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# ***Basics of Applied Microbiology***

## **Sampling & Testing**

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# Purpose of this talk

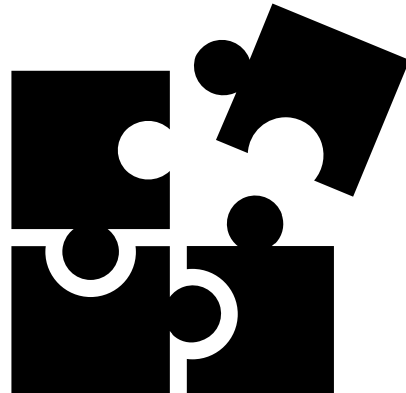
- NOT to make you a microbiologist!!



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# Why is this important then?

- Need to know what you're testing for
- Helps guide appropriate test method
- Helps interpret a result, especially a “presumptive” one

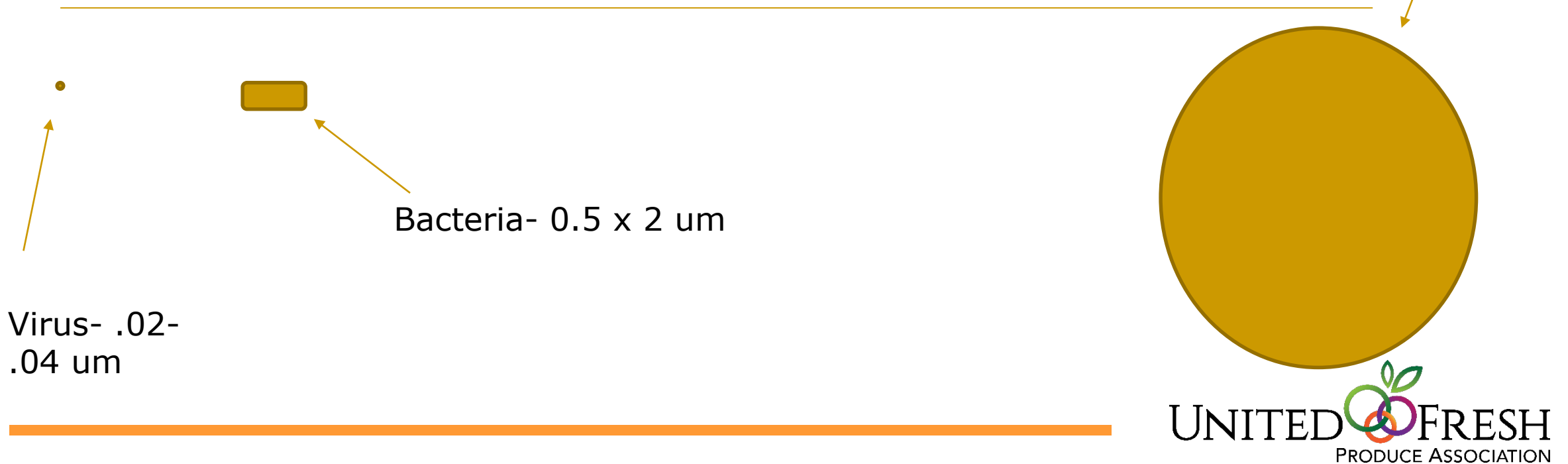


# Approach

- Compare and contrast bacteria, viruses & parasites

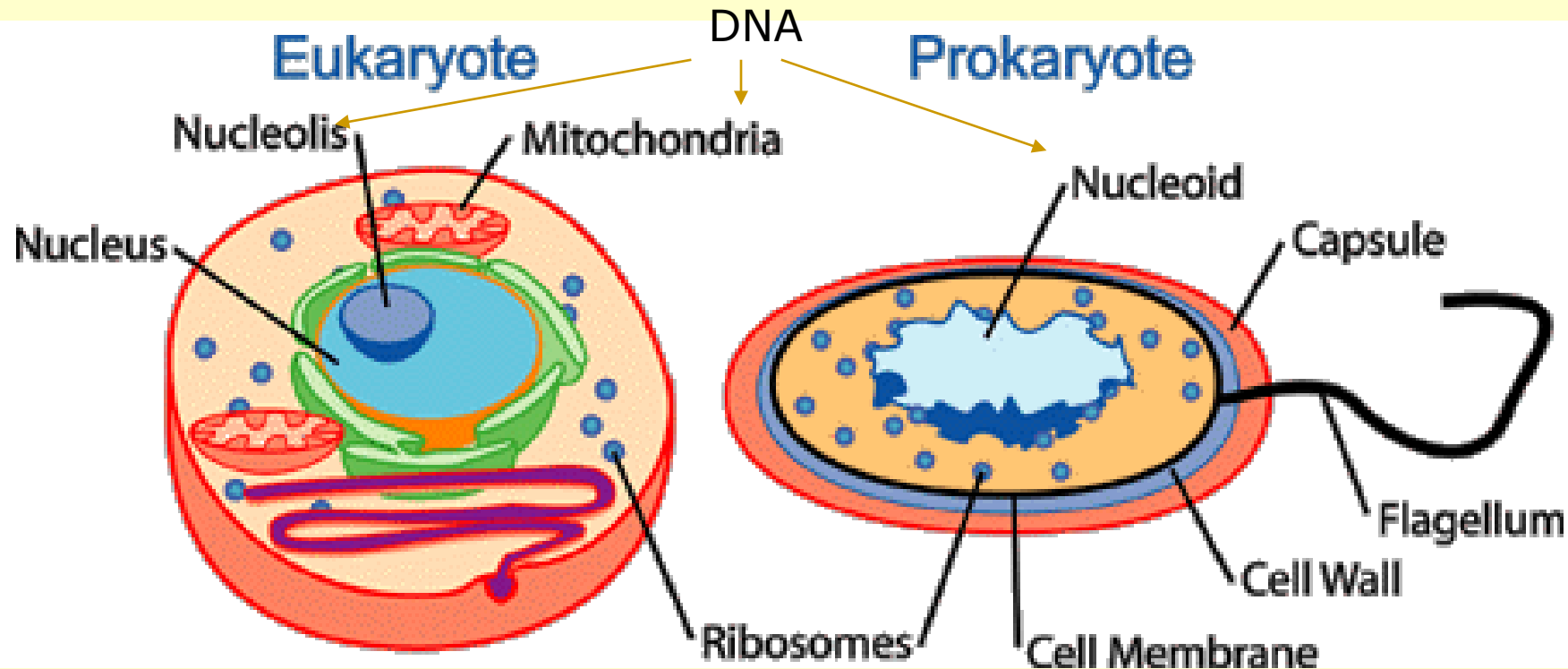
None are visible to the naked eye

