# Microbiology

# Chapter 10

# Classification of Microorganisms

本內容已由授課教師方翠筠修訂

# The Study of Phylogenetic (種系發展的) Relationships

## Taxonomy

- The science of classifying organisms
- Taxon (經分類而成的同類 ,單數); Taxa (---,複數)
- Provides universal names ( 統一的名稱) for organisms
- Provides a reference for identifying organisms

# Systematics, or Phylogeny (種系發展史)

- The study of the evolutionary history of organisms
- All Species Inventory (2001–2025)
  - To identify all species of life on Earth
  - Currently more than 1.7 million identified
  - Estimated number: 10~100 million

# Placing Bacteria

1735 Kingdoms Plantae and Animalia (Carolus Linnaeus)

1857 Bacteria and fungi put in the Kingdom Plantae –"Flora" (植物群) (Carl von Nägeli)

1866 Kingdom Protista (原生生物界) proposed for bacteria, protozoa (原生動物), algae, and fungi

1937 *Prokaryote* (原核生物) introduced for cells "without a nucleus" (Edouard Chatton)

1961 *Prokaryote* defined as cell in which nucleoplasm is not surrounded by a nuclear membrane

1959 Kingdom Fungi

1968 Kingdom Prokaryotae proposed

1969 Five kingdom systems (Robert H. Whittaker)

1978 Two types of prokaryotic cells found

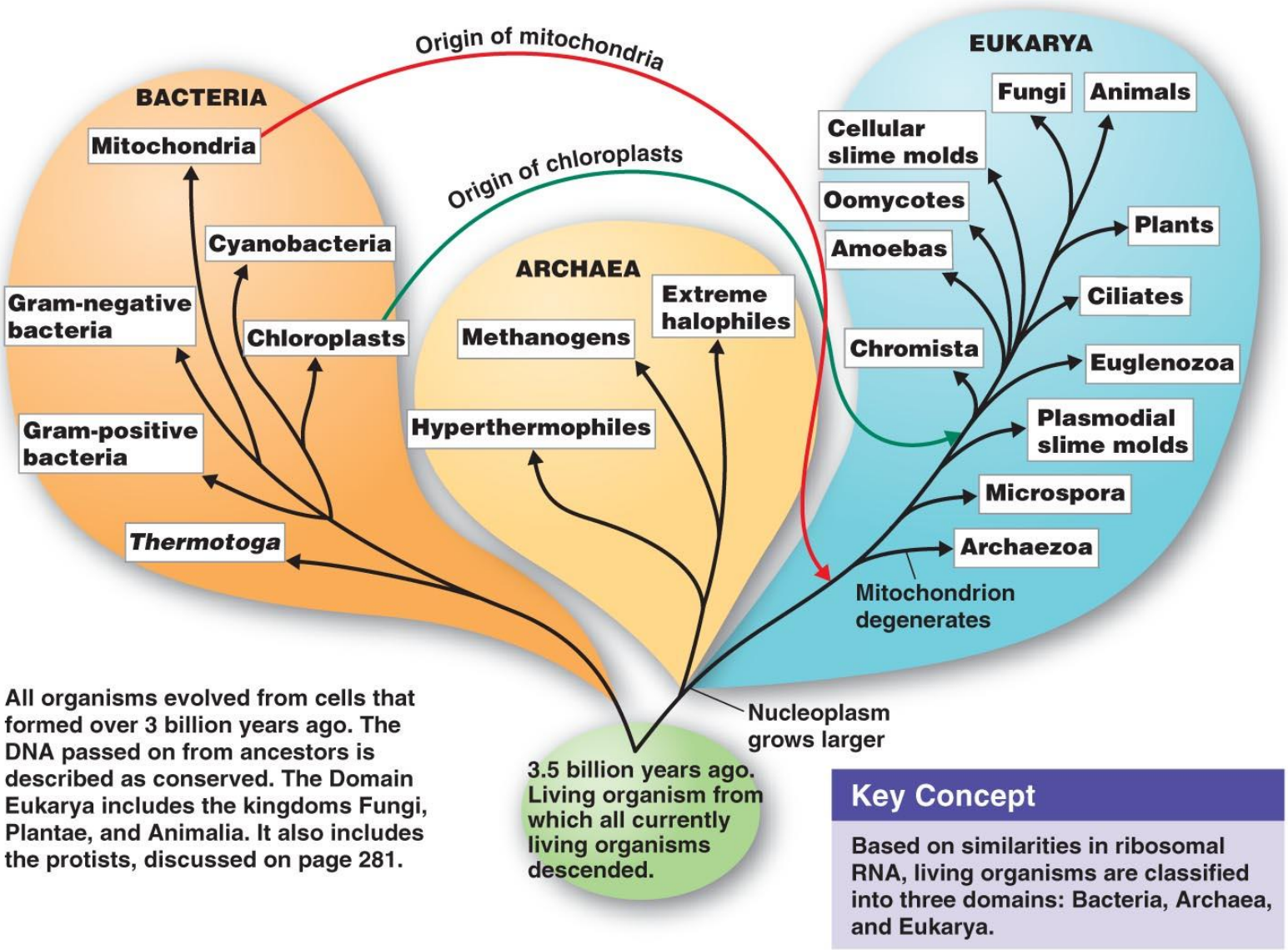
# The Three-Domain System

- Three cell types was discovered based on the observations that ribosomes are not the same in all cells.
- Carl R. Woese proposed in 1978.
- Comparing **the sequences of nucleotides in ribosomal RNA (rRNA)** from different kinds of cells shows that there are 3 different cell groups: the eukaryotes and two different types of prokaryotes (the bacteria and the archaea).

# The Three-Domain System 三域系統

- Living organisms are currently into three domains (Figure 10.1). A domain can divided into kingdoms.
  - In this system, **plants**, **animals**, **fungi**, and **protists** belong to the Domain **Eukarya**.
  - **Bacteria** (with peptoglycan) form a second domain.
  - **Archaea** (with unusual cell walls) are placed in the Domain Archaea.

# The Three-Domain System




All organisms evolved from cells that formed over 3 billion years ago. The DNA passed on from ancestors is described as conserved. The Domain Eukarya includes the kingdoms Fungi, Plantae, and Animalia. It also includes the protists, discussed on page 281.

**Key Concept**

Based on similarities in ribosomal RNA, living organisms are classified into three domains: Bacteria, Archaea, and Eukarya.



Figure 10.1

# The Three-Domain System

- Domain Archaea includes prokaryotes that often live in extreme environments and carry out unusual metabolic processes.
- Archaea include three major groups:

  1. The methanogen, strict anaerobes that produce methane ($CH_4$) from carbon dioxide and hydrogen.

  2. Extreme halophiles, which require high concentrations of salt for survival.

  3. Hyperthermophiles, which normally grow in hot environments.

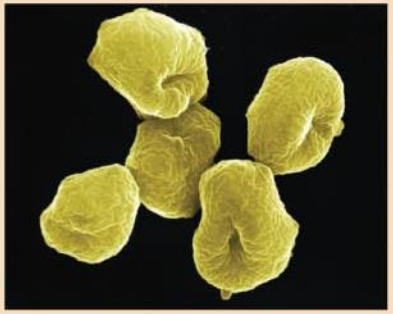
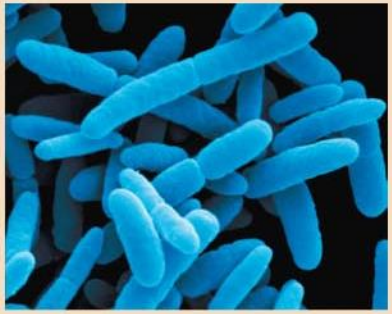
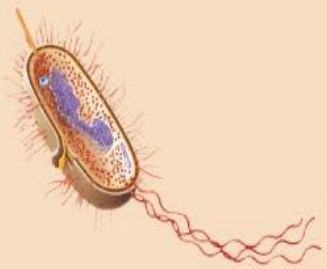
| | Archaea | Bacteria | Eukarya |
|---|---|---|---|
| |  *Sulfolobus* SEM .5 μm |  *E. coli* SEM 1 μm |  *Amoeba* SEM 10 μm |
| **Cell Type** | Prokaryotic | Prokaryotic | Eukaryotic |
| **Cell Wall** | Varies in composition; contains no peptidoglycan | Contains peptidoglycan | Varies in composition; contains carbohydrates |
| **Membrane Lipids** | Composed of branched carbon chains attached to glycerol by ether linkage | Composed of straight carbon chains attached to glycerol by ester linkage | Composed of straight carbon chains attached to glycerol by ester linkage |
| **First Amino Acid in Protein Synthesis** | Methionine | Formylmethionine | Methionine |
| **Antibiotic Sensitivity** | No | Yes | No |
| **rRNA Loop*** | Lacking | Present | Lacking |
| **Common Arm of tRNA†** | Lacking | Present | Present |



