

MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES BIOINFORMATICS WORKSHOP

Presents

Introduction to tree visualization and annotation using ggtree

INSTRUCTED BY

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INFORMATION FOR PARTICIPANTS

**All workshops are being recorded and posted to the
[MMID Bioinformatics Workshop – YouTube](#)**

**For live Q&A, go to [slido.com](#) and use participant
code #[3323315](#)**

2023 MMID Bioinformatics Workshop Schedule

DATE	INSTRUCTOR	TOPIC
March 2	Grace E. Seo	Introduction to the 2023 MMID Bioinformatics Workshop
March 9	Grace E. Seo	Introduction to conda and tool installation
March 16	Grace E. Seo	Introduction to genomics and viral data analysis
March 23	Jill Rumore	Bacterial Genomics
March 30	Jill Rumore	Reference Databases
April 6	Taylor Davedow	Beginner's Guide to Phylogenetic Trees
April 13	Taylor Davedow	Introduction to tree visualization and annotation using ggtree
April 20	-	Bfx workshop: Bring your own dataset!
April 27	-	Bfx workshop: Bring your own dataset!

April 20 and April 27 in-person sessions are open to the public (up to 100 people)!

Work with your colleagues/friends to analyze data together.

SET UP WI-FI (IN-PERSON PARTICIPANTS)

- 1. Connect to UofM-secure (if you are a student or staff)**
- Use your @myumanitoba.ca or @umanitoba.ca login and password
- 2. Connect to UofM-guest**

To access uofm-guest Wi-Fi:

1. Ensure your wireless card is active and connected to the **uofm-guest** network.
2. Open your web browser (e.g. Google Chrome, Microsoft Edge, Firefox, etc.) and browse to any website. This should redirect you to the **Acceptable Use Agreement** page.
3. Review the Acceptable Use Agreement for the unsecured wireless.
4. Select **I Agree**.

LEARNING OBJECTIVES

1. Create a tree using ggtree
2. Use geometric functions and aesthetic mappings to annotate tree
3. Learn how to customize legends and add themes

DISCLAIMER

To provide a basic working instruction, all tools will be run with default settings. HOWEVER, careful consideration of analysis parameters in the context of the research question should be taken into account when analyzing your own datasets, as default parameters do not always provide the most optimal result.

FILES FOR WORKSHOP

1. msa.fasta.tree (newick format)

- Created by downloading a subset of genome accessions listed in manuscript, skesa assembly, multiple sequence alignment and running iqtree

2. metadata.xlsx

- Information compiled from manuscript (Tables 1 & 2)

3. RScripts

- Follow along with today's workshop using our R code

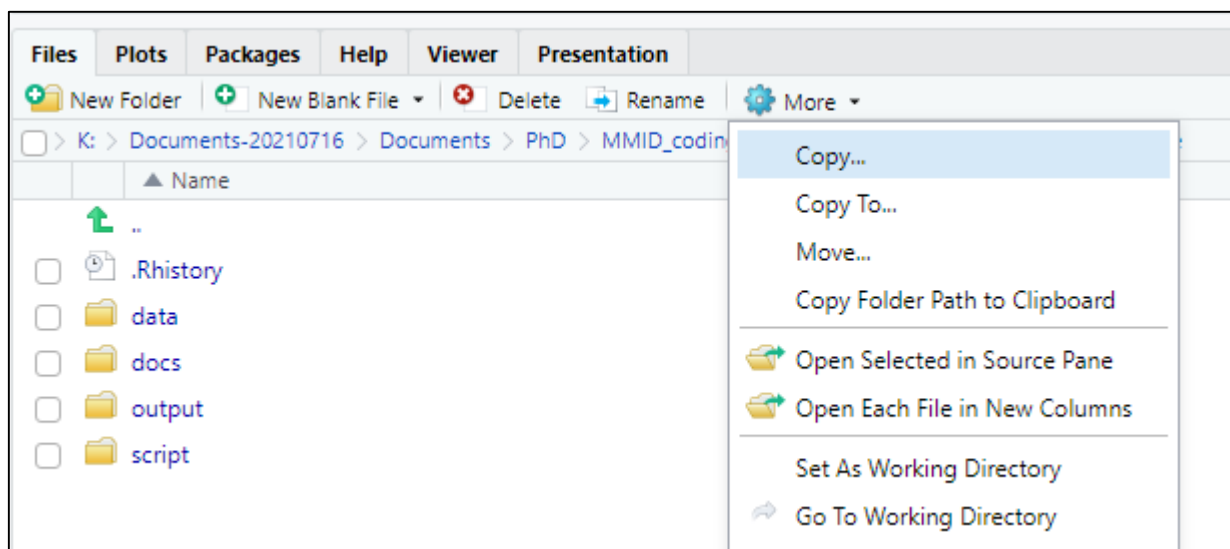
Please download and save materials to your workshop directory:

<https://github.com/mmid-bioinformatics-workshop/2023-04-13-Intro-to-tree-visualization-and-annotation-using-ggtree>

DEMONSTRATION

GETTING STARTED...

1. Open up RStudio
2. Set working directory to your workshop folder:
In the files pane, navigate to your workshop folder, then click:
more > set as working directory
3. Create four sub directories in the workshop directory:
Move the tree and metadata to “data” directory
Move script files to “script” directory



GETTING STARTED...

4. Open up Script and install packages

```
install.packages("readxl")  
install.packages("BiocManager")  
install.packages("treeio")  
install.packages("tidyverse")  
install.packages("phytools")
```

5. Load BiocManager package then install ggtree

```
library(BiocManager)  
BiocManager::install("ggtree")
```

**** Note: if you already have ggtree (or other packages) installed you can skip installation****

Detailed instructions for installing and loading packages can be found here:

<https://www.rdocumentation.org/packages/utils/versions/3.6.2/topics/install.packages>

PUBLICALLY AVAILABLE DATASET

<https://doi.org/10.3389/fpubh.2019.00066>

ORIGINAL RESEARCH article

Front. Public Health. 08 May 2019

Sec. Infectious Diseases: Epidemiology and Prevention

Volume 7 – 2019 | <https://doi.org/10.3389/fpubh.2019.00066>

Clustering of *Vibrio parahaemolyticus* Isolates Using MLST and Whole-Genome Phylogenetics and Protein Motif Fingerprinting

Kelsey J. Jesser^{1*}, Willy Valdivia-Granda², Jessica L. Jones² and Rachel T. Noble¹

¹ Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC, United States

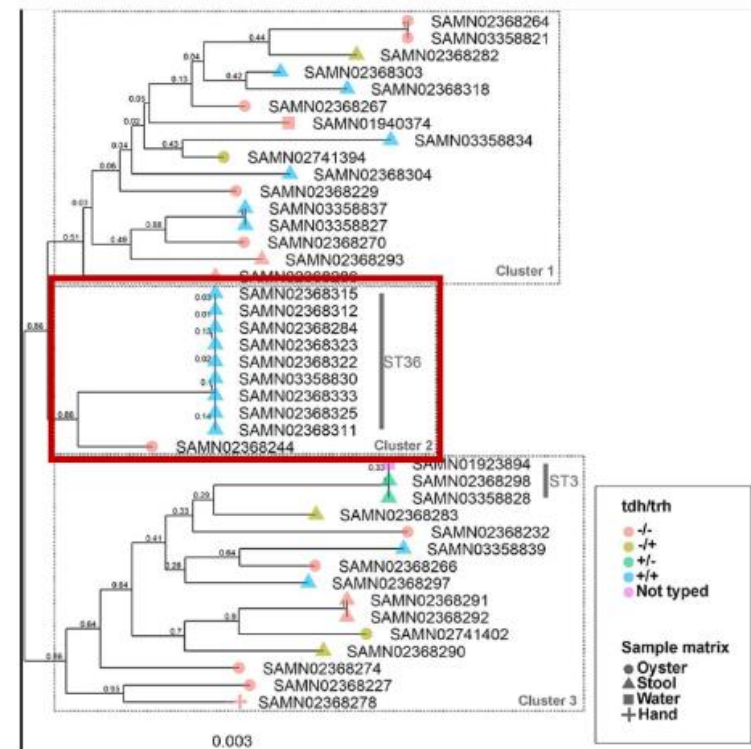
² Orion Integrated Biosciences, New Rochelle, NY, United States

³ Gulf Coast Seafood Laboratory, Division of Seafood Science and Technology, U.S. Food and Drug Administration, Dauphin Island, AL, United States

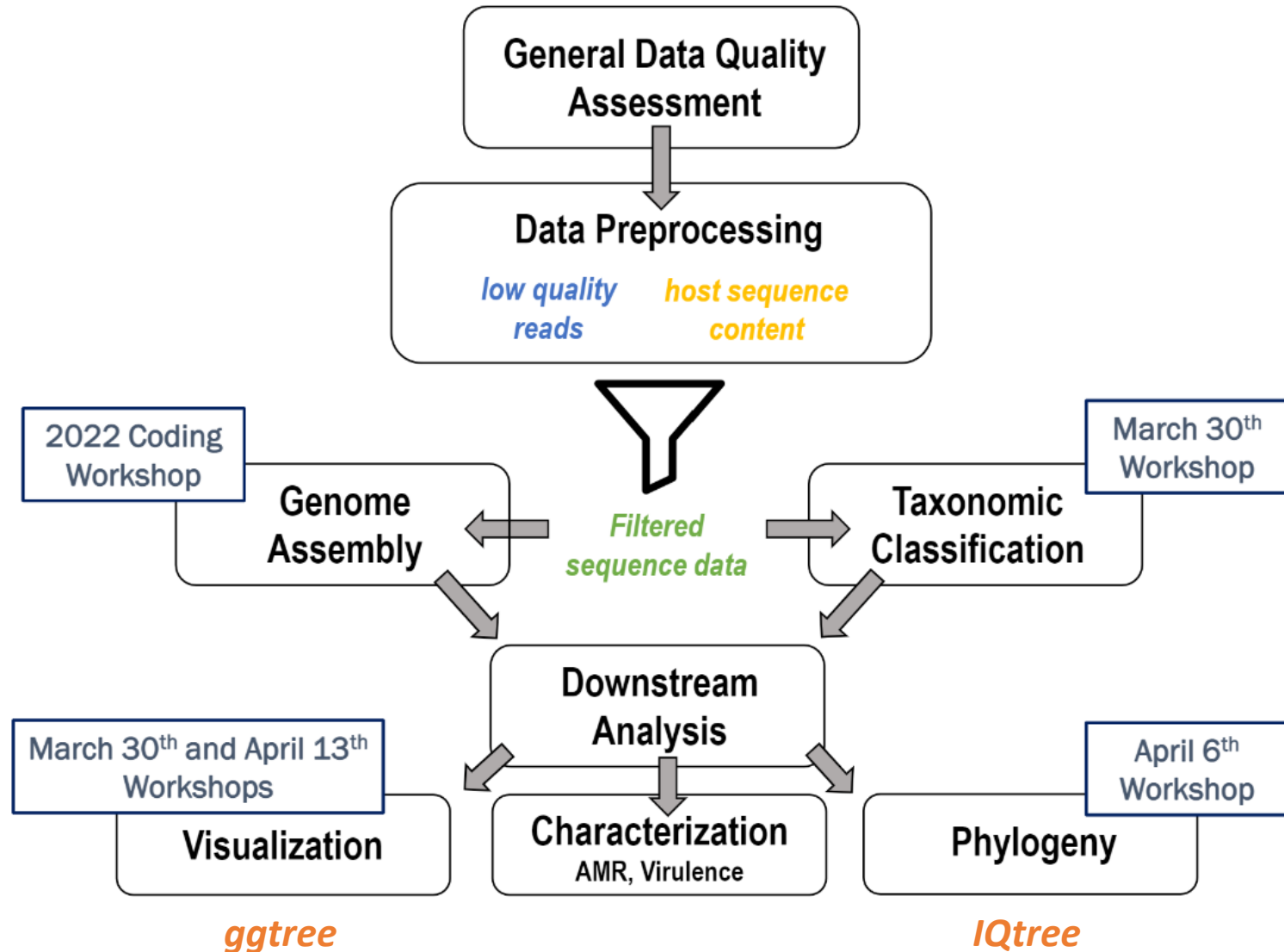
Uploaded to the MMID Bioinformatics GitHub Repository

