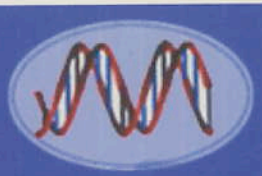
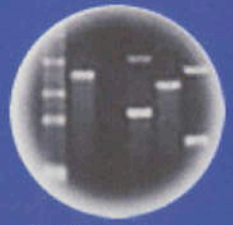


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

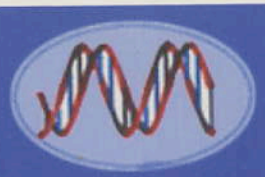
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A Winter 2008
Genetic Engineering in Medicine, Agriculture, and Law

Professor Bob Goldberg

Lecture 3
What Are Genes & How Do They Work-Part Two



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Entire Genetic Code
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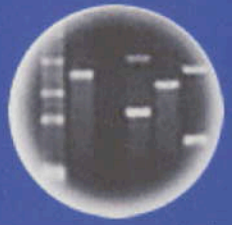


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

THEMES

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

The Beginning of a Gene is its:

a. 5' End
b. 3' End

iClicker

The Sense Strand of a Gene Is Transcribed?

a. Yes
b. No

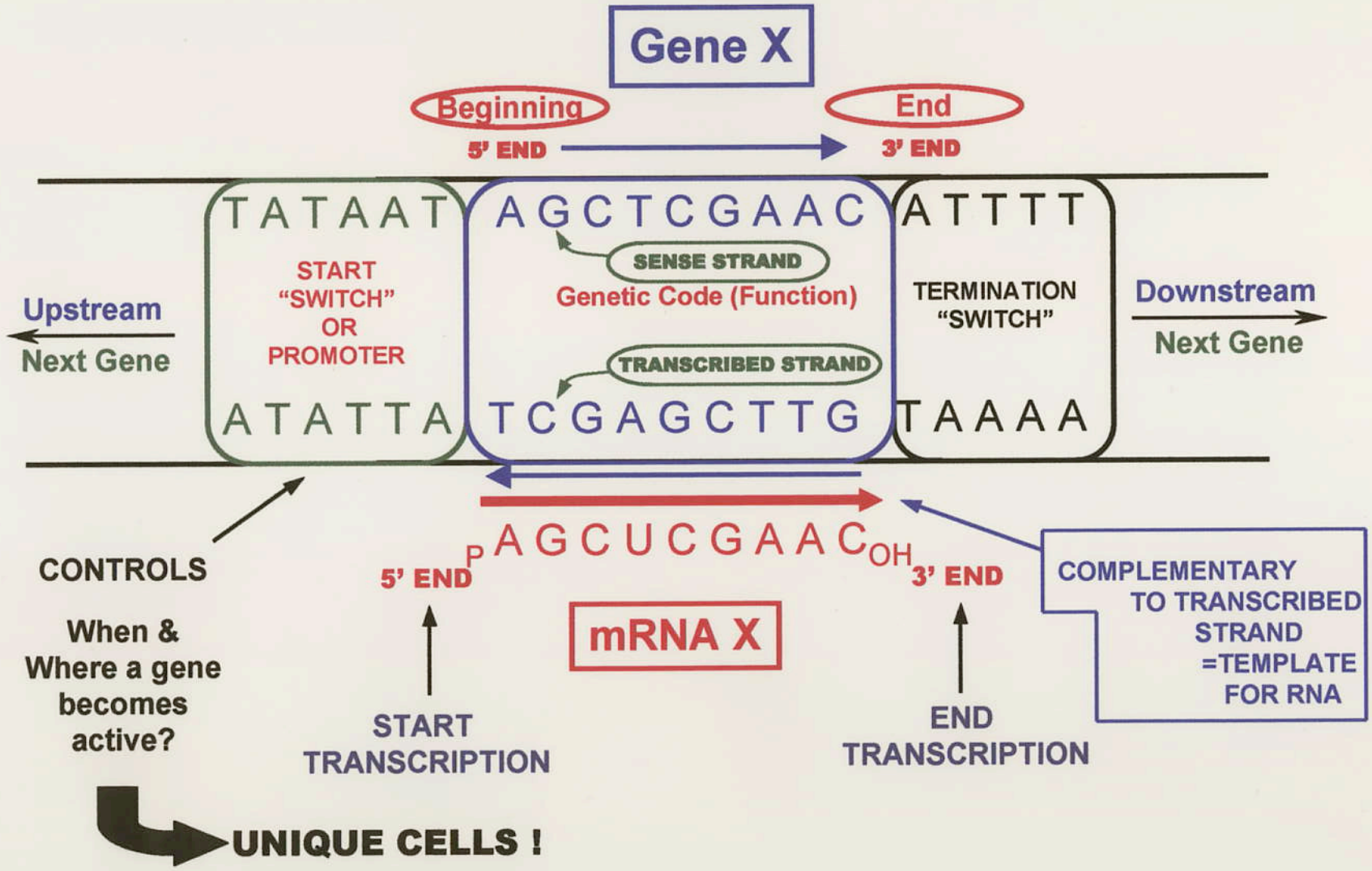
iClicker

The Sequence of a mRNA is the Same As the Sequence of the:

a. Sense Strand
b. Antisense Strand

iClicker

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

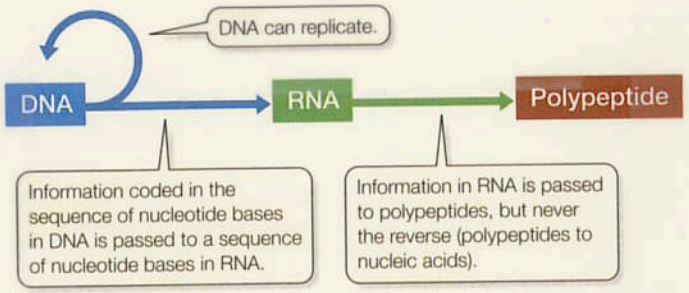


Note: *mRNA Sequence = Sense Strand Sequence*

How do genes work?

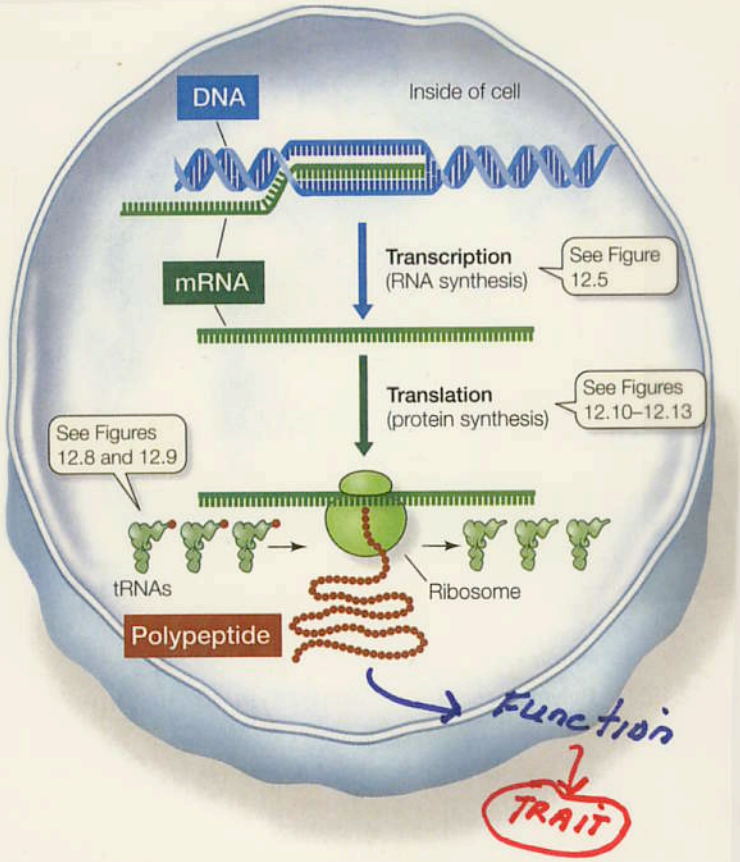
① Replication

② Gene Activity to Function



12.2 The Central Dogma Information flows from DNA to RNA to polypeptide, as indicated by the arrows.

Gene Activity → Protein → Function → Phenotype (trait)

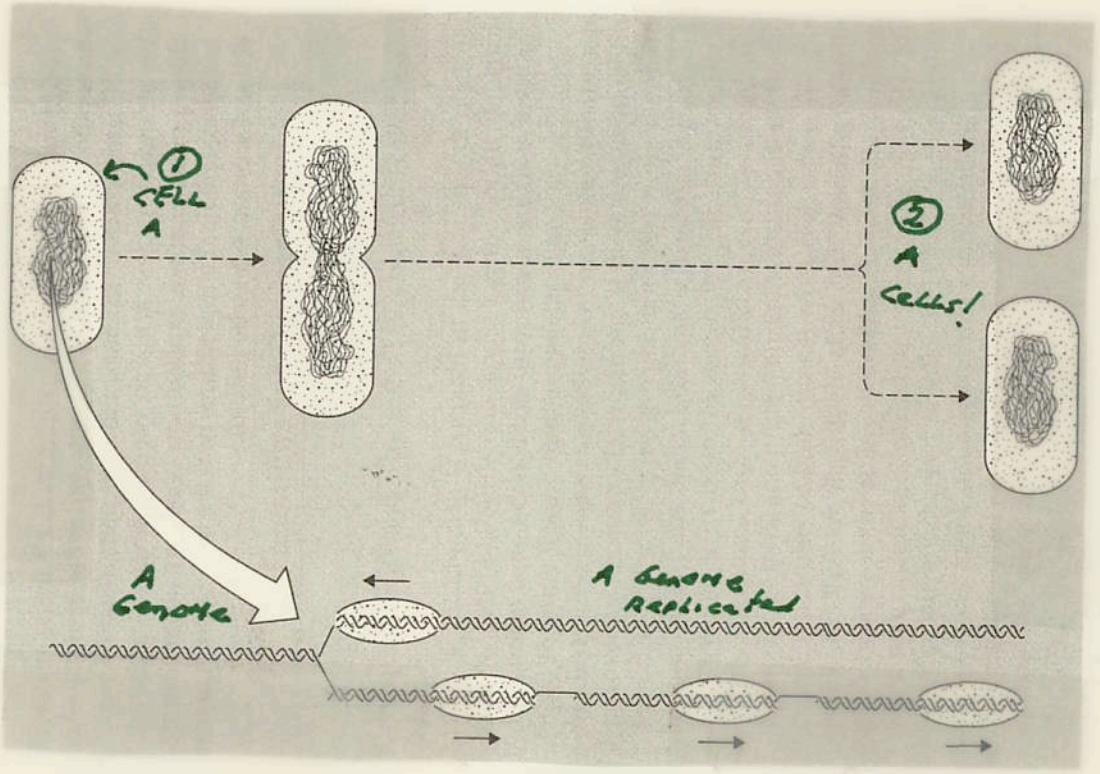


12.3 From Gene to Protein This diagram summarizes the processes of gene expression in prokaryotes. In eukaryotes, the processes are somewhat more complex.

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?

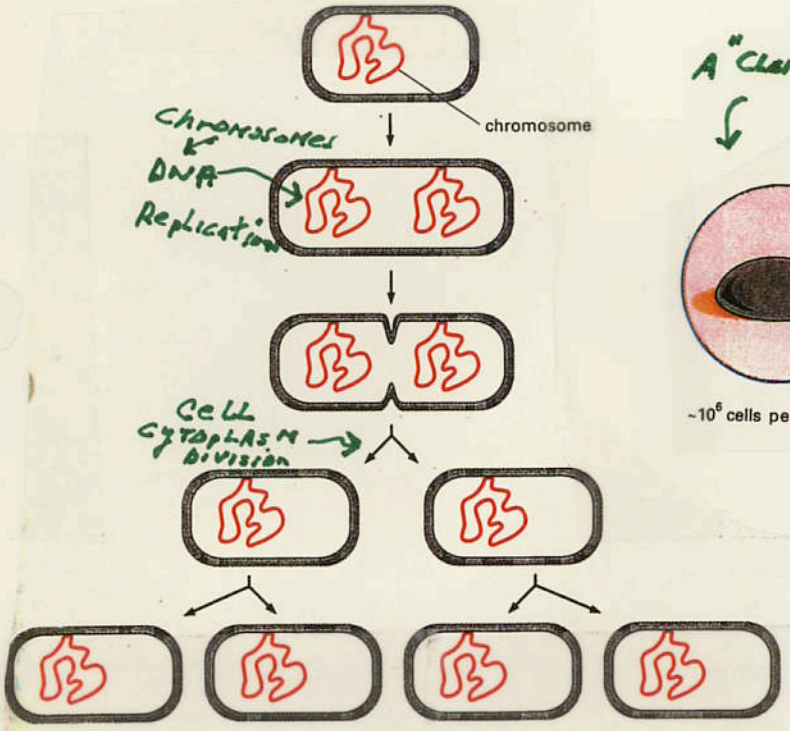
BASIS OF LIFE!

The Structure of DNA "Allows" the Genotype of a Cell To Be Passed on From Generation to Generation?

a. Yes
b. No

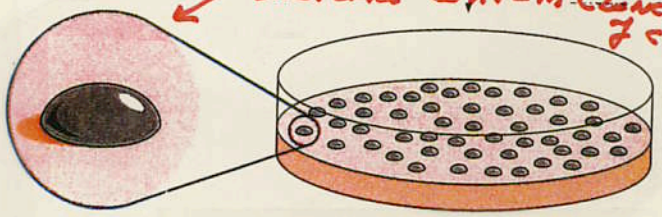
iClicker

GENES ARE REPLICATED DURING EACH CELL DIVISION



A "Clone"

Note - Each clump of bacteria contain clones of cells



~10^6 cells per colony

~50 colonies per dish

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!

MAJOR PROPERTIES OF GENETIC MATERIAL

Replication & Stability

THE SEQUENCE OF EACH DNA STRAND MUST BE MAINTAINED DIVISION AFTER DIVISION

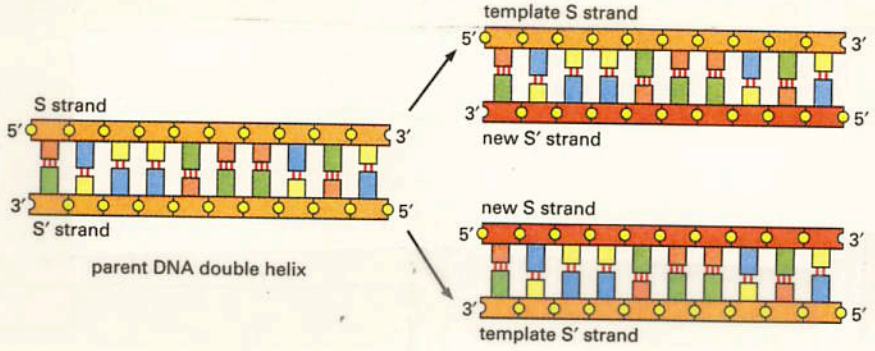


Figure 5–2 The DNA double helix acts as a template for its own duplication. Because the nucleotide A will successfully pair only with T, and G only with C, each strand of DNA can serve as a template to specify the sequence of nucleotides in its complementary strand by DNA base-pairing. In this way, a double-helical DNA molecule can be copied precisely.

HOW DOES THAT OCCUR?
PROPERTY OF THE DNA MOLECULE

NOTE → SEQUENCE + POLARITY

DNA REPLICATION OCCURS SEMI-CONSERVATIVELY

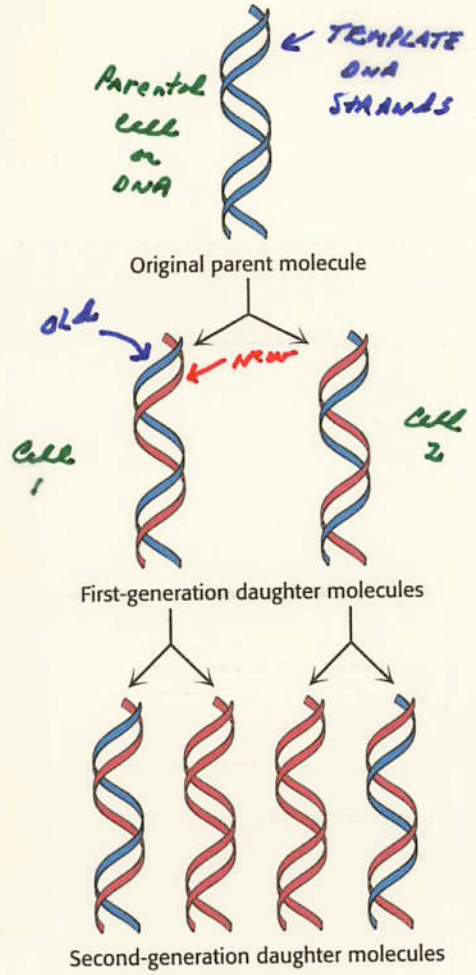


FIGURE 5.16 Diagram of semiconservative replication.

1. DNA STRUCTURE ALLOWS DNA sequence to be Maintained
   Complementary BASES
2. EACH STRAND Serves as a template for the synthesis of a complementary strand of DNA
3. New Molecules of DNA are PRECISE COPIES OF PARENTAL DNA – ONE TEMPLATE STRAND + ONE NEWLY Synthesized Complementary STRAND!

