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**Week 1-- 27 September 2016**

Daily Discussion:

Koalas, besides being one of Australia’s well-known, cuddly marsupials, are known to contract chlamydia. Recently, there have been large outbreaks of chlamydia in koala populations, sometimes affecting up to all of them. In order to treat them, they are taken into wildlife hospitals, administered antibiotics, and then released back into the wild. However in recent years, some koalas have unexpectedly been dropping dead out of trees, leaving scientists puzzled about the cause. One explanation as to why the sudden deaths are occurring is based on the idea that the antibiotics that the koalas are receiving are negatively affecting their gut bacteria. Their gut bacteria is what enables them to digest eucalyptus leaves, their main food source. Eucalyptus leaves contain tannins that can be toxic in large quantities, but the koalas’ gut bacteria is able to metabolize them.

The class will be a part of the research project that will isolate and characterize the koalas’ gut bacteria that may be critical for their digestion process. The research conducted will determine whether or not the antibiotics are affecting the gut bacteria.

Materials:

Koala poo

Vortex

Centrifuge

Centrifuge tube

Inoculation loops

Petri dishes

Procedure:

1. Received a small sample of koala poo in a centrifuge tube.
2. The sample was labeled with my initials.
3. A micropipette was utilized to add 800 μL of nanopure water to the centrifuge tube containing the fecal matter.
4. The centrifuge tube was placed onto a vortex to break up the feces as best as possible.
5. The sample was then centrifuged to separate the liquid and solid components.
6. Obtained three different Petri dishes labeled with specific conditions and added the koala’s initials, my initials, and the date.
7. The micropipette was used to place 100 μL of the liquid portion of the sample onto each plate, taking care not to lift the lid too high to prevent contamination.
8. The liquid was spread all over the plate with the larger end of the inoculation loops.
9. Placed the plates in their designated spots in the back of the room based on their specific conditions.

Data:

Koalas in study:

Zachary- Z (given)

Cynthia- C

Austin- A

Plates Worked With

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Plate ID | Media | Incubation Temperature | Aerobic? | Tannin? | Special Notes |
| LB,37,O2+,T+,Z | LB | 37℃ | O2+ | T+ |  |
| BHI,37,O2+,T+,Z | BHI | 37℃ | O2+ | T+ | Accidentally pipetted a bit of poo onto the plate and spread it around before realizing I did so. |
| LB,25,O2+,T-,Z | LB | 25℃ | O2+ | T- |  |

**Week 2-- 4 October 2016**

Daily Discussion:

The class was introduced to dilution streaking and given a tutorial on how to do it. Most of the plates from last week showed growth, including my own. In pairs, students selected specific colonies, labeled them accordingly, and placed them onto new plates using dilution streaking. The old plates were sealed with parafilm. After everyone was done streaking the new plates with the colonies, another demonstration was given on how to properly use a micropipette. This was followed by practicing pipetting water from a small centrifuge tube into another.

Materials:

Inoculating loops

Plates with colony growth

BA, BHI, and LB plates

Procedure:

1. Obtained my own plates from last session.
2. With a partner, identified 10 different colonies on plates, prioritizing plates with tannin. Circled each colony on the outer bottom of the plate and numbered them.
3. Matched the media that the colonies grew on with the media of the new plates
4. Labeled new plates according to the previous plates’ labels with the addition of colony number.
5. Used smaller end of the inoculation loop and placed selected colonies on their designated plate, using dilution streaking methods.
   1. Streaked plate on the left side using only one side of the loop.
   2. Flipped loop over, streaked from the edge of the left side towards the bottom of the plate.
   3. Used a completely new loop, and streaked from the edge of the bottom side towards the middle of the plate.
6. Placed new plates in their designated spots in the back of the room based on their specific conditions.

Data:

Plates Worked With

|  |  |  |  |
| --- | --- | --- | --- |
| Plate ID | Colony Number | Observations | Special Notes |
| LB,37,O2+,T+, Z AP | 1 | Small, round, cream, opaque | Agar slightly torn by loop |
| BHI,25,O2+,T-,Z,ALM | 1 | Round, white, smooth, opaque |  |
| BHI,25,O2+,T-,Z,ALM | 2 | Round, greenish, smooth, opaque | Agar slightly torn by loop |
| LB,25,O2+,T-,Z,AP | 1 | Round, gray, looks sporous |  |
| BHI,37,O2+,T+,Z,AP | 1 | Small, round, white, opaque | Agar slightly torn by loop |

**Week 3-- 11 October 2016**

Daily Discussion:

The plates from last week were placed under their specific conditions and were left to grow. Today the class collected their renumbered test tubes at the beginning of class. Professor Coil discussed the errors that were made during the dilution streaking last week. Some students did not follow the protocol correctly so some plates showed large amounts of growth throughout. However if one of these plates seemed to only have one type of growth on them, they were transferred to the specific media broths. Plates with more than one type of growth on them were discarded.

During the transfer of broths, another error occurred. It is unknown how, when, or with what specifically it was contaminated. The contaminant will be determined when we identify the bacteria growing (I think?).

A brief overview of the day’s plan was discussed.