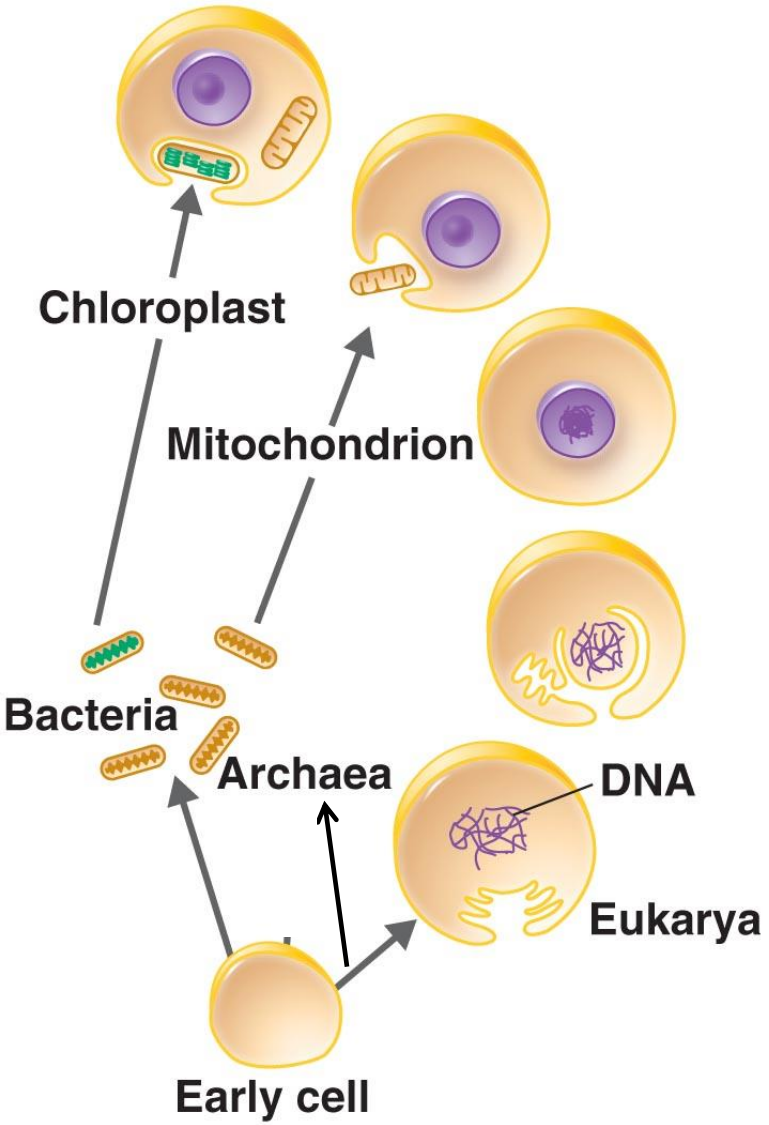
Table 10.1

## Table 10.2 Prokaryotic Cells and Eukaryotic Organelles Compared

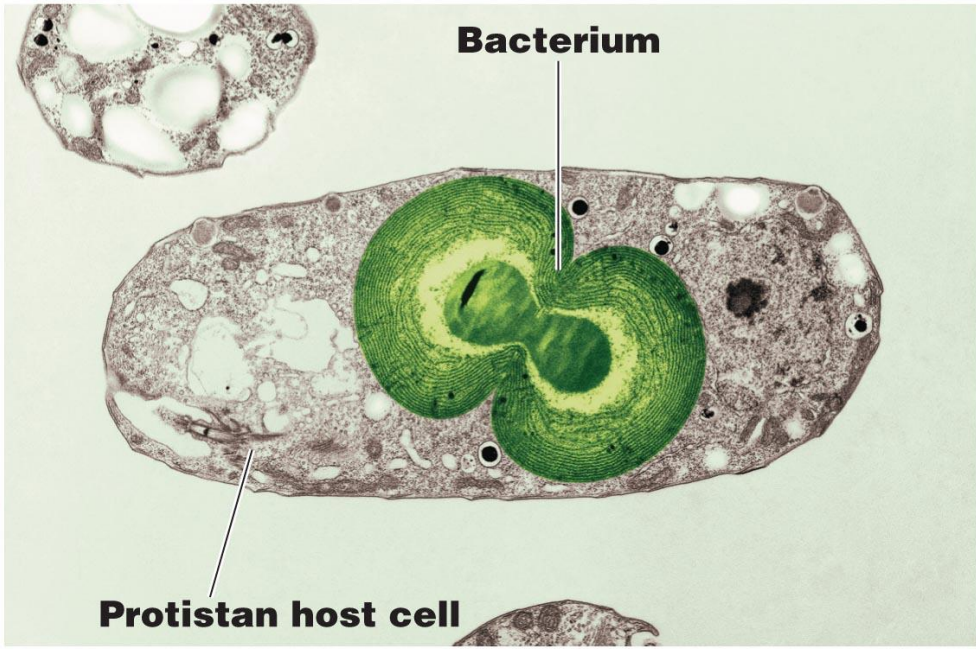
| | Prokaryotic Cell | Eukaryotic Cell | Eukaryotic Organelles (Mitochondria and Chloroplasts) |
|---|---|---|---|
| **DNA** | One circular; some two circular; some linear | Linear | Circular |
| **Histones** | In archaea | Yes | No |
| **First Amino Acid in Protein Synthesis** | Formylmethionine (bacteria) Methionine (archaea) | Methionine | Formylmethionine |
| **Ribosomes** | 70S | 80S | 70S |
| **Growth** | Binary fission | Mitosis | Binary fission |



# A Model of the Origin of Eukaryotes: Endosymbiotic Theory




Eukaryotic cells evolved from prokaryotic cells living one another, as endosymbionts.






Figures 10.2, 10.3

# Phylogenetics (系統發生學)

- Each species retains some characteristics of its ancestor
- Grouping organisms according to common properties implies that a group of organisms evolved from a common ancestor
  - Anatomy (解剖學)
  - Fossils
  - rRNA sequencing and DNA hybridization

# Fossilized Prokaryotes

**stromatolites**
疊層石

30 cm

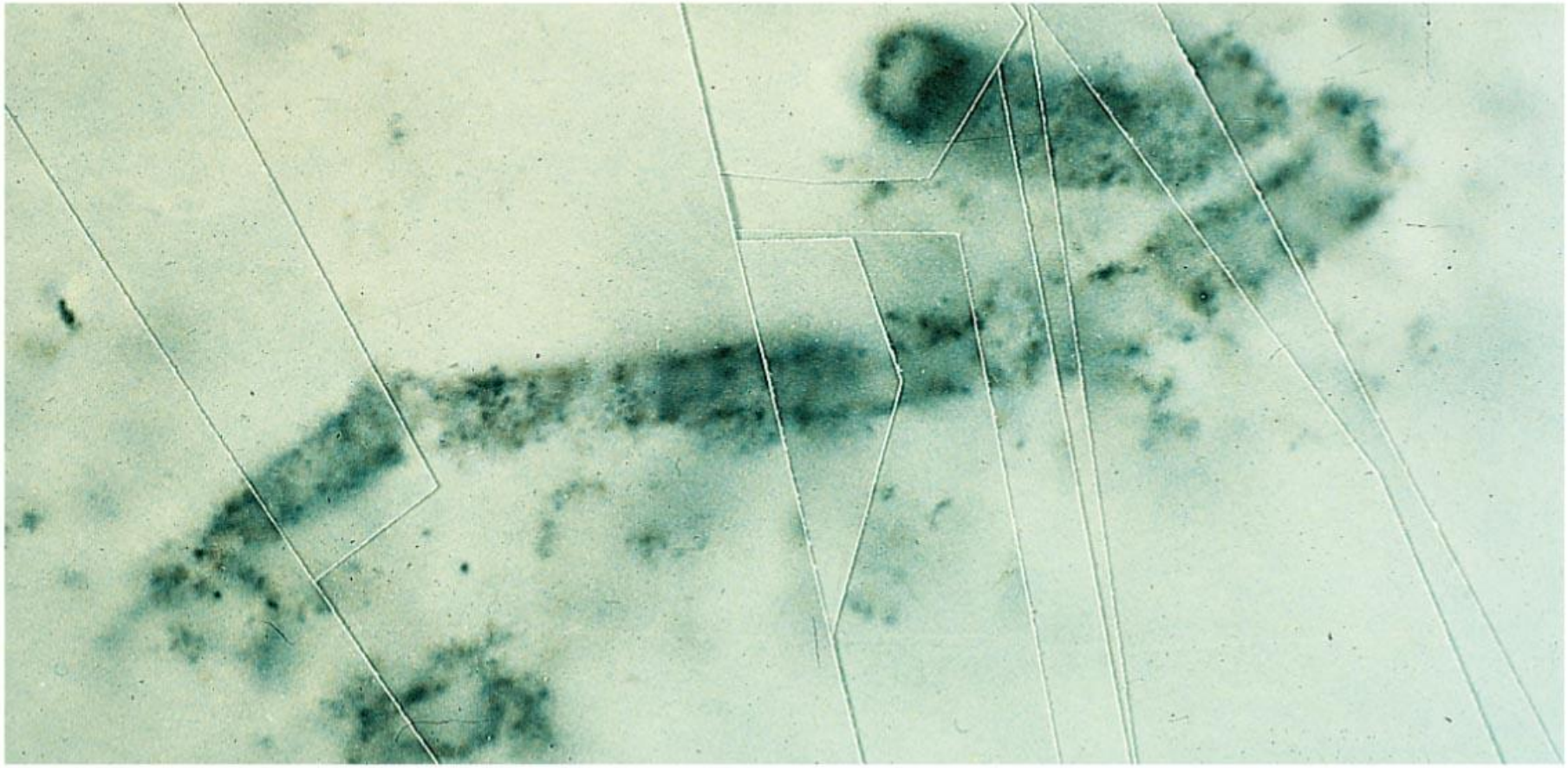
Figure 10.4a

# Fossilized Prokaryotes




Figure 10.4b

# Fossilized Prokaryotes




**Filamentous prokaryotes**



Figure 10.4c

# Classification of Organisms

## Scientific Nomenclature

- Because common names can be misleading and are in different languages, a system of scientific names, referred to as scientific nomenclature, was developed in the eighteenth century.
- Binomial (二項式 ) Nomenclature (genus + specific epithet (特徵或性質))
  - Used worldwide
    - *Escherichia coli*
    - *Homo sapiens*
  - Genus: capitalized, a noun
  - Species: lowercase, an adjective

# Scientific Names

| Scientific Binomial | Source of Genus Name | Source of Specific Epithet |
|---|---|---|
| *Klebsiella pneumoniae* | Honors Edwin Klebs | The disease |
| *Pfiesteria piscicida* | Honors Lois Pfiester | Disease in fish |
| *Salmonella typhimurium* | Honors Daniel Salmon | Stupor (麻痹;恍惚) (*typh*-) in mice (*muri*-) |
| *Streptococcus pyogenes* | Chains of cells (*strepto*-) | Forms pus (膿) (*pyo*-) |

# Taxonomic Hierarchy

Domain 域

Kingdom 界

Phylum 門

Class 綱

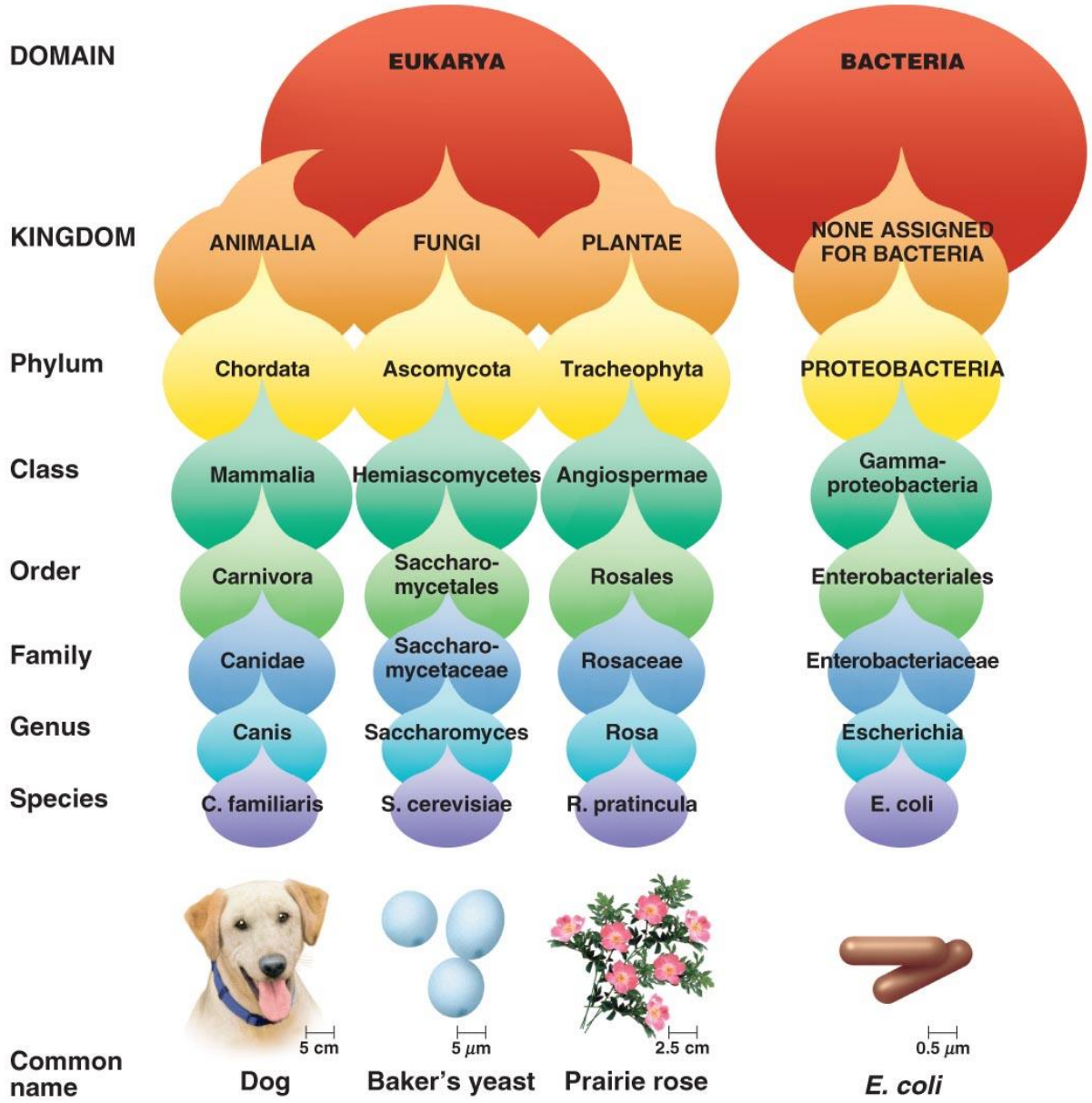
Order 目

Family 科

Genus 屬

Species 種

# The Taxonomic Hierarchy




**No kingdoms assigned for Bacteria and Archaea**

**Related species make up a genus, related genera make up a family……**

Figure 10.5

# Classification of Prokaryotes

- **Prokaryotic species**: A population of cells with similar characteristics
  - **Culture**: Grown in laboratory media
  - **Clone**: Population of cells derived from a single cell
  - **Strain**: Genetically different cells within a clone