Meselson & Stahl 1957

DNA SEQUENCE OF ONE STRAND IS A TEMPLATE FOR THE NEW STRAND

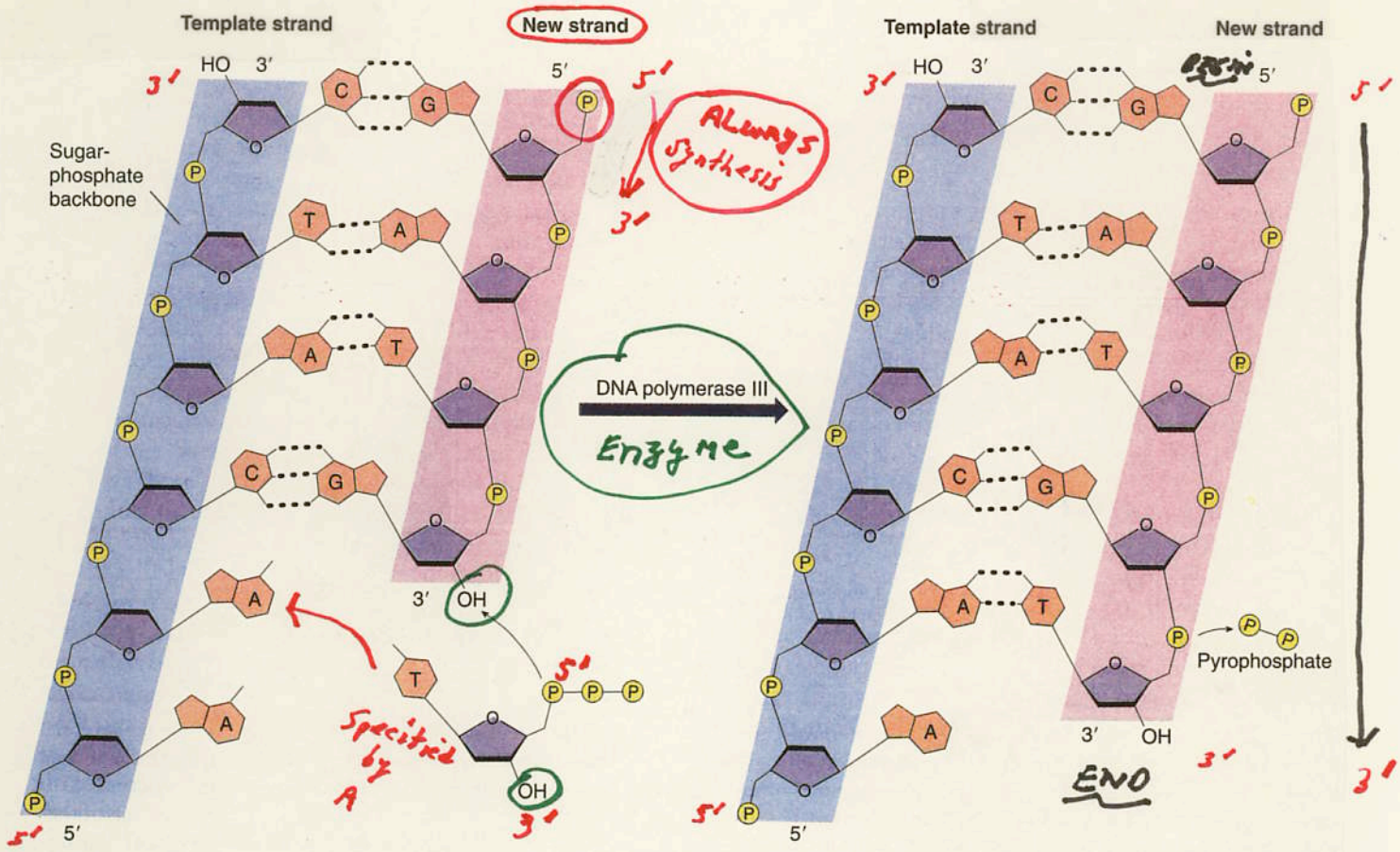


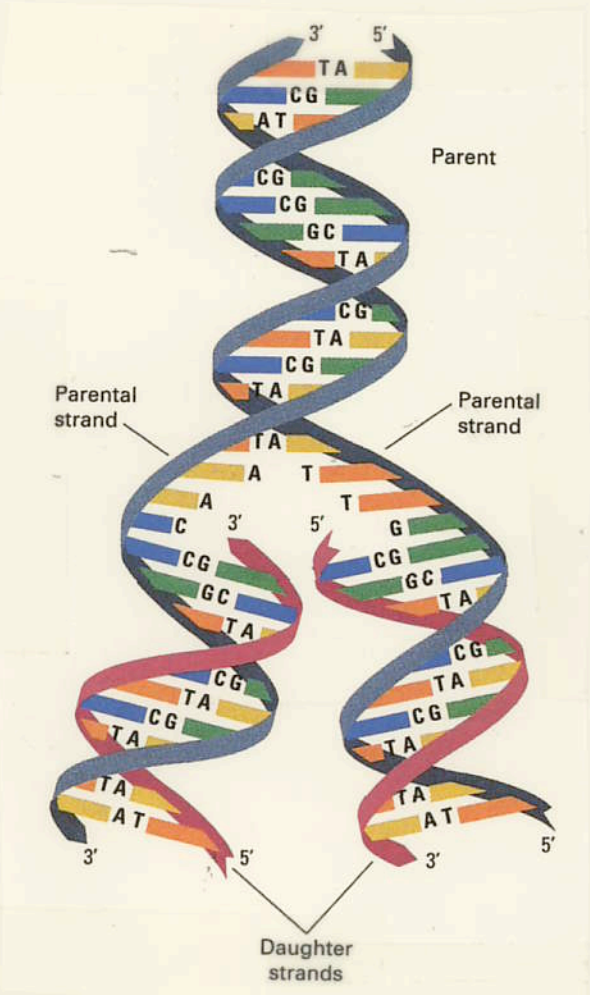
FIGURE 14.14
How nucleotides are added in DNA replication. DNA polymerase III, along with other enzymes, catalyzes the addition of nucleotides to the growing complementary strand of DNA. When a nucleotide is added, two of its phosphates are lost as pyrophosphate.

SEQUENCE IS SPECIFIED BY COMPLEMENTARY BASES

NOTE 5′ (P) × 3′ (OH)

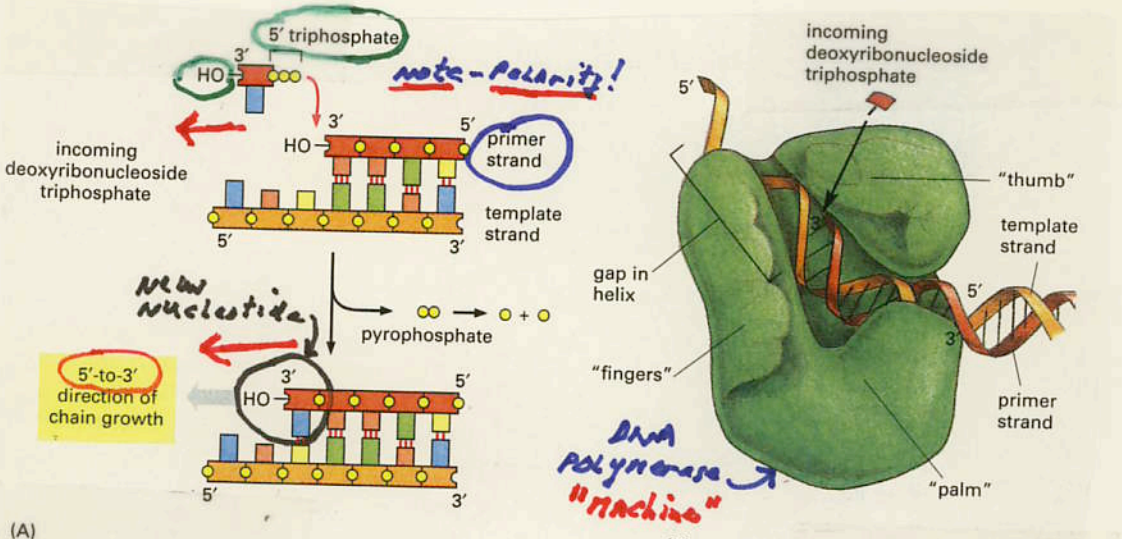
POLARITY // SEQUENCE

THE DNA SEQUENCE IS MAINTAINED GENERATION TO GENERATION



THE DNA SEQUENCE "LIVES" FOREVER!

DNA REPLICATION REQUIRES AN ENZYME - DNA POLYMERASE

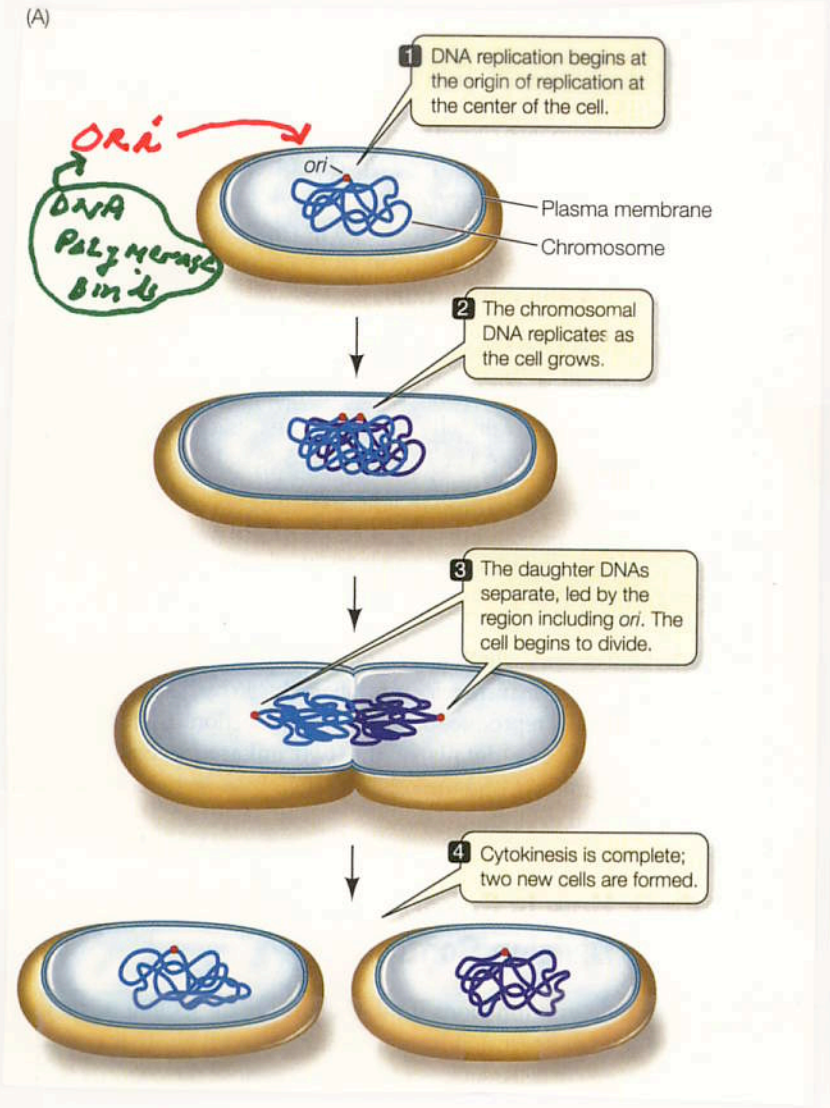


DNA POLYMERASE CATALYZES PHOSPHODIESTER BONDS AND "COPIES" THE TEMPLATE

NUCLEOTIDES ARE ALSO NEEDED

NEED A Primer + TEMPLATE + DNA Polymerase + Nt's

DNA Replication Requires AN ORIGIN of Replication



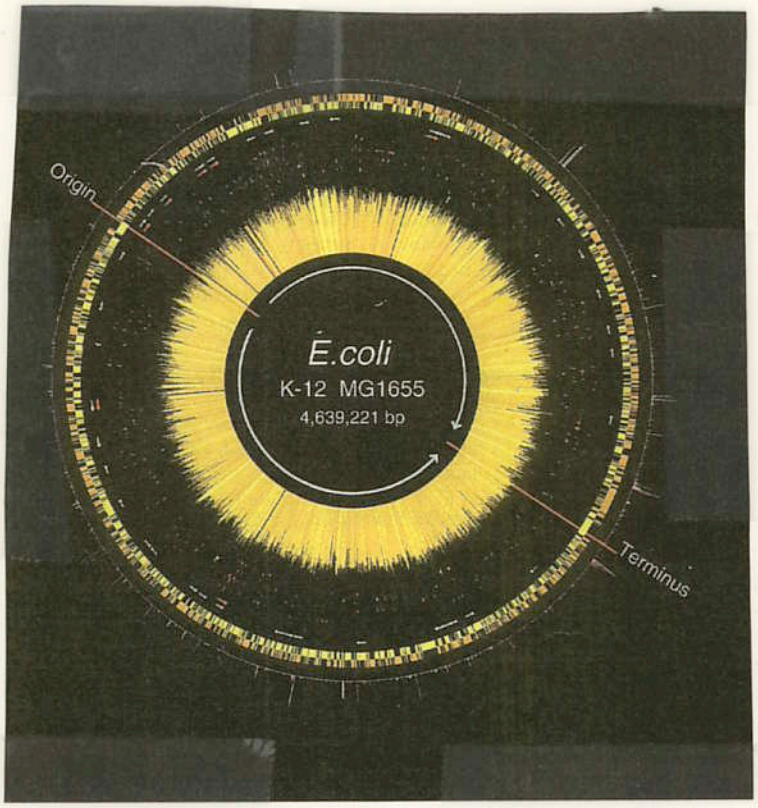
9.2 Prokaryotic Cell Division (A) The process of cell division in a bacterium. (B) These two cells of the bacterium *Pseudomonas aeruginosa* have almost completed cytokinesis.

DNA Replication ALso Requires:

1. Template
2. Nucleotides
3. DNA polymerase (Machine)
4. "primer" to Start Replication

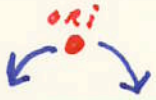
ORI

DNA Replication Starts at the ORIGIN OF REPLICATION



Key: tRNA genes; rRNA genes; — origin and terminus of replication

DNA Replication is bidirectional from the ORI!!

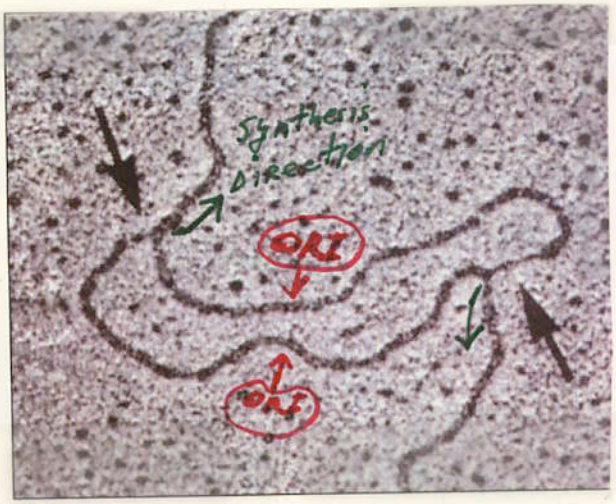


Hypothesis FOR TWO DIRECTION Synthesis?

DNA Polymerase BINDS to the ORIGIN OF Replication (ORI) TO Begin DNA Synthesis

CONTROL DIVISION?

DNA in The Process of BEING REPLICATED



Replication Moves Bidirectionally FROM ORIGIN

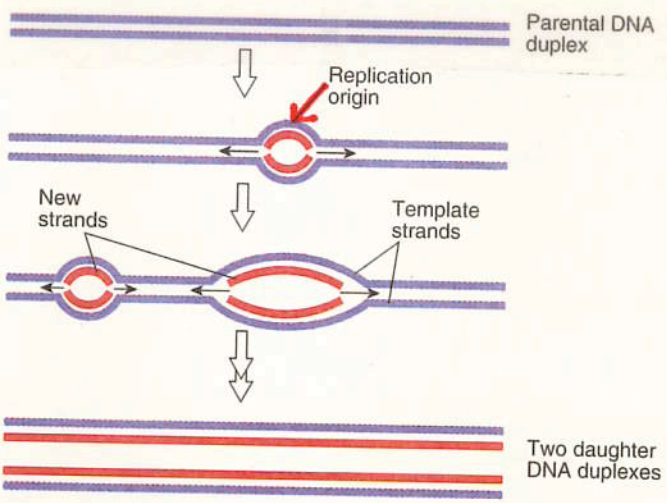


FIGURE 14.13
Origins of replication. At a site called the replication origin, the DNA duplex opens to create two separate strands, each of which can be used as a template for a new strand. Eukaryotic DNA has multiple origins of replication.

CONCEPT!

Foreign DNA segments use ori of chromosomes/DNA they are inserted into

e.g., bacteria insect^R gene → use plant ori

The ORIGEN OF REPLICATION IS A SPECIFIC SEQUENCE

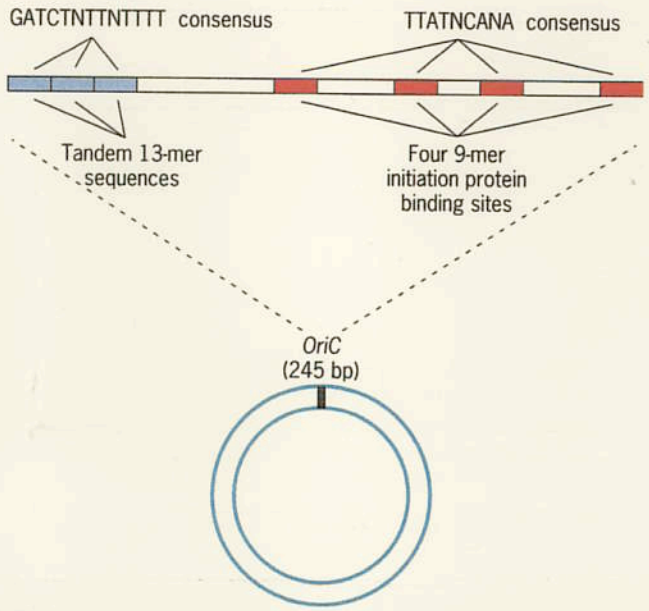


Figure 11.6 Structure of *OriC*, the single origin of replication in the *E. coli* chromosome.

