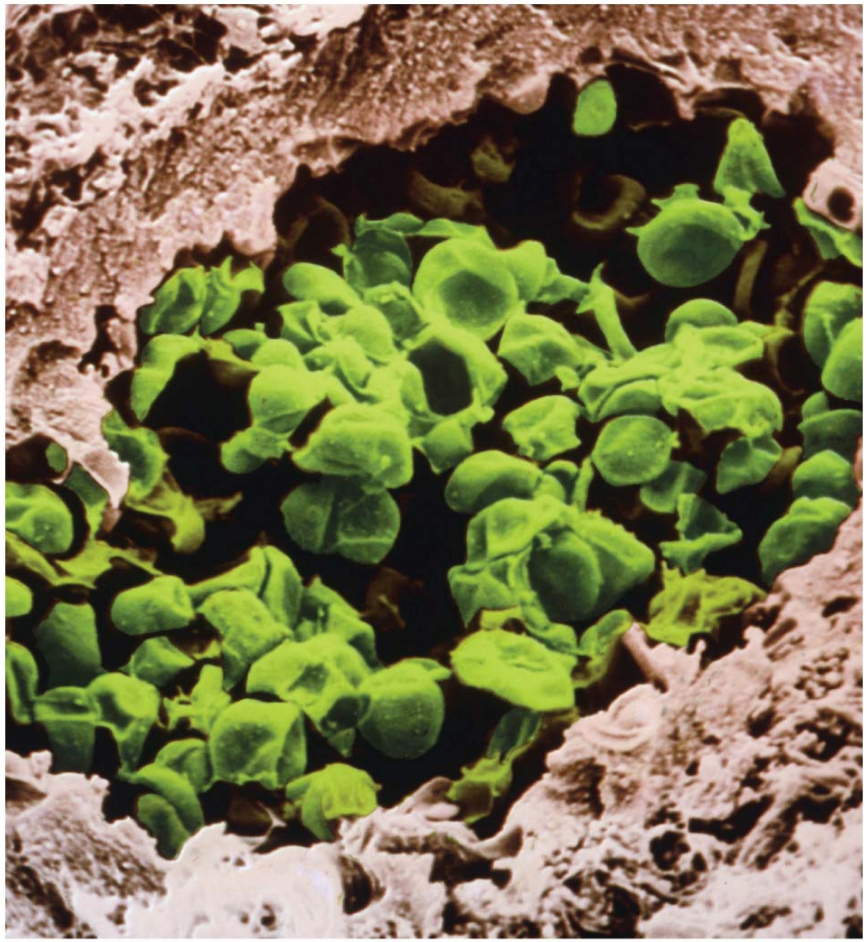
Chapter 10

Classification of Microorganisms



Taxonomy

- The science of classifying organisms
- Provides universal names for organisms
- Provides a reference for identifying organisms

Phylogeny

- The study of the evolutionary history of organisms
  - In Aristotle's time all life was categorized as either plant or animal
  - Today rRNA is used to distinguish and relate living organisms
- The goal of the “All Species Inventory” of 2001–2025 // To identify all species of life on Earth

Placing Bacteria

1735	Kingdoms Plantae and Animalia
1857	Bacteria and fungi put in the Kingdom Plantae—“Flora”
1866	Kingdom Protista proposed for bacteria, protozoa, algae, and fungi
1937	*Prokaryote* introduced for cells “without a nucleus”
1961	*Prokaryote* defined as cell in which nucleoplasm is not surrounded by a nuclear membrane
1959	Kingdom Fungi
1968	Kingdom Prokaryotae proposed
1978	Two types of prokaryotic cells found

The Three-Domain System

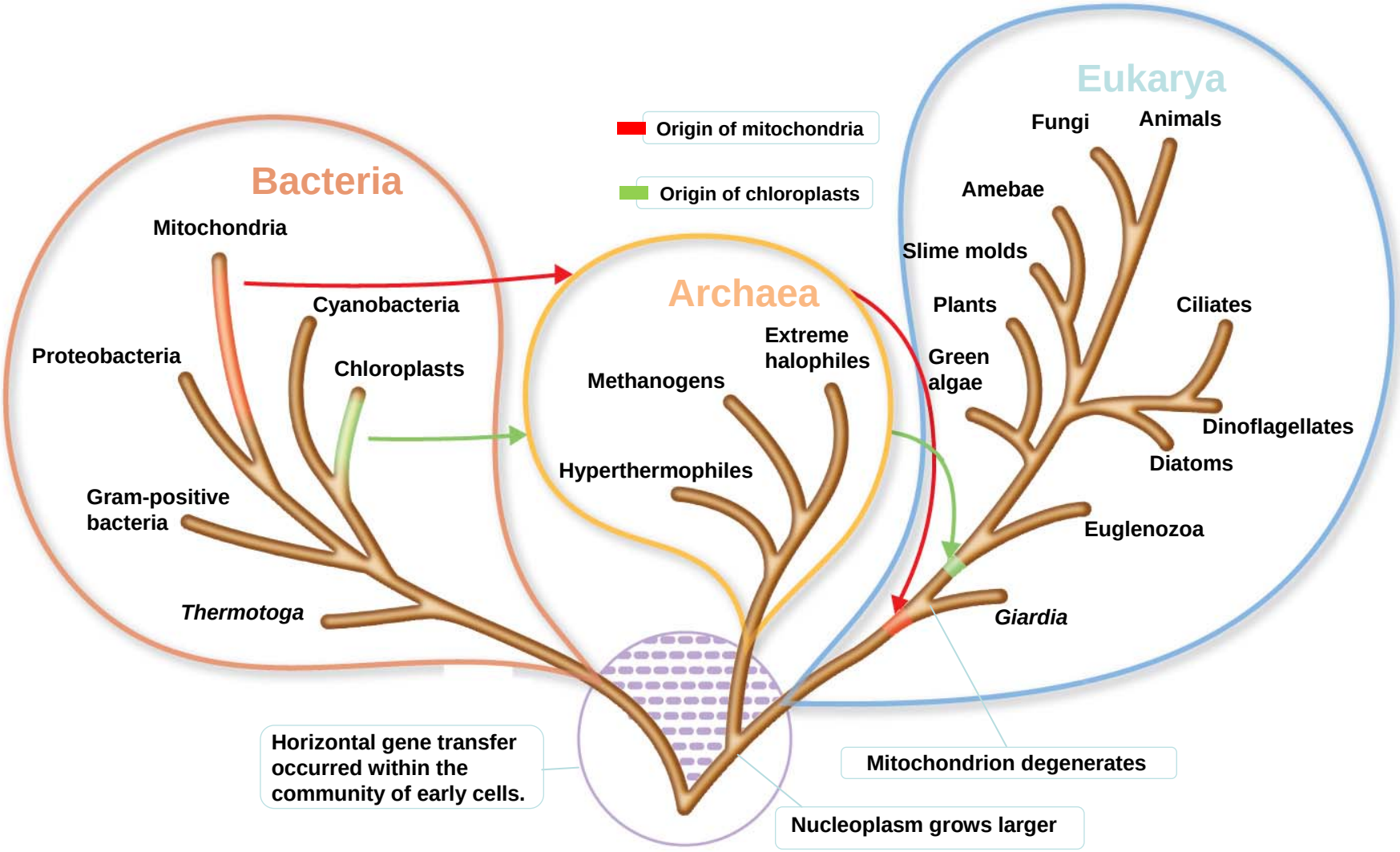
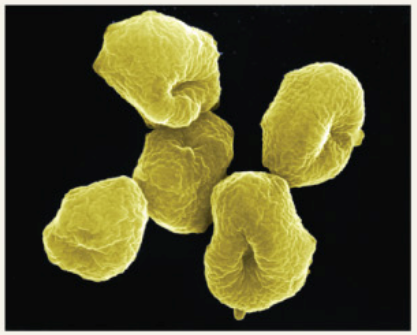
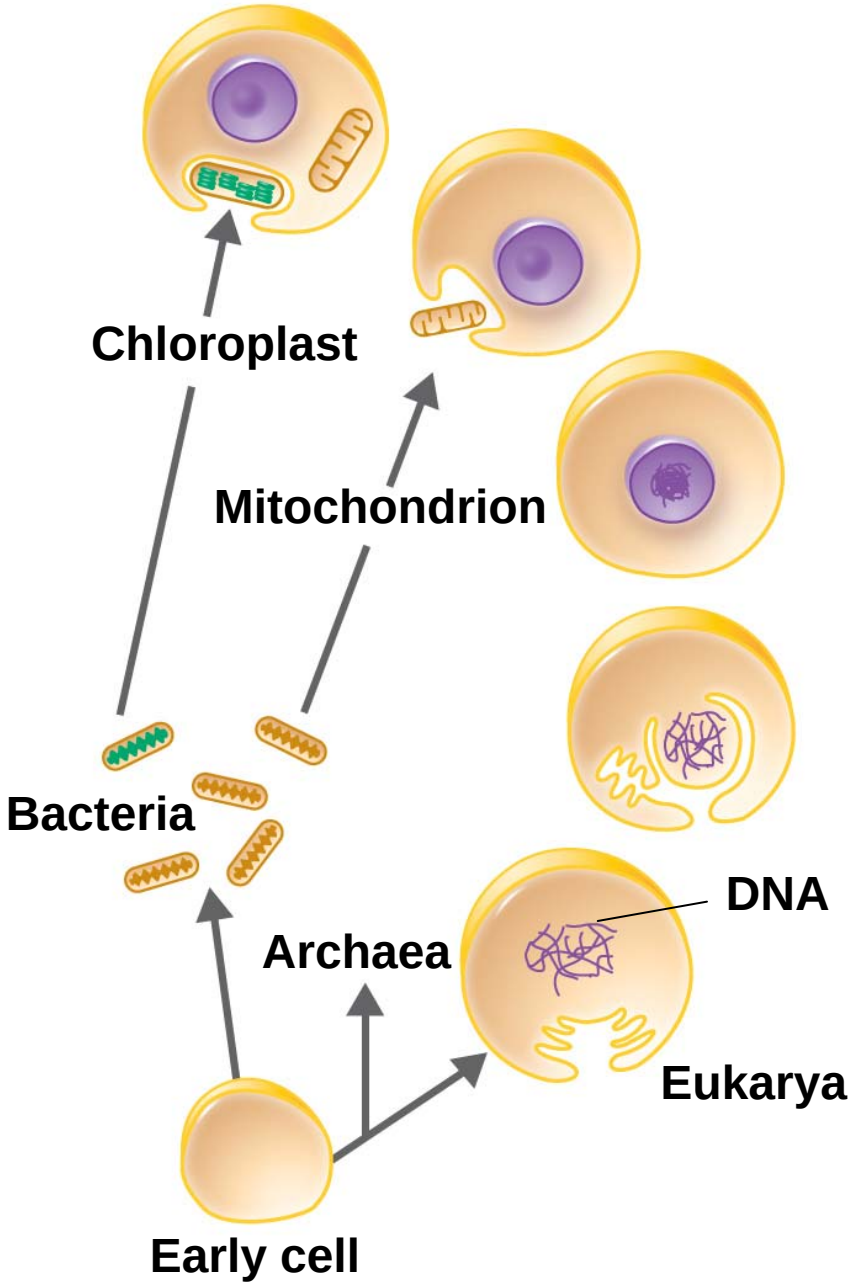


