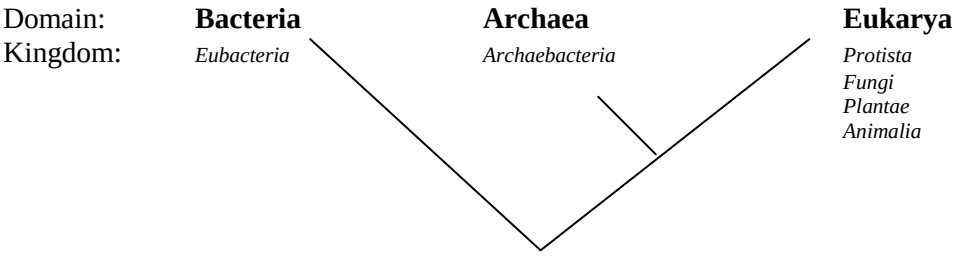
NAME: _____ DATE: _____

PARTNER: _____

DIVERSITY I
Eubacteria, Protista, Fungi

This exercise will begin the first of five laboratory sessions in which we will survey the tremendous diversity of organisms in the world around us. Based on current work in the field of taxonomy, the relationships of these organisms are commonly depicted by the following 3 domain / 6 kingdom classification system. This is based, at least at the domain level, on differences in specific cellular characteristics observed between these groups of organisms. This distinction is also used to draw a line between the two structurally different kinds of cells observed in living organisms: prokaryotic cells (Bacteria & Archaea) and eukaryotic cells (Eukarya).



In this session, we will focus our attention on representatives from the kingdoms Eubacteria, Protista and Fungi.

I. KINGDOM EUBACTERIA

The prokaryotic cells we will examine are representatives of Kingdom Eubacteria. Bacteria within this group constitute those present in our normal environment with which we have the most common contact. Bacteria are single-celled organisms which lack a nucleus and other organelles. They all have cell walls to protect them from the environment.

A. Isolation of Bacteria

Bacteria are the most prevalent organisms on Earth and can be isolated from nearly every part of our environment, including the surfaces of our bodies. In this activity, you will collect a bacterial sample from an area of your body, culture the sample and draw conclusions regarding the number and diversity of bacteria at that location.

1. Take a Petri dish containing Nutrient Agar and write your name, the date and the location you will sample along the edge of the bottom side of the dish.
2. Using a sterile moistened swab, wring out the excess water against the inside of the tube and swab the selected surface of your body (e.g., gums, ear, scalp, armpit, fingers, throat, between toes, etc.). Be sure to swab the surface thoroughly to maximize the number of bacteria recovered.
3. Rub the swab over the surface of a Petri dish containing Nutrient Agar. This is a general purpose medium that can be used to grow a variety of microorganisms. Be sure to smear the contents of the swab completely and thoroughly over the surface of the media.

4. Discard the swab directly into the biohazard container.
5. Place the dish inverted (bottom side up) in the incubator set to 37°C.
6. The plates will incubate for 24-48 h, after which your instructor will preserve them for your examination in the following lab period.

- What area did you choose to swab and why did you decide on that particular location?

_____

_____

_____

- Why were the plates incubated at 37°C and not some other temperature?

_____

_____

B. Observation of Bacteria

Next session: record and interpret results from last week's isolation of bacteria. Describe what you see on your petri dish. Consider the following questions: Do you have individual colonies (spots), or a "lawn" of bacteria? What color are your colonies? Are they smooth, shiny, fuzzy? How many do you have? What does this tell you about the location you chose to sample?

_____

_____

_____

_____

_____

A laboratory assistant made a slide preparation called a Gram stain from one colony from each plate. Look at this in the microscope – your instructor will assist you with using oil immersion microscopy for this. Describe what the cells look like. Are they purple or pink (i.e., Gram positive or Gram negative)? Are they round spheres (cocci) or rods (bacilli, which can be very long or very short, but definitely not spherical)? Are they individuals, or do they cluster in pairs, chains, or grape clusters, or another arrangement? Use the dichotomous key on the back of the page to identify the group of bacteria you may have isolated. If your bacterial strain doesn't fit, explain where in the dichotomous key it "falls off".

