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Skill Pill+: Evolutionary Genomics

Bourguignon Unit

December 2018

Visualization under the microscope

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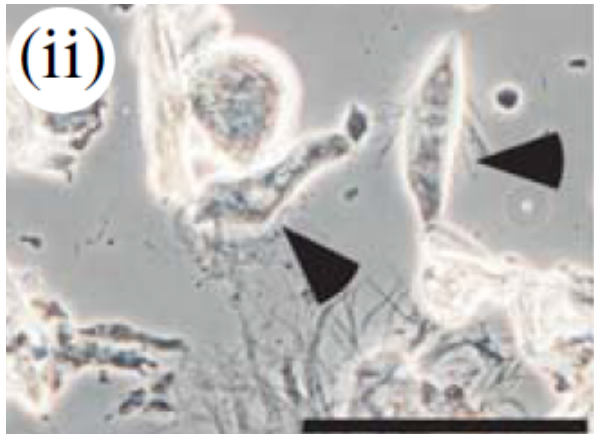
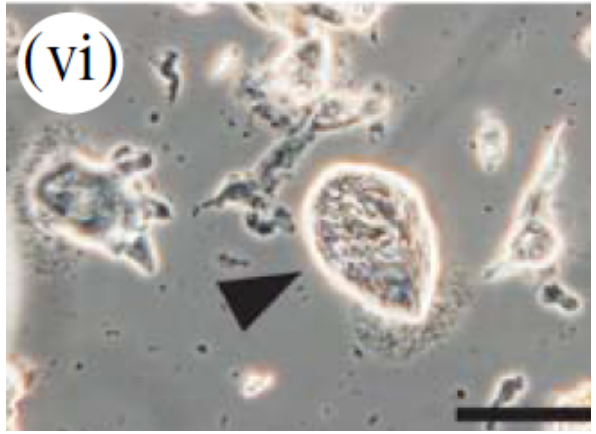
The workflow

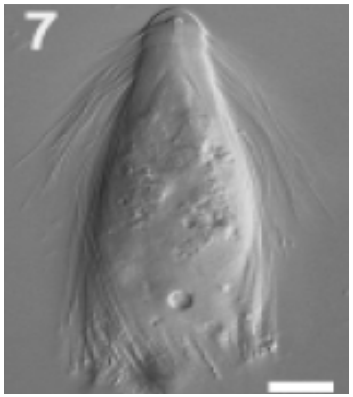
- Clean the bench with 70% ethanol and tissue
- Clean the microscope lens and platform with 70% ethanol and kim-wipe (as the lens is sensitive).
- Pour 500 ul of 70% ethanol into a petri plate.
- Add two drops of Solution U to the slide using 100 *ul* pipette.
- Transfer the termite to the petri plate containing ethanol for a few seconds to clean the termite.
- Remove the termite from petri plate, transfer it to the cover of the petri plate (or any clean surface).
- Dissection: Using two tweezers, hold the head of the termite with one tweezer, and pull the gut out of the termite from the rear side [abdomen side] using the other tweezer.
- Transfer the gut to the slide, mix the gut content in one of the drops of Solution U on the slide.
- Transfer a few drops of the gut content from one drop to the other drop, using pipette. This is called as dilution.
- Check under the microscope (80X magnification).

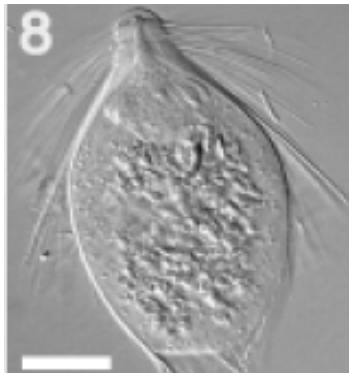
Images of termite gut content

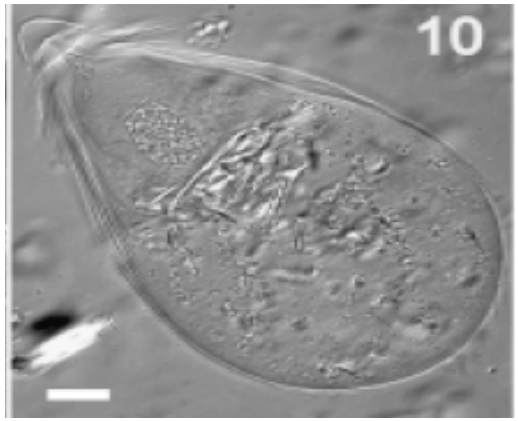




Ohkuma *et al.* 2008







Carpenter *et al*. 2009

Lets learn about the microbes in the termite gut!