TABLE 10.1 Some Characteristics of Archaea, Bacteria, and Eukarya

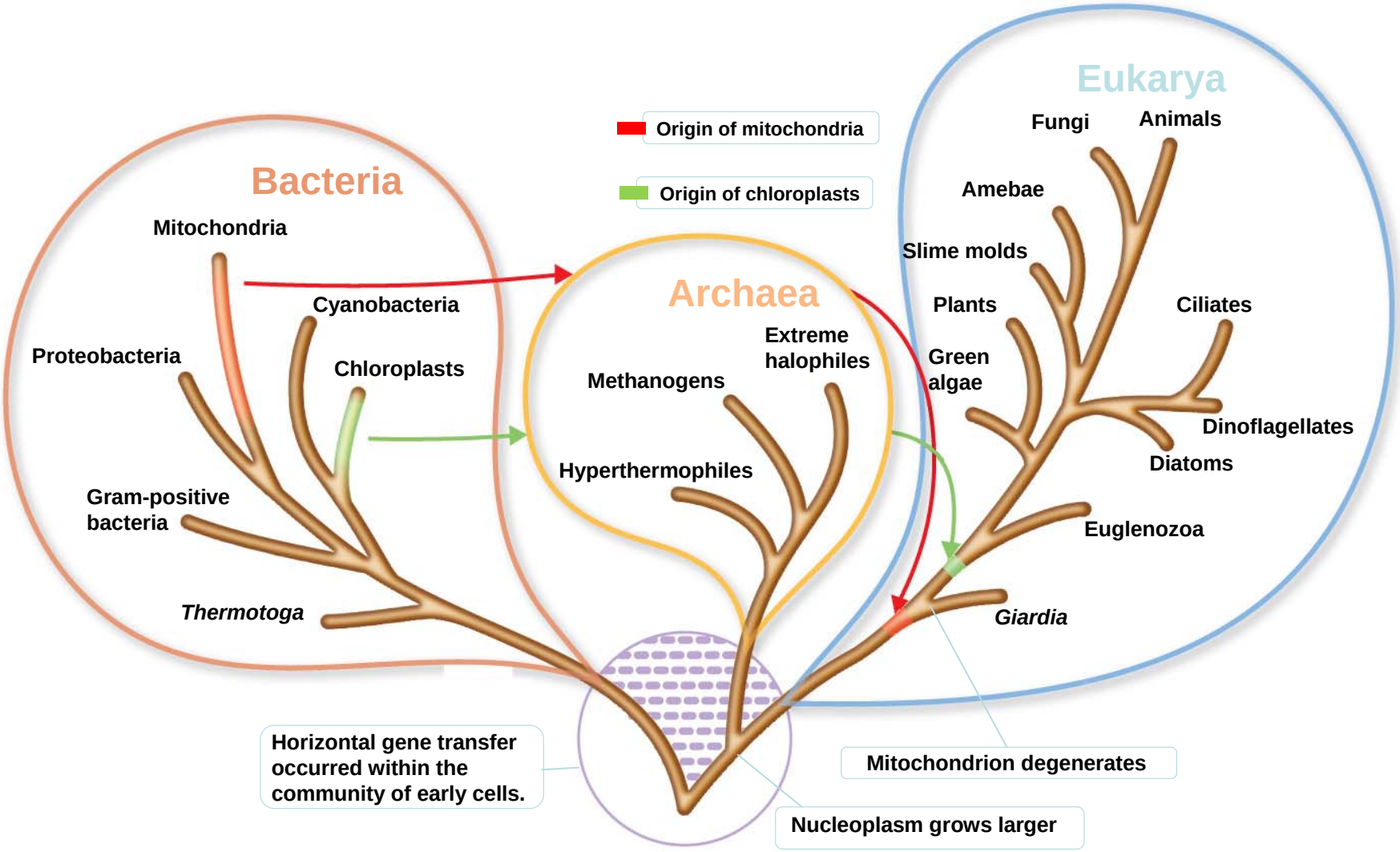
	Archaea	Bacteria	Eukarya
	 *Sulfolobus* SEM 1 μm	 *E. coll* SEM 1 μm	 *Amoeba* SEM 5 μm
Cell Type	Prokaryotic	Prokaryotic	Eukaryotic
Cell Wall	Varies in composition; contains no peptidoglycan	Contains peptidoglycan	Varies in composition; contains carbohydrates
Membrane Lipids	Composed of branched carbon 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

*Binds to ribosomal protein; found in all bacteria.
†A sequence of bases in tRNA found in all eukaryotes and bacteria: guanine-thymine-pseudouridine-cytosine-guanine.

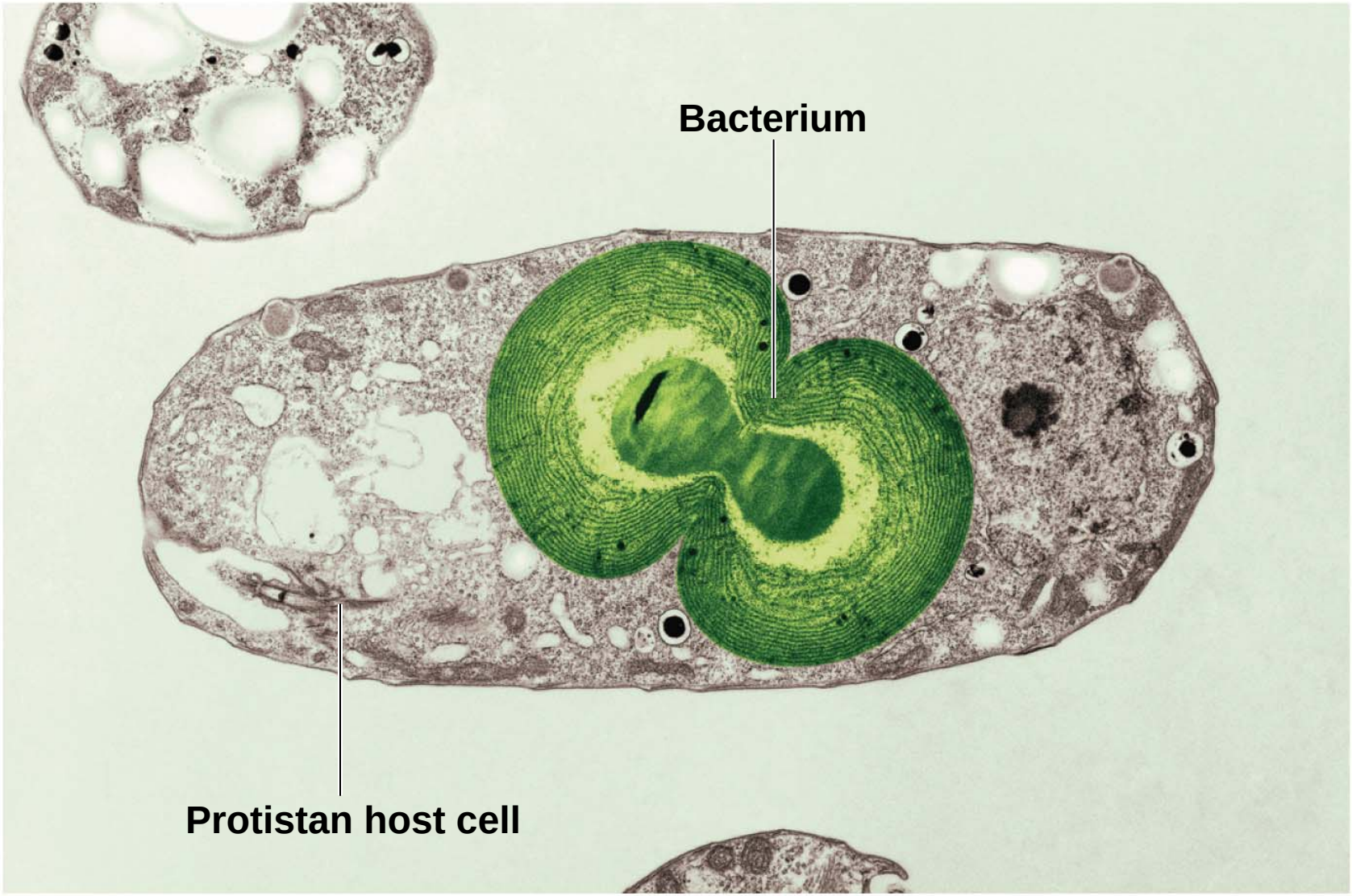
A model of the origin of eukaryotes.



The Three-Domain System



***Cyanophora paradoxa*.**



Two life forms // coexist with each other.

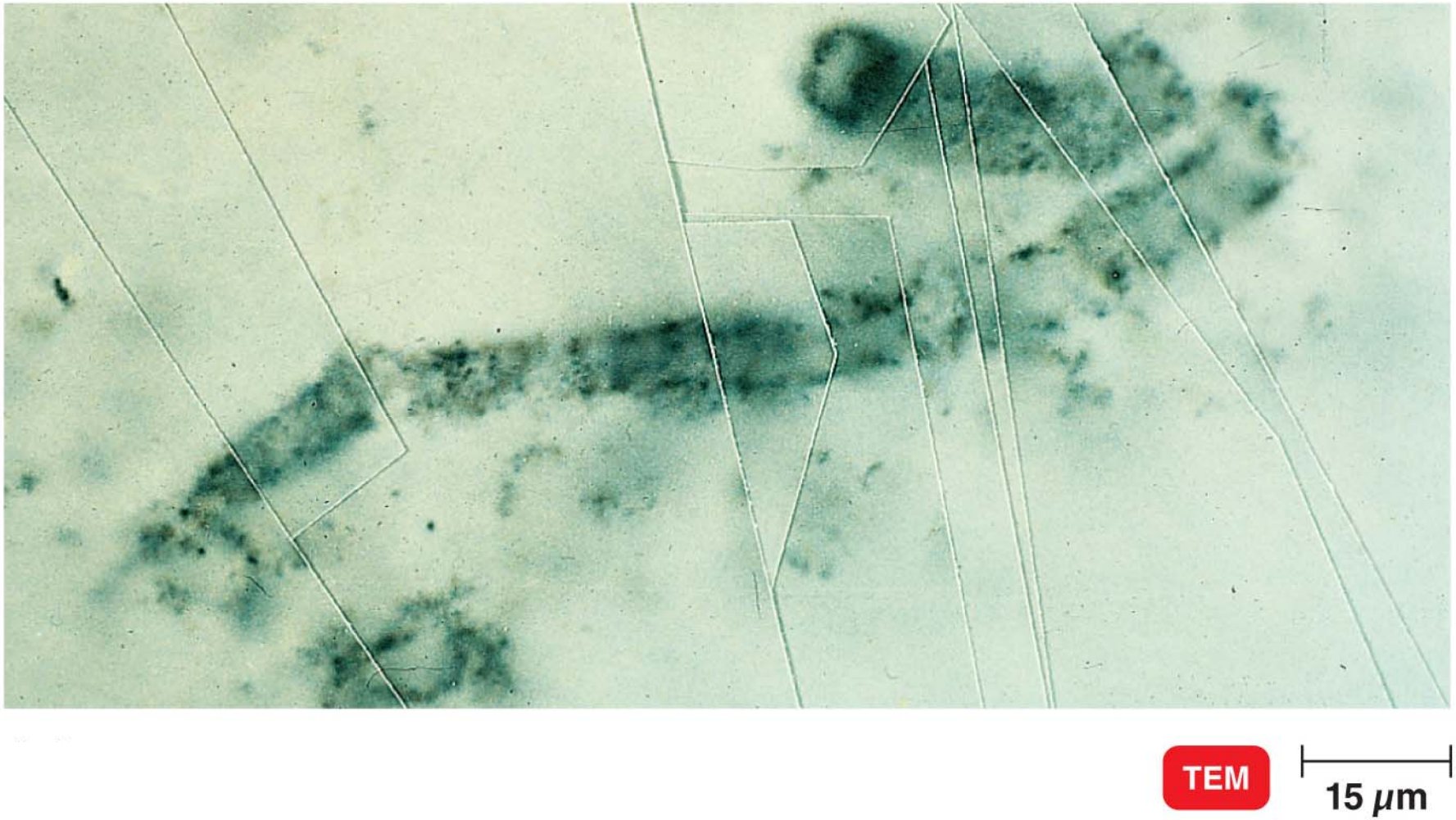
Fossilized prokaryotes.

