Worksheet: Bioinformatics Activity

For this activity you will be examining and comparing the composition of 5 different individuals' gut microbiomes. You will be using an image-based resource demonstrating gut microbiome composition and the Interactive Tree of Life (iTOL) software tool. The gut composition resource indicates the relative quantity of a subset of the microbes in each person's microbiome, to make comparisons easier. The iTOL element of this activity will be a broader exploration of the qualitative compsitions (i.e. the organisms present).

LOGIN INFO

You have been asked to create an account on the Interactive Tree of Life (iTOL) website for yourself. Based on this, we have connected a 6-week complimentary standard subscription for iTOL with this account.

To log in, please visit the iTOL website at https://itol.embl.de/
and go to "Login" on the top right hand corner.

Once your 6-week complimentary standard subscription has run out, you can continue to use iTOL in free access mode with all iTOL annotation features fully accessible.
For more information on access, please visit: https://itol.embl.de/infoReg.cgi?f=p

If you would like to run this activity with your students, you can request complimentary standard access for the duration of the assignment (see: https://itol.embl.de/pricing.cgi).

ACTIVITY 1: GUT MICROBIOME MATCHING

Read about these different people and using the information provided,
try to match them to the correct gut microbiome compositions found on the following pages.

- Kathyrn eats a healthy balanced diet with a variety of fruit and vegetables and protein from meat, fish and pulses, she has not taken any medicines recently.

- Sonesh suffers from inflammatory bowel disease, he tries to eat a well-balanced diet, but stress and some foods make his IBD worse.

- Laura is usually a healthy individual, however she's suffering from diarrhea and muscle cramps, she's recently been traveling abroad in an area with poor sanitation.