_____

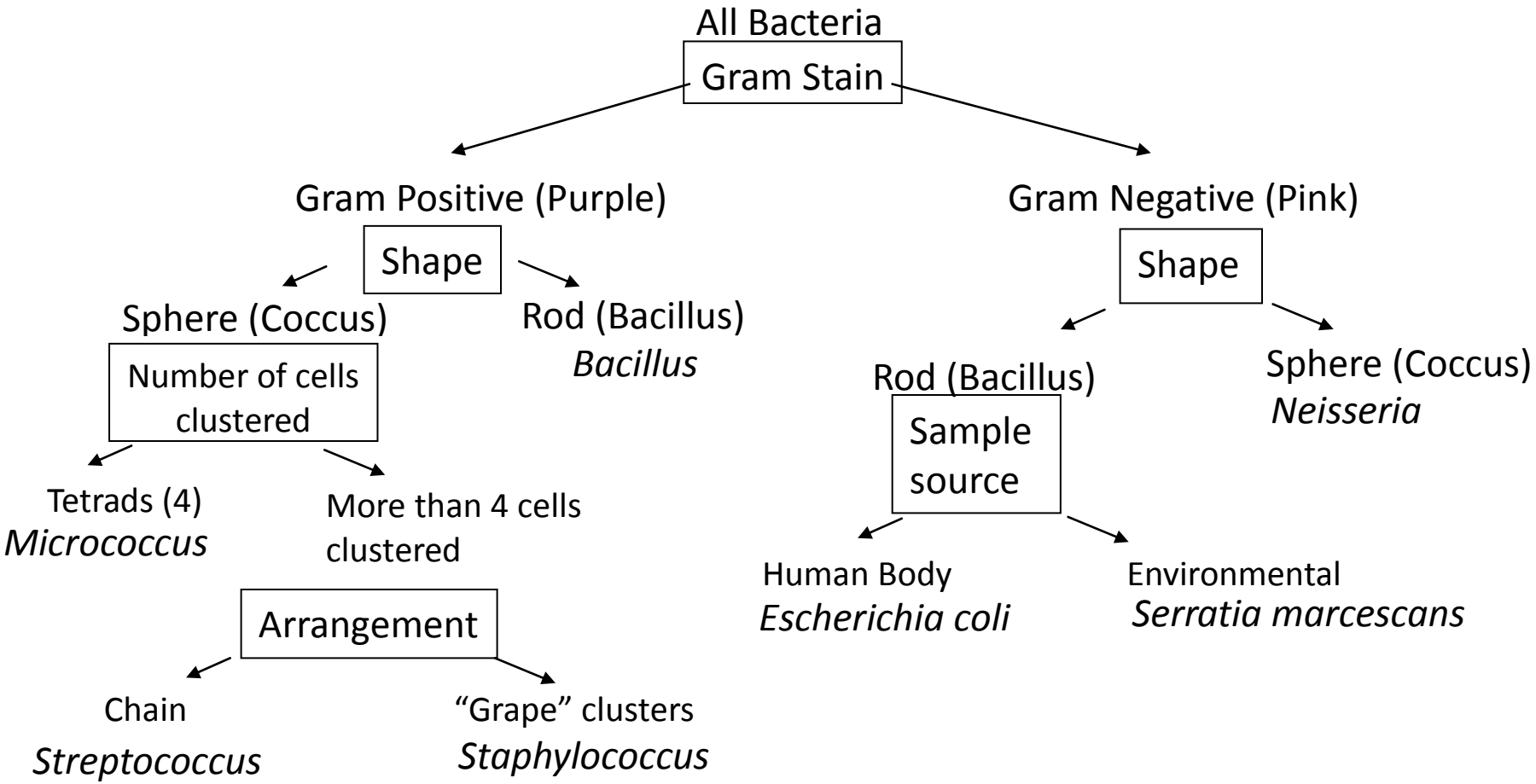
_____

_____

_____

_____

Bacterial Dichotomous Key



Please note that these are not actual positive identifications, but the likeliest bacteria given the sample sources, the method of culture, etc. Without biochemical tests, positive identification of species is not possible.