Bacterial communities form rocklike pillars called stromatolites. These began growing about 3000 years ago. However, the oldest Stromatolites have been dated as old as 3.5 billion years old!!!!!

Fossilized prokaryotes.

Cut section through a fossilized stomatolite that flourished 2 billion years ago.

Fossilized prokaryotes.



Filamentous prokaryotes from the Early Precambrian (3.5 billion years ago) of western Australia.

White Cliffs of Dover, England // Diatom cell walls

Phylogenetics

- 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
  - Fossils
  - rRNA

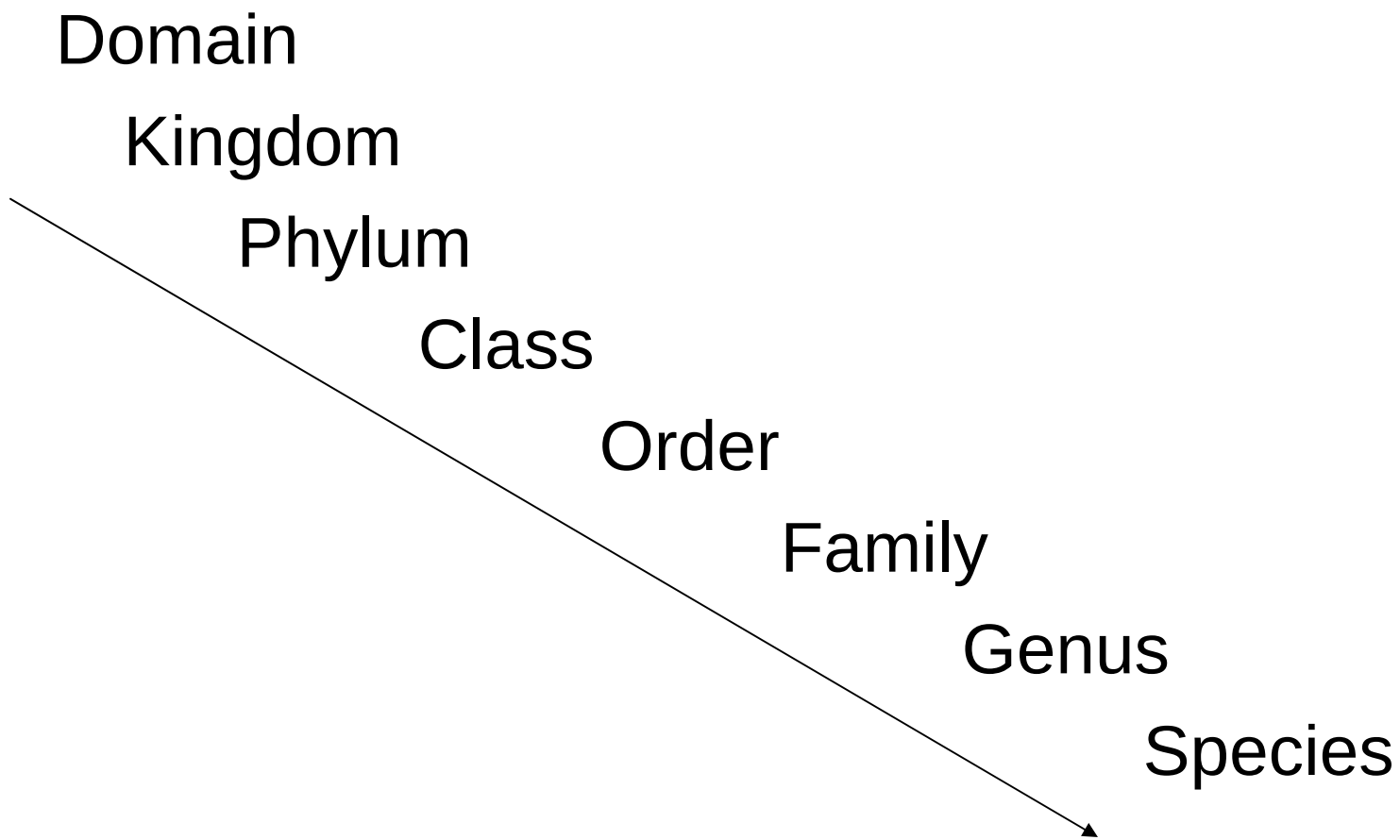
Scientific Nomenclature

- Common names
  - Vary with languages
  - Vary with geography
- Binomial nomenclature (genus + specific epithet)
  - Used worldwide // e.g. *Escherichia coli & Homo sapiens*

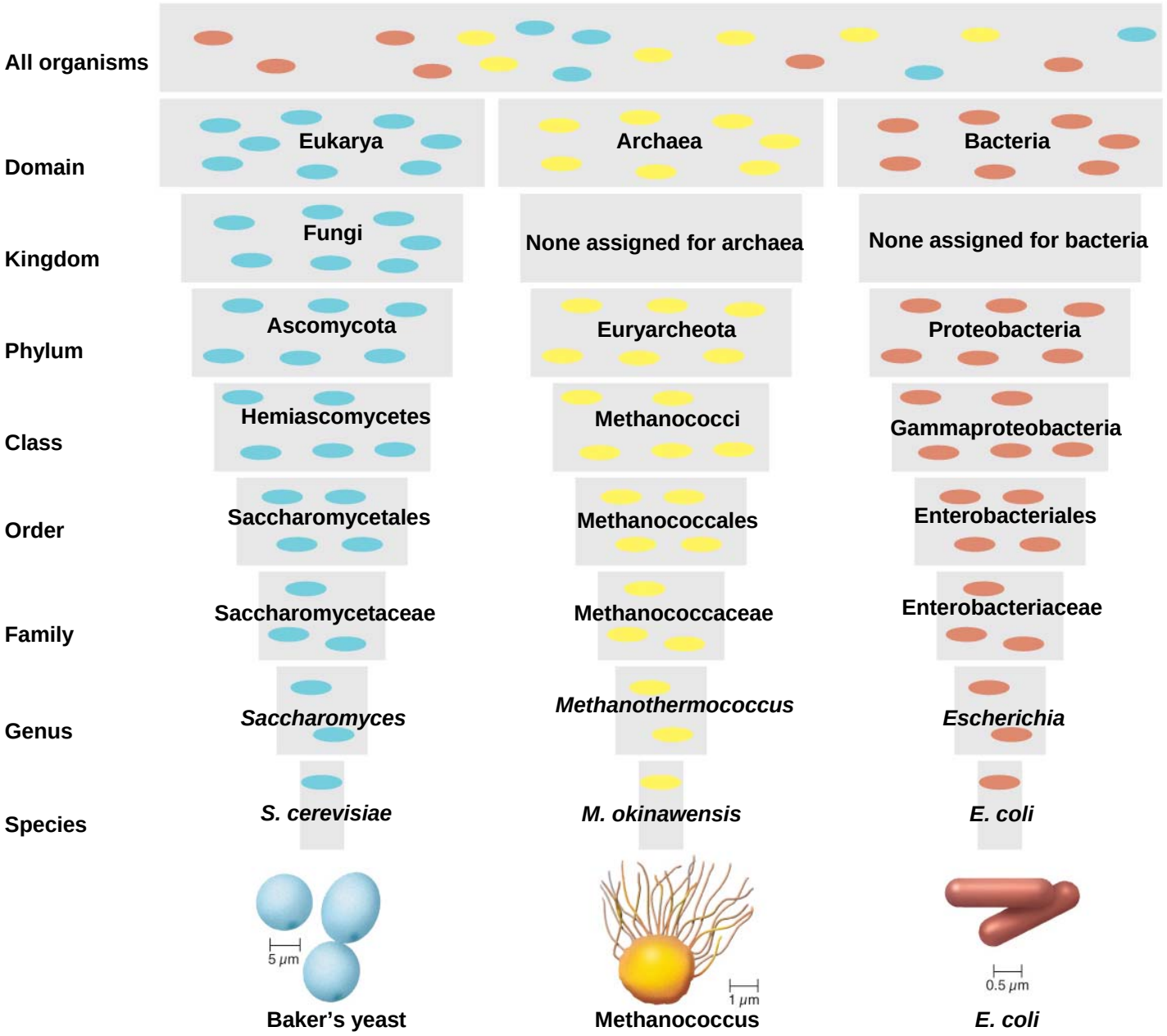
Scientific Names

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 (*typh*-) in mice (*muri*-)
Streptococcus pyogenes	Chains of cells (*strepto*-)	Forms pus (*pyo*-)
Penicillium chrysogenum	Tuftlike (*penicill*-)	Produces a yellow (*chryso*-) pigment
Trypanosoma cruzi	Corkscrew-like (*trypano* = borer; *soma* = body)	Honors Oswaldo Cruz

Taxonomic Hierarchy



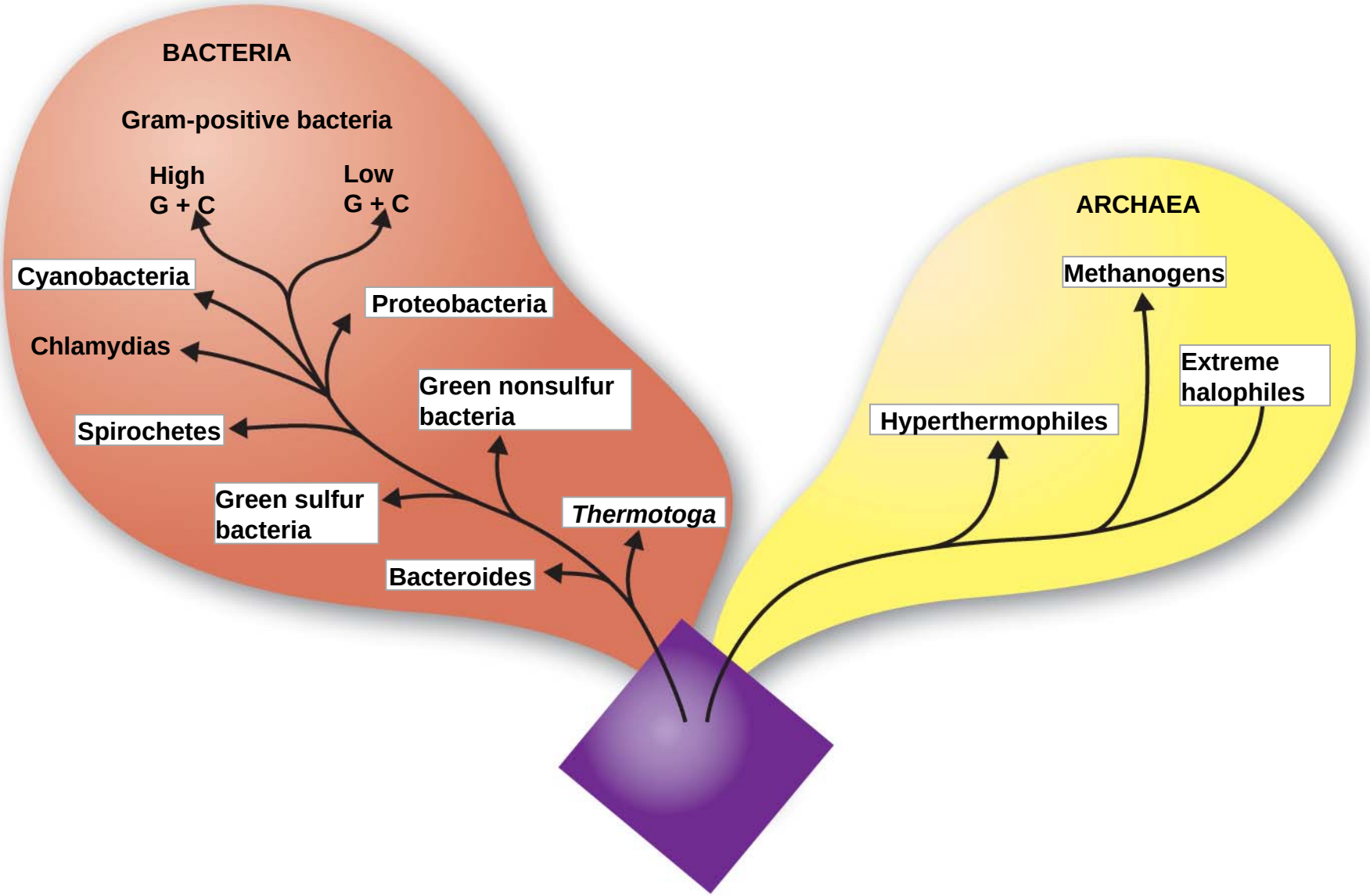
The taxonomic hierarchy.



