### Lab Book

Cientific Iniciation - Coral Metagenomes Letcia Costa Cavalcante

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> > 2018

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## May 2018

#### 1.1 13

#### 1.1.1 Learning LATEX

- Working folder: path

LATEX is a high-quality typesetting system, available as free software, which allows to produce scientific or technical documents [?]. I am using LATEX 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. Alternatevily, 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 8.1).

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 &

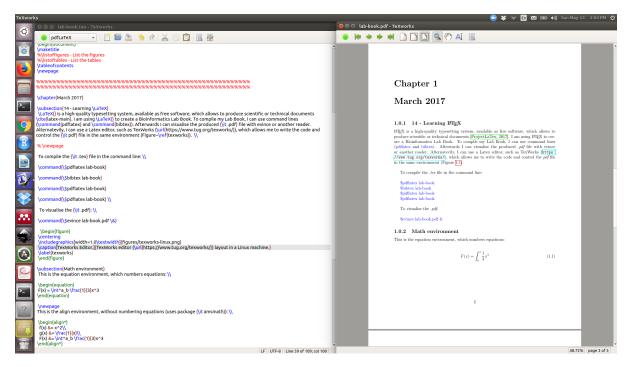


Figura 1.1: TexWorks editor (https://www.tug.org/texworks/) layout in a Linux machine.

#### 1.1.2 Math environment

This is the equation environment, which numbers equations:

$$F(x) = \int_{b}^{a} \frac{1}{3}x^{3} \tag{1.1}$$

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

$$f(x) = x^{2}$$

$$g(x) = \frac{1}{x}$$

$$F(x) = \int_{b}^{a} \frac{1}{3}x^{3}$$

#### 1.1.3 15 - Short-term project proposal

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

- $\bullet\,$  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

# 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
- $\bullet$  10.1073/pnas.0801980105
- 10.1111/1462-2920.13362
- 10.1126/science.1132690
- 10.1186/s40168-015-0077-6

Tabela 2.1: table 1

DOI	Phylum
10.1038/nature 14486	Candidatus Falkowbacteria
10.1038/nature 14486	Candidatus Kuenenbacteria
10.1038/nature 14486	Candidatus Magasanikbacteria
10.1038/nature 14486	Candidatus Uhrbacteria
10.1038/nature 14486	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 1000 / 40010	
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 Staskawiczbacteria
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
$10.1038 \rm /ncomms 13219$	Candidatus Yonathbacteria
$10.1038 \rm /ncomms 13219$	Candidatus Lloydbacteria
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
10.1038/mature14480 10.1073/pnas.0801980105	Candidatus Korarchaeota
10.1075/phas.0001930103 10.1038/nature12352	Candidatus Fervidibacteria
10.1038/nature12352 10.1038/nature12352	Candidatus Aenigmarchaeota
· ·	Candidatus Fermentibacteria
10.1038/ismej.2016.43	
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/nature 12352	Candidatus Hydrogenedentes
10.1038/nature 12352	Candidatus Acetothermia

10.1038/nature 12352
10.1038/ncomms13219
10.1186/s40168-015-0077-6
10.1038/nature 21031
10.1038/nature 21031
10 1038/nature21031

Candidatus Nanohaloarchaeota Candidatus Eisenbacteria candidate division WOR-3 Lokiarchaeota Odinarchaeota Heimdallarchaeota

#### 2.2 28

#### 2.2.1 Bibliographic search for metagenomes

The reserarch 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

mgm4694759.3mgm4694760.3SRR1275409 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

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

SRR5605611

SRR2937345 SRR2937346 SRR2937347 SRR2937348 SRR2937350 SRR2937351 SRR2937352 SRR2937353 SRR2937354 SRR2937355 SRR2937356

Espcie: Platygyra carnosa Healthy

I found other metagenomes of coral from article doi  $\ddot{1}0.3389/\text{fmars}.2018.00101\ddot{1}$  uptated 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.

## 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  $_{\dot{c}}$  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	$317.00 \; \mathrm{Mb}$	318M
SRR6785057	$364.00 \; \text{Mb}$	365M
SRR6785056	$560.00 \; \mathrm{Mb}$	561M
SRR6785055	624.00  Mb	625M

So I checked the others files:

A Bia me informou que o SDU arrendonda os valores de tamanho dos arquivos, entao at o momento nao tive problemas com o download dos arquivos do mg\_rast

Tabela 3.2: Comparing sizes of files 2

ID of of a reason a	the size in NCBI site	size of file in SDU	size of cleanned file
ID of metagenome	30M	29.1 MB	28M
mgm4440319.3.299.1			
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	$6{,}4M$	$6.4~\mathrm{MB}$	$6{,}4M$
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	$160.2~\mathrm{MB}$	163M
mgm4516694.3.299.1	193M	192.9 MB	193M
mgm4653307.3.299.1	17M	$16.0~\mathrm{MB}$	17M
mgm4694757.3.299.1	1,9G	1.8 GB	1.9G
mgm4694758.3.299.1	2,2G	$2.1~\mathrm{GB}$	$2{,}2G$
mgm4694759.3.299.1	1,7G	$1.7~\mathrm{GB}$	1,8G
mgm4694760.3.299.1	592M	1.6 GB	597M

# 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

# Quality filter

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

- trim\_qual\_left 25
- $\bullet$  trim\_qual\_right 25

## Uniformity filter (size and N bases)

#### 6.1 Command line

#### Parameters:

- min\_len 80
- $ns_max_p 2$
- out\_format 1
- Command: nohup bash slurm\_job\_prinseq\_single\_corais\_FASTA.bash & slurm\_prinseq\_corais.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

