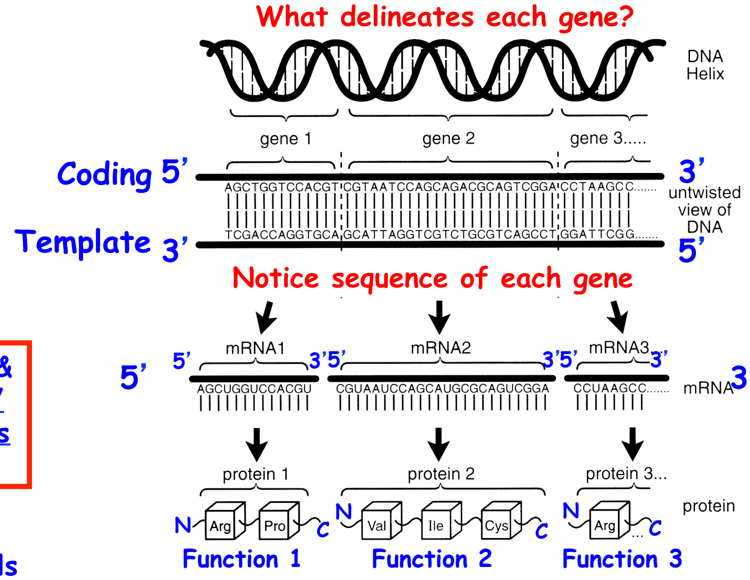


Position of Genes 1, 2, & 3 in chromosome

Discrete Units!

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

What delineates each gene?



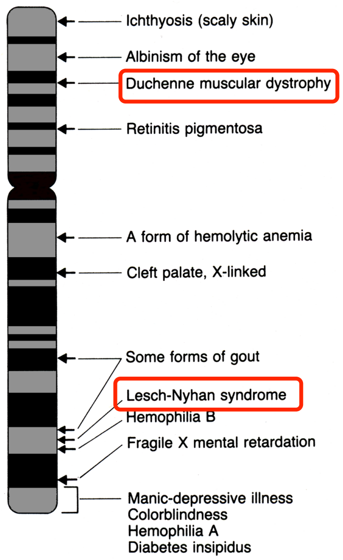
Note sequence of each protein

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

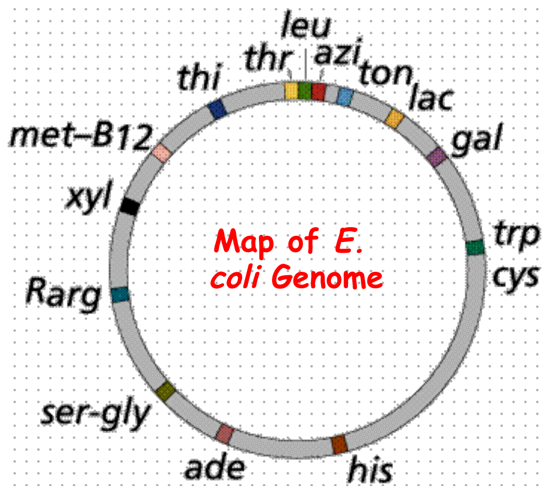
VERY IMPORTANT CONCEPT!
COLINEARITY BETWEEN GENE SEQUENCE AND PROTEIN SEQUENCE

Genes Reside at Specific Locations That Can Be Mapped

Human X Chromosome



Linear DNA
How Know?



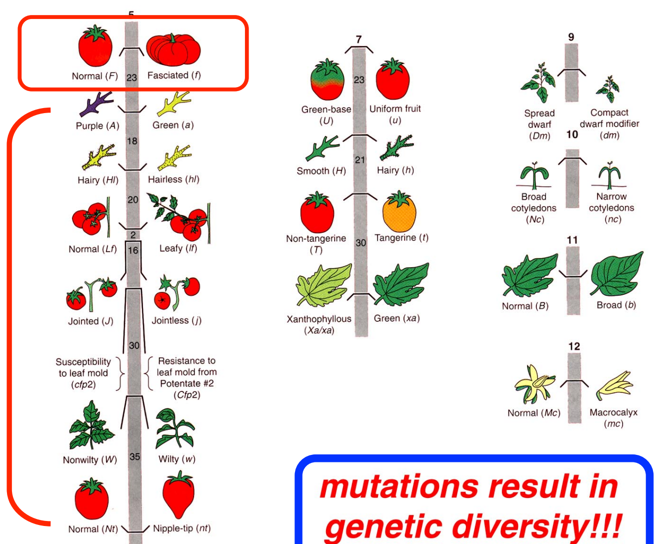
Circular DNA
How Know?

Alleles Reside at the Same Position on a Chromosome

Allele Phenotypes Specify Markers For Each Gene Location!

Alleles

Different Genes

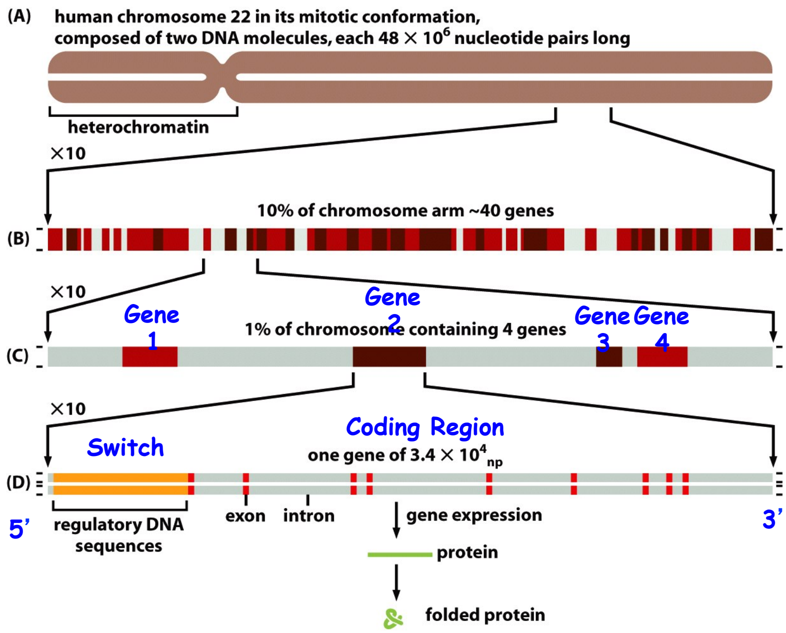


mutations result in genetic diversity!!!

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

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

Organization of Genes on Human Chromosome 22



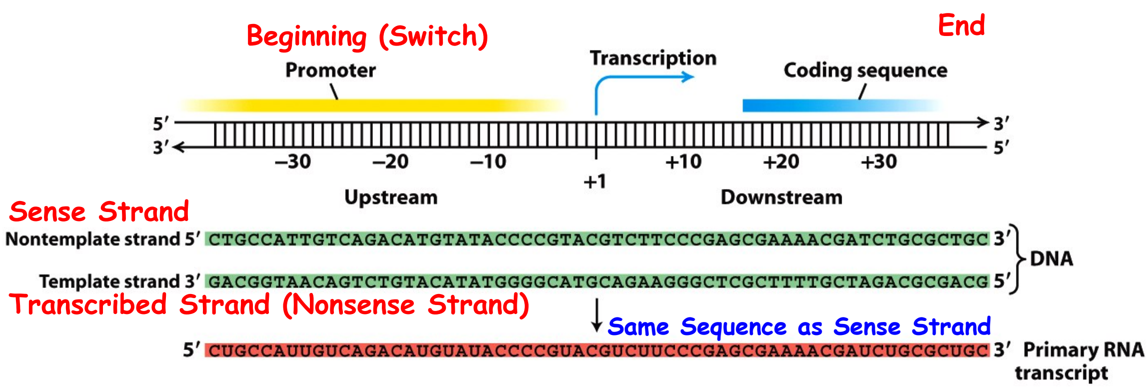
250 genes

Genes Are Defined/ Precise Regions of DNA

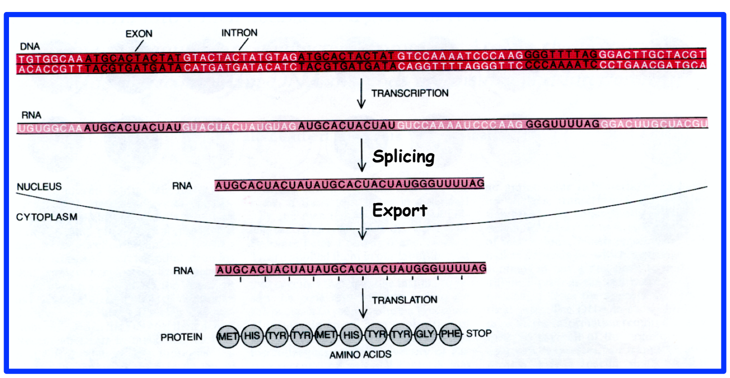
One Large Gene!
(Note Size of Introns & Exons)

