HC70A Winter 2004
Professor Bob Goldberg

Lecture #3 How Do Genes Work?

Themes / Concepts

1. Conservation of Gene Processes
2. DNA Replication (Hour class ORI Discussion)
3. PCR (Kary Mullis film)

Stop 1/20/04

4. Mutation & Human Disease Genes
5. Genes to Proteins / Colinearity of Sequences
6. Universality of Genetic Code
7. Transcription & Switches & RNAs
8. Translation & Ribosomes
9. Gene Activity in Prokaryotes Vs. Eukaryotes
10. What is Needed to Express a Gene in Foreign Cells?
11. Yo - it's all in the Sequences!
12. How to Detect Genes

Stop 1/22/04

Stop 1/27

w/hr Bacteria Experiment + Gel Electrophoresis Demo

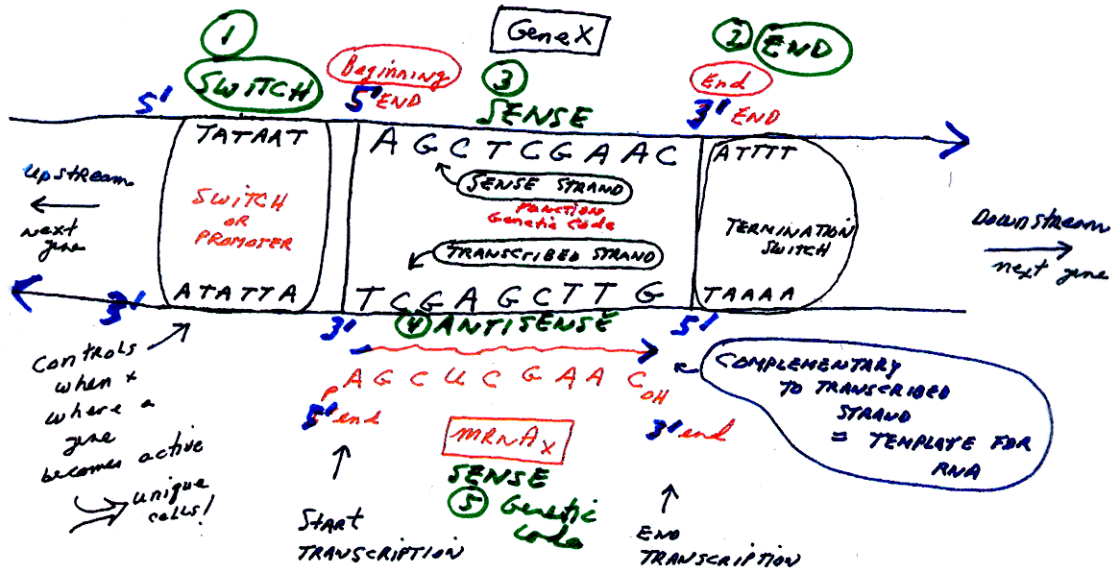
Announcements

1. Concept questions - website
2. Double-Helix Essay - Guidelines - Reading
3. Quiz - Grading Policy
4. Kyoto - who will go. No backing out.
5. Experiment - Lab Report - Lab Credit
6. Gel Electrophoresis - Demo

A Simple Gene – Revisited

A Simple Gene = A DOUBLE HELIX

ONLY ONE STRAND TRANSCRIBED



NOTICE! Specific Sequences Specify Beginning & End of Gene & CONTROL ITS ACTIVITY!

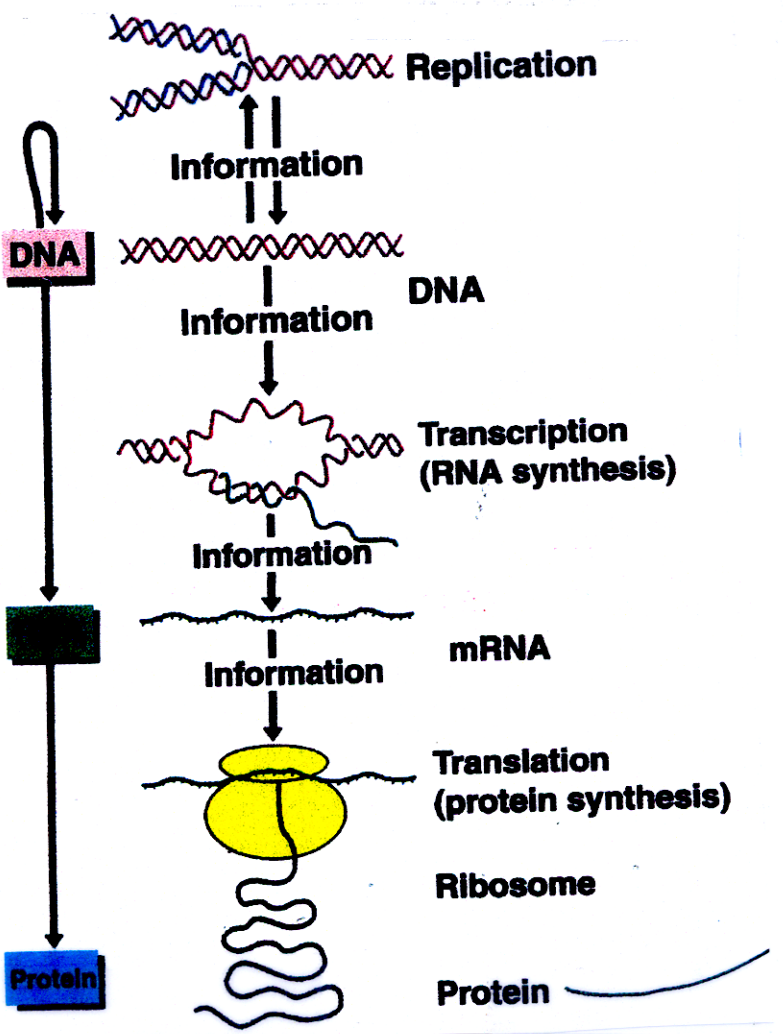
NOTICE: mRNA Sequence = SEQUENCE OF SENSE STRAND

1. Sense = Genetic code
2. Sense = 5'→3'
3. Anti-Sense TRANSCRIBED Complement of Sense 3'→5'
4. mRNA = 5'→3' SAME Sequence as Sense complementary to Anti-Sense
5. Switch TRANS gene on – Directs STRAND for TRANSCRIPTION