#### Ressubmeti o job com:

- Command: sbatch slurm\_job\_prinseq\_single\_corais\_FASTA.bash

Abholidate: Terminal\*\*

### 100-35-27.png

### 100-

Figura 6.1: Erro no job no SDU

## Profilling metagenomes

#### 7.1 Mg-Rast metagenomes

I used the following script in the following folder:

- Folder:  $scratch/ebiodiv/leticia.cavalcante/mg\_rast/filtered\_prinseq\_good$
- Command: sbatch slurm\_job\_kraken2\_corais.sh

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

Ressubmeti o job, modificando a localizaca<br/>o da DB do Kraken para a home do Rilquer. Numero do job<br/>:  $216410\,$ 

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

#### 7.2 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 fa um teste de stane "Crestien of DIOM table of abundance

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

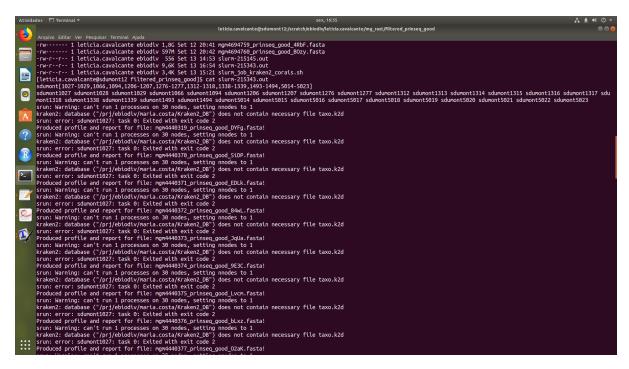


Figura 7.1: 20 erro no job no SDU

• perl filterRank.pl input table.from\_biom\_with\_taxonomy.txt -rank p ; abundance.matrix

#### 7.3 Teste com o kraken no scratch

Linha de teste:

perl select Groups.pl input mgm4440370\_prinseq\_good\_SiDP.fasta\_kraken.report –file\_groups groups.txt  $\+i$  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

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

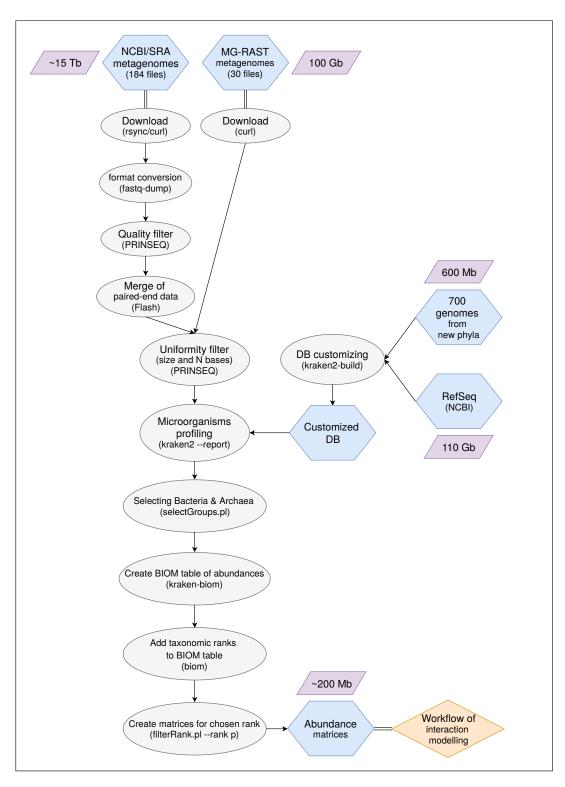


Figura 7.2: Pipeline of taxonomic annotation

# Functional annotation of metagenomes

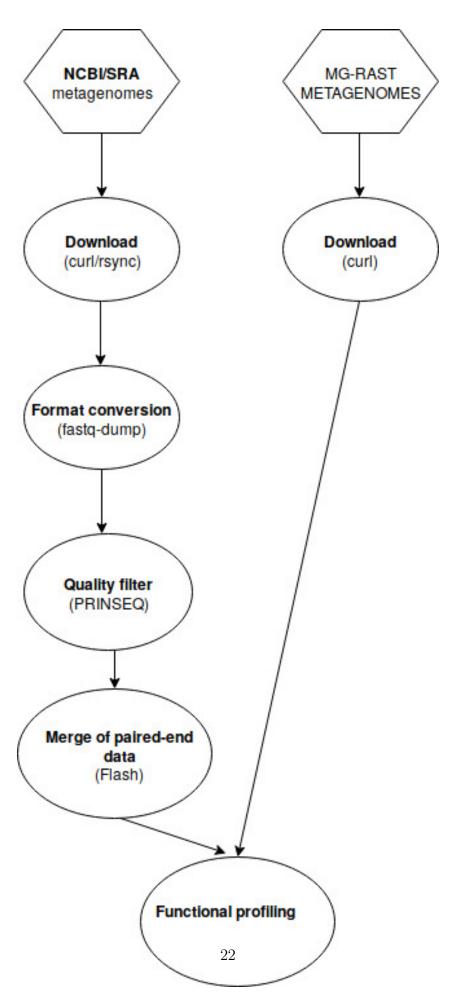


Figura 8.1: Pipeline of functional annotation

#### 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/nmicrobiol.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/nmicrobiol.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:

## Softwares, instalacao e linhas

#### 10.1 Profilling metagenomes

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:

 $maquina\ remota\ para\ local:\ scp\ leticia.cavalcante@login.sdumont.lncc.br:/scratch/ebiodiv/leticia.cavalcante@login.sdumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumonte.gr.dumon$ 

# Referências Bibliográficas