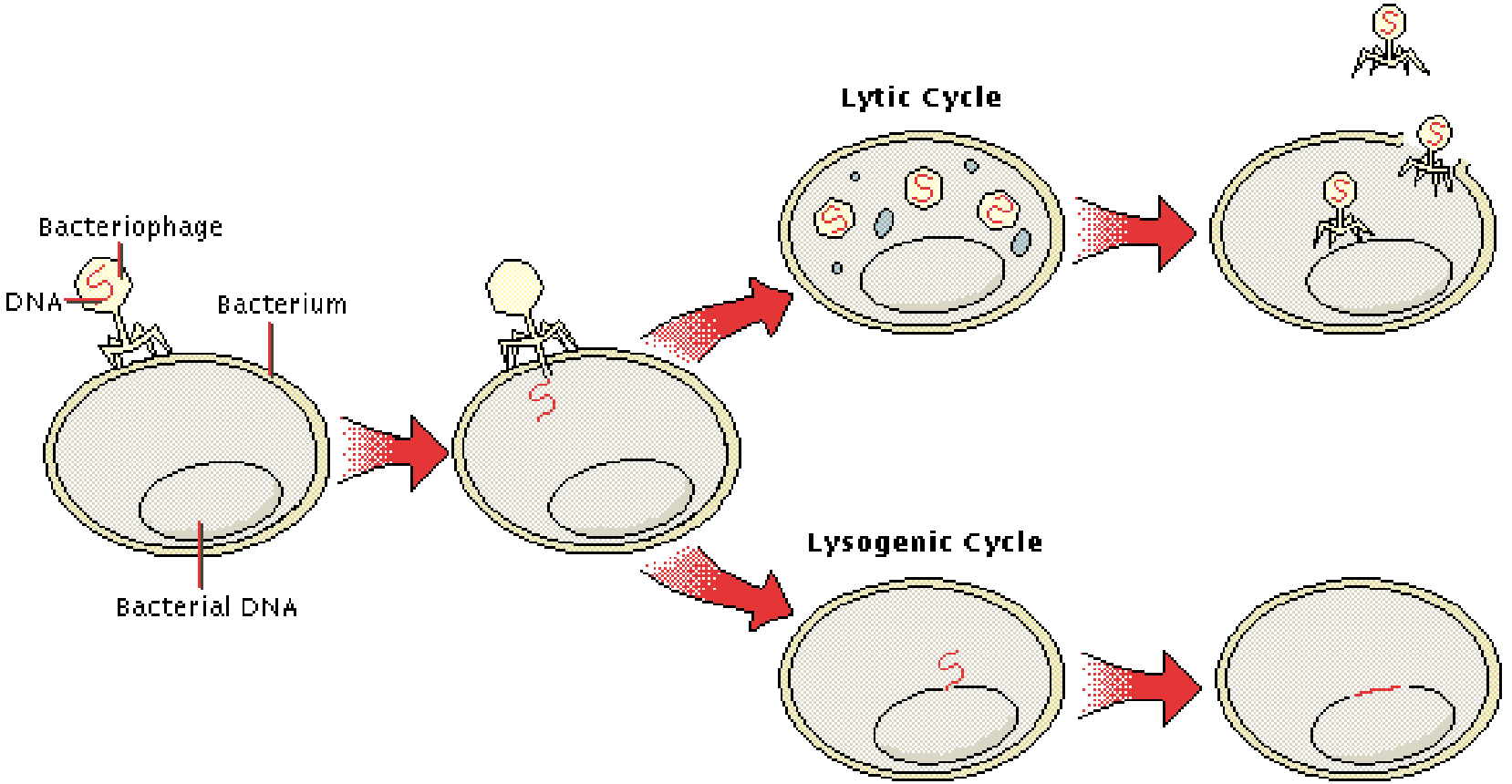
Biological characteristics of water I

Biological characteristics of water

- **Importance**
  - Microorganisms (esp. bacteria) plays a key role in the decomposition and stabilization of organic matter
  - Control of diseases caused by pathogenic organisms of human origin
- **Prokaryotes vs. Eukaryotes**
  - Key difference: the presence of membrane-bound nucleus
  - Prokaryotes: bacteria & archaea
  - Eukaryotes: protozoa, fungi, ------> , humans
- **Viruses**: a form of life or not?
  - Intracellular parasites
  - Require the machinery of a host cell to support growth
  - Contain genetic information (DNA or RNA), but cannot replicate themselves