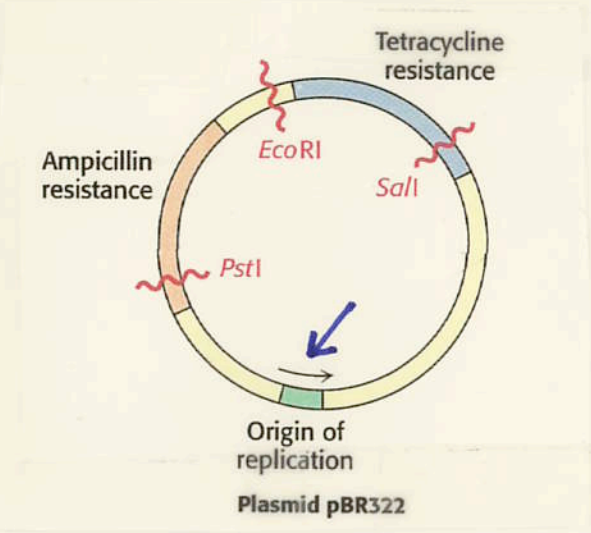
HOW CLONE AN ORIGIN OF REPLICATION?

Specific Sequence what does this mean FOR Genetic Engineering?

What is the Significance FOR Genetic Engineering?

CAN Replicating "CHROMOSOMES" Be Made?

VECTORS ARE NEEDED TO REPLICATE GENES IN SPECIFIC CELLS



1. ORI is a specific sequence
2. ORI is Genome & ORGANISM Specific
3. DNA Polymerases are specific for EACH ORGANISM

∴ 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 cell.

Yo! It's in the sequence = function

∴

Vectors CAN Be Engineered!
ORI's CAN BE CLONED/synthesized!

MODULAR

The Polymerase Chain Reaction or PCR is a Molecular Xerox Machine

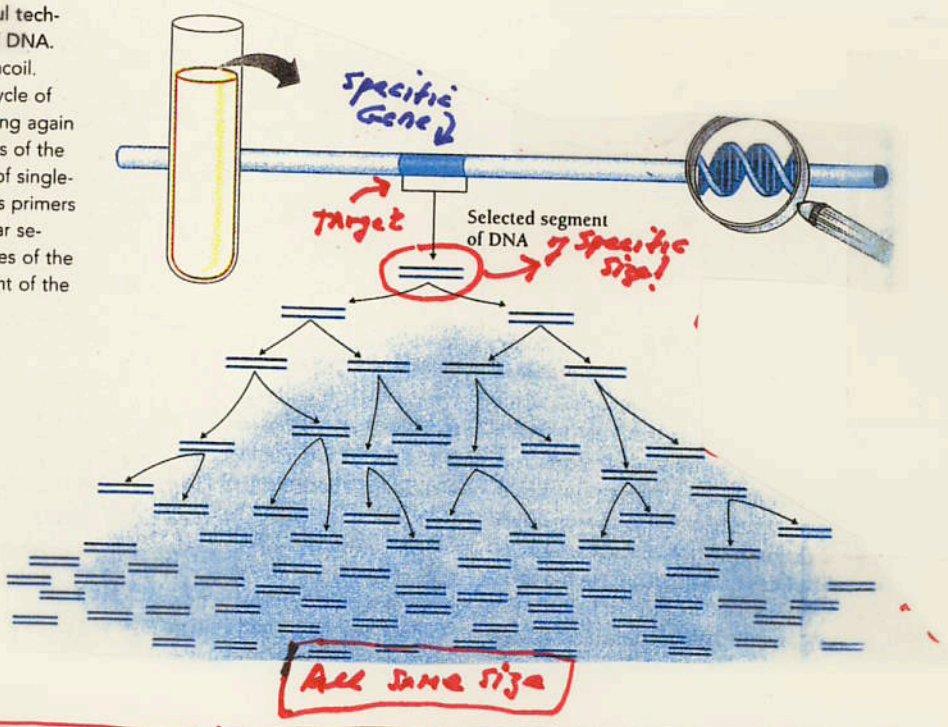


Figure 13-2 PCR is a simple, powerful technique for multiplying specific sequences of DNA. A. When DNA is heated, the two strands uncoil. They are then cooled and replicated. The cycle of heating, cooling, replicating, and then heating again is repeated until millions or billions of copies of the sequence are obtained. B. Short segments of single-stranded DNA called oligonucleotides act as primers and allow researchers to replicate a particular sequence, not just any DNA. The 20 or so bases of the oligonucleotide pair with the correct segment of the DNA and initiate replication.

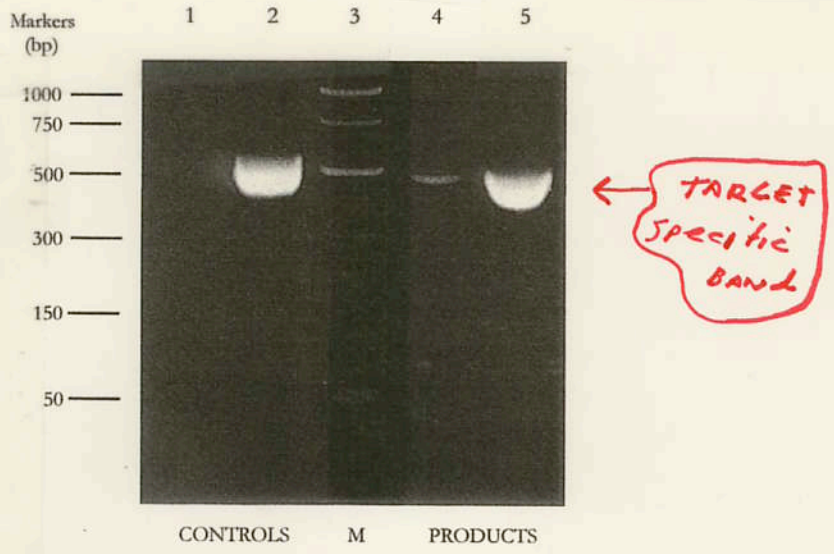
How many copies after 10 replication cycles?

PCR has revolutionized DNA analysis!
SPECIFIC DNA sequences/genes can be "copied" directly from "tiny" amounts of DNA!

NO cloning needed!
but need sequence!
∴ have to clone "gene" first

DNA Polymerase

USING GEL ELECTROPHORESIS TO VISUALISE PCR PRODUCTS



Specific Diagnostic DNA Band Unique to DNA Sequence Being Amplified

Fig. 7.8. Visualisation of PCR products of ornithine decarboxylase on an agarose gel. Lane 1 – negative control (no DNA); lane 2 – positive control (cloned ornithine decarboxylase fragment, 460 bp); lane 3 – PCR size markers; lanes 4 and 5 – PCR product using rat liver genomic DNA and the ornithine decarboxylase primers used in lane 2. Lane 4 shown product after 15 cycles, lane 5 after 30 cycles of PCR. Photograph courtesy of Dr F. McKenzie.

CAN AMPLIFY ONE DNA SEQUENCE IN A WHOLE GENOME

PCR Has MANY Uses That have Changed MANY Fields

(1) Amplify any DNA Sequence or Gene from "Tiny" Amounts of DNA. no need for Bacteria or Vector!

(2) Study DNA from Limited Sources! a single hair, an ancient insect/plant, a bone fragment, cheek cell

(3) Used in: Forensics, DNA Fingerprinting, Law, Evolution, Disease Diagnosis, Identification, Pathogen Identification, Basic Molecular Biology, Evolution studies, mRNA Detection

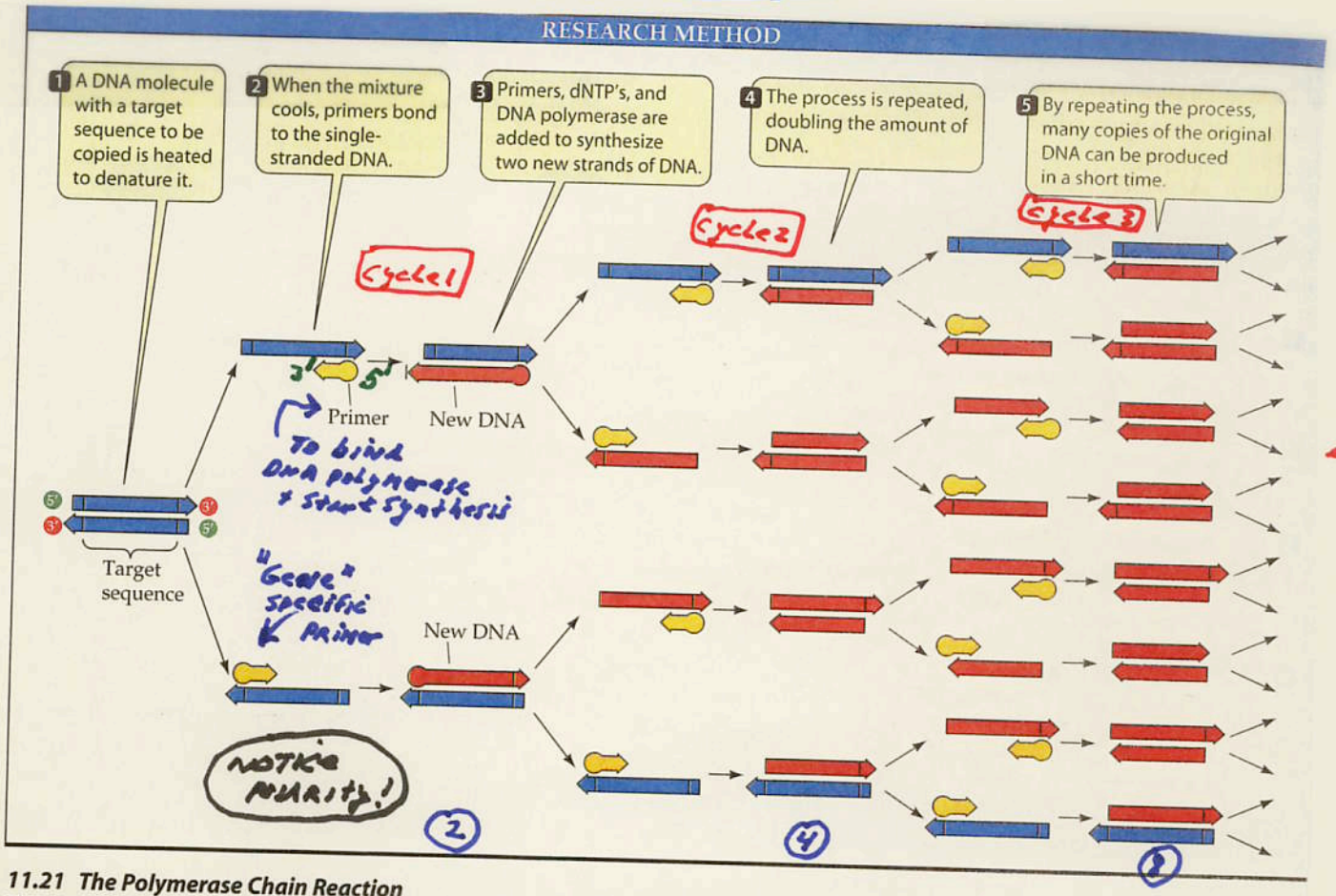
Need as Little as ONE Molecule of DNA!

CAN Make an ∞ Amount of DNA!

But- Need Sequence of DNA Segment to be used for PCR! i.e. have to clone a Sequence First!

REVOLUTIONIZED MANIPULATING DNA

PCR IS A CYCLIC PROCESS OF DNA REPLICATION



11.21 The Polymerase Chain Reaction
The steps in this cyclic process are repeated many times to produce multiple copies of a DNA sequence.

2^{n} molecules of DNA where n = # cycles

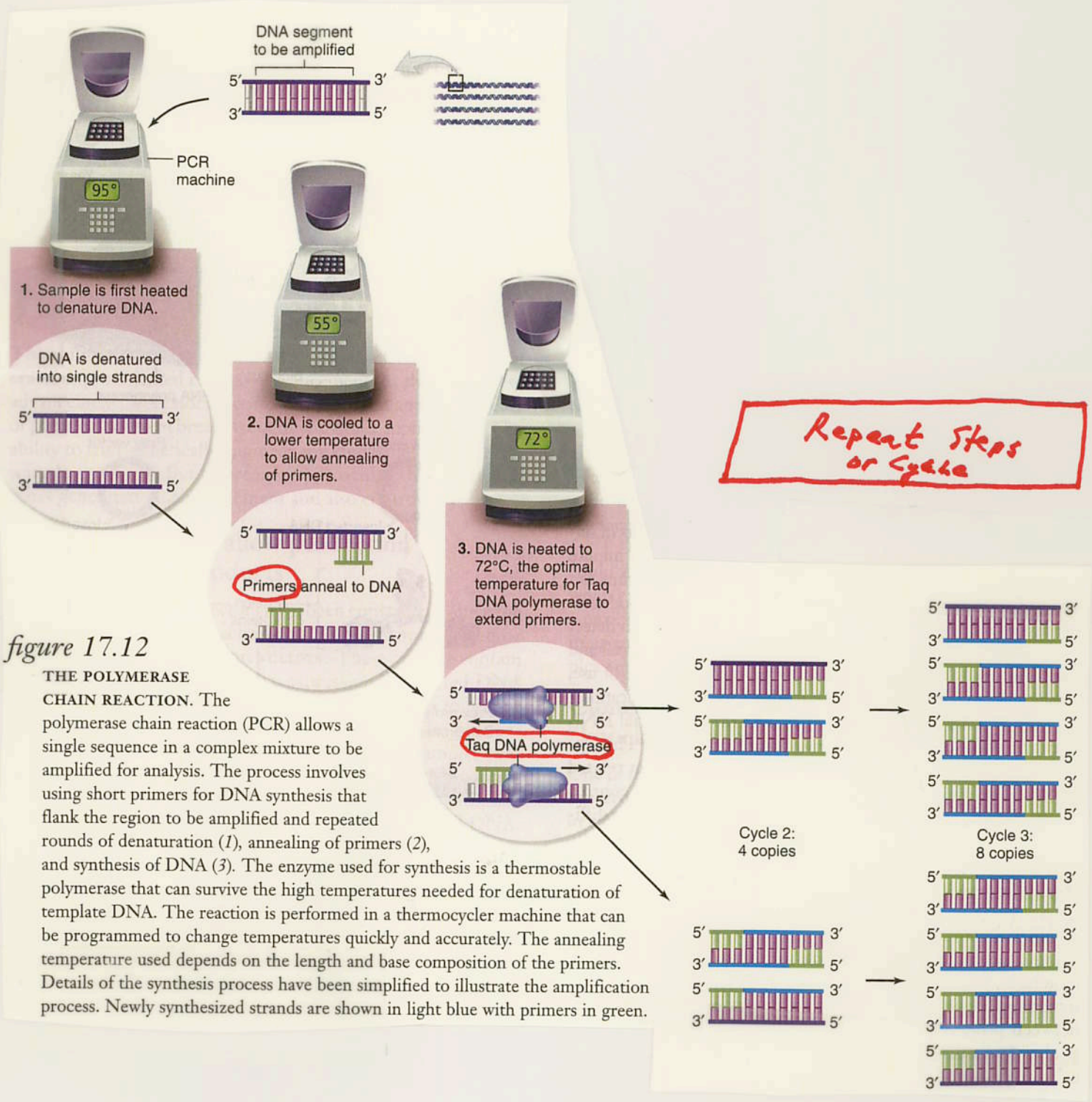
PCR Requirements

1. Knowledge of DNA Sequence :. Must clone DNA the "old fashioned way" first
2. DNA Polymerase - Heat stable to ~100°C!! (where isolated?)
3. Thermo programmer / cycler to Heat + Cool DNA in cycles - Separate strands & Allow new strands to form
4. Primers - Recognize specific DNA Sequences & Initiate DNA synthesis & Binding of DNA Polymerase Note: one not needed in test tube!

It's ALL in the DNA Sequences
Know Sequence - can "make" an infinite Amount of DNA sequence!

In 1 hour can do what took months before PCR!

PCR is a cyclical process of DNA synthesis



Repeat Steps or Cycle

figure 17.12

THE POLYMERASE CHAIN REACTION. The polymerase chain reaction (PCR) allows a single sequence in a complex mixture to be amplified for analysis. The process involves using short primers for DNA synthesis that flank the region to be amplified and repeated rounds of denaturation (*1*), annealing of primers (*2*), and synthesis of DNA (*3*). The enzyme used for synthesis is a thermostable polymerase that can survive the high temperatures needed for denaturation of template DNA. The reaction is performed in a thermocycler machine that can be programmed to change temperatures quickly and accurately. The annealing temperature used depends on the length and base composition of the primers. Details of the synthesis process have been simplified to illustrate the amplification process. Newly synthesized strands are shown in light blue with primers in green.