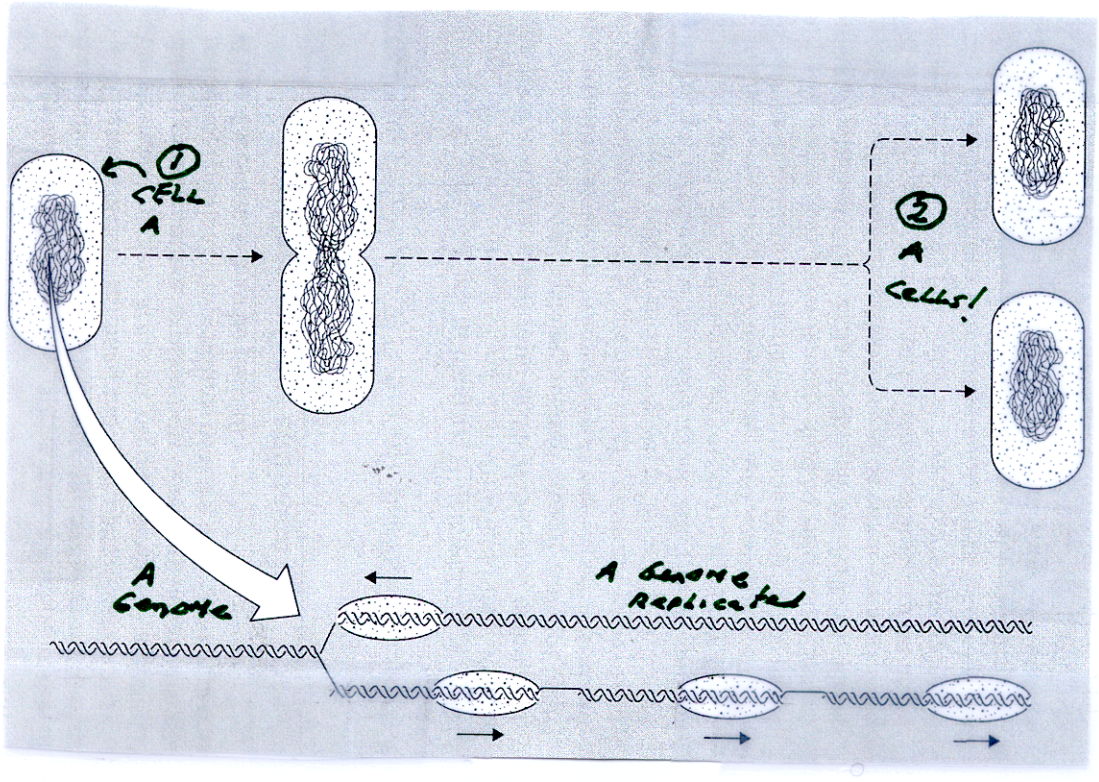
Everything Follows double-helix rules – Anti-parallel chains (RNA + DNA) – Complementary Base Pairing!

∴ Sequence in Gene → Sequence in mRNA!

HOW DO GENES WORK?

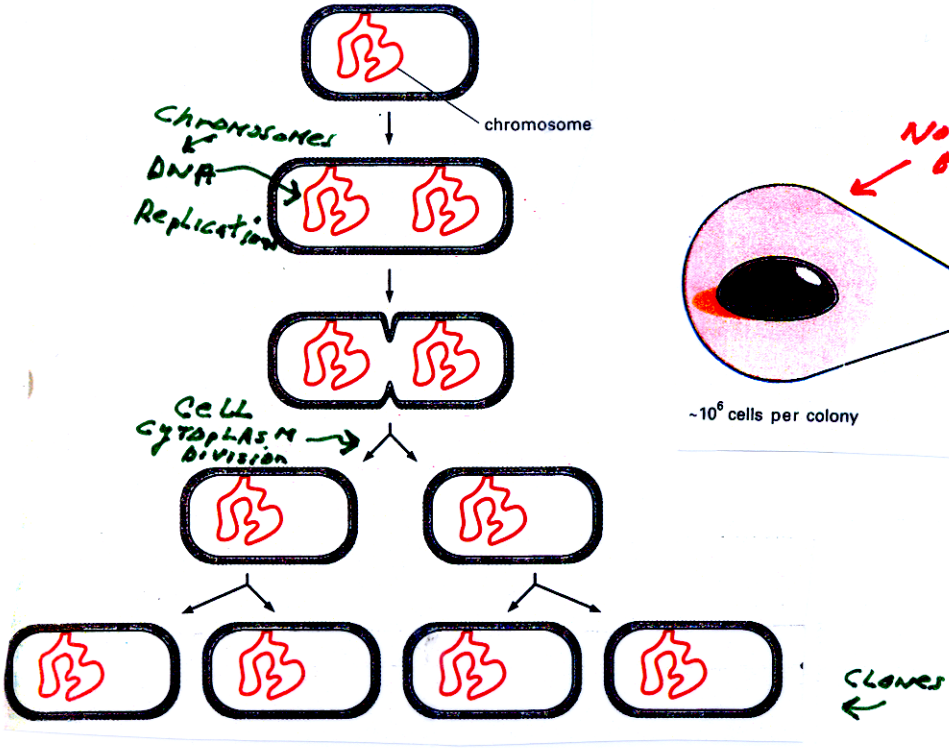


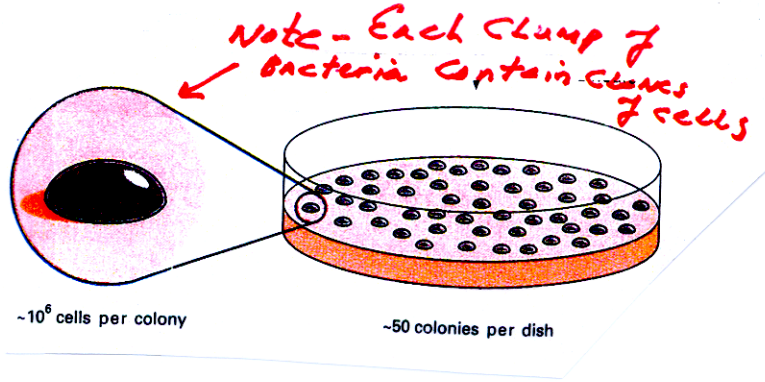
HOW ARE GENES REPLICATED EACH CELL GENERATION?



HOW IS THE DNA SEQUENCE COPIED / REPLICATED EACH CELL DIVISION?

GENES ARE REPLICATED DURING EACH CELL DIVISION





A BACTERIAL COLONY CONTAINS MANY COPIES OF SAME CELL OR CLONES WHICH ARE GENETICALLY IDENTICAL!

