

Lab Book

Cientific Initiation - Coral Metagenomes
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Capítulo 1

May 2018

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1.1.1 Learning L^AT_EX

- Working folder: *path*

L^AT_EX is a high-quality typesetting system, available as free software, which allows to produce scientific or technical documents [?]. I am using L^AT_EX to create a Bioinformatics Lab Book. To compile my Lab Book, I can use command lines ([pdflatex](#) and [bibtex](#)). Afterwards I can visualise the produced *.pdf* file with evince or another reader. Alternatively, I can use a Latex editor, such as TexWorks (<https://www.tug.org/texworks/>), which allows me to write the code and control the *pdf* file in the same environment (Figure 1.1).

To compile the *.tex* file in the command line:

```
$pdflatex lab-book  
$bibtex lab-book  
$pdflatex lab-book  
$pdflatex lab-book
```

To visualise the *.pdf*:

```
$evince lab-book.pdf &
```

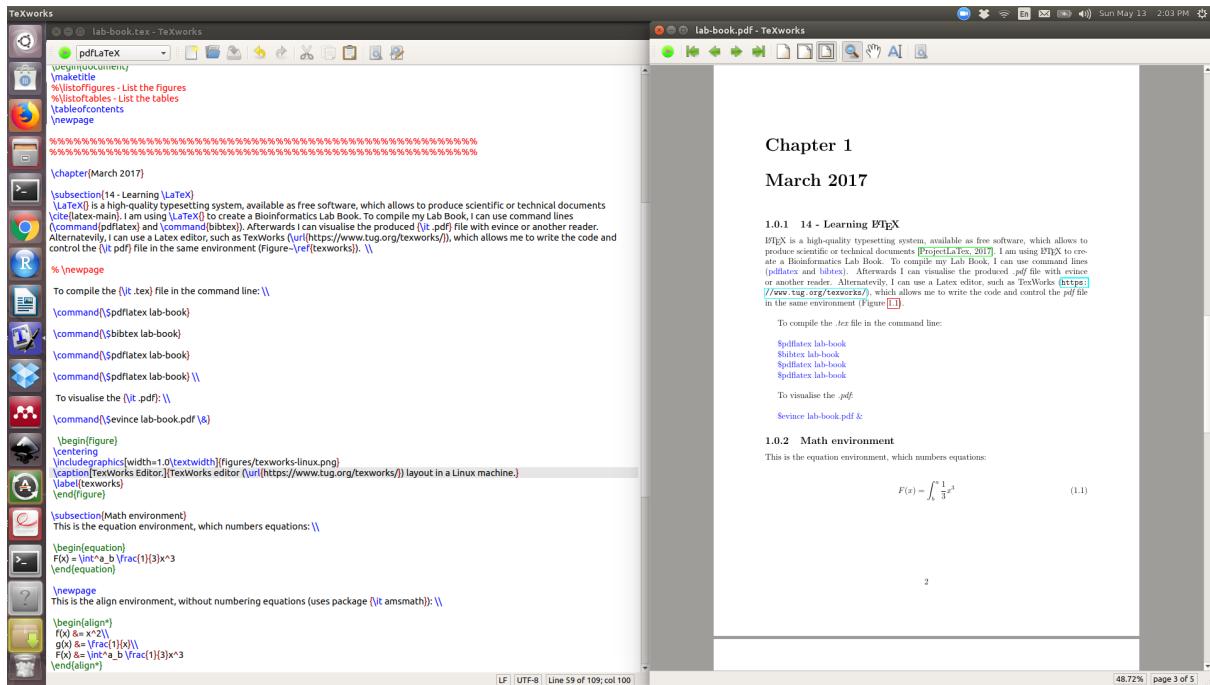


Figura 1.1: TexWorks editor (<https://www.tug.org/texworks/>) layout in a Linux machine.

1.1.2 Math environment

This is the equation environment, which numbers equations:

$$F(x) = \int_b^a \frac{1}{3}x^3 \quad (1.1)$$

This is the align environment, without numbering equations (uses package *amsmath*):

$$\begin{aligned}f(x) &= x^2 \\g(x) &= \frac{1}{x} \\F(x) &= \int_b^a \frac{1}{3}x^3\end{aligned}$$

1.1.3 15 - Short-term project proposal

Some text here. Including and referencing a table (table 1.1).

- First numbered list item
- Second numbered list item

Tabela 1.1: table0

species	changes	score
Macaque	4	0.0
Human	2	14.9
Orangutan	0	0.0
Pan	0	0.0
Gorilla	0	0.0

Capítulo 2

Creation of data base of metagenomes and genomes

2.1 28

2.1.1 Bibliographic search for genomes

Found a new possibility of phyla list. Because of this, there are four possibilities of list of microorganisms phyla, one of them, the SILVA database, is based in RNA sequences:

- The list of Prokariotic names with stading nomenclature <http://www.bacterio.net/-classifphyla.html>
- SILVA database LSU(large subunit of ribosome) <https://www.arb-silva.de/browser/lSU/>
- SILVA database SSU(small subunit of ribosome) <https://www.arb-silva.de/browser/ssu/>
- PATRIC GENOMES https://www.patricbrc.org/view/Taxonomy/2#view_tab=taxontree

The list of articles used until now is:

- 10.1038/nature14486
- 10.1038/ismej.2013.111
- 10.1038/ismej.2013.174
- 10.1038/ismej.2016.43
- 10.1038/nature12352
- 10.1038/nature14486
- 10.1038/nature21031
- 10.1038/ismej.2015.233

- 10.1038/ncomms13219
- 10.1073/pnas.0801980105
- 10.1111/1462-2920.13362
- 10.1126/science.1132690
- 10.1186/s40168-015-0077-6

The list os correspondent phyla and articles is above

Tabela 2.1: table 1

DOI	Phylum
10.1038/nature14486	Candidatus Falkowbacteria
10.1038/nature14486	Candidatus Kuenenbacteria
10.1038/nature14486	Candidatus Magasanikbacteria
10.1038/nature14486	Candidatus Uhrbacteria
10.1038/nature14486	Candidatus Moranbacteria
10.1038/nature14486	Candidatus Azambacteria
10.1038/nature14486	Candidatus Yanofskybacteria
10.1038/nature14486	Candidatus Jorgensenbacteria
10.1038/nature14486	Candidatus Wolfebacteria
10.1038/nature14486	Candidatus Giovannonibacteria
10.1038/nature14486	Candidatus Nomurabacteria
10.1038/nature14486	Candidatus Campbellbacteria
10.1038/nature14486	Candidatus Adlerbacteria
10.1038/nature14486	Candidatus Kaiserbacteria
10.1038/nature14486	C. S. yataiensis
10.1038/nature14486	Pacebacteria
10.1038/nature14486	Candidatus Collierbacteria
10.1038/nature14486	Candidatus Beckwithbacteria
10.1038/nature14486	Candidatus Roizmanbacteria
10.1038/nature14486	Candidatus Saphirobacteria
10.1038/nature14486	Candidatus Amesbacteria
10.1038/nature14486	Candidatus Woesebacteria
10.1038/nature14486	Candidatus Gottesmanbacteria
10.1038/nature14486	Candidatus Levybacteria
10.1038/nature14486	Candidatus Daviesbacteria
10.1038/nature14486	Candidatus Curtissbacteria
10.1038/nature14486	WWE3
10.1038/nature14486	CPR3
10.1038/nature14486	WS6
10.1038/nature14486	Candidatus Berkelbacteria
10.1038/nature14486	Candidatus Peregrinibacteria
10.1038/nature14486	Candidatus Gracilibacteria
10.1038/nature14486	CPR2
10.1038/nature14486	Kazan
10.1038/nature14486	Saccharibacteria (TM7)
10.1038/nature14486	SR1
10.1038/ncomms13219	Candidatus Kerfeldbacteria
10.1038/ncomms13219	Candidatus Komeilibacteria
10.1038/ncomms13219	Candidatus Andersenbacteria
10.1038/ncomms13219	Candidatus Ryanbacteria
10.1038/ncomms13219	Candidatus Niyogibacteria

10.1038/ncomms13219	Candidatus Tagabacteria
10.1038/ncomms13219	Candidatus Terrybacteria
10.1038/ncomms13219	Candidatus Vogelbacteria
10.1038/ncomms13219	Candidatus Zambryskibacteria
10.1038/ncomms13219	Candidatus Taylorbacteria
10.1038/ncomms13219	Candidatus Sungbacteria
10.1038/ncomms13219	Candidatus Brennerbacteria
10.1038/ncomms13219	Candidatus Spechtbacteria
10.1038/ncomms13219	Candidatus Staskawiczibacteria
10.1038/ncomms13219	Candidatus Wildermuthbacteria
10.1038/ncomms13219	Candidatus Portnoybacteria
10.1038/ncomms13219	Candidatus Woykebacteria
10.1038/ncomms13219	Candidatus Blackburnbacteria
10.1038/ncomms13219	Candidatus Chisholmbacteria
10.1038/ncomms13219	Candidatus Buchananbacteria
10.1038/ncomms13219	Candidatus Jacksonbacteria
10.1038/ncomms13219	Candidatus Veblenbacteria
10.1038/ncomms13219	Candidatus Nealsonbacteria
10.1038/ncomms13219	Candidatus Colwellbacteria
10.1038/ncomms13219	Candidatus Liptonbacteria
10.1038/ncomms13219	Candidatus Harrisonbacteria
10.1038/ncomms13219	Candidatus Yonathbacteria
10.1038/ncomms13219	Candidatus Lloydibacteria
10.1038/ncomms13219	Candidatus Abawacabacteria
10.1038/ncomms13219	Candidatus Doudnabacteria
10.1038/ismej.2013.111	Candidatus Poribacteria
10.1111/1462-2920.13362	Candidatus Desantisbacteria
10.1038/nature12352	Candidatus Omnitrophica
10.1038/nature12352	Candidatus Aminicenantes
10.1126/science.1132690	Candidatus Micrarchaeota
10.1038/nature14486	Candidatus Magasanikbacteria
10.1073/pnas.0801980105	Candidatus Korarchaeota
10.1038/nature12352	Candidatus Fervidibacteria
10.1038/nature12352	Candidatus Aenigmarchaeota
10.1038/ismej.2016.43	Candidatus Fermentibacteria
10.1038/ismej.2013.174	Candidatus Bathyarchaeota
10.1016/j.cub.2015.01.014	Candidatus Woesearchaeota
10.1016/j.cub.2015.01.014	Candidatus Kryptonia
10.1038/nature12352	Candidatus Diapherotrites
10.1038/nature12352	Candidatus Latescibacteria
10.1038/nature21031 10.1038/ismej.2015.233	Candidatus Thorarchaeota
10.1038/ncomms13219	Candidatus Lindowbacteria
10.1038/nature12352	Candidatus Parvarchaeota
10.1038/nature12352	Candidatus Cloacimonetes
10.1038/nature12352	Candidatus Hydrogenedentes
10.1038/nature12352	Candidatus Acetothermia

10.1038/nature12352	Candidatus Nanohaloarchaeota
10.1038/ncomms13219	Candidatus Eisenbacteria
10.1186/s40168-015-0077-6	candidate division WOR-3
10.1038/nature21031	Lokiarchaeota
10.1038/nature21031	Odinarchaeota
10.1038/nature21031	Heimdallarchaeota

2.2 28

2.2.1 Bibliographic search for metagenomes

The research for coral metagenomes started last year. The actual list is:

Tabela 2.2: table 1

IDs
mgm4440319.3
mgm4440370.3
mgm4440371.3
mgm4440372.3
mgm4440373.3
mgm4440374.3
mgm4440375.3
mgm4440376.3
mgm4440377.3
mgm4440378.3
mgm4440379.3
mgm4440380.3
mgm4440381.3
mgm4445755.3
mgm4445756.3
mgm4480739.3
mgm4480740.3
mgm4480741.3
mgm4480748.3
mgm4480750.3
mgm4487909.3
mgm4487910.3
mgm4487911.3
mgm4516541.3
mgm4516694.3
mgm4653307.3
mgm4694757.3
mgm4694758.3

mgm4694759.3
mgm4694760.3
SRR1275409
SRR1275449
SRR1283349
SRR1283371
SRR1283377
SRR1283433
SRR1283435
SRR1283437
SRR1286223
SRR1286225
SRR1286226
SRR1286227
SRR1286229
SRR1286232
SRR1822488
SRR1822516
SRR3499156
SRR3569370
SRR3694369
SRR3694370
SRR3694371
SRR3694372
SRR5215424
SRR5215454
SRR5215455
SRR5215456
SRR5215457
SRR5215458
SRR5215462
SRR5605611

I found these metagenomes in the article: "Metagenomic analysis reveals a green sulfur bacterium as a potential coral symbiont"

SRR2937345
SRR2937346
SRR2937347
SRR2937348
SRR2937349
SRR2937350
SRR2937351
SRR2937352

SRR2937353
SRR2937354
SRR2937355
SRR2937356

Espcie: Platygyra carnosa Healthy

I found other metagenomes of coral from article doi [10.3389/fmars.2018.00101](https://doi.org/10.3389/fmars.2018.00101) I updated the file pmc_results_1.txt in the repository Lab_book. I continue to look the articles in results. Estou atualizando a lista pmc_results_2.txt Na pesquisa bibliografica olhando o ttulo ja me faz perceber se devo descartar e olhar. E olho aqueles que marquei para olhar. Ao olhar, leio o resumo procurando por metodos. E vou para os metodos do artigo para checar. Checking the sizes of metagenomes files. The mg-rast metagenomes base have 72 Gb.

The pipeline of bioinformatic is different for MGRAST and NCBI. The size of NCBI should be superestimated, because the ncbi says the file size of sra file, but most of them is paired-end metagenomes, so when we apply fastq-dump, its generate two files fastq.

2.2.2 Amostras do MG-RAST indicadas pelo professor e Miguel

Na reuniao feita em 30 de outubro, o professor e Miguel indicaram amostras existentes de Madracis para serem analisadas. O artigo entitulado: "Turbulence-driven shifts in holobionts and planktonic microbial assemblages in St. Peter and St. Paul Archipelago, Mid-Atlantic Ridge, Brazil". DOI: [10.3389/fmicb.2015.01038](https://doi.org/10.3389/fmicb.2015.01038). Amostras inclusas:

- mgm4486661.3
- mgm4486662.3
- mgm4486663.3
- mgm4486664.3
- mgm4486665.3
- mgm4486666.3
- mgm4486667.3
- mgm4486668.3
- mgm4486669.3

O estado de saude dessas amostras nao esta claramente indicado no MG-RAST, mas pelo nome da amostra. No artigo, as amostras de Madracis branqueadas esto nomeadas com madble.

O professor indicou procurar pelo laboratorio do professor Alexandre Rosado, para encontrar mais amostras de metagenomas de corais feitas no Brasil, especificamente nos trabalho de Raquel Peixoto (nao estava encontrando antes porque pensava que o nome era Raquel Rosado). Indo em seu perfil no google academic, utilizei o recurso de ctrl f, pesquisando por coral. Os trabalhos que continham 'coral' no ttulo no so de metagenoma.

Capítulo 3

Download of metagenomes

3.1 Download of mg-rast files

Espao no SDU Disponvel para o ebiodiv: 10Tb Bia: 5Tb Rilquer: 2T Remanescente: 3Tb

- Working folder: *scratch/ebiodiv/leticia.cavalcante/mg_rast*

I insert the list of metagenomes in the files before using it. After this, I used the following command line:

- Command: *nohup bash download_curl_mgrast_corais.sh & download_curl_mgrast_corais.nohupout &*

3.2 Download of NCBI metagenomes

I use the script *download_sra_wget_corais.sh*, libs folder. I used the wget, because the curl is getting some problem in SDU. I noted that the size of the files is different:

Tabela 3.1: Comparing sizes of files

ID of metagenome	the size in NCBI site	size of file in SDU
SRR6785058	317.00 Mb	318M
SRR6785057	364.00 Mb	365M
SRR6785056	560.00 Mb	561M
SRR6785055	624.00 Mb	625M

So I checked the others files:

Tabela 3.2: Comparing sizes of files 2

ID of metagenome	the size in NCBI site	size of file in SDU	size of cleanned file
mgm4440319.3.299.1	30M	29.1 MB	28M
mgm4440370.3.299.1	3,6M	3.5 MB	3,5M
mgm4440371.3.299.1	5,0M	4.9 MB	4,8M
mgm4440372.3.299.1	6,0M	6.0 MB	5,9M
mgm4440373.3.299.1	6,2M	6.1 MB	6,0M
mgm4440374.3.299.1	4,1M	4.1 MB	4,0M
mgm4440375.3.299.1	3,8M	3.7 MB	3,7M
mgm4440376.3.299.1	3,9M	3.9 MB	3,8M
mgm4440377.3.299.1	3,5M	3.5 MB	3,4M
mgm4440378.3.299.1	6,2M	6.2 MB	6,1M
mgm4440379.3.299.1	7,0M	7.0 MB	6,9M
mgm4440380.3.299.1	5,2M	5.2 MB	5,2M
mgm4440381.3.299.1	6,4M	6.4 MB	6,4M
mgm4445755.3.299.1	158M	157.0 MB	155M
mgm4445756.3.299.1	150M	149.9 MB	147M
mgm4480739.3.299.1	8,0M	7.9 MB	7,9M
mgm4480740.3.299.1	12M	11.3 MB	12M
mgm4480741.3.299.1	8,5M	8.5 MB	8,5M
mgm4480742.3.299.1	10M	12.9 MB	10M
mgm4480743.3.299.1	15M	10.0 MB	14M
mgm4484839.3.299.1	13M	14.1 MB	13M
mgm4487909.3.299.1	17M	16.5 MB	17M
mgm4487910.3.299.1	36M	35.6 MB	36M
mgm4487911.3.299.1	12M	11.4 MB	12M
mgm4516541.3.299.1	161M	160.2 MB	163M
mgm4516694.3.299.1	193M	192.9 MB	193M
mgm4653307.3.299.1	17M	16.0 MB	17M
mgm4694757.3.299.1	1,9G	1.8 GB	1,9G
mgm4694758.3.299.1	2,2G	2.1 GB	2,2G
mgm4694759.3.299.1	1,7G	1.7 GB	1,8G
mgm4694760.3.299.1	592M	1.6 GB	597M

A Bia me informou que o SDU arredonda os valores de tamanho dos arquivos, entao at o momento nao tive problemas com o download dos arquivos do mg_rast

3.2.1 Amostras indicadas por Miguel na reuniao de 30 de novembro

Fiz um loop para baixar as amostras chamado: loop_download_6_november.sh, funcionou no computador local, mas nao no servidor por algum motivo ligado a internet. Por isso, fiz o download das amostras no computador local e as transferi para o cluster Atlantico.

Capítulo 4

Format Conversion of NCBI metagenomes

Adaptei o script da Bia para fazer a conversao do dos arquivos .sra
Inicialmente submeti apenas um na cpu_dev para testar:

```
Script: teste_slurm_job_fastq_dump_corais.sh
Numero do job: 220896
```

Deu certo.

O Rilquer me ajudou a criar um script que cria jobs de anotacao com o kraken2 para cada dois metagenomas.

O nome do script 'creatijobfile.sh', ele unir dois scripts: 'header' e 'ending'
Adaptei para criar jobs do fastq-dump para cada 2 arquivos .sra, haja visto que no tenho
uma boa ideia do quanto cada fastq-dump demorar.

Jobs submetidos dia 27/09/2018:

- job_0.sh - job 221475
- job_100.sh - job 221476
- job_102.sh - job 221477
- job_104.sh - job 221478
- job_106.sh - job 221480
- job_108.sh - job 221481
- job_10.sh - job 221482
- job_110.sh - job 221483
- job_112.sh - job 221484
- job_114.sh - job 221485

- job_116.sh - job 221486
- job_118.sh - job 221487
- job_120.sh - job 221488
- job_122.sh - job 221489
- job_124.sh - job 221490
- job_126.sh - job 221493
- job_12.sh - job 221495

Jobs submetidos dia 09/10/2018:

- job_14.sh - slurm 226902
- job_16.sh - slurm 226903
- job_18.sh - slurm 226904
- job_20.sh - slurm 226905
- job_22.sh - slurm 226906
- job_24.sh - slurm 226907
- job_26.sh - slurm 226908
- job_28.sh - slurm 226909
- job_2.sh - slurm 226910
- job_30.sh - slurm 226911
- job_32.sh - slurm 226912

Jobs submetidos dia 19/10/2018:

- job_34.sh - slurm 231744
- job_36.sh - slurm 231745
- job_38.sh - slurm 231746
- job_40.sh - slurm 231747
- job_42.sh - slurm 231748
- job_44.sh - slurm 231749
- job_46.sh - slurm 231750
- job_48.sh - slurm 231751

- job_4.sh - slurm 231752
- job_50.sh - slurm 231753
- job_52.sh - slurm 231754
- job_54.sh - slurm 231755

4.0.1 Taxonprofiling

Essa subsecao foi escrita apos o Rilquer construir um script que unifica as etapas de bioinformatica em um script unico. Com script do Rilquer chamando Taxonprofiling, todas as etapas de bioinformatica esto unidas em um so script. Os jobs acimas esto guardados, mas com a utilizacao do script que o Rilquer construiu, as amostras estao sendo analisadas no Atlantico, cluster da UFBA

Capítulo 5

Adaptacao dos identificadores

Esse passo fez-se necessrio nas anlises anteriores, pois quando eu fazia a limpeza, os arquivos de outputs que saiam eram apenas os singletons. The output files were named as SRAXXX_good_singletons_1 and SRAXXX_good_singletons_2 and there arent any other output files beyond these and the files with bad sequences. O Pablo fez a seguinte sugesto:

```
cat SRR1275409_pass_1.fastq | sed -r 's/(SRR1275409.[0-9]+)\.([0-9]+)/\1_left /' > SRR1275409_pass_1.corr.fastq
```

```
cat SRR1275409_pass_2.fastq | sed -r 's/(SRR1275409.[0-9]+)\.([0-9]+)/\1_right /' > SRR1275409_pass_2.corr.fastq
```

Capítulo 6

Quality filter

This step is only required for NCBI metagenomes. The command line was proposed by Bia:

- trim_qual_left 25
- trim_qual_right 25

6.0.1 Taxonprofiling

Essa subsecao foi escrita apos o Rilquer construir um script que unifica as etapas de bioinformatica em um script unico. A etapa do quality filter foi modificada pelo Rilquer, apos ele insistir com o professor sobre qual seriam os melhores parametros de qualidade.