<https://github.com/mmidi-bioinformatics-workshop>

Data was sequenced using the Illumina HiSeq 2000



BASIC WORKFLOW



GGTREE



- Package for R programming language
- Under Bioconductor project
- Creator: Guangchuang Yu
- Extension of ggplot2
- Data integration, manipulation and visualization of phylogenetic trees
- Customized annotation of tree

More information about ggtree can be found here:

- <https://yulab-smu.top/treedata-book/>
- <https://github.com/YuLab-SMU/ggtree>

FOLLOW ALONG IN RSCRIPT

GETTING STARTED...

Load packages

```
library(readxl)    # for reading in xl files
library(ggtree)     # for building tree
library(treeio)     # for read.newick function
library(phytools)  # for midpoint.root (also has read.newick option)
library(tidyverse) # to assist with data tidying
```

**** We will also be using ggplot2 which should automatically load in with ggtree****

LOAD IN FILES

Load tree and metadata file from data directory

```
# tree file
tree <- read.newick("data/msa.fasta.tree")

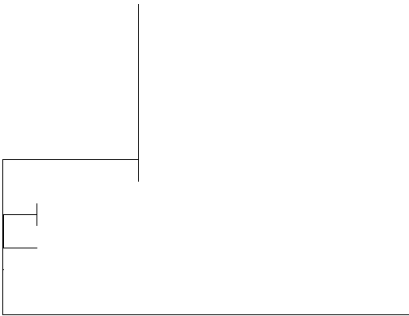
# metadata file
metadata <- read_excel("data/metadata.xlsx")
```


CHANGING TREE LAYOUT

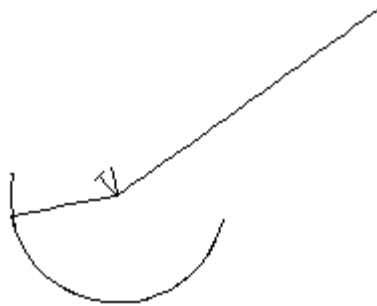
You can change the tree layout by using the “layout” argument

```
ggtree(tree, layout = “rectangular”)
```

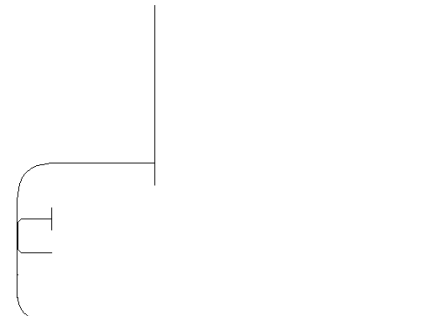
rectangular



circular



roundrect



CHANGING TREE LAYOUT

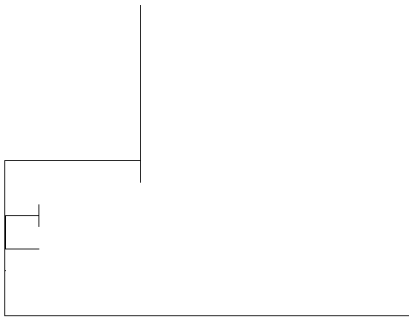
A **cladogram** will show topology without branch length information

```
ggtree(tree, branch.length = "none")
```

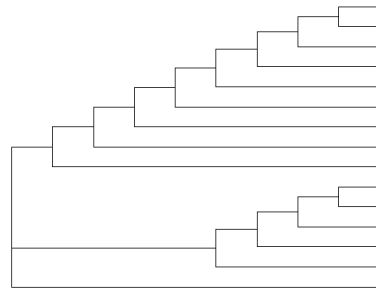
Midpoint root: roots the tree at the midpoint of the longest point between two tips.

```
ggtree(midpoint.root(tree))
```

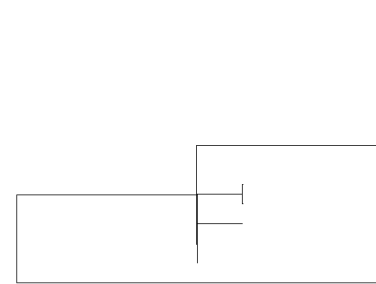
rectangular



cladogram



midpoint.root



You could also try changing position of the root node using **root.position** argument

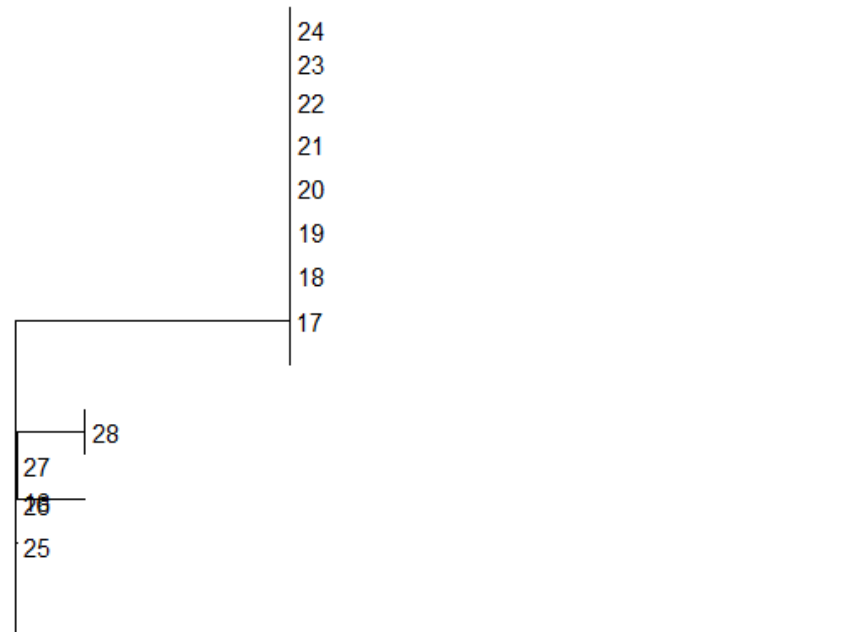
****Caution: rooting *can* drastically change tree topology and you must use an appropriate method based on your data ****

IDENTIFYING NODES

We can display the node numbers by using `geom_text2` argument:

```
ggtree(tree) +  
  geom_text2(aes(subset=!isTip,  
                 label=node),  
             hjust = -.3)
```

This will help us refer to a specific nodes while using other functions later on



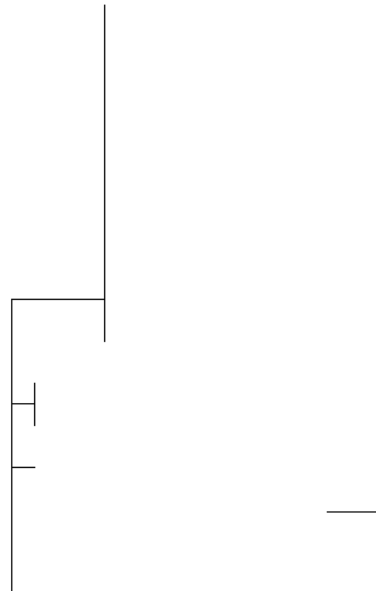
TREE MANIPULATION

- To view a particular clade, we can use `viewClade()`
- Notice the difference b/w operators
 - `+` **Plus**
 - `%>%` **Pipe**

```
> ggtree(tree) %>%  
  viewClade(node = 17)
```

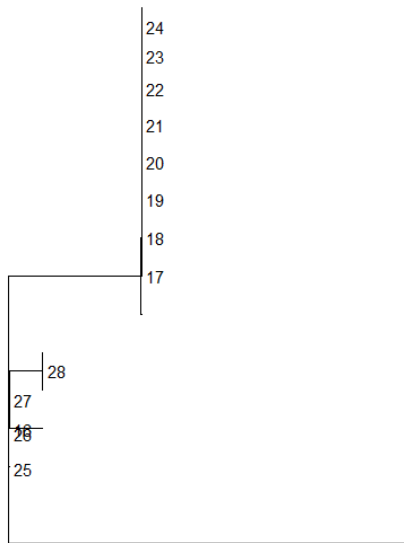


```
> ggtree(tree) +  
  viewClade(node = 17)
```

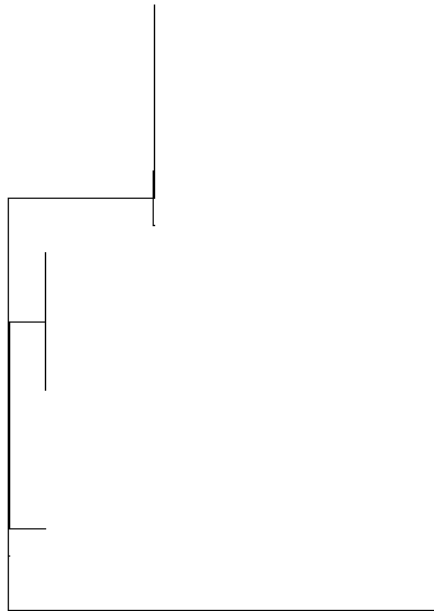


TREE MANIPULATION

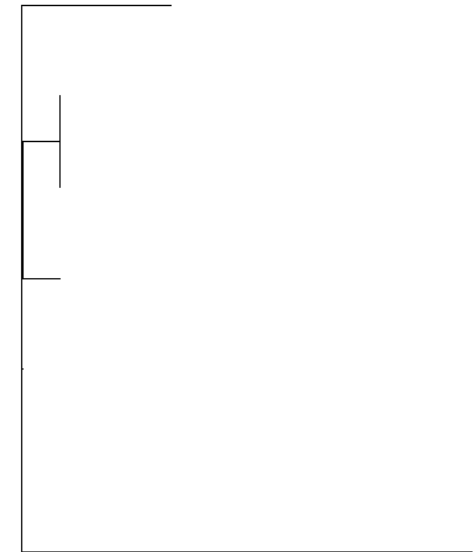
Original tree with
node labels



```
> ggtree(tree) %>%  
  scaleClade(node = 27,  
             scale = 5)
```



```
> ggtree(tree) %>%  
  collapse(node = 17)
```