Materials:

Heat block

Vortex

Centrifuge

Micropipette

Spin columns

Extra collection tubes

AE buffer

AL buffer

AW1 buffer

AW2 buffer

Ethanol

PBS solution

Proteinase K

Procedure:

(copy and pasted from “Week 3: DNA Extraction Protocol” from the Protocols for Wet Lab folder)

**Prep**

1. Assign test tube to students via number label.
2. Send notification to students informing them if their test tube numbers
3. Organize test tubes in numerical order for easy recovery
4. Remind students to label the LID of the eppendorf tubes and spin columns - not the side!

**Lysis**

1. Pipette 1ml **(1000ul)** vortexed/well shaken broth into 1.5ml tube, with the lid correctly labeled
2. Centrifuge for 2 min at max rpm, remove supernatant (the liquid on the top of the ‘pellet’)

*The first centrifuge is to condense the bacterial cells into a pellet. We pipet out the nutrient broth.*

1. Resuspend pellet in **200ul** PBS onto pellet.

*It is easiest to pipette the liquid up and down a couple of times over the pellet to resuspend. Stubborn pellets can be vortexed briefly.*

1. Pipette **20ul** Proteinase K into working tube.

*Proteinase K is added to lyse the cells, or to break them open and release the contents*

1. Pipette **200ul** of AL buffer into working tube

*The buffers added during this step are to provide conditions (pH, salts) to maximize enzyme activity.*

1. Vortex well.

*Double check that there is no trace of a pellet at the bottom of the tube.*

1. Put tube in the heat block (56C) for 10 minutes

*The incubation step is performed at the optimal temperature for comprehensive lysis of bacterial cells.*

**Precipitate**

1. Add **200ul** EtOH to the tube
2. Vortex

*Ethanol is added to precipitate the DNA molecules. The way this works is that normally, the negatively charged phosphate backbone of DNA is shielded by the water molecules. Note that the buffer solution also contains positive ions such as sodium. Adding ethanol disrupts the charge shielding, allowing the positive ions to bind both to the negative phosphate backbone and to the negatively charged silica column. Silica is what’s in the spin column. - Adam wrote this up from last quarter!*

\*Note: Precipitants may not be visible but doesn’t mean utter failure.

**Stick to the membrane**

1. Pipette the mixture into a spin column
2. **LABEL TOP OF SPIN COLUMN VIA RESPECTIVE NUMBER, NOT THE COLLECTION TUBE**
3. Centrifuge 1 min at 8000rpm

*The centrifugation step after adding ethanol passes the solution through the column and allows the DNA to stick to the column.*

1. Discard collection tube and liquid
2. Place spin column into **NEW** collection tube

**Wash part 1**

1. Pipette **500u**l Buffer AW1 into spin column
2. Centrifuge 1 min at 8000rpm
3. Discard collection tube and liquid
4. Place spin column into **NEW** collection tube

**Wash part 2**

1. Pipette **500u**l Buffer AW2 into spin column
2. Centrifuge 3 min at 14,000rpm
3. Discard collection tube and liquid

*The washes wash away any compounds that are not DNA while the DNA still sticks to the column.*

**Elute**

1. Place spin column on a new, correctly labeled, 1.5 centrifuge tube
2. Pipette **200ul** Buffer AE into spin column
3. Wait 1 minute
4. Centrifuge 1 min at 8000rpm
5. Collect the liquid and throw out the spin column

*The elution step uses a solution that releases the DNA from the silica column, so that the only compound in the solution that we keep is DNA. Buffer AE is composed of 10 mM of Tris-HCl and 0.5 mM EDTA with an overall pH of 9.0. The reason pH is important is because a lower pH can affect the DNA sample and make it unstable. DNA should NOT be rinsed with water because it has a pH of 4-5 but RNA is fine when being diluted with water.*

Data:

Collected tubes 27-31

Started incubation of tubes at 4:30pm. Took out of heat block at 4:40pm.

I was only able to stay until 5pm, where my group and I made it to step 12 collectively. They were able to stay later to finish the extractions.

At the point I stopped at, all the tubes were all at ~620 microliters.

**Week 4-- 18 October 2016**

Daily Discussion:

At the beginning of class, the technique polymerase chain reaction (PCR) was briefly discussed. The purpose of preparing the tubes for PCR today was so that the DNA collected could be amplified and used to sequence 16s rRNA gene. The master mix utilized in the preparation was made at the beginning of class to prevent primer dimers. It contains, amongst water, buffers, and other components, the universal primers 27 F and 1391 R which will prime that specific gene. After everyone and their partner completed their PCR preparation, the class did an inventory of all the lab materials that have been utilized throughout the course in order to start the grant-writing process. The class ended with an introduction to EndNote.

Materials:

Micropipettes (2 uL and 100 uL)

Disposable micropipette tips

Strip tubes

Master Mix

DNA from last week’s tubes

Procedure:

(copy and pasted from “Week 4: PCR Prep” from the Protocols for Wet Lab folder)

**PCR prep**

1. Find your DNA (it’s numbered very nicely)
2. Strips have 8 tubes, try to find a partner where your combined samples make 8 – you will be sharing a tube.
3. Label each tube in your strip with the sample number you will be putting in there.
4. Add 48 ul Master Mix.
5. Add 2 ul DNA.
6. Place in appropriate location.