# Phylogenetic Relationships of Prokaryotes

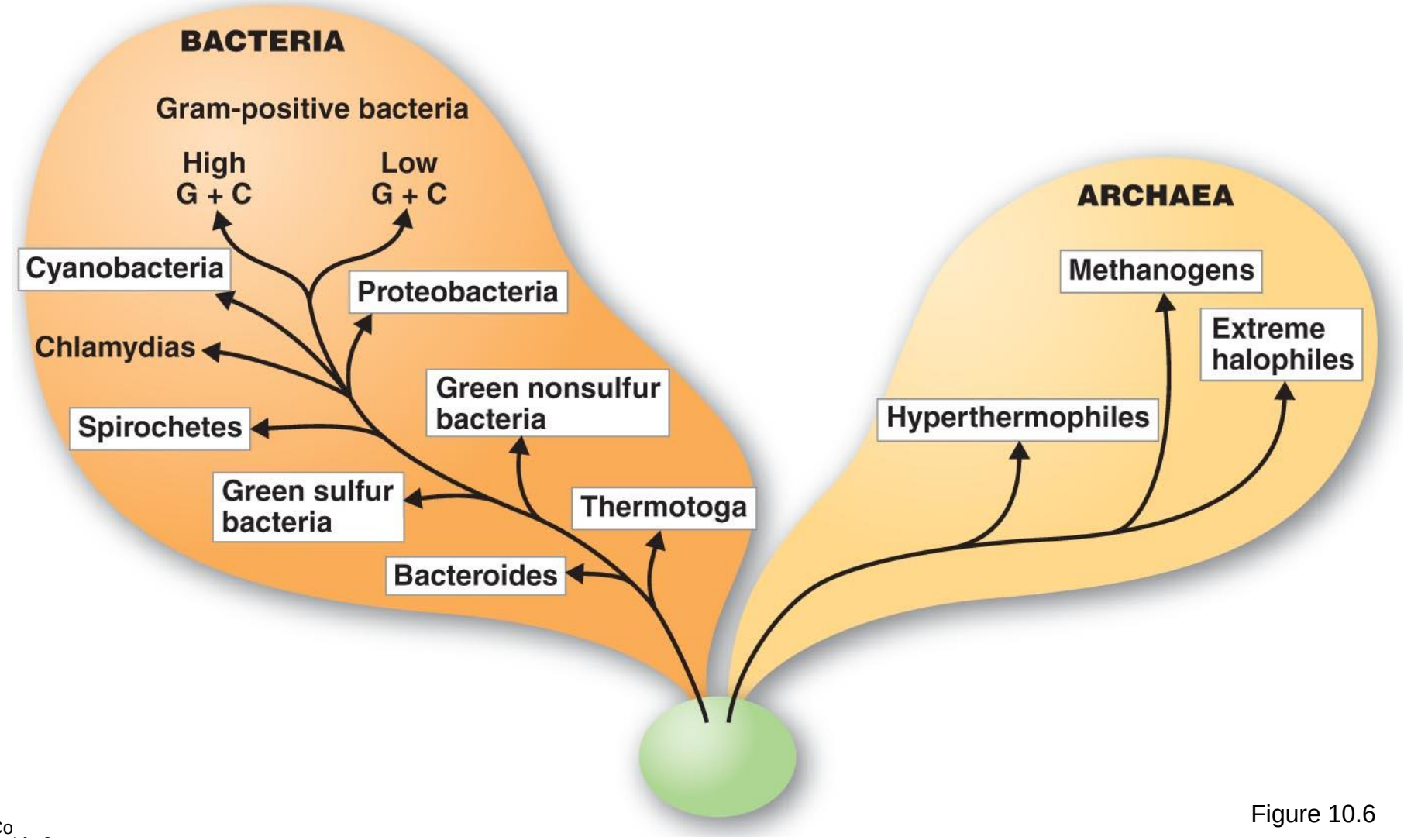



Figure 10.6

# Classification of Eukaryotes

**Eukaryotic species**: A group of closely related organisms that breed among themselves

- **Animalia**: Multicellular; no cell walls; chemoheterotrophic
- **Plantae**: Multicellular; cellulose cell walls; usually photoautotrophic (光自養)
- **Fungi**: Chemoheterotrophic (化學異營).; unicellular or multicellular; cell walls of chitin (幾丁質;甲殼素); develop from spores or hyphal fragments (菌絲片段)
- **Protista (**原生生物界): A catchall kingdom for eukaryotic organisms that do not fit other kingdoms
  - Grouped into **clades (**分化枝) based on rRNA

# clade

- A **clade** (from Ancient Greek κλάδος, *klados*, "branch") or **monophylum** (see monophyletic) is a group consisting of an ancestor and all its descendants, a single "branch" on the "tree of life". The ancestor may be an individual, a population or even a species (extinct or extant).

From Wikipedia, the free encyclopedia

# Classification of Viruses

- **Viral species**: Population of viruses with similar characteristics (including morphology, genes, and enzymes) that occupies a particular ecological niche (生態棲位)

- Viruses are not placed in a kingdom. They are not composed of cells and cannot grow without a host cell.

# References

| | |
|---|---|
| ***International Journal of Systematic and Evolutionary Microbiology*** | Articles with evidence of new species or classification |
| ***Bergey's Manual of Systematic Bacteriology*** | Provides phylogenetic (系統發生的) information on bacteria and archaea |
| ***Approved Lists of Bacterial Names*** | Lists species of known prokaryotes<br>Based on published articles |

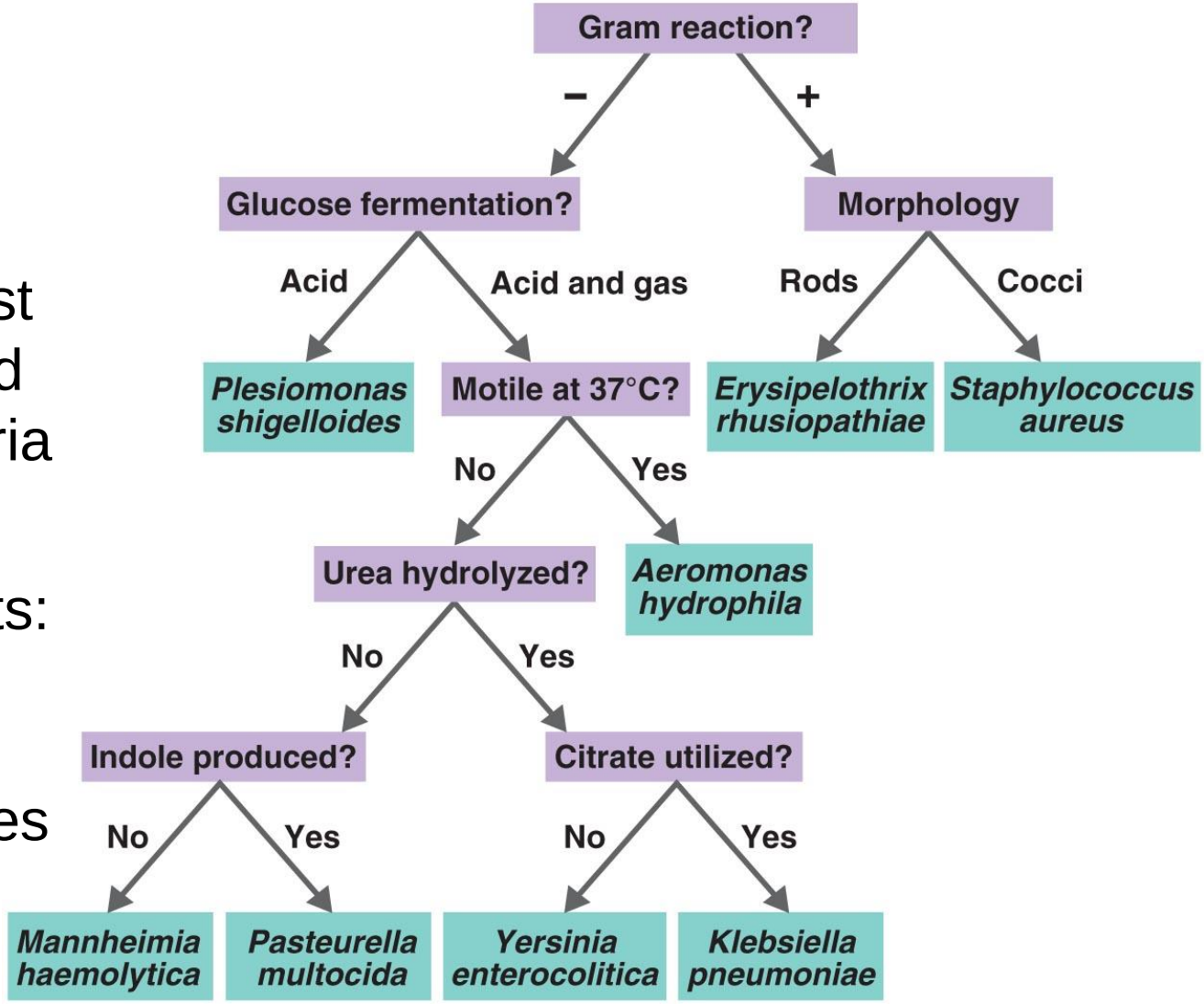
# Classifying & Identifying Microorganisms

## Identification Methods

- **Morphological characteristics**: Especially useful for identifying eukaryotes
- **Differential staining**: Gram staining, acid-fast staining
- **Biochemical tests**: Determines presence of bacterial enzymes

# Identifying Bacteria

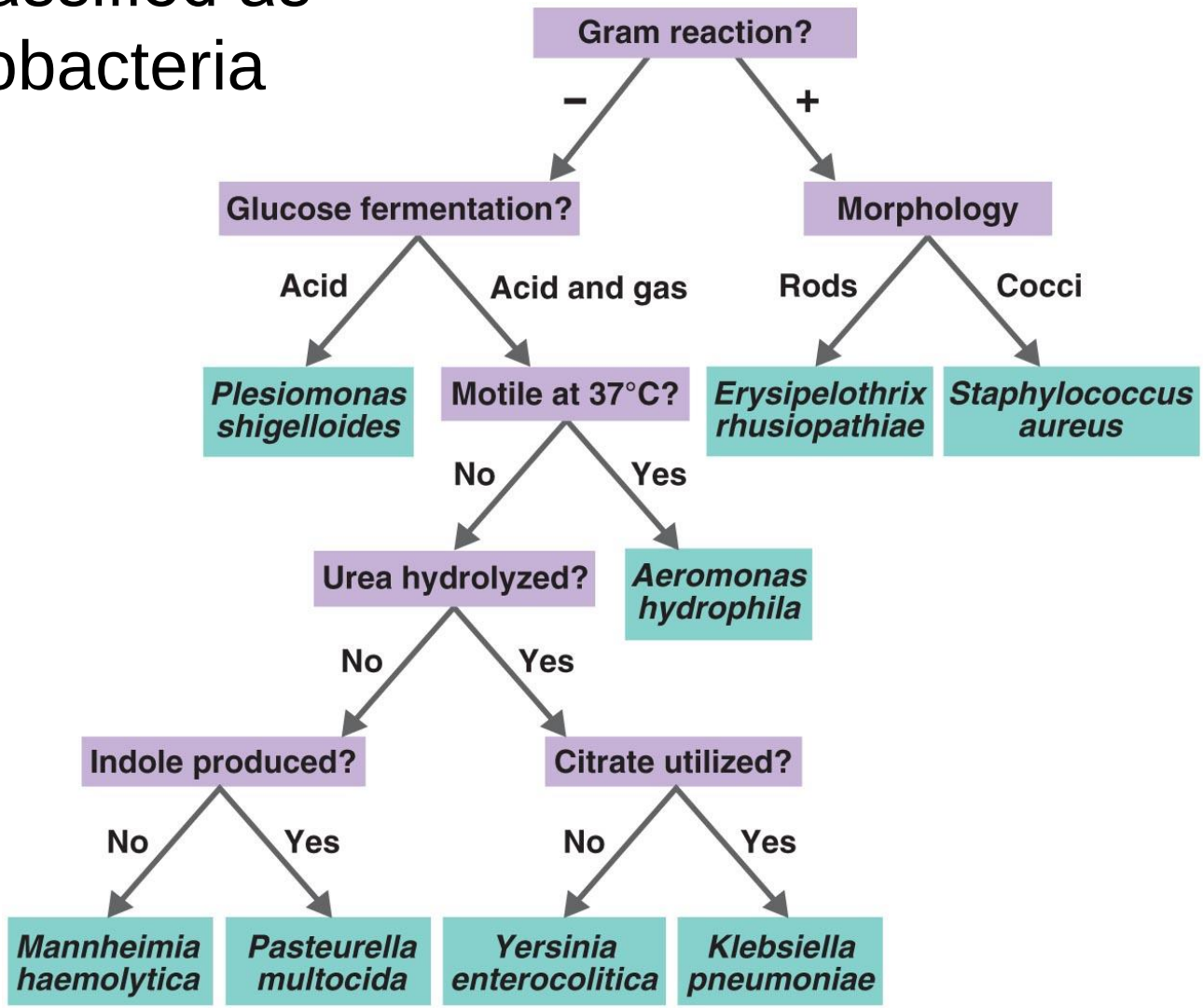
- Differential staining: Gram staining, acid-fast staining are used to identify bacteria
- Biochemical tests: Determines presence of bacterial enzymes



