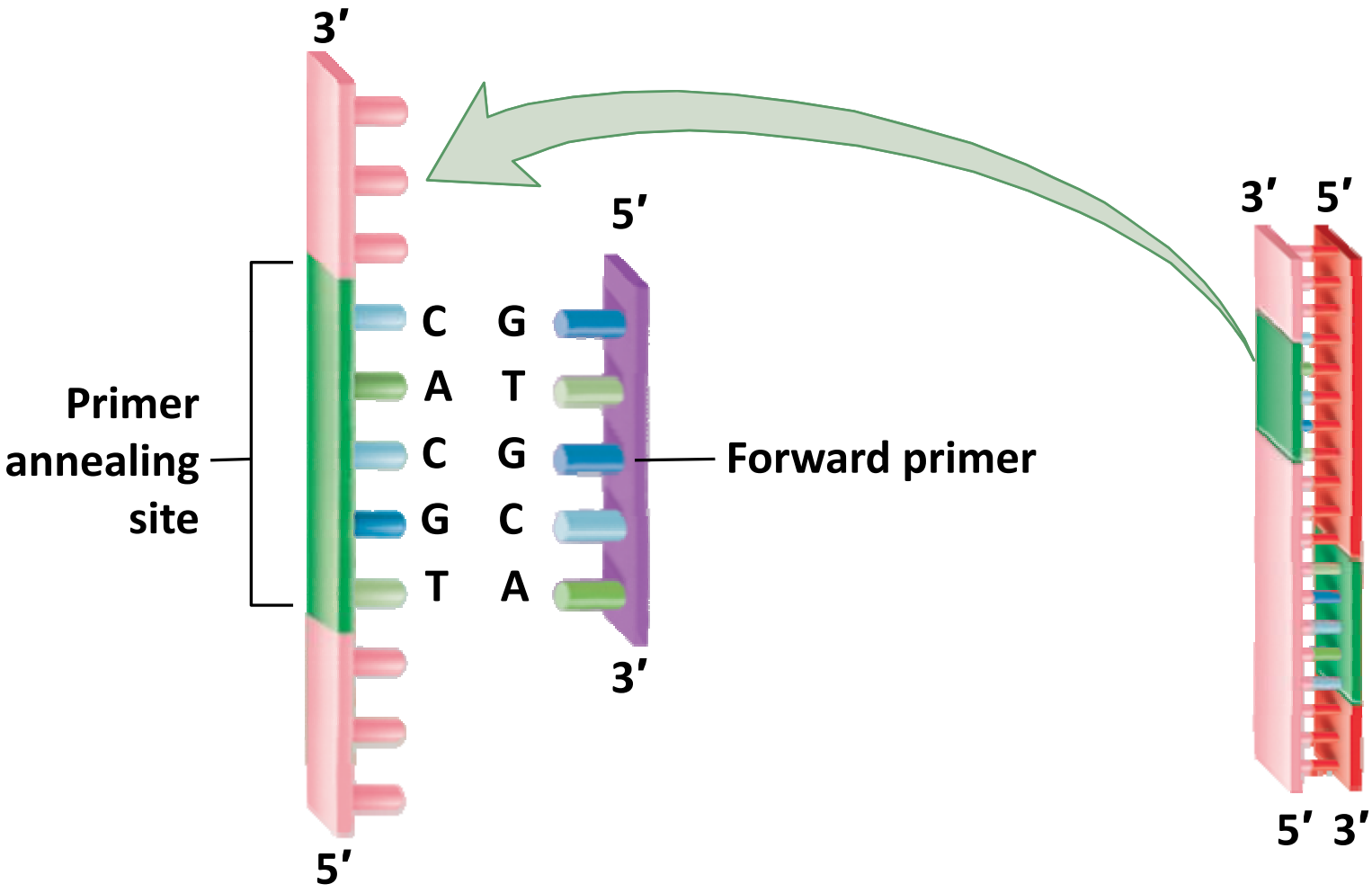