Data:

Partner: Abigail Mende

Tubes collected: 15, 27, 28, 43

Abigail’s tubes: 10, 14, 29, 30

**Week 5-- 25 October 2016**

Daily Discussion:

Today the class performed gel electrophoresis on the DNA samples that went through PCR last week. The original samples that were prepared in class last week underwent the incorrect PCR procedure and had to be tossed out. More DNA was prepared and then underwent the right PCR procedure. Demonstrations were done for properly loading the wells and using the parafilm and micropipettes in order to mix the DNA samples and loading dye together. The class divided up into five groups to make the agarose gel needed for the process. Some gels prepared had more wells than others so people had to switch around accordingly. After the samples went through gel electrophoresis they were examined in the lightbox and discovered that most samples did not have any banding.

Materials:

125 mL Erlenmeyer flask

Agarose

Weigh boat

SYBR safe DNA gel stain

Casting tray

Comb

Parafilm

10 uL micropipette

Disposable micropipette tips

Loading dye

Gel box

DNA samples

Lightbox

Procedure:

(copy and pasted from “Gel Protocol” from the Protocols for Wet Lab folder)

1. Making Gel Mixture
2. Weigh 0.5 g agarose with weigh boat
3. Put into clean 125 ml flask
4. Add 50 ml of 1X TAE (fill to 50ml line in the flask) \*\*\*Toxic-don’t get on skin and clothes
5. Microwave (we will figure out time with 1st group, microwave until agarose is completely dissolved)-- about 1 minute
6. DO NOT BOIL OVER
7. Add the proper amount of invitrogen SYBR safe DNA gel stain (WE WILL TALK ABOUT THIS)

\*\*\*Don’t get this on your skin - contains DMSO, a solvent.

1. Swirl to mix
2. Pour gel into assembled casting tray (make sure comb is present)
3. Let cool until totally opaque and solid

II. Prep samples

1. Find your samples (i.e. 1-20, 21-40, …) → Follow Demo
2. Get a 6-inch strip of parafilm
3. For each sample, Add 2 ul loading dye to a spot on the parafilm
4. Pipette out 10 ul of sample onto parafilm with loading dye
5. Pipette up and down to mix

-make sure NO BUBBLES are present while mixing

1. Transferring gel

-Remove comb

-Slide gel from casting tray into gel box

III. Running the gel

1. Fill the gel box - completely covering gel - with 1X TAE (do NOT overfill)
2. Load samples with a new pipette tip for each sample (WITH EXTREME CARE)

-Tip of the pipette should be in the well but don’t stab the gel - this is easier said than done.

1. Load 5 ul of DNA ladder to first well on the gel
2. Add all 12 ul of DNA/dye mixture to well, 1 well per sample! Note in your lab notebook what samples are where - don’t lose track
3. Attach lid
4. Run at 150 volts until the dye is about 50% down the gel or when instructor says

-Ask instructor when to terminate/turn off gel - we will need to eyeball it for you :)

IV. Analyzing the gel

1. Take out gel
2. Visualize on the light box
3. Turn on lightbox - USE the Shield!!!!!! \*\*\*Directly looking at UV rays can damage eyes
4. Take a picture - add to lab notes
5. Record in lab notebook

* Was there a nice band? Talk about quality of the band
* Presence/Absence (David will talk about what faint bands mean)
* Indicate which samples will proceed to cleanup for next week’s class

Data:

Gel Electrophoresis Results

|  |  |  |
| --- | --- | --- |
| DNA Sample | PCR (+/-) | Band Description |
| 15 | + | Weak band |
| 27 | - | Weak band |
| 28 | + | No band |
| 43 | + | Medium band |