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

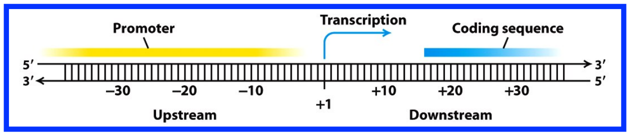
A Conceptualized Gene

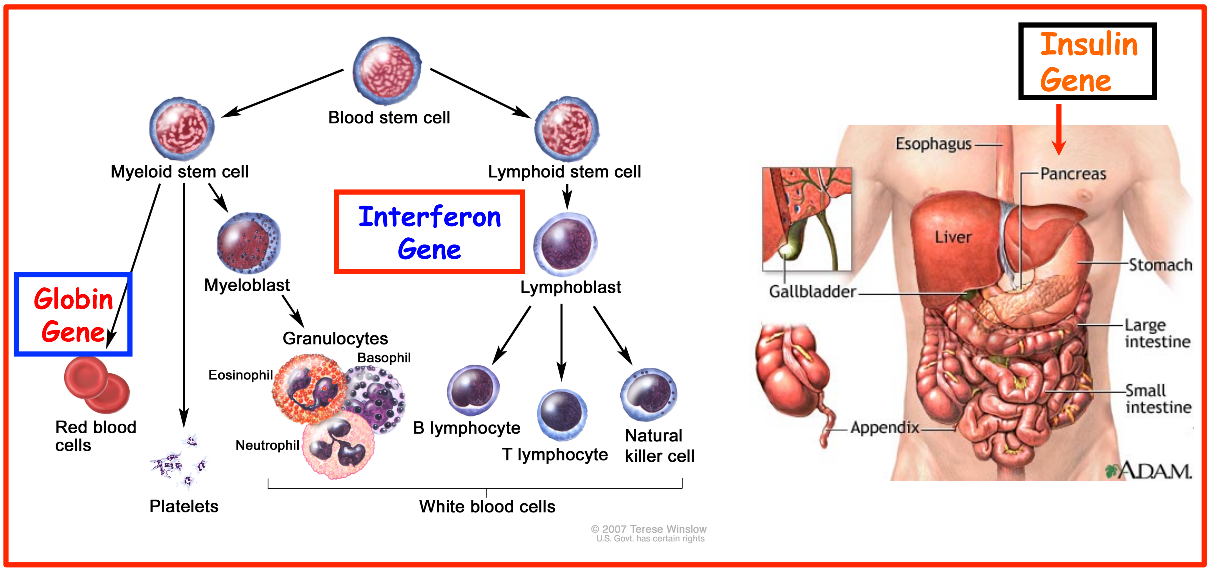


DNA to Protein



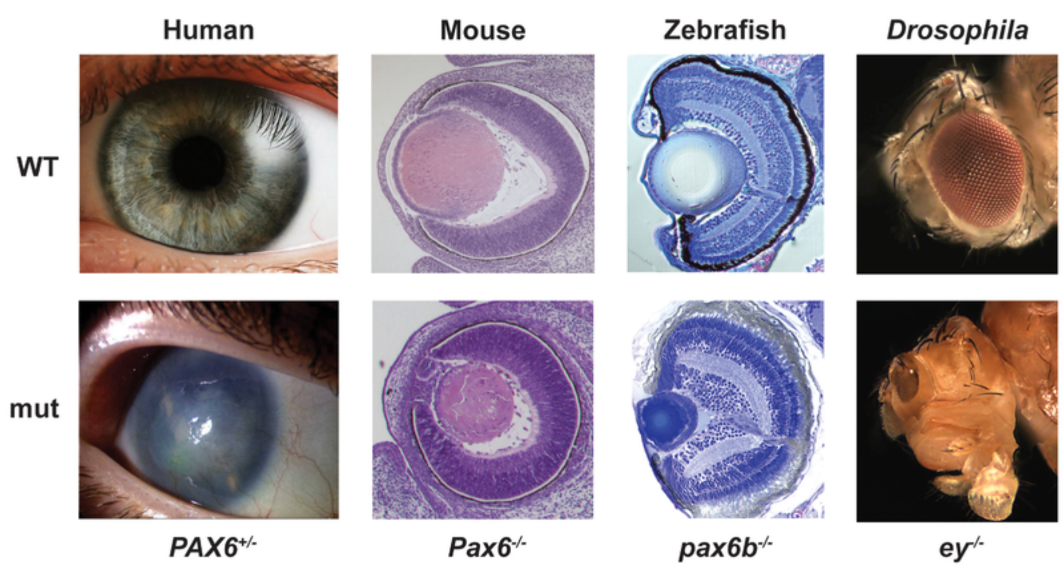
Switches Control Where & When A Gene Is Active
Unique Functions → Unique Cells





Different Switches!!!!

Using Genetic Engineering to Change Body Architecture-Engineering Eyes on a Fly's Leg With a Single Gene!

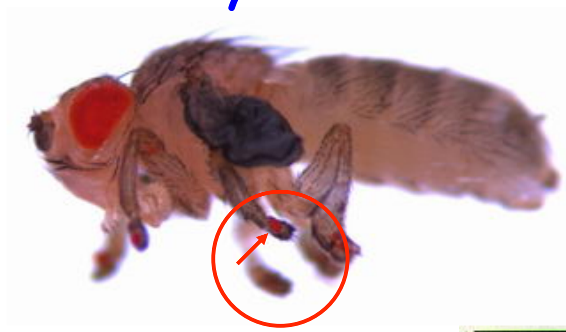


	Human	Mouse	Zebrafish	*Drosophila*
EQs	cornea opaque iris absent retina degenerate lens opaque aqueous humor of eyeball increased pressure	eye decreased size lens fused_to cornea iris morphology anterior chamber absent	eye decreased size lens decreased size retina malformed	eye absent

Regulatory Genes – Turns on Gene Switches

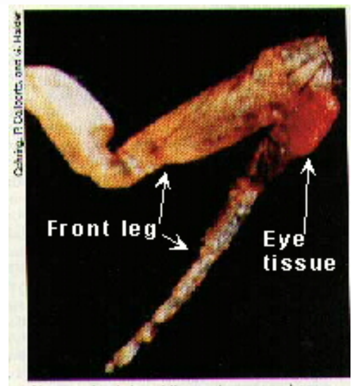
The Eye Gene Can Be Expressed in Different Parts of the Fly by Engineering the Eye Switch

Eye Gene

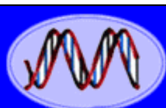


Replace the Head Switch With the Leg Switch by Genetic Engineering

Eye Gene
+
Leg Switch



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



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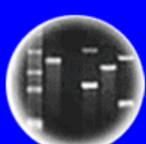

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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Eye Regulatory Network

Control Genes Like The Eye Gene Control The Activity of Other Genes By Coding For a Protein That Interacts With Switches of Other Genes and Switches These Genes On!

5' | Eye Switch | Eye Gene | 3' — Eye Protein → Gene 1, Gene 2, Gene 3, Gene 4 → Eye Proteins: Protein 1, Protein 2, Protein 3, Protein 4

Works in Head!

Eye Protein Binds to Switches to Turn Genes On!

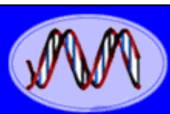
Eye on Head!

Leg Switch | Eye Gene — Eye Protein → Gene 1, Gene 2, Gene 3, Gene 4 → Eye Proteins: Protein 1, Protein 2, Protein 3, Protein 4

Normally Eye Gene is OFF in Leg. Switch only Works in Leg.

Genes Act Like Legos!

Eye on Leg!



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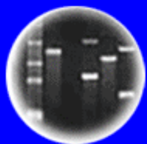

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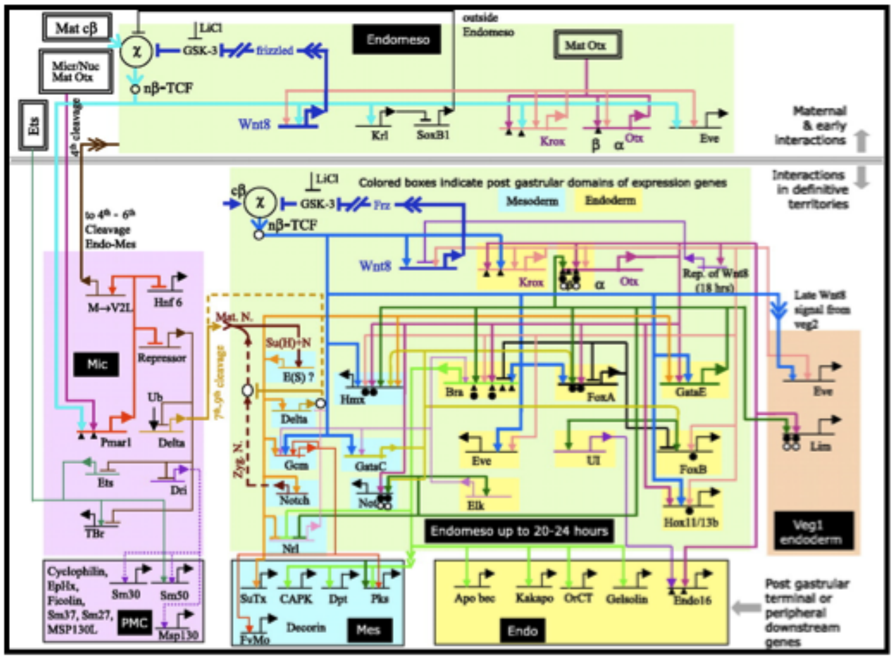
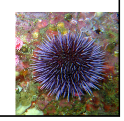
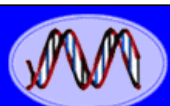
<u>Ultimate Goal</u>:To Dissect Genetic Regulatory Networks Programming Human Development From Birth to Death!



Genetic Networks Programming Early
Sea Urchin Development





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Entire Genetic Code
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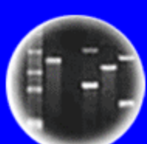

DNA Fingerprinting

Cloning: Ethical Issues
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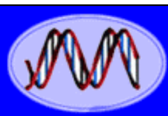
GENES AND SWITCHES ARE UNIQUE DNA SEQUENCES

1. They Can Be Cloned & "Shuffled" & Engineered Creating New Genes That Have No Counterparts in Nature

2. These New Genes Can Be Transcribed in New Cell Types (Switch Change) &/or Organisms &/or Both (e.g., <u>Human Genes in Bacteria</u>)

3. All Genes are Regulated & Controlled by Switches Genetic Engineering Can Uncover Genes & their Switches & the Wiring Together of All Switches in All Genes ⇨ <u>Program of Life From Birth to Death</u>

Yo! It's in the Sequences!!



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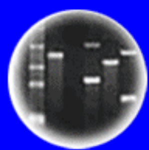

DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

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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?

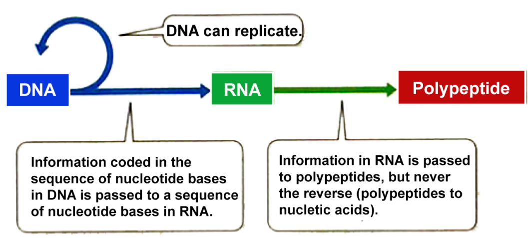
We Will Know at the DNA Level What Biological Information Programs Life to Death!

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!

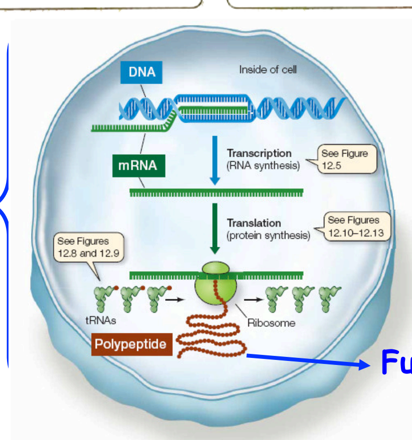
How Do Genes Work?

① Replication



② Gene Activity to Function & Phenotype

Gene Activity
↓
Protein
↓
Function
↓
Phenotype (Trait)



Function →

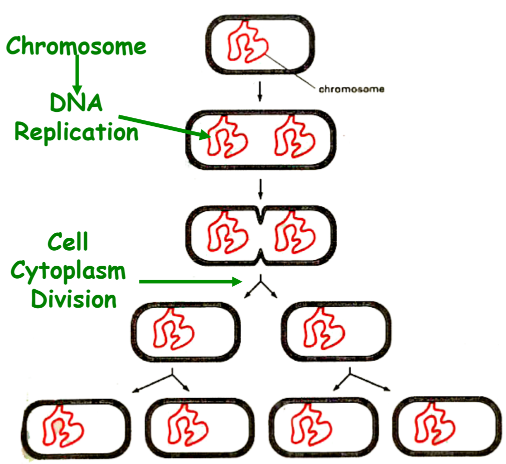
A Gene is NOT Expressed Unless A Functional Protein Produced!