How viruses maintain their life - bacteriophage



#1

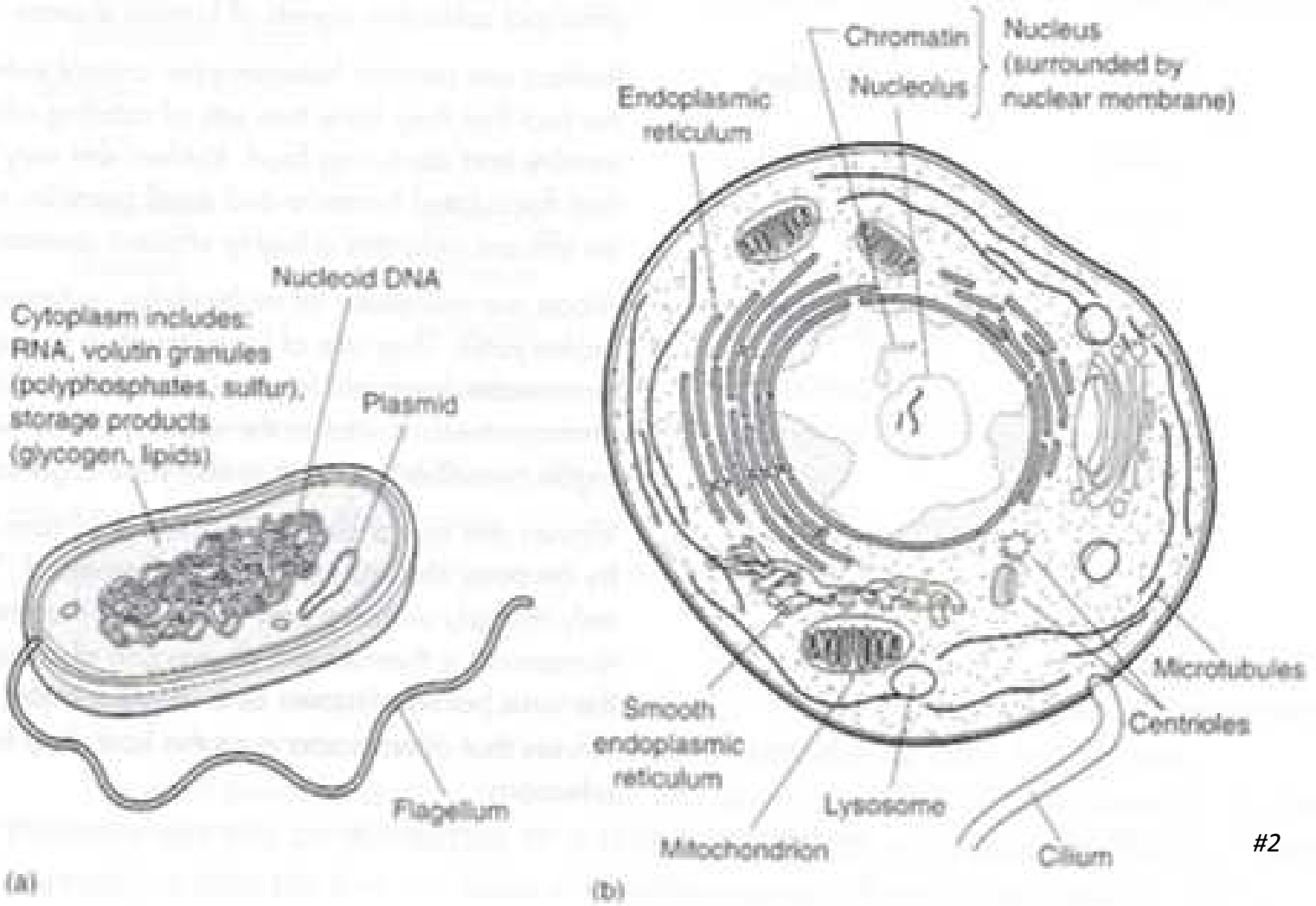
Types of microorganisms

- **Bacteria**
  - Single-cell prokaryotes
  - Typically 0.5-5 µm in size
  - Plays a key role in organic matter decomposition
  - Some examples of pathogenic bacteria: *Salmonella typhi* (typhoid fever), *Vibrio cholera* (cholera), *Campylobacter jejuni* (diarrhea)
- **Archaea**
  - Similar to bacteria in size and basic cell components
  - Important in anaerobic processes (methanogenesis)
  - Found under extreme conditions (e.g., high temp., high salinity, etc.)
- **Fungi**
  - Multicellular, non-photosynthetic, heterotrophic eukaryotes
  - Ability to degrade some large organic molecules (e.g., cellulose, lignin)

Types of microorganisms

- **Protozoa**
  - Eukaryotes, usually single cells
  - Predators of bacteria
  - Generally an order of magnitude larger than bacteria
  - Well-known pathogenic protozoa in water: *Cryptosporidium parvum* and *Giardia lamblia*
- **Algae**
  - Unicellular or multicellular, autotrophic, photosynthetic eukaryotes
  - Important role in the ecology of water environment: supply oxygen by photosynthesis

Prokaryotes vs. Eukaryotes



#2

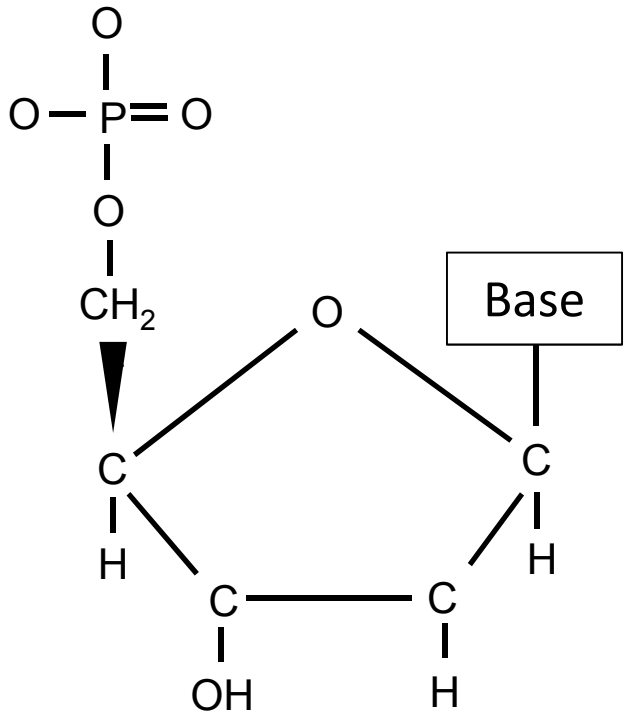
Prokaryotic cell components

Cell component	Function
Cell wall	Provides strength to maintain the cell shape and protects the cell membrane
Cell membrane	Controls the passage of dissolve organics and nutrients into the cell and the waste materials and metabolic byproducts out of the cell
Cytoplasm	Contains the material within the cell to carry out cell functions and includes water, nutrients, enzymes, ribosomes, and small organic molecules
Cytoplasmic inclusions	Contains storage material that can provide carbon, nutrients, or energy
Deoxyribonucleic acid (DNA)	A double-stranded helix-shaped molecule that contains genetic information which determines the nature of the cell protein and enzymes that are produced