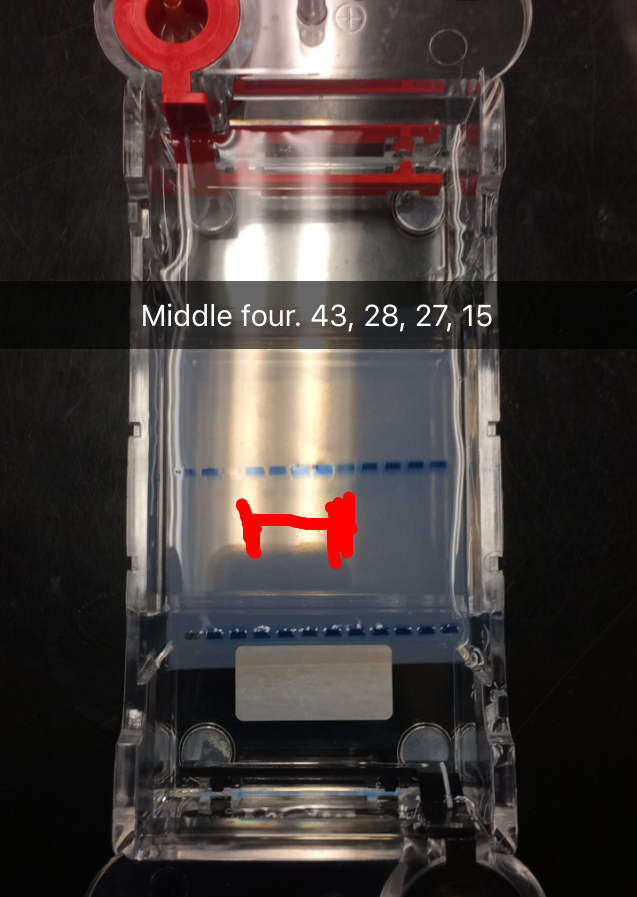
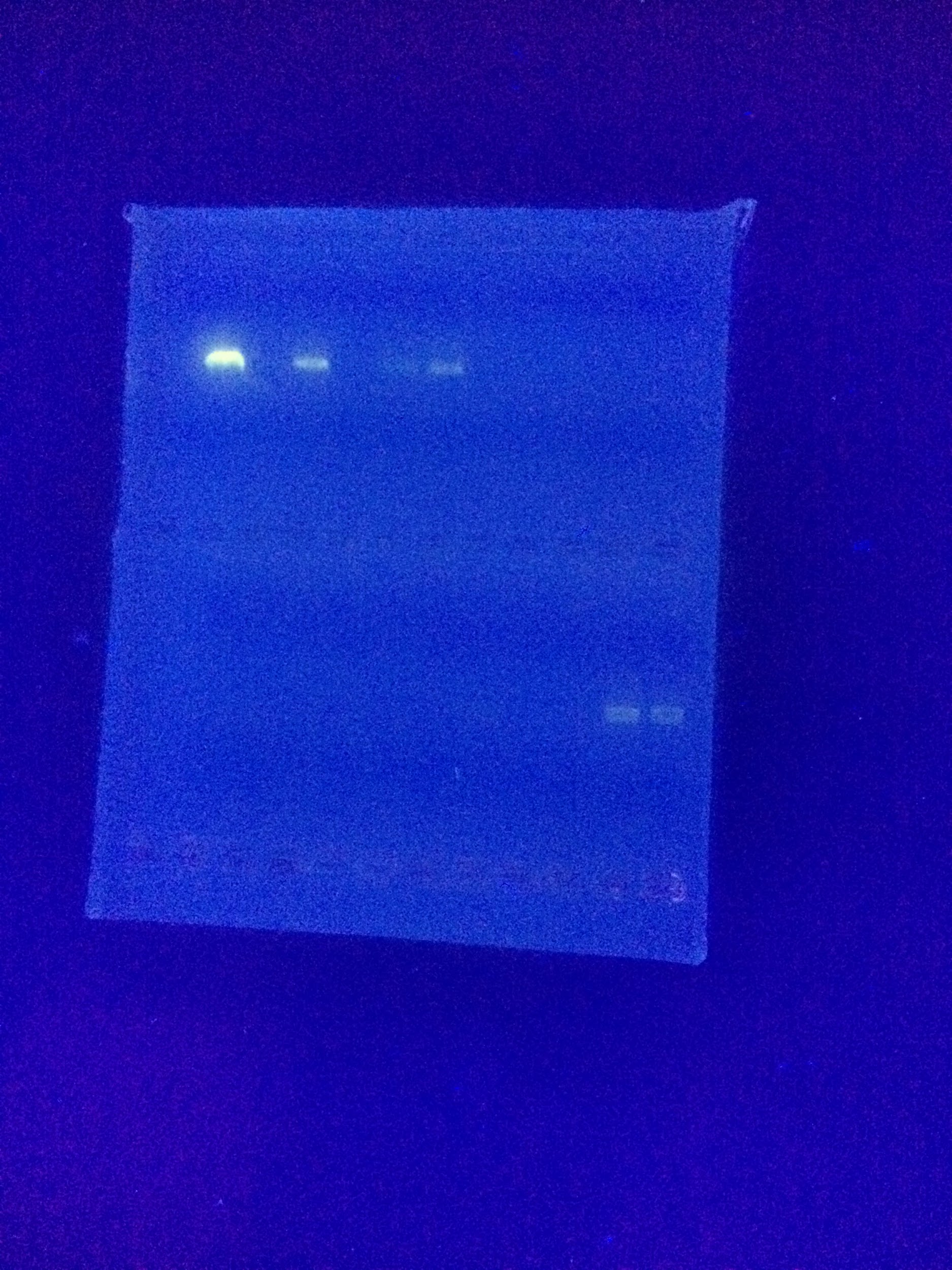
 

Figure 1: DNA loaded into wells. Figure 2: Results shown in the lightbox. Gel

placed exactly how it was in Figure 1.

**Week 6-- 1 November 2016**

Daily Discussion:

Today we discussed how the samples that failed PCR last week were rerun and approximately half worked. Then we gathered our samples and performed PCR clean up in groups. At the end of class we had a discussion about the literature review.

Materials:

PCR samples

Micropipettes

Disposable micropipette tips

Buffers NT3, NE, NTI

Centrifuge

Spin columns

Microcentrifuge tubes

Procedure:

(copy and pasted from “PCR product clean-up protocol (week 6)” from Protocols for wet lab)

Buffering

Start by making sure your tube is labeled.