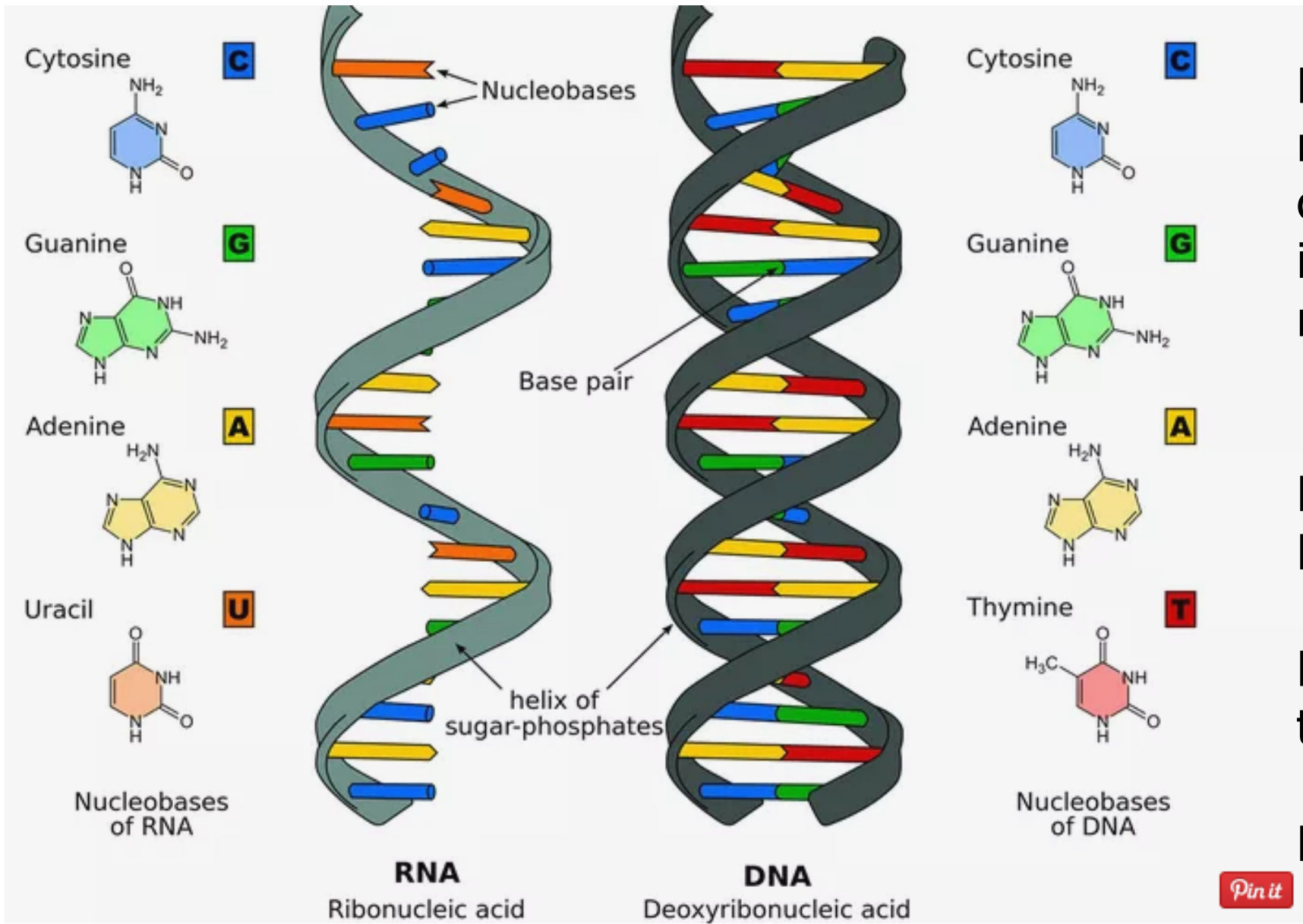
Parasites- variable size-  
produce issues are 4-10 um



# Eukaryotic and Prokaryotic Cells

Is your method looking for the actual cells, or DNA, or some other part of the cell?





DNA is your genetic material. RNA helps convert those genes into the proteins that make life happen.