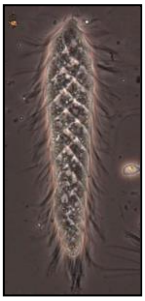
II. KINGDOM PROTISTA

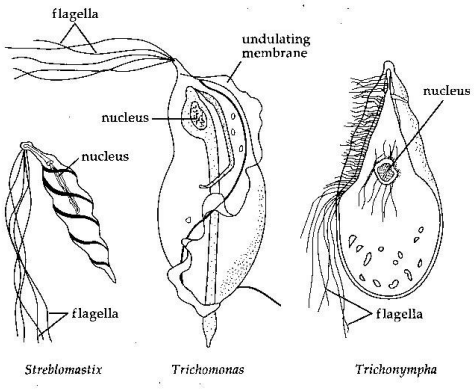
This will begin our examination of organisms with eukaryotic cellular organization. Among other characteristics, cells of eukaryotes will contain nuclei and other membrane bound organelles within their cells. Members of Kingdom Protista include the "animal-like" protistans (e.g., amoebas, ciliates, flagellates, sporozoans), the "plant-like" protistans (e.g., euglenoids, dinoflagellates, other algae) and the "fungi-like" protistans (e.g., cellular slime molds). There is no unifying characteristic of Kingdom Protista; they all are eukaryotes that simply do not fit into another kingdom. We will focus on just a few examples.

A. Isolation of Protists from the Gut of Termites

Although termites (*Zootermopsis*) are known to consume a diet of wood, they cannot actually digest the cellulose component of this product – at least not on their own. Within the gut of termites live symbiotic protistans (particularly members of the Zoomastigophora), some of which possess the enzymes necessary to break down cellulose into simpler carbohydrates that both the host and symbiont can use for energy. (Which is the host? _____ Which is the symbiont? _____)

1. Place a drop of saline solution on the center of a microscope slide.
2. Collect a termite and place it in the drop of saline.
3. Using two dissecting needles, place one through the anterior end (head), one through the posterior end and gently pull apart.
4. Locate the intestine (long, tubular structure) and move the other materials aside.
5. Tear open the intestine with the dissecting needles and cover the preparation with a cover slip.
6. Observe under the microscope and try to identify flagellates or other microorganisms (see atlas pictures on display).





- What observations can you make about the <u>types</u> and <u>numbers</u> of protistans present in the termite gut? _____

_____

_____

_____

_____

_____

- How do both the termite and flagellates benefit from this relationship? Explain for each one. _____

_____

_____

_____

_____

_____

_____

- The term symbiosis typically refers to two different species that live together and closely interact. However, this interaction may have beneficial, detrimental or insignificant impact on one or both of the organisms involved. What type of symbiotic relationship is represented in this example (give the scientific term) and how would this relationship be defined? You may need to use your text.

_____

_____

_____

_____

7. Place the microscope slides in the sanitizing solution and clean your workspace.

B. Form & Function of some Protista

Euglena are unicellular flagellated protistans that, because of their photosynthetic (autotrophic) abilities, are sometimes confused with algae. However, another interesting trait of *Euglena* is their ability to absorb nutrients from their environment and gain energy through heterotrophic mechanisms when light is not available. This dual ability makes them very efficient "feeders" that can thrive in nearly any condition.

1. Make a wet mount of *Euglena* and observe the organisms under the microscope (look at preserved specimens as well, and see pictures on next page of this packet).
   a. Place a drop of specimen on a microscope slide.
   b. Add a drop of Protoslo and cover with a coverslip.
   c. Begin at low power and progress to using the 40x objective for best viewing.