(you have ~ 40 uL of PCR product to clean up)

Add 80uL Buffer NTI to your PCR product

Mix by pipetting up and down a few times in the tube

Bind DNA to membrane

Put whole mixture in the spin column (Tube with a removable short tube in the center)

Label the cap and the tube

Centrifuge 30 sec at 11,000xg

Wash and Dry

Add 700uL buffer NT3 to spin column

Centrifuge 30 sec at 11,000xg

Dump out washed product (at the bottom of the long tube)

Centrifuge 1 min at 11,000xg

Wash and Dry...Again

Add 700uL buffer NT3 to spin column

Centrifuge 30 sec at 11,000xg

Dump out washed product (at the bottom of the long tube)

Centrifuge 1 min at 11,000xg

Elute

Move spin column to a NEW 1.5uL microcentrifuge tube (NOT A COLLECTION TUBE!!! When you finish, you will need to cap it. Once you remove the spin column from the collection tube, there’s no cap. Think people.)

Label your tube and cap

Add 20 uL NE buffer

Incubate at room temp for 1 min

Centrifuge 1 min at 11,000xg

Again, make sure everything is labeled.

Data:

PCR Results

|  |  |  |
| --- | --- | --- |
| Sample Number | PCR Round 1 (+/-) | PCR Round 2 (+/-) |
| 15 | + | + |
| 27 | + | + |
| 28 | - | - |
| 43 | + | + |

DNA Qubit Results

|  |  |
| --- | --- |
| Sample Number | DNA Qubit Results (ug/mL) |
| 15 | 9.66 |
| 27 | 7.19 |
| 43 | 23.6 |

**Week 7: 8 November 2016**

Daily Discussion:

Instead of being in the usual classroom in Storer Hall, class was held right next door in 93 Hutchison Hall so that the class could access computers and specific programs needed to the perform the day’s tasks. The samples from last week were sent to the Sanger Lab to be sequenced and then sent back. The sample sequences were separated into two different computer files--one is a forward read and the other is a reverse read. SeqTrace and BLAST were then utilized to identify the bacteria.

Materials:

Computer files of samples

SeqTrace program

BLAST program

Procedure:

(copy and pasted from “Copy of SeqTrace and BLAST Instructions--DAC” from the Protocols for Computer Lab folder”)

**Background** The Sanger facility sends us forward and reverse reads… we need to:

1. cut off the crappy end parts,
2. “overlap” the trimmed sequences by matching up the base-pairs,
3. get a “consensus” sequence from the two reads

**How to open the SeqTrace program**

Go to your folder on the desktop

Got to the class software folder

Double click on SeqTrace

**Get your files on the desktop**

Download all your sequence files from the Google Drive. Make sure to unzip the downloaded folder.

(1 pair for every bug that you're working on; 27F and 1391R)

Example: For sample 94, download 008\_**94+27F**\_A02.ab1 001\_**94+1391R**\_H02.ab1

(Read number\_samplenumber+primer name\_plate position.ab1)

\*\*\*\*You can control click to highlight lots of files and right click to download all at once

Now go to SeqTrace

**Edit and create a consensus sequence with seqTrace**

1. Create new project (File → New Project) → Okay \*\*\*don’t change the defaults
2. To add files, go to “Traces” and click on “Add trace files”, then select the reads from the folder on the desktop (.ab1 files) you want to work with.
3. Click the arrow icon, in front of your REVERSE read file (1391R) (this designates it’s to be reverse in SeqTrace) make the arrow look like this: ←
4. Group your forward and reverse reads by highlighting both of them and clicking “Group selected forward/reverse files” (under “Traces”). **Name the group with the sample name!** Press Ok.
5. Under “Sequences” go to “Generate Finished Sequences” and click on “for all trace files”. (if it asks you to overwrite, say YES)
6. To view your consensus sequence, click on the read pair group and then click on the **magnifying glass icon** at the top of the page.

*The “Trace View” shows the quality scores, the chromatogram (trace) display, and the raw base calls from both the forward and reverse reads, as well as the consensus sequence. The consensus sequence is at the bottom.*

1. To export the consensus, go to “Sequences”, hover on “Export Sequences”, “From all Traces”

and then...

Find the desktop, then save.

Name it: **[lastname\_sample1\_sample2\_sample3].fasta**

**Example: Coil\_25\_26\_48.fasta**

*This will create a file containing the consensus sequence, which can then be used for analysis such as searching for closely related sequences using the BLAST program (Altschul, 1990) which can be used to identify the organism.*

**Fix up the first line of each FASTA sequence**

1. Open your Fasta file (saved to the desktop) using Notepad

2. Cleanup/Rename the 1st line to “>[sample#]” For example here is the before and after of a line in the sequence file:

Before:

>..\..\..\dnasequencingresults24765\003\_100+27F\_F02.ab1, ..\..\..\dnasequencingr

After

>100

**Copy your FASTA file back into the “Consensus” Google Drive Folder for consensus sequences (or we will lose it when you log out)**

**Also save your SeqTrace project and put it into the “SeqTrace” Google Drive Folder**

**BLAST your consensus Sequence**

1. Navigate to the Standard Nucleotide BLAST at NCBI:
2. <http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome>.Upload your consensus fasta file.
3. Check the box to exclude Uncultured/environmental sample sequences, since these will not be informative for identification. Be sure the nucleotide collection (nr/nt) is selected under database and click the “BLAST” button (Fig. 6).
4. Record your results i.e. list the **species** you observe on the first page of hits in your individual lab notebook - in order. In the compiled lab notebook, list only the first 4 (there might not be 4).

RDP - upload instructions

1. Go to<http://rdp.cme.msu.edu>.
2. Create an account.
3. Click on “my RDP/login”.
4. Upload the fasta file containing your 16S rDNA sequences.
5. Assign it a group name (your name is fine).