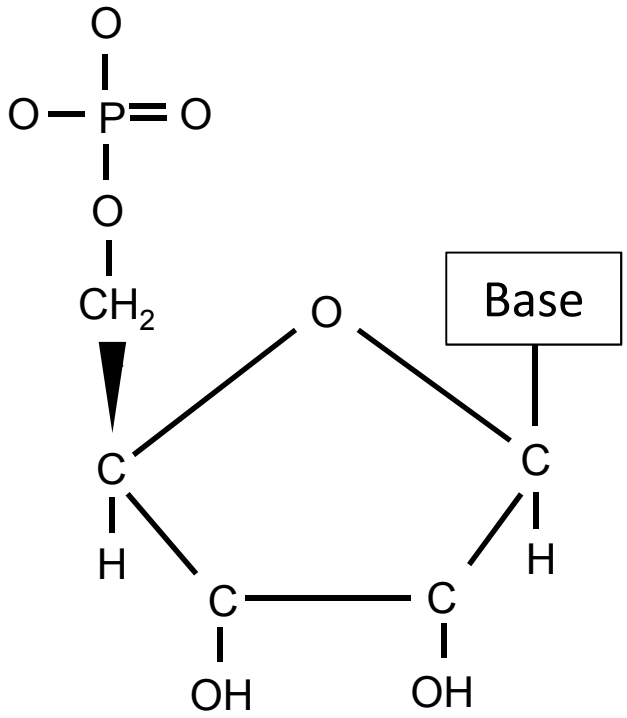
Prokaryotic cell components

Cell component	Function
Plasmid DNA	Small circular DNA molecules that can also provide genetic characteristic for the bacteria
Ribosomes	Particles in the cytoplasm that are composed of ribonucleic acid (RNA) and protein and are the sites where proteins are produced
Flagella	Protein hair-like structures that extend from the cytoplasm membrane several bacteria lengths out from the cell and provide mobility by rotating at high speeds
Fimbriae and pili	Short protein hair-like structures (pili is longer) that enable bacteria to stick to surfaces. Pili also enable bacteria to attach to each other