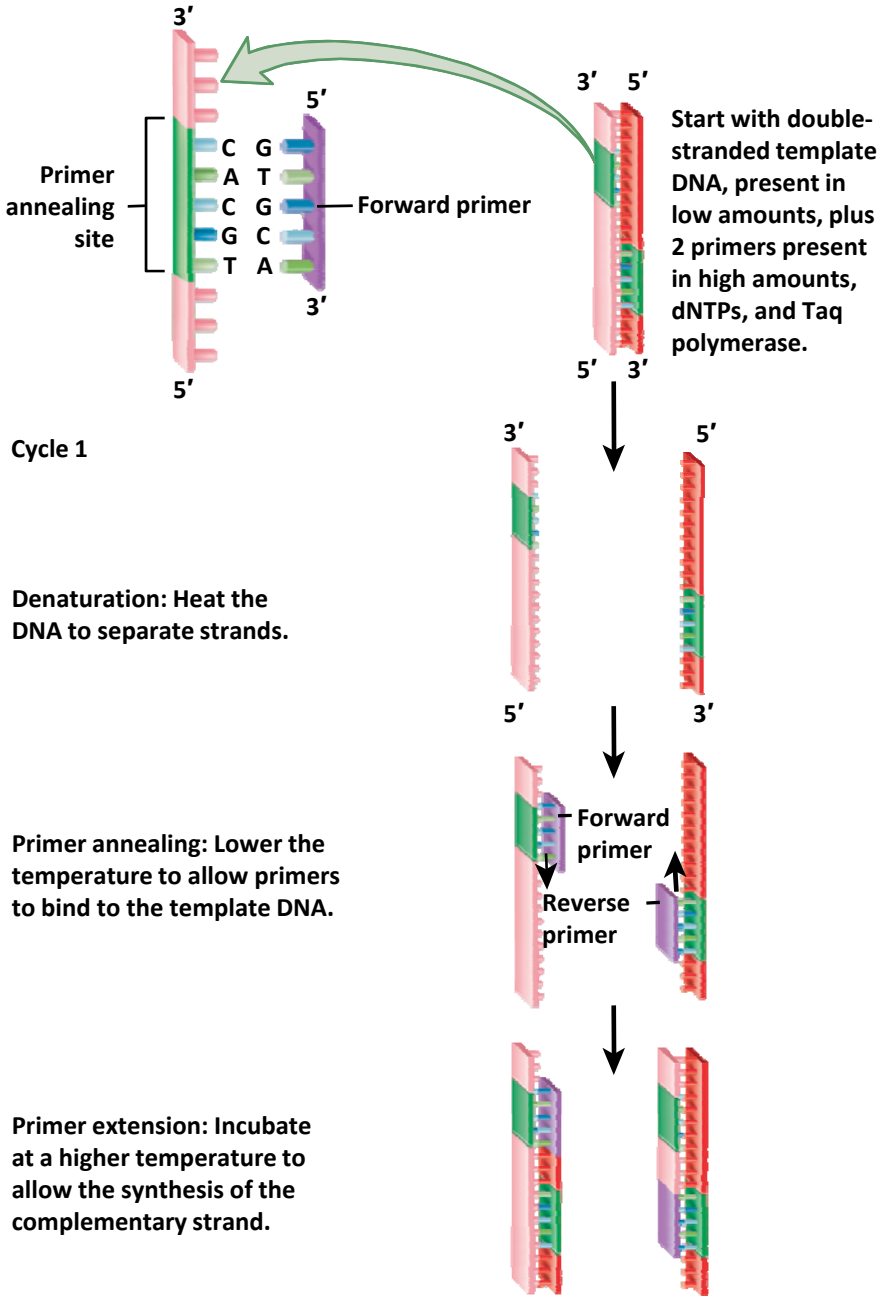
# PCR reaction