Data:

Blast Results--

Sample 15

Max score, total score, query cover, E value, Ident, Accession

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Select seq gb|CP016497.1|** | [Escherichia coli strain UPEC 26-1, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1046573820) | 1123 | 7839 | 69% | 0.0 | 85% | [CP016497.1](https://www.ncbi.nlm.nih.gov/nucleotide/1046573820?report=genbank&log$=nucltop&blast_rank=1&RID=24W73TTJ01R) |
| **Select seq gb|CP015074.2|** | [Escherichia coli strain Ecol\_745, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1043308734) | 1123 | 7850 | 69% | 0.0 | 85% | [CP015074.2](https://www.ncbi.nlm.nih.gov/nucleotide/1043308734?report=genbank&log$=nucltop&blast_rank=2&RID=24W73TTJ01R) |
| **Select seq gb|CP016358.1|** | [Escherichia coli strain K-15KW01, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1043194029) | 1123 | 7833 | 69% | 0.0 | 85% | [CP016358.1](https://www.ncbi.nlm.nih.gov/nucleotide/1043194029?report=genbank&log$=nucltop&blast_rank=3&RID=24W73TTJ01R) |
| **Select seq gb|KU362661.1|** | [Shigella sp. 80B.5 16S ribosomal RNA gene, partial sequence](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_973456952) | 1123 | 1123 | 69% | 0.0 | 85% | [KU362661.1](https://www.ncbi.nlm.nih.gov/nucleotide/973456952?report=genbank&log$=nucltop&blast_rank=4&RID=24W73TTJ01R) |

Sample 43

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Select seq gb|CP016497.1|** | [Escherichia coli strain UPEC 26-1, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1046573820) | 2156 | 15043 | 98% | 0.0 | 97% | [CP016497.1](https://www.ncbi.nlm.nih.gov/nucleotide/1046573820?report=genbank&log$=nucltop&blast_rank=1&RID=24V2CKNJ01R) |
| **Select seq gb|CP015074.2|** | [Escherichia coli strain Ecol\_745, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1043308734) | 2156 | 15070 | 98% | 0.0 | 97% | [CP015074.2](https://www.ncbi.nlm.nih.gov/nucleotide/1043308734?report=genbank&log$=nucltop&blast_rank=2&RID=24V2CKNJ01R) |
| **Select seq gb|CP016358.1|** | [Escherichia coli strain K-15KW01, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1043194029) | 2156 | 14987 | 98% | 0.0 | 97% | [CP016358.1](https://www.ncbi.nlm.nih.gov/nucleotide/1043194029?report=genbank&log$=nucltop&blast_rank=3&RID=24V2CKNJ01R) |
| **Select seq gb|CP014667.1|** | [Escherichia coli strain ECONIH2, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1039701615) | 2156 | 14993 | 98% | 0.0 | 97% | [CP014667.1](https://www.ncbi.nlm.nih.gov/nucleotide/1039701615?report=genbank&log$=nucltop&blast_rank=4&RID=24V2CKNJ01R) |

**Week 8--15 November 2016**

Daily Discussion:

Today’s discussion focused on the process of building phylogenetic trees from the data that we saved last week. A phylogenetic tree is a branching diagram that shows the evolutionary relationship between species based on their physical and genetic traits. The literature review papers were also briefly discussed at the end.

Materials:

Saved RDP file

FastTree program

Dendroscope program

Virtual Machine

Procedure:

**Getting into the Virtual Machine:**

Login to the computer with kerberos

click on user icon in upper left corner

click on class software

click on FRS 2 Virtual Machine

wait - be patient...

Don’t touch anything until the window turns blue and prompted to put in password: frs2

click login

click away the two pop-up windows

click on little weird mouse talking thing upper left hand corner

click on terminal emulator

STOP DO NOT PROCEED WITHOUT INSTRUCTOR APPROVAL

**Meanwhile:**

Start by re-BLASTing your .fasta file from last week. You’ll want the BLAST results handy as you attempt to build the phylogenetic tree.

<http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq>.

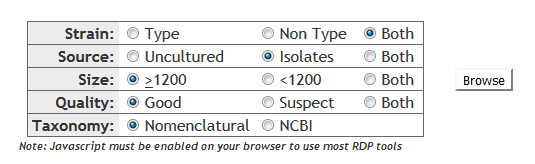
As a reminder, please make sure under “Choose Search Set” that:

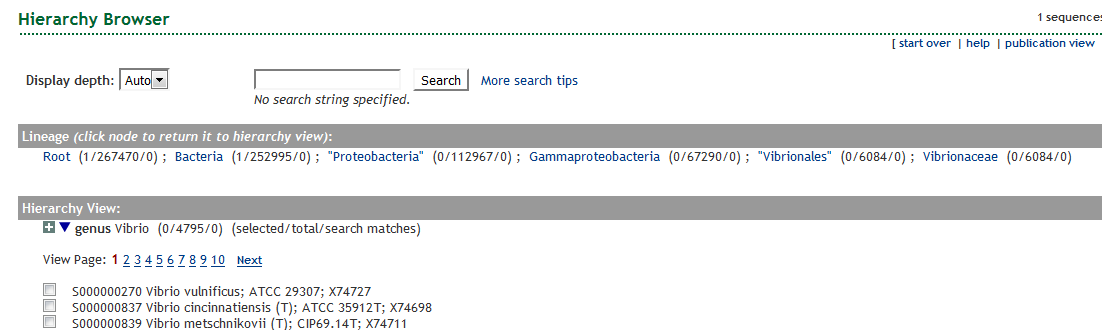
“Nucleotide collection (nr/nt)” is chosen for database