ADDING TIP LABELS

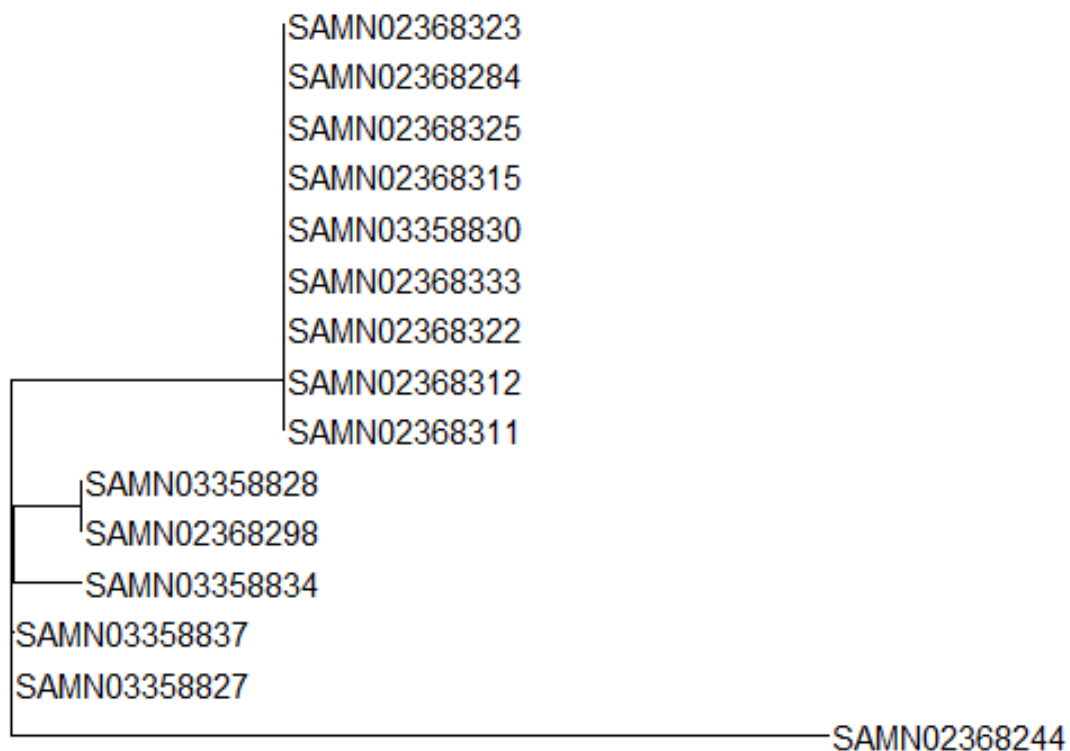
1. You can check the name of the tip labels using head()

```
head(tree$tip.label)
```

2. Add tip labels using geom_tiplab

```
ggtree(tree) +  
  geom_tiplab(size = 4) + # displaying tip labels  
  coord_cartesian(clip = 'off') + # allows us to draw outside the  
plot  
  theme(plot.margin = margin(1,3,1,1, "cm")) # add space around  
the plot
```

ADDING TIP LABELS



CUSTOMIZING TIP LABELS

1. Since we will be linking the metadata file to the tree, we can use a vector to check if there are any biosample_id observations that are not in the tree:

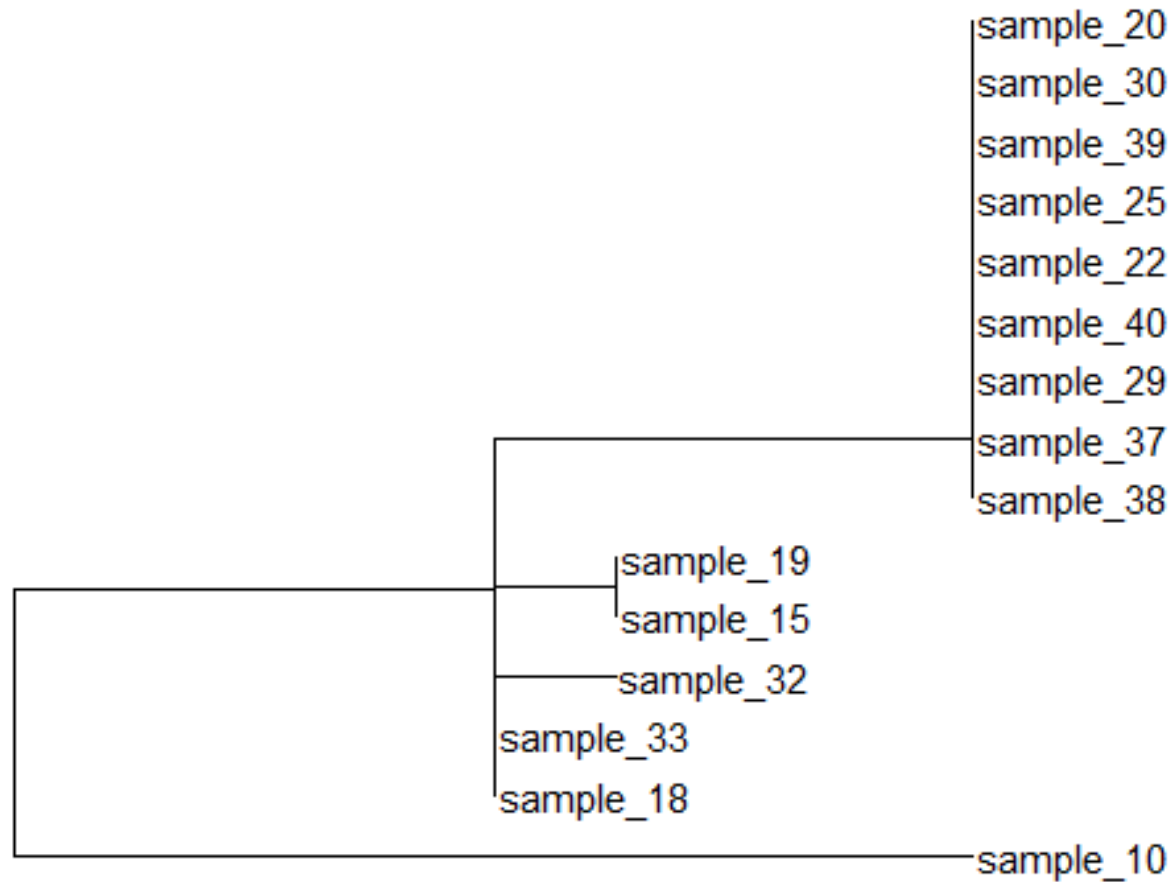
```
metadata$biosample_id[!tree$tip.label %in% metadata$biosample_id]
[1] character(0)
# all observations match b/w tree and metadata
```

2. Link metadata and change the tip label using geom_tiplab:

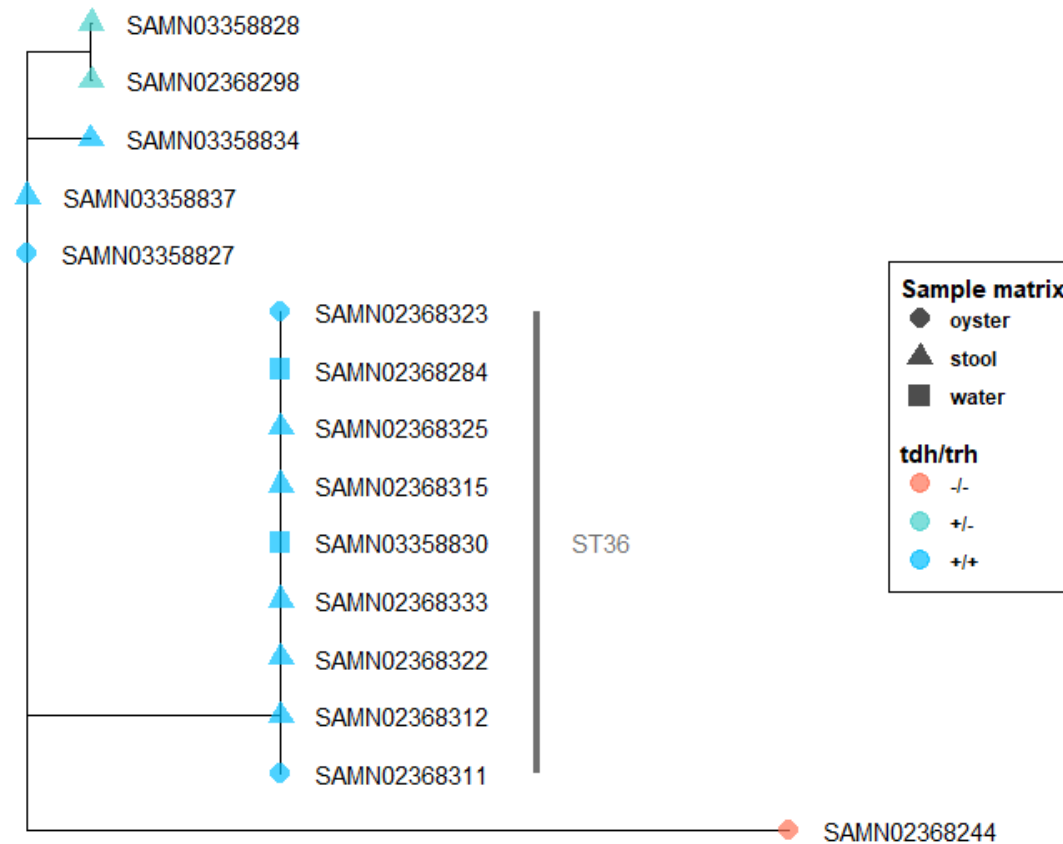
```
ggtree(tree) %<+% # to attach annotation data to tree
  metadata + # our metadata
  geom_tiplab(aes(label = sample_id)) + # change to strain_ID
  coord_cartesian(clip = 'off')+
  theme(plot.margin = margin(1,3,1,1, "cm"))
```

Any data we want linked to the tree must have a column that **EXACTLY** matches the tip.label