DNA- double stranded;  
RNA single strand

DNA is more stable  
than RNA

DNA is longer than RNA



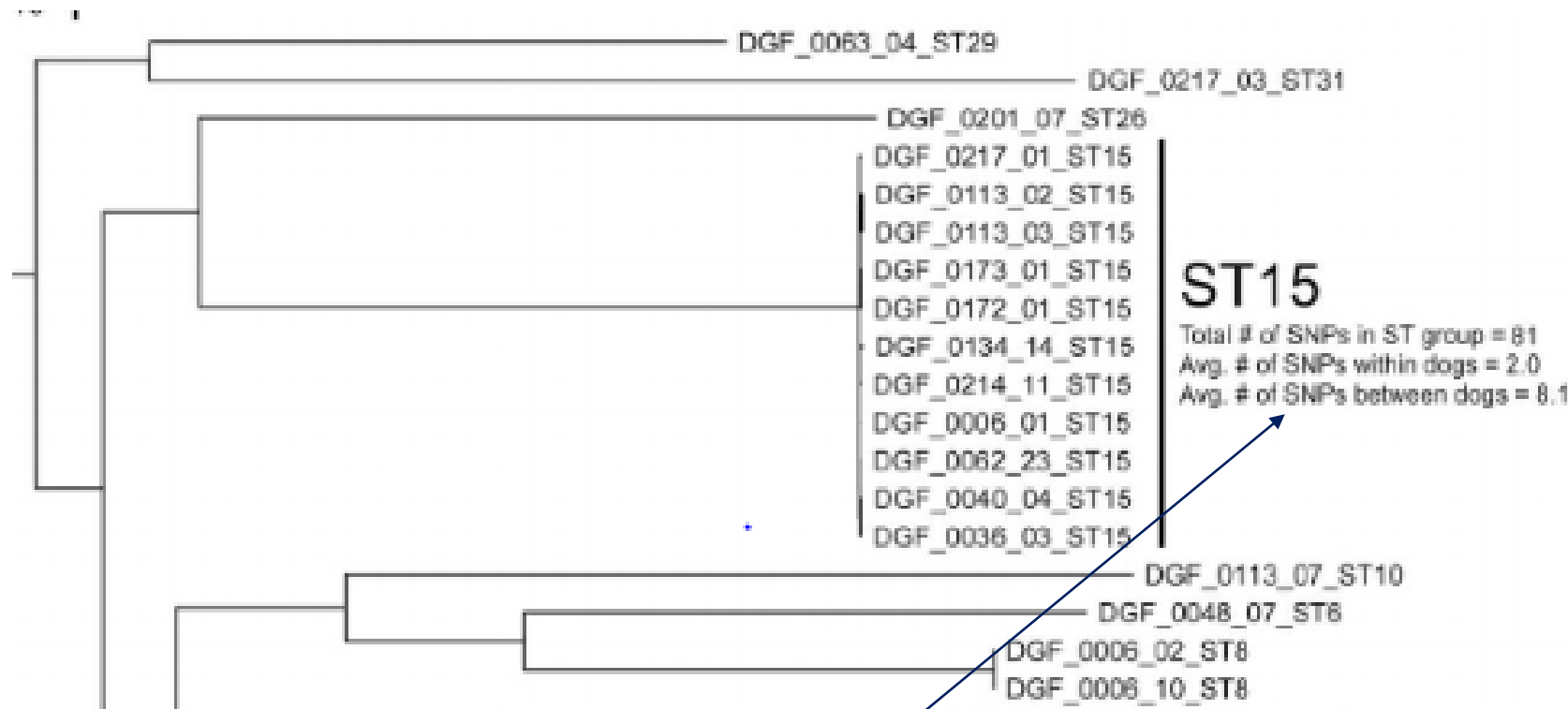
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<https://www.thoughtco.com/dna-versus-rna-608191>

# Side note on Whole Genome Sequencing

WGS collects the DNA from cells, and through a combination of the lab and computers, figures out the sequence of the DNA strand. For example, Listeria is 3M letters long. Then you can compare how similar or different strains are.



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# Bacteria pre-test

- What are some examples?
- What do you know about bacteria?