Segue criterios de qualidade:

- min_qual_score 13
- trim_qual_left 13
- trim_qual_right 13

Linha parcial abaixo:

```
-min_qual_score 13 -trim_qual_left 13 -trim_qual_right 13 -  
ns_max_n 2
```

Capítulo 7

Uniformity filter (size and N bases)

7.1 Command line

Parameters:

- min_len 80
- ns_max_p 2
- out_format 1

- Command: *nohup bash slurm_job_prinseq_single_cora1s_FASTA.bash & slurm_prinseq_cora1s.out &*

Deu erro o job nohup: ignorando entrada

Location of PRINSEQ dir and scripts: /scratch/app/prinseq/0.20.4/bin srun Warning: can't run 1 processes on 21 nodes, setting nnodes to 1 srun Requested partition configuration not available now srun job 212425 queued and waiting for resources srun Force Terminated job 212425 srun Job has been cancelled srun error: Unable to allocate resources: No error srun Warning: can't run 1 processes on 21 nodes, setting nnodes to 1 srun Requested partition configuration not available now srun job 212428 queued and waiting for resources srun Force Terminated job 212428 srun Job has been cancelled

09-35-27.png

Figura 7.1: Erro no job no SDU

Ressubmeti o job com:

- Command: *sbatch slurm_job_prinseq_single_cora1s_FASTA.bash*

7.1.1 Utilizacao do Taxonprofiling

Essa subsecao foi escrita apos o Rilquer construir um script que unifica as etapas de bioinformatica em um script unico. Os criterios foram modificados para:

- -min_len 50
 - -ns_max_n 2

Linha total:

```
prinseq-lite -verbose -fastq ${OUTDIR}/fastqdump_output/${id}_  
_pass_1.fastq -fastq2 ${OUTDIR}/fastqdump_output/${id}_pass_2  
.fastq -min_len 50 -min_qual_score 13 -trim_qual_left 13 -  
trim_qual_right 13 -ns_max_n 2 -out_format 1
```

Capítulo 8

Profiling metagenomes

8.1 Mg-Rast metagenomes

I used the following script in the following folder:

- Folder: *scratch/ebiodiv/leticia.ca valcante/mg_rast/filtered_prinseq_good*
- Command: *sbatch slurm-job_kraken2_corais.sh*

The job doesn't work, o erro aparece na proxima figura

Ressubmeti o job, modificando a localizacao da DB do Kraken para a home do Rilquer.

Numero do job: 216410

Esse problema foi resolvido modificando o endereco da base para o scratch do Rilquer.

8.2 Kraken-biom

Pasta onde est instalado kraken-biom:

/home/leticia/.local/bin

Para executar: python2.7 .kraken-biom

Executar o help do kraken-biom:

kraken-biom -h

Abrir no vim o arquivo .bashrc e inserir:

```
export PATH=$PATH:/home/leticia/.local/bin/kraken-biom
```

Executar o help do kraken-biom:

kraken-biom -h

Eu fiz um teste da etapa "Creation of BIOM table of abundances" da pipeline da bia com os seguintes passos: Na pasta /home/leticia/Documentos/libs/leticia_profiling_metagenomes:

- kraken-biom selected_file -o table.biom –max D –min P
- biom convert -i table.biom -o table.from_biom_with_taxonomy.txt –to-tsv –header-key taxonomy

```
Atividades Terminal sex, 16/35
leticia.cavalcante@sdumont12:~/scratch/eblodiv/leticia.cavalcante/mg_rast/filtered_prinseq_good

Arquivo Editar Ver Pesquisar Terminal Ajuda

-rw-r--r-- 1 leticia.cavalcante eblodiv 1,8G Set 12 20:41 mgm4694759_prinseq_good_4RF.fasta
-rw-r--r-- 1 leticia.cavalcante eblodiv 597M Set 12 20:42 mgm4694760_prinseq_good_BOZY.fasta
-rw-r--r-- 1 leticia.cavalcante eblodiv 556 Set 13 14:53 slurm-215145.out
-rw-r--r-- 1 leticia.cavalcante eblodiv 9,6K Set 13 16:54 slurm-215343.out
-rw-r--r-- 1 leticia.cavalcante eblodiv 3,4K Set 13 15:21 slurm_job_kraken2_corals.sh
[leticia.cavalcante@sdumont12 filtered_prinseq_good]$ cat slurm-215343.out
sdumont1027-1029,1066,1094,1206-1207,1276-1277,1312-1318,1338-1339,1493-1494,5014-5023]
sdumont1027 sdumont1028 sdumont1029 sdumont1066 sdumont1094 sdumont1266 sdumont1207 sdumont1276 sdumont1277 sdumont1312 sdumont1313 sdumont1314 sdumont1315 sdumont1316 sdumont1317 sdumont1318 sdumont1333 sdumont1339 sdumont1493 sdumont1494 sdumont5014 sdumont5015 sdumont5016 sdumont5017 sdumont5018 sdumont5019 sdumont5020 sdumont5021 sdumont5022 sdumont5023
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440319_prinseq_good_DVF.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440320_prinseq_good_SDP.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440321_prinseq_good_EDLk.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440322_prinseq_good_B4W.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440323_prinseq_good_84W.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440324_prinseq_good_4Qa.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440325_prinseq_good_9E3C.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440326_prinseq_good_lVcm.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440327_prinseq_good_bLxz.fasta!
srun: Warning: can't run i processes on 30 nodes, setting nnodes to 1
kraken2: database ('/prj/eblodiv/maria.costa/Kraken2_DB') does not contain necessary file taxo.k2d
srun: error: sdumont1027: task 0: Exited with exit code 2
Produced profile and report for file: mgm4440328_prinseq_good_02ak.fasta!
```

Figura 8.1: 2o erro no job no SDU

- perl filterRank.pl input_table.from_biom_with_taxonomy.txt -rank p,i,abundance.matrix

8.3 Teste com o kraken no scratch

Linha de teste:

```
perl selectGroups.pl input mgm4440370_prinseq_good_SiDP.fasta_kraken.report -file_groups groups.txt > selected_file
```

- First Command: `sbatch slurm_job_kraken2_corais.sh`
- Second Command:
`kraken2 -db /prj/ebiodiv/rilquer.silva/Serrapilheira`
`/Kraken2_custom_DB/ mgm4440370_prinseq_good_SiDP.fasta`
`-output mgm4440370_prinseq_good_SiDP.fasta_kraken.profiled`
`-use-names -report mgm4440370_prinseq_good_SiDP.fasta_kraken.report`

Já testei o comando acima na home do SDU e agora no scratch

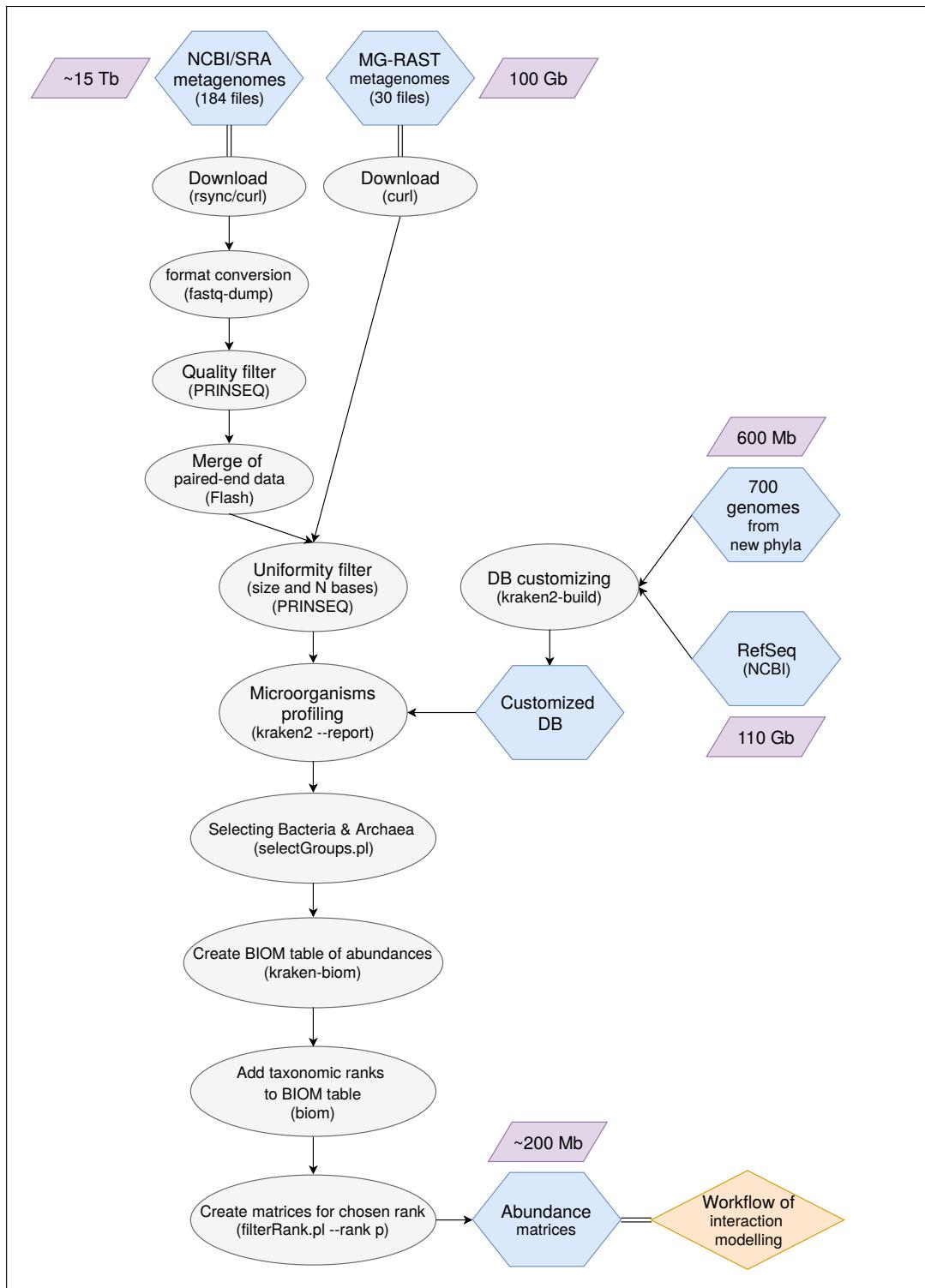


Figura 8.2: Pipeline of taxonomic annotation

8.4 Profiling no Atlantico com a ajuda do Rilquer

8.4.1 MG RAST metagenomes

O Rilquer fez um script que automatiza o processo, em que a limpeza e anotação ocorrem simultaneamente. Fiz um teste com esse script para um metagenoma com a seguinte linha:

- Comand: *taxonprofiling -s mgm4440378.3.299.1 -f MGRAST -k /home/pedro/-Kraken2_custom_DB*
- Script: taxonprofiling
- Folder: /fsprofpedro/holobionts/mgrast
- Para chamar o script: taxonprofiling

Sairam 4 outputs:

- mgm4440378_kraken_class
- mgm4440378_kraken_output
- mgm4440378_kraken_report
- mgm4440378_kraken_unclass

De acordo com a pipeline da Bia, o arquivo a ser usado é o report. Teste a seguir com a pasta com os metagenomas do mg-rast inteiro:

- Comand: *taxonprofiling -d /fsprofpedro/holobionts/mgrast -f MGRAST -k /home/-pedro/Kraken2_custom_DB*
- Folder: /fsprofpedro/holobionts

No email do Rilquer, vi que tenho que submeter o job para uma fila que no tem acesso ao fsprofpedro. Então criei uma pasta temporária chamada mgrast_temp, no home/pedro

- Job: *profiling_metagenomes_corais_mgrast.sh*
- Folder temporário em que as amostras foram copiadas: /home/pedro/mgrast_temp
- Folder de submissão: /home/pedro/

Na pasta /fsprofpedro/holobionts, tem um exemplo de job chamado jobexample. Submissão:

- Folder de submissão: /home/pedro/mgrast_temp
- Numero: 122296.atlantico
- Command: qsub profiling_metagenomes_corais_mgrast.sh

I have noticed that the files of abundance matrix is empty, I need to warn Rilquer. I had transferred the report files to this computer, to the following folder: /home/leticia/-Documentos/dados/report_atualizado_23_10_2018.