Diagnostic for Amplified DNA Sequence

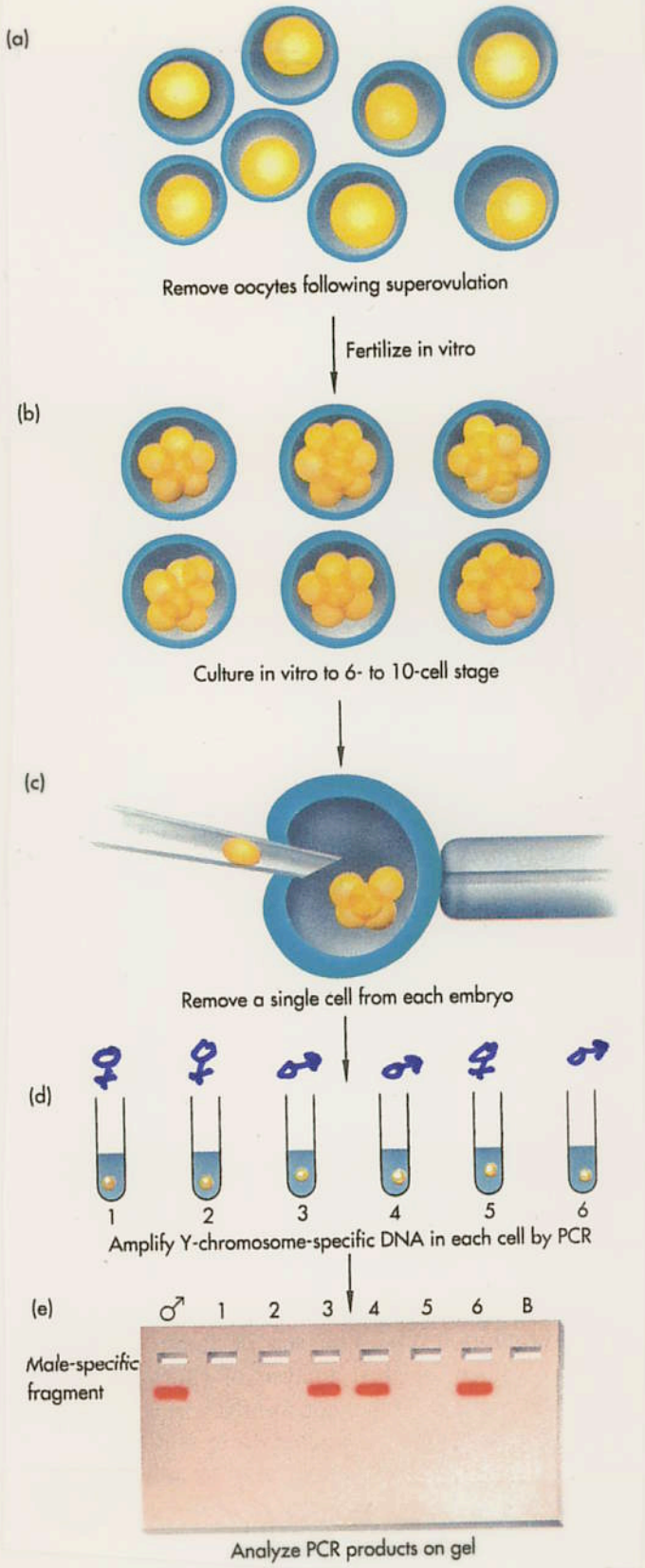
DNA fragments ALL The Same Size Primer Sequence Primer

PCR APPLICATIONS

1. Amplify known DNA sequence for study +/or Engineering (e.g., with another DNA)
   - specific gene, specific switch, ORI
2. Genetic Diagnosis to determine Genotype (e.g., disease genes)
   - PGD or pre-implantation genetic testing diagnosis
3. Ancient DNA (e.g., sources where DNA present in small amounts +/or degraded)
   - bones, fossils (Neanderthal), mummies
4. DNA forensics (i.e., DNA fingerprints)
   - crime scenes, mass graves, criminal suspects, innocent people wrongly convicted
5. Identity Testing (i.e., DNA fingerprints)
   - paternity, family relationships, immigration individuals (tracing)
6. Pathogen Identity (e.g., Medicine / Agriculture)

PCR CAN BE USED TO ANALYZE Genes in A Single Embryo Cell

PGD
pre-implantation genetic diagnosis



WHAT IS THE IMPLICATION OF THIS PROCEDURE Considering THAT The Human Genome Has Been Sequenced!

SEX determination in 8-cell Embryos!

Parents Should Be Allowed to Use PGD To Test Their Embryos For Any Gene and Select Those With the Gene Combination They Want To Become Their Child?

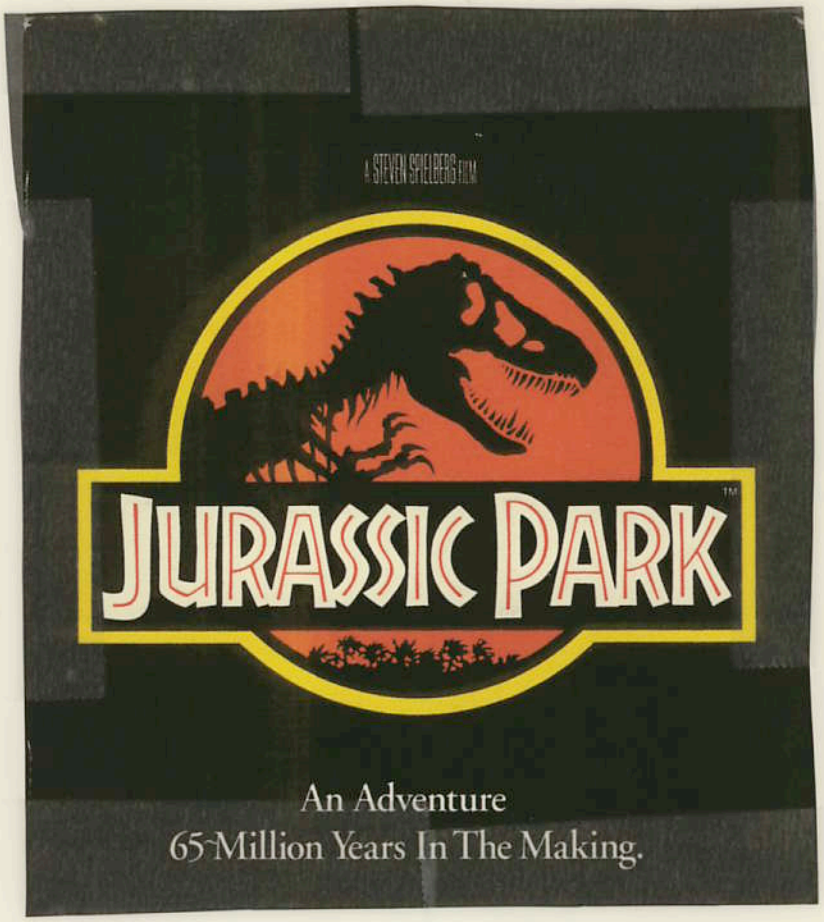
a. Yes
b. No

iClicker

Parents Should Be Allowed to Use PGD To Test Their Embryos For Any Gene and Select the "Best" One To Become Their Child?

a. Yes
b. No

iClicker


A STEVEN SPIELBERG FILM
JURASSIC PARK
An Adventure
65 Million Years In The Making.

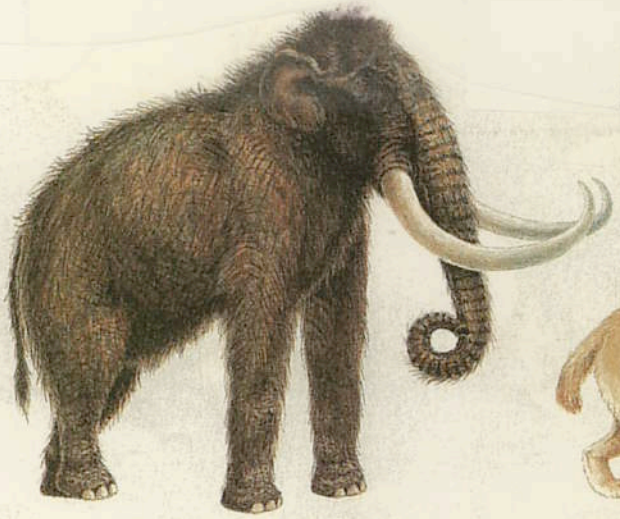
USING PCR TO DETECT GENES IN ANCIENT DNA

Ancient DNA Milestones

These extinct organisms have yielded meaningful genetic sequences.

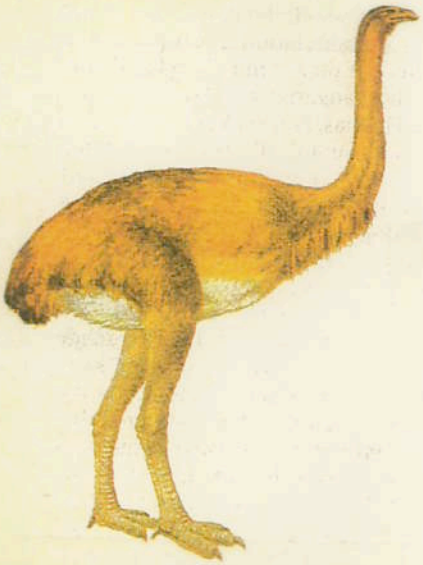
AMBER INSECTS
40 MILLION YEARS OLD

FOSSIL LEAVES
17 MILLION



MAMMOTH
40,000

SMILODON
13,000



MOA
4,300

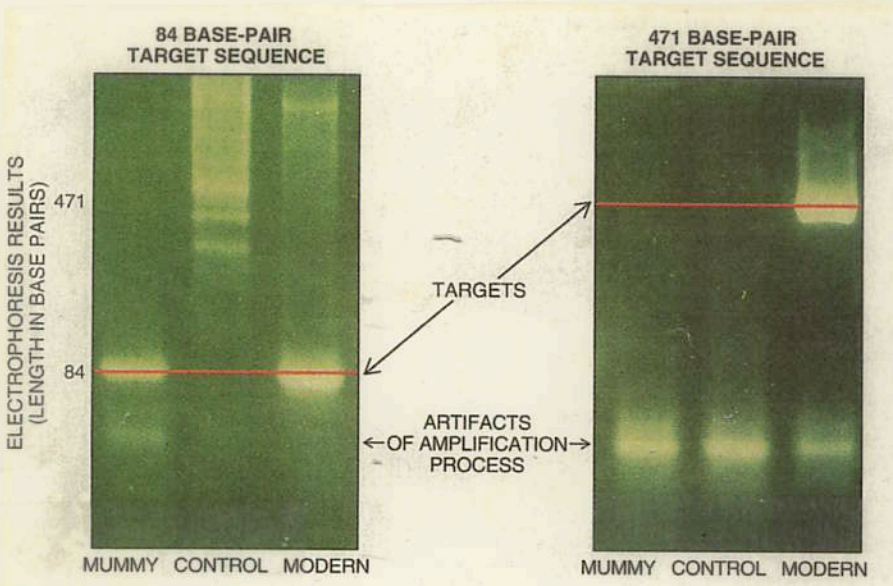
QUAGGA
140

THYLACINE
80

PRESENT

→ JUST NEED ONE MOLECULE OF DNA ←

Using PCR to detect genes in mummy DNA



Using PCR to amplify Neanderthal bone DNA & sequence the ENTIRE genome!

Neanderthal DNA vs. Homo sapien (Human) DNA

Sequence to determine relationships

USING PCR in CRIME SCENES

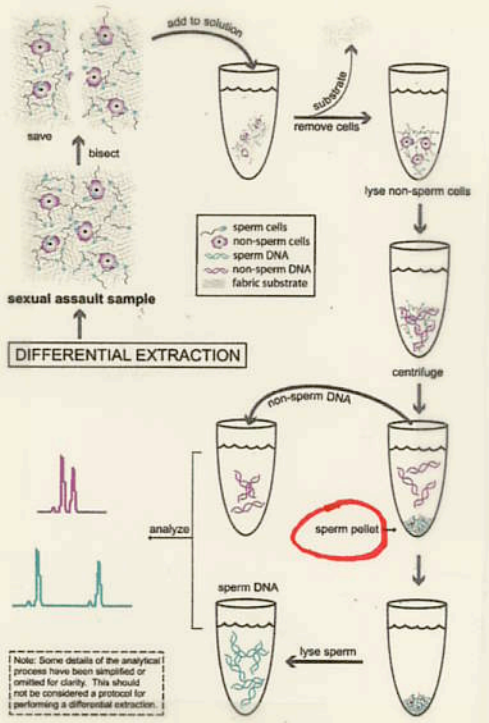
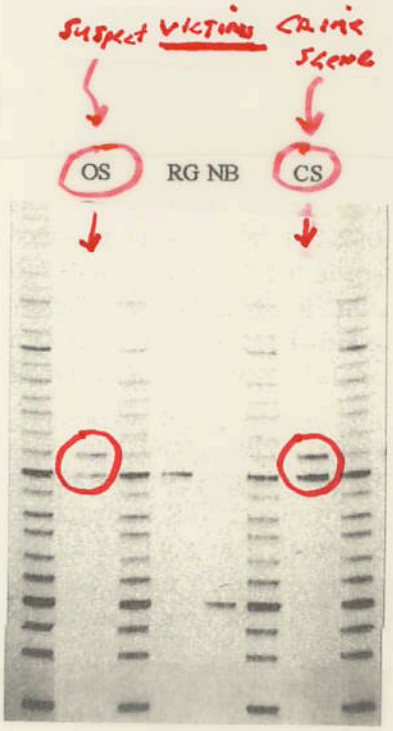


Plate 4 Differential extraction.

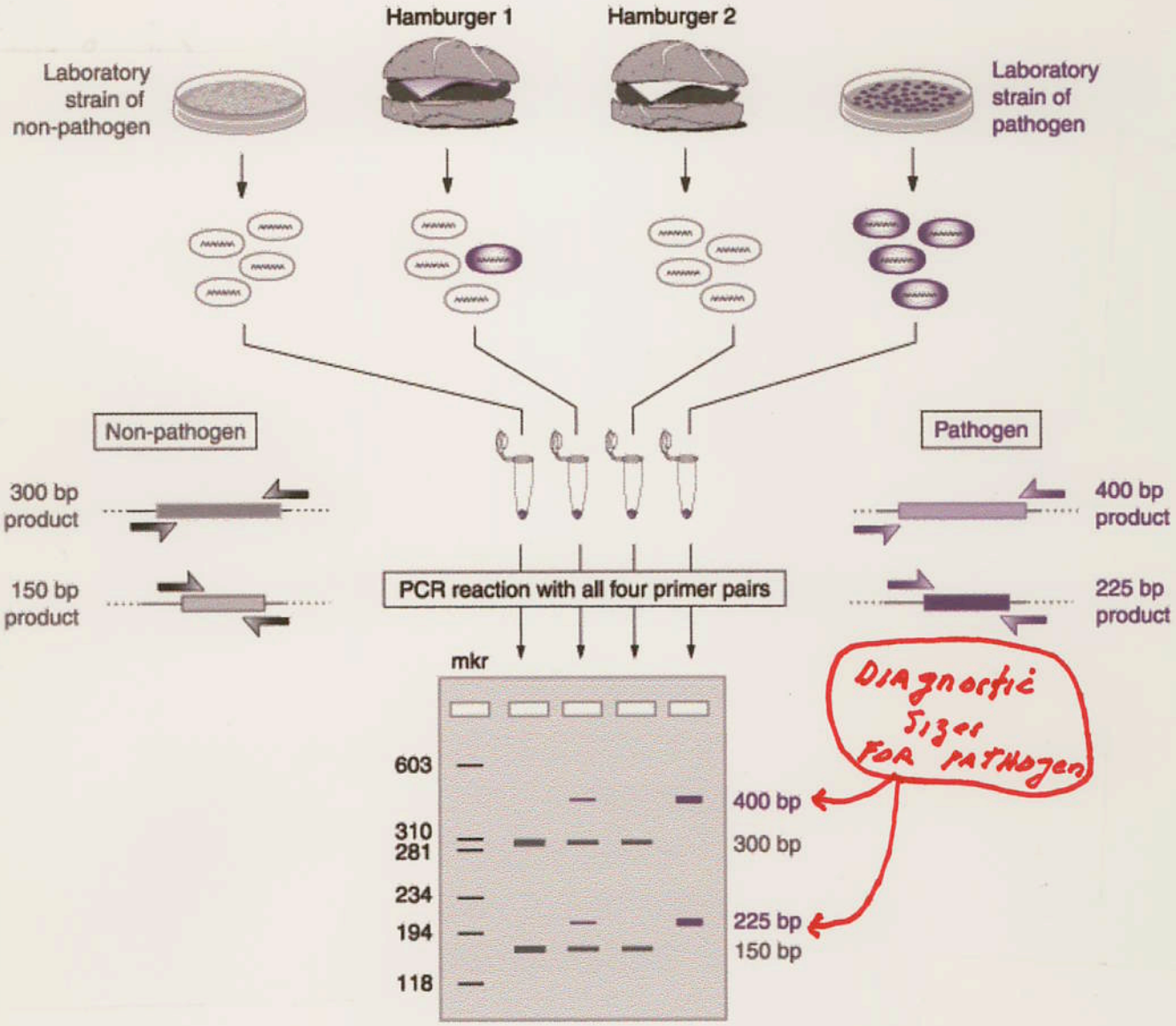


"Match" what is probability that this will occur by chance?

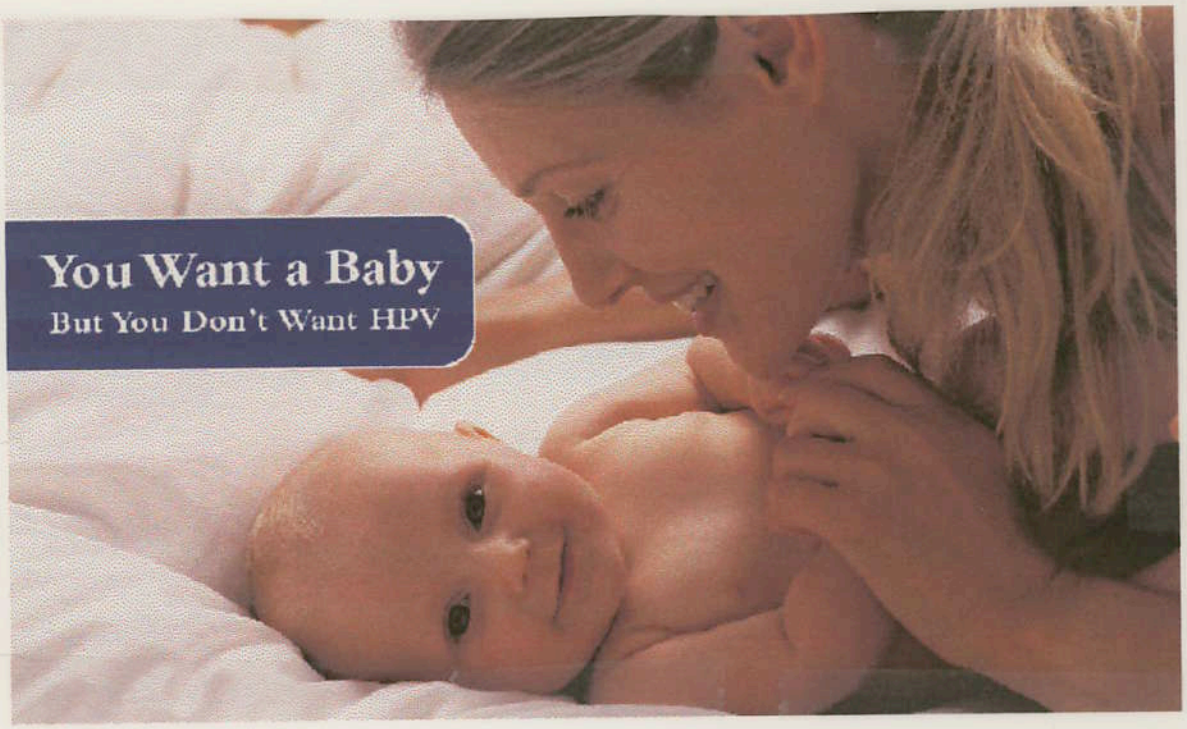
OS = Suspect
CS = CRIME SCENE
RG + NB = VICTIMS

DNA DOESN'T "LIE"!!