DNA vs. RNA



DNA

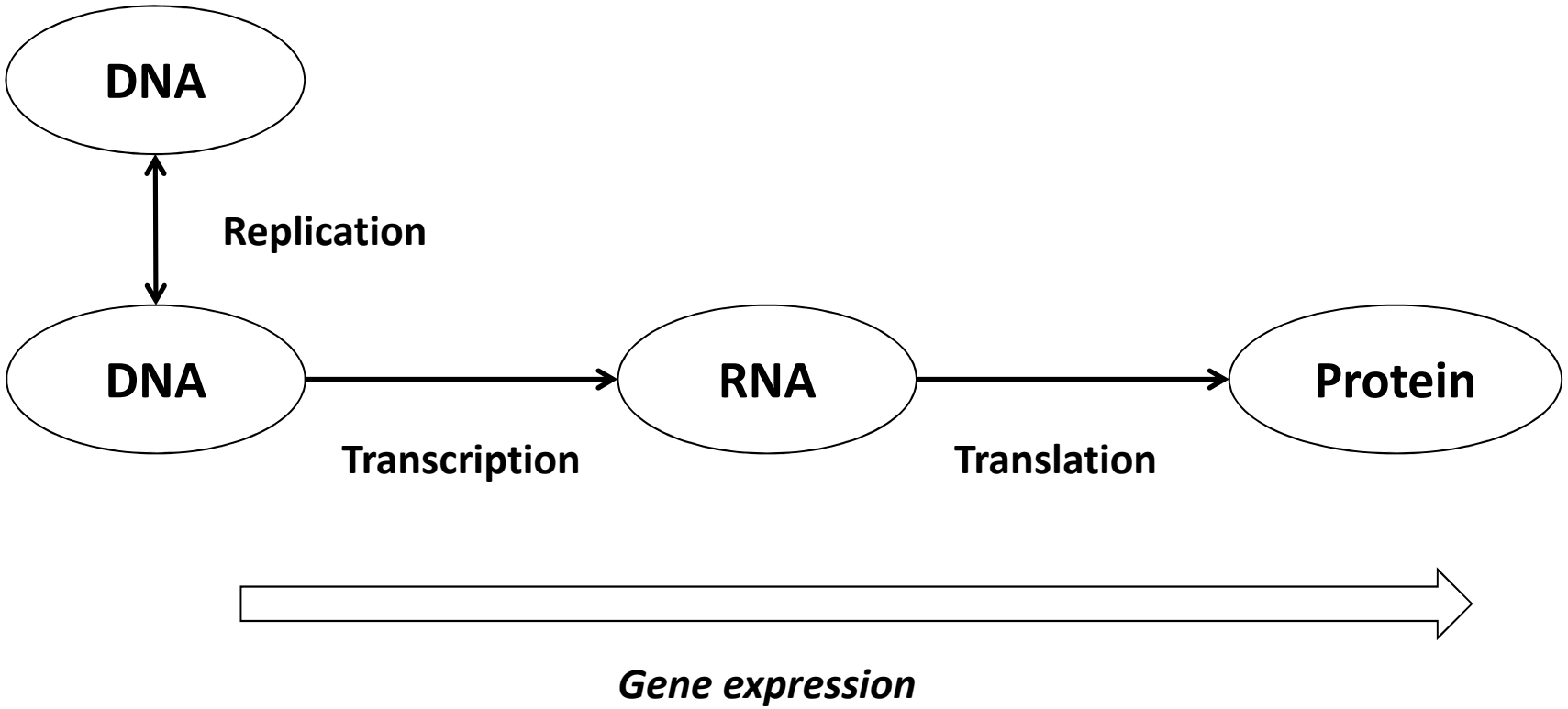


RNA

DNA vs. RNA

	DNA (deoxyribonucleic acid)	RNA (ribonucleic acid)
Sugar	deoxyribose	ribose
Strand	double-stranded	single-stranded
Base	adenine (A), thymine (T), guanine (G), cytosine (C)	adenine (A), uracil (U), guanine (G), cytosine (C)
Function	Long-term storage of genetic information; transmission of genetic information to new cells	Transfer the genetic code from the DNA to ribosomes to make proteins

DNA & RNA: gene expression



Enzymes

- Catalyze biological reactions necessary for cell functions
- Mostly works inside the cell membrane, but cells may also produce enzymes for activity outside the cell (extracellular enzymes)
- Constitutive vs. inducible
  - ***Constitutive***: produced continuously
  - ***Inducible***: produced in response to the presence of a particular compound

Cell composition

Typical composition of bacterial cells

Major cellular material	% of dry weight
Protein	93.8
Polysaccharide	28.0
Lipid	13.7
DNA	30.2
RNA	20.5
Other organics (sugars, amino acids)	6.3
Inorganic ions	1.0

Cell elements	% of dry weight	Cell elements	% of dry weight
C	50.0	K	1.0
O	22.0	Na	1.0
N	12.0	Ca	0.5
H	9.0	Mg	0.5
P	2.0	Cl	0.5
S	1.0	Fe	0.2

Other trace elements: 0.3% of dry weight

- Generic cell formula: $C_5H_7O_2N$
  - Formula weight: 113 g/mole; COD value: 1.42 mg COD/mg cells

Taxonomy vs. phylogeny

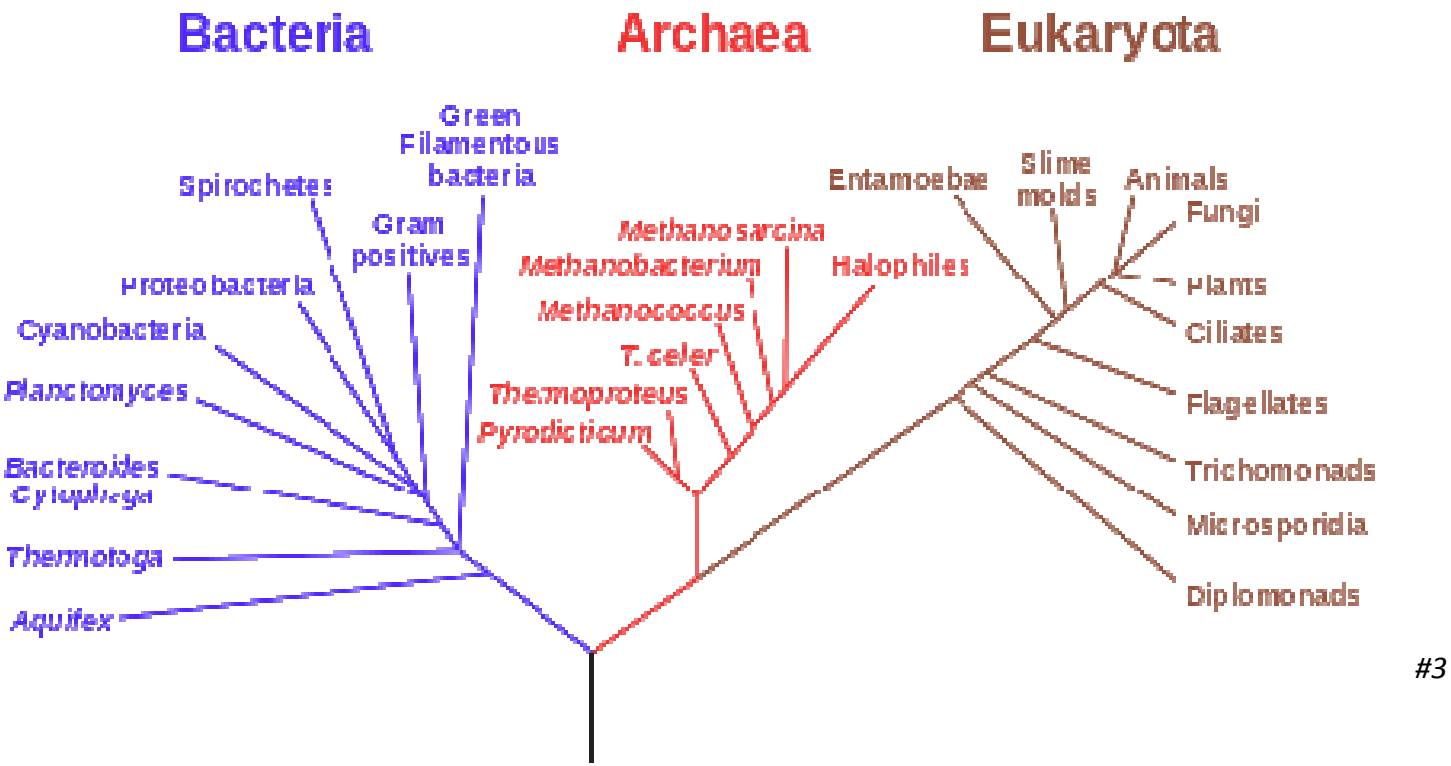
- **Taxonomic classification**
  - Classification of microorganisms based on the physical properties and metabolic characteristics
  - Types of tests to characterize a pure culture
    - Microscopic observation (size & shape)
    - Gram staining: if the bacteria cell wall will absorb crystal violet dye
    - Type of electron acceptor (O_2, NO_3^-, CO_2, etc.)
    - Type of carbon source used for cell growth
    - The ability to use N and S sources
    - Nutritional needs
    - Cell wall chemistry
    - Cell characteristics including pigments, segments, cellular inclusions, and storage products
    - Resistance to antibiotics
    - Environmental effects of temperature and pH