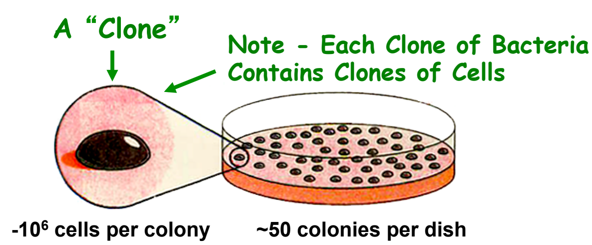
WHAT ARE THE PROPERTIES OF A GENE?

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

1

How Are Genes Replicated Each Cell Generation?





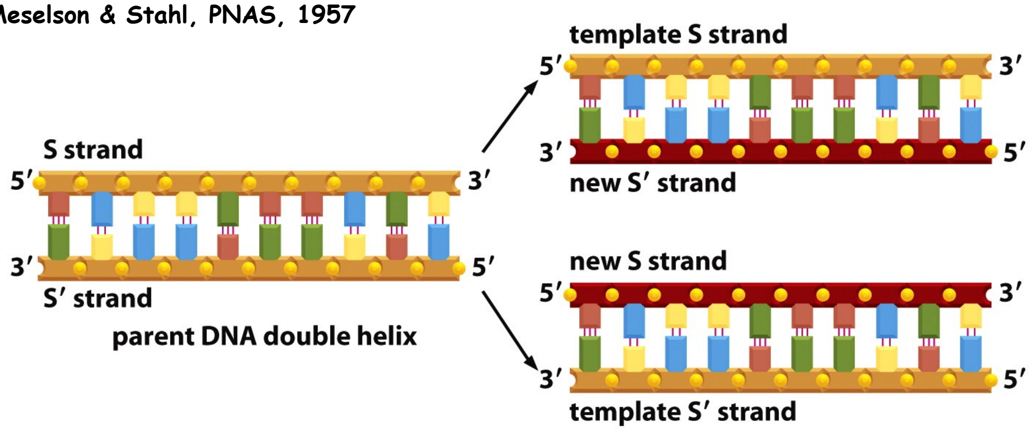
A Bacterial Colony Contains Many Copies of Same Cell, or Clones, Which are Genetically Identical!

Each Daughter Cell Contains The Same Collection of Genes

Clones!

DNA Replication Occurs Semi-Conservatively

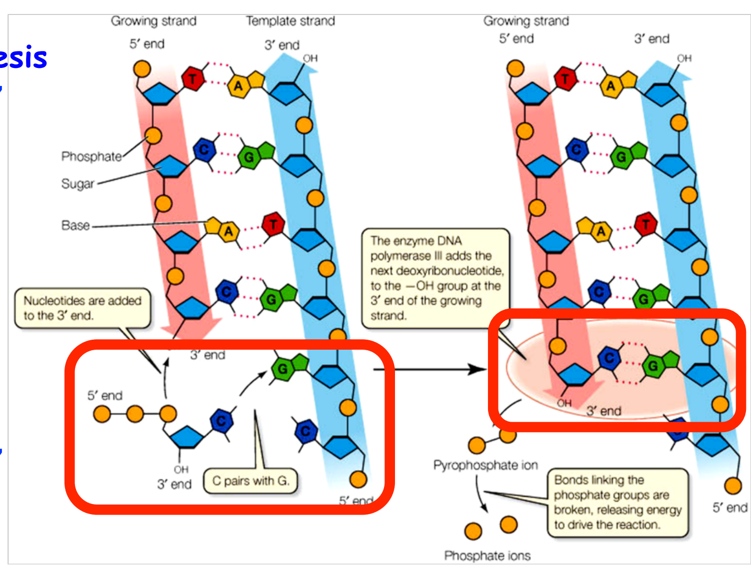
Meselson & Stahl, PNAS, 1957



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'

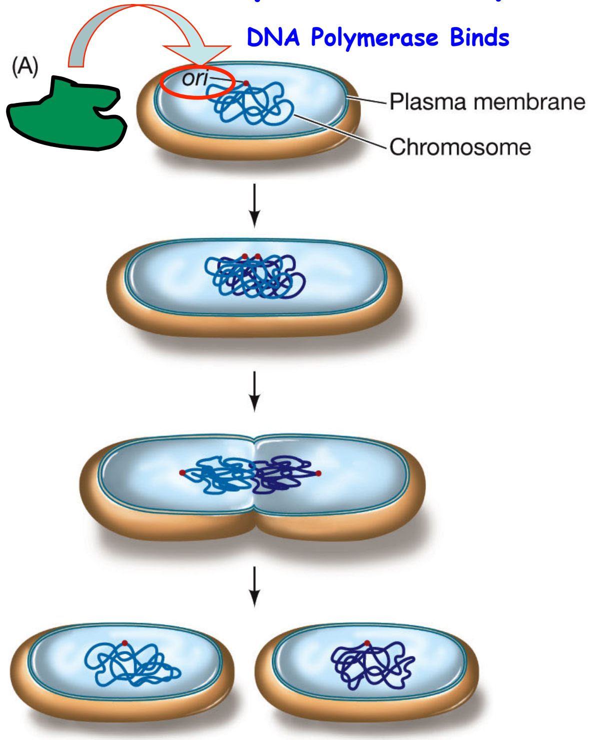


Sequence is Specified by Complementary Bases

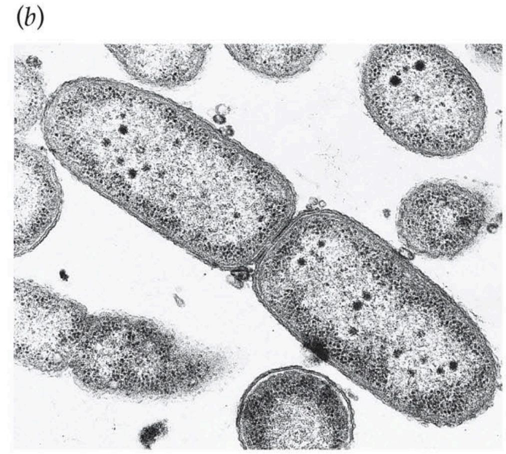
Note: 5' (P) & 3' (OH)

5' to 3' Polarity Specifies Sequence

DNA Replication Requires An Origin of Replication

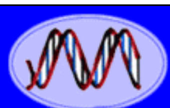


Two IDENTICAL Cells - Phenotypically & Genotypically - From One



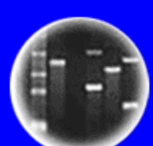
DNA Replication Also Requires:
1. Template
2. Nucleotides
3. DNA Polymerase (Machine)
4. "Primer" to Start Replication

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



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

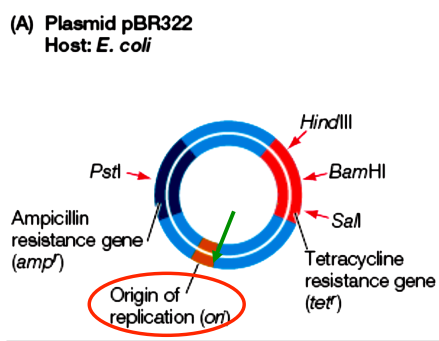


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Vectors Are Needed To Replicate Genes In Transformed Cells



1. Ori is a specific sequence
2. Ori is Genome & Organism Specific
3. DNA Polymerases are Specific For Each Organism Therefore, Need Correct Ori to Replicate Gene in a Specific Organism!

Note → Need Bacterial Ori to clone human gene in bacteria. Need human Ori to replicate a bacterial gene in human cells.

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

Yo! It's in the Sequence= Function

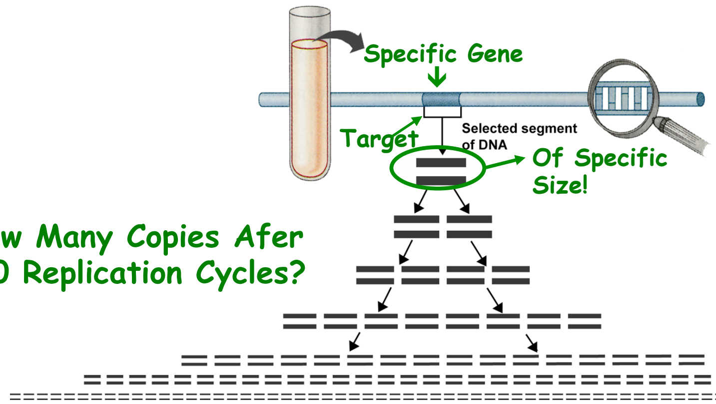
∴ Vectors can be Engineered!

Ori's can be cloned/synthesized!

MODULAR!!

The Second Genetic Engineering Revolution - The Polymerase Chain Reaction (PCR) is a Molecular Xerox Machine That Can Amplify DNA Sequences in a Test Tube Without Cloning!

How Many Copies Afer 10 Replication Cycles?



DNA Copies All The Same Size

Kary Mullis

1993

Based on Knowledge of DNA Synthesis

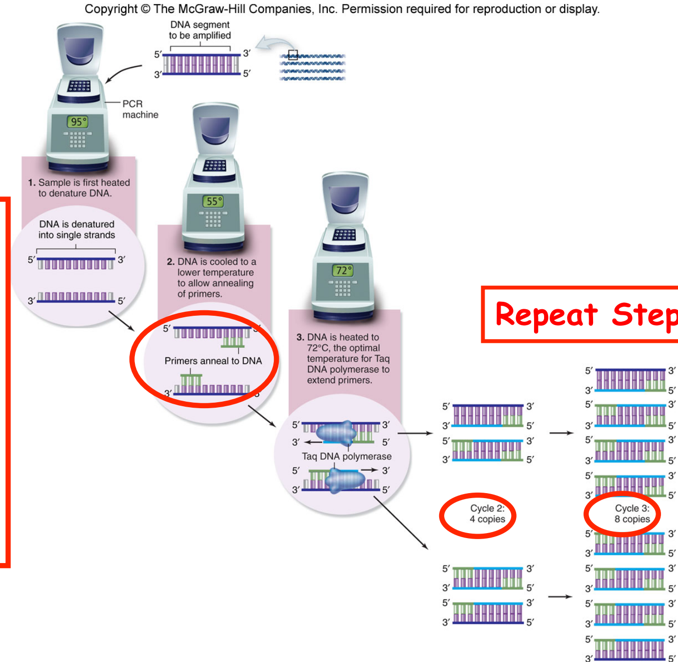
1. PCR Has **Revolutionized** DNA Analysis!