CUSTOMIZING TIP LABELS



EXAMPLE 1



DEMONSTRATION

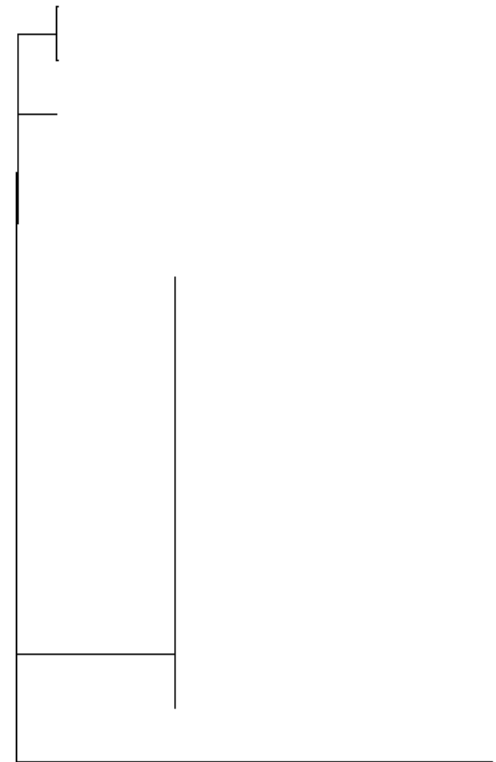
EXAMPLE 1

1. create a simple tree and save it as an object

```
gg_simple <- ggtree(tree) %+%  
  metadata + # link our metadata file  
here  
  coord_cartesian(clip = 'off')+  
  theme(plot.margin = margin(1,4,1,1,  
"cm"))
```

2. Reorient the tree using flip(), and save as new object

```
gg_flip <- gg_simple %>%  
  flip(25, 17)
```



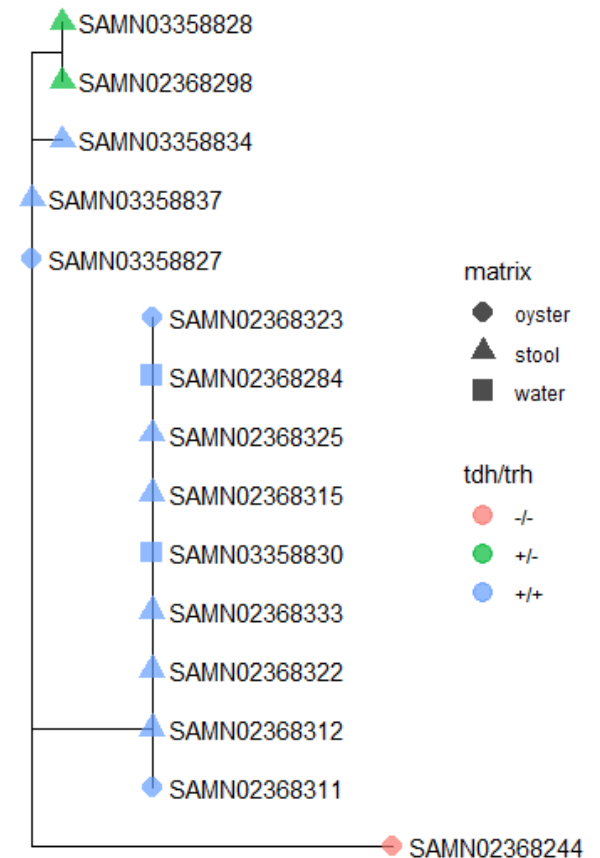
EXAMPLE 1

3. Add tip labels

```
gg_flip +  
  geom_tiplab(offset = 0.0001)
```

4. Add tip points

```
gg_flip +  
  geom_tiplab(offset = 0.0001) +  
  geom_tippoint(aes(color = `tdh/trh`,  
    shape = matrix),  
    size = 4,  
    alpha = 0.7)
```



SCALE_MANUAL

Create your own discrete scale

cornsilk3	dodgerblue4	gray45	gray3	gray69	lemonchiffon2	mediumorchid	palevioletred4	slateblue	
cornsilk2	dodgerblue3	gray44	gray2	gray68	lemonchiffon1	mediumblue	palevioletred3	skyblue4	
cornsilk1	dodgerblue2	gray43	gray1	gray67	lemonchiffon	mediumaquamarine	palevioletred2	skyblue3	
cornsilk	dodgerblue1	gray42	gray0	gray66	lawngreen	maroon4	palevioletred1	skyblue2	yellowgreen
cornflowerblue	dodgerblue	gray41	gray	gray65	lavenderblush4	maroon3	palevioletred	skyblue1	yellow4
coral4	dimgray	gray40	greenyellow	gray64	lavenderblush3	maroon2	paleturquoise4	skyblue	yellow3
coral3	dimgray	gray39	green4	gray63	lavenderblush2	maroon1	paleturquoise3	sienna4	yellow2
coral2	deeppink4	gray38	green3	gray62	lavenderblush1	maroon	paleturquoise2	sienna3	yellow1
coral1	deeppink3	gray37	green2	gray61	lavenderblush	magenta4	paleturquoise1	sienna2	yellow
coral	deeppink2	gray36	green1	gray60	lavender	magenta3	paleturquoise	sienna1	whitesmoke
chocolate4	deeppink1	gray35	green	gray59	khaki4	magenta2	palegreen4	sienna	wheat4
chocolate3	deeppink	gray34	gray100	gray58	khaki3	magenta1	palegreen3	seashell4	wheat3
chocolate2	deeppink4	gray33	gray99	gray57	khaki2	magenta	palegreen2	seashell3	wheat2
chocolate1	deeppink3	gray32	gray98	gray56	khaki1	linen	palegreen1	seashell2	wheat1
chocolate	deeppink2	gray31	gray97	gray55	khaki	limegreen	palegreen	seashell1	wheat
chartreuse4	deeppink1	gray30	gray96	gray54	ivory4	lightyellow4	palegoldenrod	seashell	violetred4
chartreuse3	deeppink	gray29	gray95	gray53	ivory3	lightyellow3	orchid4	seagreen4	violetred3
chartreuse2	darkviolet	gray28	gray94	gray52	ivory2	lightyellow2	orchid3	seagreen3	violetred2
chartreuse1	darkturquoise	gray27	gray93	gray51	ivory1	lightyellow1	orchid2	seagreen2	violetred1
chartreuse	darkslategray	gray26	gray92	gray50	ivory	lightyellow	orchid1	seagreen1	violetred
cadetblue4	darkslategray4	gray25	gray91	gray49	indianred4	lightsteelblue4	orchid	seagreen	violet

0	1	2	3	4
□	○	△	+	×
5	6	7	8	9
◇	▽	⊠	*	⊞
10	11	12	13	14
⊕	⊗	⊞	⊗	⊞
15	16	17	18	19
■	●	▲	◆	●
20	21	22	23	24
●	●	■	◆	▲

To find codes for ggplot2 colors and shapes, go to:

<http://sape.inf.usi.ch/quick-reference/ggplot2/colour>

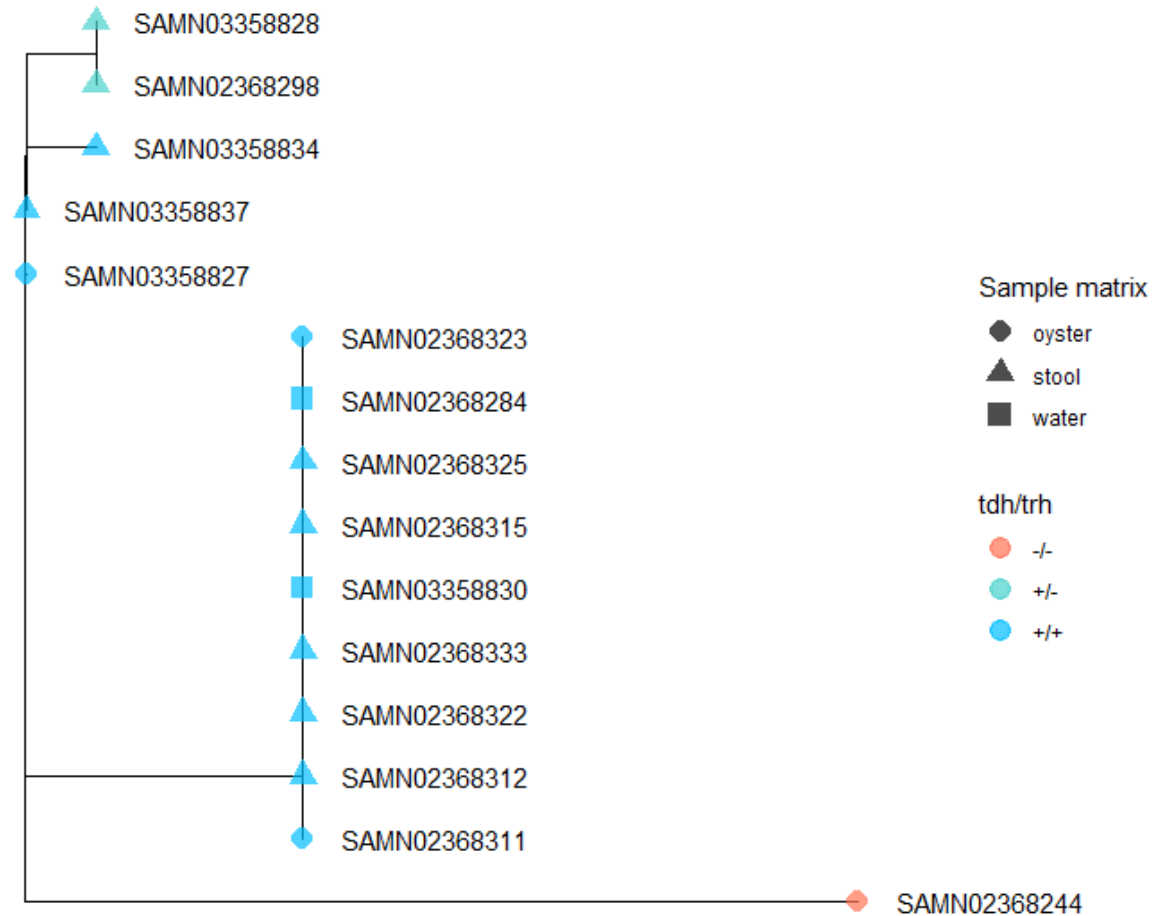
Or search “ggplot2 colors” in google

EXAMPLE 1

5. Use `scale_manual` to specific shape and color

```
gg_flip +  
  geom_tiplab(offset = 0.0001)+  
  geom_tippoint(aes(color = `tdh/trh`, shape = matrix),  
               size = 4,  
               alpha = 0.7)+  
  scale_color_manual(values = c("+/+" = "deepskyblue1",  
                                "+/-" = "mediumturquoise",  
                                "-/-" = "coral1"))+  
  scale_shape_manual(values = c("oyster" = 16,  
                                "stool" = 17,  
                                "water" = 15),  
                    name = "Sample matrix")
```