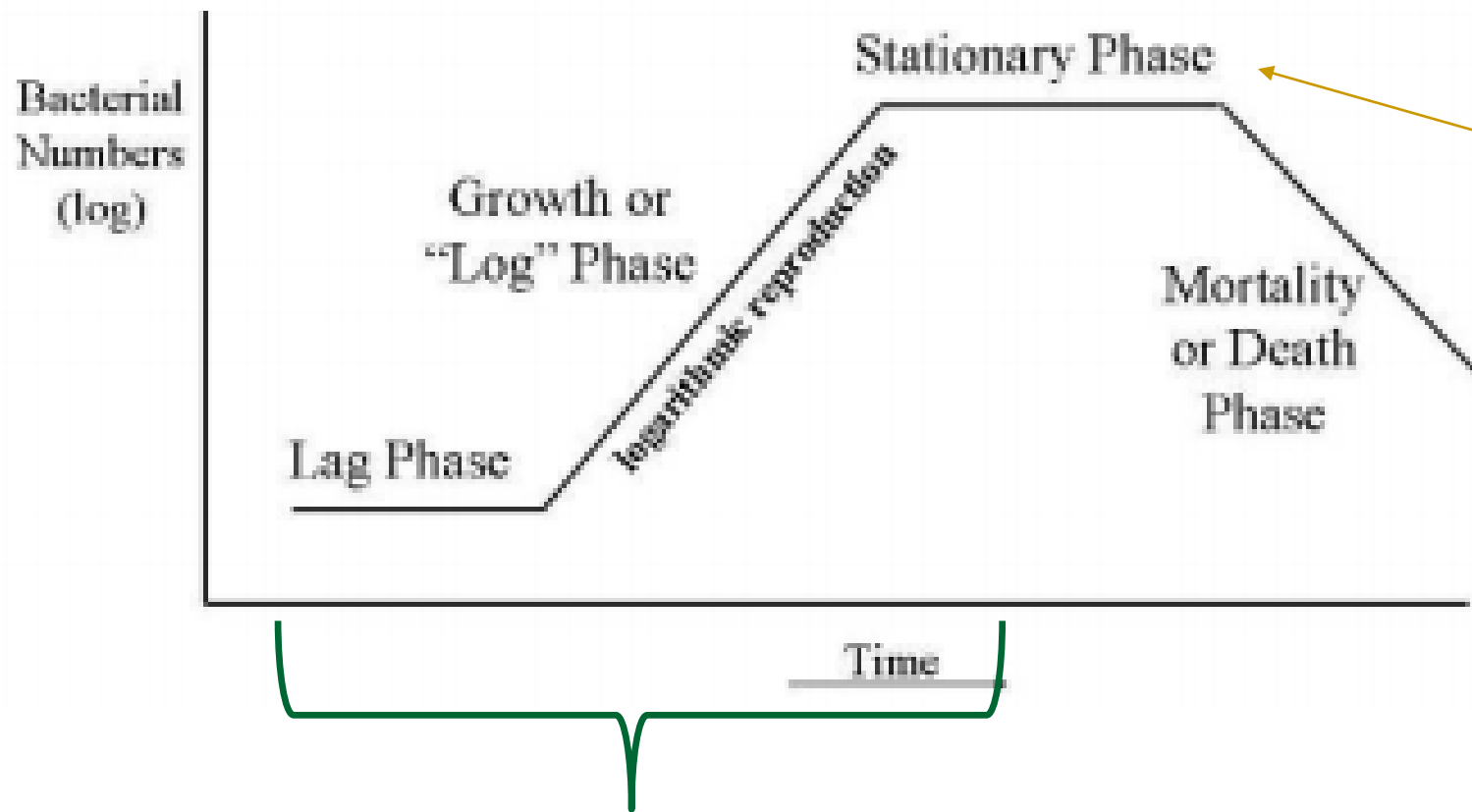
# Bacteria

- Single celled
- Genetics: DNA and RNA
  - Single chromosome
  - (test: how many do humans have?)
- Replication: asexual reproduction
  - 1, 2, 4, 8, 16, 32 that are theoretically identical
  - *If cells replicate every hour, how many cells are there after 24 hours?*
  - Whole genome sequencing is useful because you can see minor changes in DNA over generations

# Bacterial growth

- Influenced by
  - Food (they eat too)
  - Acid (the more neutral, the better)
  - Temperature (most pathogens like body temperature)
  - Time
  - Oxygen (some like oxygen, some hate it, some don't care)
    - For this reason, modified atmosphere packaging changes the balance of microbes
  - Moisture (they like moisture)

*Bacteria can **survive** under harsher/ less hospitable conditions compared to the conditions where they will actually replicate*



Detection is easy at this point

What is your enrichment time?  
What is the limit of detection of the method?