# Classification and Identification

- **Classification**: Placing organisms in groups of related species. Lists of characteristics of known organisms.
- **Identification**: Matching characteristics of an "unknown" organism to lists of known organisms.
  - Clinical lab identification

- Identifying *Klebsiella* doesn't tell you it's classified as gammaproteobacteria



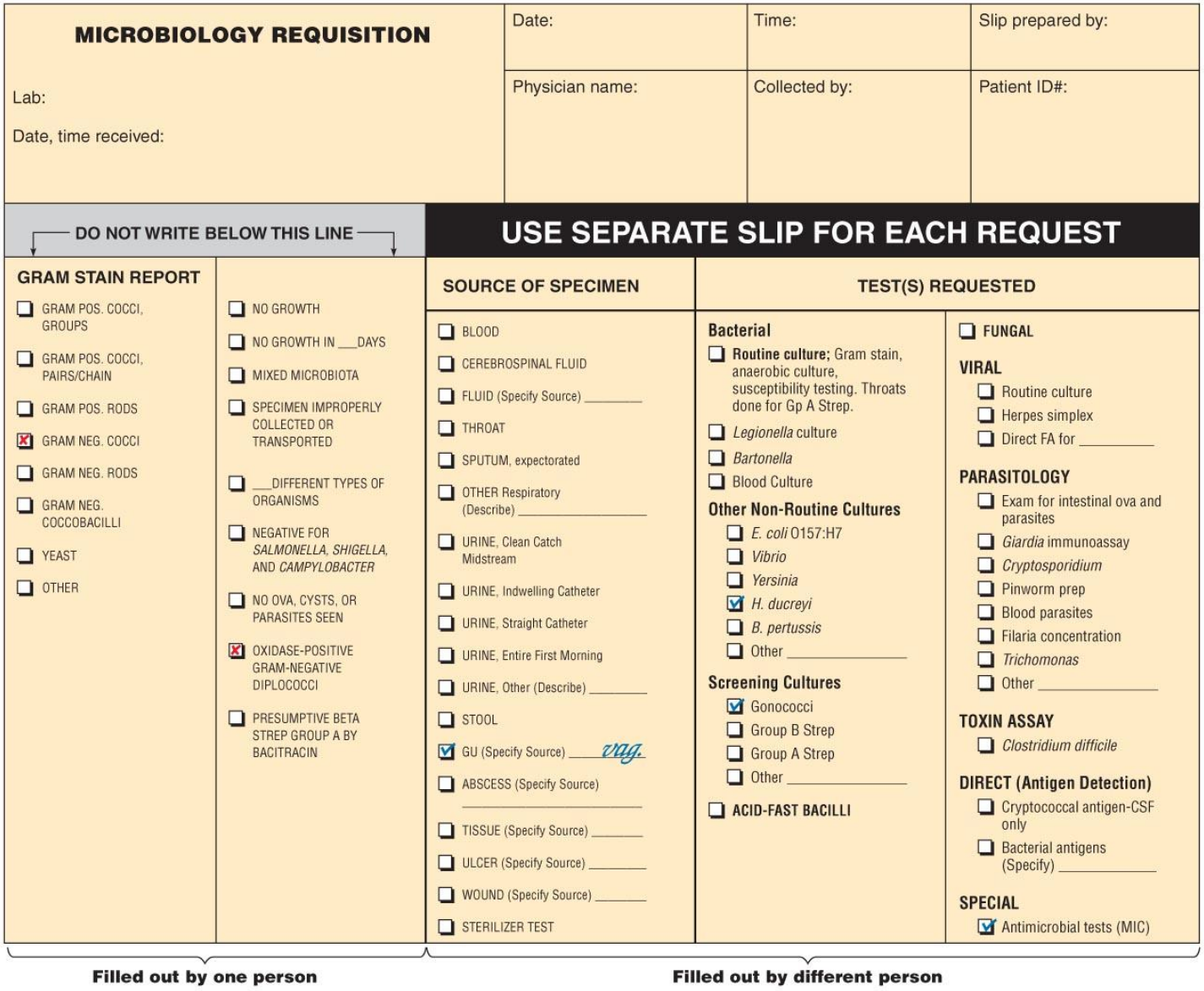



Applications, p. 283

# References

| | |
|---|---|
| ***Bergey's Manual of Determinative Bacteriology***<br>Provides *identification* schemes for identifying bacteria and archaea | Morphology, differential staining, biochemical tests |
| ***Bergey's Manual of Systematic Bacteriology***<br>Provides *phylogenetic* information on bacteria and archaea | Based on rRNA sequencing |

# A Clinical Microbiology Lab Report Form



**MICROBIOLOGY REQUISITION**

Lab:

Date, time received:

Date:

Time:

Slip prepared by:

Physician name:

Collected by:

Patient ID#:

DO NOT WRITE BELOW THIS LINE

**USE SEPARATE SLIP FOR EACH REQUEST**

**GRAM STAIN REPORT**

- ☐ GRAM POS. COCCI, GROUPS
- ☐ GRAM POS. COCCI, PAIRS/CHAIN
- ☐ GRAM POS. RODS
- ☒ GRAM NEG. COCCI
- ☐ GRAM NEG. RODS
- ☐ GRAM NEG. COCCOBACILLI
- ☐ YEAST
- ☐ OTHER

- ☐ NO GROWTH
- ☐ NO GROWTH IN ___DAYS
- ☐ MIXED MICROBIOTA
- ☐ SPECIMEN IMPROPERLY COLLECTED OR TRANSPORTED
- ☐ ___DIFFERENT TYPES OF ORGANISMS
- ☐ NEGATIVE FOR *SALMONELLA, SHIGELLA,* AND *CAMPYLOBACTER*
- ☐ NO OVA, CYSTS, OR PARASITES SEEN
- ☒ OXIDASE-POSITIVE GRAM-NEGATIVE DIPLOCOCCI
- ☐ PRESUMPTIVE BETA STREP GROUP A BY BACITRACIN

**SOURCE OF SPECIMEN**

- ☐ BLOOD
- ☐ CEREBROSPINAL FLUID
- ☐ FLUID (Specify Source) _____
- ☐ THROAT
- ☐ SPUTUM, expectorated
- ☐ OTHER Respiratory (Describe) _____
- ☐ URINE, Clean Catch Midstream
- ☐ URINE, Indwelling Catheter
- ☐ URINE, Straight Catheter
- ☐ URINE, Entire First Morning
- ☐ URINE, Other (Describe) _____
- ☐ STOOL
- ☑ GU (Specify Source) ___*vag.*___
- ☐ ABSCESS (Specify Source) _____
- ☐ TISSUE (Specify Source) _____
- ☐ ULCER (Specify Source) _____
- ☐ WOUND (Specify Source) _____
- ☐ STERILIZER TEST

**TEST(S) REQUESTED**

**Bacterial**

- ☐ **Routine culture;** Gram stain, anaerobic culture, susceptibility testing. Throats done for Gp A Strep.
- ☐ *Legionella* culture
- ☐ *Bartonella*
- ☐ Blood Culture

**Other Non-Routine Cultures**

- ☐ *E. coli* O157:H7
- ☐ *Vibrio*
- ☐ *Yersinia*
- ☑ *H. ducreyi*
- ☐ *B. pertussis*
- ☐ Other _____

**Screening Cultures**

- ☑ Gonococci
- ☐ Group B Strep
- ☐ Group A Strep
- ☐ Other _____

- ☐ **ACID-FAST BACILLI**

- ☐ **FUNGAL**

**VIRAL**

- ☐ Routine culture
- ☐ Herpes simplex
- ☐ Direct FA for _____

**PARASITOLOGY**

- ☐ Exam for intestinal ova and parasites
- ☐ *Giardia* immunoassay
- ☐ *Cryptosporidium*
- ☐ Pinworm prep
- ☐ Blood parasites
- ☐ Filaria concentration
- ☐ *Trichomonas*
- ☐ Other _____

**TOXIN ASSAY**

- ☐ *Clostridium difficile*

**DIRECT (Antigen Detection)**

- ☐ Cryptococcal antigen-CSF only
- ☐ Bacterial antigens (Specify) _____

**SPECIAL**

- ☑ Antimicrobial tests (MIC)

**Filled out by one person**

**Filled out by different person**

Figure 10.7

# Identifying a Gram – Negative

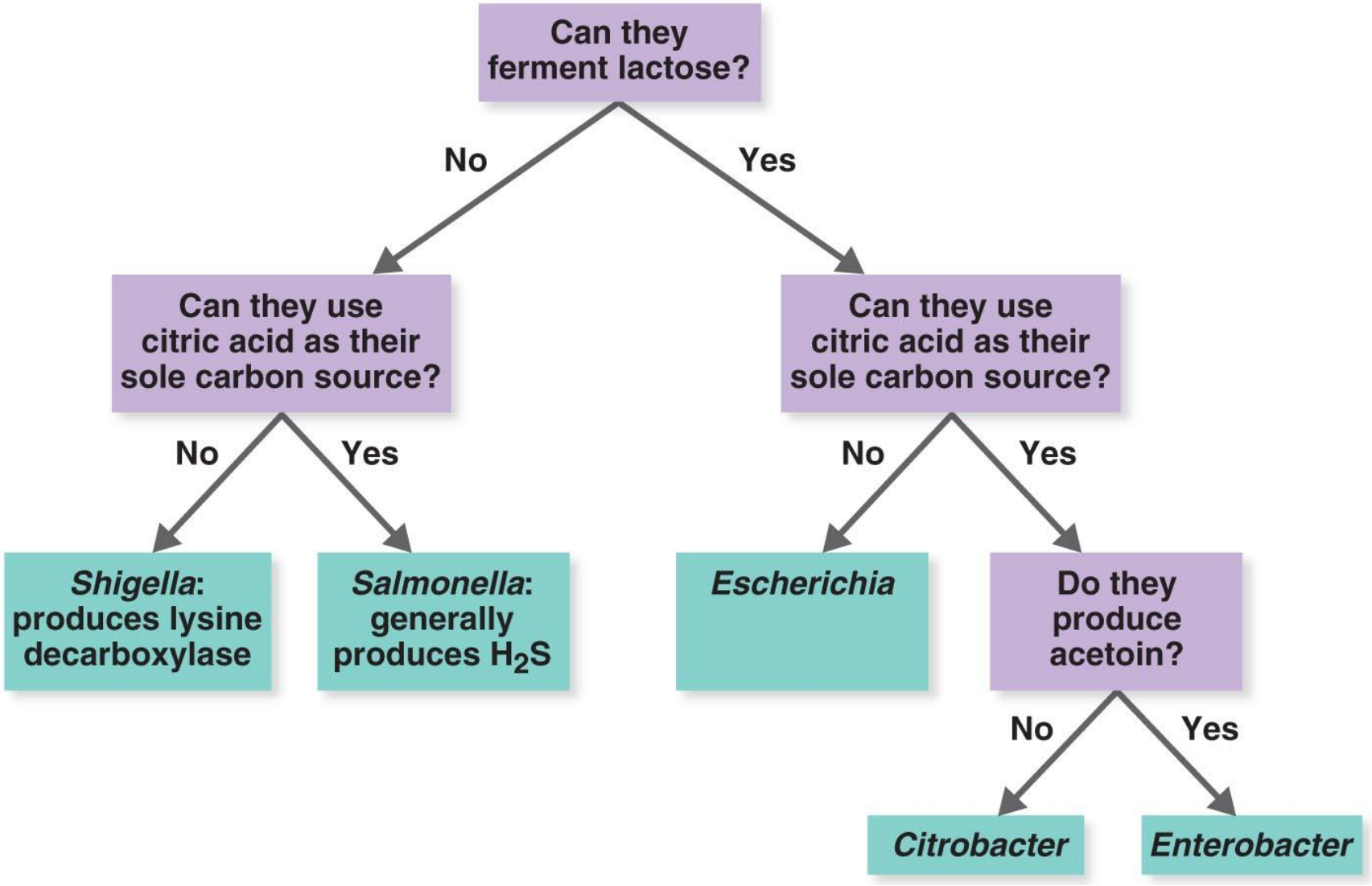
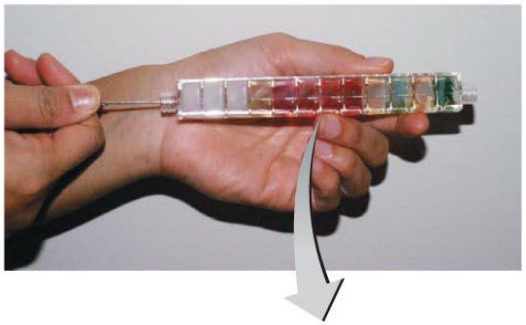