EXAMPLE 1

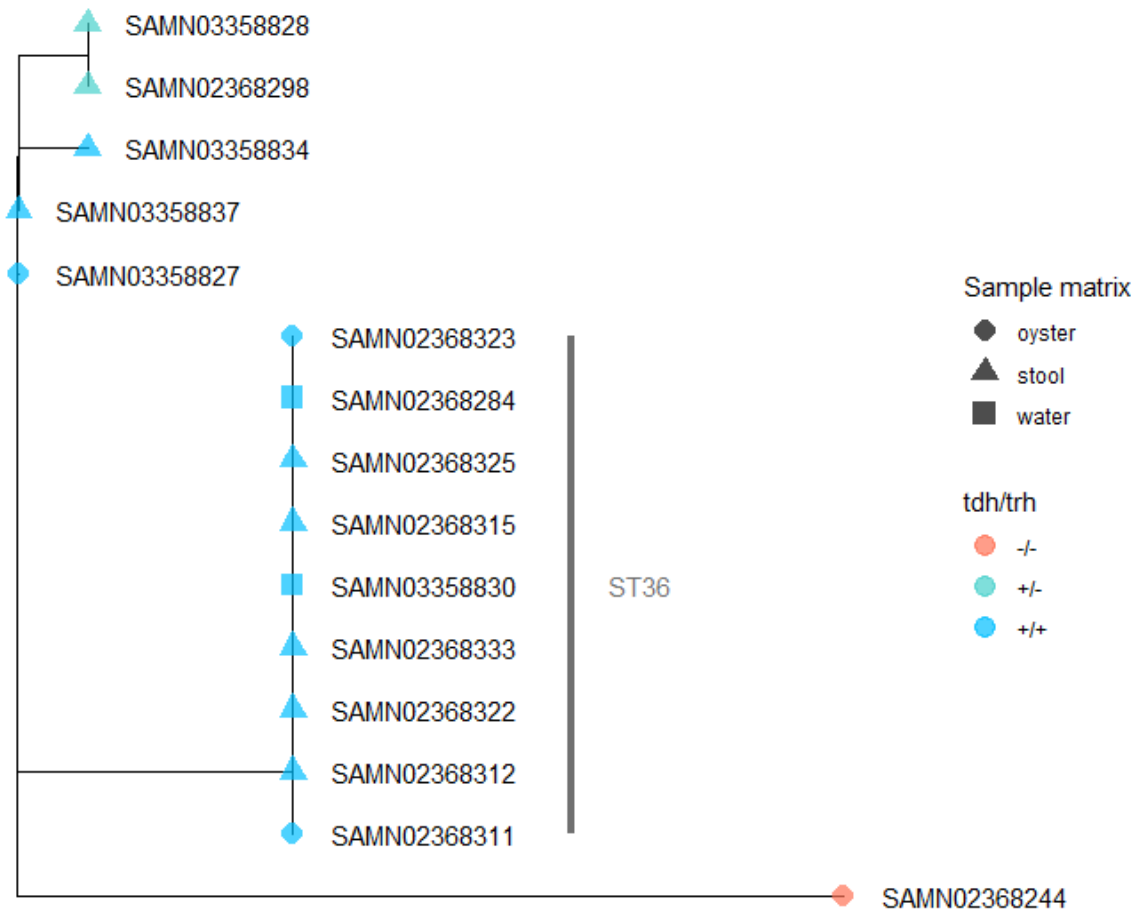


EXAMPLE 1

6. Add a clade label

```
gg_flip +  
  geom_tiplab(offset = 0.0001)+  
  geom_tippoint(aes(color = `tdh/trh`, shape = matrix),  
               size = 4,  
               alpha = 0.7)+  
  scale_color_manual(values = c("+/+" = "deepskyblue1",  
                                "+/-" = "mediumturquoise",  
                                "-/-" = "coral1"))+  
  scale_shape_manual(values = c("oyster" = 16,  
                                "stool" = 17,  
                                "water" = 15),  
                     name = "Sample matrix") +  
  geom_cladelab(node = 17, label = "ST36",  
               offset = 0.0008,  
               barsize = 1.5,  
               barcolor = 'grey44',  
               textcolor = 'grey44',  
               offset.text = 0.0001)
```

EXAMPLE 1

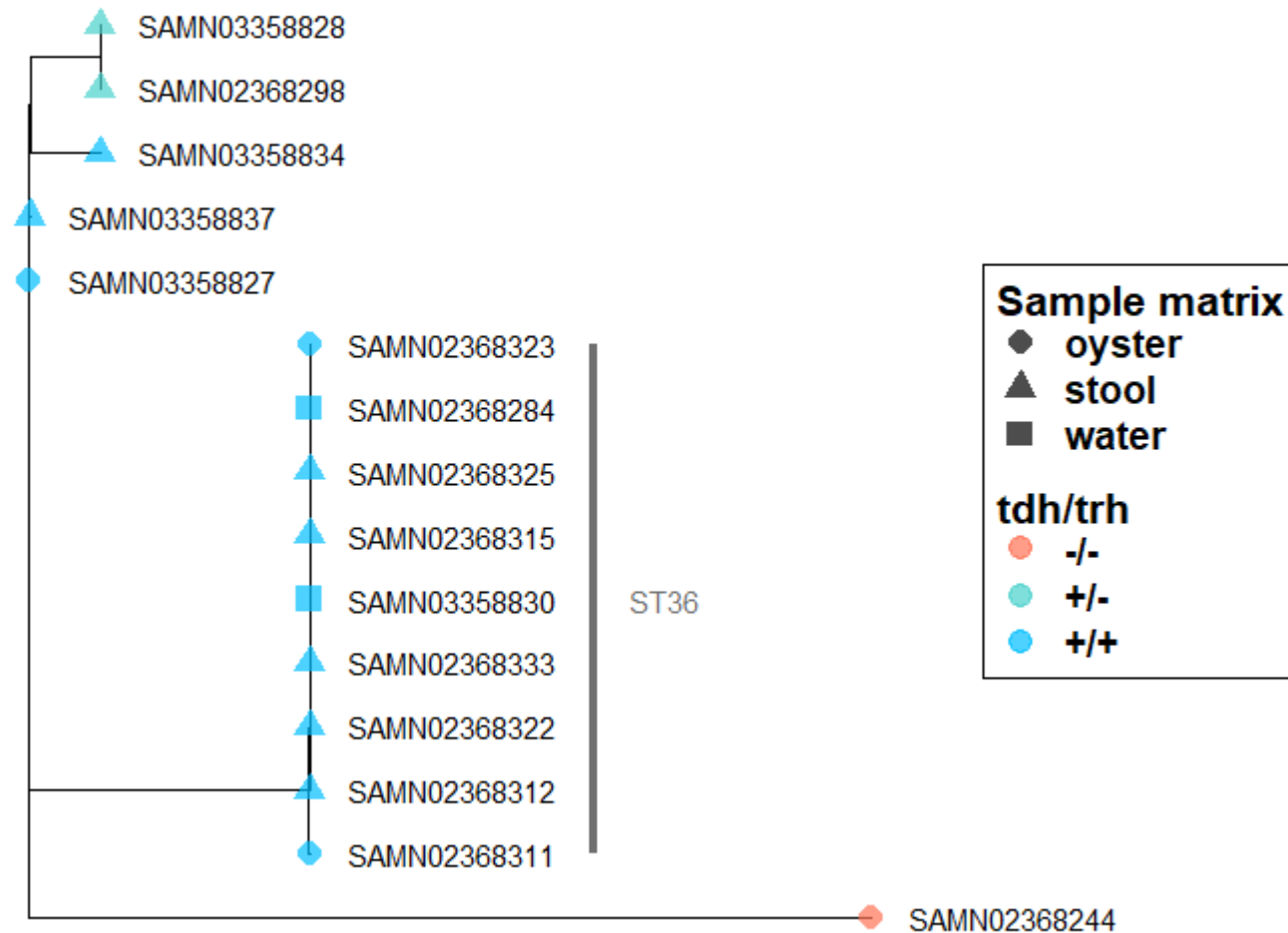


EXAMPLE 1

7. Use theme() to add final touches to the plot & save as new object

```
example1 <- gg_flip +  
  geom_tiplab(offset = 0.0001)+  
  geom_tippoint(aes(color = `tdh/trh`, shape = matrix),  
    size = 4,  
    alpha = 0.7)+  
  scale_color_manual(values = c("+/+" = "deepskyblue1",  
    "+/-" = "mediumturquoise",  
    "-/-" = "coral1"))+  
  scale_shape_manual(values = c("oyster" = 16,  
    "stool" = 17,  
    "water" = 15),  
    name = "Sample matrix") +  
  geom_cladelab(node = 17, label = "ST36",  
    offset = 0.0008,  
    barsize = 1.5,  
    barcolor = 'grey44',  
    textcolor = 'grey44',  
    offset.text = 0.0001)+  
  theme(legend.text = element_text(size = 14, face = "bold"),  
    legend.title = element_text(size = 16, face = "bold"),  
    legend.spacing.y = unit(0, "mm"),  
    panel.border = element_blank(),  
    aspect.ratio = 1, axis.text = element_text(colour = 1, size = 12),  
    legend.background = element_blank(),  
    legend.box.background = element_rect(colour = "black"))
```

EXAMPLE 1



EXAMPLE 1

8. Export final tree into output directory

```
ggsave("output/example1.jpeg", example1, dpi = 300)
```

Additional arguments

- Scale, width, height, units, dpi

Details:

- Currently supports pdf, jpeg, tiff, png and more...