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- What are protists?
- What are the function of protists in the termite gut?
- Types of protists in the gut
- How to identify the protists in the termite gut?

Protists

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- Protists are eukaryotes, which are neither plant, animal, bacteria or fungi.
- They can have long tails called flagellates on their surface, to help them move.
- They can eat by photosynthesis, enzyme secretions outside the body, or by eating pre-digested food. Termite gut protists are the latter ones.

https://www.ducksters.com/science/biology/protists.php

Functions of protists

- They can be parasites, causing disease or symbiont, helping the host organism.
- The termite gut protists are symbionts. They help digest cellulose eaten up by termites.

Types of protists in termite gut

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- There are more than 400 different species of protists in the termite gut.
- They can be classified into two groups:

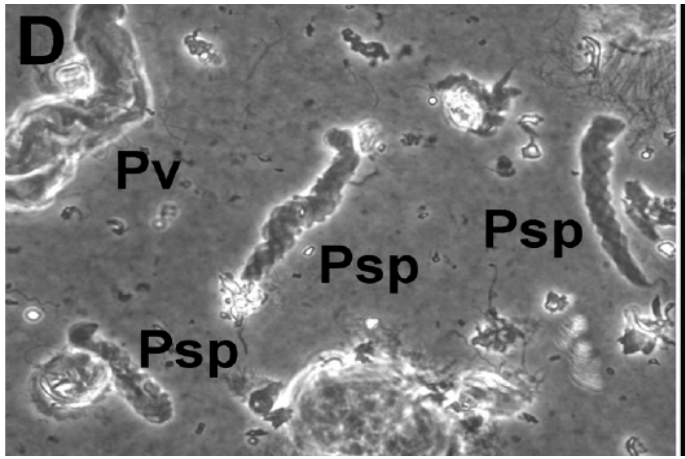
Parabasalids - They are larger in size, they have parabasal filaments.

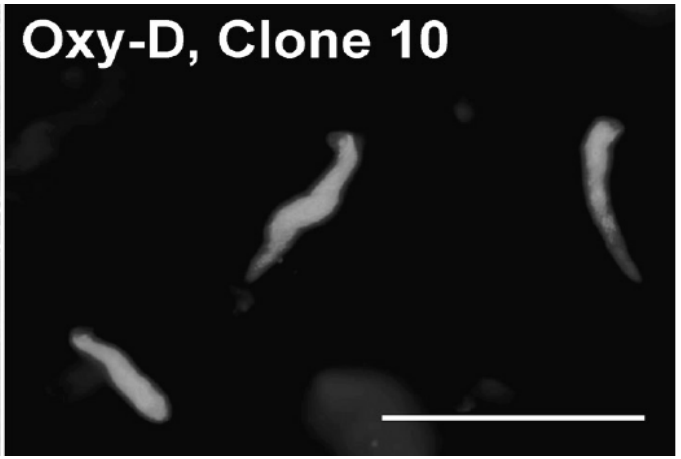
Oxymonads -They are smaller in size, they lack these filaments.

Parabasalids

Carpenter *et al*, 2009

Oxymonads





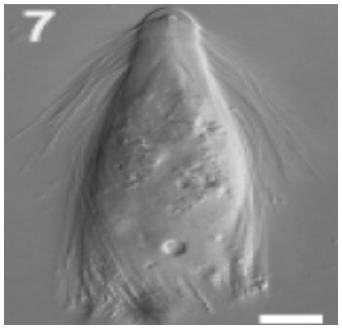
Stingl and Brune, 2003

Identifying the protists in the termite gut
Method I - Using the eye: Morphological analysis

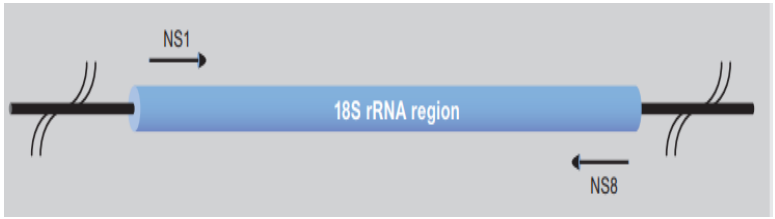
We already did this under the microscope.