- Ricardo is suffering from diarrhea and abdominal pain, he's not travelled abroad recently but has drunk unpasteurised milk.
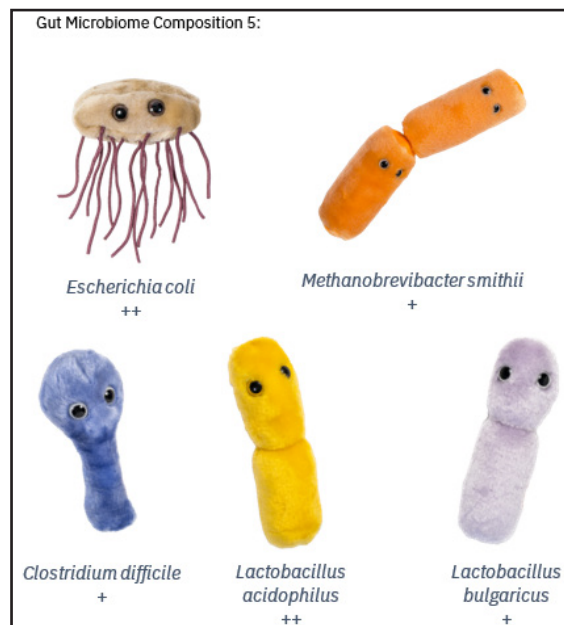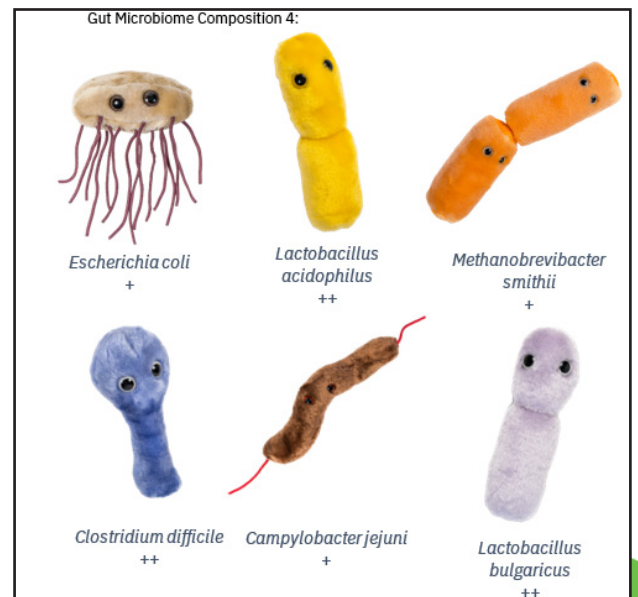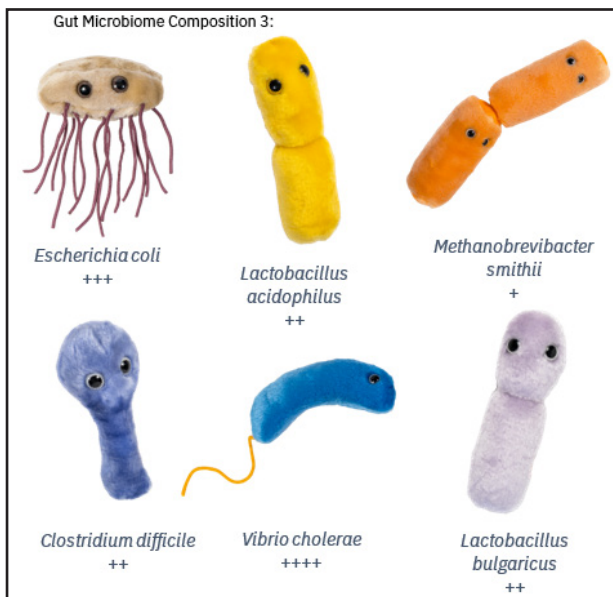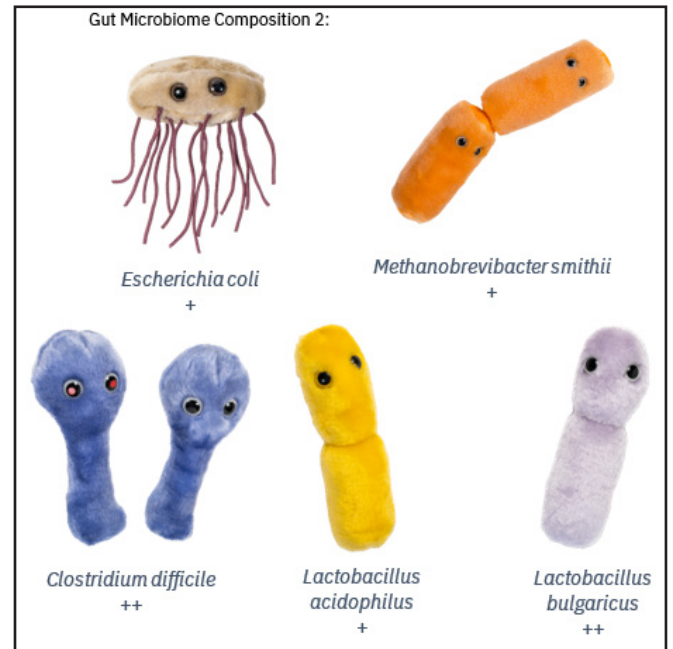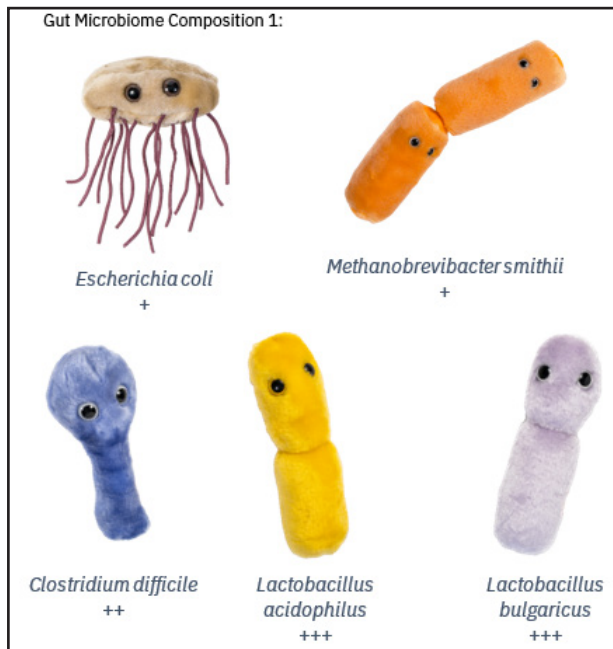
- Alex has had a bad tooth infection and is currently close to finishing a course of antibiotics.

GUT MICROBIOME COMPOSITIONS

The + symbols are indicative of relative abundance in the whole microbiome.

Gut Microbiome Composition 1:

Escherichia coli
+

Methanobrevibacter smithii
+

Clostridium difficile
++

Lactobacillus acidophilus
+++

Lactobacillus bulgaricus
+++

Gut Microbiome Composition 2:

Escherichia coli
+

Methanobrevibacter smithii
+

Clostridium difficile
++

Lactobacillus acidophilus
+

Lactobacillus bulgaricus
++

Gut Microbiome Composition 3:

Escherichia coli
+++

Lactobacillus acidophilus
++

Methanobrevibacter smithii
+

Clostridium difficile
++

Vibrio cholerae
++++

Lactobacillus bulgaricus
++

Gut Microbiome Composition 4:

Escherichia coli
+

Lactobacillus acidophilus
++

Methanobrevibacter smithii
+

Clostridium difficile
++

Campylobacter jejuni
+

Lactobacillus bulgaricus
++

Gut Microbiome Composition 5:

Escherichia coli
++

Methanobrevibacter smithii
+

Clostridium difficile
+

Lactobacillus acidophilus
++

Lactobacillus bulgaricus
+

MORE INFORMATION ON DIFFERENT GUT MICROBES

- *Lactobacillus acidophilus*
  https://en.wikipedia.org/wiki/Lactobacillus_acidophilus

- *Lactobacillus bulgaricus*
  https://en.wikipedia.org/wiki/Lactobacillus_delbrueckii_subsp._bulgaricus

- *Clostridium difficile*
  https://en.wikipedia.org/wiki/Clostridioides_difficile_(bacteria)

- *Vibrio cholerae*
  https://en.wikipedia.org/wiki/Vibrio_cholerae

- *Methanobrevibacter smithii*
  https://en.wikipedia.org/wiki/Methanobrevibacter_smithii

- *Escherichia coli*
  https://en.wikipedia.org/wiki/Escherichia_coli

- *Campylobacter jejuni*
 *https://en.wikipedia.org/wiki/Campylobacter_jejuni*

ACTIVITY 2: EXPLORING THE GUT MICROBIOME WITH ITOL

Once you've matched the microbiomes with the individuals, you can take a closer look at the microbiome composition of each individual by using the Interactive Tree of Life tool.

Before you start, gain a basic overview of the iTOL's user interface, basic functions and how to annotate trees interactively and with external data sets by watching the "User interface," "iTOL functions" and "Tree annotation" video tutorials, respectively, which can be accessed via the following link: https://itol.embl.de/video_tutorial.cgi

INSTRUCTIONS

1. Go to https://itol.embl.de/itol.cgi
2. Upload data set trees for each person. Please see the "How to upload a data set tree to iTOL" page for a detailed explanation on how to do so.
3. Explore the trees to find the microorganism of interest. You can also use the search tool on the left hand side of the screen.
4. Based on your observations, answer the questions on the following page.
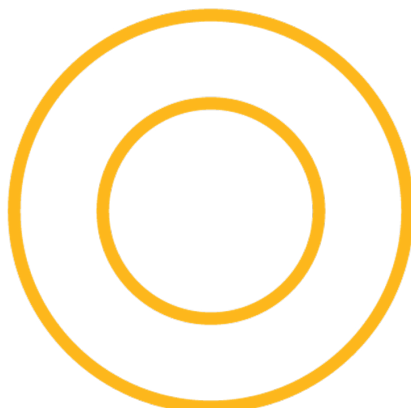
1. Which microorganism is consistently present across the gut microbiome profiles and why do you think this is?
2. Sonesh is suffering from a *C. difficile* infection. How many *C. difficile* strains are present?
3. How does his composition compare to the healthy individual and what does this tell you about the importance of *C. difficile*?
4. Which organism is most abundant in Laura's gut microbiome and could indicate the cause of illness?
5. Ricardo has similar symptoms to Laura - comparing their gut microbiomes are they suffering from the same disease or is there a different cause?
6. Compare Alex's gut microbiome composition to Kathryn's - has the microbe composition been affected by taking antibiotics?