“Uncultured/environmental sample sequences” is checked

You may have to uncheck “Align two or more sequences” to access these options

* Navigate to RDP and login (using the account you made last week):
* <http://rdp.cme.msu.edu/myrdppub>
* Find your uploaded sequence and click the “+” to add it to your cart
* Click on “BROWSERS” (on top of the webpage)
* Click on “Isolates” to select only isolates for further analysis. Then click “Browse” ([Fig. 10](https://peerj.com/articles/960/#fig-10)).



* Search genus/species of interest (whatever came up as the closest genus match in BLAST). Type in the full name of interest (genus and species epithet) in the search box. 

*Now select all the species you think could represent your sample (these are going to become branches of your tree). Select by clicking on the checkbox or “+” to select all the listed names. Select those that are most similar in name to your top 3-4 BLAST results. Start by searching for the species, if there are less than 20-30 sequence matches for that species then you can select them all. If there are many more, you should manually select ~20 representatives from that species. Do this for each of the species that matched at the highest level of* ***identity*** *in your BLAST results. Note: click “refresh” whenever a series of selections is made.* This adds each species to your SEQCART. A SeqCart of 10-50 species (depending on your genus) will work well for tree building.

* Next you need to choose an outgroup to root your tree. (from the genus closest matched in your BLAST result, go back by one classification hierarchy *e.g. genus--> family* and choose an outgroup that you like) --This outgroup should be selected as in the previous step above when choosing the species that closely represent your sample. **Do not forget to click “refresh” as well after selecting a few species for the outgroup.**

*As discussed at the start of class, the best outgroup is as close as possible to the group of interest without falling within the group of interest. Best is to choose a type strain of a species from a genus within the same family as your group of interest.*

* View your selection by clicking on the SEQCART tab on top of the webpage (This will show you how many comparative sequences you have selected.)
* Click on “download”, leave the download options as the defaults (fasta, aligned, uncorrected), and then click on the appropriate download button (the appropriate download button is on the bottom of the page with 2 other selections, **DO NOT click the download button on the top right of the page**). Save the file to your **desktop** and then rename it something informative. Afterwards, upload the file to the appropriate folder in the Google Drive.

Repeat this process for each isolate

**Step 2: Clean up your new FASTA file.**

The RDP alignment will have taxon names that most of the downstream software tools will not tolerate because they include special text characters. So, we have written a little Perl script (cleanup.pl) that will remove those special characters and replace them with underscores.

This script has been placed in the Virtual Machine on these computers. The first step is to get the file into the Virtual Machine as well. Open a web browser \*WITHIN THE VIRTUAL MACHINE\* by clicking the weird mouse thing to find the web browser icon in the dropdown. Navigate to your Google Drive and download the file into the virtual machine.

To run cleanup.pl:

* Go to the Terminal Emulator window that you already opened
* Navigate to the Desktop by using the cd (current directory commands)

username$ cd Desktop/

* Call the perl script by entering the following command.

username$ cleanup.pl

-i **myrdp\_download\_𝓧𝓧\_seqs.fa** -o **FRS##clean**.fa

(𝓧𝓧 => look on Desktop to get number, ## => your sample)

*Your input file is denoted by –i, your output file is denoted by –o*

*Put clean somewhere in your output file name.* The new file will appear on the Desktop within the virtual machine if done correctly.

This will give you a new and improved file that FastTree can read!

Repeat Step for each isolate

**Step 3: Build a Tree with FastTree**

* Use the following command in the terminal to run FastTree

username$ FastTree -nt FRS##clean.fa > FRS##clean.tre

*Note: FRS##clean.fa should be your cleaned FASTA file from the previous step and may have a different name if you renamed it.*

*FRS##clean.tre will be your new output file and you should rename it.*

*(nt = nucleotide tree) input file, “>” means write to the output file.*

*You better name it .tre or dendroscope won’t recognize it.*

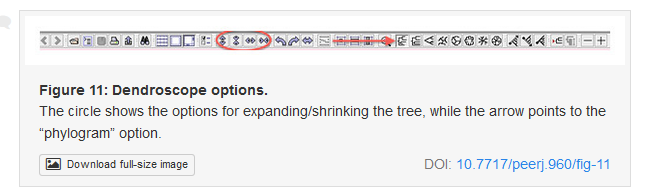
*Repeat Step for each isolate*

Then you need to move the .tre file back into Google Drive. \*\*Be sure you use the Google Drive that is open in the Virtual Machine\*\*!

**Step 4: View your Tree in Dendroscope**

* Find Dendroscope by typing “Dendroscope” in the search bar under the strange mouse icon
* Open from File → open → your file on the desktop
* Select “Interpret as node labels” when asked (if this does not come up, you did something wrong.)