How many different shapes and sizes of protists did you observe?

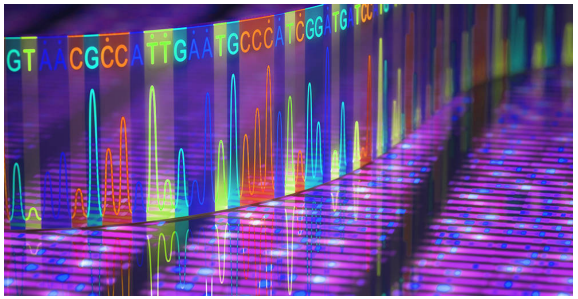
Method 2 - 18S sequence analysis



Isolate protists from the gut



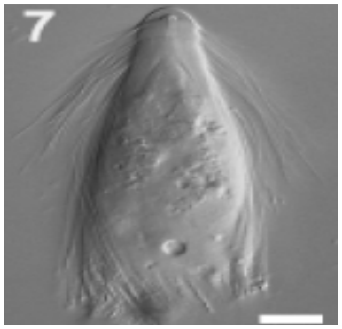
Amplify the 18S region using primers



Next generation sequencing

- Amplify the 18S ribosomal RNA sequence of the protists observed under the microscope.
- 18S ribosomal RNA is ~13,105 base pairs long. The primers are selected to represent the diversity of each eukaryotic order. For termite gut Parabaslid species, Ohkuma *et al.* (1998) primers were used. These primers target regions ~19-42 using forward primer and ~1772-1795 using reverse primer.

Method 2 - 18S sequence analysis



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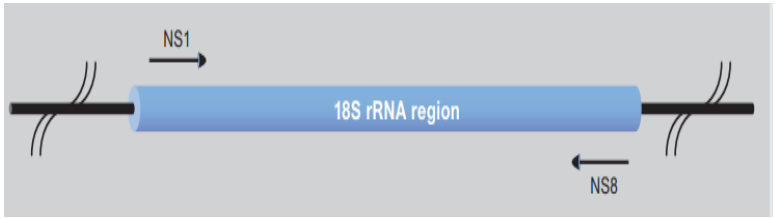

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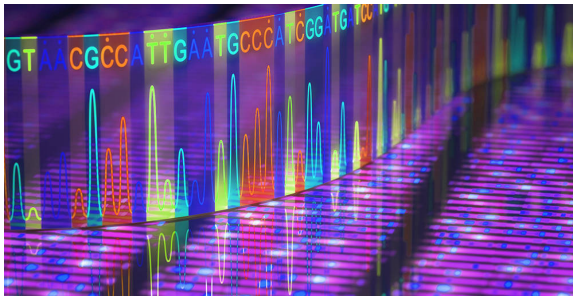
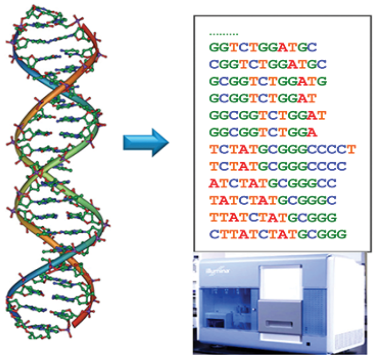


Next generation sequencing



Data analysis

Today we will be making sense of the data we usually get from the sequencing machine.