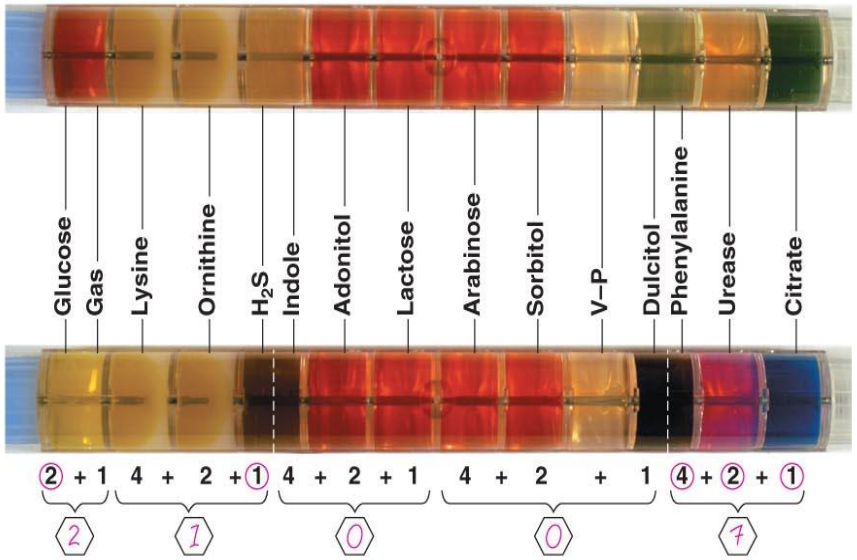

Figure 10.8

# Numerical Identification

1. One tube containing media for 15 biochemical tests is inoculated with an unknown enteric bacterium.



2. After incubation, the tube is observed for results.




3. The value for each positive test is circled, and the numbers from each group of tests are added to give the ID value.

4. Comparing the resultant ID value with a computerized listing shows that the organism in the tube is *Proteus mirabilis*.

| ID Value | Organism | Atypical Test Results | Confirmatory Test |
|---|---|---|---|
| 21006 | *Proteus mirabilis* | Ornithine$^-$ | Sucrose |
| 21007 | *Proteus mirabilis* | Ornithine$^-$ | |
| 21020 | *Salmonella choleraesuis* | Lysine$^-$ | |

A rapid identification method which is designed to perform several biochemical tests simultaneously and can identify bacteria within 4-24 hours.

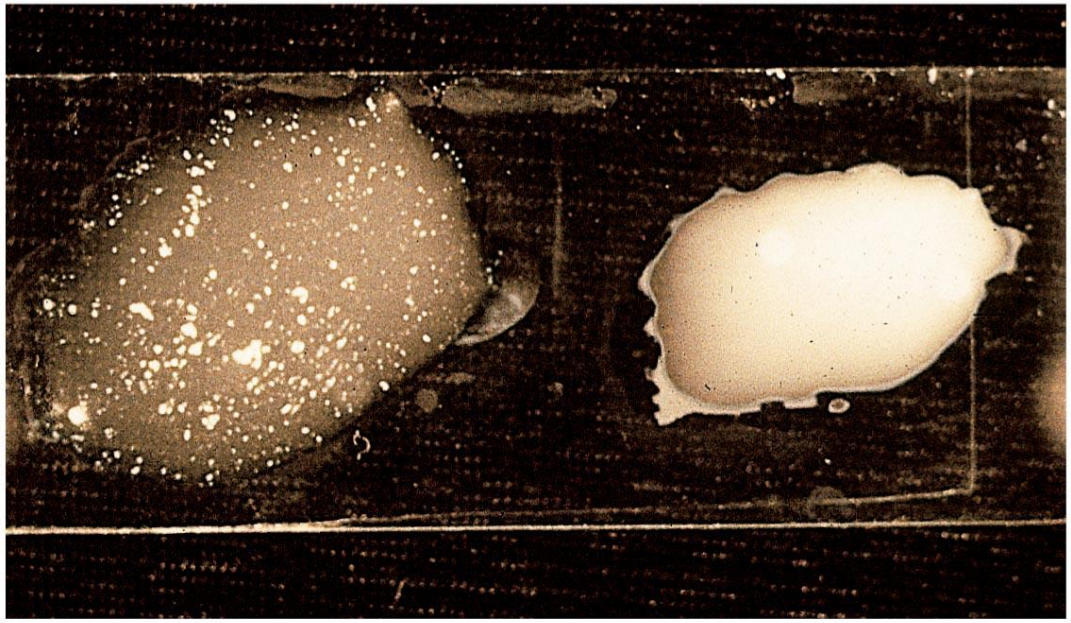
The results of each test are assigned a number.



Figure 10.9

# Serology

- Combine known antiserum plus unknown bacterium
- **Slide agglutination test**



**(a)** Positive test **(b)** Negative test

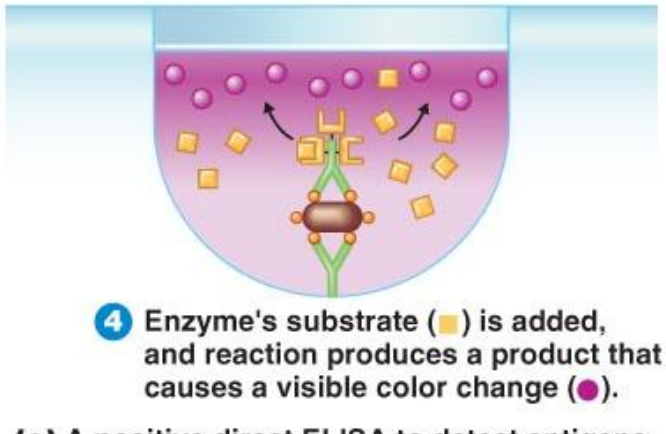
*Grainy appearance is due to the agglutination of the bacteria.*



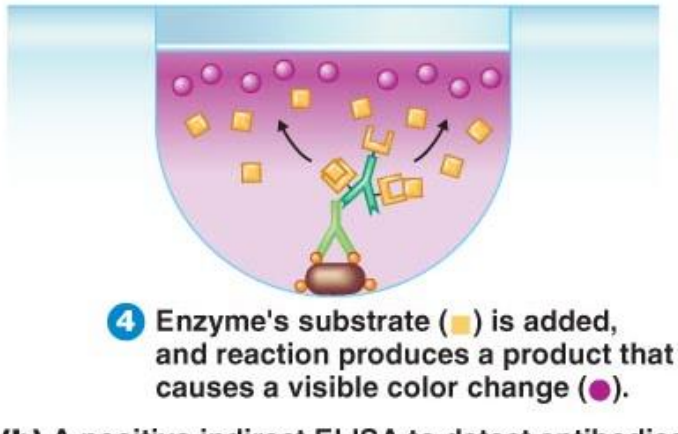
Figure 10.10

# ELISA

- **Enzyme-linked immunosorbent assay**
- Known antibodies
- Unknown type of bacterium (detect antigens)
- Antibodies linked to enzyme
- Enzyme substrate




**(a)** A positive direct ELISA to detect antigens

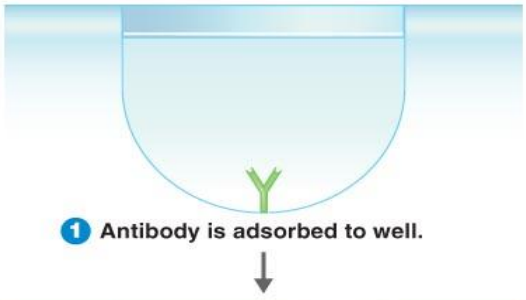



**(b)** A positive indirect ELISA to detect antibodies

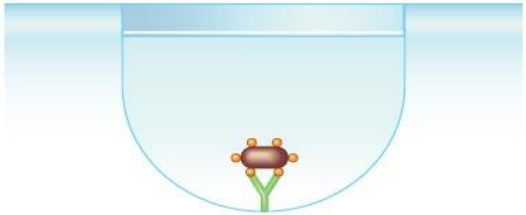


Figure 18.14

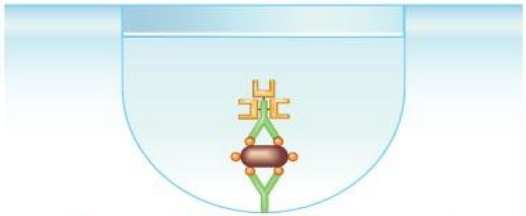
# ELISA



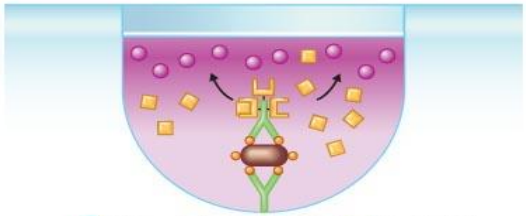
1 Antibody is adsorbed to well.



2 Patient sample is added; complementary antigen binds to antibody.

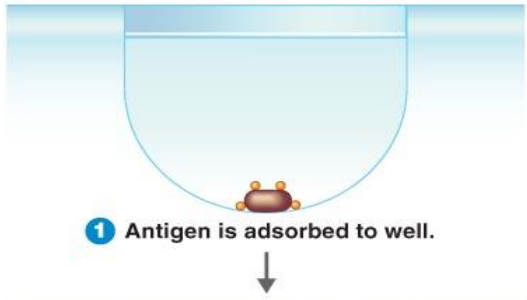


3 Enzyme-linked antibody specific for test antigen is added and binds to antigen, forming sandwich.

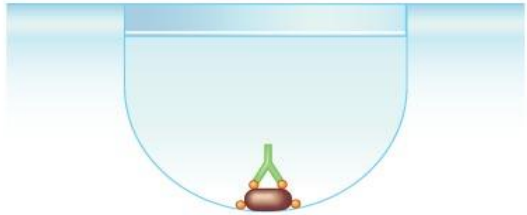


4 Enzyme's substrate (■) is added, and reaction produces a product that causes a visible color change (●).

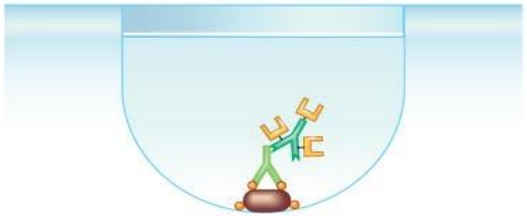
**(a)** A positive direct ELISA to detect antigens



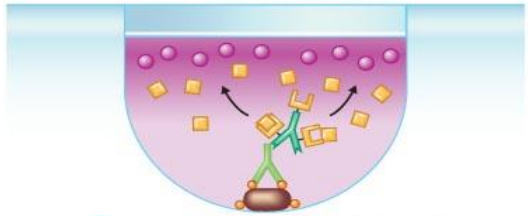
1 Antigen is adsorbed to well.