Taxonomy vs. phylogeny

- **Phylogenetic classification**
  - Phylogeny: the characterization of microorganisms based on genetic information and evolutionary location in time
  - Current method of identification and classification
  - Use of ribosomal RNA for phylogenetic classification:
    16S rRNA for prokaryotes (18S rRNA for eukaryotes)
    - Significant for evolution
    - Present in all known forms of life
    - Well conserved across broad phylogenetic distances
    - Contains sufficient nucleotide sequences so that similarity in sequences between two organisms indicates a phylogenetic relationship

Taxonomy vs. phylogeny

Phylogenetic tree



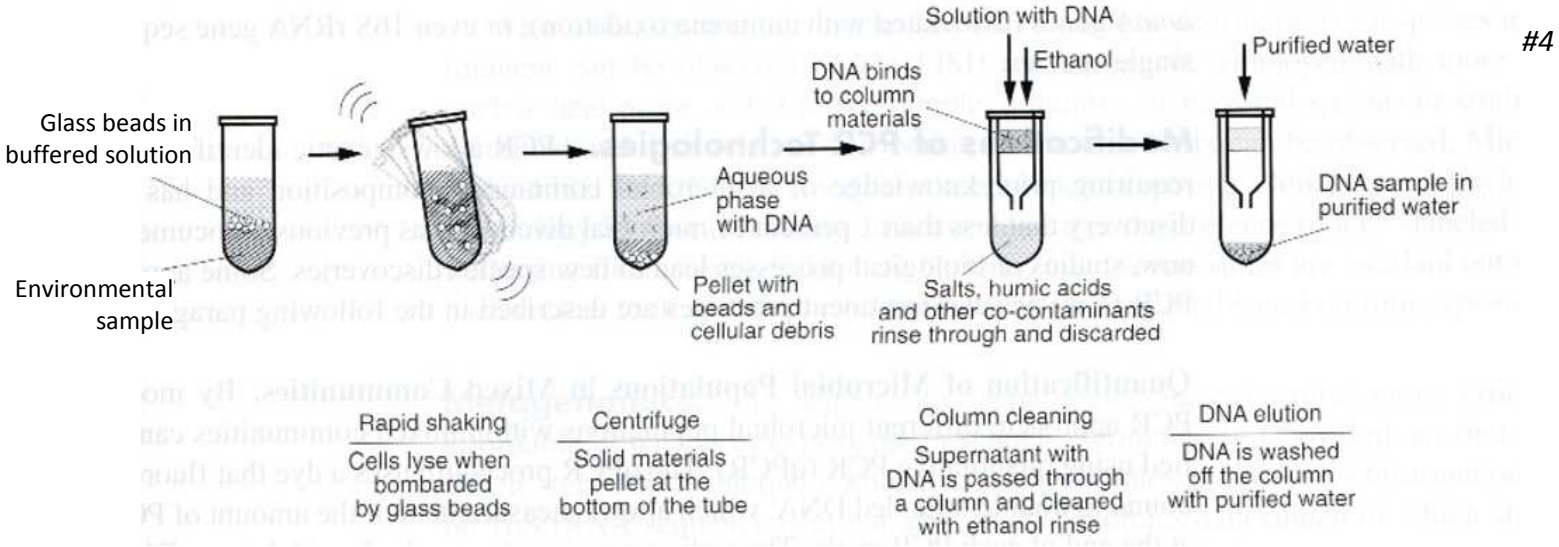
#3

Life divided into three domains: bacteria / archaea / eukarya

Molecular tools

- Use DNA, RNA, and proteins to identify, track, and quantify the presence and activities of microorganisms

- Extraction of DNA from environmental sample
  - Disrupt the cells either physically chemically to recover DNA
  - Column cleaning for clean-up and concentration of DNA
  - Elution into purified water



#4

Polymerase chain reaction (PCR)