# Pathogens vs indicators vs index organisms

- The bacteria don't know they are pathogens! We humans have decided that.
- **Indicator-** “Assess the adequacy of a... process ..., therefore indicating process failure or success; assess the hygienic status of the production environment and processing conditions; ... assess the overall quality of the food product.”
- **Index-** “suggest the possibility of a microbial hazard without actually testing for specific pathogens. ...serve a predictive function. The absence of the index organism does not always mean that the food is free from ... pathogens.”

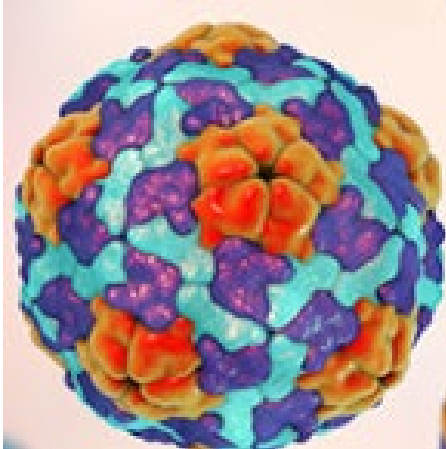
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# “Zoonotic” organisms

- Animals are the “natural” host, even though they can live elsewhere
- What are some examples?

# Virus pre-test

- What do you know about viruses?
- How are they different from bacteria?
- What are some examples?



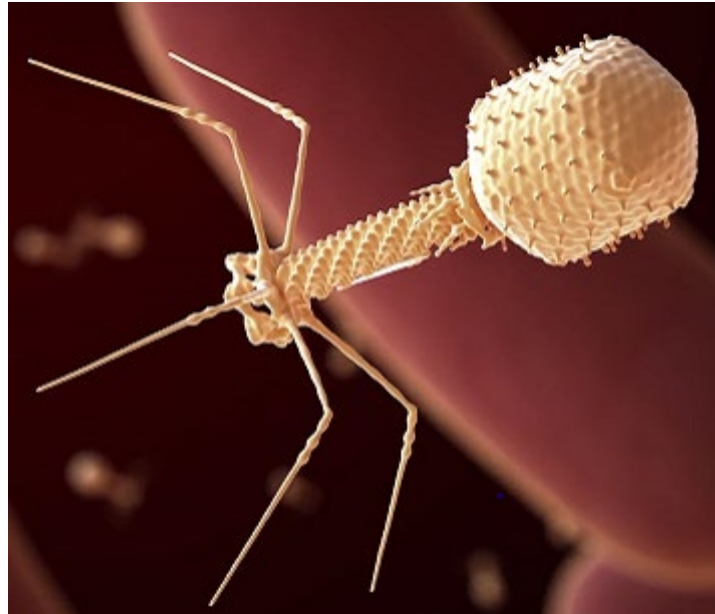
# Viruses

- Are they even alive?
- Genetics- don't always have DNA
- Need a host to replicate- will NOT grow outside a host
  - Human host- can cause illness
  - Bacterial host

<https://www.cdc.gov/features/viralhepatitis/index.html> and  
<https://www.cdc.gov/norovirus/about/index.html>