2 Patient serum is added; complementary antibody binds to antigen.



3 Enzyme-linked anti-HISG (see page 513) is added and binds to bound antibody.



4 Enzyme's substrate (■) is added, and reaction produces a product that causes a visible color change (●).

**(b)** A positive indirect ELISA to detect antibodies



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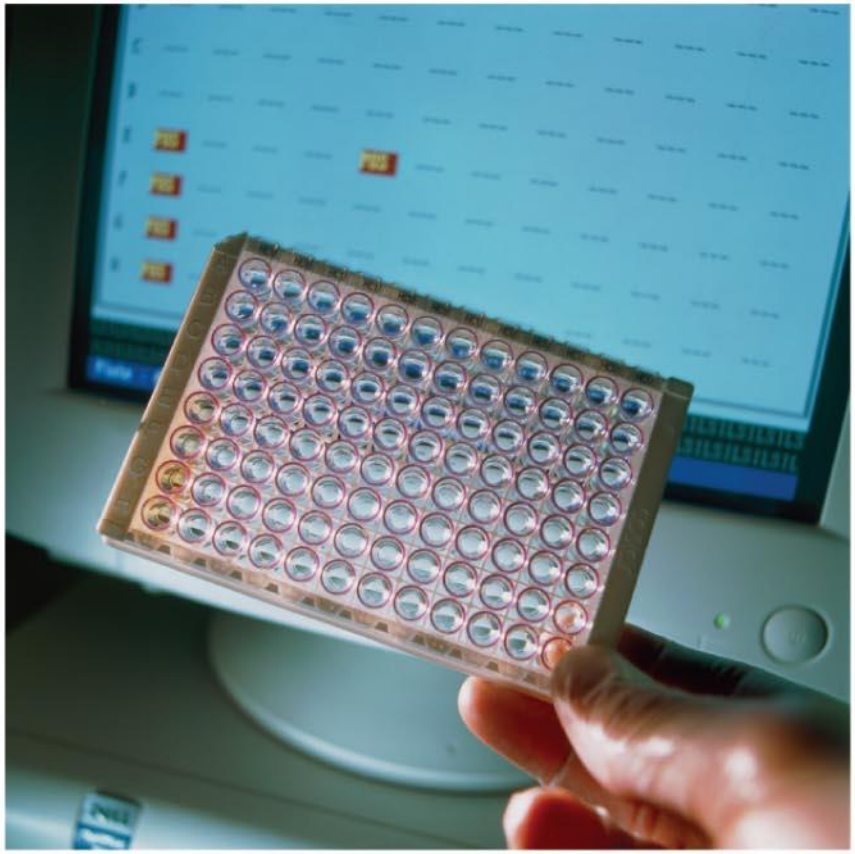
# An ELISA Test


(a)



(b)



Figure 10.11

# The Western Blot

1. If Lyme disease is suspected in a patient: Electrophoresis is used to separate *Borrelia burgdorferi* proteins. Proteins move at different rates based on their charge and size when the gel is exposed to an electric current.

2. The bands are transferred to a nitrocellulose filter by blotting. Each band consists of many molecules of a particular protein (antigen). The bands are not visible at this point.

3. The proteins (antigens) are positioned on the filter exactly as they were on the gel. The filter is then washed with patient's serum followed by antihuman antibodies tagged with an enzyme. The patient antibodies that combine with their specific antigen are visible (shown here in red) when the enzyme's substrate is added.

4. The test is read. If the tagged antibodies stick to the filter, evidence of the presence of the microorganism in question—in this case, *B. burgdorferi*—has been found in the patient's serum.

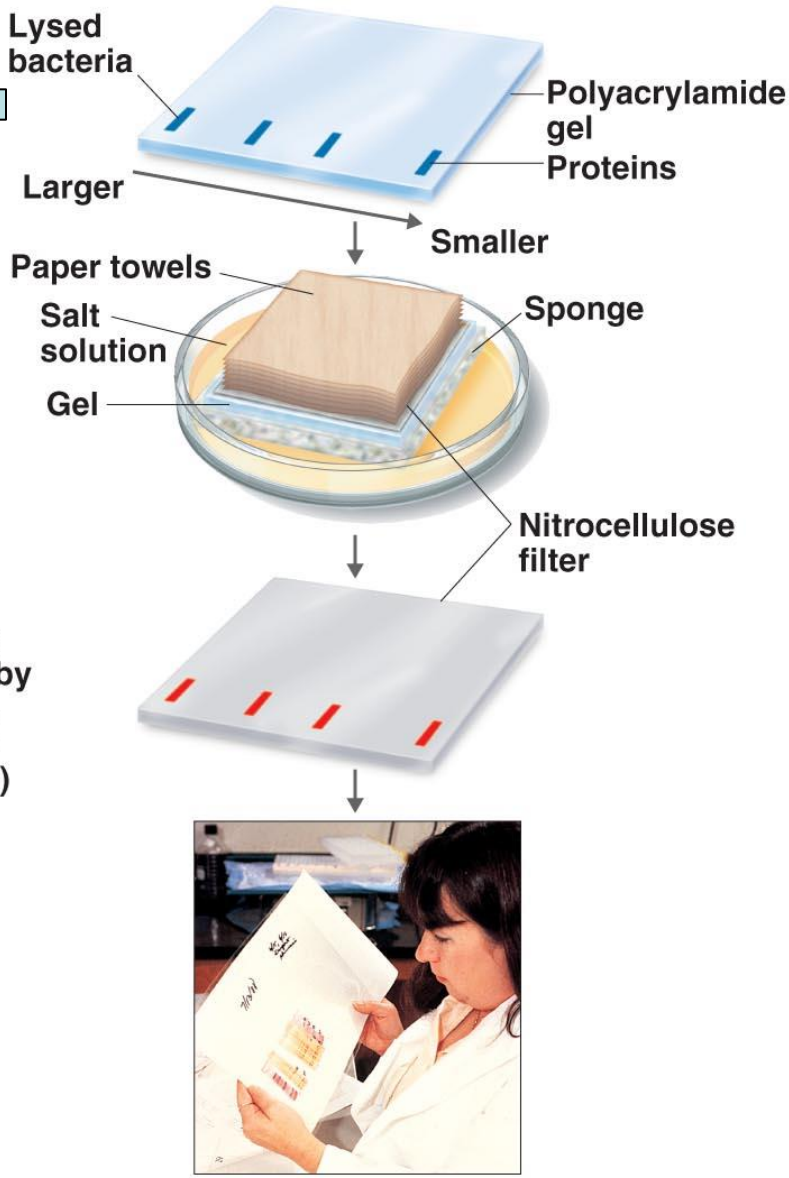


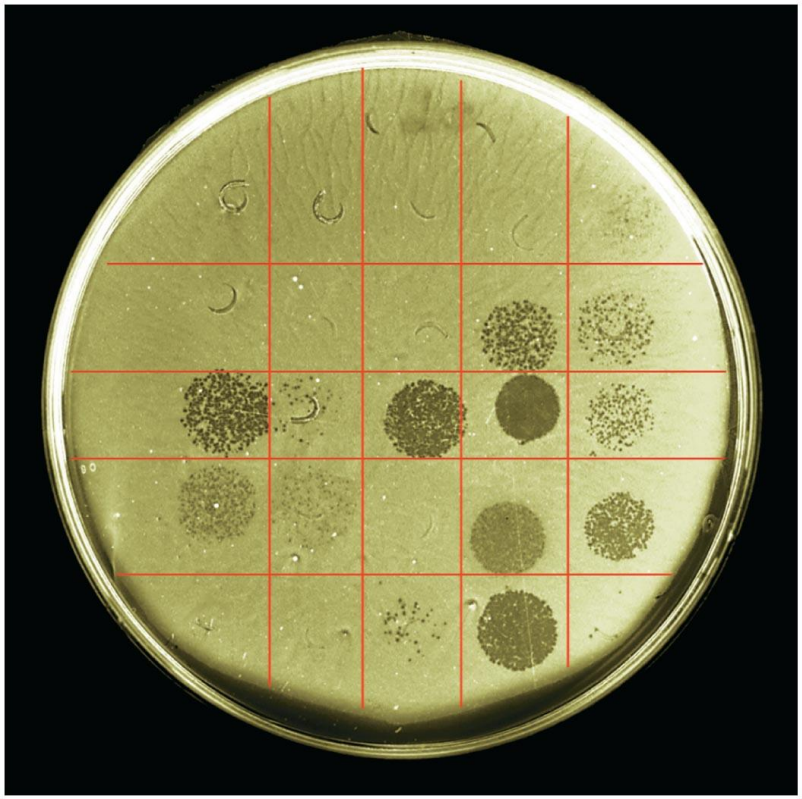



Figure 10.12

# Lyme disease

- 1975年美國康乃迪克州萊姆鎮，許多幼童或成人同時出現不明原因的復發性類風濕關節炎，當時將該病稱為萊姆關節炎（Lyme arthritis）。經調查追蹤後，才發現是寵物身上的伯氏疏螺旋體（*Borrelia burgdorferi*）所引起的人畜共通傳染疾病，故命名為萊姆病。

# Phage Typing of *Salmonella enterica*



The identification of bacterial species and strains by the determination of their susceptibility to various phages.

1. A plate covered with bacteria growing on agar.
2. A drop of each different phage type is then placed on the bacteria.
3. Wherever the phages are able to infect and lyse the bacterial cells, clearings in the bacterial growth (called plaques) appear.



Figure 10.13

# Flow Cytometry

- Identify bacteria in a sample without culturing the bacteria.
- In a flow cytometer, a moving fluid containing bacteria is forced through a small opening.
- Uses differences in **electrical conductivity** between species
- **Fluorescence** of some species, e.g. *Pseudomonas*
- Cells selectively tagged with antibody + fluorescent dye

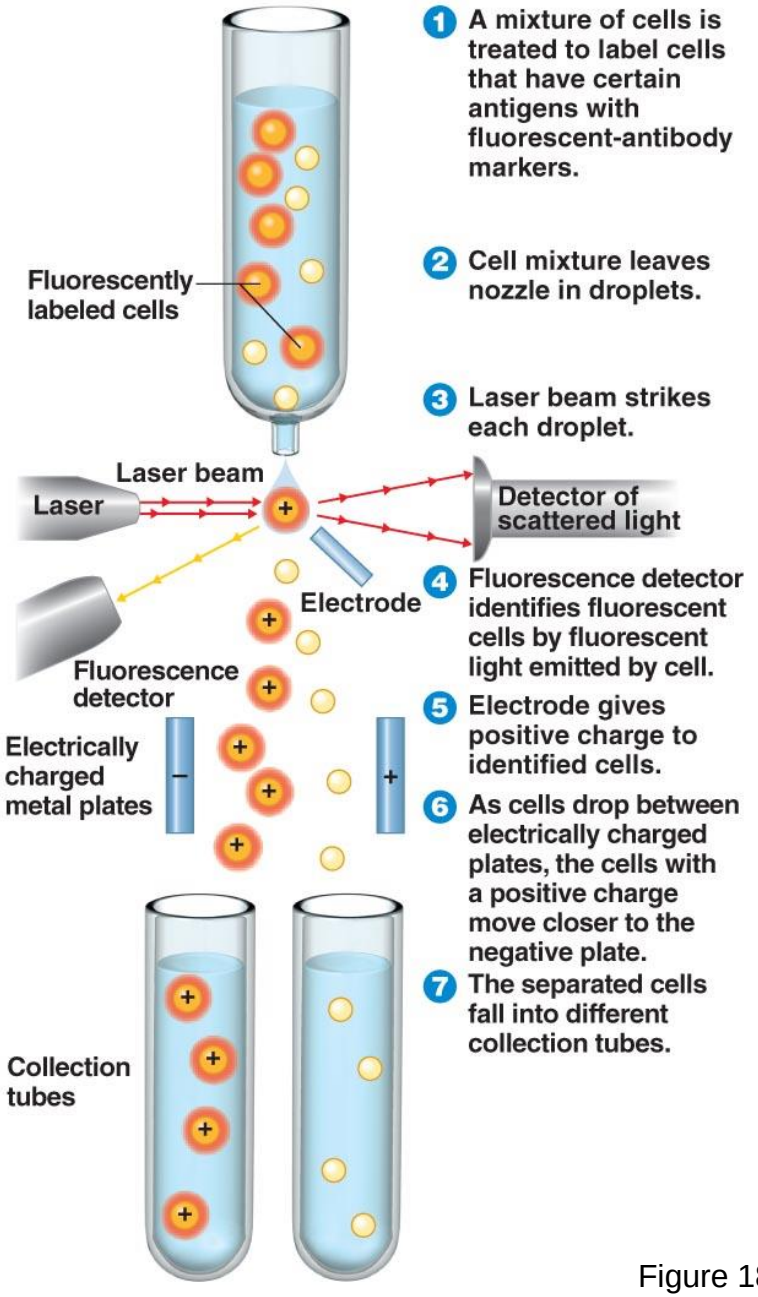





Figure 18.12

# Genetics

- DNA base composition
  - Guanine + cytosine moles% (GC)
  - GC+AT = 100%
  - Difference in GC% lager than 10%➔ probably not related
- DNA fingerprinting (Fig. 10.14)
  - Electrophoresis of restriction enzyme digests
  - Determine genetic similarities: the more similar the patterns the more closely related the organisms.
- rRNA sequencing
- Polymerase Chain Reaction (PCR)
  - If a primer for a specific MO is used, the presence of amplified indicates that MO is present.