Learn more about `ggsave()` in r documentation:

<https://www.rdocumentation.org/packages/ggplot2/versions/0.9.0/topics/ggsave>

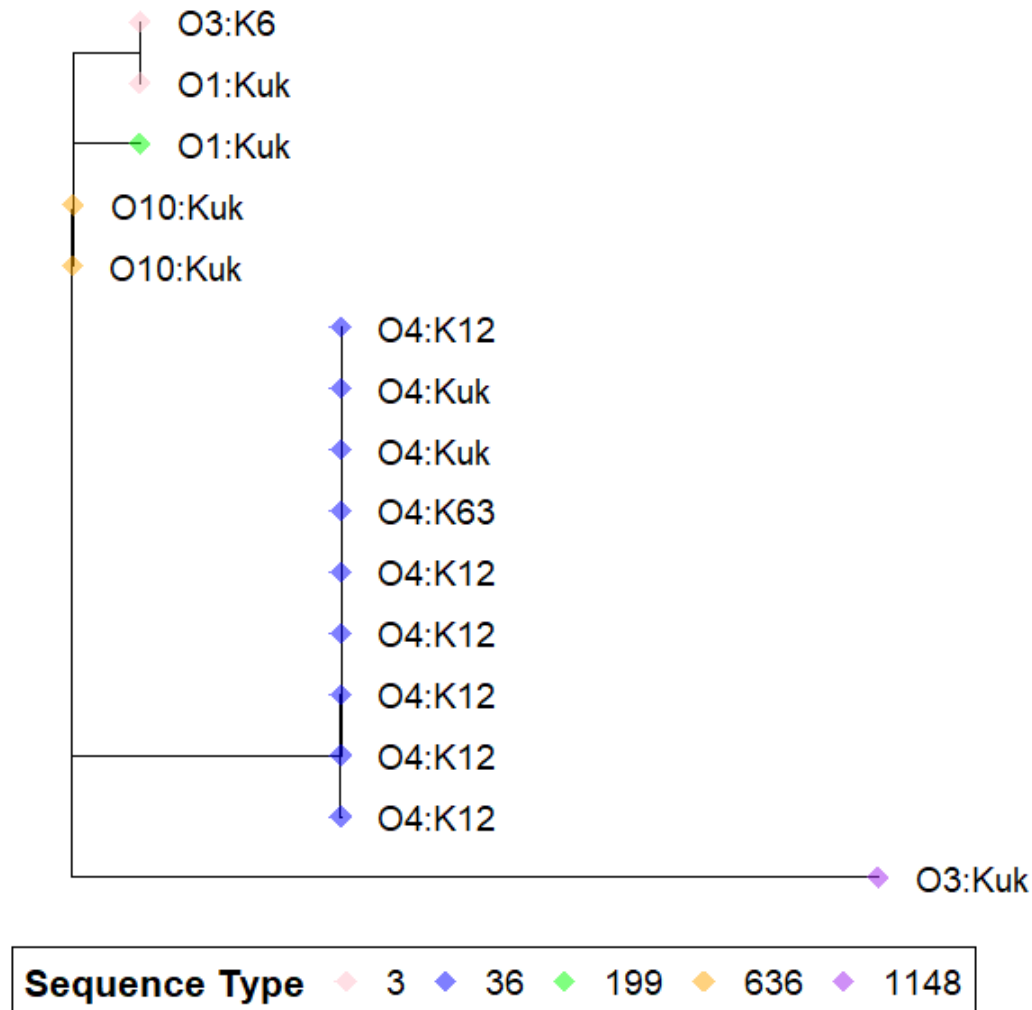
EXERCISE 1

Let's apply everything we've learned so far:

- 1) Create a tree using the serovar as the tip labels
- 2) Set your own color of the nodes based on sequence type (st) and change their shape
- 3) Change the font size of the tip labels and legend
- 4) (Bonus) Move the legend to the bottom of the tree (hint, use help page)

Time: 10 minutes

EXERCISE 1



EXERCISE 1

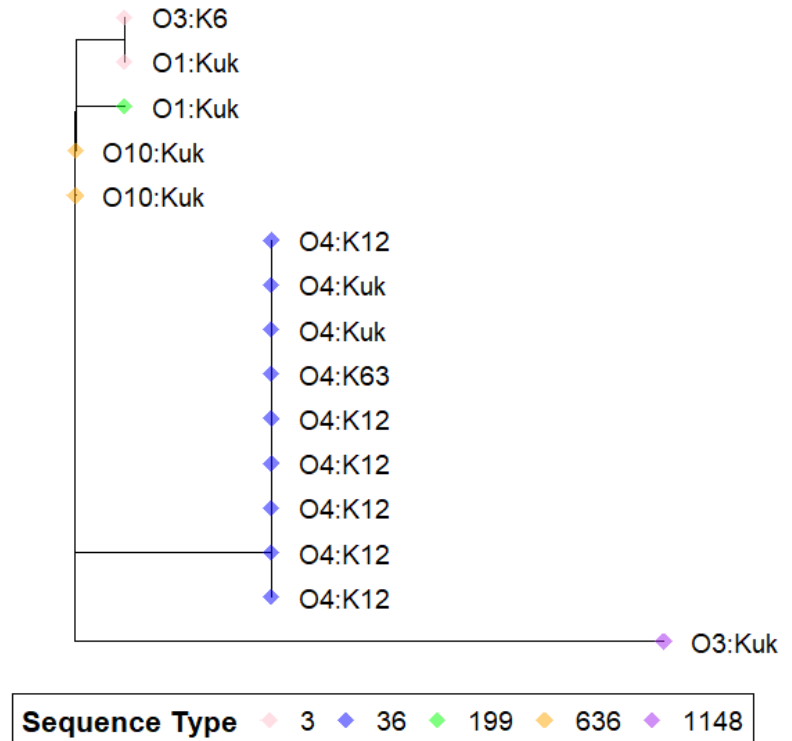
Let's apply everything we've learned so far:

- 1) Create a tree using the serovar as the tip labels
- 2) Set your own color of the nodes based on sequence type (st) and change their shape
- 3) Change the font size of the tip labels and legend
- 4) (Bonus) Move the legend to the bottom of the tree

Time: 10 minutes

HINT: use `AS.FACTOR()` around a continuous variable to read as a discrete scale

To find codes for ggplot2 colors and shapes, go to:
<http://sape.inf.usi.ch/quick-reference/ggplot2/colour>
Or search “ggplot2 colors” in google



DEMONSTRATION

EXERCISE 1 ANSWER

```
exercisel <- gg_flip +  
  geom_tiplab(aes(label = serovar),  
             offset = 0.0001,  
             size = 5)+  
  geom_tippoint(aes(color = as.factor(st)),  
               shape = 18,  
               size = 4,  
               alpha = 0.5)+  
  scale_color_manual(values =  
    c("pink", "blue", "green", "orange", "purple"),  
                    name = "Sequence Type")+  
  theme(legend.text = element_text(size = 14),  
        legend.title = element_text(size = 16, face = "bold"),  
        legend.position = "bottom",  
        legend.spacing = unit(0, "cm"),  
        panel.border = element_blank(),  
        aspect.ratio = 1, axis.text = element_text(colour = 1,  
        size = 12),  
        legend.background = element_blank(),  
        legend.box.background = element_rect(colour = "black"))
```

FOLLOW ALONG IN RSCRIPT

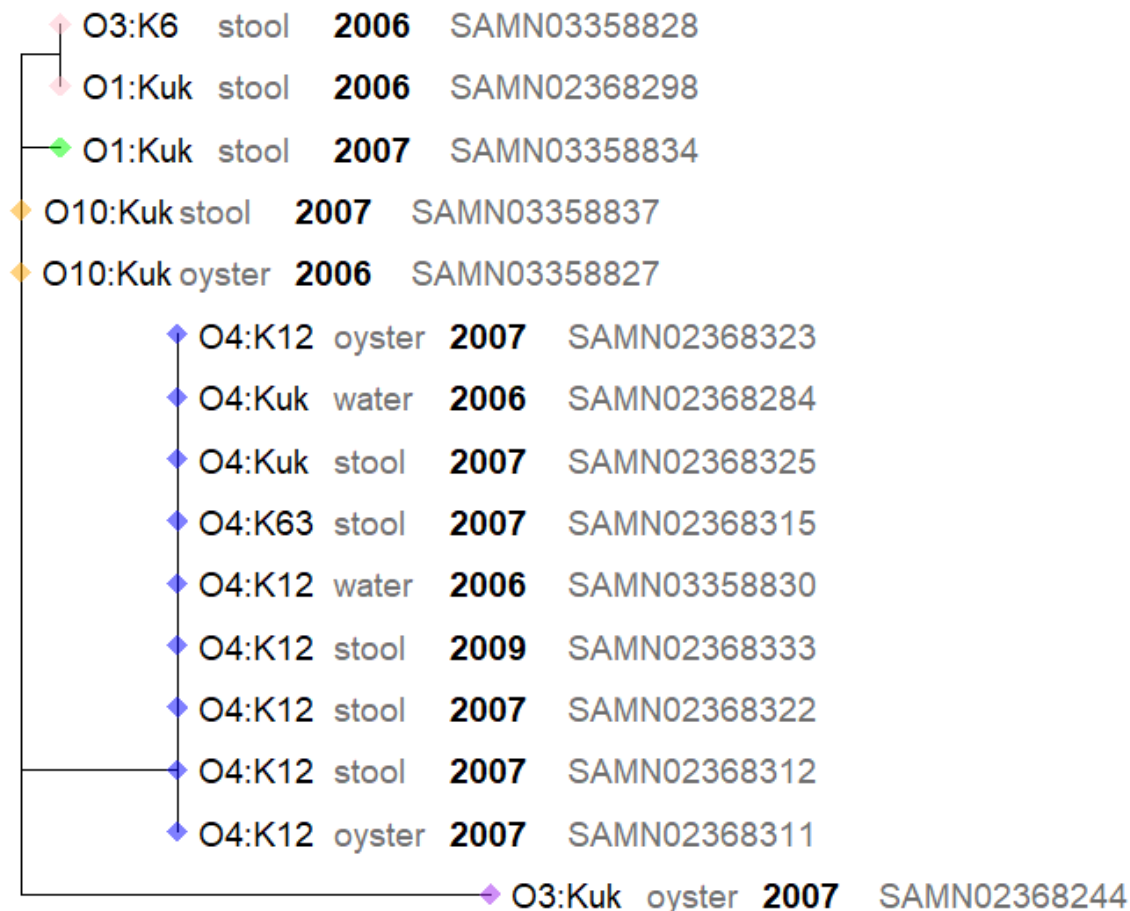
LAYERS FOR TREE ANNOTATION

Add `geom_tiplab` layer onto the tree from exercise 1

```
exercisel +  
  geom_tiplab(aes(label = matrix),  
             offset = 0.0006,  
             size = 5,  
             color = "grey44")
```