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. Thermo Cycler

Repeat Steps or Cycle

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

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

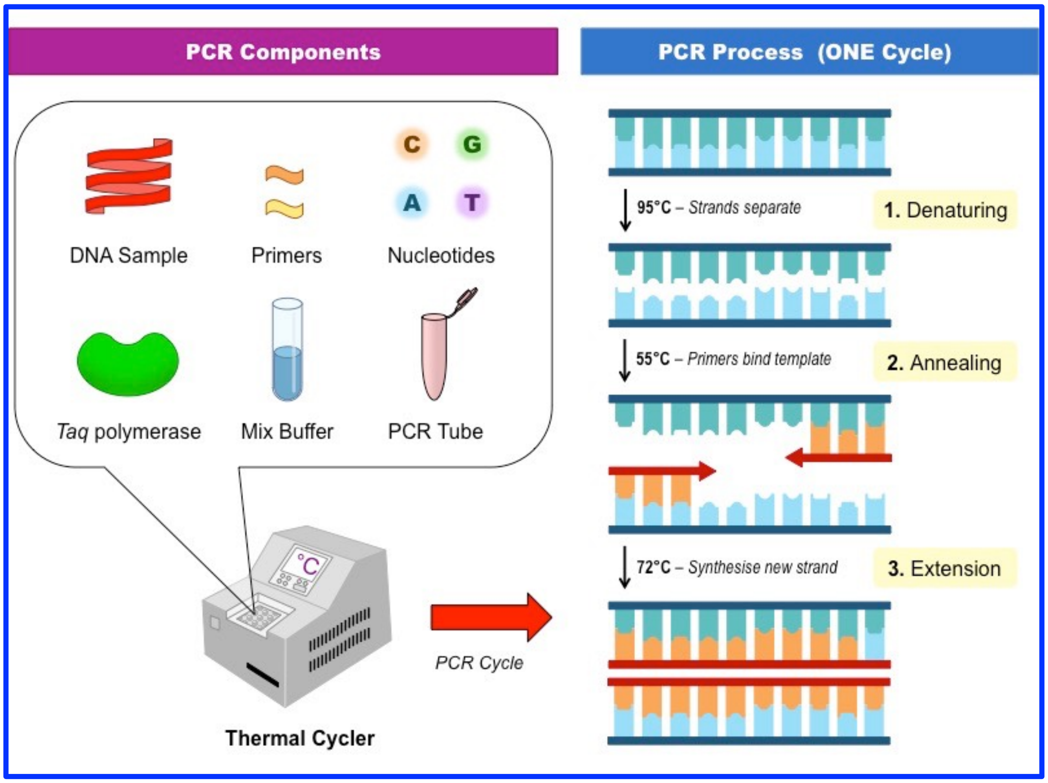
Using Gel Electrophoresis to Visualize PCR Products

Specific Diagnostic DNA Band Unique to DNA Sequence Being Amplified

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

Can Amplify One DNA Sequence From An Entire Genome *or an Entire Genome*!!!

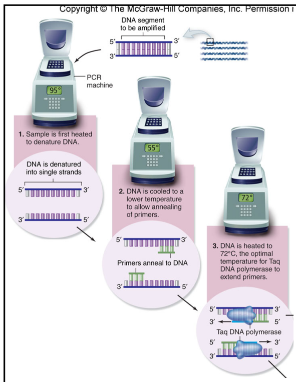
Requirements For the Polymerase Chain Reaction

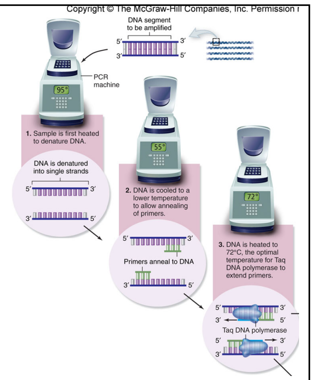


Requirements For PCR

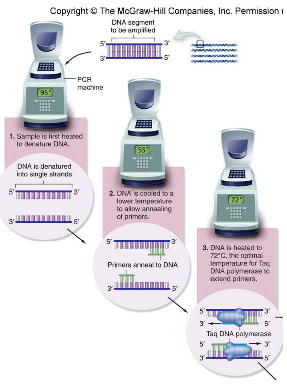
1. Knowledge of a <u>Specific Sequence</u> to Amplify (e.g.,insulin gene)
    a) Must Have First Cloned & Sequenced DNA of Interest the "Old-fashioned Way"
2. <u>Primers That Recognize Specific DNA Sequences</u> & Initiate DNA Synthesis & DNA Polymerase Binding To Template
3. <u>Template</u> (e.g., DNA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

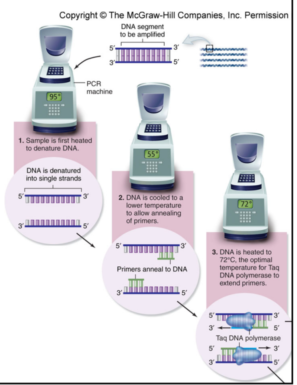
It's All in the DNA Sequences - Know Sequence & Can Synthesize an Infinite Amount of Specific DNA Sequences. It now Takes One Hour to Do What Used to Take YEARS!





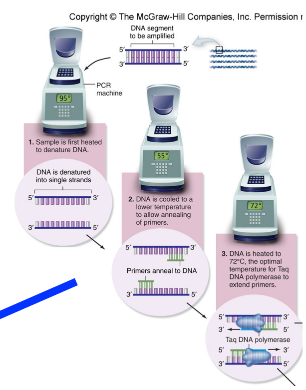
Examples of PCR Applications



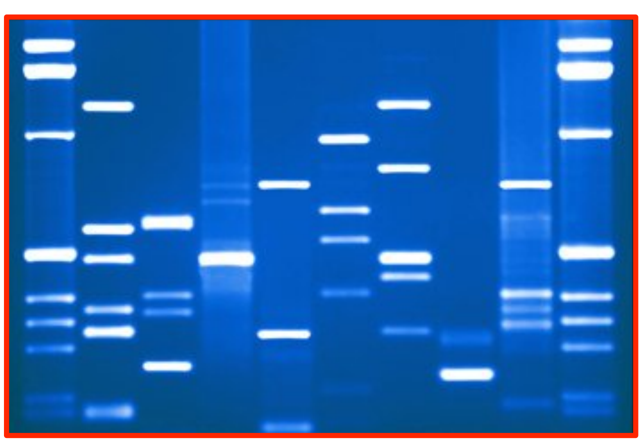


Using PCR to Determine Your DNA Fingerprint & Identity

What is YOUR DNA Fingerprint?

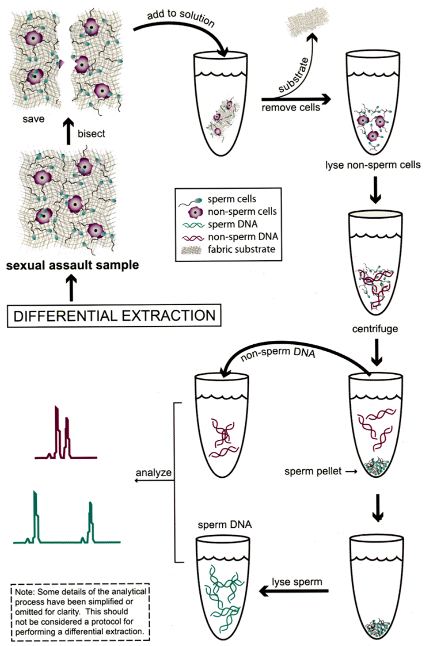


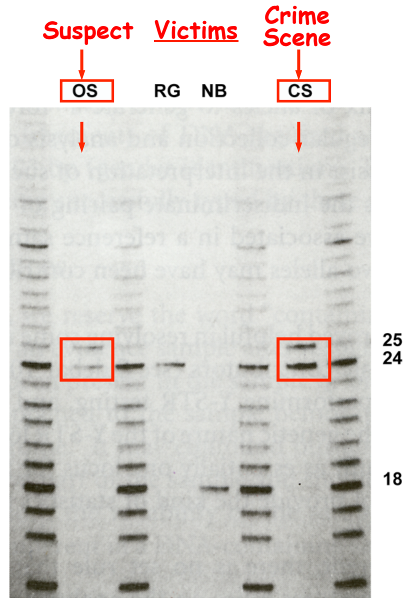
Unique Pattern of DNA Bands = Fingerprint



Using DNA Fingerprints to Identify Individuals & Genes They Don't "Lie"

Using PCR in Crime Scenes



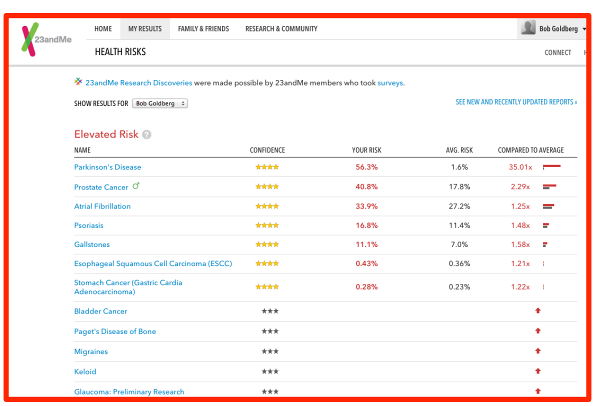


"Match" What is Probability That This Will Occur by Chance?

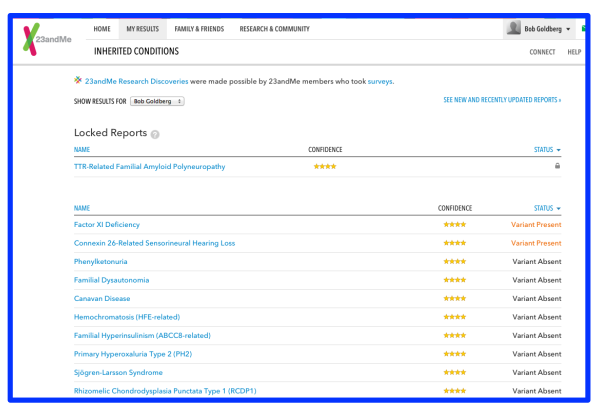
OS = Suspect
CS = Crime Scene
RG & NB = Victims

DNA Doesn't "Lie" !!