EACH DAUGHTER CELL CONTAINS THE SAME COLLECTION OF GENES

MAJOR PROPERTIES OF GENETIC MATERIAL

Replication & Stability

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

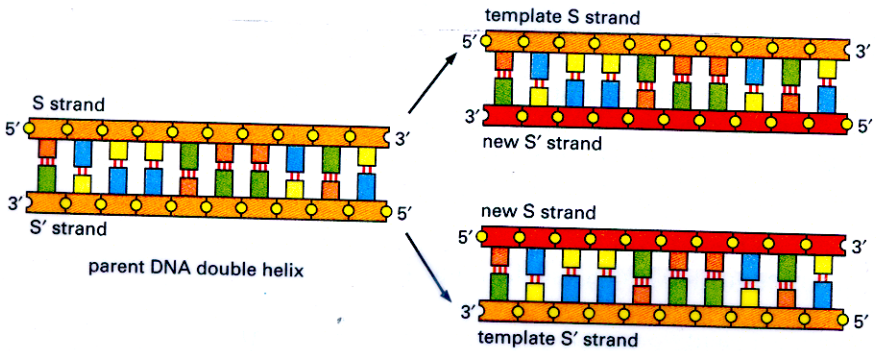


Figure 5–2 The DNA double helix acts as a template for its own duplication. Because the nucleotide A will successfully pair only with T, and G only with C, each strand of DNA can serve as a template to specify the sequence of nucleotides in its complementary strand by DNA base-pairing. In this way, a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

HOW DOES THAT OCCUR?
PROPERTY OF THE DNA MOLECULE

DNA REPLICATION OCCURS SEMI-CONSERVATIVELY

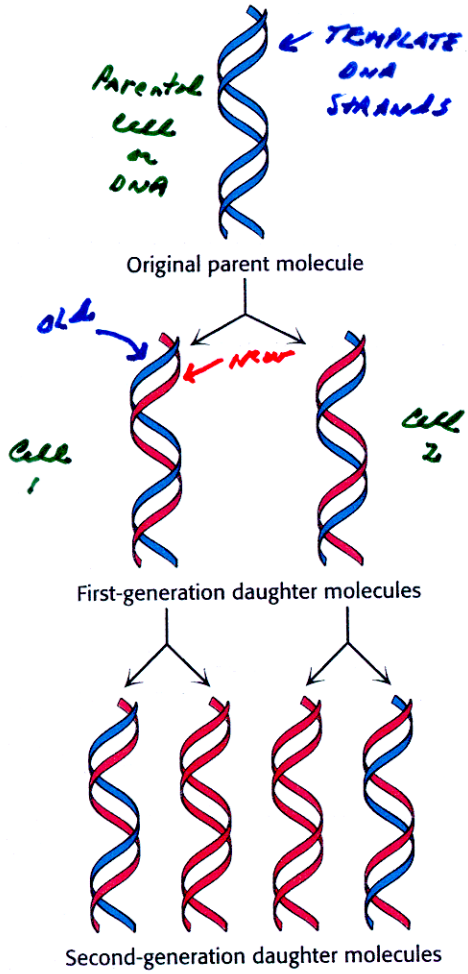


FIGURE 5.16 Diagram of semiconservative replication.

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!

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

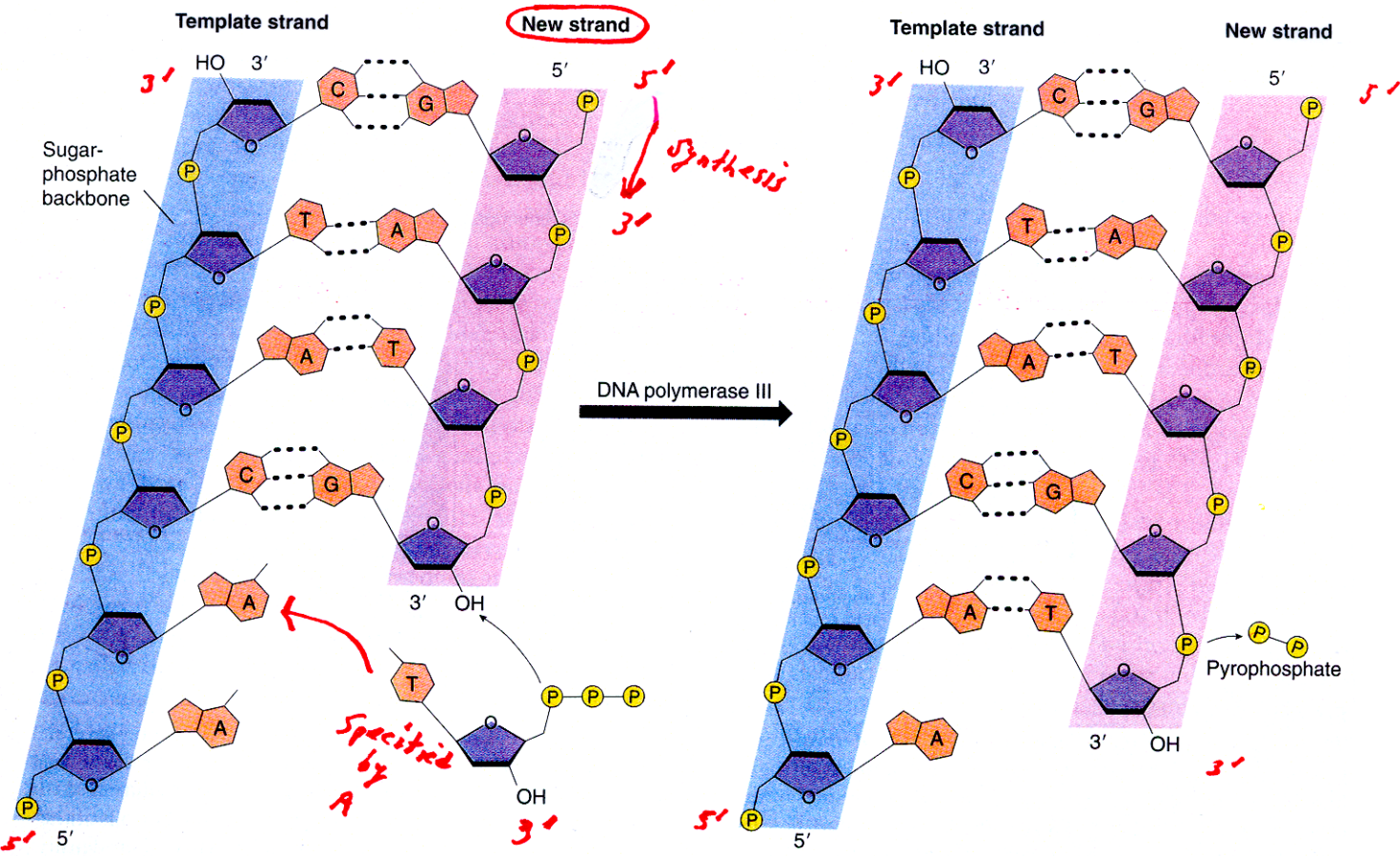
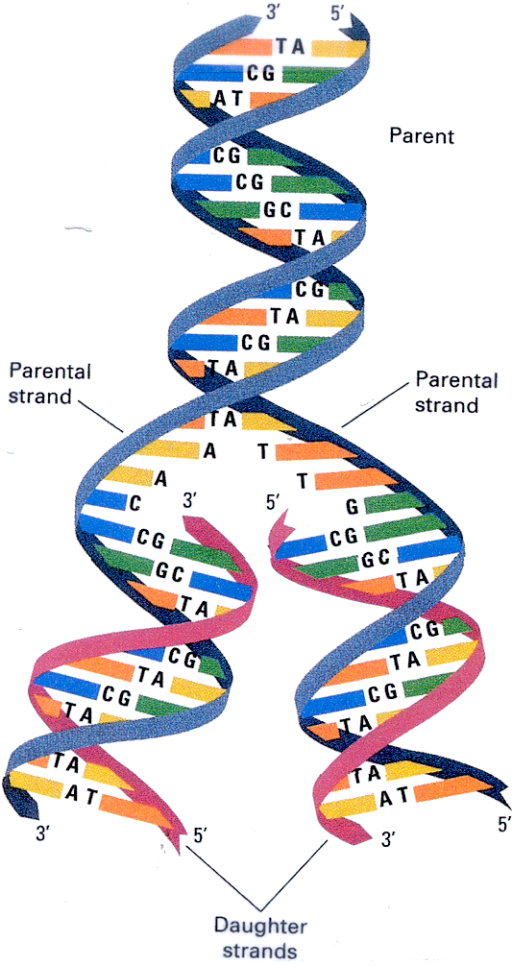