USING PCR TO DETECT FOOD PATHOGENS



USING PCR TO TEST FOR HUMAN PATHOGENS



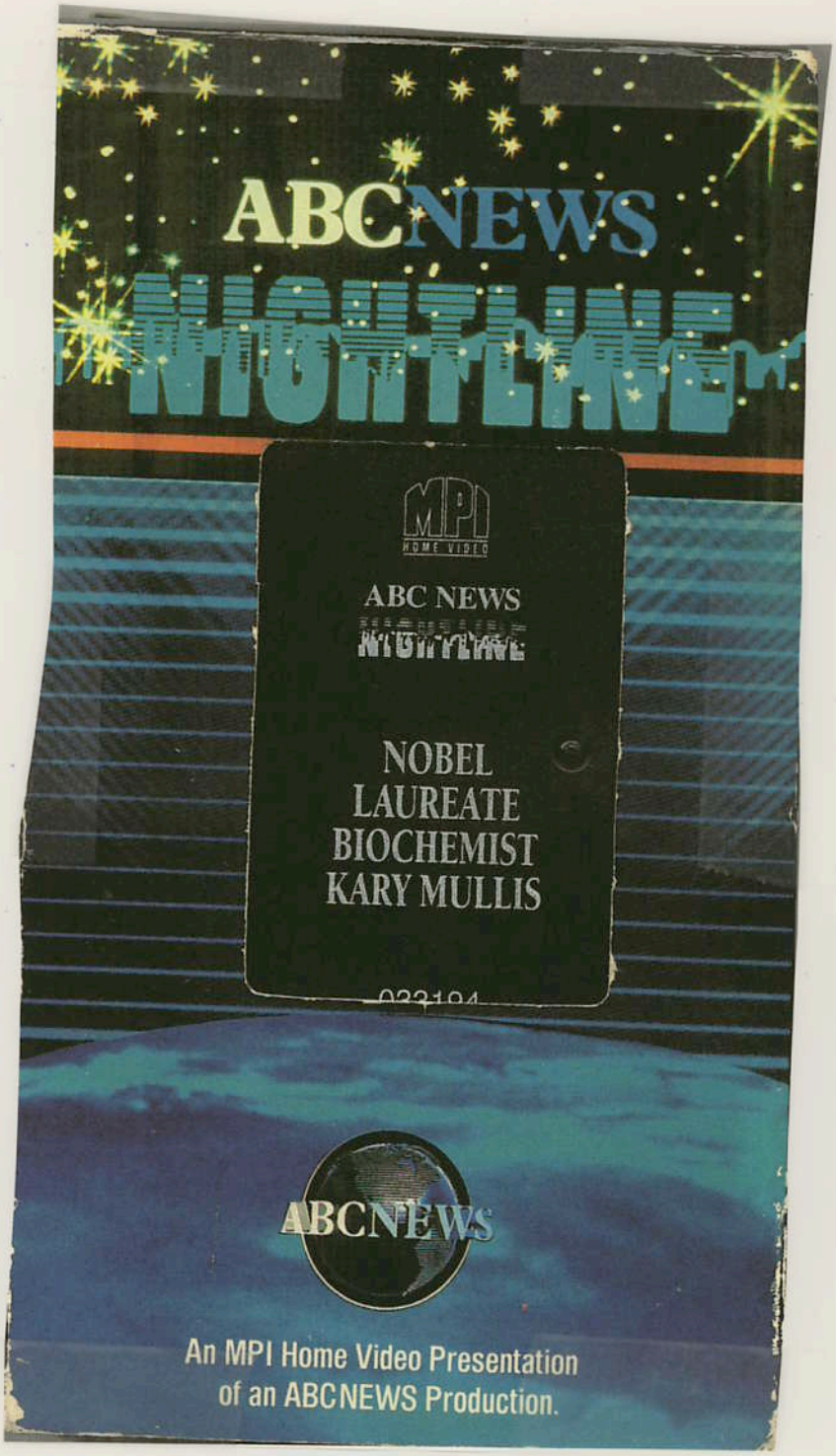
Identify Specific Pathogenic Bacteria, Viruses, Fungi

Each Genome Has Specific DNA Sequences & therefore DIAGNOSTIC DNA FRAGMENTS

The Polymerase Chain Reaction Has Made DNA Cloning and Recombinant DNA Obsolete?

a. Yes
b. No

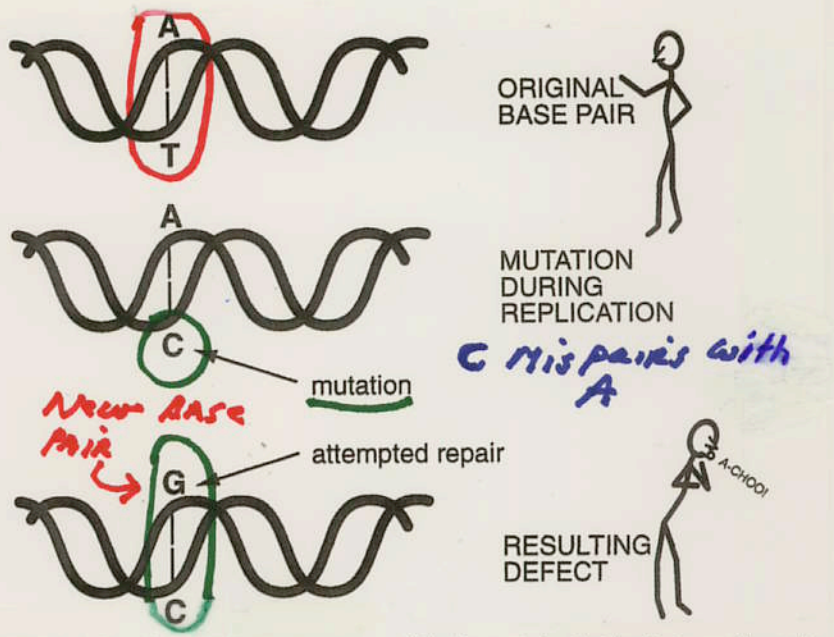
iClicker


ABCNEWS
NIGHTLINE
MPI
HOME VIDEO
ABC NEWS
NIGHTLINE
NOBEL
LAUREATE
BIOCHEMIST
KARY MULLIS
ABCNEWS
An MPI Home Video Presentation
of an ABCNEWS Production.

DNA REPLICATION IS PRECISE BUT MISTAKES OR MUTATIONS CAN OCCUR!

GENE A

Replication (1)



C Mispairs with A

RARE BASE MISMATCH

Replication (2)

GENE A' Allelic variant

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

MUTATIONS IN GENES ARE RARE BUT ARE INHERITED

ONE gene per gamete ♂ + ♀

TWO genes per somatic cells

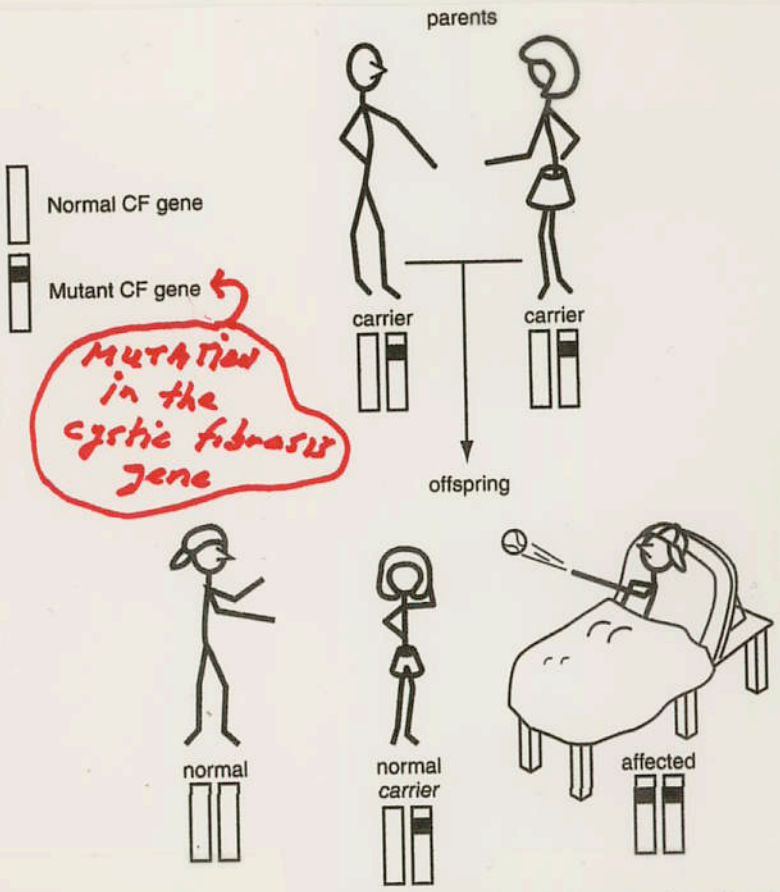


FIGURE 1.4 Two parents heterozygous for CF and producing normal or affected kids.

HOW FOLLOW INHERITANCE?

WHAT ALLOWS DISEASE TO BE FOLLOWED?

MARKER!

Alleles Are Different Forms of the Same Gene?

a. Yes
b. No

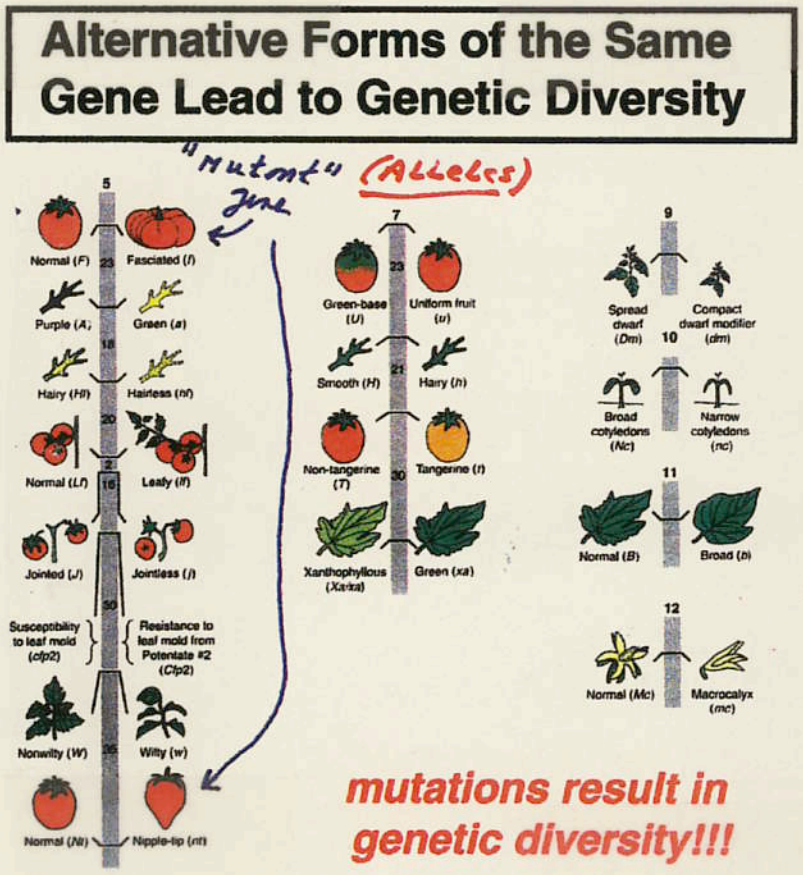
iClicker

Alleles Are Generated By Mutations and Lead to Genetic Diversity in a Population?

a. Yes
b. No

iClicker

MUTATIONS GIVE RISE TO GENETIC DIVERSITY



Change in DNA Sequence!

How know Sequence changed?

Different forms of SAME Gene

SNP - Single nucleotide polymorphism!

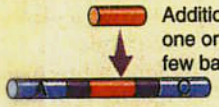
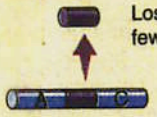
VARIABILITY Acted on x
used By "Nature" x
By our ANCESTORS / EARLY Gene ENGINEERS!

SAME PROCESS → DIVERSITY OF HUMAN GENES!

HOW KNOW MUTATION? ARE MUTATIONS GOOD, BAD, NEUTRAL?

MUTATIONS CAN OCCUR DIFFERENT WAYS

Table 18.1 Types of Mutation

Mutation	Example result
NO MUTATION	
	Normal B protein is produced by the *B* gene.
POINT MUTATION	
Base substitution	B protein is inactive because changed amino acid disrupts function.
	
Insertion	B protein is inactive because inserted material disrupts proper shape.
	
Deletion	B protein is inactive because portion of protein is missing.
	
CHANGES IN GENE POSITION	
Transposition	*B* gene or B protein may be regulated differently because of change in gene position.
	
Chromosomal rearrangement	*B* gene may be inactivated or regulated differently in its new location on chromosome.
	

(1) BASE-PAIR CHANGE

(2) ADD/DELETE BASE PAIRS

BASE SEQUENCE OF GENE CHANGES!

(3) MOVE GENE OR PART OF GENE TO NEW LOCATION!
SWITCHES CHANGE!

LOSS OF FUNCTIONAL PROTEIN

MUTATIONS CAN OCCUR BY CHANGING, ADDING TO, OR DELETING SPECIFIC NUCLEOTIDES

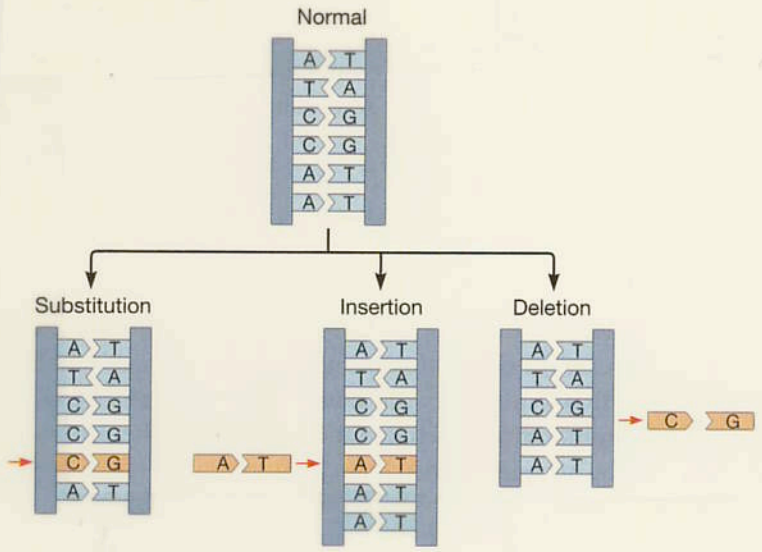
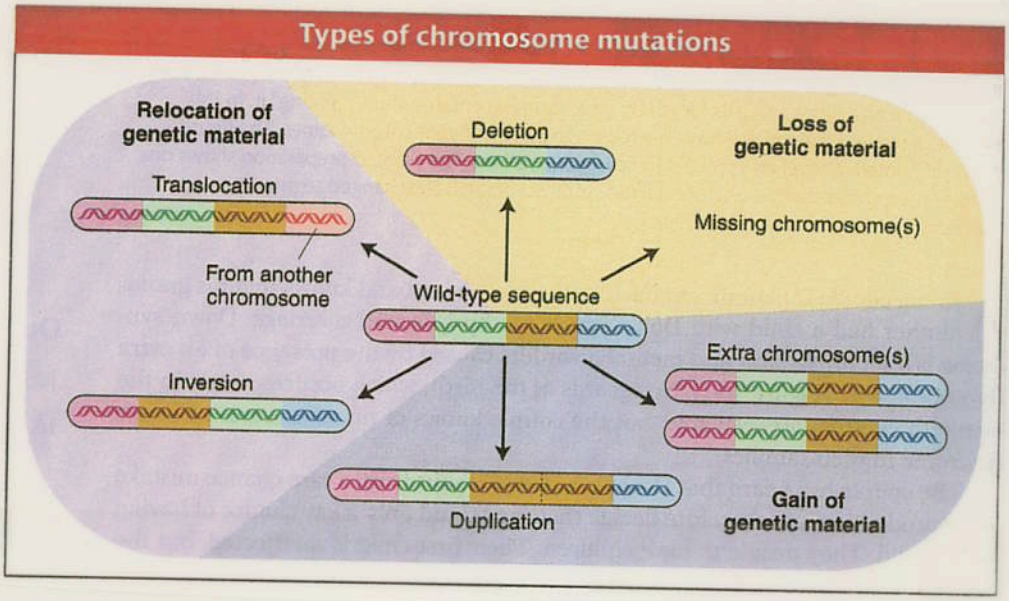


FIGURE 2-10

The three types of mutation. They are substitution, addition, and deletion of a base pair in a DNA strand.