Using PCR to Determine Bobg's Genotype



NAME	CONFIDENCE	YOUR RISK	AVG. RISK	COMPARED TO AVERAGE
Parkinson's Disease	★★★★	56.3%	1.6%	35.01x
Prostate Cancer ♂	★★★★	40.8%	17.8%	2.29x
Atrial Fibrillation	★★★★	33.9%	27.2%	1.25x
Psoriasis	★★★★	16.8%	11.4%	1.48x
Gallstones	★★★★	11.1%	7.0%	1.58x
Esophageal Squamous Cell Carcinoma (ESCC)	★★★★	0.43%	0.36%	1.21x
Stomach Cancer (Gastric Cardia Adenocarcinoma)	★★★★	0.28%	0.23%	1.22x
Bladder Cancer	★★★			
Paget's Disease of Bone	★★★			
Migraines	★★★			
Keloid	★★★			
Glaucoma: Preliminary Research	★★★			



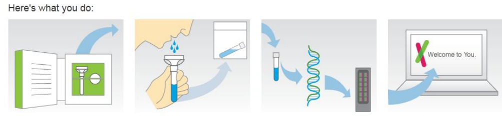
NAME	CONFIDENCE	STATUS
TTR-Related Familial Amyloid Polyneuropathy	★★★★	

NAME	CONFIDENCE	STATUS
Factor XI Deficiency	★★★★	Variant Present
Connexin 26-Related Sensorineural Hearing Loss	★★★★	Variant Present
Phenylketonuria	★★★★	Variant Absent
Familial Dysautonomia	★★★★	Variant Absent
Canavan Disease	★★★★	Variant Absent
Hemochromatosis (HFE-related)	★★★★	Variant Absent
Familial Hyperinsulinism (ABCC8-related)	★★★★	Variant Absent
Primary Hyperoxaluria Type 2 (PH2)	★★★★	Variant Absent
Sjögren-Larsson Syndrome	★★★★	Variant Absent
Rhizomelic Chondrodysplasia Punctata Type 1 (RCDP1)	★★★★	Variant Absent

Personal Genome Service™

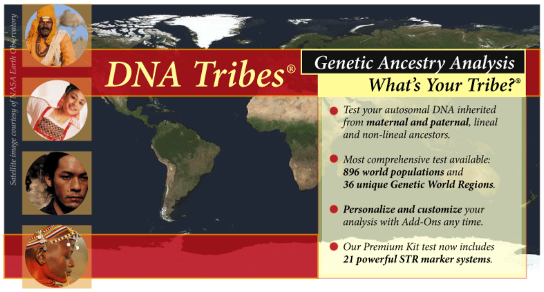
Get to know your DNA. All it takes is a little bit of spit.

Here's what you do:



1. Order a kit from our online store.
2. Register your kit, spit into the tube, and send it to the lab.
3. Our CLIA-certified lab analyzes your DNA in 6-8 weeks.
4. Log in and start exploring your genome.

Using PCR To Determine an Individual's Ancestry

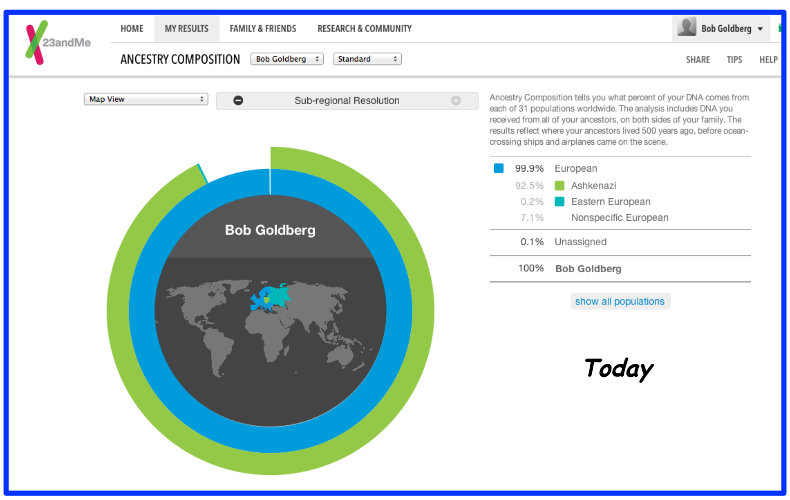


PCR Started a New Industry

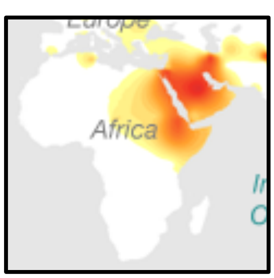
DNA can reveal ancestors' lies and secrets

LA Times, January 18, 2009

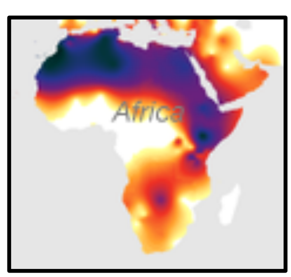
Bobg's Ancestry



♂



500 Years Ago



♀

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

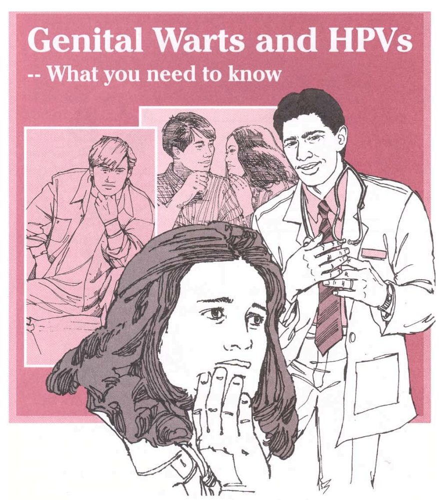
Analysis of one million base pairs of Neanderthal DNA

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

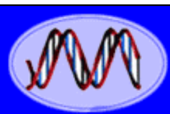
Using PCR To Detect Human Pathogens
(Viruses, Fungi, Bacteria)



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



DNA
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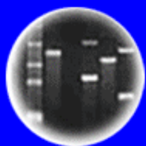
Entire Genetic Code of a Bacteria



DNA Fingerprinting

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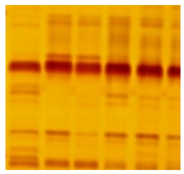
Plants of Tomorrow

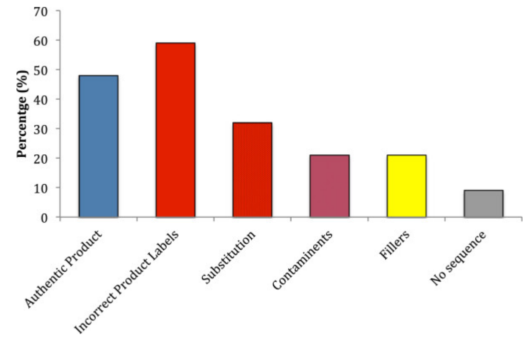
And Consumer Fraud in the Natural Food Industry

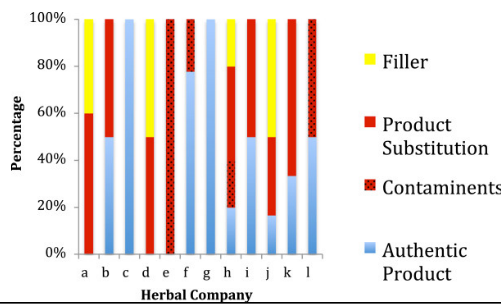
DNA barcoding detects contamination and substitution in North American herbal products

BMC Medicine, 11, 222, 2013

Barcoding = DNA Fingerprinting!



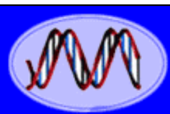




PCR Has Many Uses, Has Changed Many Fields, and Lead To New Ones That Have Had a Big Impact On Our Lives

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



DNA
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Entire Genetic Code
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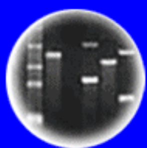

DNA Fingerprinting

Cloning: Ethical Issues
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Plants of Tomorrow

WHAT ARE THE PROPERTIES OF A GENE?

STOP!!!!!

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

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

Think Tautomers!

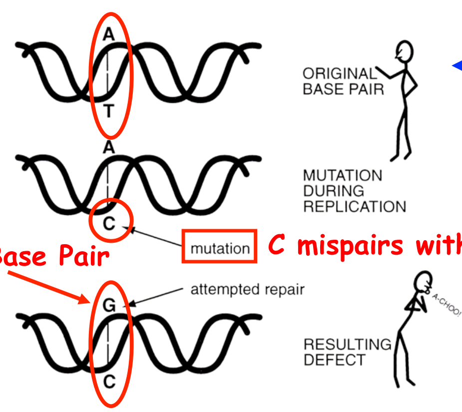
Rare Base Mismatch

Gene A

Replication①

New Base Pair

Replication②
Gene A'
Allelic Variant

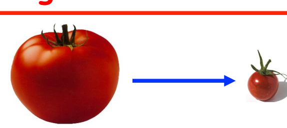


C mispairs with A

See Mutation As Change in Phenotype Creates Alleles

Change DNA Sequence From A = T to G ☰ C

∴ Change Protein Amino Acid Sequence ⇨ Alter Function!

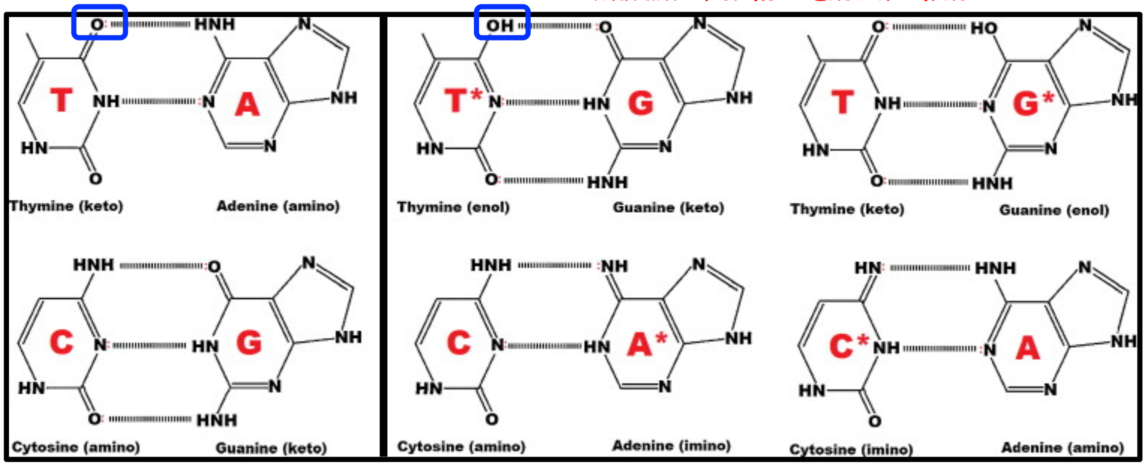


Big Tomato to Small Tomato

TAUTOMERS CHANGE BASE PAIRING RULES

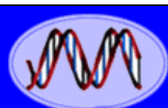
Normal Forms – Keto & Amino

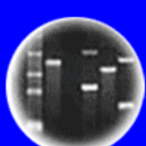
"Mutant" Forms – Enol & Imino



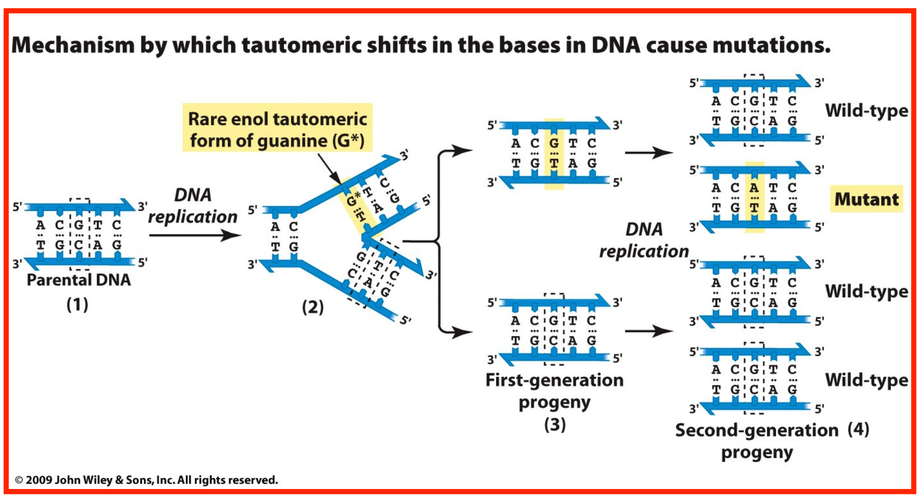
And Lead To Mistakes in DNA Replication & Mutations ➤ Genetic Diversity

Chemistry Leads to Biology!!