18S analysis

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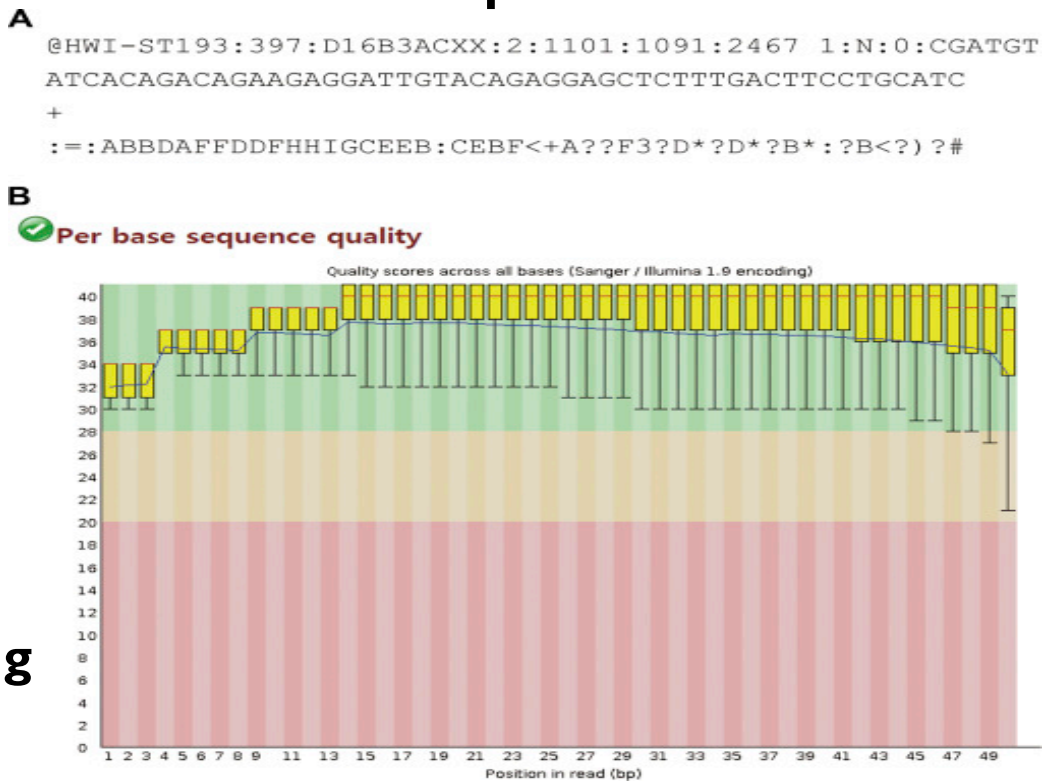
- The sequenced data is in fastq format. This is not in human readable format.
- To check the quality of the reads, we will use Fastqc software.

https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

NOTE-

(Taxonomy analysis can be done with fastq or fasta files. I have already converted the fastq files to fasta files for ease of analysis. The overall steps are the same for both file formats.)