Chapter 20

# Polymerase chain reaction (PCR)

- Copy DNA without vectors and host cells
- Goal to make many copies of DNA in a defined region
- Uses high concentration of two primers that are complementary to sequences at the ends of the DNA region to be amplified, deoxynucleoside triphosphates (dNTPs), and a heat-stable form of DNA polymerase called *Taq* polymerase
- Sample of DNA taken through repeated cycles of denaturation, annealing and synthesis
  - Thermocycler automates this process
- After 30 cycles of amplification, a DNA sample will increase $2^{30}$-fold





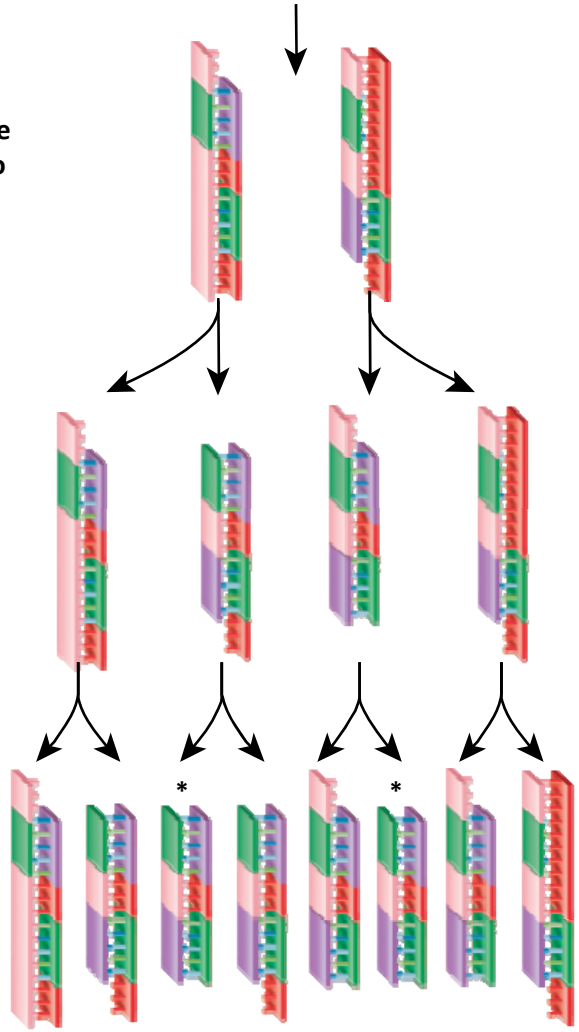
Primer extension: Incubate at a higher temperature to allow the synthesis of the complementary strand.

Cycle 2

Repeat denaturation, primer annealing, and primer extension.

Cycle 3

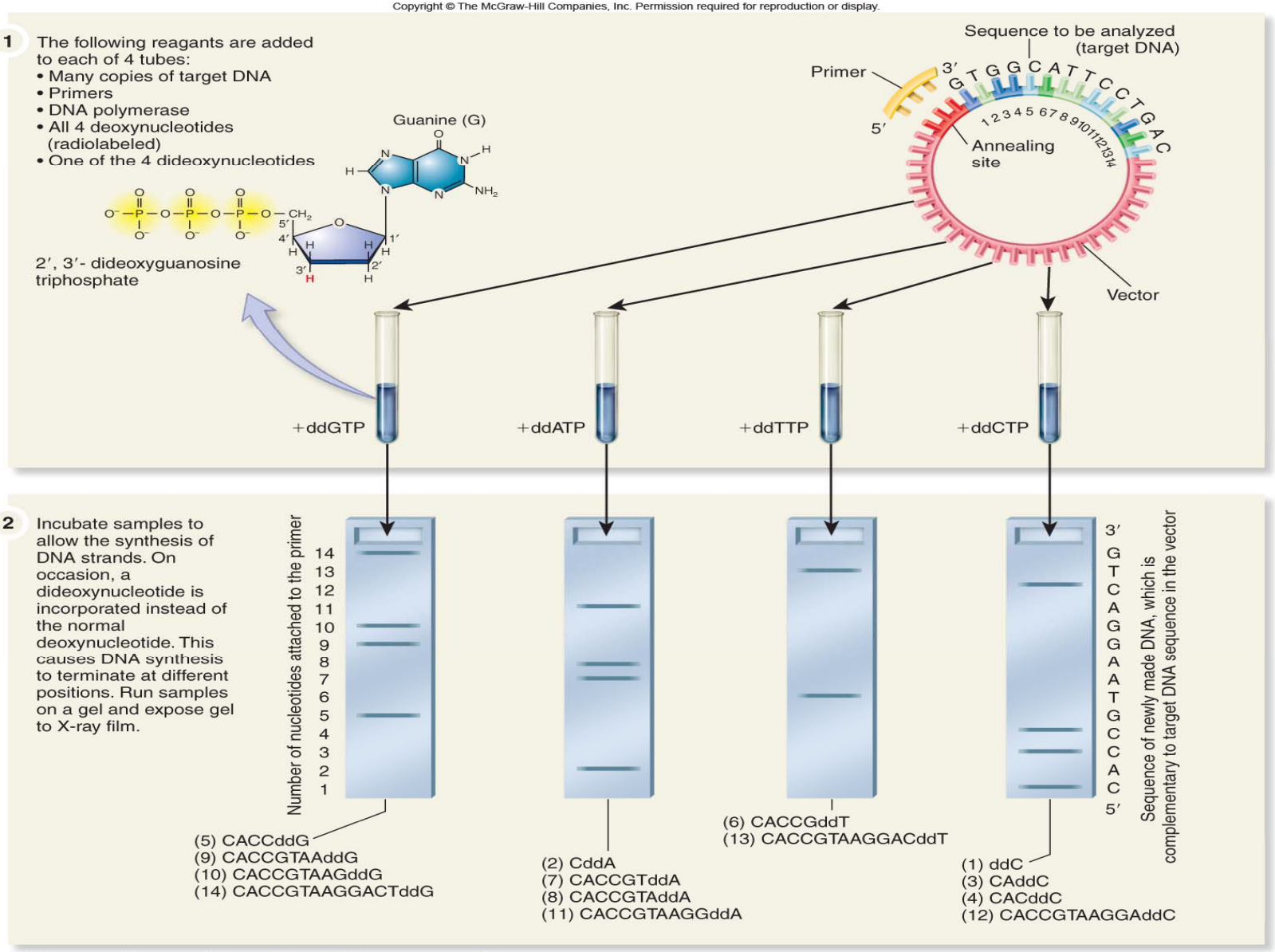
Repeat denaturation, primer annealing, and primer extension.