Sequence of command lines:

```
- Folder: /home/leticia/Documentos/libs/leticia_profiling_metagenomes  
Command 1: nohup bash select_groups_perl_corais.sh & select_groups_corais_perl.nohupout &  
Command 2: biom convert -i table_corais_mg_rast.biom -o table_corais_mg_rast.from_biom_with_taxonomy.txt -to-tsv -header-key taxonomy  
Command 3: perl filterRank.pl --input table_corais_mg_rast.from_biom_with_taxonomy.txt --rank p & abundance_corais_mgrast_2.matrix
```

Output gerado: **abundance_corais_mgrast_2.matrix**. O output gerado foi modificado no R, transposto, colocado em porcentagem e com os detalhes de genero, especie e estado de saude e retirei a linha gerada quando transformei em data frame, com X1, X2 e etc.

Coloquei para rodar novamente as amostras do MG-RAST para as matrizes de familia serem geradas. Job: 123190.atlantico

8.4.2 SRA metagenomes

Originalmente, os arquivos esto no folder:**/fsprofpedro/holobionts/SRA**. O total de arquivos 158764768. Para fazer o trabalho de fastq dump, limpeza e anotacao, os arquivos devem estar no seguinte folder:

```
- Folder: /home/pedro/holobionts_temp/SRA  
- Command: qsub profiling_metagenomes_corais_SRA.sh  
- Linha: taxonprofiling -d /home/pedro/holobionts_temp/SRA -f SRA -t /home/pedro/sratoolkit.2.9.2-ubuntu64 -k /home/pedro/DB -o /home/pedro/holobionts_temp/SRA/ -r p,f
```

Submeti o job para amostras do SRA no Atlantico, job 123186.atlantico. Notei que o job gerou os arquivos limpos, mas no encontro as matrizes de abundancias. Pedi ajuda do Rilquer. Testei uma linha isoladamente.

```
taxonprofiling -s /home/pedro/holobionts_temp/SRA/SRR6793730.sra  
-f SRA -t /home/pedro/sratoolkit.2.9.2-ubuntu64 -k /home/pedro/DB -o /home/pedro/holobionts_temp/SRA/ -r p,c,o,f,g
```

[breaklines]

```
Atividades Terminal
Arquivo Editar Ver Pesquisar Terminal Ajuda
Good bases (singletons file 1): 376,852
Good mean length (singletons file 1): 301.00
Good sequences (singletons file 2): 5 (0.00%)
Good bases (singletons file 2): 1,505
Good mean length (singletons file 2): 301.00
Bad sequences (file 1): 875,252 (99.86%)
Bad bases (file 1): 263,450,852
Bad mean length (file 1): 301.00
Bad sequences (file 2): 876,499 (100.00%)
Bad bases (file 2): 263,826,199
Bad mean length (file 2): 301.00
Sequences filtered by specified parameters:
min_qual_score: 1751751
Prinseq successfully run on sample SRR6793730
/home/pedro/bin/taxonprofiling: line 438: [: !=: unary operator expected
/home/pedro/bin/taxonprofiling: line 438: [: !=: unary operator expected
Loading database information... done.
5 sequences (0.00 Mbp) processed in 0.114s (2.6 Kseq/m, 1.59 Mbp/m).
5 sequences classified (100.00%)
0 sequences unclassified (0.00%)
Kraken successfully run for sample SRR6793730
Traceback (most recent call last):
  File "/home/pedro/miniconda2/bin/kraken-biom", line 11, in <module>
    sys.exit(main())
  File "/home/pedro/miniconda2/lib/python2.7/site-packages/kraken_biom.py", line 377, in main
    biomT = create_biom_table(sample_counts, taxa)
  File "/home/pedro/miniconda2/lib/python2.7/site-packages/kraken_biom.py", line 196, in create_biom_table
    generated_by=gen_str, input_is_dense=True)
  File "/home/pedro/miniconda2/lib/python2.7/site-packages/biom/table.py", line 508, in __init__
    errcheck(self)
  File "/home/pedro/miniconda2/lib/python2.7/site-packages/biom/err.py", line 472, in errcheck
    raise ret
biom.exception.TableException: Duplicate sample IDs!
Usage: biom convert [OPTIONS]
Try "biom convert -h" for help.

Error: Invalid value for "-i" / "--input-fp": File "/home/pedro/holobionts_temp/SRA//SRR6793730_table.biom" does not exist.
It was not possible to open file /home/pedro/holobionts_temp/SRA//SRR6793730_table.biom.tsv
```

Figura 8.3: Erro com o script taxon profiling se manifestando ao no gerar as matrizes de abundncia

8.5 Analises e obtencao de figuras

Apliquei o tutorial do professor para obtemo de figuras no R para visualizao dos resultados.

- Script: *analisys.R*
- Folder: */home/leticia/Documentos/libs/R*

Figuras obtidas:

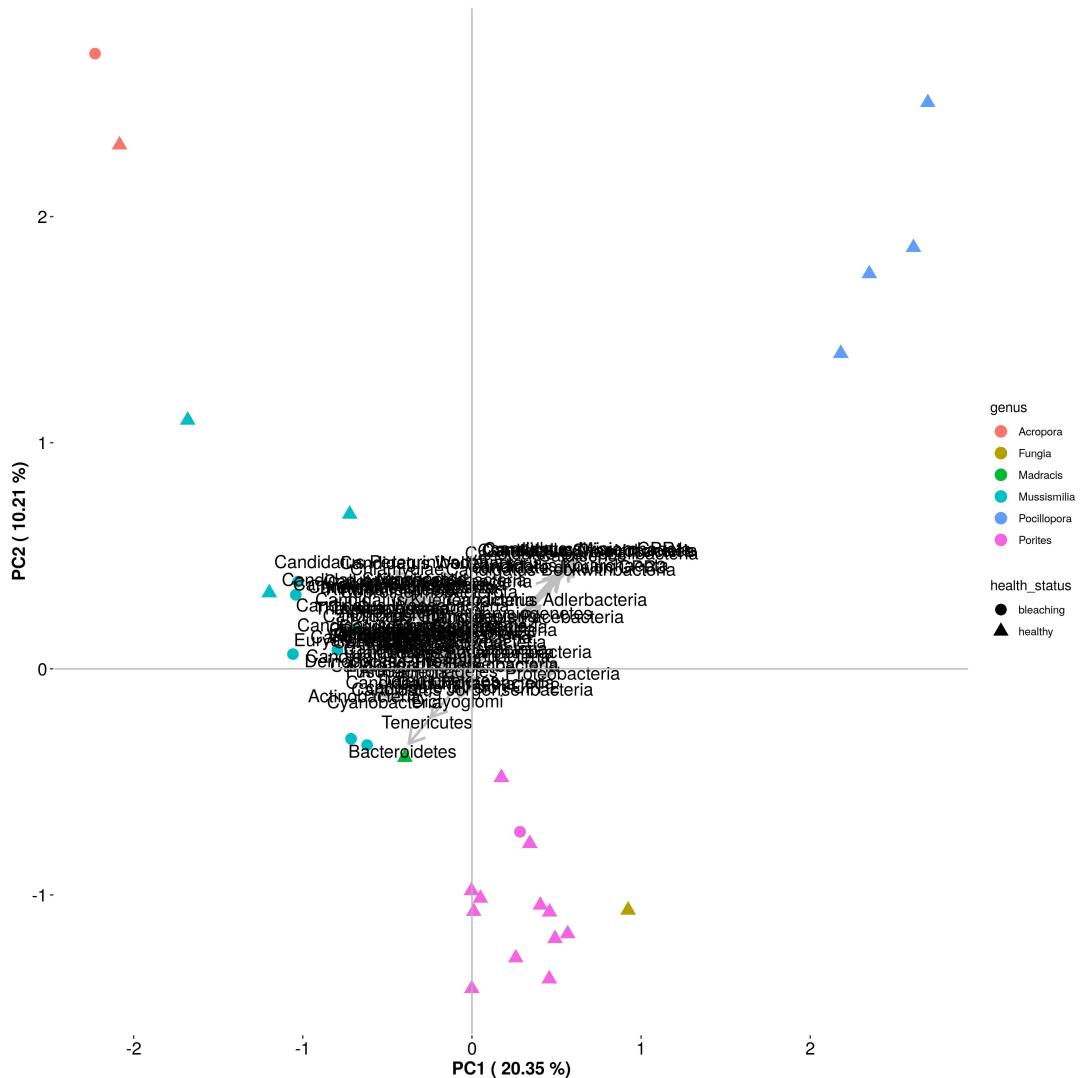


Figura 8.4: Anlise de componentes principais com todos os filos como variaveis

Na analise acima, as variaveis so muitas e ficam muito sobrepostas, fazendo com que haja grande poluicao visual. O professor recomendou em marco a utilizar uma analise de Random Forest, para que as variaveis mais importantes para os metagenomas que trabalho sejam ranqueadas. O random forest um algoritmo de machine learning que, a partir das duas categorias de sede (categorias de supervisao), elencar as variveis mais importantes para classificar as amostras nesses dois estados. o random forest su-

pervisionado por estado de saude

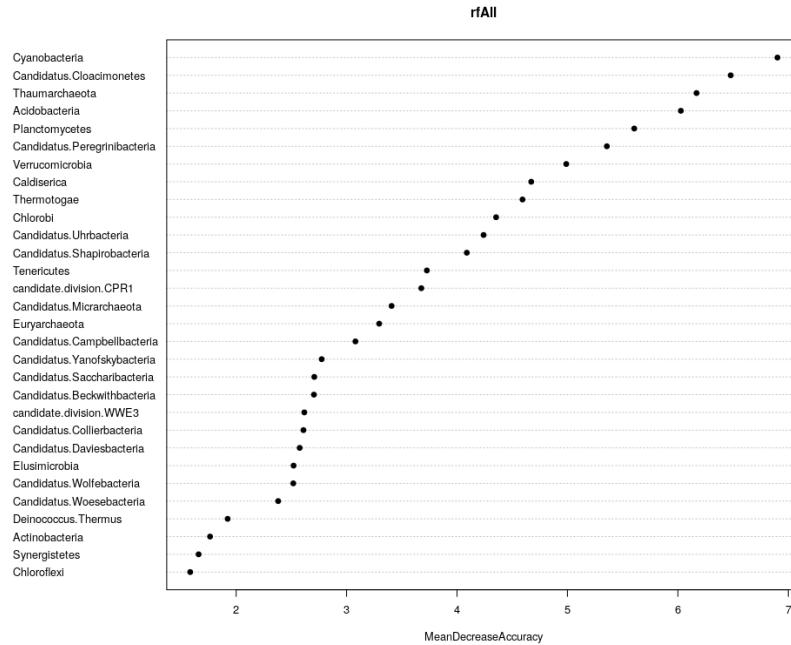


Figura 8.5: Random Forest ranqueando filos

Eu utilizei os 20 primeiros filos ranqueados para fazer o PCA. Segue esse PCA abaixo:

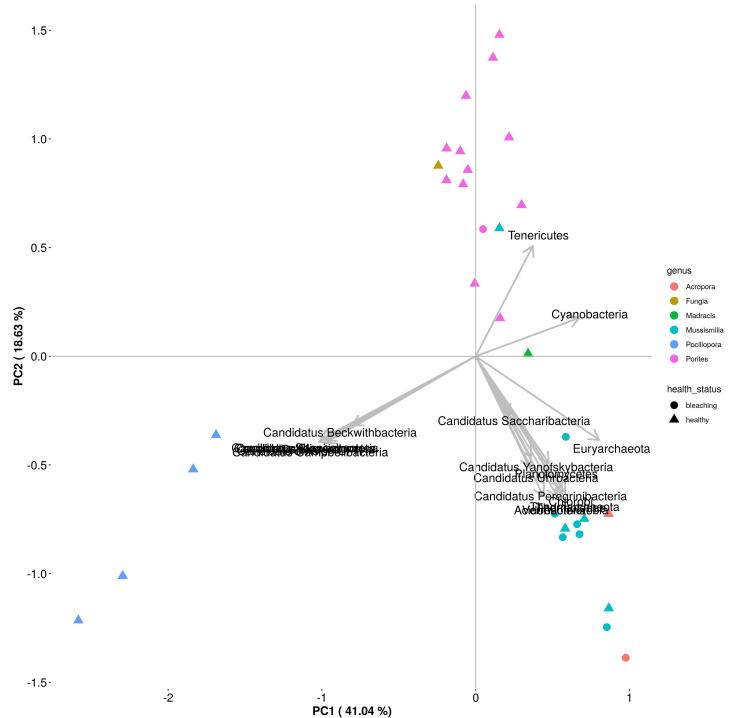


Figura 8.6: PCA com 20 filos utilizados no PCA

Uma tendencia se manteve: foi a separao das amostras por genero. Mas a poluicao visual ainda continuou, por isso fiz um random forest com os 15 primeiros filos indicados pelo random forest. Segue abaixo:

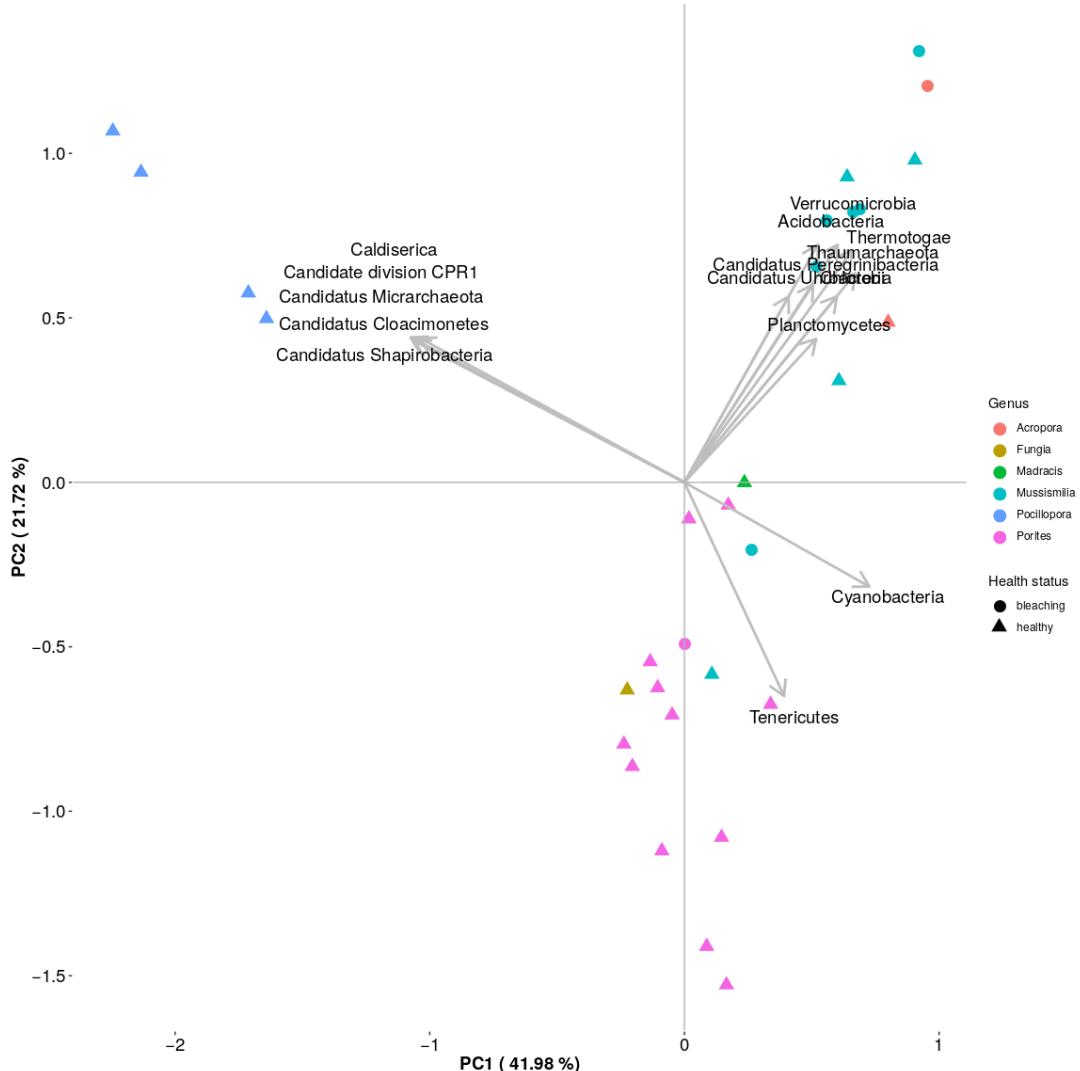


Figura 8.7: PCA com 15 filos como variaveis

Algumas tendencias tambem se mantiveram e a explicao dos eixos melhorou levemente. As amostras agrupadas no quadrante direito superior so mais diferentes das que esto no quadrante esquerdo do que das que esto no quadrante inferior direito. Na reuniao feita no dia 03/10/2018, o Amaro, o professor e Miguel me sinalizaram que existe uma separacao forte entre generos, indicando que os grupos candidatos podem ser genero - especficos. Surgiu a sugesto de leitura de textos em core microbiome e especificidade de filos entre generos e o professor sugeriu fazer um random forest nao supervisionado que segue abaixo. O professor Garcia no congresso sugeriu utilizar os auto valores do PCA para ver quais podem ser mais relevantes (?).

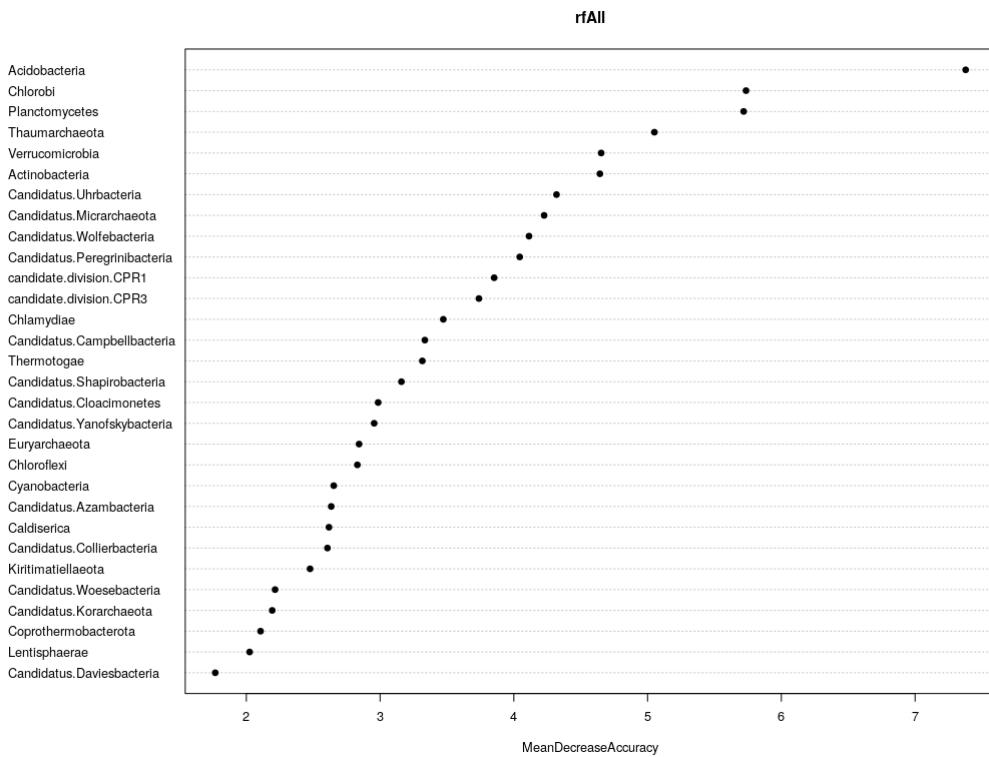


Figura 8.8: Random Forest nao supervisionado

Fiz um pca (libs/R/analisis.R) a partir dos primeiros 15 filos indicados no Random Forest nao supervisionado acima. Segue abaixo:

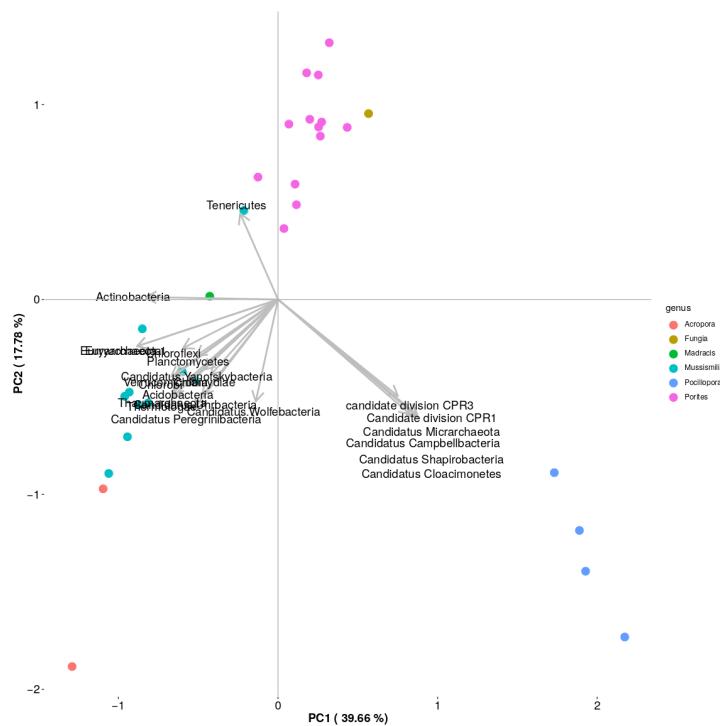


Figura 8.9: PCA a partir dos 20 filos primeiros filos que aparecem acima no random forest