Fastq format



Quality check using Fastqc software

Taxonomy analysis - Method 1- Using Blast

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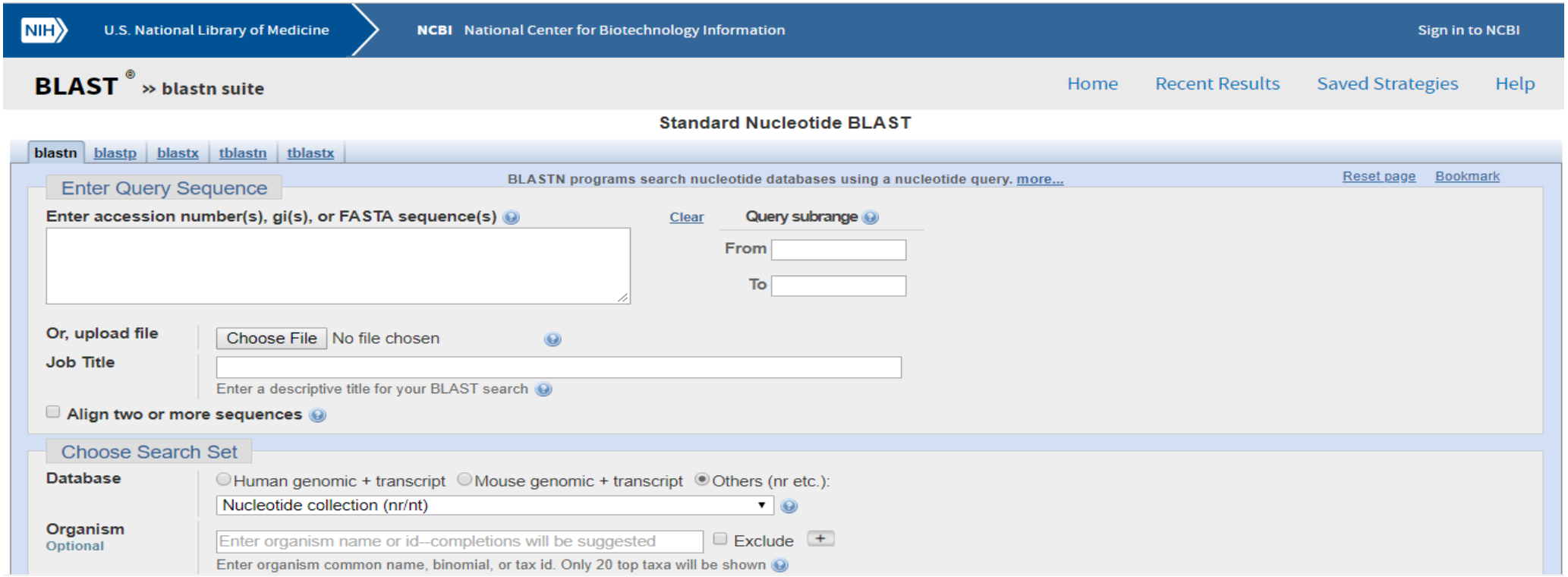
- Blast search can be done on the web or on command line.

The web interface



What are the advantages and disadvantages of web based blast search?

Method 2 - Phylogenetic tree

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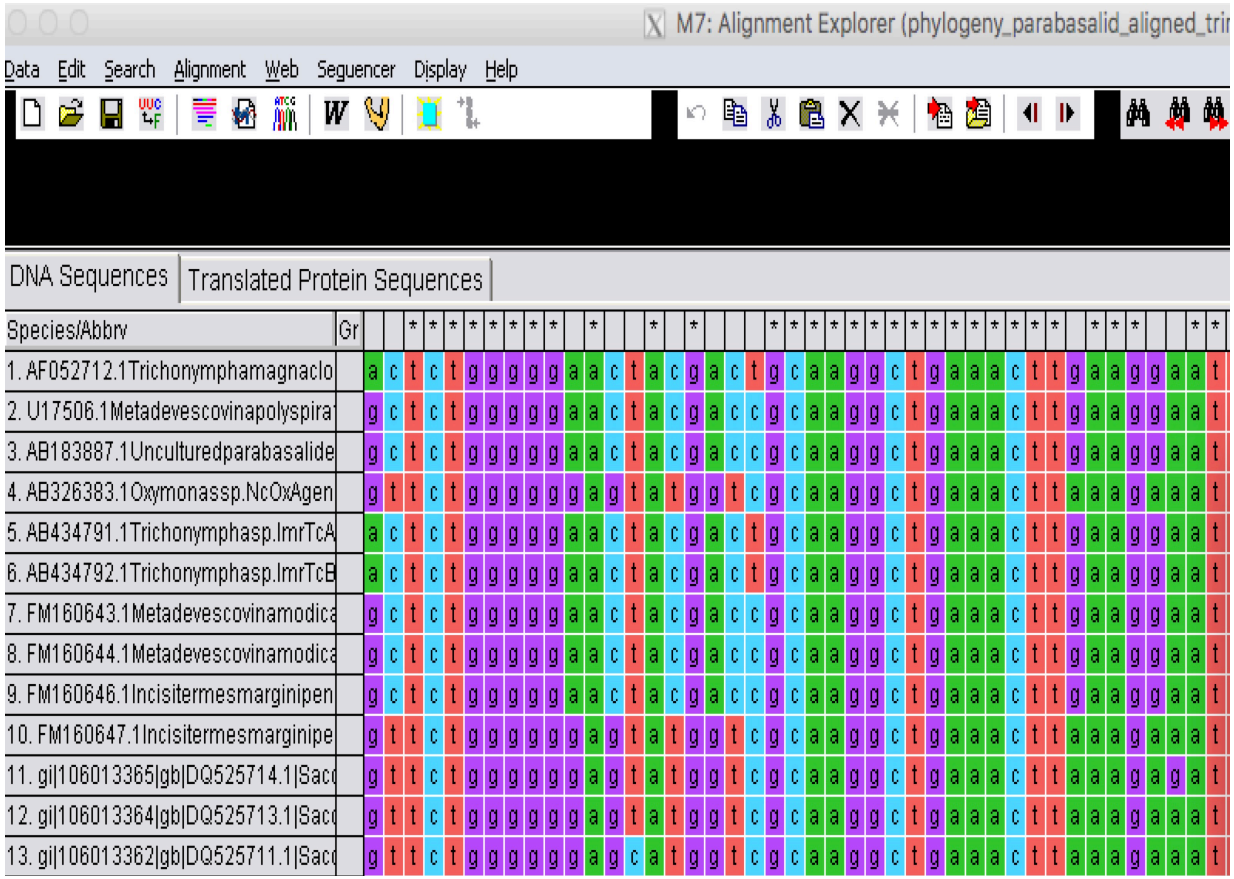
- Align DNA sequences with MEGA7 or with MAFFT

```
mkdir MAFFT
cd MAFFT
```