You can continue to add layers, while changing the offset each time

LAYERS FOR TREE ANNOTATION



Sequence Type	3	36	199	636	1148

HIGHLIGHTING CLADES

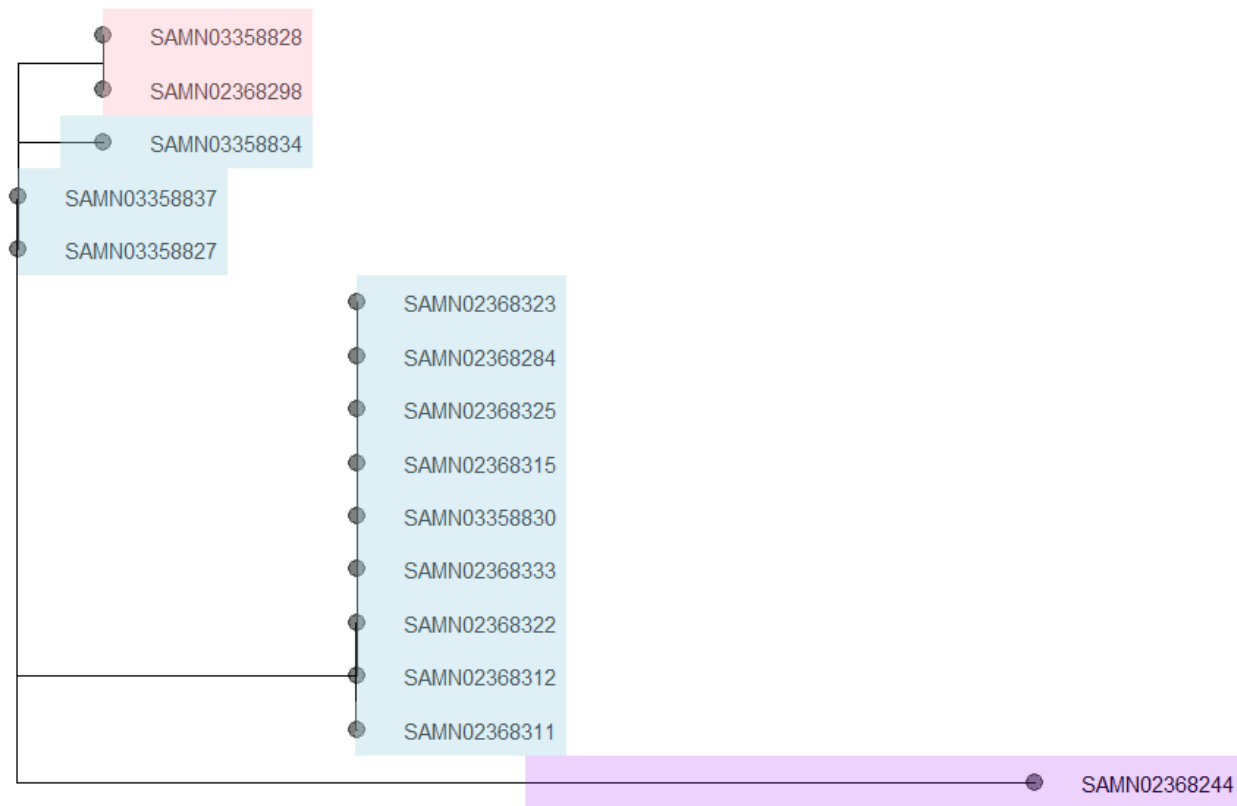
1. By specifying node

```
gg_flip +  
  geom_tiplab(offset = 0.0001)+  
  geom_tippoint(color = 'black',  
                size = 4,  
                alpha = 0.5)+  
  geom_hilight(node = c(11,12),  
               fill = "pink",  
               alpha = 0.4,  
               extend = 0.0005)
```

2. By subsetting by a condition

```
gg_flip +  
  geom_tiplab(offset = 0.0001)+  
  geom_tippoint(color = 'black',  
                size = 4,  
                alpha = 0.5)+  
  geom_hilight(mapping=  
aes(subset = wg_cluster %in% 1),  
   fill = "pink",  
   alpha = 0.4,  
   extend = 0.0005)
```

HIGHLIGHTING CLADES



MORE EXAMPLES !

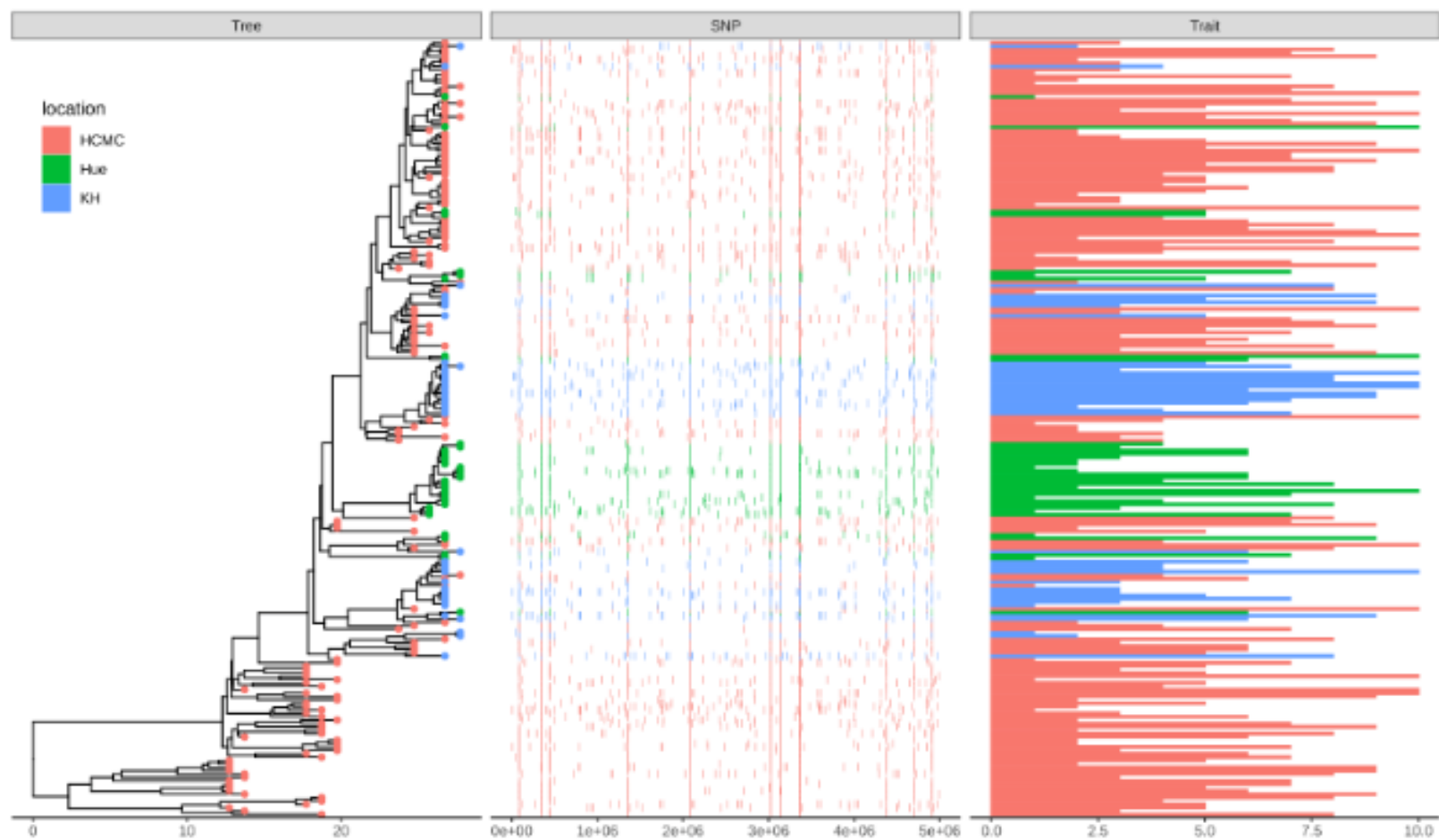


FIGURE 7.2: **Example of plotting SNP and trait data.** The 'location' information was attached to the tree and used to color tip symbols (Tree panel), and other datasets. SNP and Trait data were visualized as dot chart (SNP panel) and bar chart (Trait panel).

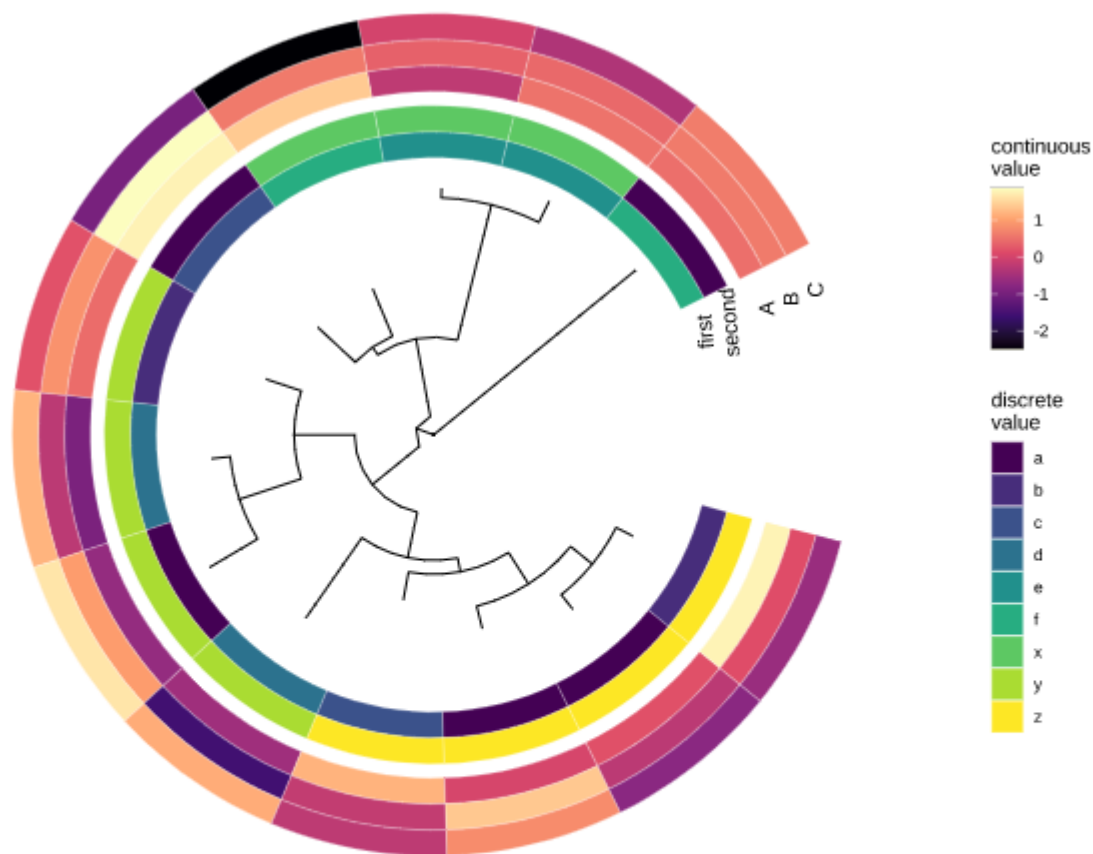
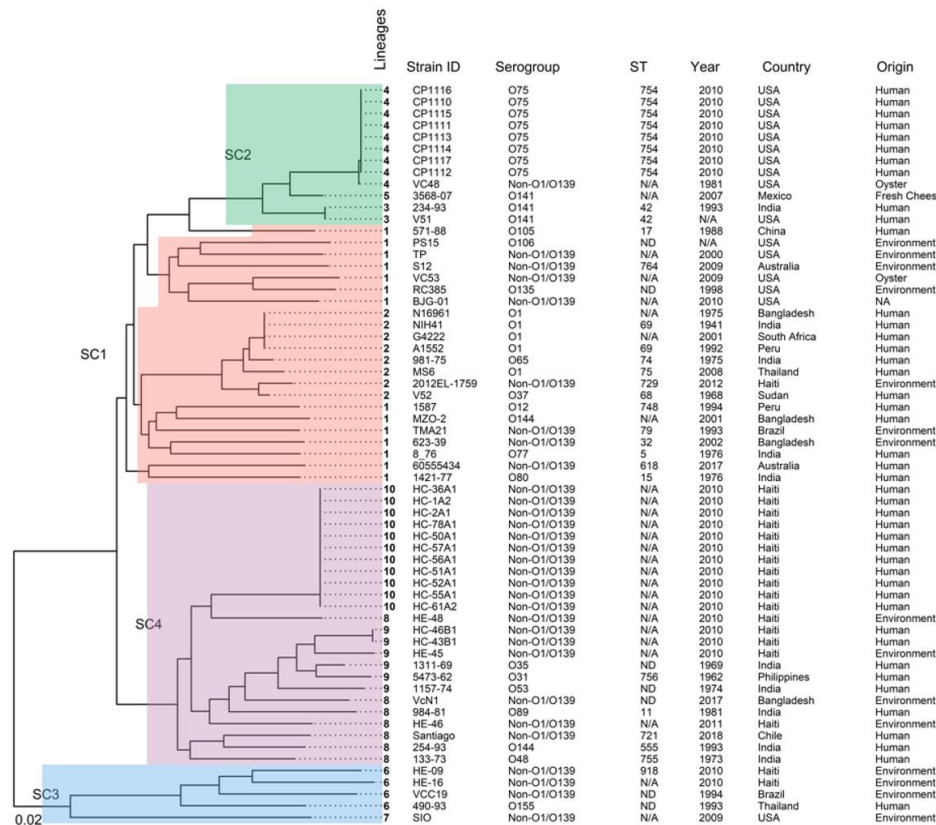


FIGURE 7.4: **Example of plotting matrix with `gheatmap()`** . A H3 influenza tree with a genotype table visualized as a heatmap (A). Tips were aligned and with a tailored x-axis for divergence times (tree) and genomic segments (heatmap) (B).