FIGURE 14.14
How nucleotides are added in DNA replication. DNA polymerase III, along with other enzymes, catalyzes the addition of nucleotides to the growing complementary strand of DNA. When a nucleotide is added, two of its phosphates are lost as pyrophosphate.

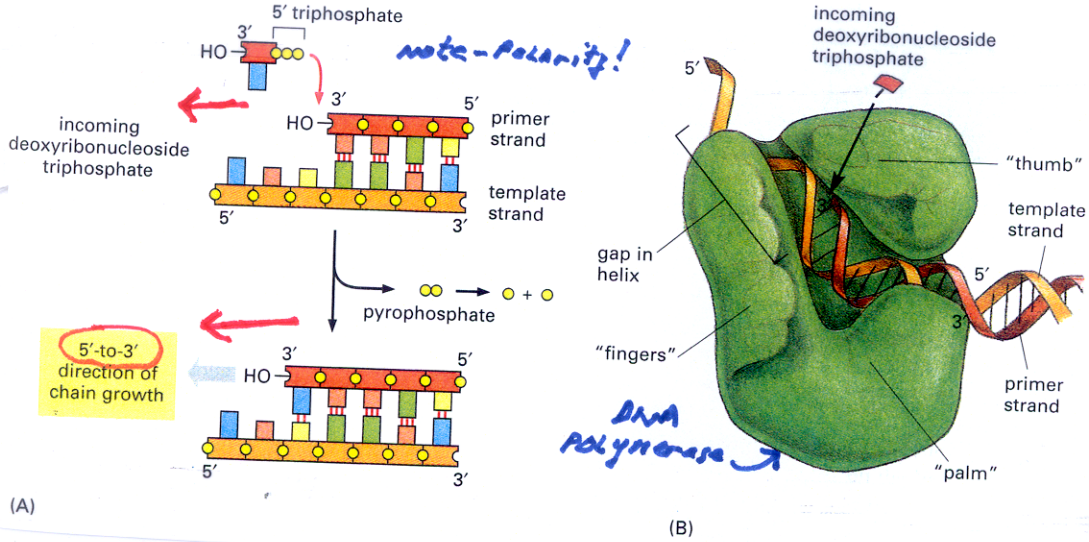
SEQUENCE IS SPECIFIED BY COMPLEMENTARY BASES

THE DNA SEQUENCE IS MAINTAINED GENERATION TO GENERATION



THE DNA SEQUENCE "LIVES" FOREVER!