## Side note on viruses

- Viruses can infect bacteria- called phage



<https://www.niaid.nih.gov/news-events/video-fighting-infection-phages>



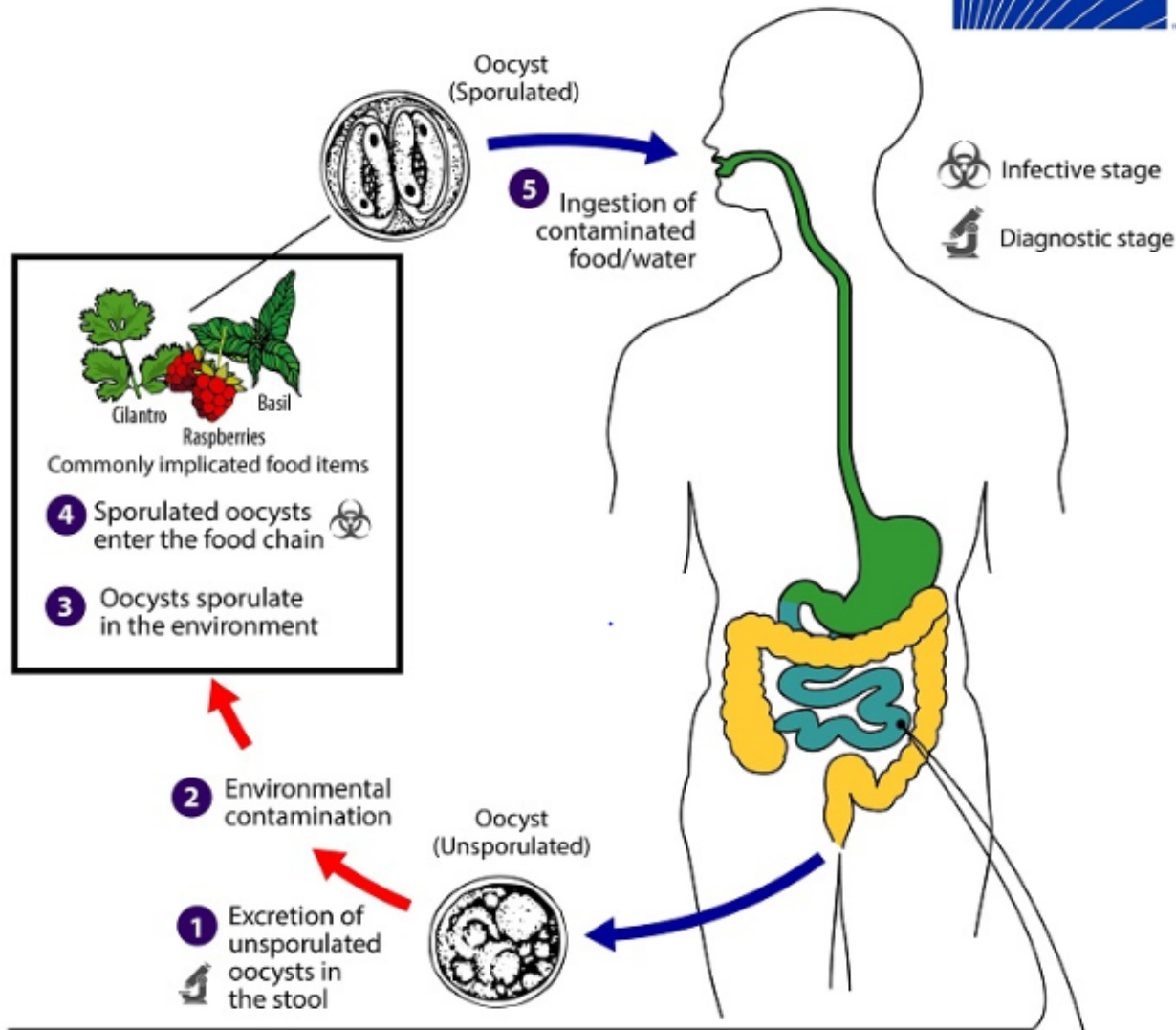
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# Parasite pre-test

- How are they different from bacteria and viruses?
- What are some parasites of concern to fresh produce?