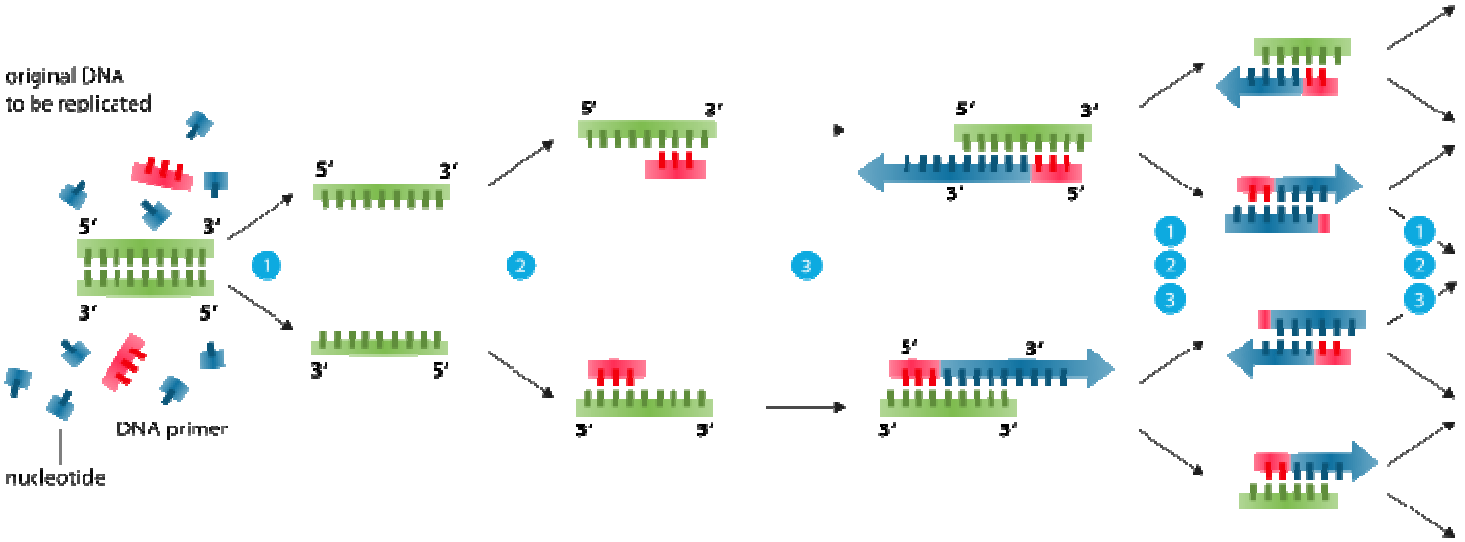
- Small sections of the extracted DNA are amplified using naturally occurring enzymes involved in cellular DNA replication

- **PCR ingredients**
  - The sample DNA (template DNA)
  - PCR primers: short oligonucleotides that complement a section of the target DNA sequence
  - DNA polymerase: a naturally occurring enzyme that creates copies of DNA during cell replication
  - Mixture of nucleotides: building blocks for new DNA
  - pH buffer containing Mg^{2+}

Polymerase chain reaction (PCR)

- **Typical procedure** (1 cycle)
  - Heating to about 95°C to separate double stranded DNA into single strands
  - Lower the temp. to 45~65°C to allow PCR primers to anneal to the DNA template
  - Increase the temperature to about 72°C and DNA polymerase extends the copy of the template DNA

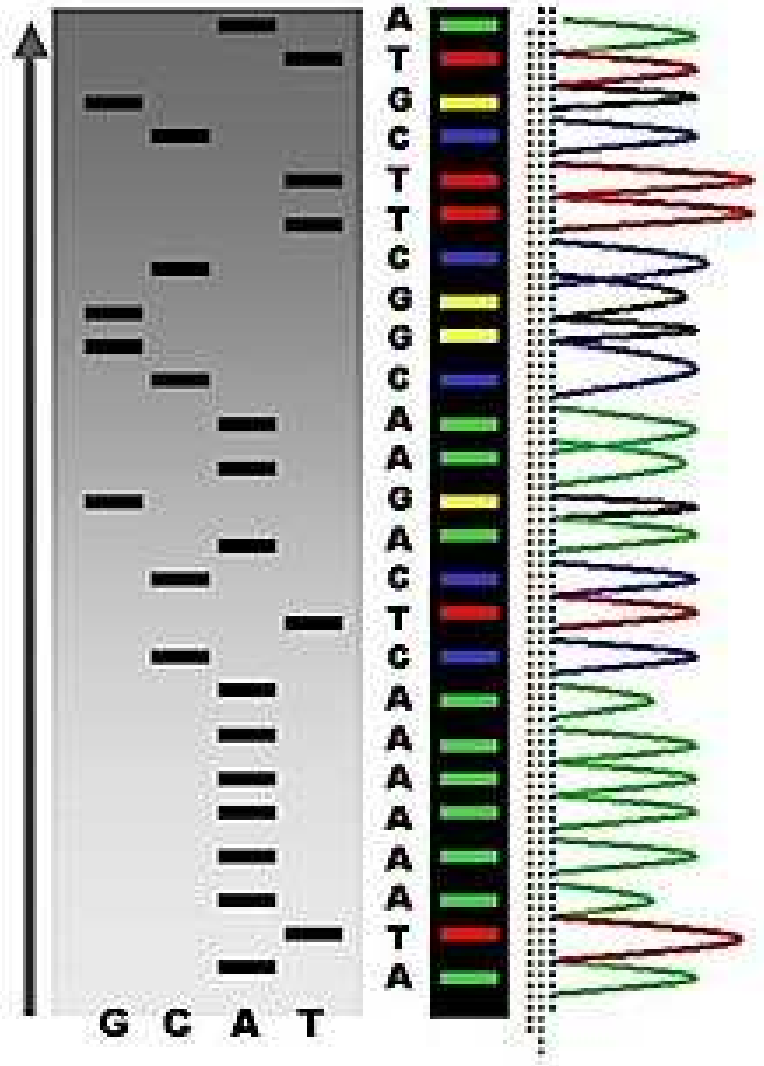
#5



1. **Denaturation** at ~95°C
2. **Annealing** at 45~65°C
3. **Elongation** at ~72°C