- MAFFT command#copy fas file to MAFFT folder

```
cp <path to phylogeny_parabasalid.fasta> .
module load mafft/7.305
mafft --maxiterate 1000 --globalpair
            phylogeny_parabasalid.fasta >
            phylogeny_parabasalid_aligned.fasta
```

Phylogenetic tree



View alignment in MEGA7

copy alignment to your local directory, then open alignment in MEGA7

1) `scp -r <path to alignment> <local directory>`

2) view alignment and trim if needed

3) save trimmed fasta file if possible

Phylogenetic tree

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- Determine best model, and partitioning strategy with PartitionFinder2
- #partitionfinder requries .phy format and .cfg format files

1) make .phy file. go to geneious >> import .fasta >> export .phy file

Also see example in

`/work/spp_dec2018/Menglin/FILE/phylogeny_parabasalid_alignedTrim.phy`

2) make .cfg file see example in `/work/spp_dec2018/Menglin/FILE/partition_finder.cfg`

3) copy all two files in one directory

```
mkdir Partitionfinder
cp <path to .phy file> Partitionfinder/
cp <path to .cfg file> Partitinfinder/
```

4) Partitionfinder command (slurm script)

```
module load partitionfinder/2.1.1
PartitionFinder.py Partitionfinder/
```

5) check result

```
cd analysis
less best_scheme.txt
```

Phylogenetic tree

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- Identify the best tree, with RAxML. That tree will be used as the tree.

```
module load raxml/8.2.11
raxmlHPC -m GTRGAMMAI -p 12345 -# 20 -k -q partitions.txt
         -s phylogeny_parabasalid_alignedTrim.phy -n theTree
```

- Carry out bootstrapping. This command makes 1000 trees with bootstrap method

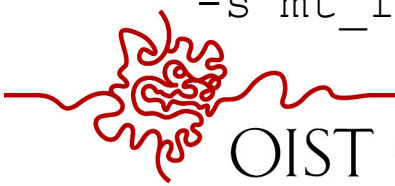
```
raxmlHPC -f d -# 1000 -p 1000 -x 1000 -k -q partitions.txt -s
       phylogeny_parabasalid_alignedTrimm.phy -n RAxML_bootstrap.trees
       -m GTRGAMMAI
```