CHANGE ADD DELETE

"MUTATIONS" CAN ALSO OCCUR BY LARGE CHROMOSONAL CHANGES



These Changes Affect MANY Genes!

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

HUMAN GENETIC DISORDERS OCCUR AS A RESULT OF MUTATIONS

TABLE 13.2 Some Important Genetic Disorders

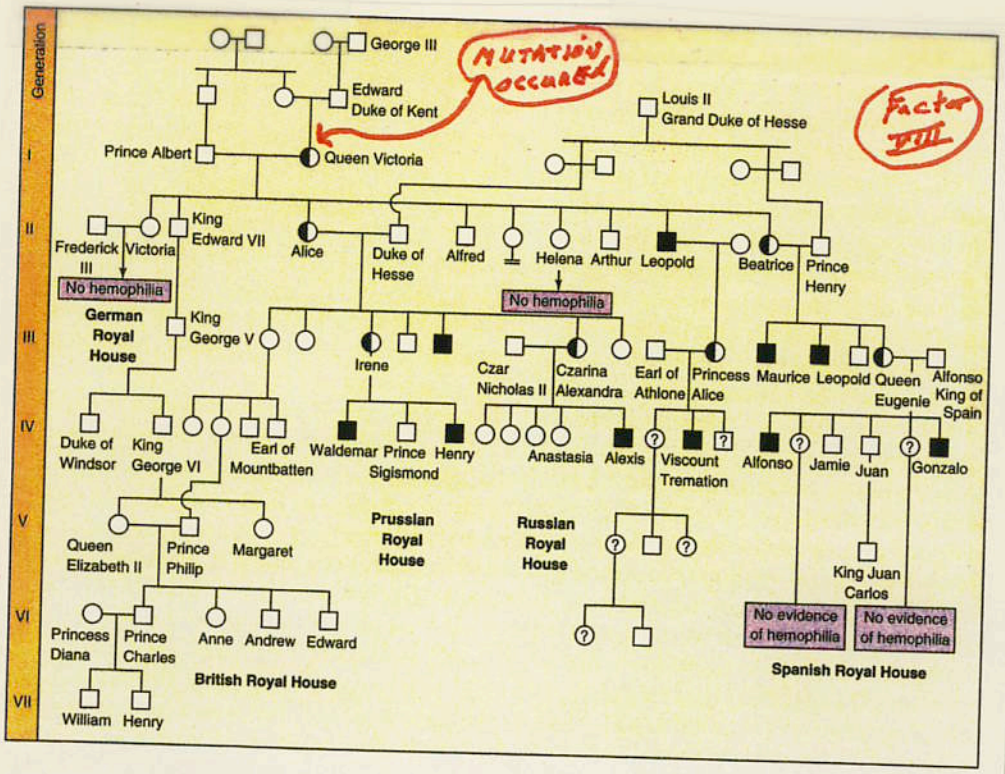
Disorder	Symptom	Defect	Dominant/ Recessive	Frequency Among Human Births
Cystic fibrosis	Mucus clogs lungs, liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cell anemia	Blood circulation is poor	Abnormal hemoglobin molecules	Recessive	1/600 (African Americans)
Tay–Sachs disease	Central nervous system deteriorates in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazi Jews)
Phenylketonuria	Brain fails to develop in infancy	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12,000
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

DIPLOID GENOTYPE	Wild type /	Dominant /	Dominant / Dominant	Recessive /	Recessive / Recessive
DIPLOID PHENOTYPE	Wild type	Mutant	Mutant	Wild type	Mutant

▲ **FIGURE 5-2 Effects of dominant and recessive mutant alleles on phenotype in diploid organisms.** A single copy of a dominant allele is sufficient to produce a mutant phenotype, whereas both copies of a recessive allele must be present to cause a mutant phenotype. Recessive mutations usually cause a loss of function; dominant mutations usually cause a gain of function or an altered function.

PEDIGREES CAN BE USED TO FOLLOW DISEASE Genes in HUMAN FAMILIES

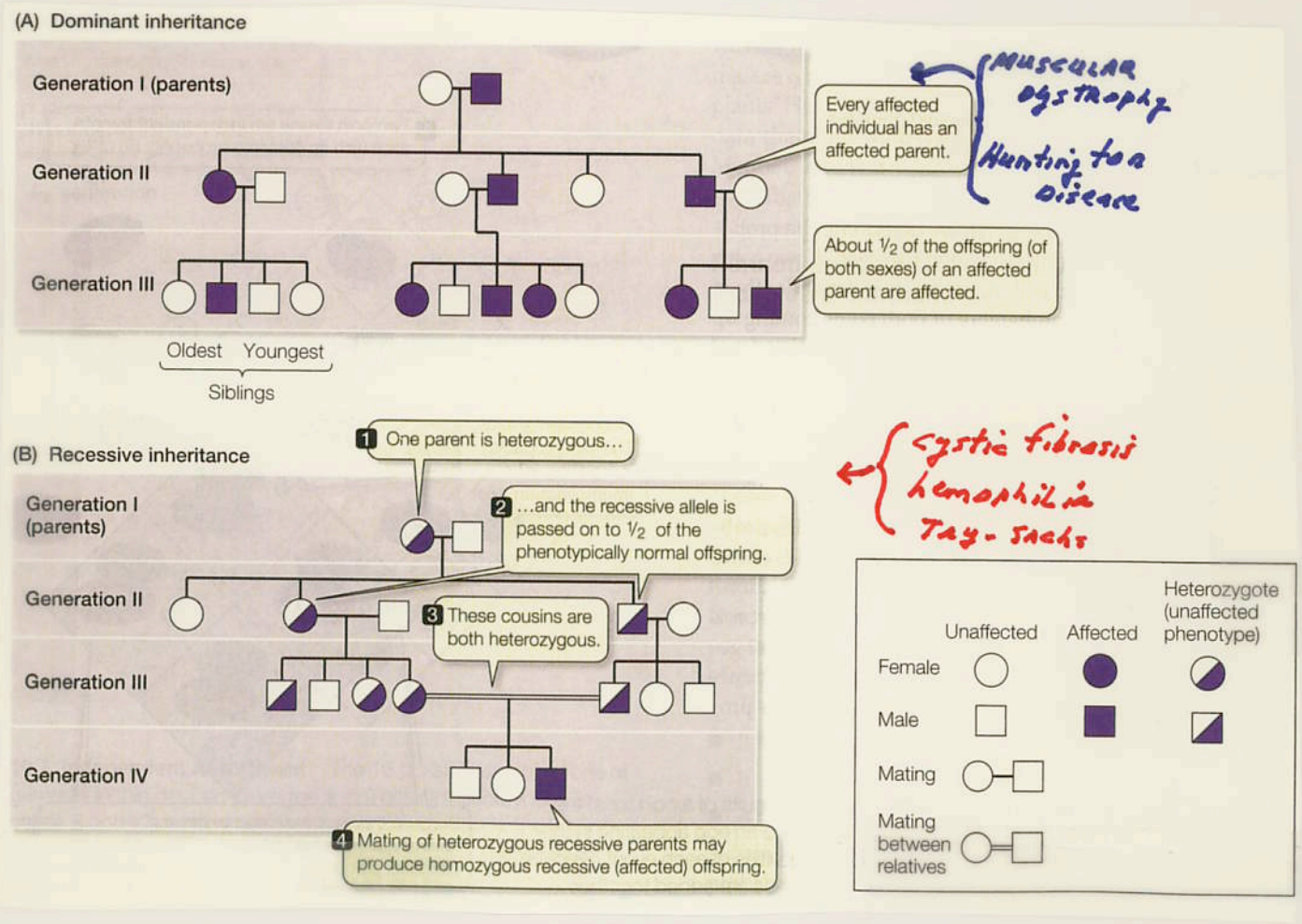


Hemophilia

Followed by Bleeding Phenotype

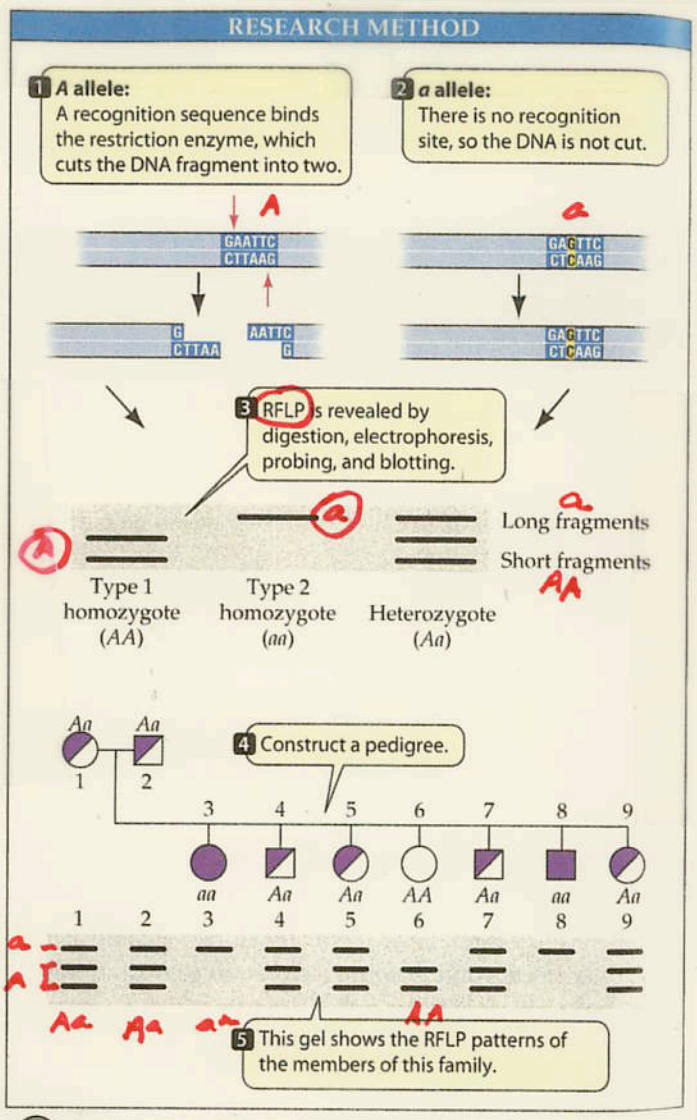
FIGURE 13.26
The Royal hemophilia pedigree. Queen Victoria's daughter Alice introduced hemophilia into the Russian and Austrian royal houses, and Victoria's daughter Beatrice introduced it into the Spanish royal house. Victoria's son Leopold, himself a victim, also transmitted the disorder in a third line of descent. Half-shaded symbols represent carriers with one normal allele and one defective allele; fully shaded symbols represent affected individuals.

PEDIGREES CAN BE USED TO DETERMINE IF TRAIT IS DOMINANT OR RECESSIVE



EACH TYPE OF INHERITANCE PREDICTS SPECIFIC RESULTS IN EACH GENERATION

OR FOLLOW BY DNA TESTS USING MOLECULAR METHODS (e.g., PCR)



17.7 RFLP Mapping Restriction fragment length polymorphisms are differences in DNA sequences that serve as genetic markers. Thousands of such markers have been described for the human genome.

CUT - A

a - NOT CUT

use PCR &/or other method

← DNA Fingerprint

RFLP

←FOLLOW in FAMILY

Implications? Combined with sequence of Human Genome, Embryotesting (PGD), & PCR ?!!!

HOW DOES A GENE LEAD TO A PHENOTYPE?

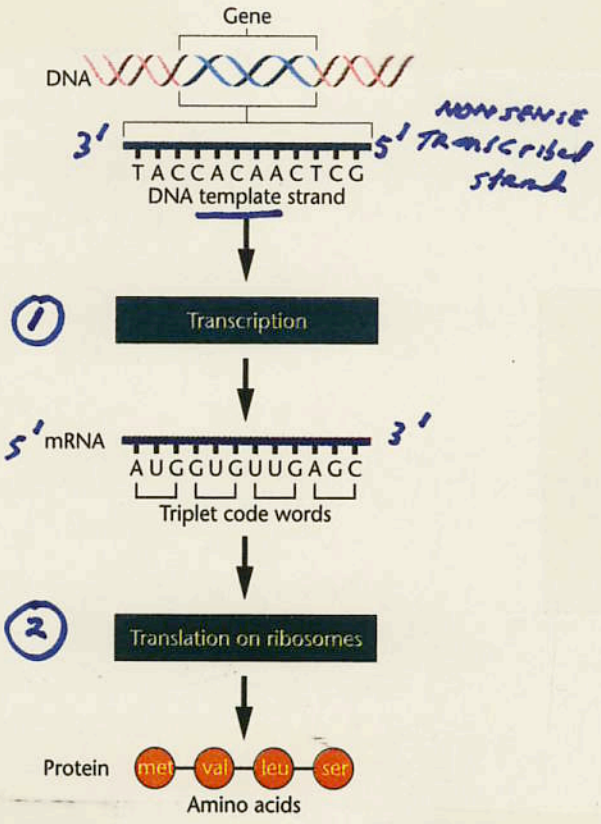


FIGURE 13.1 An overview of the concept of the flow of genetic information encoded in DNA to messenger RNA to protein.

1. mRNA synthesized by TRANSCRIPTION
   - COMPLEMENTARY to TRANSCRIBED, NONSENSE 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

KNOW SEQUENCE
KNOW PROTEIN
→ ENGINEER NEW PROTEINS

MUTATIONS - CHANGE DNA SEQUENCE & CHANGE CORRESPONDING PROTEIN SEQUENCE!!

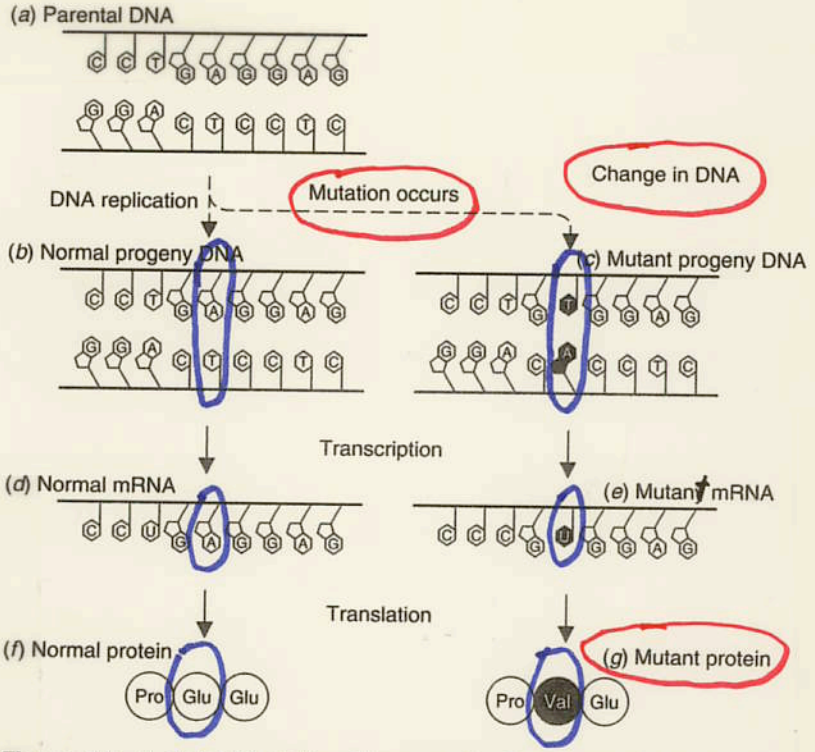
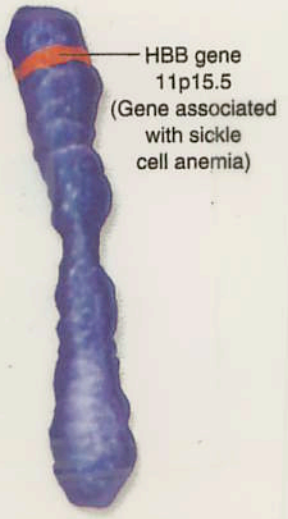


Figure 3-5. A Point Mutation Changes the Sequence of Amino Acids in a Protein. DNA replication is very accurate, so the nucleotide sequence in the progeny DNA **(b)** is usually identical to that of normal parental DNA **(a)**. Occasionally an error is made. In this example, a particular A · T base pair in parental DNA changes to a T · A pair in the mutant, progeny DNA **(c)**. During transcription, the information in DNA is converted into messenger RNA. The mutation in DNA results in a conversion of particular GAG codon in normal messenger RNA **(d)** into a GUG codon in mutant messenger RNA **(e)**. During translation of the information into protein, GAG codes for the amino acid glutamic acid (Glu) **(f)**, while GUG codes for valine (Val) **(g)** (see Figure 2-6). The two amino acids have very different chemical properties. Since the structure of the resulting protein is determined by the precise order of the amino acids, the mutant protein will differ significantly from the normal protein. The differences between the normal and mutant molecules shown are identical to those found between healthy people and patients suffering from sickle-cell disease.

Change in DNA
↓
Change in mRNA
↓
Change in Protein
↓
Change in Phenotype

GENE MUTATION LEADING TO SICKLE CELL ANEMIA



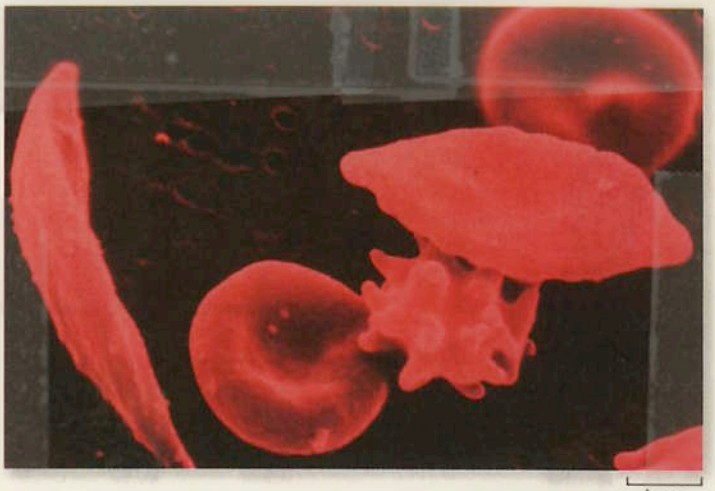


figure 13.11

SICKLE CELL ANEMIA. In individuals homozygous for the sickle cell trait, many of the red blood cells have sickled or irregular shapes, such as the cell on the far right.