*Once the tree is visible, the first step is to re-root the tree to the outgroup. Expand the tree by clicking the expansion button (labeled in*[*Fig. 11*](https://peerj.com/articles/960/#fig-11)*), then scroll through the tree to locate the outgroup. Click on the beginning of the taxon name, to select it, and re-root the tree by going to edit and selecting “re-root”. (If you don’t get a tree, you probably made a mistake in what file you inputted into the terminal. Start from calling the perl script again and make sure you clean up the RDP file. Don’t give up, you can do this!)*



*We recommend viewing the tree as a phylogram, which can be accomplished by clicking on the phylogram button (labeled in*[*Fig. 11*](https://peerj.com/articles/960/#fig-11)*). From this tree it should be possible to determine the phylogenetic placement of the candidate sequence, and in some cases to give it a name with more certainty than a simple BLAST search.*

Repeat Step for each isolate

Save Dendroscope files to the FRS 2 Folder.

**Summary of Unix/Linux commands and terms**

$ **ls** lists files and directories (folders). If left as just “ls” this command will list the files and directories in your current location. If a “path” is added afterwards (e.g., ls /usr) this command will list the files and directories in that location.

$ **cd** use to change directories

$ **cd ..** use to move up one directory

$ **cd directory\_name** use to move to that directory

$ **cd** ∼ use to move to the home directory of the current user

$ **less file\_name** view a file, type q to exit

A few quick definitions:

*command line*—the command line is where you type commands in a terminal window

*script*—a computer program. Usually computer programs are called scripts when they perform relatively simple functions that are limited in scope. Scripts are typically only run from the command line

*directory*—a folder

*compile*—turning a human-readable file into a computer-executable program

Data:

Outgroup chosen-- Citrobacter

Sample 43 genus species: E. coli

**Week 9-- 22 November 2016**

Daily Discussion:

Today we briefly discussed the antibiotic resistance test we were to set up for our samples. It is also called the Kirby Bauer test. At the end of class we went over what is expected of us for the literature review peer reviews.

Materials:

tubes of overnight culture of your sample

Sterile swabs

Forceps

Lighter

BHI agar plates

Antibiotic disks:

* Ampicillin disks
* Chloramphenicol disks
* Tetracycline disks
* Penicillin disks
* Gentamicin disks
* Enrofloxacin disks
* Neomycin disks
* Streptomycin disks

Procedure:

(copy and pasted from “Week 9, Antibiotic Sensitivity” from the Protocols for wet lab folder)

1. Gently shake your overnight culture to resuspend the bacteria into the broth. This is your stock culture.
2. Dip a sterile swab into the liquid culture, use the swab to spread the liquid culture **evenly** over the entire plate.
3. Allow the liquid to dry
4. Using flame sterilized forceps, aseptically place 4 different antibiotics disks onto each labeled quadrant of a plate. FLAME THE FORCEPS BETWEEN EACH DISK. Press down \*gently\* to make sure the disks stick to the agar and don’t fall off when the plate is inverted. Repeat for second plate.
5. Plate your perfectly labeled plates into the appropriate pile

Data:

Samples and Conditions

|  |  |
| --- | --- |
| Sample Number | Plate Conditions |
| 15 | BA, 37, O2-, T+ Z-01 |
| 43 | BHI25O2-T+CGTY4OCT16Colony6 |

**Week 10--29 November 2016**

Daily Discussion:

Today we wrapped up the class by discussing the literature review and overviewing what we did throughout the course of the quarter. But first we measured the length of the diameter of the rings around the antibiotic disk to determine the bacteria’s susceptibility to the antibiotic. The larger the diameter, the more effective the antibiotic is against the bacteria. If there is no ring present, then the bacteria is resistant to the antibiotic. After everyone was finished measuring the diameter lengths of the rings, everyone went around the room and gave a brief summary of their findings from their lit review. We finished off the class by describing what we did each week on the chalkboard.

Materials:

Ruler

Plates from last week

Procedure:

1. Take a ruler and measure the diameter of the susceptibility zone in millimeters, if present.
2. Record measurements in the individual and group lab notebooks.
3. If no diameter, mark “R” for resistant.

Data:

Length of Susceptibility Zones

|  |  |  |  |
| --- | --- | --- | --- |
| Sample Number | Antibiotic | Diameter Length in mm  R stands for resistant | Notes |
| 15 | Ampicillin | 23 mm |  |
| 15 | Chloramphenicol | R |  |
| 15 | Tetracycline | 21 mm |  |
| 15 | Penicillin | R |  |
| 15 | Gentamicin | 11 mm |  |
| 15 | Enrofloxacin | 22 mm |  |
| 15 | Neomycin | 14 mm |  |
| 15 | Streptomycin | 12 mm |  |
| 43 | Ampicillin | 24 mm |  |
| 43 | Choramphenicol | 46 mm | rings for Chloramphenicol and Tetracycline overlapped a bit so roughly estimated the diameter |
| 43 | Tetracycline | 32 mm | rings for Chloramphenicol and Tetracycline overlapped a bit so roughly estimated the diameter |
| 43 | Penicillin | R |  |
| 43 | Gentamicin | 13 mm |  |
| 43 | Enrofloxacin | 26 mm |  |
| 43 | Neomycin | 21 mm |  |
| 43 | Streptomycin | 15 mm |  |