- Create bipartition tree that can be read with Figuretree

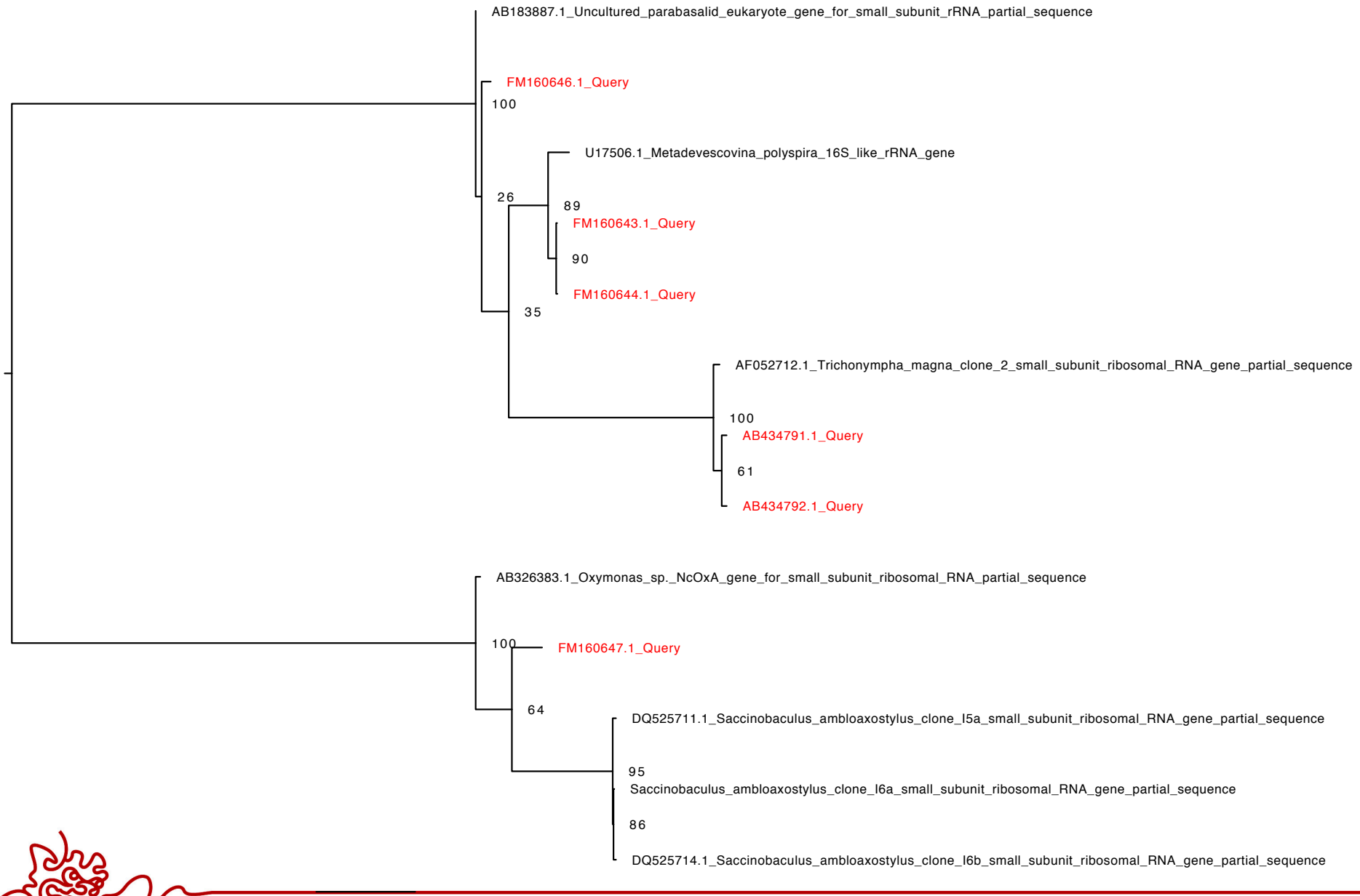
```
raxmlHPC -m GTRGAMMAI -p 12345 -f b -t RAxML_bestTree.theTree
       -z RAxML_bootstrap.trees -n tree
```

- ALL IN ONE

```
raxmlHPC -f a -m GTRGAMMA -q partitions.txt
     -s mt_for_Menglin.phy -n tree -p 12345 -x 12345 -# 1000
```



Phylogenetic tree



Downloading some software for Sunday

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- Tracer-

https://github.com/beast-dev/tracer/releases/tag/v1.7.1

- BEAST-

http://beast.community/installing