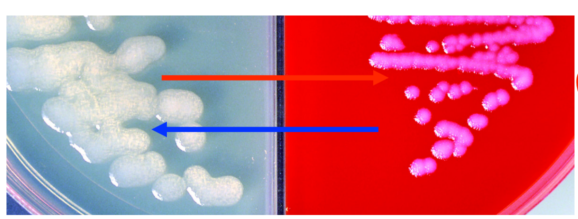




How Tautomeric Shifts Cause Mutations

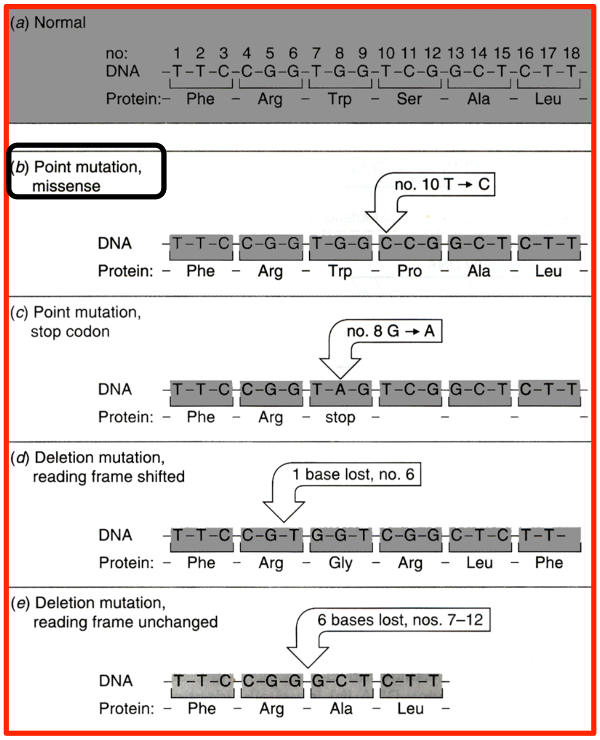


Smooth Virulent Form



Rough Avirulent Form

Mutations Can Occur Different Ways



1. Base-Pair Change (SNP) - Vast Majority of Mutations (>99%)
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

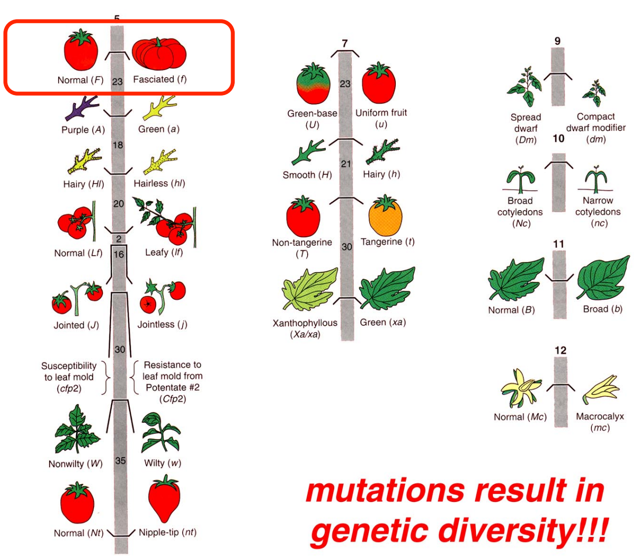
∴

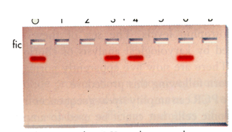
Phenotype Changes

Alleles!

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)

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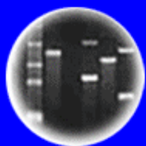

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Plants of Tomorrow

ARTICLE

1000 Genomes
A Deep Catalog of Human Genetic Variation

doi:10.1038/nature09534

A map of human genome variation from population-scale sequencing

Nature, October 10, 2010

The 1000 Genomes Project Consortium*

The 1000 Genomes Project aims to provide a deep characterization of human genome sequence variation as a foundation for investigating the relationship between genotype and phenotype. Here we present results of the pilot phase of the project, designed to develop and compare different strategies for genome-wide sequencing with high-throughput platforms. We undertook three projects: low-coverage whole-genome sequencing of 179 individuals from four populations; high-coverage sequencing of two mother–father–child trios; and exon-targeted sequencing of 697 individuals from seven populations. We describe the location, allele frequency and local haplotype structure of approximately 15 million single nucleotide polymorphisms, 1 million short insertions and deletions, and 20,000 structural variants, most of which were previously undescribed. We show that, because we have catalogued the vast majority of common variation, over 95% of the currently accessible variants found in any individual are present in this data set. On average, each person is found to carry approximately 250 to 300 loss-of-function variants in annotated genes and 50 to 100 variants previously implicated in inherited disorders. We demonstrate how these results can be used to inform association and functional studies. From the two trios, we directly estimate the rate of *de novo* germline base substitution mutations to be approximately 10^{-8} per base pair per generation. We explore the data with regard to signatures of natural selection, and identify a marked reduction of genetic variation in the neighbourhood of genes, due to selection at linked sites. These methods and public data will support the next phase of human genetic research.

- Sequenced Genomes of 2500 individuals & From 26 Different Global Populations
- Found 84 Million Variants (SNPs) & <0.5% Unique to a Population!
- Evidence For Common Ancestry of All Humans
- Found 250-300 Loss-Of-Function Mutations (KOs) Per Person
- Found 50-100 Mutations Implicated in Genetic Disorders Per Person
- 10^{-8} bp Mutations Per Generation (30 per Genome)

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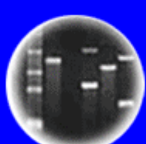

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ARTICLE

Nature, October 1, 2015

OPEN

doi:10.1038/nature15393

A global reference for human genetic variation

26 Populations

The 1000 Genomes Project Consortium*

The 1000 Genomes Project set out to provide a comprehensive description of common human genetic variation by applying whole-genome sequencing to a diverse set of individuals from multiple populations. Here we report completion of the project, having reconstructed the genomes of 2,504 individuals from 26 populations using a combination of low-coverage whole-genome sequencing, deep exome sequencing, and dense microarray genotyping. We characterized a broad spectrum of genetic variation, in total over 88 million variants (84.7 million single nucleotide polymorphisms (SNPs), 3.6 million short insertions/deletions (indels), and 60,000 structural variants), all phased onto high-quality haplotypes. This resource includes >99% of SNP variants with a frequency of >1% for a variety of ancestries. We describe the distribution of genetic variation across the global sample, and discuss the implications for common disease studies.

doi:10.1038/nature15394

An integrated map of structural variation in 2,504 human genomes

26 Populations

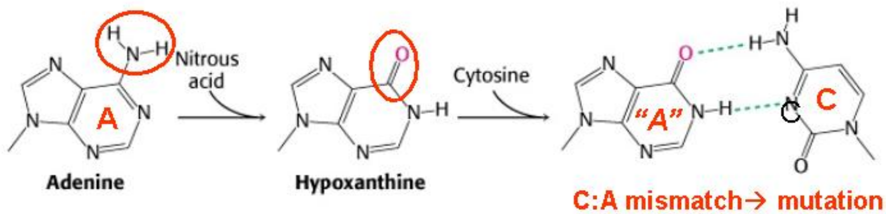
A list of authors and their affiliations appears at the end of the paper.

Structural variants are implicated in numerous diseases and make up the majority of varying nucleotides among human genomes. Here we describe an integrated set of eight structural variant classes comprising both balanced and unbalanced variants, which we constructed using short-read DNA sequencing data and statistically phased onto haplotype blocks in 26 human populations. Analysing this set, we identify numerous gene-intersecting structural variants exhibiting population stratification and describe naturally occurring homozygous gene knockouts that suggest the dispensability of a variety of human genes. We demonstrate that structural variants are enriched on haplotypes identified by genome-wide association studies and exhibit enrichment for expression quantitative trait loci. Additionally, we uncover appreciable levels of structural variant complexity at different scales, including genic loci subject to clusters of repeated rearrangement and complex structural variants with multiple breakpoints likely to have formed through individual mutational events. Our catalogue will enhance future studies into structural variant demography, functional impact and disease association.

Chemicals Can Cause Mutations

Chemical Mutagen : Nitrous acid (HNO_2)

Deamination causes A:T to G:C transitions

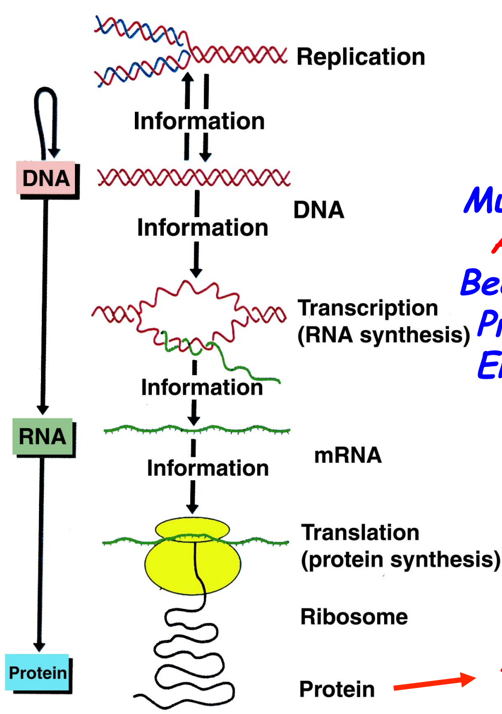


* HNO_2 also deaminates C to U: causes G:C to A:T transitions

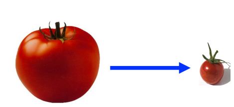
By Altering Bases and Base Pairing Rules

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

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

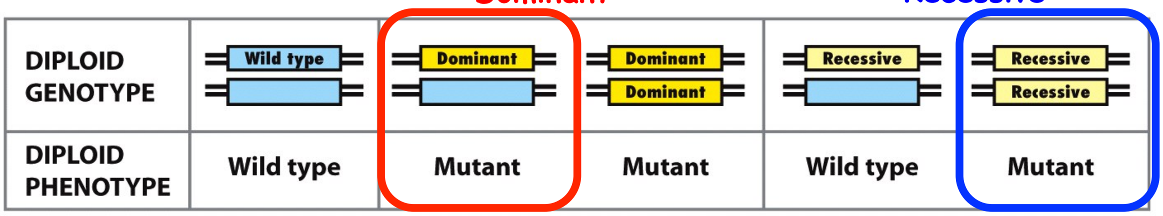


Figure 5-2
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company

Need One Allele

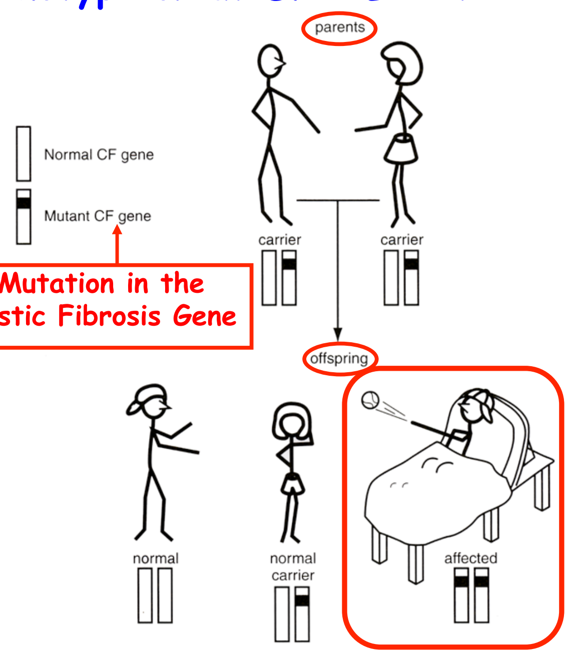
Need Two Alleles

Mutations in Genes Are Rare But Are Inherited & Can Be Followed in Families By Phenotype or at DNA Level!