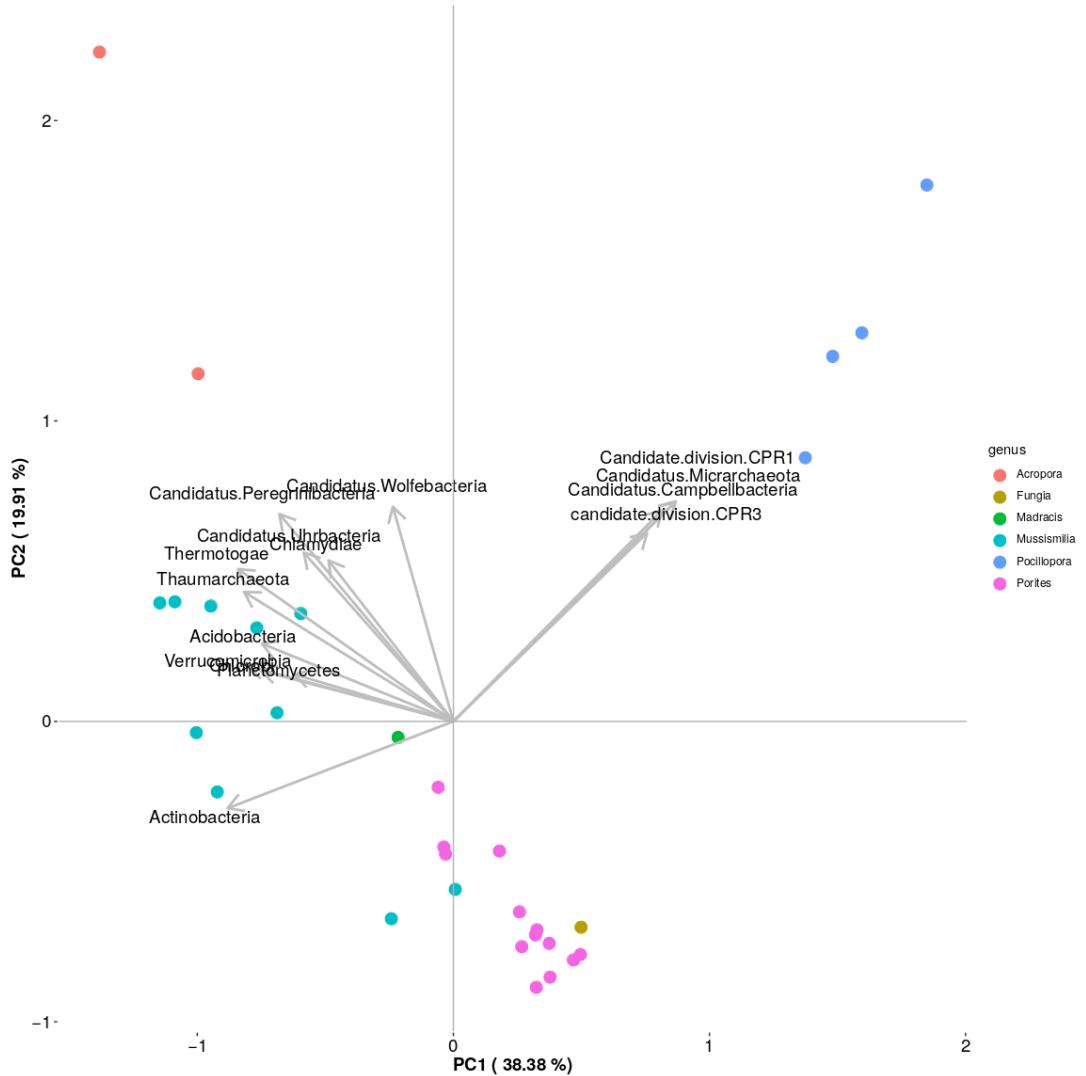


Figura 8.10: PCA a partir dos 15 filos primeiros filos que aparecem acima no random forest nao supervisionado

8.6 Obtencao de figuras com o segundo profiling feito com a ajuda do Rilquer - 23/10/2018

As figuras a seguir foram feitas com dados de abundancia gerados com a ajuda do Rilquer. As primeiras serao com abundancia dos metagenomas do MG-RAST.

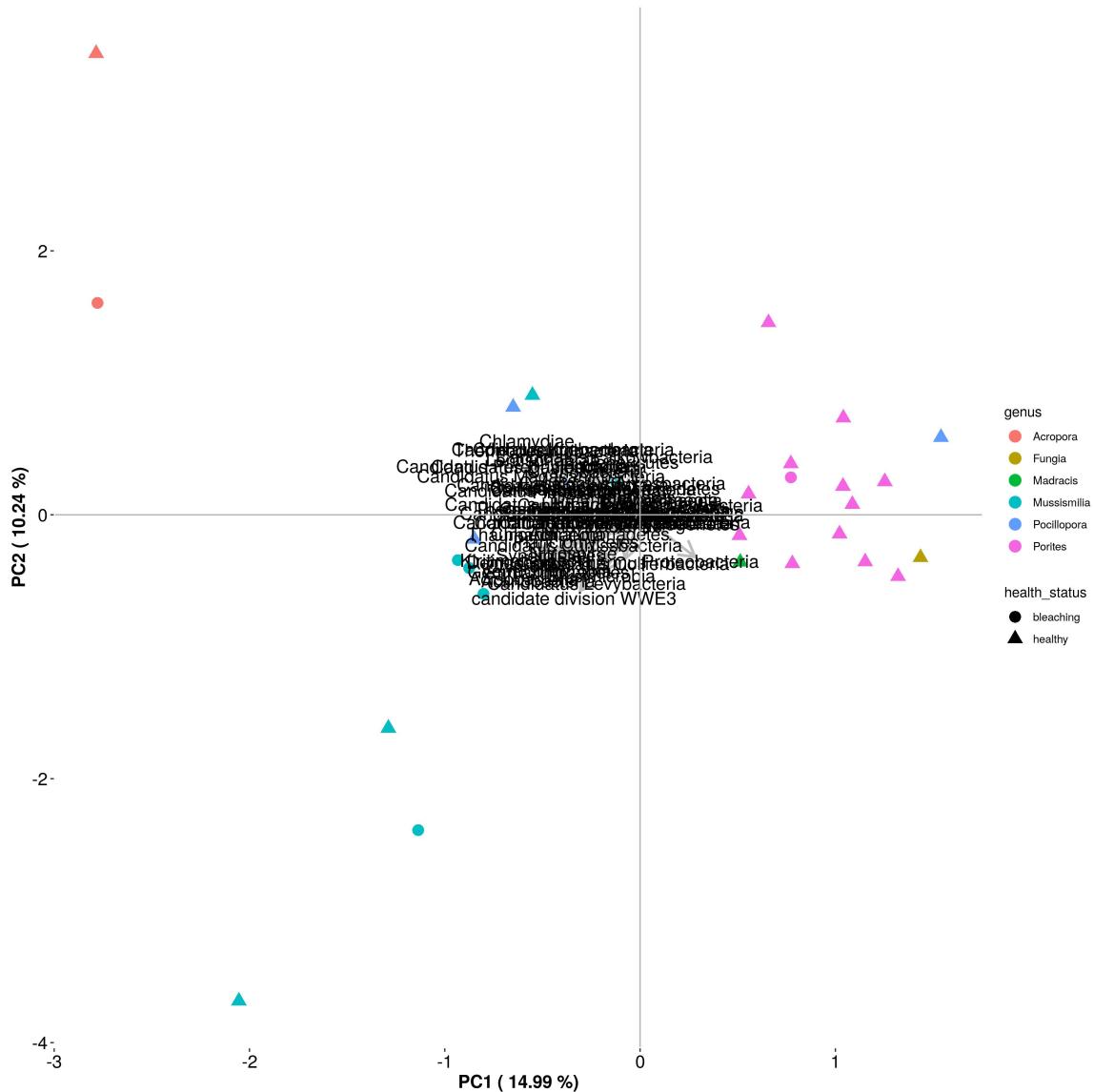


Figura 8.11: PCA das amostras de metagenomas do mg rast com todos os filos

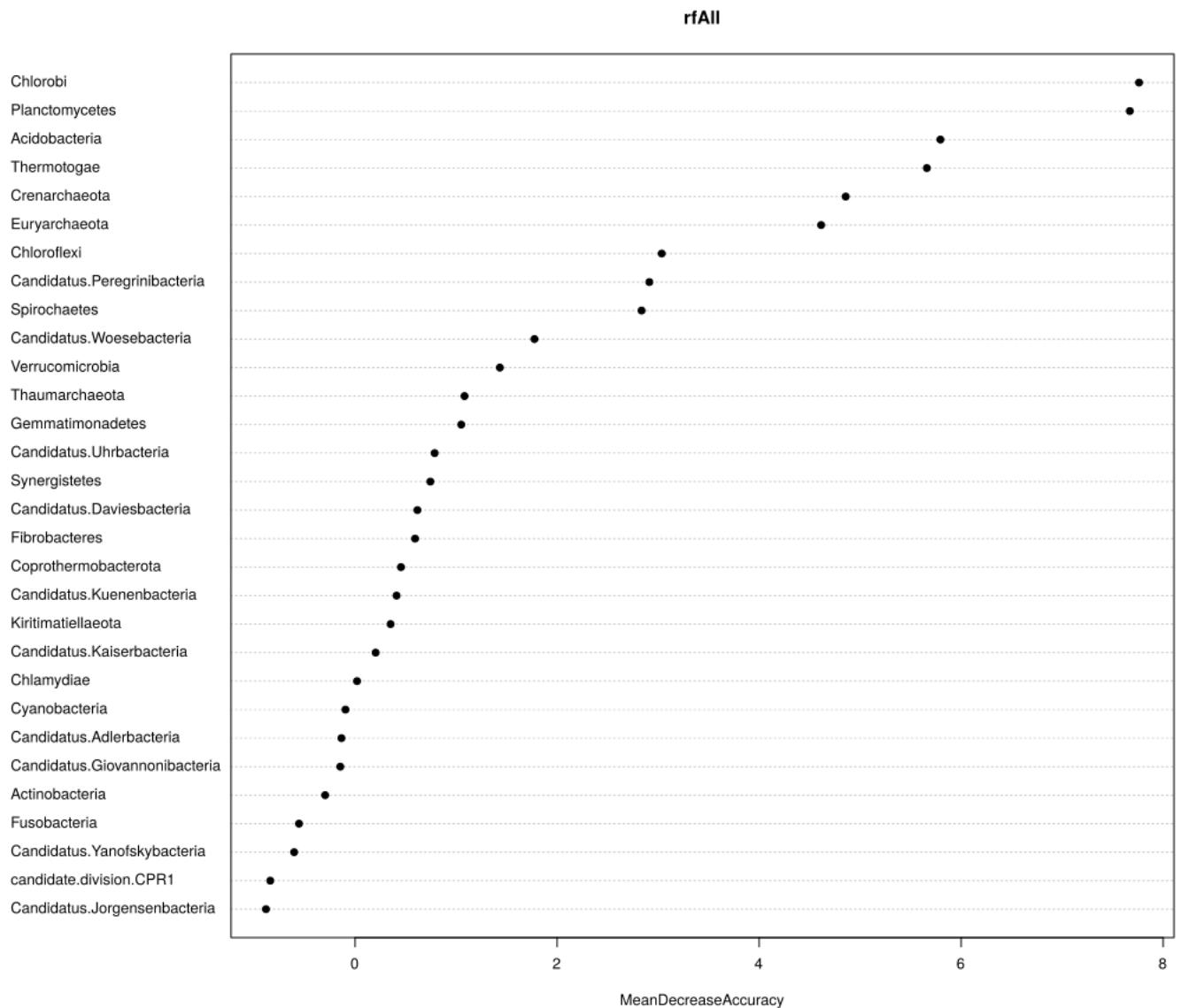


Figura 8.12: Random Forest no supervisionado dos metagenomas de corais do MG-RAST analisadas com a base definitiva