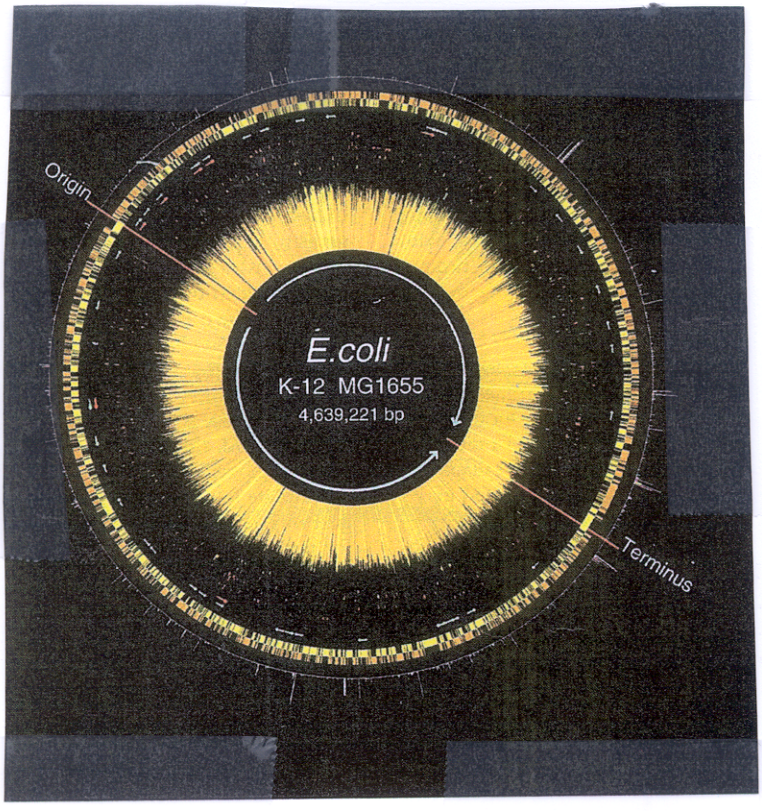
DNA REPLICATION REQUIRES AN ENZYME DNA POLYMERASE



DNA POLYMERASE CATALYZES PHOSPHODIESTER BONDS AND "COPIES" THE TEMPLATE

NUCLEOTIDES ARE ALSO NEEDED

DNA Replication Starts at the ORIGIN OF REPLICATION



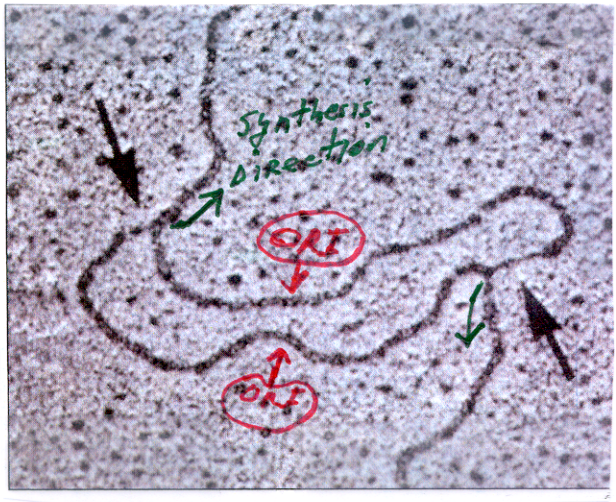
Key: tRNA genes; rRNA genes; origin and terminus of replication

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

DNA in The Process of Being Replicated



Replication Moves Bidirectionally FROM ORIGIN

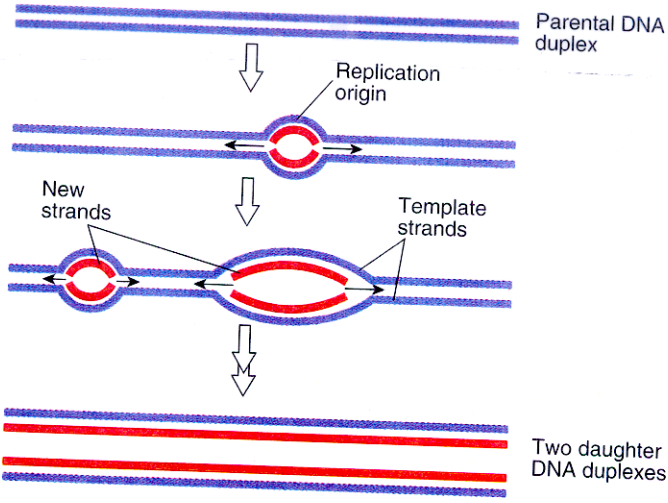


FIGURE 14.13
Origins of replication. At a site called the replication origin, the DNA duplex opens to create two separate strands, each of which can be used as a template for a new strand. Eukaryotic DNA has multiple origins of replication.

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

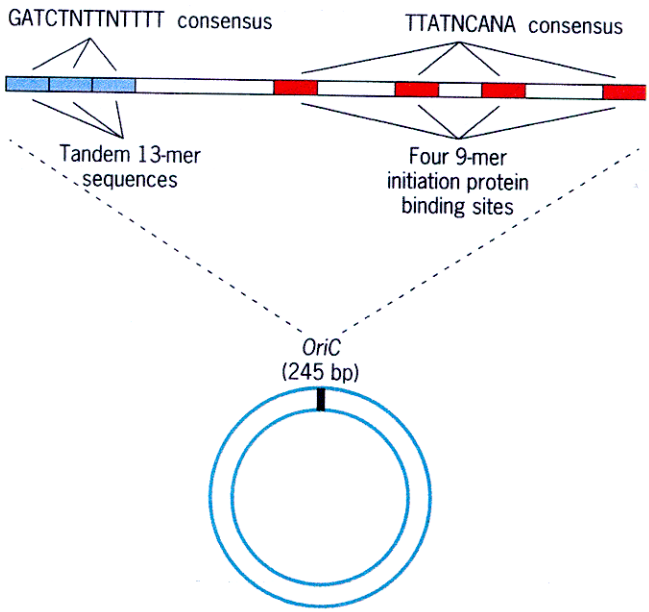
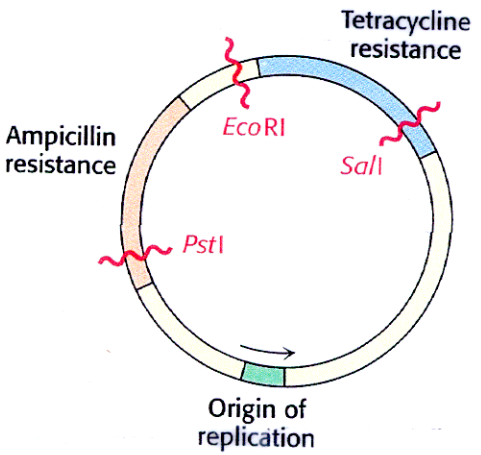


Figure 11.6 Structure of *OriC*, the single origin of replication in the *E. coli* chromosome.

What is the significance for Genetic Engineering?

CAN Replicating "Chromosomes" Be Made?

VECTORS ARE NEEDED TO REPLICATE GENES IN SPECIFIC CELLS



Plasmid pBR322

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!

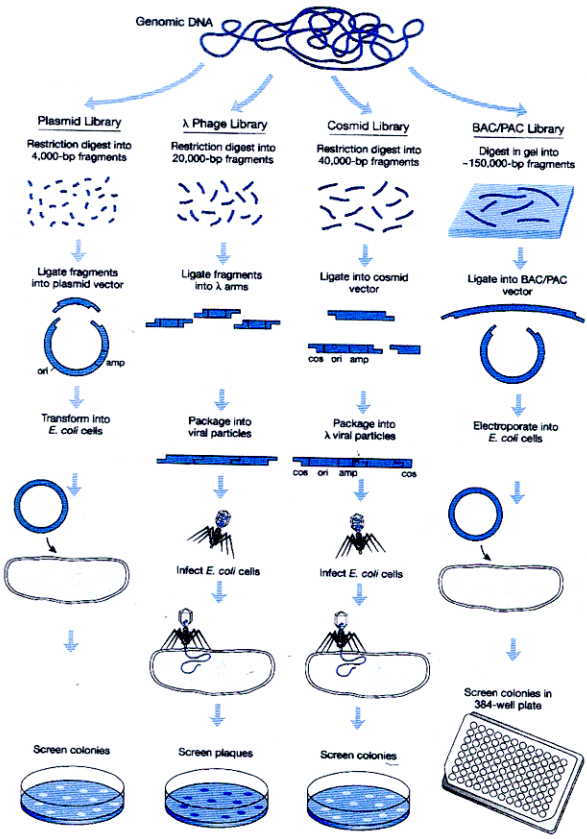
Need bacterial ori to clone human gene in bacteria. Need human ori to replicate a bacterial gene in human cell.