      - Note the presence of the pigmented "eye spot" and a single flagellum. How might these structures contribute to the autotrophic feeding practices of this organism?

_____

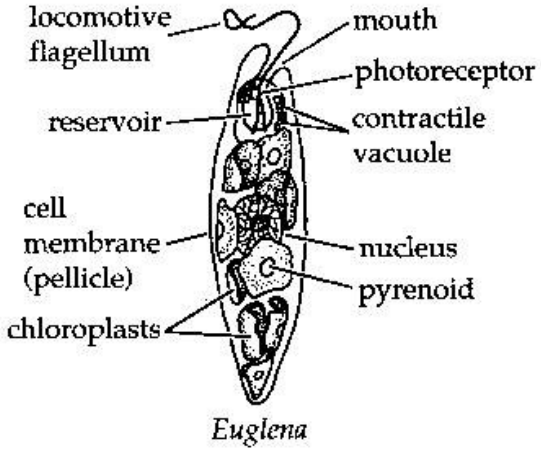
_____

_____

_____

_____

_____



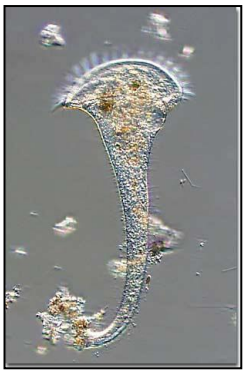
Labeled drawing of *Euglena*

Photomicrograph of *Euglena*

2. *Paramecium* and *Stentor* are unicellular ciliated heterotrophic protistans. *Paramecium* feeds primarily on bacteria, while *Stentor*, being larger, feeds on other protists and algae as well.

As before, make separate wet mounts of both *Paramecium* and *Stentor* and observe the organisms under the microscope (look at preserved specimens as well).

Paramecium



Stentor

- Describe how the arrangement of cilia differs between these two organisms.

  _____

  _____

  _____

- How does this difference in arrangement relate to differences in the way the cilia are used by these organisms? _____