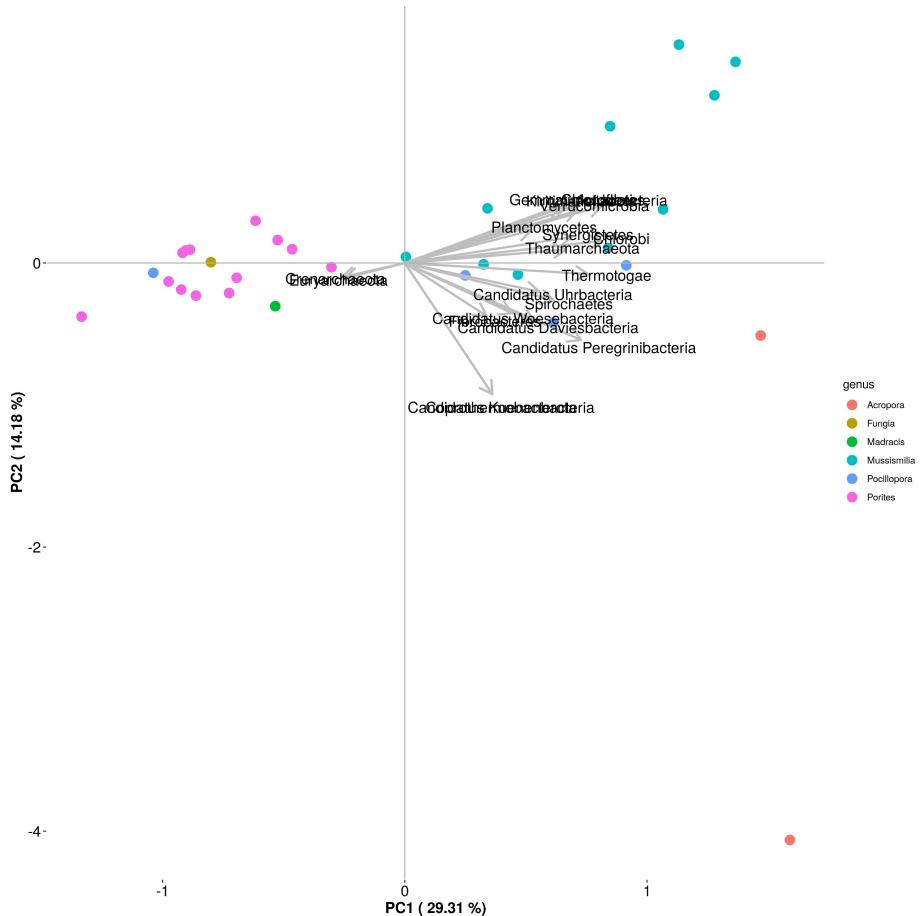


Figura 8.13: PCA GERADO COM OS PRIMEIROS 20 FILOS DO RANDOM FOREST NAO SUPERVISIONADO ACIMA

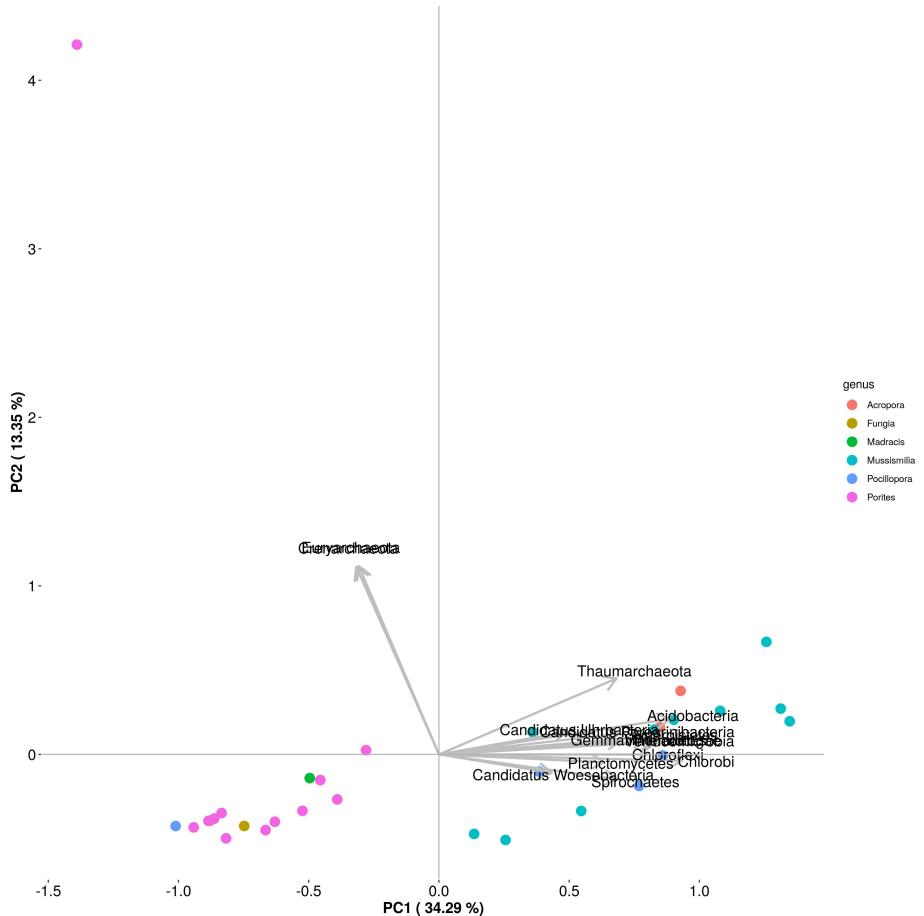


Figura 8.14: PCA GERADO COM OS PRIMEIROS 15 FILOS DO RANDOM FOREST NAO SUPERVISIONADO

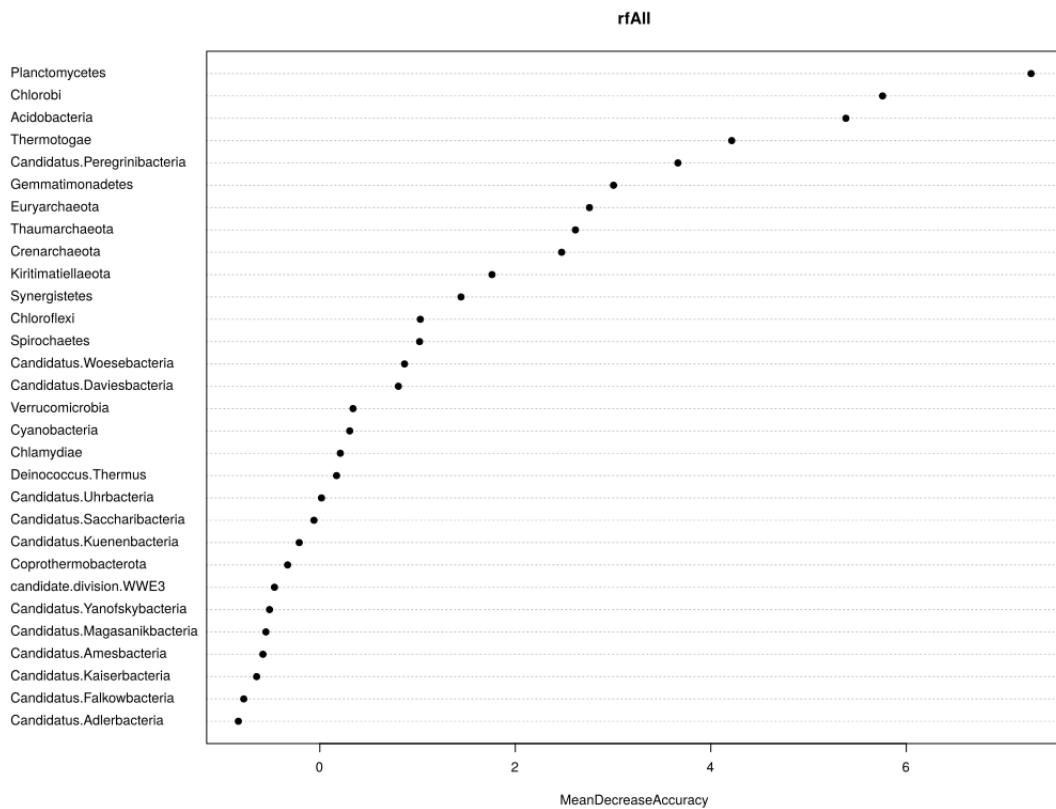


Figura 8.15: RANDOM FOREST SUPERVISIONADO POR GENERO DOS METAGENOMAS DE CORAIS DO MG RAST ANALISADOS COM A BASE DEFINITIVA