Yo! It's in the sequence = function

∴

Vectors can be engineered!

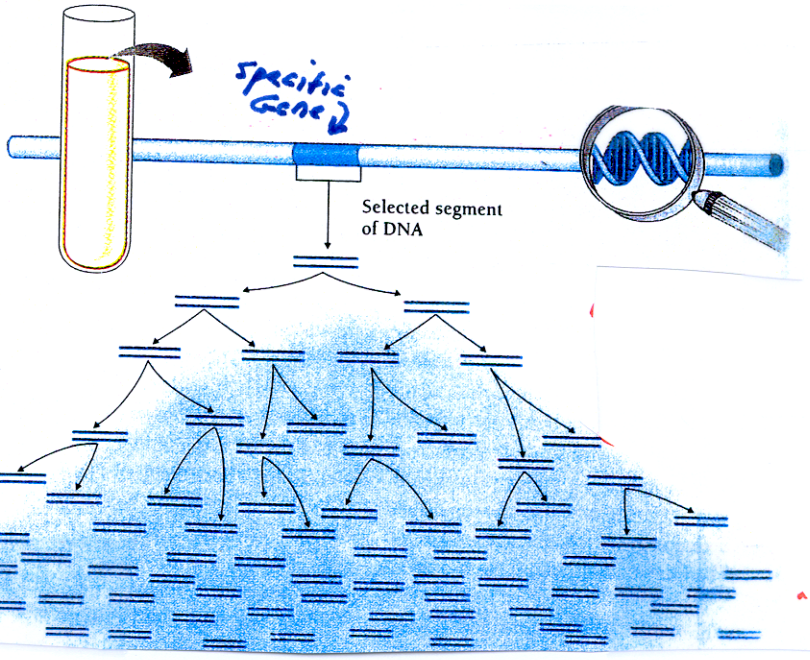
There ARE MANY Types of Vectors
ALL REQUIRE AN ORI



Making a Genomic Library

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

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!

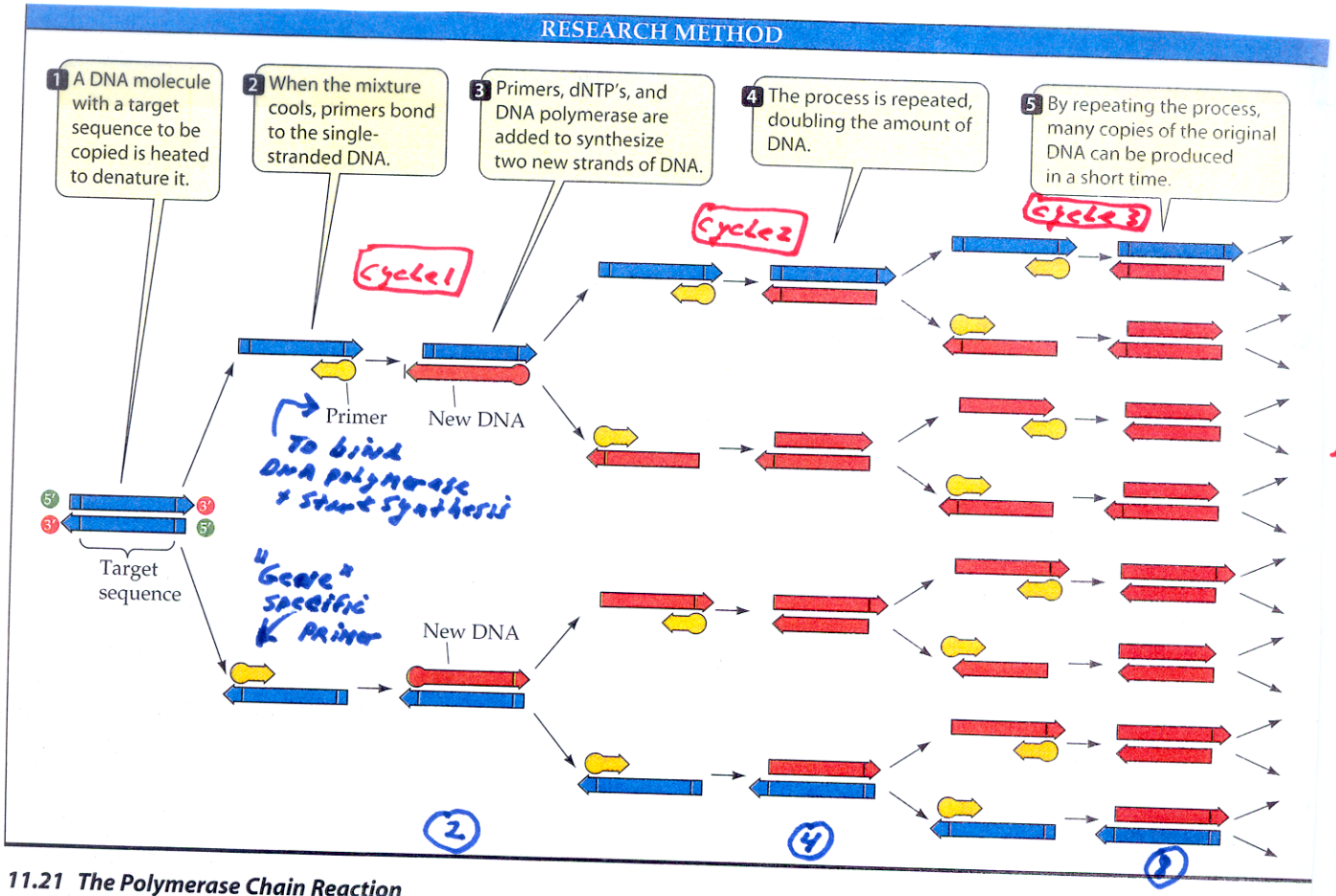
2. Study DNA from Limited Sources! a single hair, an ancient insect/plant, a bone fragment, cheek cell

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!