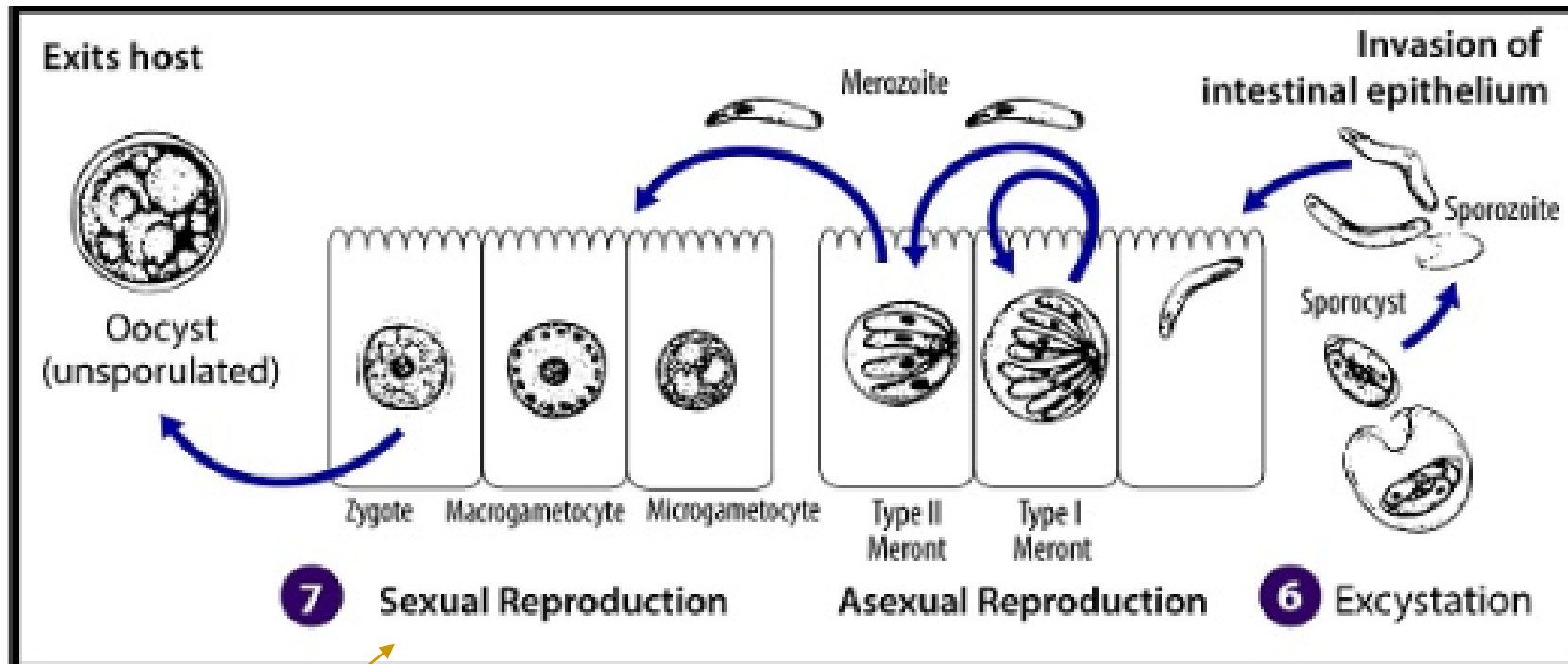
# Parasites

- Single or multi-cell
- More complex organization inside the cell
- Need a host
  - Some parasites require a very specific host, some are more flexible
- Sexual and/ or asexual reproduction



Will NOT grow outside the host, so you cannot grow it in a lab.

To test produce or water, you have to be able to detect whatever is in the product/ water



Makes whole genome sequencing really hard- DNA is recombined

# Summary

- Bacteria, viruses and parasites are extremely different
- It's a lot easier to detect more of something compared to less
  - If something grows in a lab, it's easier to detect
    - Plus, you know it's alive!
- The options to test for them are very different
  - Only bacteria grow well without a host
    - Parasites and viruses don't

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# Questions?

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