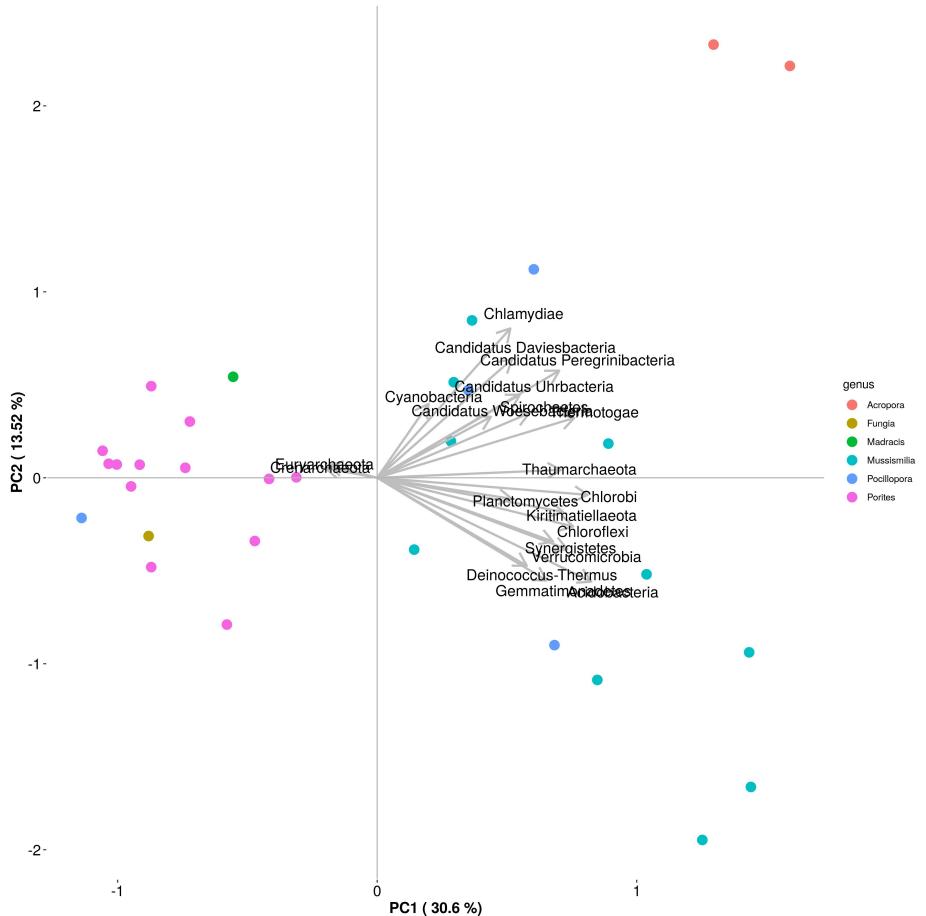


Figura 8.16: PCA GERADO COM OS PRIMEIROS 20 FILOS DO RANDOM FOREST SUPERVISIONADO POR GENERO

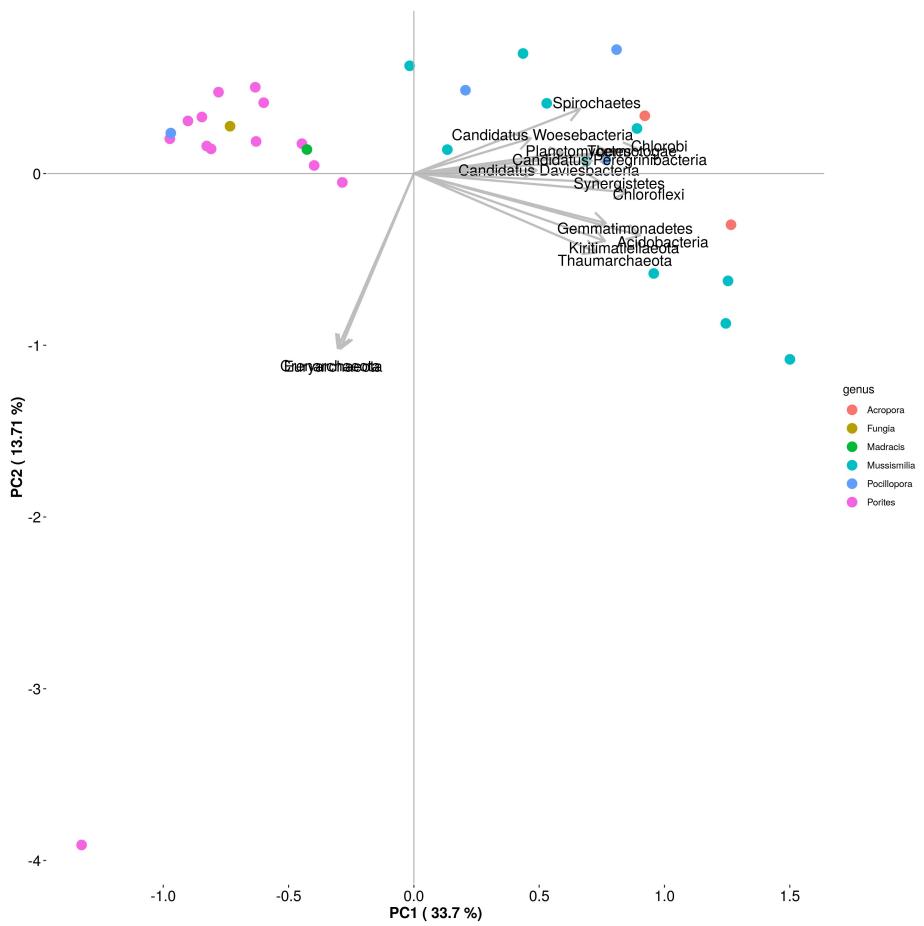


Figura 8.17: PCA GERADO COM OS PRIMEIROS 15 FILOS DO RANDOM FOREST SUPERVISIONADO POR GENERO

Capítulo 9

Functional annotation of metagenomes

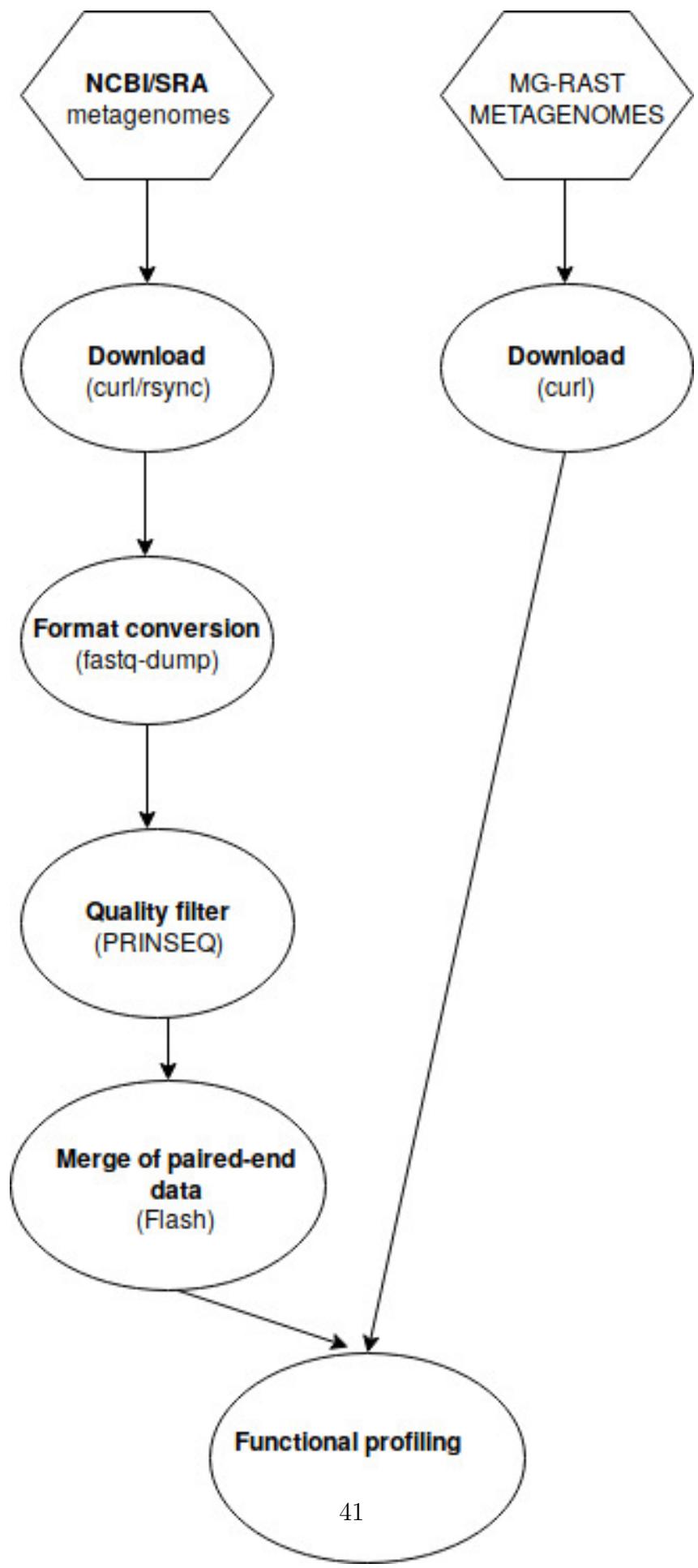


Figura 9.1: Pipeline of functional annotation

Capítulo 10

references

Articles list:

- 10.1371/journal.pone.0071301: Relata resultados que eu acreditava ter sido a primeira a encontrar
- 10.1038/nature14486: reconstruction of microorganism's genomes we use
- 10.1038/nm microbiol.2016.48: three of life, including the Candidate Phyla Radiation
- 10.1146/annurev.micro.57.030502.090759: speaks about the uncultured majority of microorganisms
- 10.1038/ismej.2016.174: revision of rare biosphere
- 10.1038/nrmicro3400: another revision of rare biosphere
- 10.1126/science.1224041: metabolic activities of *Candidatus Parcubacteria*, one of super-phyla of CPR
- 10.1128/MMBR.00009-08: Revision of bioinformatic methods and steps for metagenomic
- 10.1186/s40168-018-0428-1: Sponge as holobiont. Note: This article has a important information about microbial ecology: "Network and modeling analyses aim to disentangle the strength and nature (positive, negative, or neutral) of the interactions and predict their dynamics. Bacteria-bacteria network analysis of the core microbiota in different sponge species has revealed a low connective network with very few strong and many weak unidirectional interactions (i.e., amensalism [/0] and commensalism [+/0] prevailed over cooperation [+/+] and competition [/]. These findings are consistent with mathematical models that predict that weak and non-cooperative interactions help to stabilize highly diverse microbial communities, whereas cooperation yields instability in the long term by fueling positive feedbacks"
- 10.1016/j.tim.2009.09.004: Microbial disease and the coral holobiont
- 10.3389/fmicb.2017.00618: Comparative Metagenomics of the Polymicrobial Black Band Disease of Corals

- 10.1038/nrmicro1643: The role of ecological theory in microbial ecology
- 10.1038/nrmicro3218: Explaining microbial genomic diversity in light of evolutionary ecology
- 10.1111/j.1462-2920.2009.01935.x: Metagenomic analysis of stressed coral holobionts
- 10.1038/nature06810: Functional metagenomic profiling of nine biomes
- 10.3389/fcimb.2014.00176: Microbes in the coral holobiont: partners through evolution, development, and ecological interactions
- 10.1038/ismej.2015.39: The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts
- 10.1111/j.1462-2920.2007.01383.x: Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*
- 10.1038/nm microbiol.2015.32: Metagenomics uncovers gaps in amplicon-based detection of microbial diversity
- 10.1038/ismej.2016.45: Challenges in microbial ecology: building predictive understanding of community function and dynamics
- 10.1111/j.1462-2920.2009.02113.x: Microbial functional structure of *Montastraea faveolata*, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies
- 10.1111/j.1758-2229.2010.00234.x:
- 10.1038/ismej.2011.116: Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges

Capítulo 11

Softwares, instalacao e linhas

11.1 Profiling metagenomes

Capítulo 12

Fundamentos teoricos

Capítulo 13

meetings

Instalar o kraken-biome

- Folder: `/home/leticia`
- Command: `pip install kraken-biom`
- Site: <https://github.com/smdabdoub/kraken-biom>

Para atualizacao:

git commit

git push origin master

Para transferencia:

maquina remota para local: `scp leticia.cavalcante@login.sduumont.lncc.br:/scratch/ebiodiv/leticia.cavalcante@192.168.1.111:/home/leticia/Documentos/dados`

13.0.1 30 de outubro 2018

Na reuniao com Amaro, Miguel e o professor, mostrei os slides contendo os resultados obtidos com analises dos metagenomas utilizando a base de dados definitiva. Foram levantadas as seguintes questoes:

- As analises por familias no nos disseram muita coisa, alem de nao conter familias dos grupos nao cultivados por nao existir tal resolucao taxonomico. Por isso, utilizaremos apenas filo para trabalhar
- Fazer um PCA utilizando especies de coral sem identificar status de saude visualmente
- Fazer um PCA utilizando apenas amostras de corais saudaveis, identificando apenas genero

Tipo, se quero ver a diferenca existente entre as comunidades de generos de corais diferentes, devo fazer a analise s utilizando saudaveis em um caso e em outro sem identificar o estado de saude.

- Fazer um pca identificando apenas corais saudaveis e doentes, sem identificar visualmente o genero

- Fazer um pca utilizando apenas corais doentes
- utilizar mais filos indicados pelo random forest para fazer o pca
- PCA por especie
- Procurar por amostras de madracis que o professor trabalho - FEITO
- fazer uma planilha simplificada para Miguel e Amanda - FEITO (re-enviar a planilha, visto que encontrei as amostras e as incluirei na planilha de metagenomas)