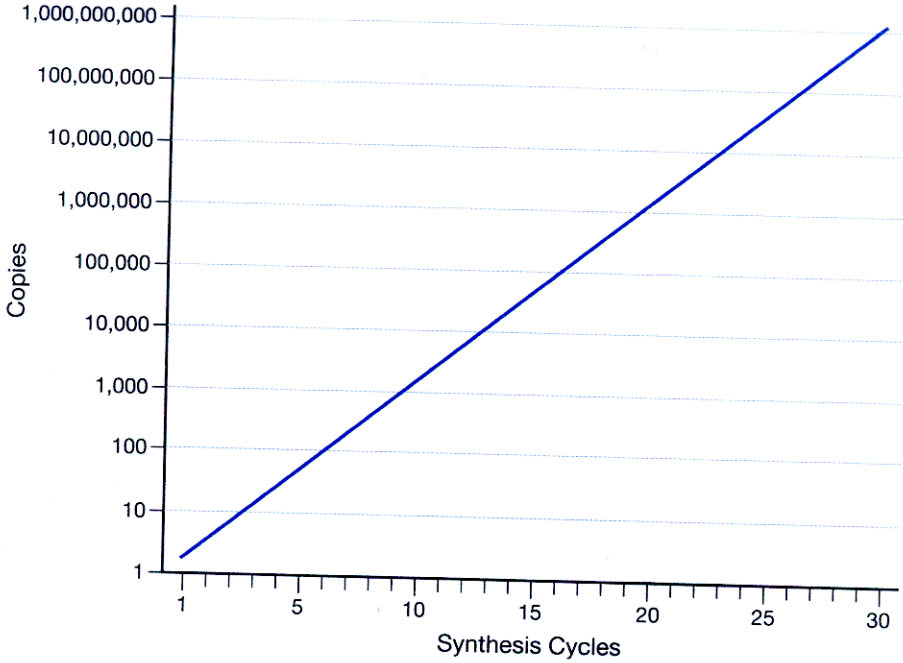
PCR is a cyclic process of DNA replication



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^{∞} molecules of DNA where ∞ = # cycles

MAKING LARGE AMOUNTS OF DNA USING PCR



Polymerase Chain Reaction Theoretical Amplification

PCR Requirements

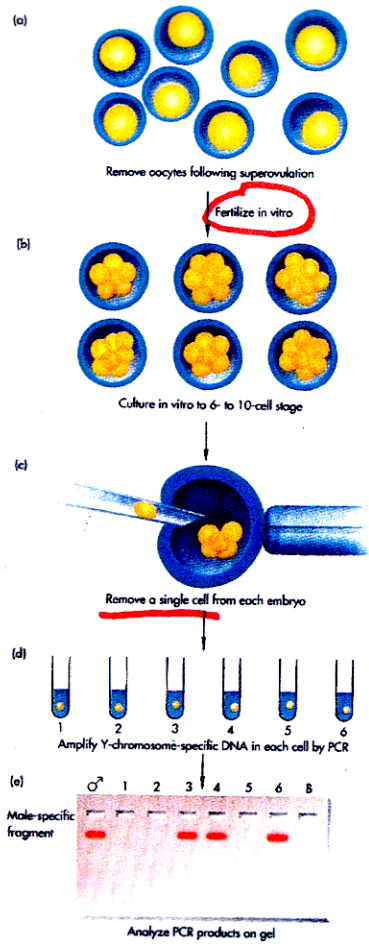
1. Knowledge of DNA Sequence :: Must clone DNA the "old fashioned way" first
2. DNA Polymerase - Heat Stable to ~100°C!! (where isolated?)
3. Thermo programmer/Cycler to Heat + Cool DNA in cycles - Separate Strands + Allow new strands to form
4. Primers - Recognize specific DNA Sequences + Initiate DNA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

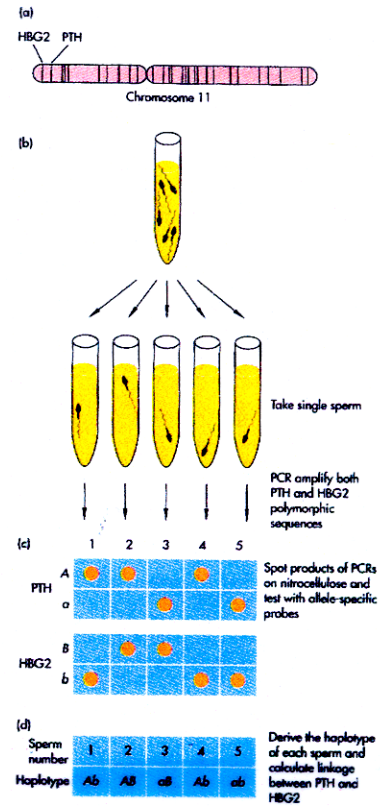
It's ALL in the DNA Sequences
Know Sequence - can "make" an infinite Amount of DNA Sequence!

In 1 hour can do what took months before PCR!

A PCR Machine!

PCR CAN BE USED TO ANALYZE GENES IN A single Human Embryo cell or sperm!





SEX determination in 8-cell EMBRYOS!

Genotypes of specific sperm!

What Are the implications of this procedure considering that Human Genome Has Been Sequenced?

A STEVEN SPIELBERG FILM

An Adventure
65 Million Years In The Making.

19a

USING PCR TO DETECT GENES IN ANCIENT DNA

Ancient DNA Milestones

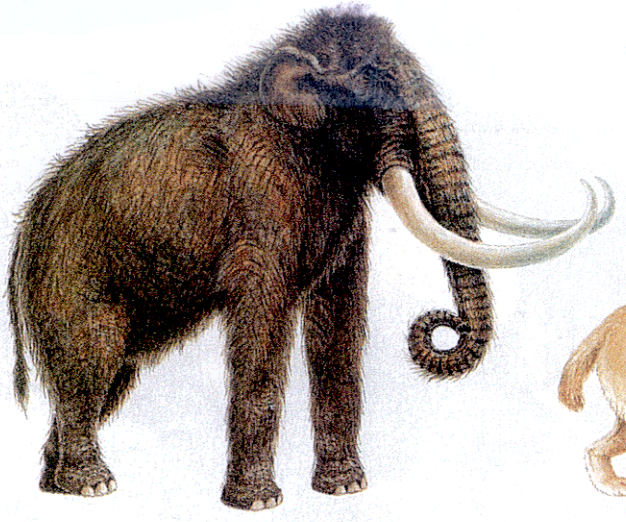
These extinct organisms have yielded meaningful genetic sequences.