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.



Classification of Eukaryotes

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

Classification of Eukaryotes

- **Animalia**: multicellular; no cell walls; chemoheterotrophic // helminths & arthropods
- **Plantae**: multicellular; cellulose cell walls; usually photoautotrophic
- **Fungi**: chemoheterotrophic; unicellular or multicellular; cell walls of chitin; develop from spores or hyphal fragments
- **Protista**: a catchall kingdom for eukaryotic organisms that do not fit other kingdoms // Grouped into **clades** based on rRNA

Classification of Viruses

- **Viral species**: population of viruses with similar characteristics that occupies a particular ecological niche
- Obligate parasite
- Viruses should not be described as alive or dead
- Viruses should be described as active or inactive

References

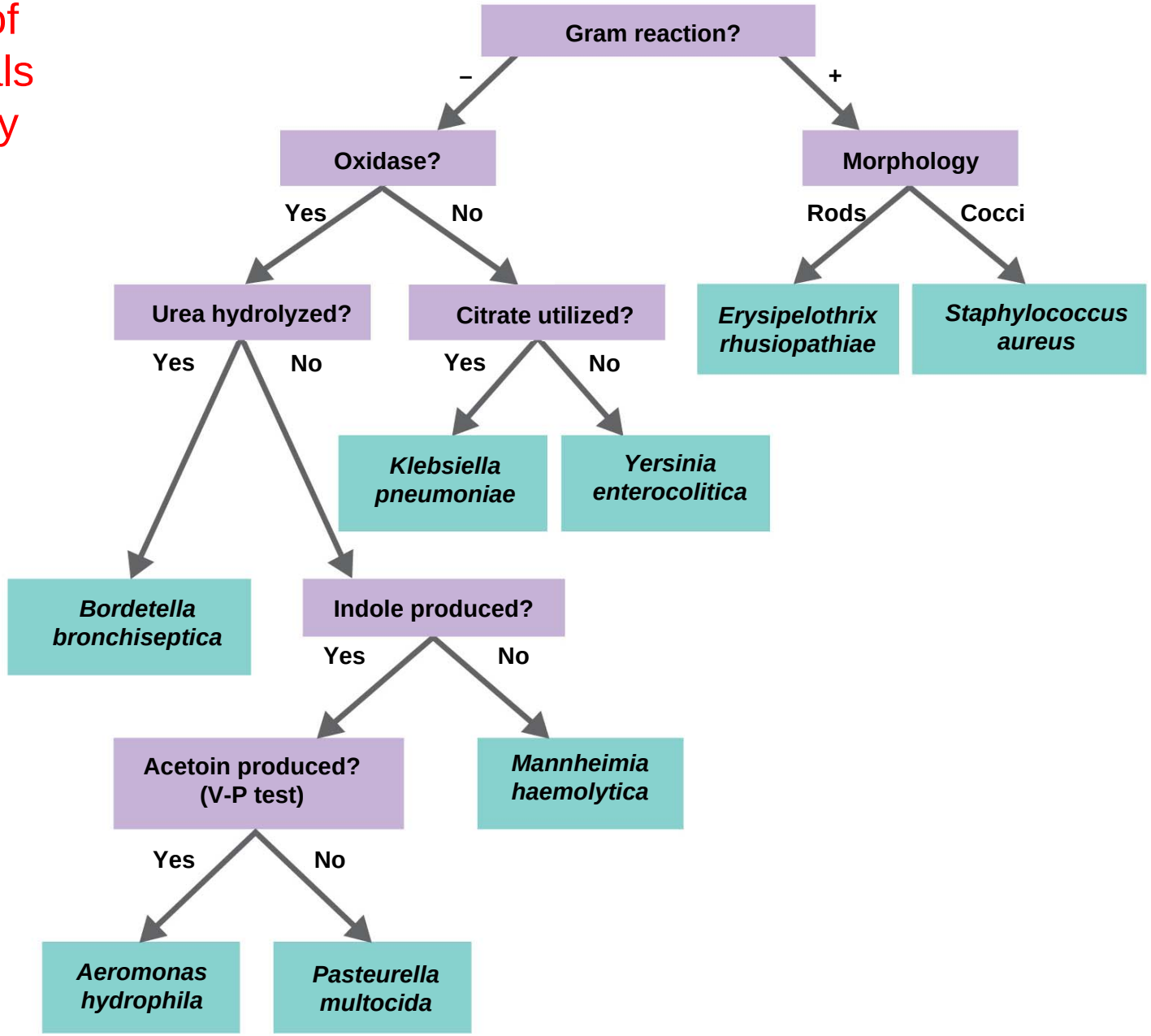
International Journal of Systematic and Evolutionary Microbiology	Articles with evidence of new species or classification
Bergey's Manual of Systematic Bacteriology	Provides phylogenetic and identification information on bacteria and archaea
Approved Lists of Bacterial Names	Lists species of known prokaryotes Based on published articles

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

Mass Deaths of Marine Mammals Spur Veterinary Microbiology



Classification and Identification

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

References

Bergey's Manual of Determinative Bacteriology Provides *identification* schemes for identifying bacteria and archaea	Morphology, differential staining, biochemical tests
Bergey's Manual of Systematic Bacteriology 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, 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)

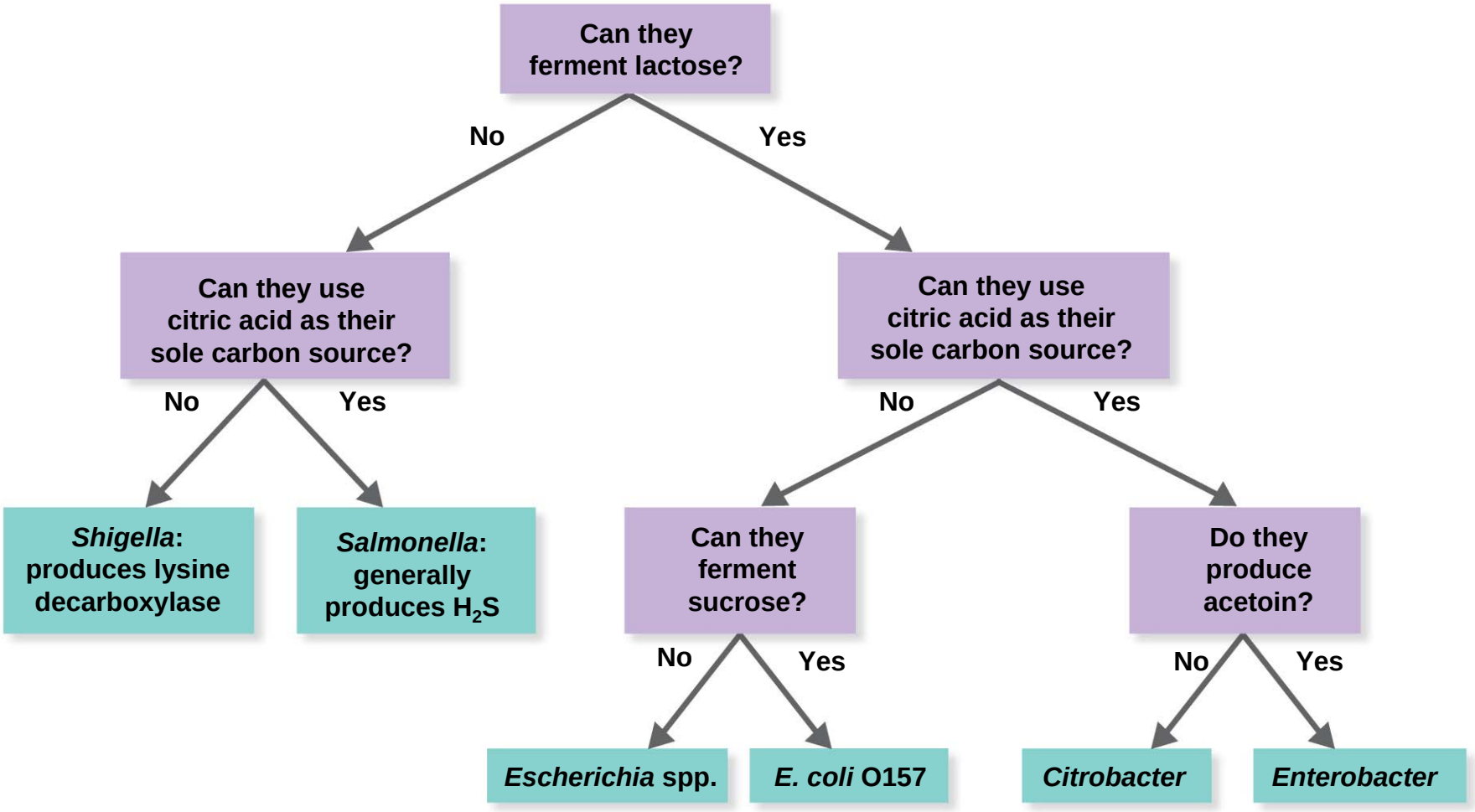
Filled out by one person

Filled out by different person

Identification Methods

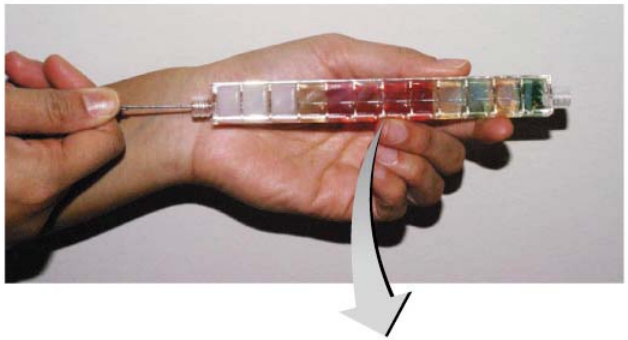
- **Morphological characteristics**: useful for identifying eukaryotes
- **Differential staining**: Gram staining, acid-fast staining
- **Biochemical tests**: determines presence of bacterial enzymes

The use of metabolic characteristics to identify selected genera of enteric bacteria.