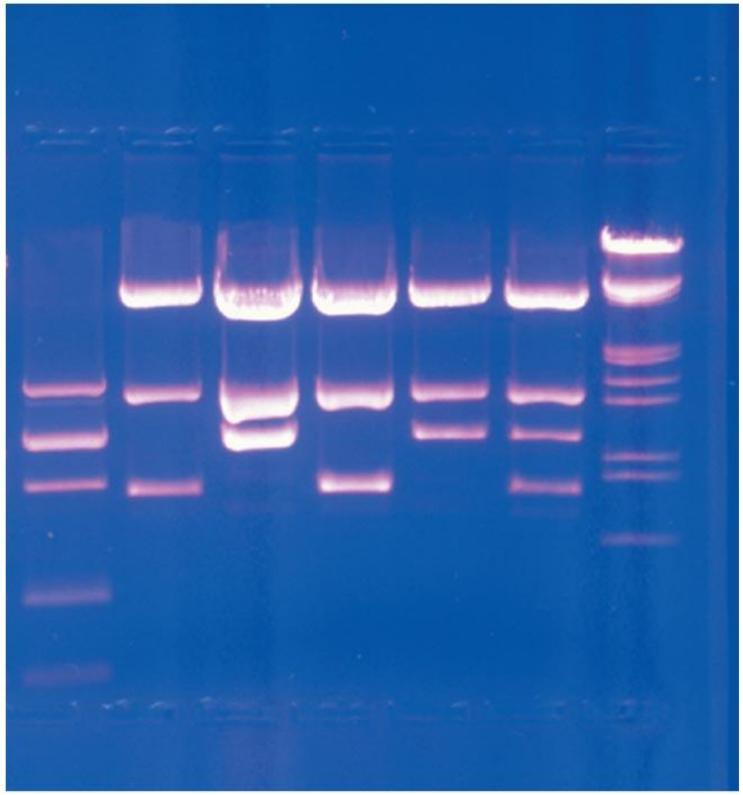




Figure 10.14

# Nucleic Acid Hybridization

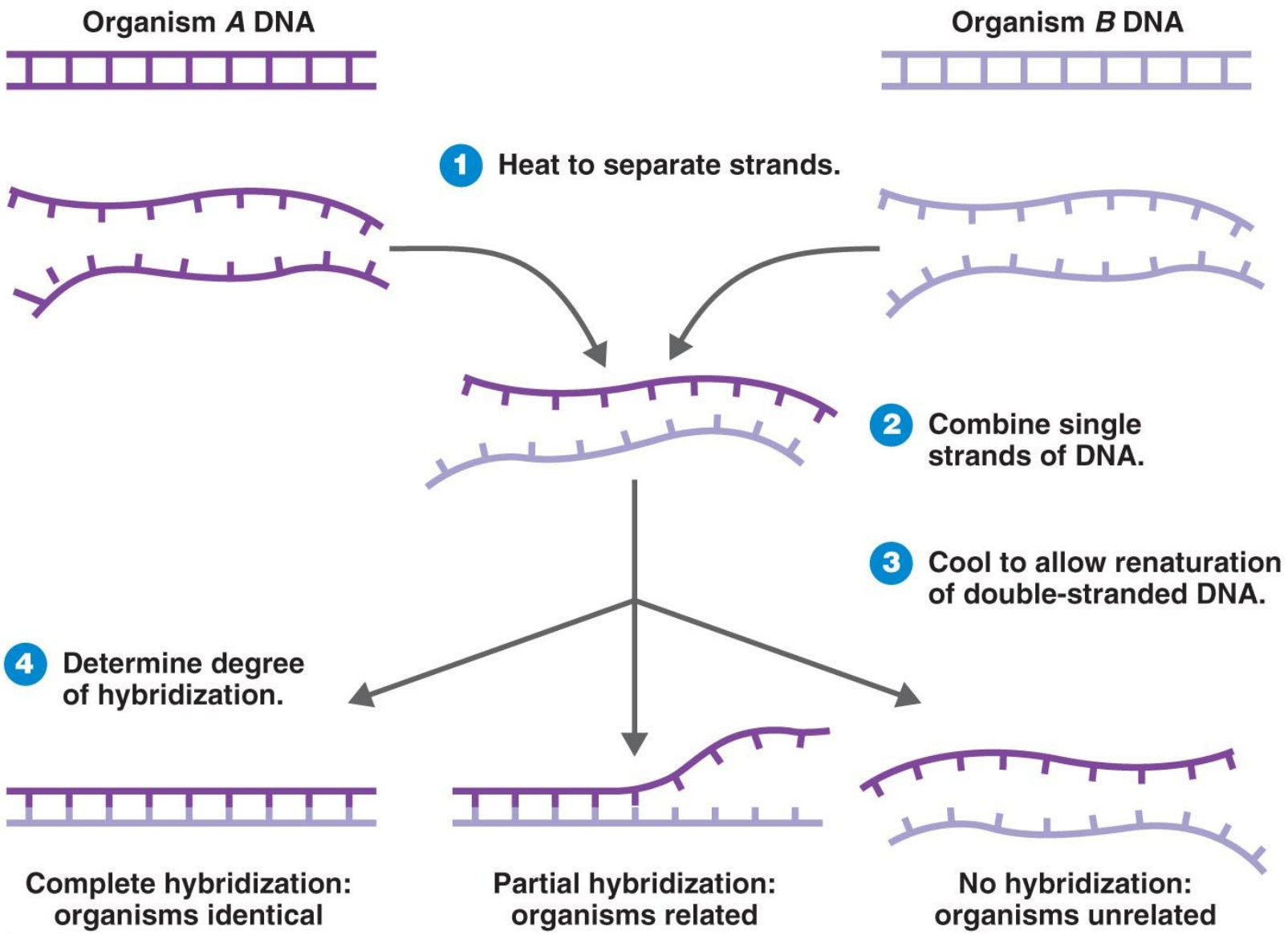





Figure 10.15

# A DNA Probe Used to Identify Bacteria

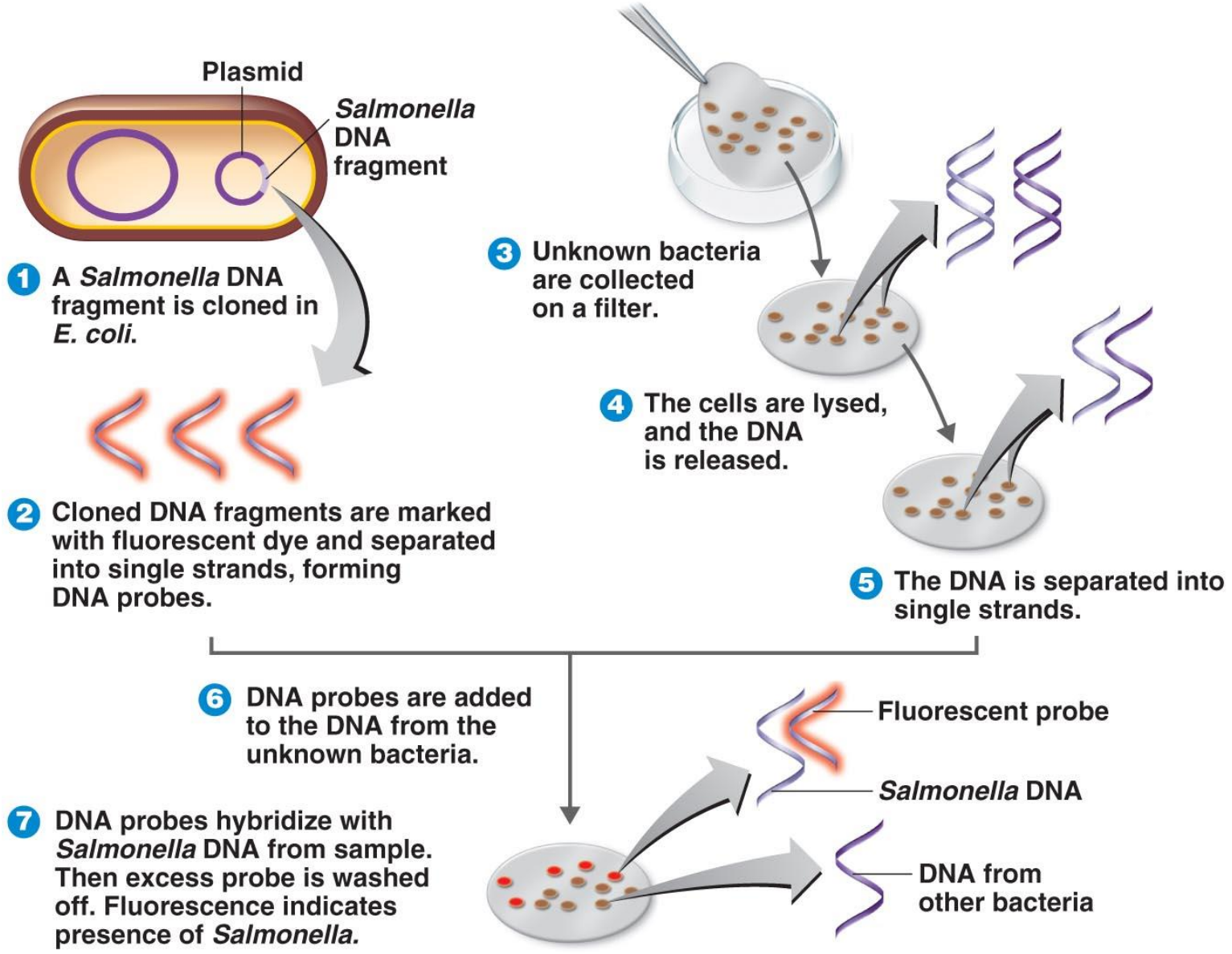
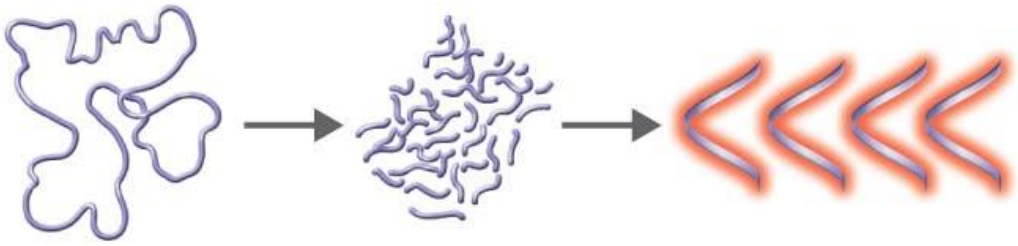





Figure 10.16

# DNA Chip Technology


**(a)** A DNA chip can be manufactured to contain hundreds of thousands of synthetic single-stranded DNA sequences. Assume that each DNA sequence was unique to a different gene.

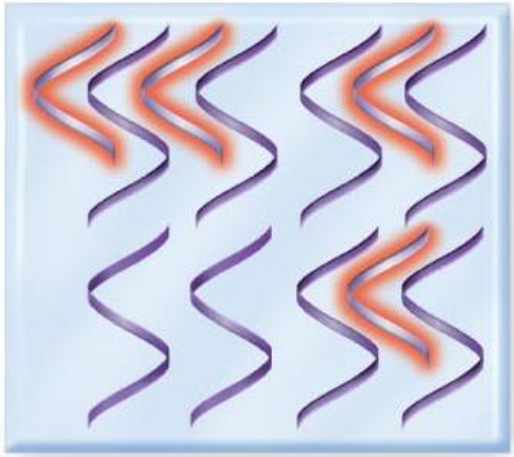


**(b)** Unknown DNA from a patient is separated into single strands, enzymatically cut, and labeled with a fluorescent dye.