AMBER INSECTS

40 MILLION YEARS OLD

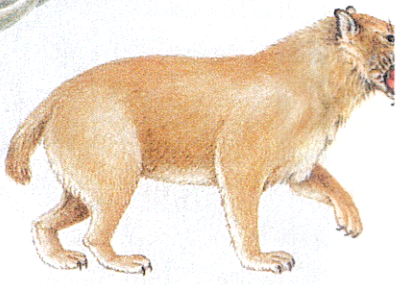
FOSSIL LEAVES

17 MILLION



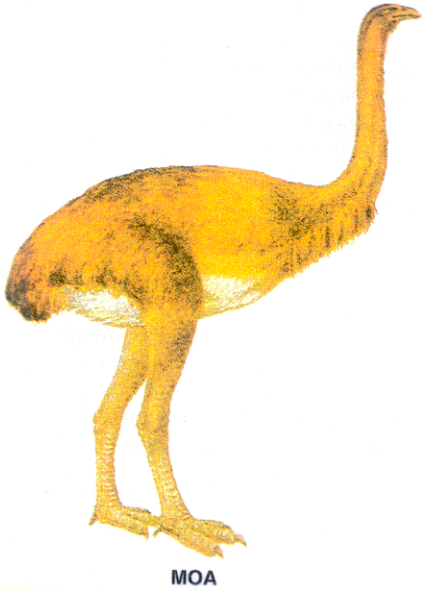
MAMMOTH

40,000



SMILODON

13,000



MOA

4,300

QUAGGA

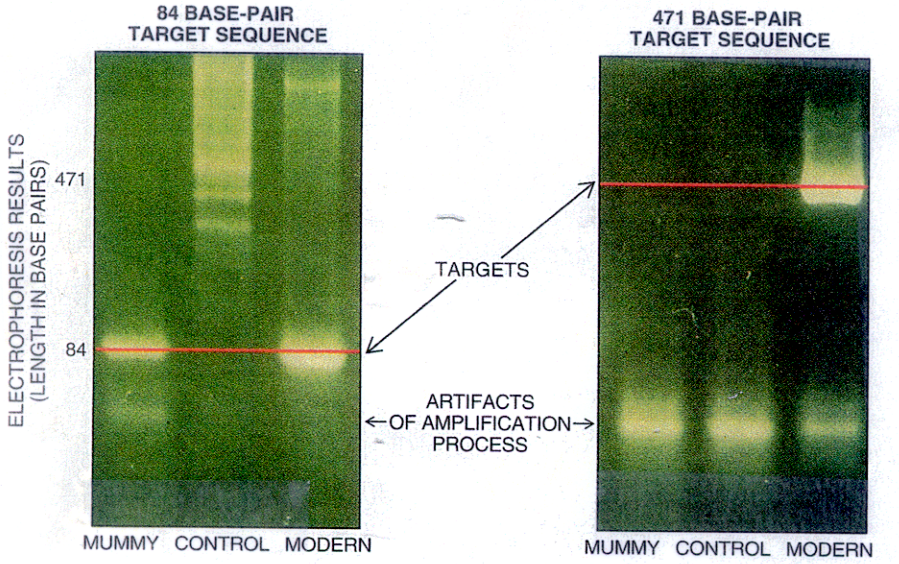
140

THYLACINE

80

PRESENT

USING PCR TO DETECT GENES IN MUMMY DNA



USING PCR in CRIME SCENES

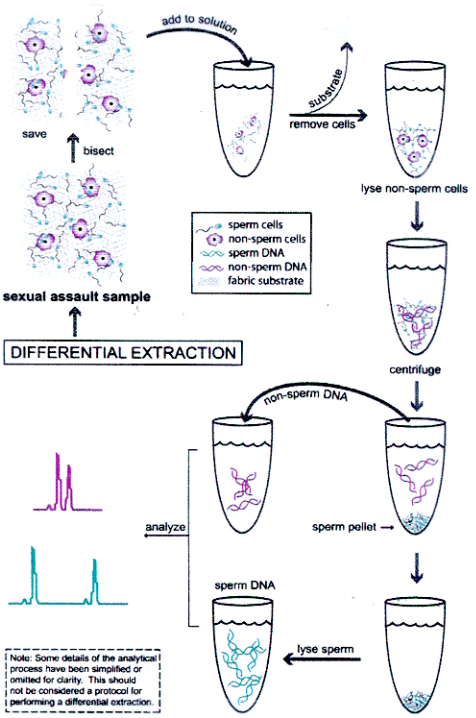
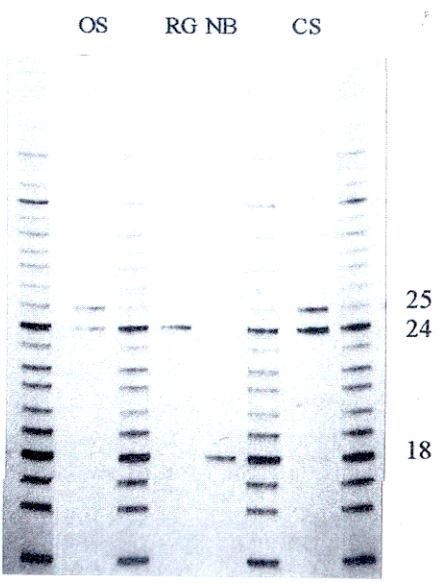


Plate 4 Differential extraction.



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

DNA REPLICATION IS PRECISE BUT MISTAKES OCCUR!

Gene A

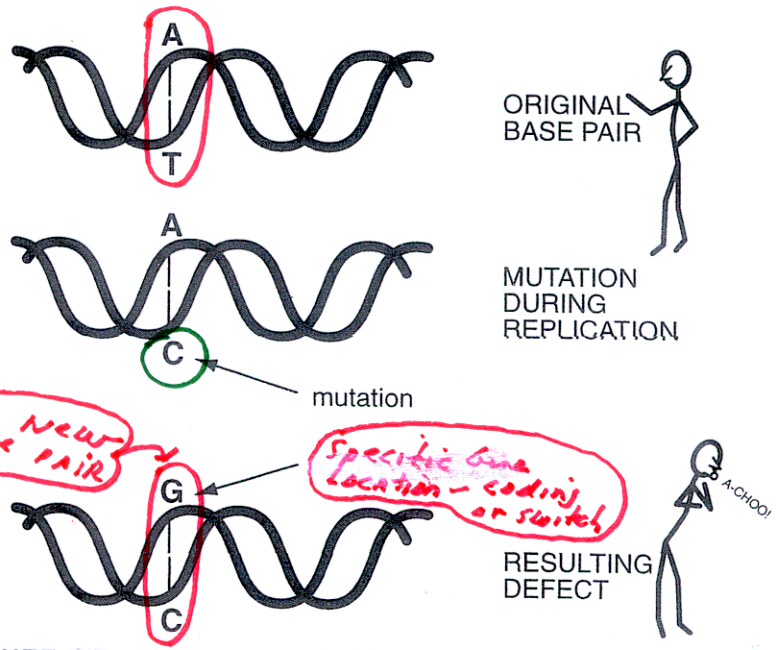


FIGURE 1.3 A mutated gene makes a defective protein that leads to an illness in an individual.

Gene A' VARIANT OF SAME Gene!

SEE MUTATION AS change in Phenotype

CHANGE IN DNA SEQUENCE
↳ CHANGE IN PROTEIN ∴ FUNCTION