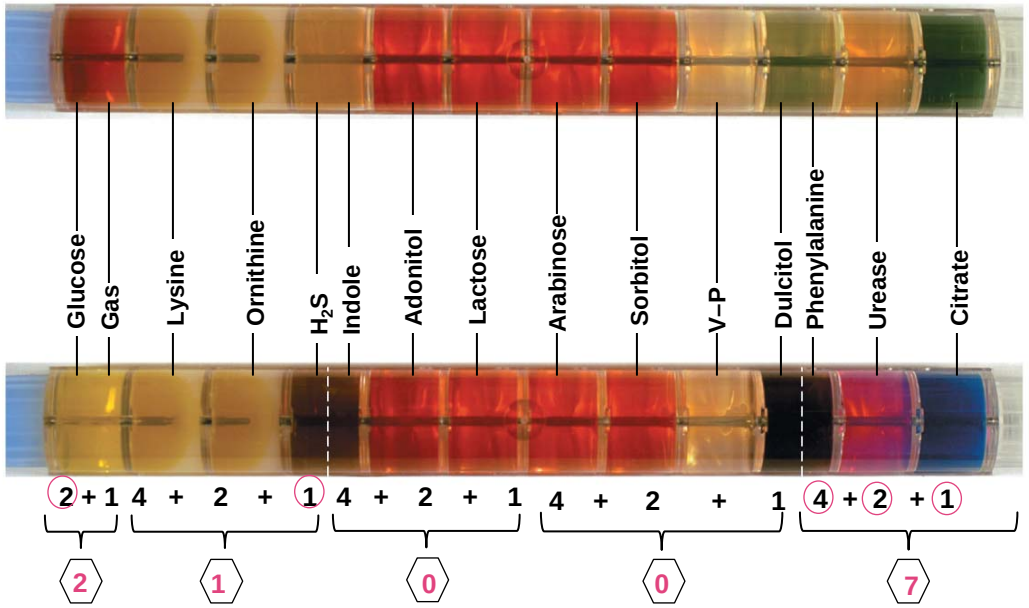


One type of rapid identification method for bacteria: Enterotube II from Becton Dickinson.

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.**

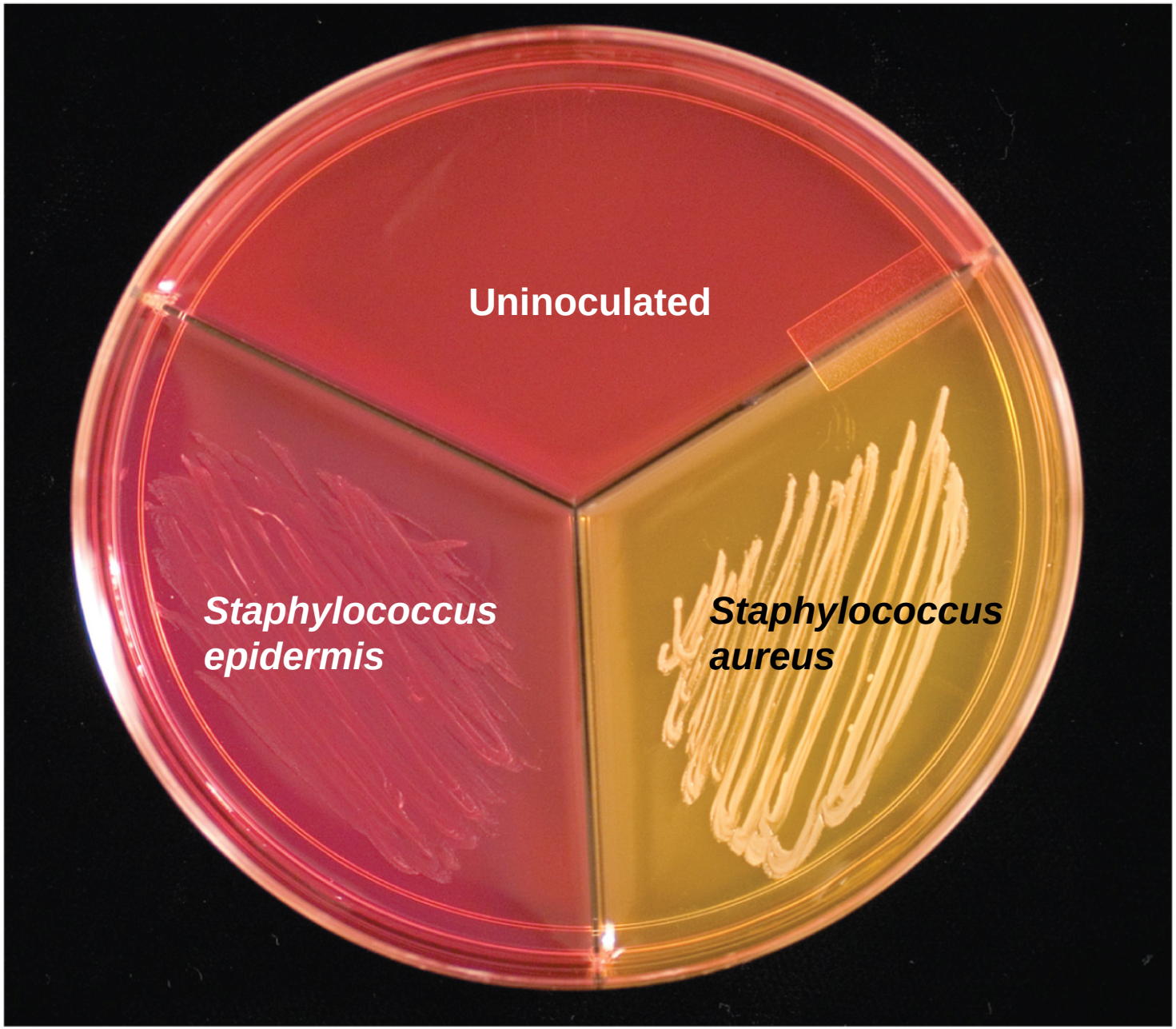


3. **The value for each positive test is circled, and the numbers from each group of tests are added to give the ID value.**

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$^-$	

Differential medium.

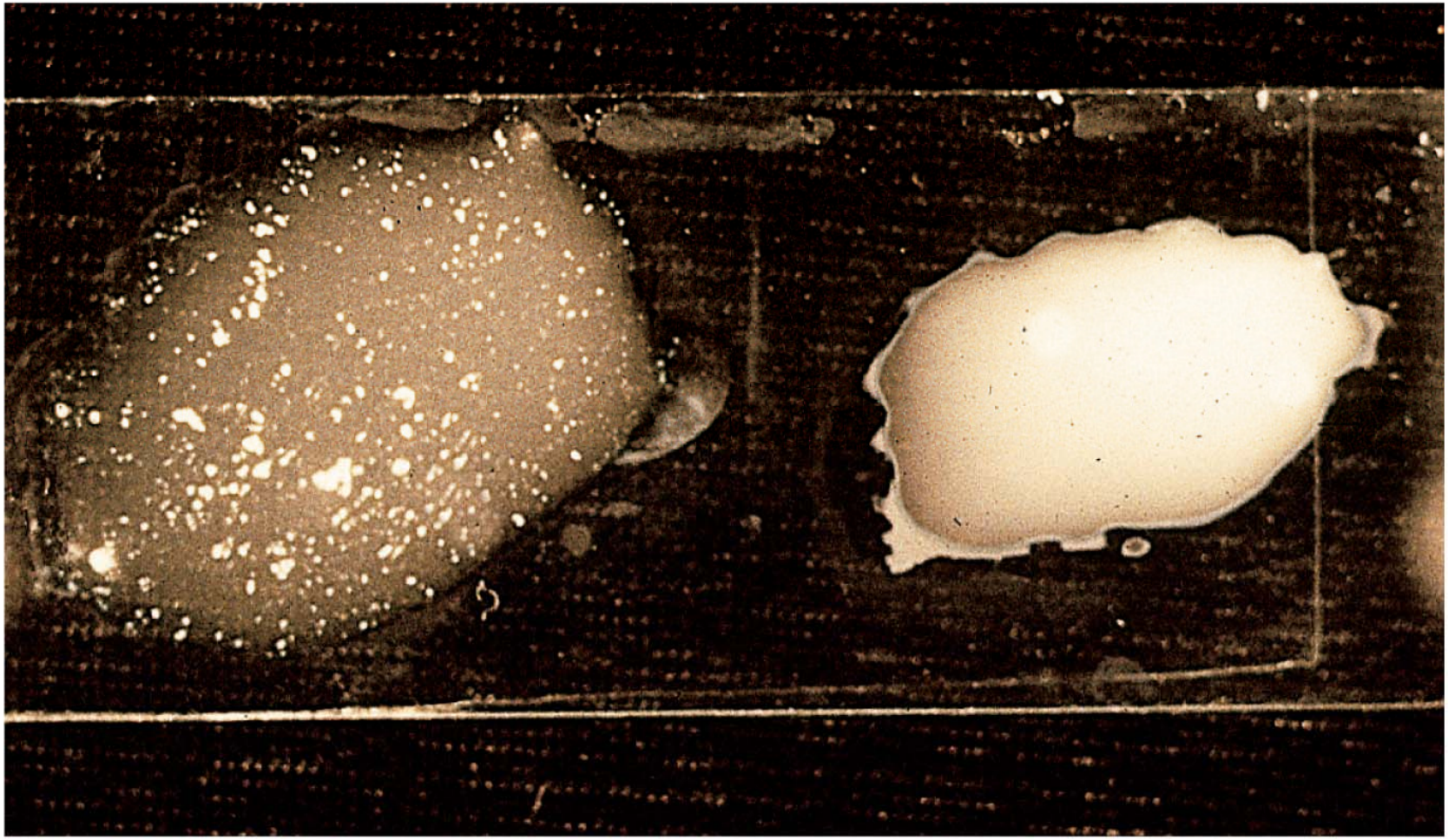


Serology

- Combine known antiserum plus unknown bacterium

- **Slide agglutination test**

A slide agglutination test.



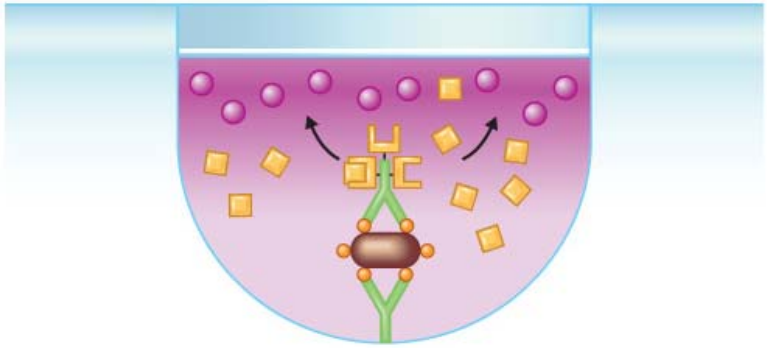
(a) **Positive test**

(b) **Negative test**

ELISA

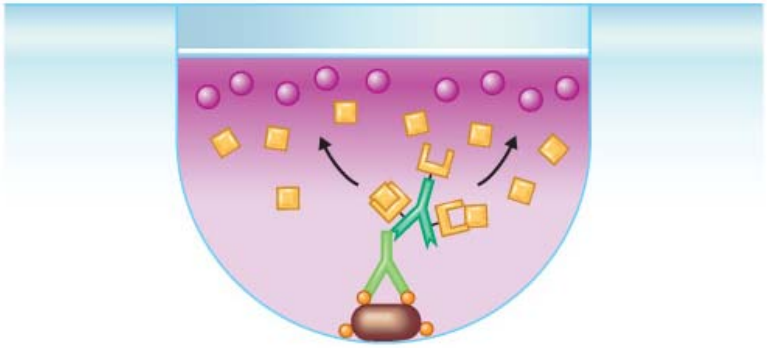
- **Enzyme-linked immunosorbent assay**
- Known antibodies
- Unknown type of bacterium
- Antibodies linked to enzyme
- Enzyme substrate

The ELISA method // **Enzyme-linked immunosorbent assay**



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**



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**

An ELISA test.