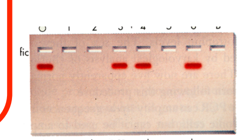
One Gene Per Gamete

♀ + ♂

Two Genes per Somatic Cells



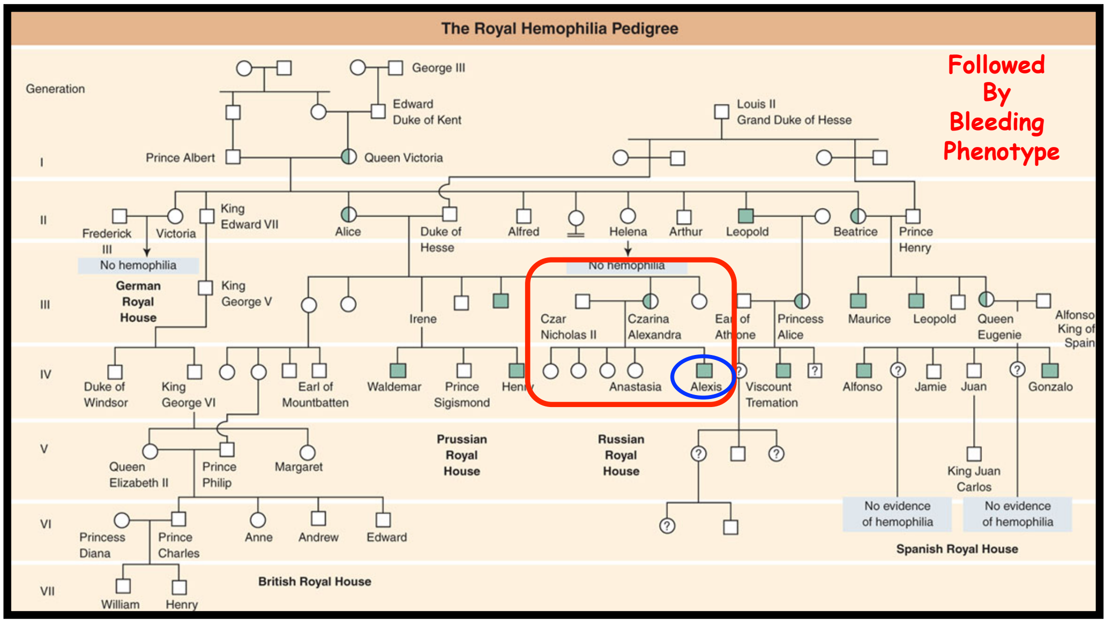
Mutation in the Cystic Fibrosis Gene



DNA Marker or Fingerprint!

How Follow Inheritance?
What Allows Disease To Be Followed?

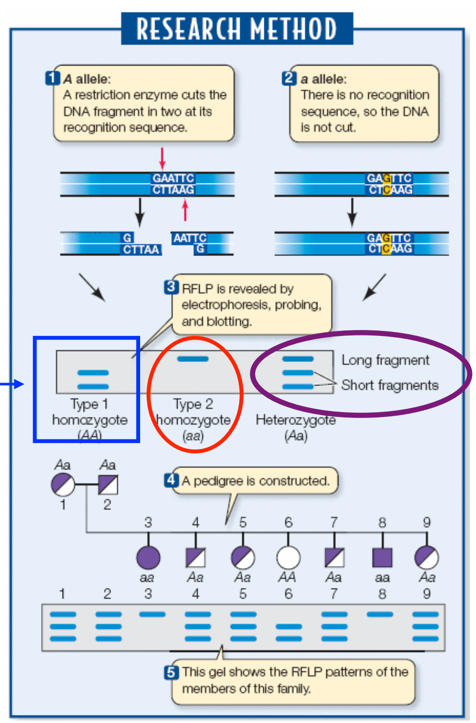
Pedigrees Can Be Used To Follow Disease Genes in Human Families



Recessive Sex Linked

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

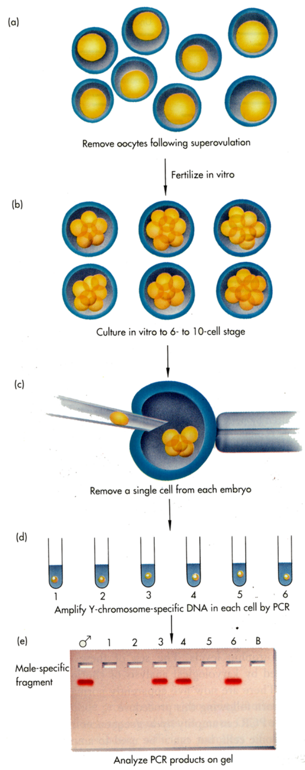
DNA Fingerprints →



← RFLP – Restriction Fragment Length Polymorphism

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

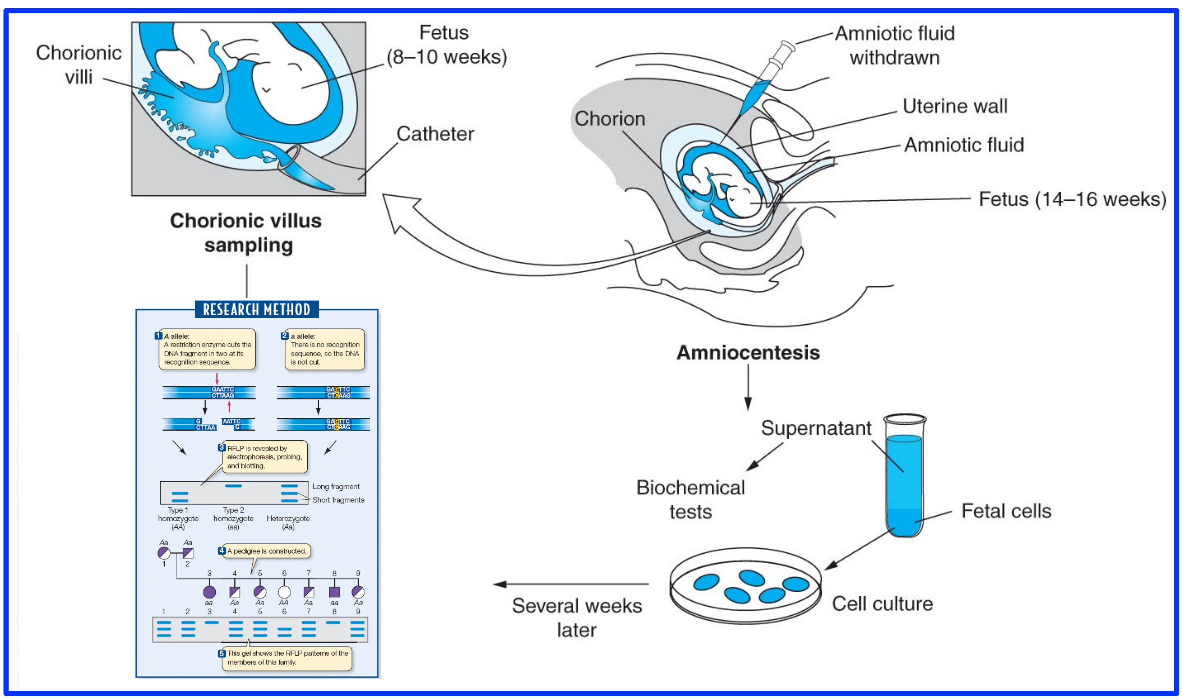
PGD
Pre-
Implantation
Genetic
Diagnosis



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

Sex Determination in 8-cell Embryo!

DNA Testing Can Be Carried Out Before Child Birth During Pregnancy



RESEARCH ARTICLE New Non-Invasive DNA Tests Are Available Based on PCR

PRENATAL DIAGNOSIS

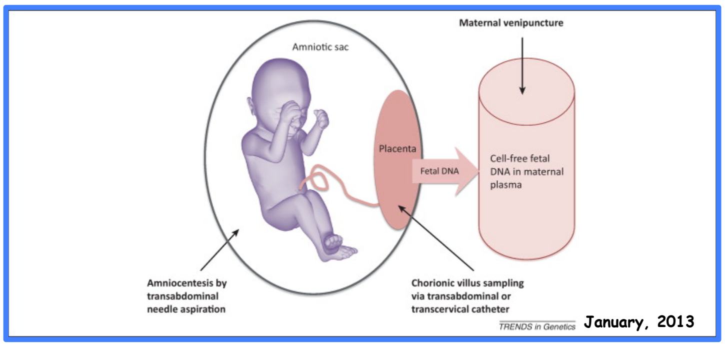
Maternal Plasma DNA Sequencing Reveals the Genome-Wide Genetic and Mutational Profile of the Fetus

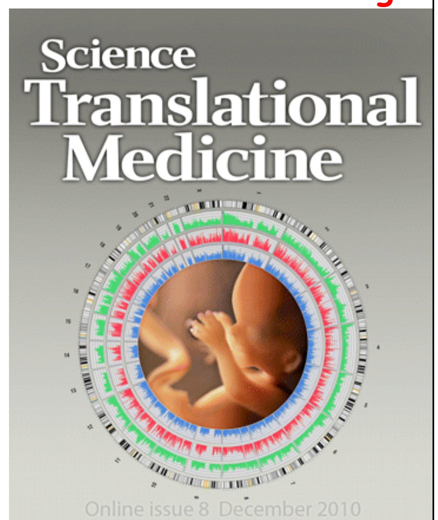
Science Translational Medicine, December 8, 2010 (61,1-12)

Sequencing DNA From the Blood of a Pregnant Woman Allows the Complete Genome Of the Fetus to Be Decoded!