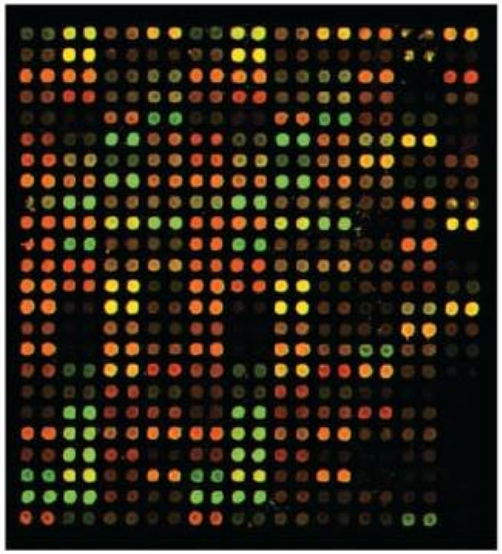


Figure 10.17

# DNA Chip Technology



**(c)** The unknown DNA is inserted into the chip and allowed to hybridize with the DNA on the chip.



**(d)** The tagged DNA will bind only to the complementary DNA on the chip. The bound DNA will be detected by its fluorescent dye and analyzed by a computer. The red light is a gene expressed in normal cells; green is a mutated gene expressed in tumor cells; and yellow, in both cells.



Figure 10.17

# FISH

- **Fluorescent in situ hybridization (螢光染色體原位雜合法: 利用螢光標定之DNA探針，藉由hybridization的過程，在染色體上將基因或DNA定位 )**
- Add DNA probe for *Staphylococcus aureus*

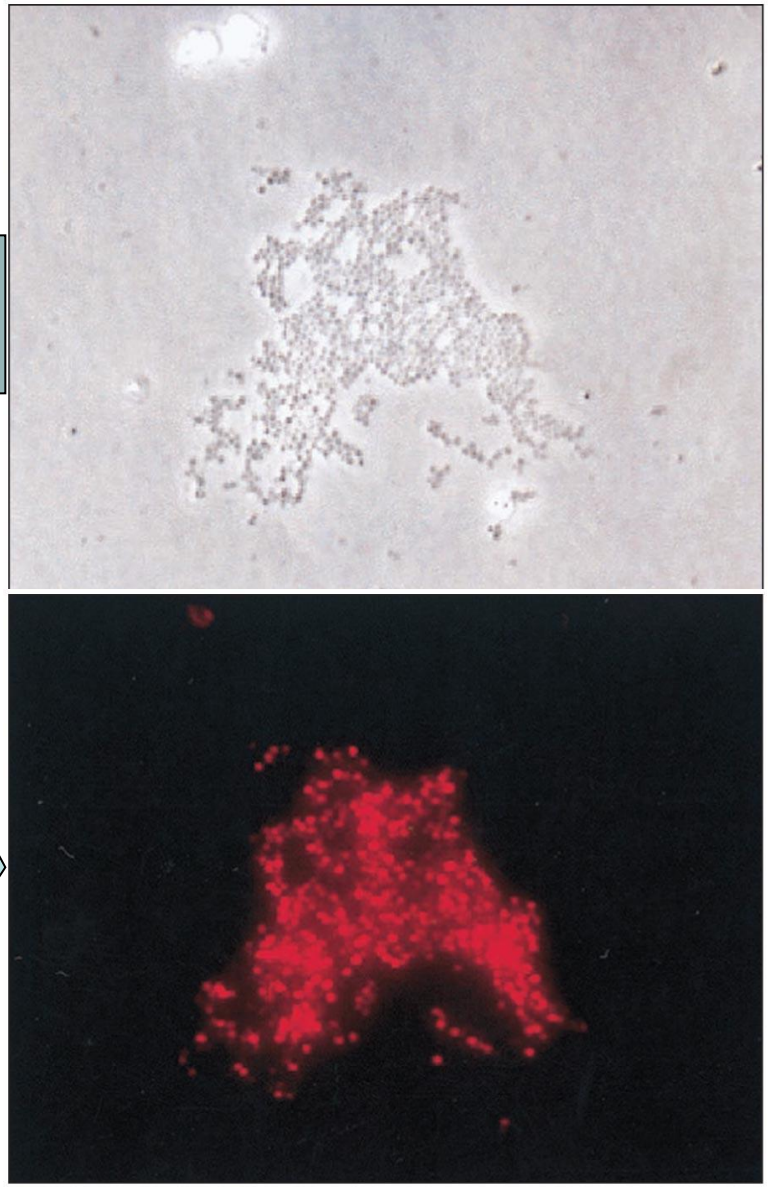




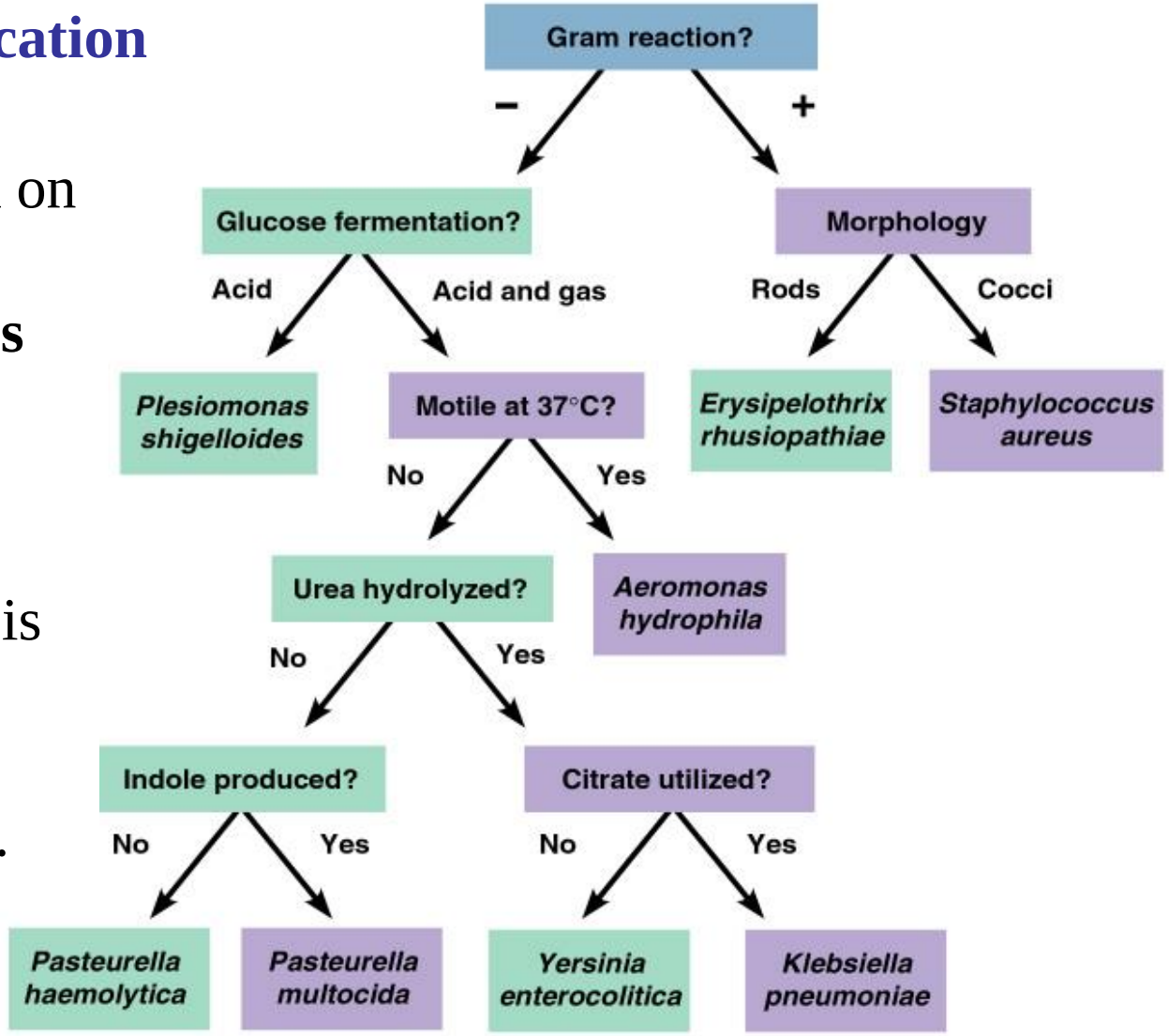
Figure 10.18

# Dichotomous Key (二叉式檢索表 )

Widely used for **identification**

Identification is based on **successive questions**, and **each question has two possible answer**.

After answering one question, the investor is directed to another question until an organism is identified.




*P287*

# Building a Cladogram (進化分支圖 · 進化樹)

Cladograms are maps that show evolutionary relationships among organisms (clado-means branch)(Fig. 10.19)

**1** Determine the sequence of bases in an rRNA molecule for each organism. Only a short sequence of bases is shown for this example.

| | |
|---|---|
| *Lactobacillus brevis* | AGUCCAGAGC |
| *L. sanfranciscensis* | GUAAAAGAGC |
| *L. acidophilus* | AGCGGAGAGC |
| *L. plantarum* | ACGUUAGAGC |

**2** Calculate the percentage of similarity in the nucleotide bases between pairs of species. For example, there is a 70% similarity between the sequences for *L. brevis* and *L. acidophilus*.

| | Percent similarity |
|---|---|
| *L. brevis* → *L. sanfranciscensis* | 50% |
| *L. brevis* → *L. acidophilus* | 70% |
| *L. brevis* → *L. plantarum* | 60% |
| *L. sanfranciscensis* → *L. acidophilus* | 50% |
| *L. sanfranciscensis* → *L. plantarum* | 50% |
| *L. plantarum* → *L. acidophilus* | 60% |



Figure 10.19

# Building a Cladogram

**3** Construct a cladogram. The length of the horizontal lines corresponds to the percent similarity values. Each branch point, or node, in the cladogram represents an ancestor common to all species beyond that node. Each node is defined by a similarity in rRNA present in all species beyond that branch point.

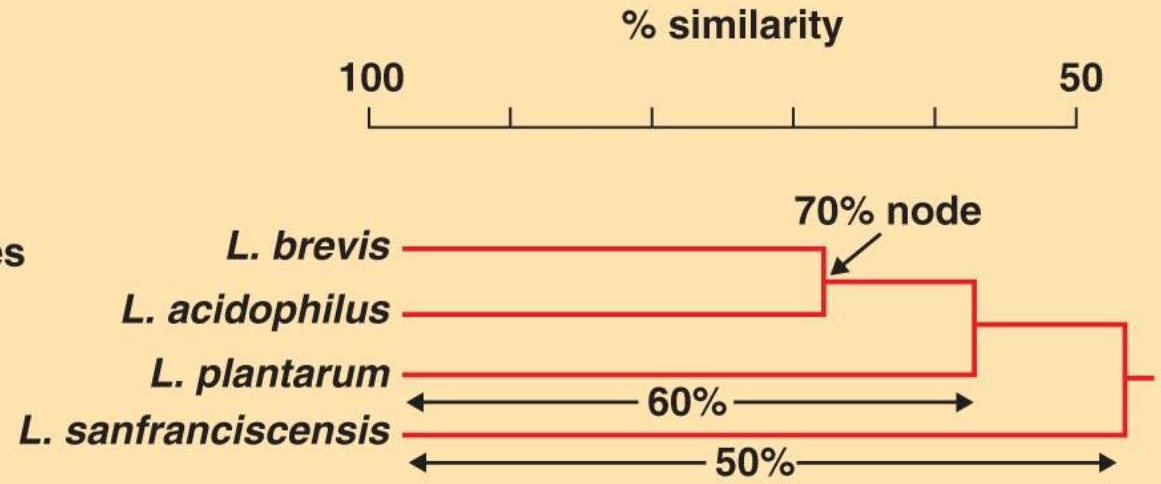





Figure 10.19