(a) **A technician uses a micropipette to add samples to a microplate for an ELISA.**

(b) **ELISA results are then read by the computer scanner.**

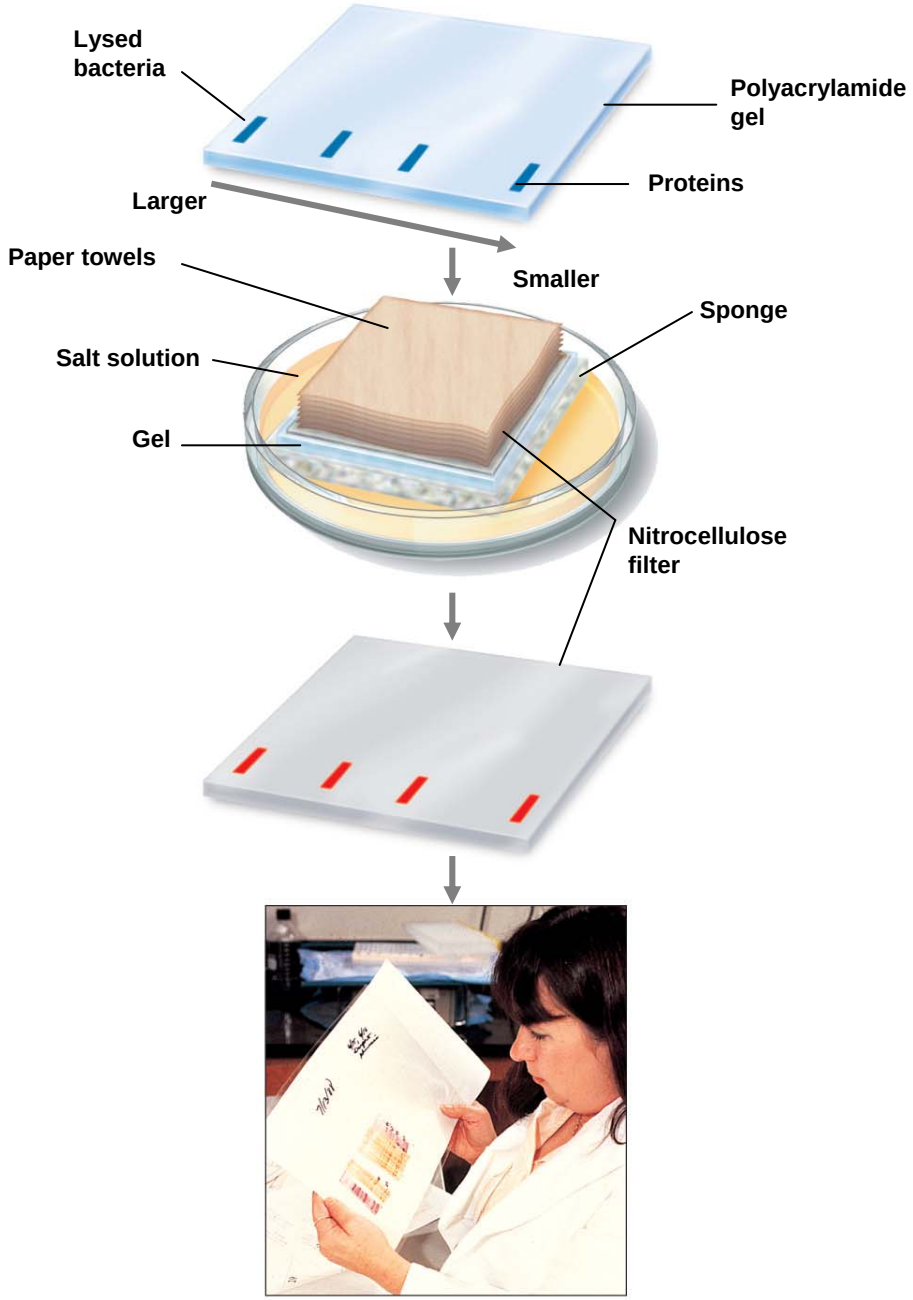
The Western blot.

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

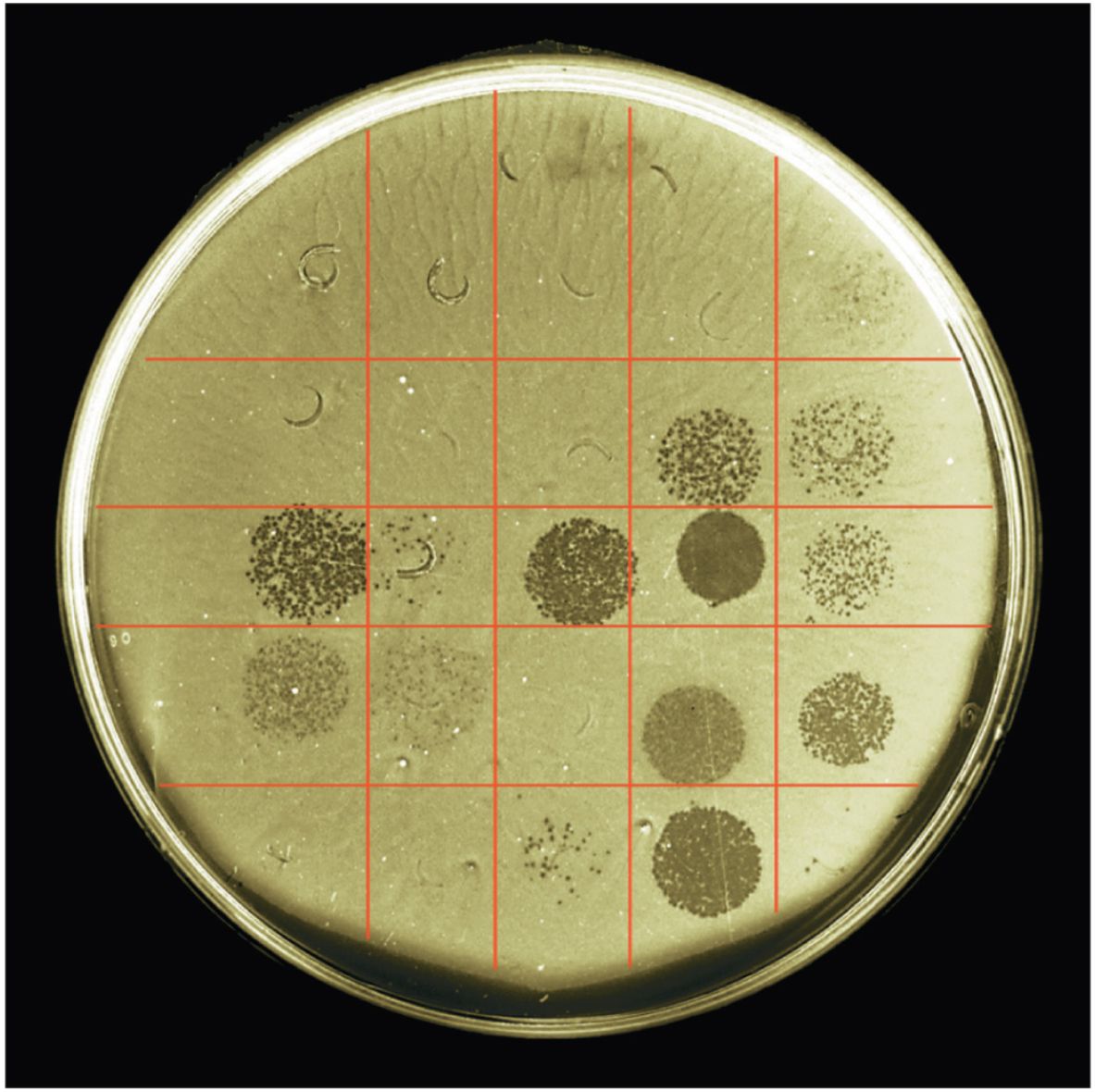
2. The bands are transferred to a nitrocellulose filter by blotting. Each band consists of many molecules of a particular protein (antigen). The bands are not visible at this point.

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 anti-human 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.



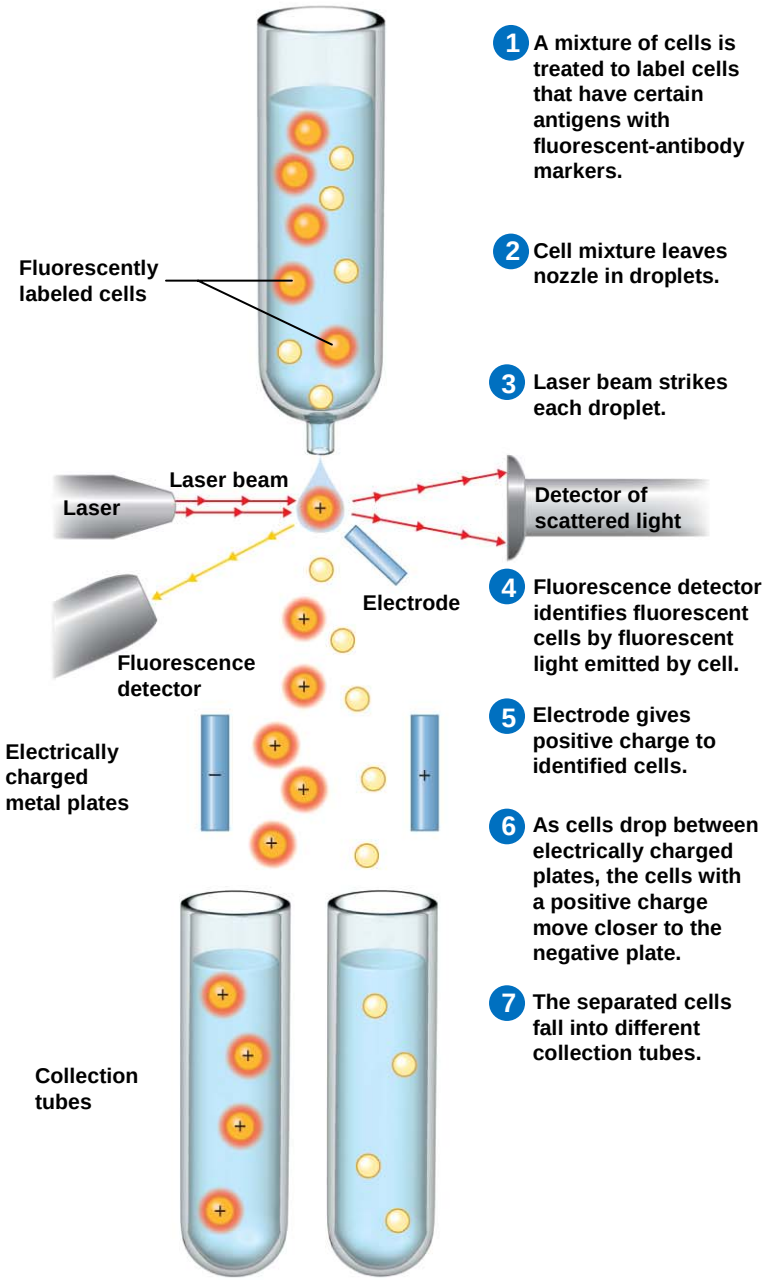
Phage typing of a strain of *Salmonella enterica.*



Flow Cytometry

- Uses differences in electrical conductivity between species
- Fluorescence of some species
- Cells selectively stained with antibody plus fluorescent dye