  _____

  _____

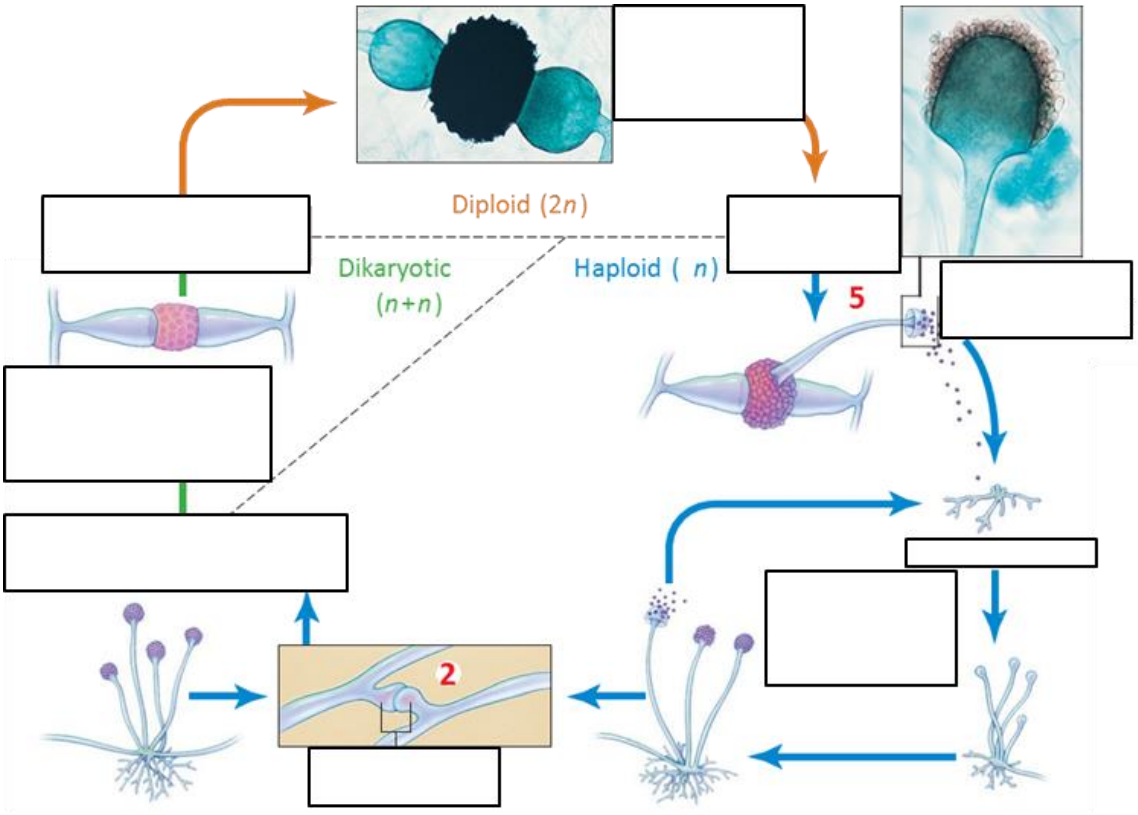
  _____

  _____

III. KINGDOM FUNGI

Fungi are heterotrophic eukaryotes with cell walls made of chitin (a polysaccharide produced only in fungi and a few other organisms. Fungi live off decaying organic matter (saprobes) or living organisms (parasites). Although some may appear to be plant-like, they lack chlorophyll and cannot photosynthesize; this is why they are heterotrophs. Most are multicellular (yeasts are an exception), and fungi can have complex life cycles characterized by spore dispersion for reproduction of new individuals, in which the haploid stage is dominant.

A. Study of Typical Fungal Life Cycle



photo, Micrographs Ed Reschke; art, © 2016 Cengage Learning

Use your text as a guide to follow the life cycle of *Rhizopus* (bread mold), a typical zygomycote. Fill in the blanks in the following diagram using the terms below.
Terms:

a. asexual reproduction	b. fusion of cytoplasm	c. fusion of nuclei
d. gametangia	e. meiosis	f. mature zygospore
g. mycelium	h. spores at tip of aerial hypha	i. young zygospore

B. Identification of Fungal Structures

Rhizopus, a member of the Zygomycota, is a common mold that is often seen growing on bread. Using the live cultures and diagrams on display, locate (if present) the following fungal structures: hyphae, mycelium, sporangium.

1. What structures are contained within the sporangium? What is their purpose?

_____

_____

_____

2. What is the relationship between a mycelium and a hypha?

_____

_____

_____

C. Identification of Miscellaneous Groups of Fungi

Members of the Fungi are commonly divided into four major groups: 1) Zygomycota (e.g., Rhizopus), 2) Ascomycota (the "sac fungi"; e.g., cup fungi, morels, truffles, yeasts), 3) Basidiomycota (the "club fungi"; e.g., mushrooms, toadstools, puff balls, shelf fungi), 4) Fungi Imperfecti (no known sexual cycle). In this exercise you will observe and compare a few examples of these organisms.

1. Using your text and the guides on display, associate the fungal specimens with their taxonomical group.

Specimen #	Common Name / Divisional Group
1	
2	
3	
4	
5	

2. What "functional" region of these fungi were you looking at (i.e., what is its role in the life cycle of the fungus; hint: it's the same for all of them)?

_____

_____

D. Fungal Symbionts

Certain members of the Fungi have formed symbiotic relationships with other organisms. Lichens represent associations between a fungus and an alga or cyanobacterium. Mycorrhizae reflect beneficial relationships between a fungus and the roots of plants.

3. Describe the different morphologies of the specimens on display. Be sure to make note of their shapes: crustose (encrusting), foliose (leaf-like), fruticose (branching) and colors.

Specimen #	Description
1	
2	
3	

4. How would you account for the different colors observed?

_____

_____

_____

5. For lichens, describe how each of the organisms involved is able to benefit from this mutually symbiotic relationship?

_____

_____

_____

_____

_____

_____

_____

_____

_____