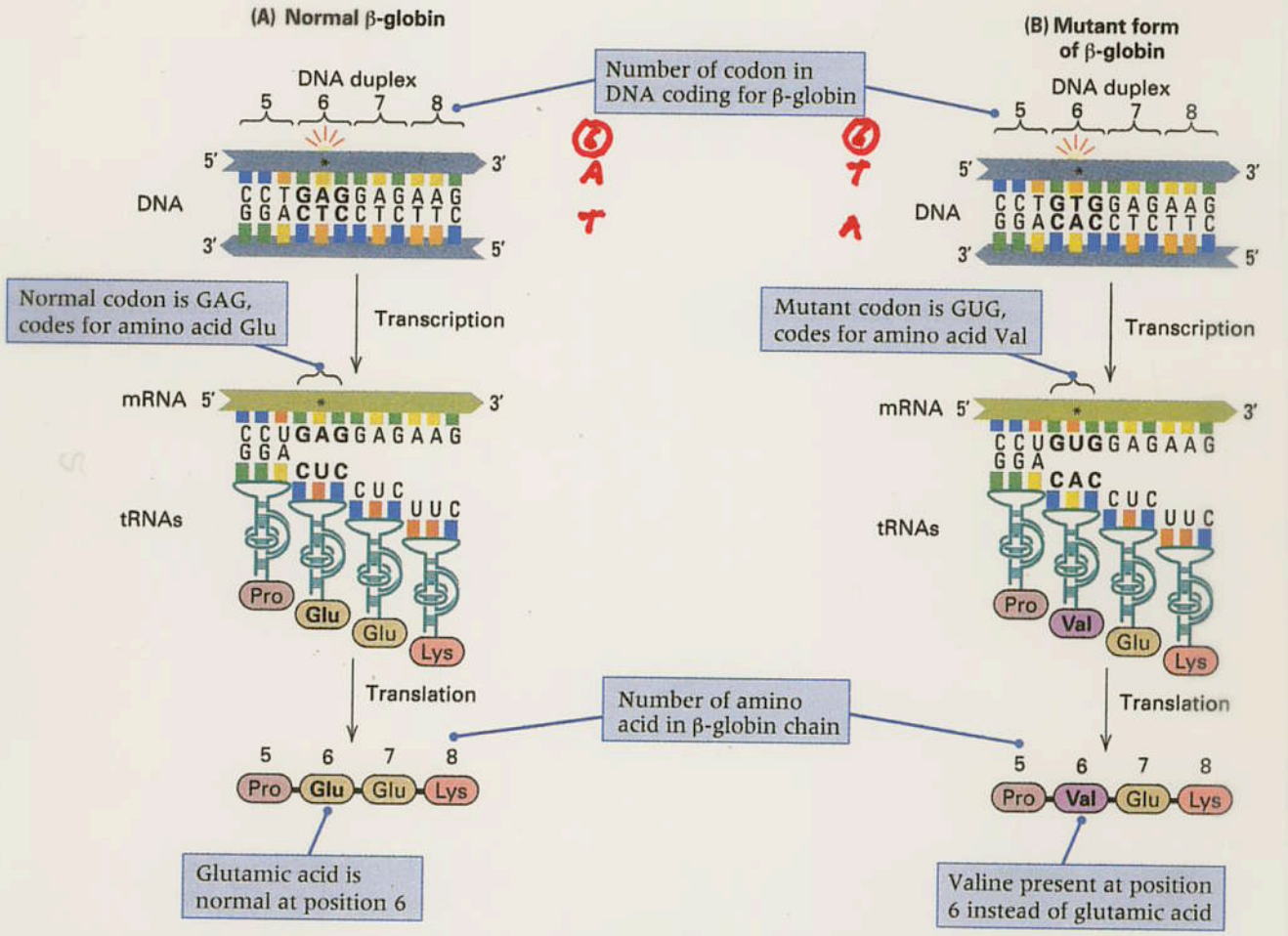
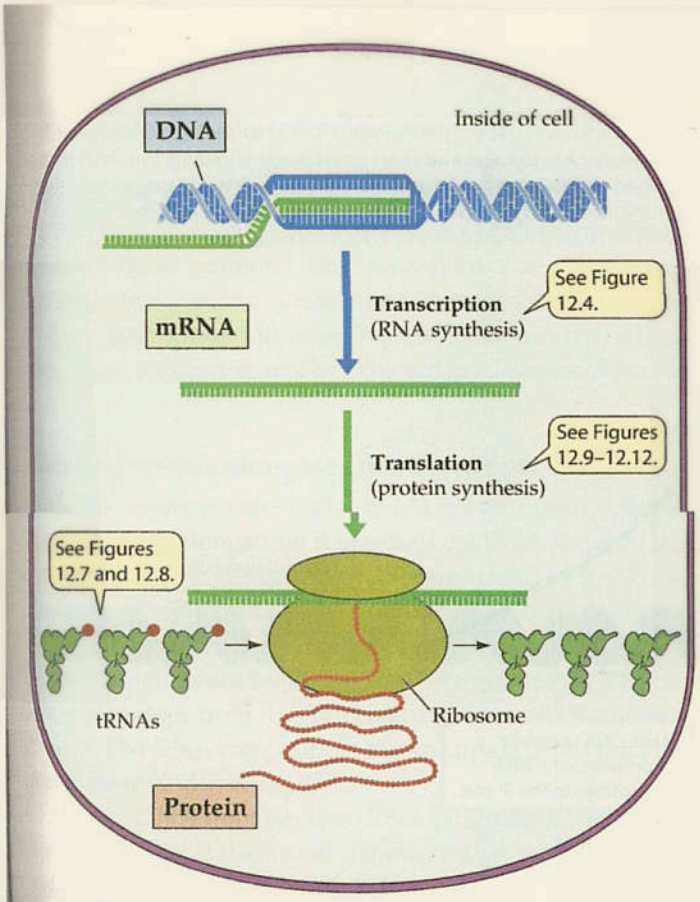


Figure 7.3 Molecular basis of sickle-cell anemia. (A) Part of the DNA in the normal β-globin gene is transcribed into a messenger RNA coding for the amino acid sequence Pro–Glu–Glu–Lys. The T in the marked A–T base pair is transcribed as the A in the GAG codon for Glu (glutamic acid). (B) Mutation of the normal A–T base pair to a T–A base pair results in the codon GUG instead of GAG. The codon GUG codes for Val (valine), so the polypeptide sequence in this part of the molecule is Pro–Val–Glu–Lys. The resulting hemoglobin is defective and tends to polymerize at low oxygen concentration.

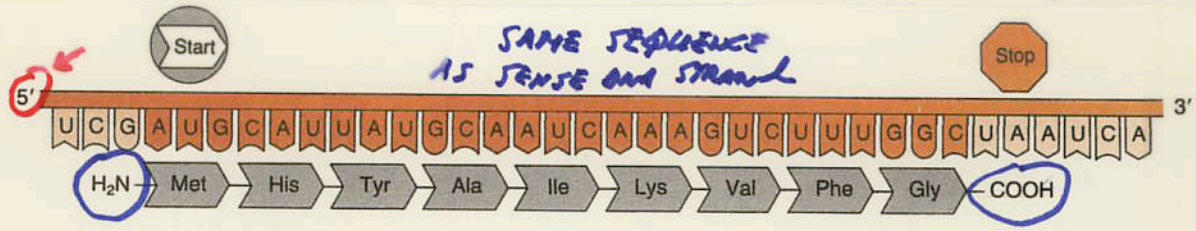
AN ELABORATE CELLULAR MACHINARY REQUIRING THOUSANDS OF GENES IS REQUIRED TO PRODUCE PROTEINS ENCODED BY SPECIFIC GENES!



12.3 **From Gene to Protein** This diagram summarizes the processes of gene expression in prokaryotes. In eukaryotes, the processes are somewhat more complex.

IT TAKES GENES TO EXPRESS (and replicate) A GENE!!

GENETIC CODE ALLOWS THE SEQUENCE of NUCLEOTIDES in mRNA / Sense STRAND of Gene to be TRANSLATED into Sequence of Amino Acids in Proteins



mRNA
Protein

Proteins have Ends too!

Figure 3.4 Decoding a messenger RNA sequence into a polypeptide.

NOTE: SEQUENCE in mRNA (= Sense Gene STRAND) IS TRANSLATED 5'→3' (= beginning of sense strand to end) & protein made in N→C direction ∴ order nts in gene = order aa in protein!

The Genetic Code is Universal!

How Know?

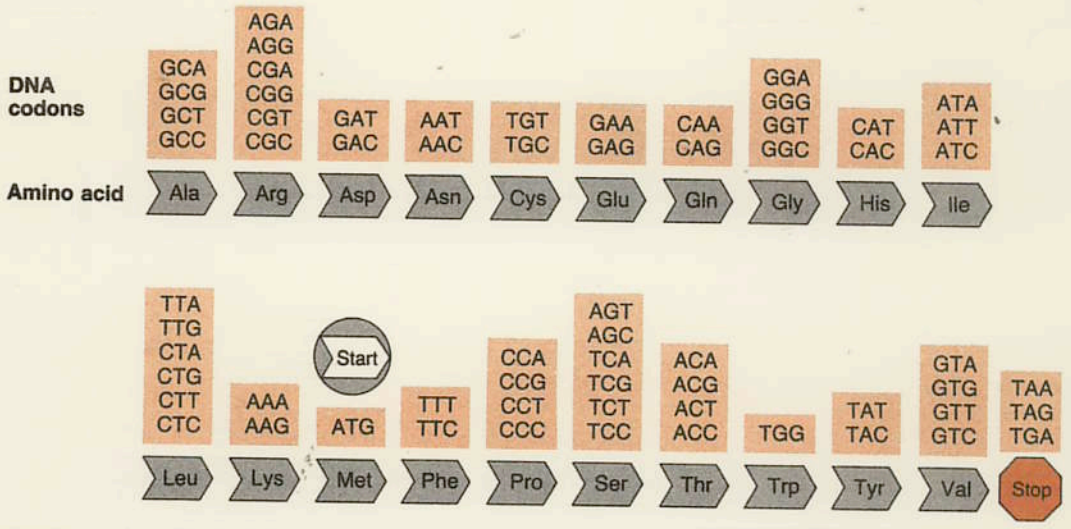


Figure 3.3 The genetic code. The codons shown for each amino acid are those for DNA. For RNA, the Ts are replaced by Us.

(1) Universal (2) Triplet (3) Punctuation
(4) Degenerate

KNOW SEQUENCE OF GENE - KNOW SEQUENCE OF PROTEIN USING GENETIC CODE

Big Implication For Genetic Engineering! CAN MAKE Genes, Genomes & Specify Proteins Wanted!
CAN Express Genes FROM ONE Organism in Another!

DESIGN AN EXPERIMENT TO SHOW UNIVERSAL!

EXPRESSION OF JELLY FISH GREEN FLUORESCENCE PROTEIN (GFP) IN PIGS SHOWS THAT Genetic code is Universal!!

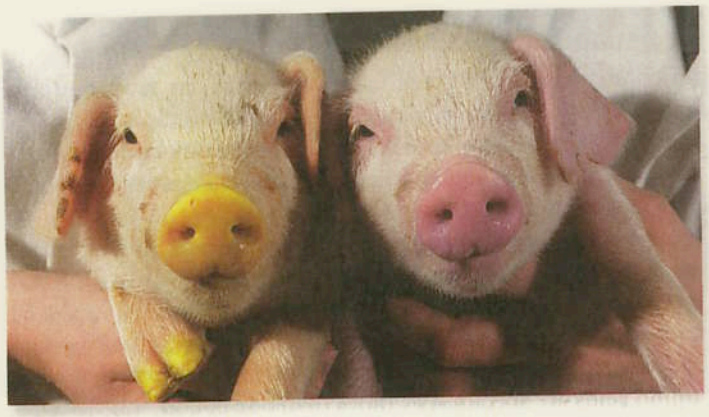


figure 15.3

TRANSGENIC PIG. The piglet on the right is a conventional piglet. The piglet on the left was engineered to express a gene from jellyfish that encodes green fluorescent protein. The color of this piglet's nose is due to expression of this introduced gene. Such transgenic animals indicate the universal nature of the genetic code.

THERE IS A COLINEARITY BETWEEN THE DNA SEQUENCE OF A GENE & THE AMINO ACID SEQUENCE OF A PROTEIN

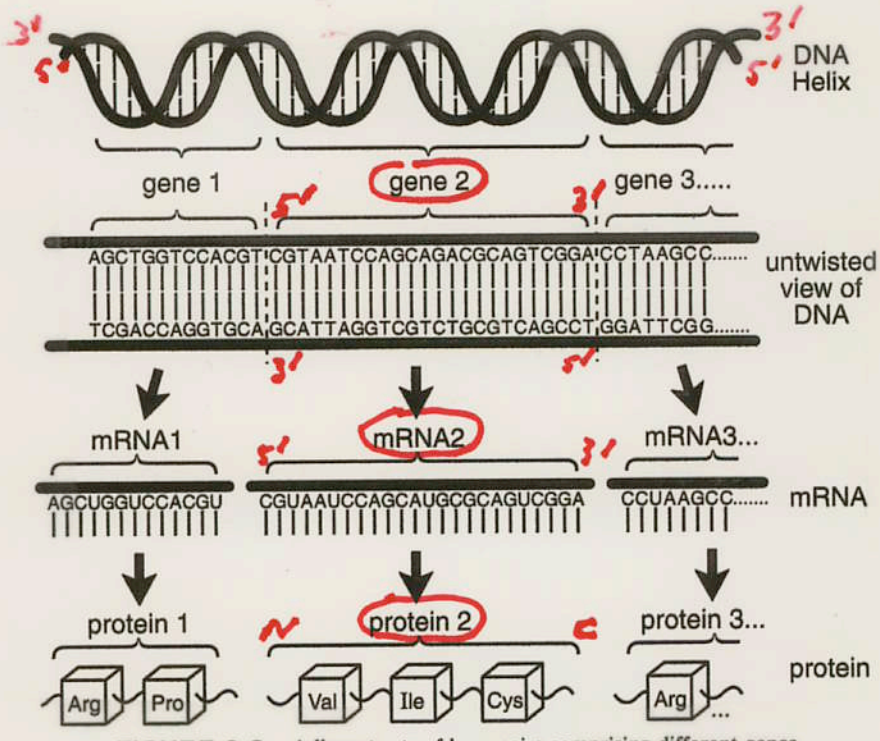


FIGURE 2.6 Adjacent sets of base pairs comprising different genes.

FUNCTION 2

→ SPECIFIC TRAITS

GENES FUNCTION AS INDIVIDUAL UNITS!

PROTEINS ARE MADE OF AMINO ACIDS AND.....

AMINO ACIDS ARE JOINED BY Peptide Bonds

ORDER!

Corresponds to 5' & 3' ends of mRNA / sense strand of gene!

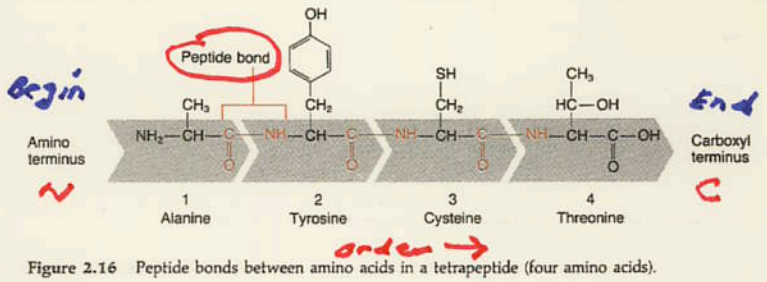
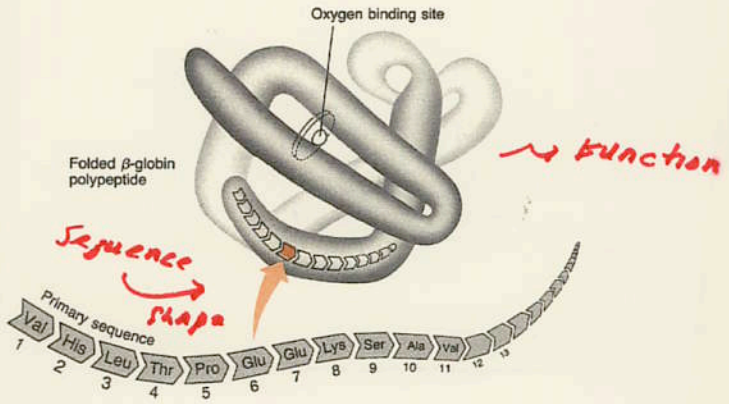


Figure 2.16 Peptide bonds between amino acids in a tetrapeptide (four amino acids).

Mutations!