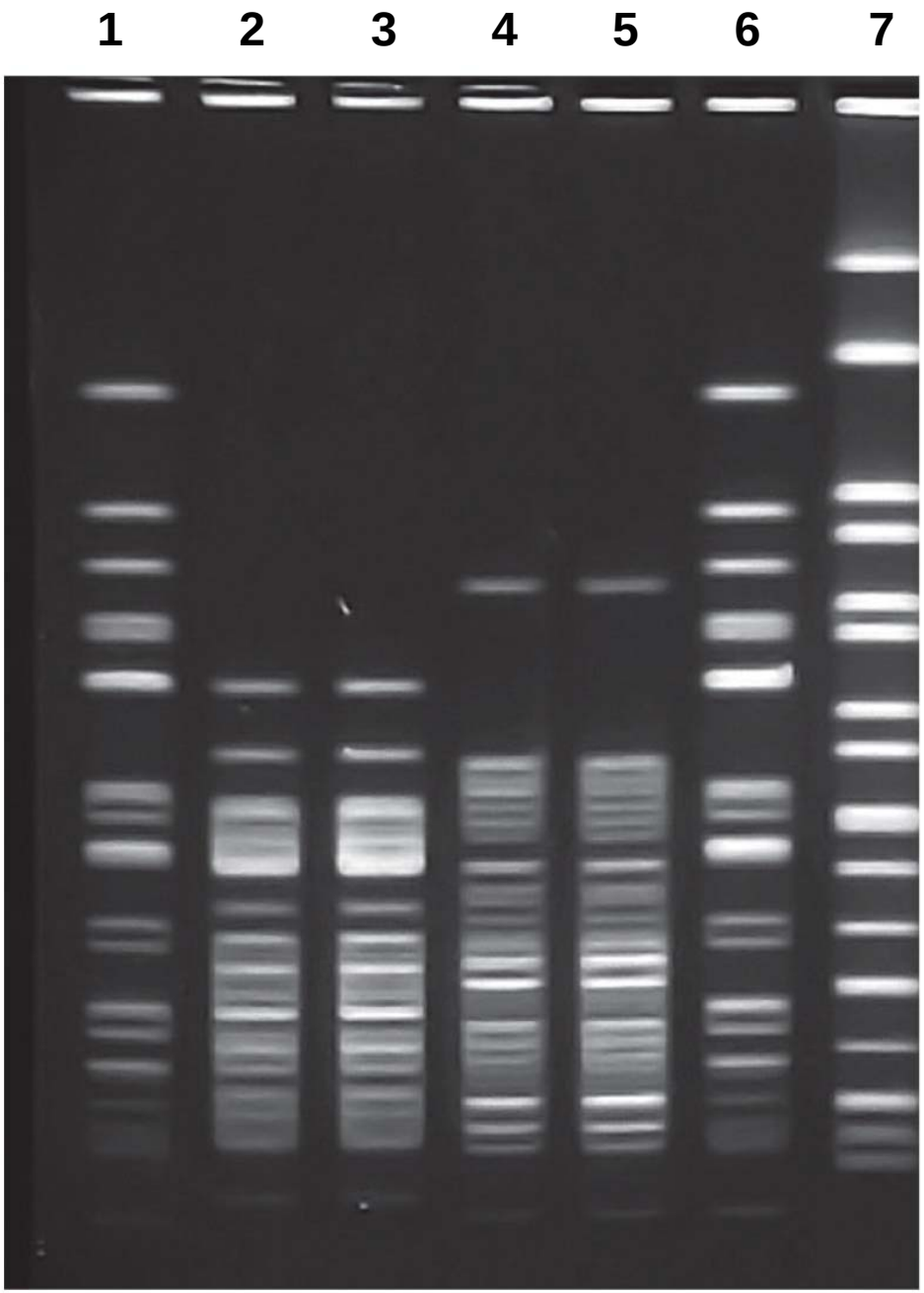
The fluorescence-activated cell sorter (FACS).



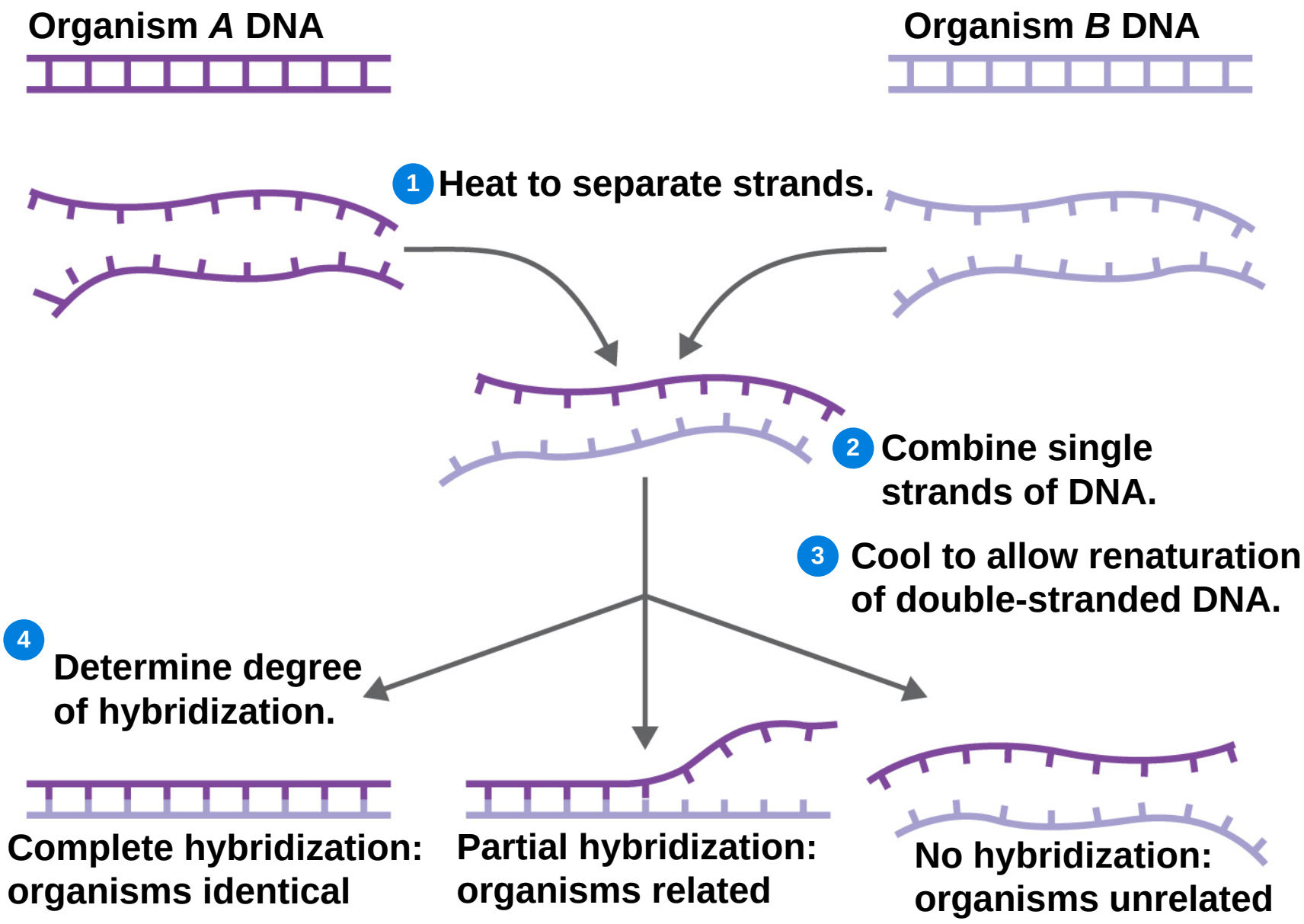
Genetics

- DNA base composition // Guanine + cytosine moles% (GC)
- DNA fingerprinting // Electrophoresis of restriction enzyme digests
- rRNA sequencing
- Polymerase chain reaction (PCR)

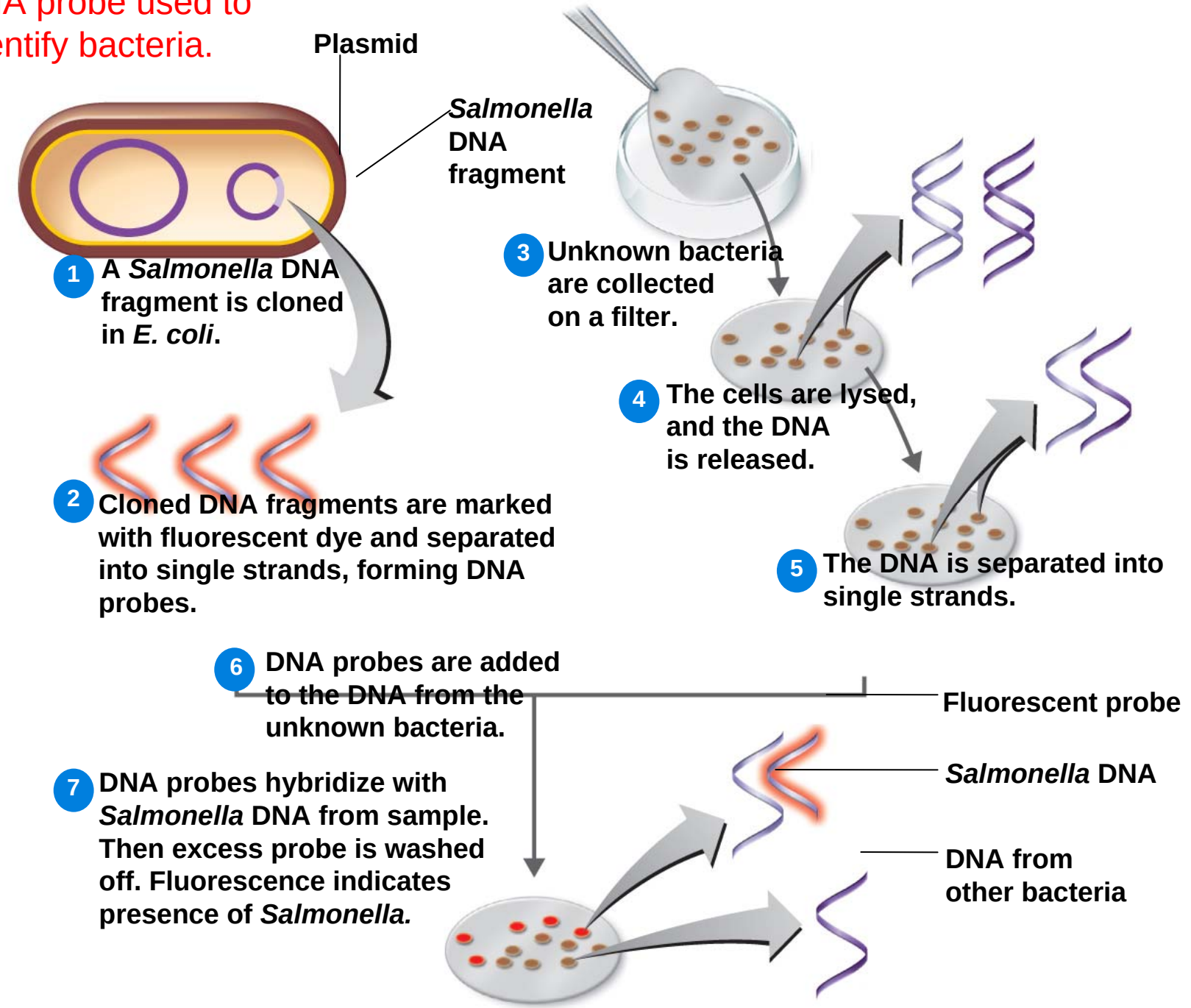
DNA Fingerprints



DNA-DNA hybridization.