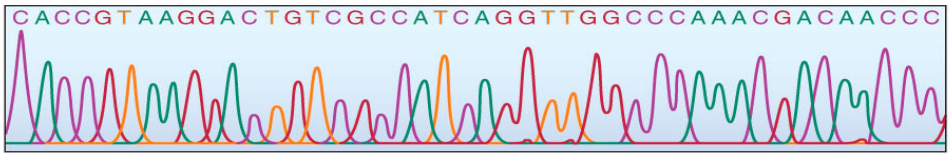

With each successive cycle, the relative amount of DNA fragments that end exactly at both primer sites (marked *) increases. Therefore, after many cycles, the vast majority of DNA fragments contain only the region that is flanked by the 2 primer sites.

# DNA sequencing

- Determines base sequence of DNA
- Dideoxy chain-termination method or dideoxy sequencing
  - Dideoxynucleoside triphosphates (ddNTPs) are missing the 3' –OH group and will terminate the chain
  - 4 tubes with many copies of single stranded DNA of interest
    - Each tube has a different radiolabelled dNTP
  - DNA polymerase will make complementary strand until dNTP inserted and chain terminates
  - After electrophoresis, DNA sequence can be read by reading which base is at the end of the DNA strand
- Procedure has been automated using fluorescent dyes in one tube