DNA sequencing

- The DNA sample amplified by PCR is sequenced to identify the microorganism



#6

Metagenomics

- Study of genetic material recovered directly from environmental samples
- Limitations of traditional methods for microorganisms in environmental samples
  - Cultivation-based (microorganisms are grown for analysis): a majority of microbial diversity can be lost during cultivation
  - PCR of 16S rRNA: genetic information from 16S rRNA may not be enough!
- Advancements in molecular tools enables obtaining all genes from all members (microorganisms) in a sample
  - Obtain non-biased result
  - Useful for microbial community analysis in environmental samples
  - Can obtain full genomic data for a microbial species

Classification of microorganisms

- **By carbon sources**
  - **Heterotrophs**: use organic carbon to produce new cells
  - **Autotrophs**: derive cell carbon from inorganic source (CO_2, HCO_3^-, ..)

- **By energy sources**
  - **Phototrophs**: Use light as an energy source
  - **Chemotrophs**: derive energy from chemical reactions

Classification of microorganisms

- **By growth in the presence/absence of O_2**

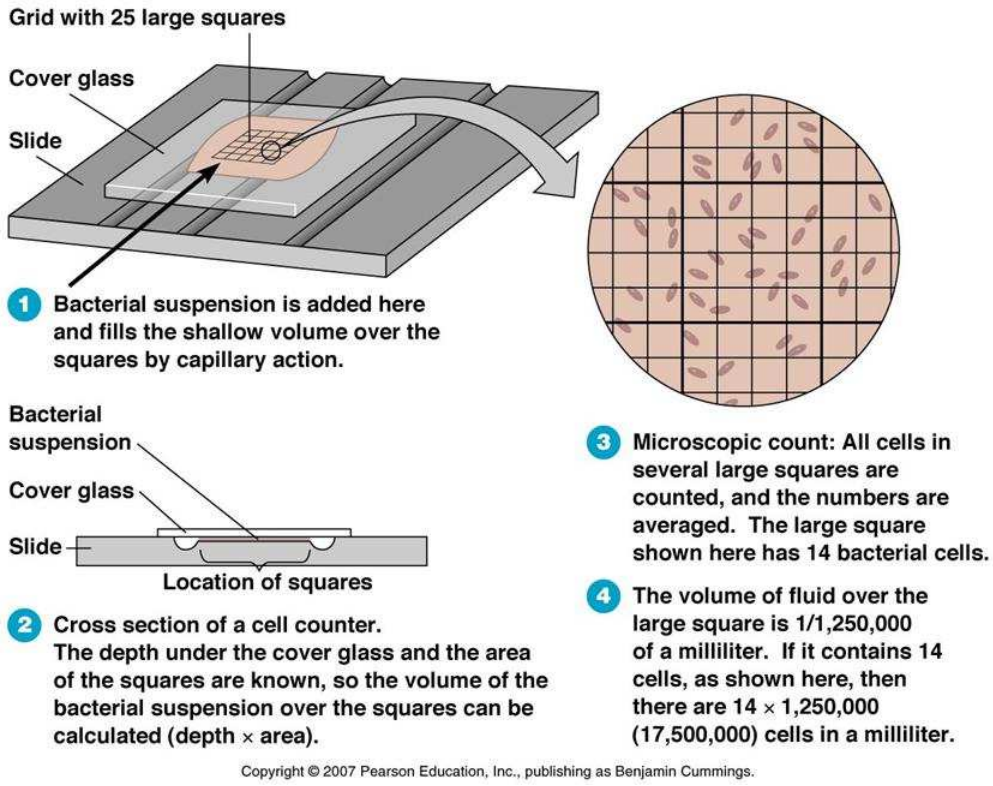
  Aerobes: use O_2 as an e^- acceptor / **Anaerobes**: Use other e^- acceptors

  * possible e^- acceptors: O_2, NO_3^-, Fe^{3+}, Mn^{4+}, SO_4^{2-}, CO_2, …

  – **Obligate aerobes**: can use only O_2 as an e^- acceptor
  – **Facultative aerobes**: prefer O_2, but can use NO_3^- or NO_2^- in the absence of O_2
  – **Obligate anaerobes**: can survive only in the absence of O_2
  – **Facultative anaerobes**: can shift from fermentative to aerobic respiratory metabolism depending on the presence of O_2
  – **Aerotolerant anaerobes**: cannot use O_2 as an e^- acceptor, but can survive under aerobic condition

Enumeration and identification of bacteria

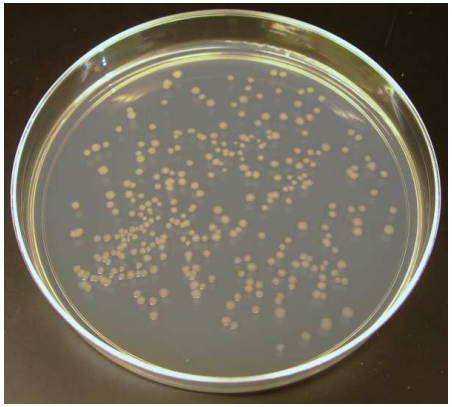
- **Direct counts**
  - Number of cells are counted by a microscope
  - Acridine orange stain may be used to differentiate between live and dead cells