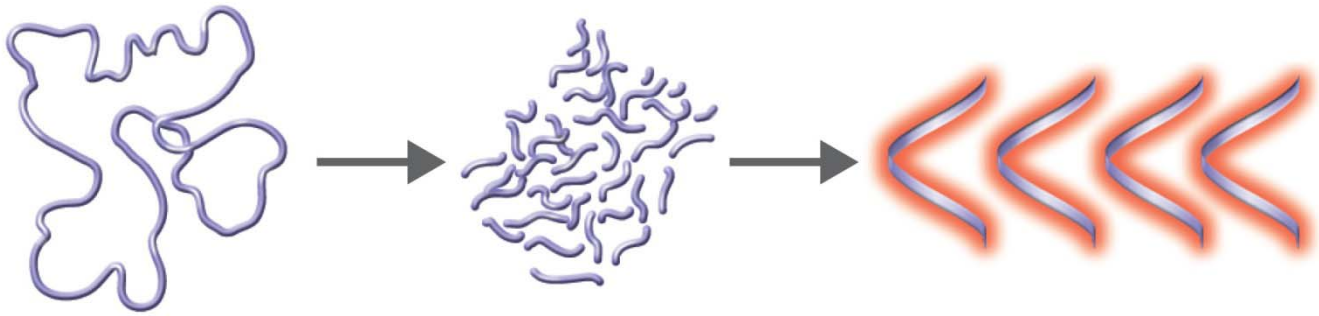


A DNA probe used to identify bacteria.



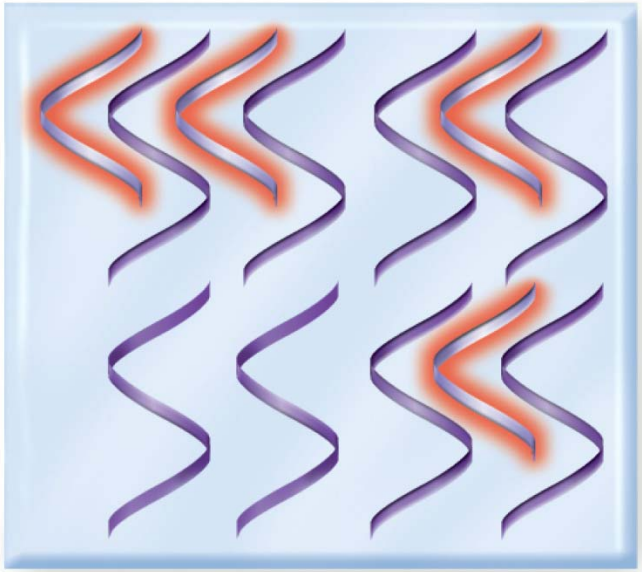
DNA Chip

(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.**

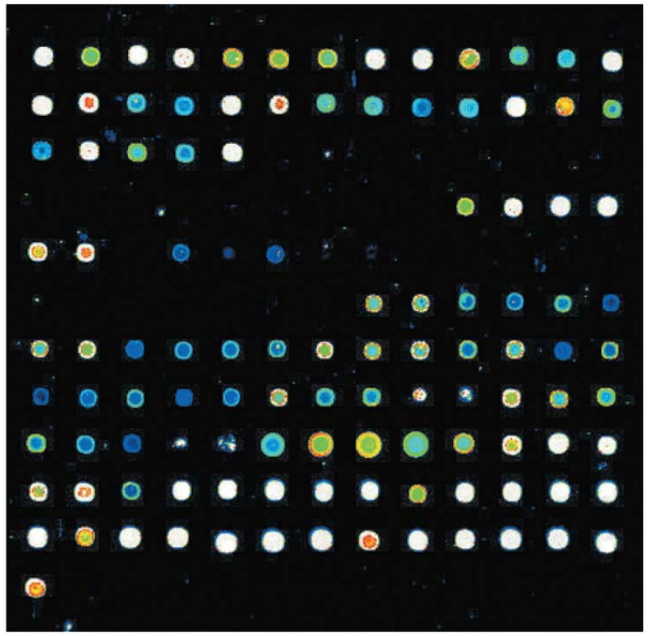


(b) **Unknown DNA from a sample is separated into single strands, enzymatically cut, and labeled with a fluorescent dye.**

DNA Chip



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

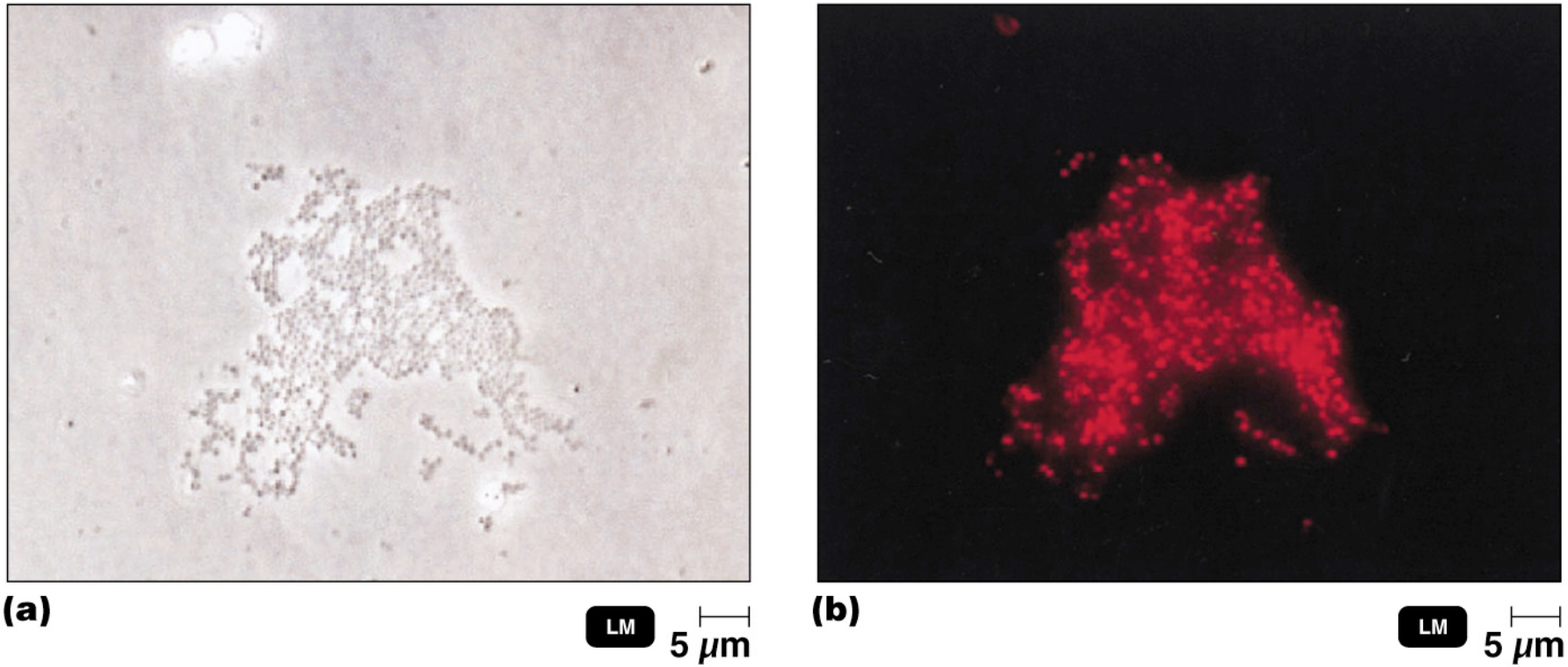


(d) **The tagged DNA will bind only to the complementary DNA on the chip. The bound DNA will be detected by its fluorescent dye and analyzed by a computer. In this *Salmonella* antimicrobial resistance gene microarray, *S. typhimurium*-specific antibiotic resistance gene probes are green, *S. typhi*-specific resistance gene probes are red, and antibiotic-resistance genes found in both serovars appear yellow/orange.**

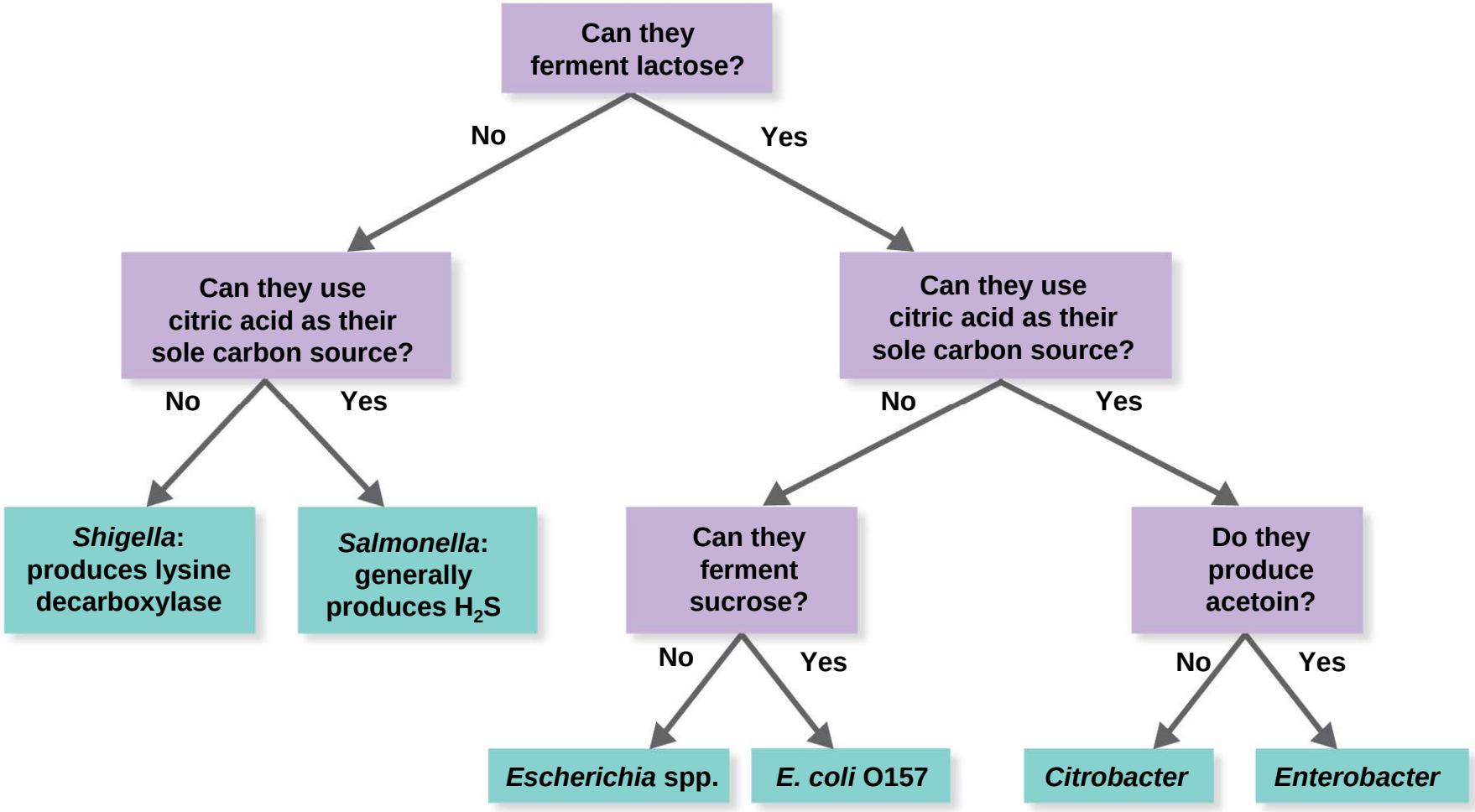
FISH

- **Fluorescent in situ hybridization**
- Add DNA probe for *S. aureus*

FISH, or fluorescent in situ hybridization.



The use of metabolic characteristics to identify selected genera of enteric bacteria.



Building a Cladogram

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%

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.**