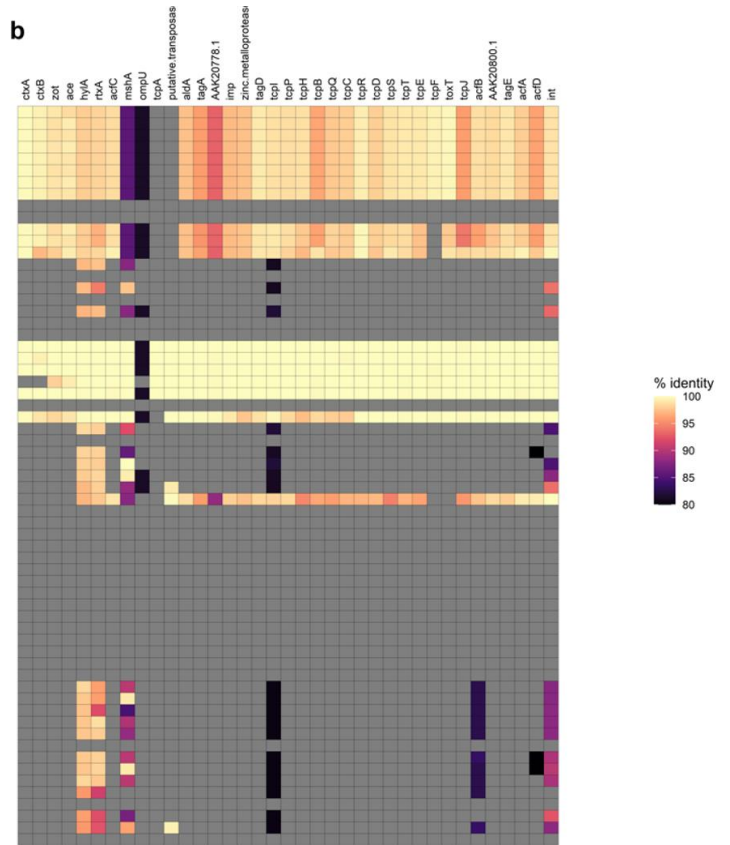
Genomic characterization of the non-O1/non-O139 *Vibrio cholerae* strain that caused a gastroenteritis outbreak in Santiago, Chile, 2018

Mónica Arteaga^{1†}, Juliana Velasco^{1‡}, Shelly Rodriguez¹, Maricel Vidal², Carolina Arellano³, Francisco Silva⁴, Leandro J. Carreño^{5,6}, Roberto Vidal^{3,6,*} and David A. Montero^{3,5,*}

a



b



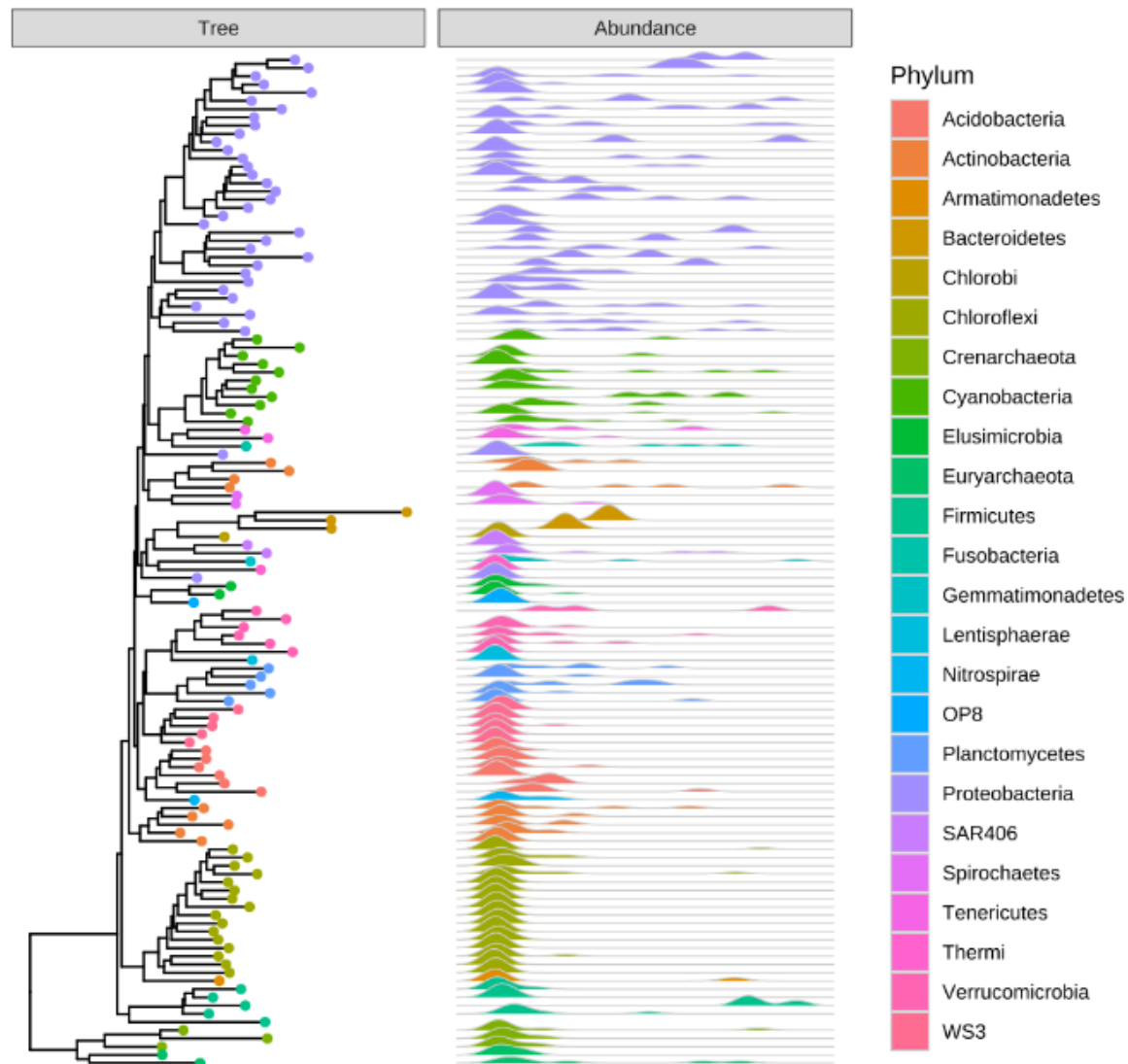


FIGURE 9.4: **Phylogenetic tree with OTU abundance densities.** Tips were colored by Phylum, and the corresponding abundances across different samples were visualized as density ridgelines and sorted according to the tree structure.

HELPFUL RESOURCES

Data integration, manipulation and visualization of phylogenetic trees:

<https://yulab-smu.top/treedata-book/index.html>

ggtree github:

<https://github.com/YuLab-SMU/ggtree>

Enhanced annotation practice:

<http://www.randigriffin.com/2017/05/11/primate-phylogeny-ggtree.html>

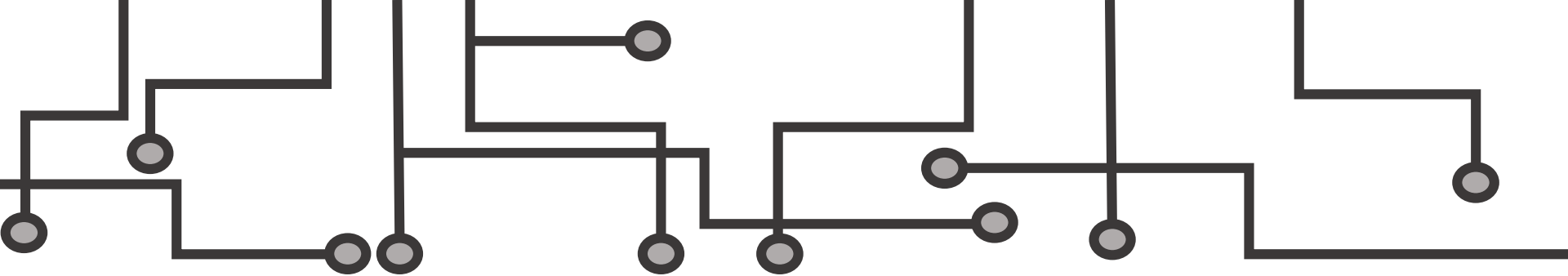
ggtreeExtra:

<https://yulab-smu.top/treedata-book/chapter10.html>

<https://github.com/YuLab-SMU/ggtreeExtra>

NEXT WEEK...

Bring your own data set!



THANK YOU FOR ATTENDING!

*Please make sure to fill out the [Exit Survey at](https://docs.google.com/forms/d/e/1FAIpQLScSMU-AOMMrU9CUz-UP1pJhrz0npverR6PpDcvF6jdrw-QDSA/viewform)
[https://docs.google.com/forms/d/e/1FAIpQLScSMU-
AOMMrU9CUz-UP1pJhrz0npverR6PpDcvF6jdrw-QDSA/viewform](https://docs.google.com/forms/d/e/1FAIpQLScSMU-AOMMrU9CUz-UP1pJhrz0npverR6PpDcvF6jdrw-QDSA/viewform)
We value your feedback!*

*More questions? Please email us at
mmid.bioinformatics.workshop@gmail.com or post them to the workshop [slack channel](#)*