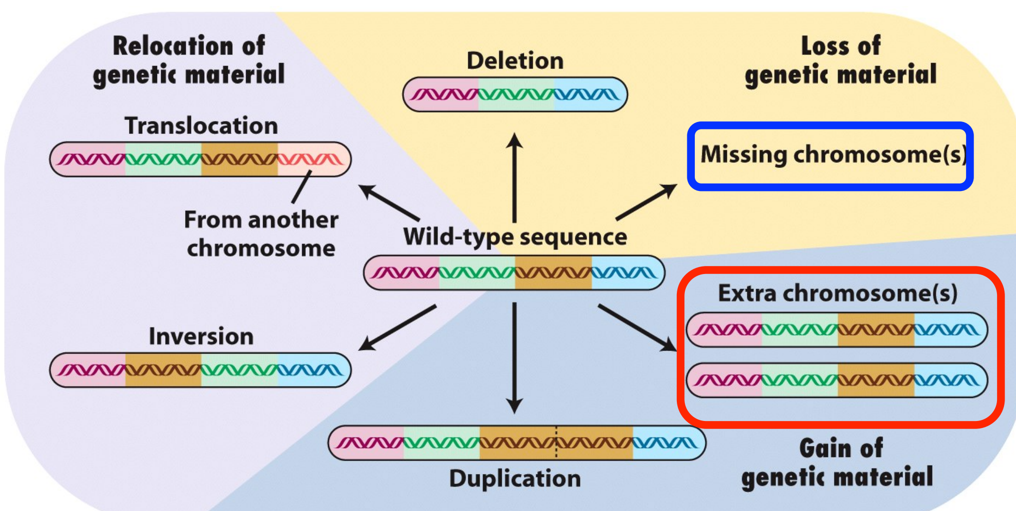
A New Era in DNA Testing!!

~10% of DNA in Maternal Plasma is From the Fetus





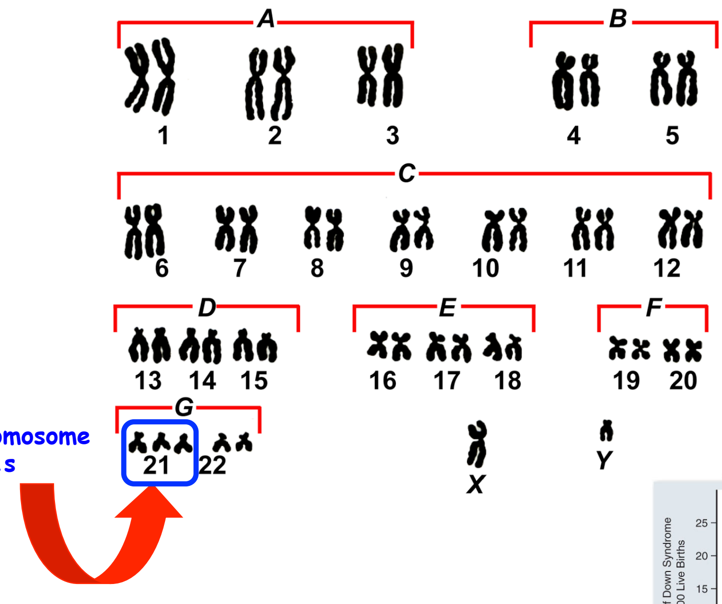
"Mutations" Can Also Occur By Large Chromosomal Changes



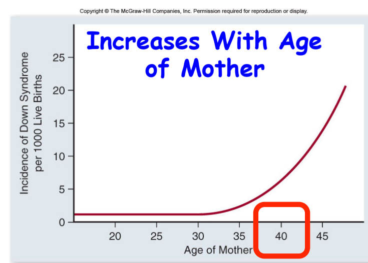
These changes affect many genes!

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

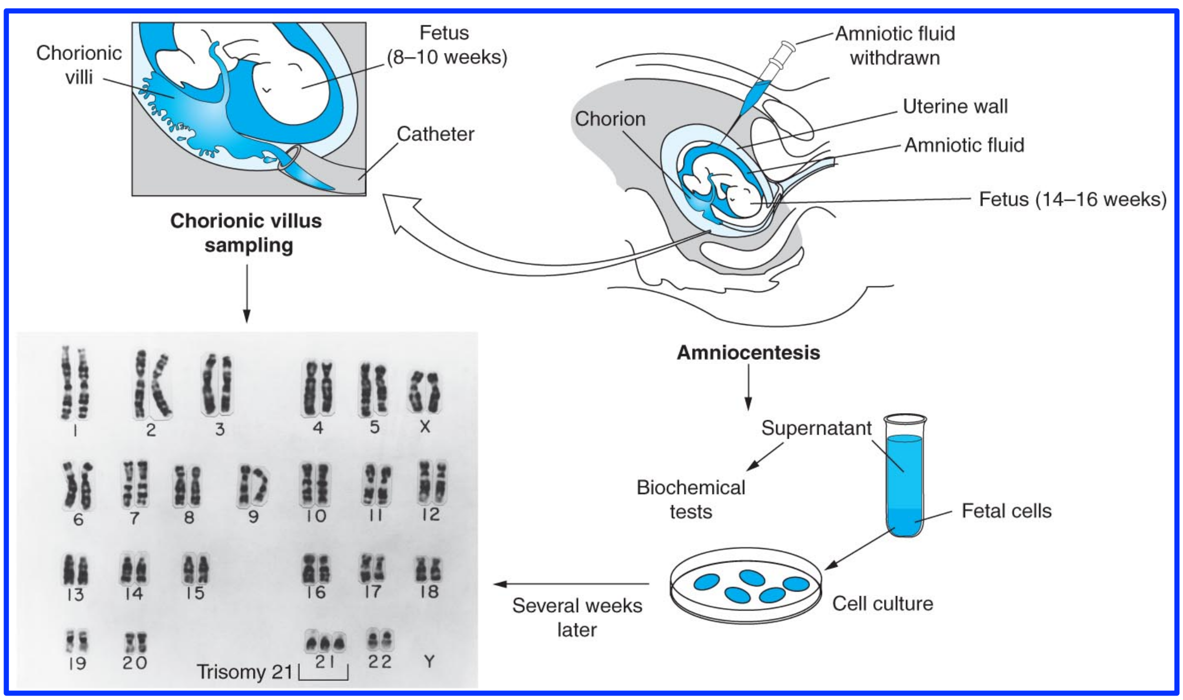
A Down's Syndrome Karyotype

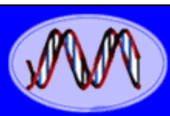


Three Chromosome # 21s



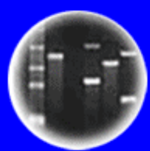
Chromosome Testing Can Be Carried Out During Pregnancy or Before (New DNA Tests)





DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

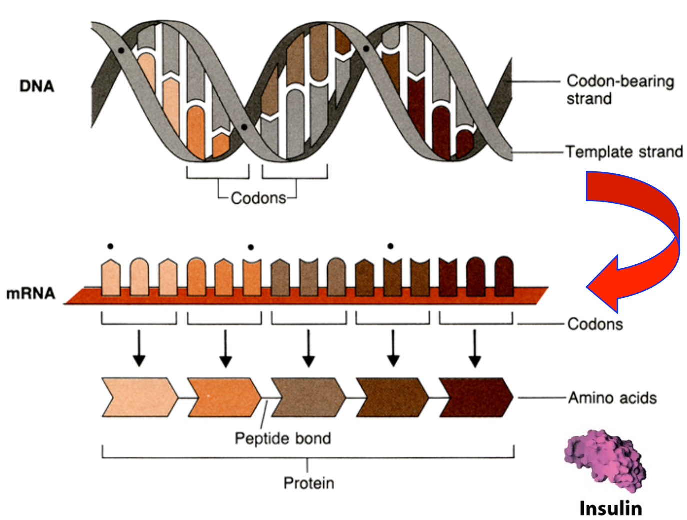
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

WHAT ARE THE PROPERTIES OF A GENE?

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

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 The Translation of The Genetic Code

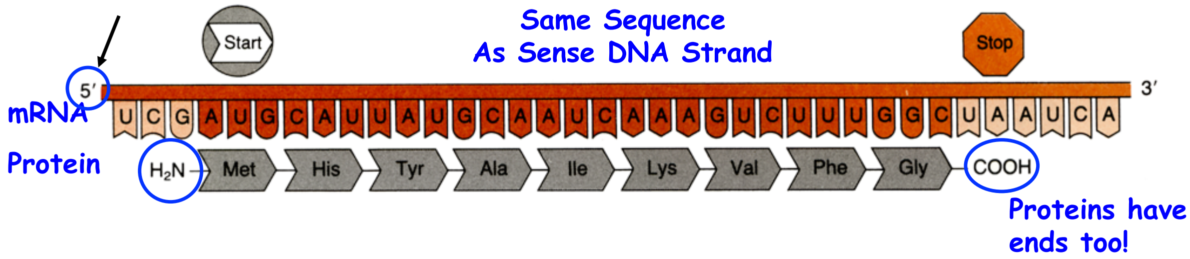
Genetic Code on mRNA Translated to Protein Sequence

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

Colinearity of Sequences!

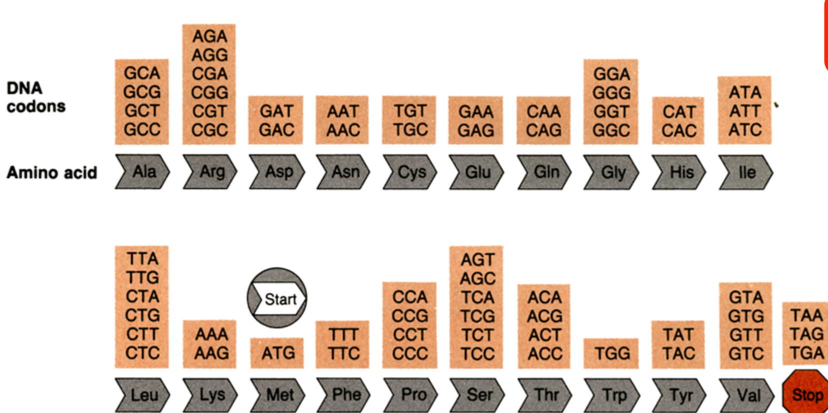
Know Sequence
Know Protein
→ Engineer New Protein

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



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

The Genetic Code is Universal!



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!

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

Implications For Genetic Engineering - "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!

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

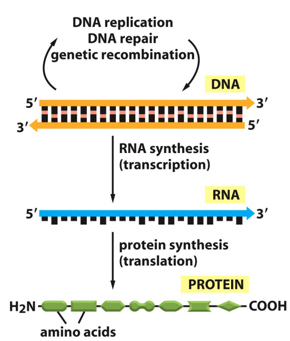
Bacteria Transcription On Switch + Human Insulin Coding Sequence + Bacteria Transcription Off Switch

→ *Human Insulin in Bacteria!!*

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!

"Behind" All Traits!

Same Processes!

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