#7

Enumeration and identification of bacteria

- **Pour and spread plate method**
  - Water sample is diluted serially
  - **Pour plate method**: a small amount of each dilution is mixed with a warmed, liquefied agar medium containing nutrients, poured into a culture dish, allowed to solidify



#8

  - **Spread plate method**: the agar medium containing nutrients is placed and solidified in a culture dish, a small amount of each dilution is placed and spread on the surface of the medium
  - The plates (prepared by either of the two methods) are incubated
  - When colonies are formed on the plates, the number of colonies are counted
  - Results are reported as “colony-forming units (CFUs)” per unit volume of sample (usually CFU/mL)

Enumeration and identification of bacteria

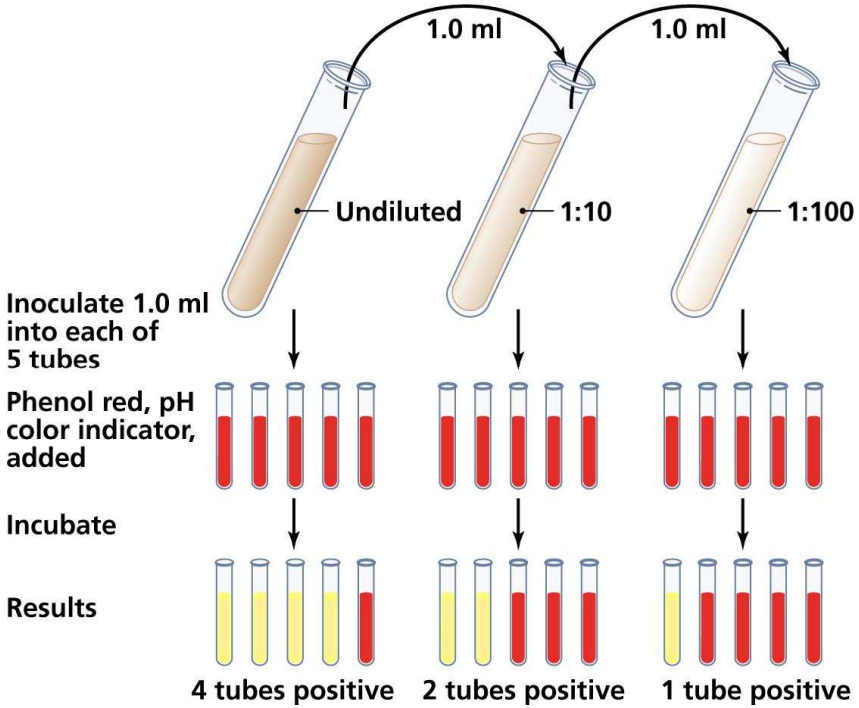
- **Membrane filter technique**
  - A known volume of water sample is passed through a membrane filter
  - Bacteria are retained on the filter
  - The membrane filter is then placed on an agar that contains nutrients necessary for the growth of the target bacteria
  - After incubation, the number of colonies formed on the surface of the filter is counted




#9

Enumeration and identification of bacteria

- **Multiple-tube fermentation**
  - Generally for the determination of total coliforms
  - Unit: most probable number per 100 mL (MPN/100 mL)
  - MPN is not the absolute concentration, but a statistical estimate of that concentration



Enumeration and identification of bacteria

Q: A simplified plate counting method employs the following procedure. A wastewater sample is serially diluted to 10^{-1} ~ 10^{-8}. An agar plate is divided into 8 sections and 0.03 mL for each diluted sample is dropped into the corresponding sections. The agar plate is incubated until visible colonies are formed. The number of colonies are counted to calculate the bacterial concentration.

If the result of the above procedure is obtained as follows, calculate the concentration of bacterial in a wastewater sample.

Dilution	No. of colonies	Dilution	No. of colonies
10^{-1}	uncountable	10^{-5}	>200
10^{-2}	uncountable	10^{-6}	27
10^{-3}	uncountable	10^{-7}	4
10^{-4}	uncountable	10^{-8}	None

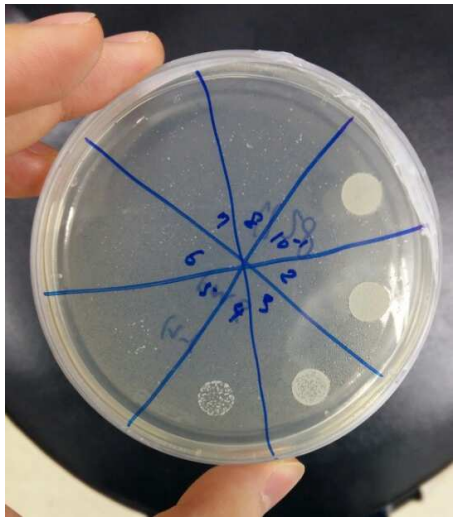


Photo: Moonkyung Kim

Enumeration and identification of bacteria

Take a result of a diluted sample that gives 10-100 colonies

(too few colonies → significant error;

too many colonies → possible overlapping of colonies)

$$\frac{27\ colonies}{0.03\ mL\ \times 10^{-6}} = 9.0 \times 10^{8}\ CFU/mL$$

References

#1) https://www.slideserve.com/howell/life-cycles-of-viruses

#2) Metcalf & Eddy, Aecom (2014) Wastewater Engineering: Treatment and Resource Recovery, 5th ed. McGraw-Hill, p. 141.

#3) https://en.wikipedia.org/wiki/Phylogenetic_tree

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