# HOW TO UPLOAD A DATA SET TREE TO ITOL (WITH AN ITOL ACCOUNT)

1. Download the data files, including the *itol_taxa* and *itol_status* files.
2. After logging into iTOL, click on the "My Trees" tab along the top toolbar.
3. Once there click on the "Upload tree files" button and select all the tree files you downloaded from eCampus and upload.
4. Once uploaded, the tree files will appear in the "Sample project" window. Click on a file to view a tree. To add the color labels, click and drag the *itol_taxa* and *itol_status* files from their downloaded location to the tree window (you can select and drag both files at once).
5. For some trees you will get the following error message pop-up. Click "OK" to dismiss the message, this is due to the nature of the file set-up.
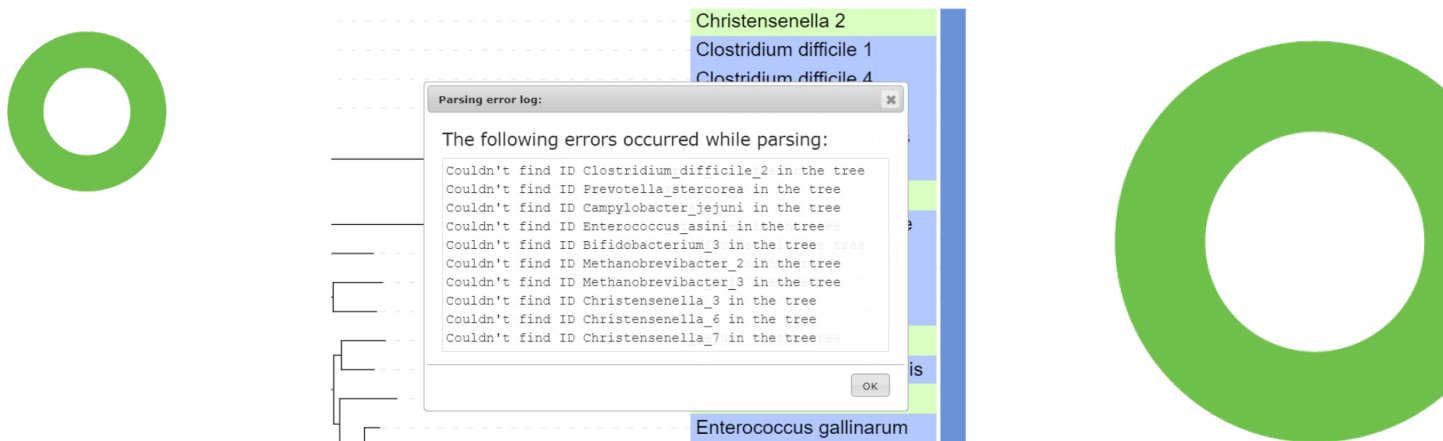
6. Change the tree "display mode" to circular view. You can refer back to the tutorial videos for guidance on how to explore the tree or change the tree display mode. You are now ready to answer the activity questions.
7. To make visualisation and exploration of the tree easier, you can label the datasets within your tree and create a Legend key. Select the "Dataset" tab in the "Controls" window. Next to "Edit:" click the "Legend" button, this opens a pop-up where you can label the taxonomy datasets within the tree and change the label colours. The "Legend title" will be *Taxonomy*, you can then name each dataset by adding text to the "Label" field, i.e. Archaea and change the colour of each set.
8. To switch the order of the microorganisms in each node you can "rotate nodes", right click on a tree branch (it highlights slightly when the cursor is in the correct place) and select "Rotate Node" from the menu.
9. Play around with the layout and colour ranges to adapt the visualisation of your trees. When you are happy with them you can "Save all changes" and export them as PDF files. To do this, select the "Export" tab in the "Controls" window menu, select PDF from the "Format" drop-down menu and click the "Export" button. The file will automatically download. Please note you will need to upload one exported tree as part of your assignment. Please note the tree files for each individual show a representative set of ~50 gut microbiota, in reality there are closer to ~200.

HOW TO UPLOAD A DATA SET TREE TO ITOL (WITHOUT AN ITOL ACCOUNT)

1. Go to https://itol.embl.de/upload.cgi
2. Enter a tree name - we'd suggest the name of the person the tree is related to.
3. Click on the "Choose File" button and upload the relevant tree. Click the "Upload" button and the tree will open automatically. You can refer back to the tutorial videos for guidance on how to explore the tree or change the tree display mode. You are now ready to answer the activity questions.
4. If you want to view multiple trees simultaneously, simply open https://itol.embl.de/upload.cgi in a new tab and repeat steps above.
5. Once you have the tree opened, you can add the colour labels. To do this, click and drag the *itol_taxa* and *itol_status* files from their downloaded location to the tree window (you can select and drag both files at once).
6. For some trees you will get the following error message pop-up. Click "OK" to dismiss the message, this is due to the nature of the file set-up.
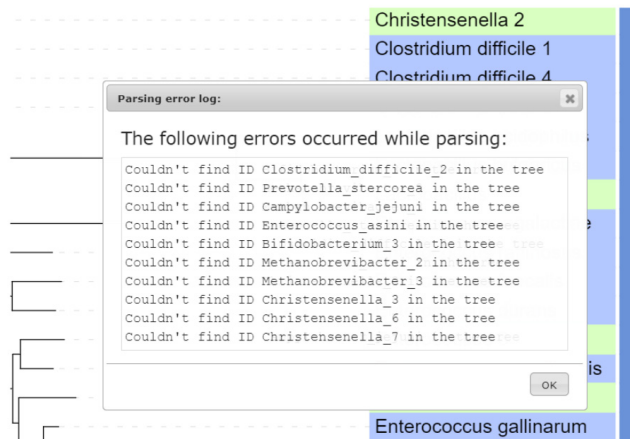
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9. To switch the order of the microorganisms in each node you can "rotate nodes", right click on a tree branch (it highlights slightly when the cursor is in the correct place) and select "Rotate Node" from the menu.
10. Play around with the layout and colour ranges to adapt the visualisation of your trees. When you are happy with them you can export them as PDF files. You won't be able to save changes to you trees in iTOL unless you are logged in.
11. To do this select the "Export" tab in the "Controls" window menu, select PDF from the "Format" drop-down menu and click the "Export" button. The file will automatically download. Please note you will need to upload one exported tree as part of your assignment. Please note the tree files for each individual show a representative set of ~50 gut microbiota, in reality there are closer to ~200.