Change Shape
Change Function
Change Phenotype



In sickle-cell hemoglobin, the Glu at position 6 is replaced by Val

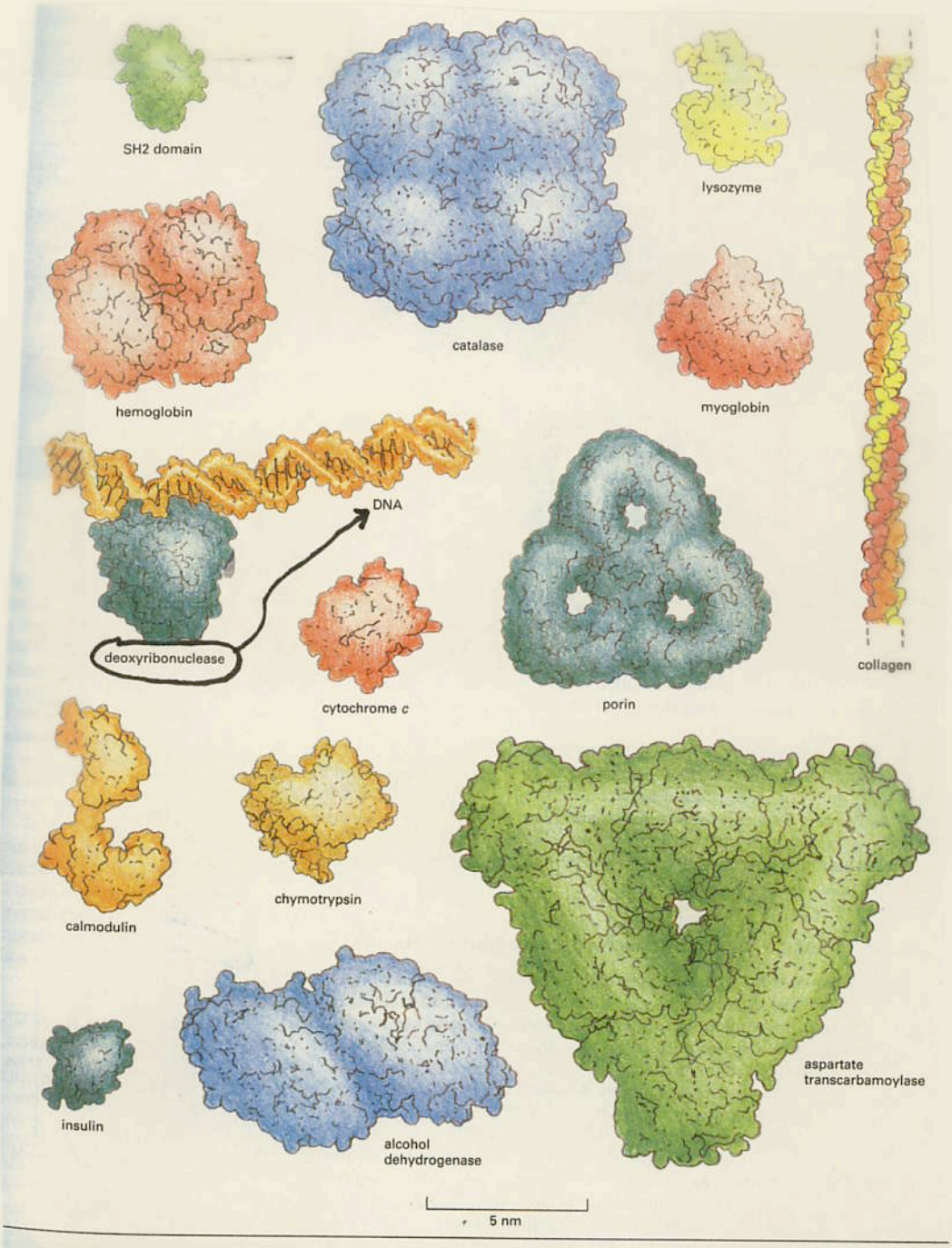
Figure 2.17 A portion of the primary structure of the β-globin polypeptide and its location in the folded, complete polypeptide. Also shown is the amino acid that is altered in the β-globin polypeptide in sickle-cell disease.

CHEMISTRY → BIOLOGY!

ORDER OF AMINO ACIDS → Specific Protein Shape & Function

SHAPE → FUNCTION → Phenotype (specific)

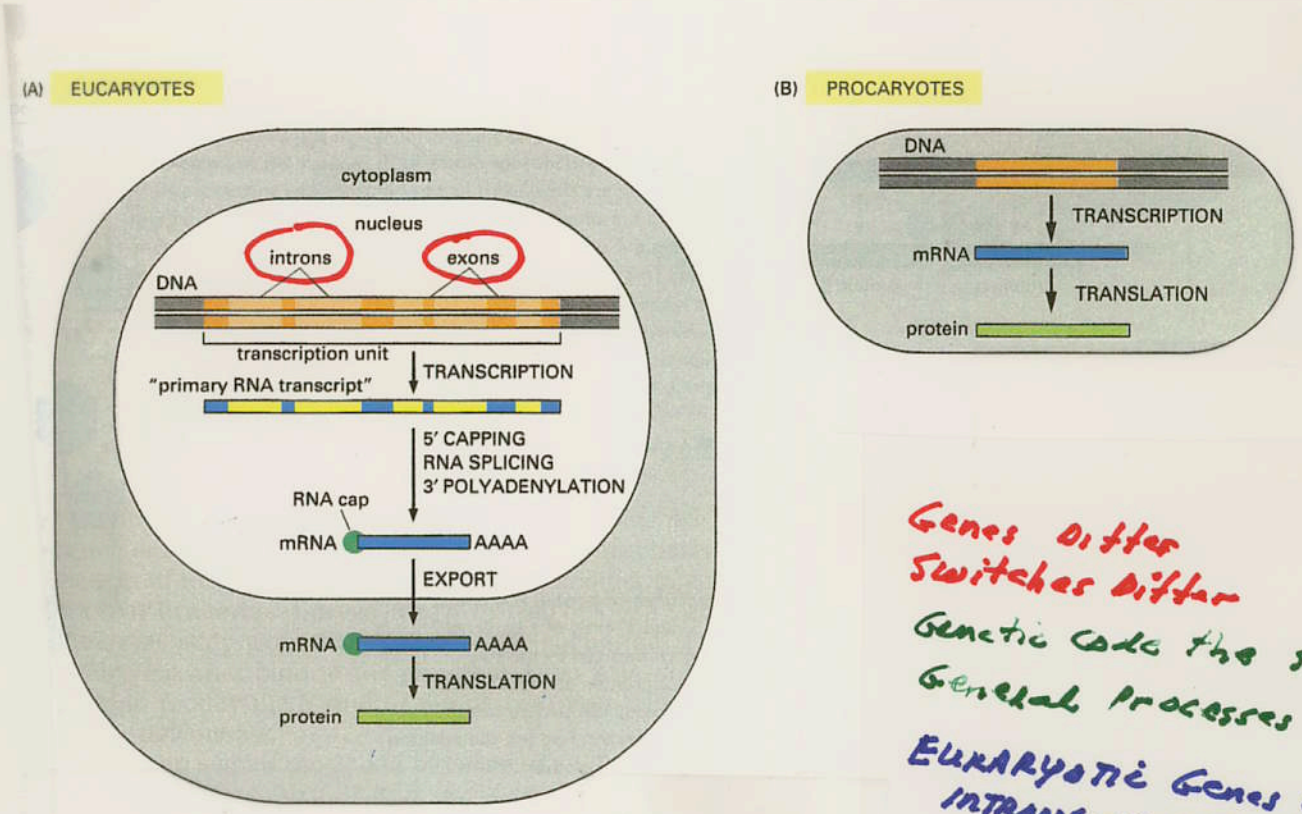
UNIQUE PROTEINS HAVE A UNIQUE COMPOSITION & ORDER OF AMINO ACIDS & HAVE UNIQUE SIZES, SHAPES, & FUNCTIONS



→ SPECIFIC TRAITS!

gure 3–24 A collection of protein molecules, shown at the same scale. For comparison, a DNA molecule bound to a protein is also istrated. These space-filling models represent a range of sizes and shapes. Hemoglobin, catalase, porin, alcohol dehydrogenase, and aspartate inscarbamoylase are formed from multiple copies of subunits. The SH2 domain *(top left)* is presented in detail in Panel 3–2 (pp. 138–139). (After vid S. Goodsell, Our Molecular Nature. New York: Springer-Verlag, 1996.)

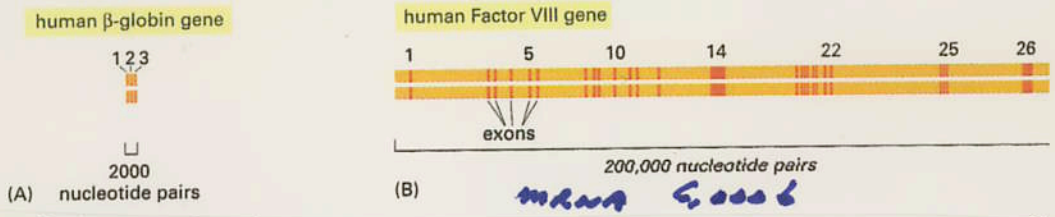
EUKARYOTIC AND PROKARYOTIC GENE EXPRESSION PROCESSES DIFFER SLIGHTLY



Genes differ
Switches differ
Genetic code the same
General processes same

EUKARYOTIC Genes HAVE INTRONS - NON-CODING Region in Gene!

GENES CAN VARY IN SIZE!



mRNA Codes

mRNA Codes

HUMAN Genes mostly introns!

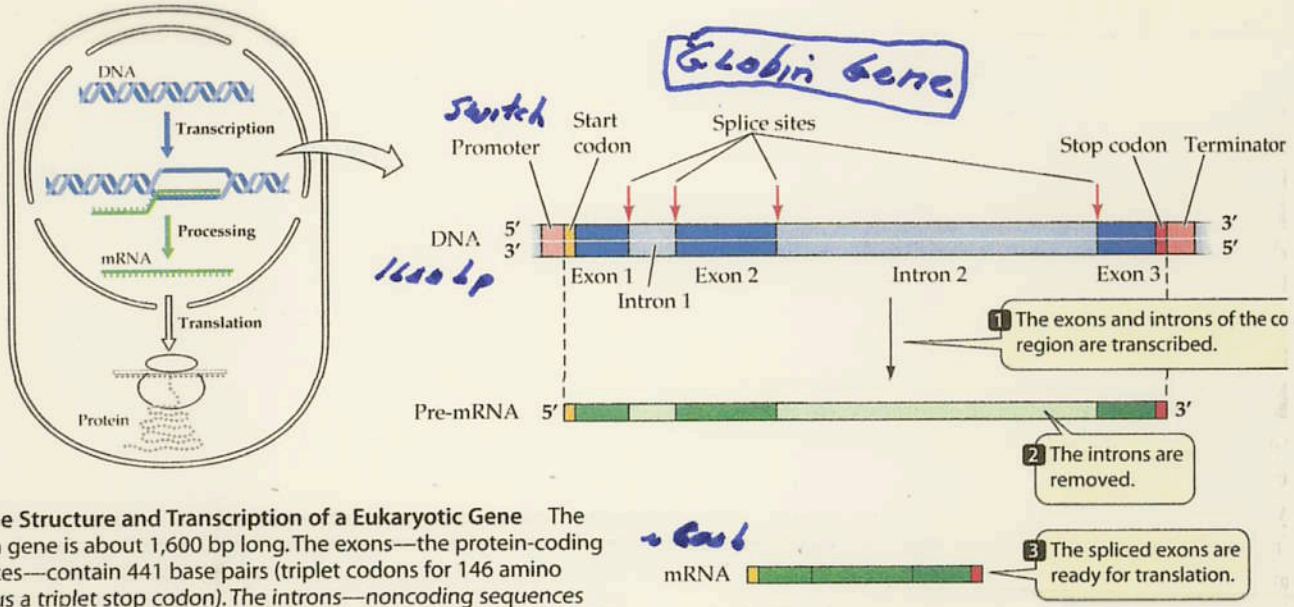
EUKARYOTIC CELLS MUST REMOVE NON-CODING REGIONS OF RNA BEFORE Genetic CODE CAN BE TRANSLATED Continuously!

The Modular Organization of Genes and Gene Function Implies That There is No Limit to How Genes Can be Functionally Rearranged and Recombined Using Genetic Engineering?

a. Yes
b. No

iClicker

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



14.4 The Structure and Transcription of a Eukaryotic Gene The β-globin gene is about 1,600 bp long. The exons—the protein-coding sequences—contain 441 base pairs (triplet codons for 146 amino acids plus a triplet stop codon). The introns—noncoding sequences of DNA—between codons 30 and 31 (130 bp long) and 104 and 105 (850 bp long), are initially transcribed, but are spliced out of the initial mRNA transcript.

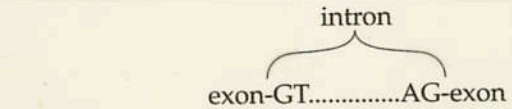
Mutations → Blood Disorders
Where can these occur?

MUTATIONS CAN OCCUR IN CODING REGIONS, SWITCH, & RNA SPLICE SITES

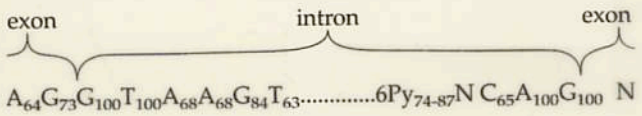
→ MUTANT PHENOTYPE

IMPLICATIONS FOR ENGINEERING EUKARYOTIC GENE IN BACTERIAL CELL FOR EXPRESSION?

Yo! It's in the Sequences!



The sequences shown here are for the DNA nontemplate strand (equivalent to the RNA transcript, but with T rather than U). In addition, there are short consensus sequences at the exon–intron junctions. For nuclear genes, the consensus junctions are

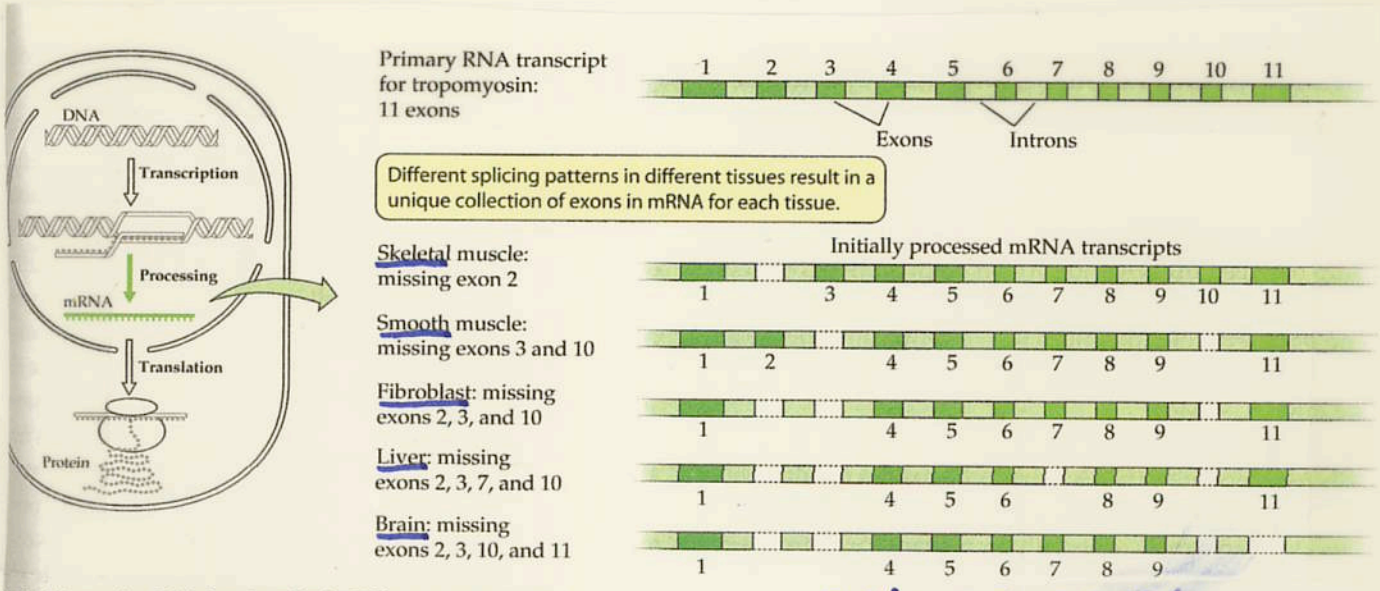


Specific Sequences required FOR RNA Splicing!

What happens if these Sequences Are Mutated in a Gene?

Alternative Splicing – One Gene ↳ Several RNAs & Proteins!

Gene active in variety of cells but ... !!!



4.20 Alternative Splicing Results in Different mRNAs and Proteins In mammals, the protein tropomyosin is encoded by a gene that has 11 exons. Tropomyosin pre-mRNA is spliced differently in different tissues, resulting in five different forms of the protein.

5 different mRNAs!!

different mRNAs = different proteins = different functions!

IMPLICATION – HUMAN Genome Has only 30,000 Genes but Can Give Rise to Many More Proteins which are Responsible for producing the Phenotype! ∴ human gene ∴ 3+ transcripts

∴ Reason why Human Genome can contain same # of genes as the Fly & Plant Genomes!

IMPLICATIONS for Genetic Engineering? Use Specific cDNA!

IMPLICATIONS FOR "YO-IT's 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 genetically!

ENGINEERING GENES REQUIRES:

1. The GENE & its DNA Sequence
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

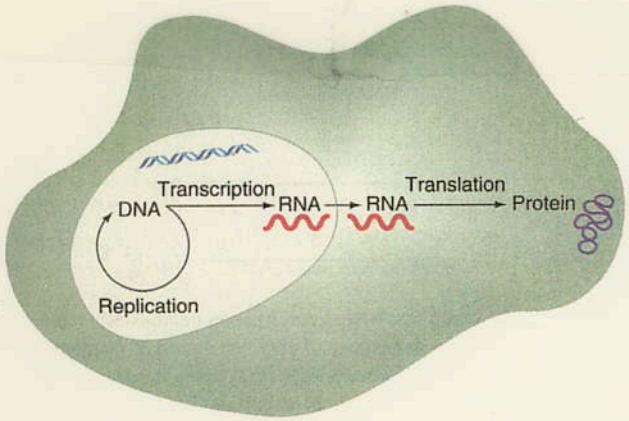


Figure 10-2 The three processes of information transfer: replication, transcription, and translation.

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

There's NO HOCUS POCUS all hypotheses are Testable!!

"Behind" ALL TRAITS!

SAME PROCESSES!