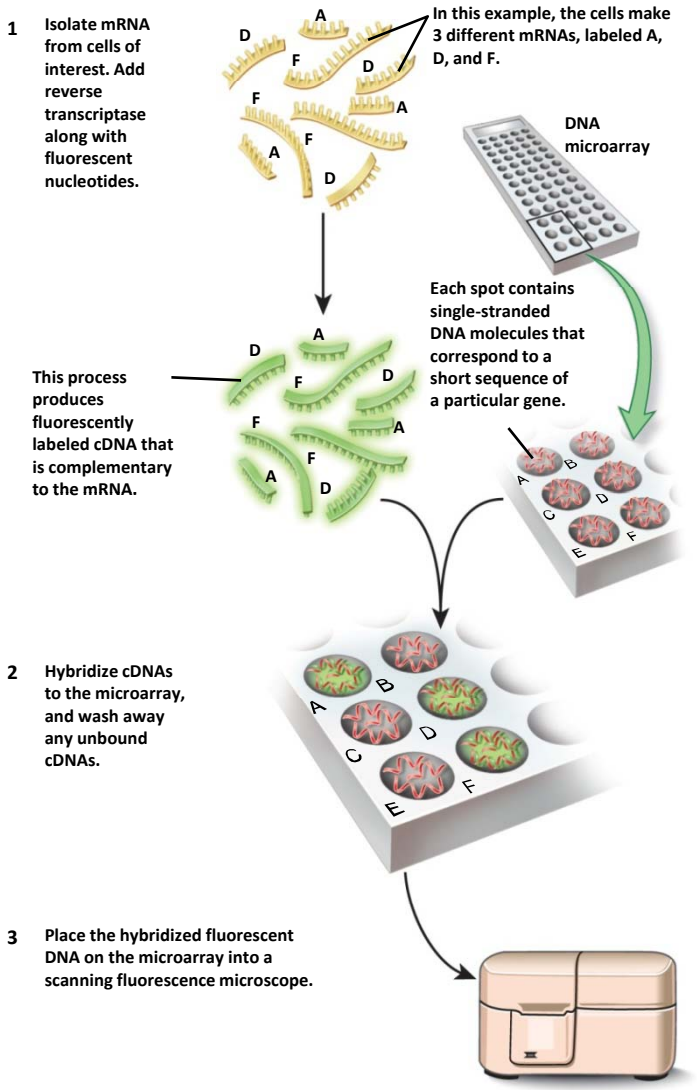

**(a) The procedure used in traditional dideoxy sequencing**




**(b) Output from automated dideoxy sequencing**

# A Microarray Can Identify Which Genes Are Transcribed by a Cell

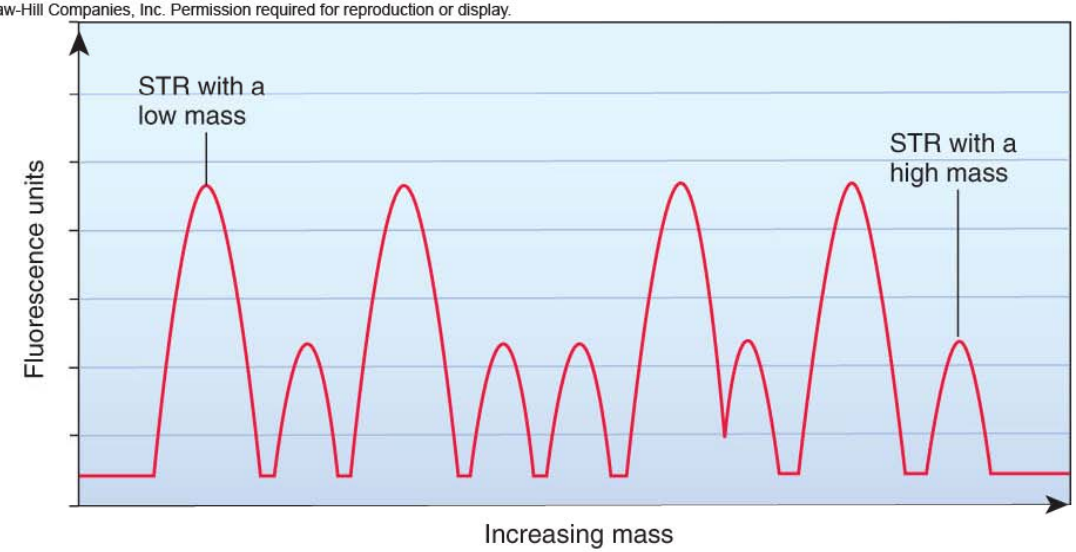
- DNA microarray or gene chip
- Used to monitor the expression of thousands of genes simultaneously
- Short sequences of known genes attached to spots on slide
- Goal to find out which genes are transcribed into mRNA in particular sample of cells
- mRNA isolated from those cells and used to make fluorescently labeled cDNA
- cDNAs that are complementary to the DNAs in the microarray will hybridize
- If the fluorescence intensity in a spot is high, a large amount of cDNA was in the sample that hybridized to the DNA at this location


1
Isolate mRNA from cells of interest. Add reverse transcriptase along with fluorescent nucleotides.
In this example, the cells make 3 different mRNAs, labeled A, D, and F.
DNA microarray
Each spot contains single-stranded DNA molecules that correspond to a short sequence of a particular gene.
This process produces fluorescently labeled cDNA that is complementary to the mRNA.
2
Hybridize cDNAs to the microarray, and wash away any unbound cDNAs.
3
Place the hybridized fluorescent DNA on the microarray into a scanning fluorescence microscope.
4
A computer generates an image that indicates the relative fluorescence intensity of each spot. In this case, spots A, D, and F are highly fluorescent.
A
B
C
D
E
F

# DNA fingerprinting

- Identifies and distinguishes among individuals based on variations in their DNA
- Chromosomal DNA produces series of bands on a gel
- Unique pattern of bands used
- Automated using PCR to amplify short tandem repeat sequences (STRs) aka microsatelite DNA
  - Such tandem repeat sequences are found at specific locations in the genomes of all species, and the number of repeats at each spot tends to vary from one individual to the next


(a) Traditional DNA fingerprinting




(b) Automated DNA fingerprinting

a: Leonard Lessin/Peter Arnold, Inc.

- Uses
  - Identify different species of bacteria and fungi
  - Forensics – 1986 first use in US court system
  - Paternity testing and other family relationships