WGS Taxonomic binning

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Contents

1 Whole Genome Shotgun metagenomics: Taxonomic Binning Pipeline

We have two fastqc files to process: 1. *Microbiome1_200k* that contains 200,000 paired end reads from total DNA extracted from human saliva. So this is a microbiome. We tag the related information as **Bact**.

2. *Vir1_100k* that contains 100,000 paired end reads from the same saliva sample but after purification of viral particles. So this is a virome. We tag the related information as **Vir**.

1.1 Taxonomic composition

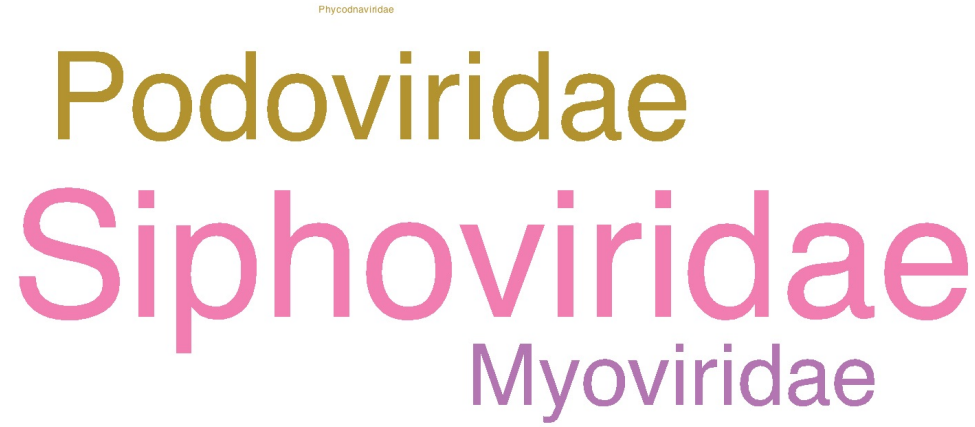
Vir_R1

Family	Abundance
Myoviridae	338.0
Podoviridae	823.0
Siphoviridae	5677.0
unclassified Caudovirales	11.0
Phycodnaviridae	9.0
unclassified archaeal dsDNA viruses	9.0
unclassified dsDNA phages	21.0
unclassified bacterial viruses	862.0
Not assigned	4375.0

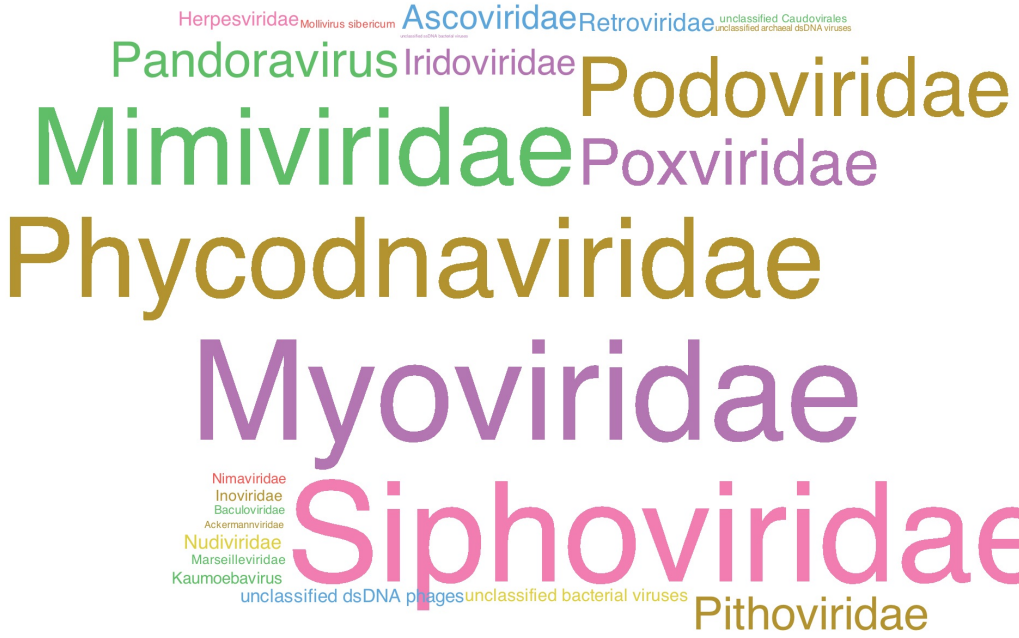
Bact_R1

Family	Abundance
Baculoviridae	1.0
Ackermannviridae	1.0
Myoviridae	343.0
Podoviridae	37.0
Siphoviridae	225.0
unclassified Caudovirales	3.0
Herpesviridae	3.0
Iridoviridae	8.0
Marseilleviridae	2.0
Mimiviridae	62.0
Nimaviridae	1.0
Nudiviridae	2.0
Phycodnaviridae	123.0
Poxviridae	18.0
unclassified archaeal dsDNA viruses	4.0
unclassified dsDNA phages	9.0
unclassified dsDNA viruses	28.0
Pandoravirus	15.0
Pithoviridae	13.0
Retroviridae	4.0
Inoviridae	1.0

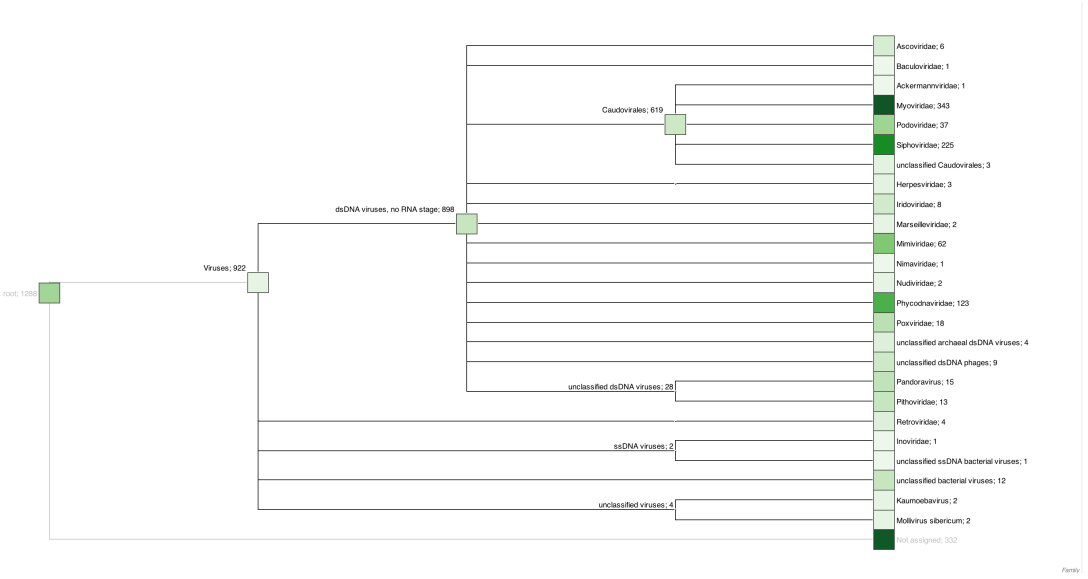
Family	Abundance
unclassified ssDNA bacterial viruses	1.0
unclassified bacterial viruses	12.0
unclassified viruses	4.0
Not assigned	332.0



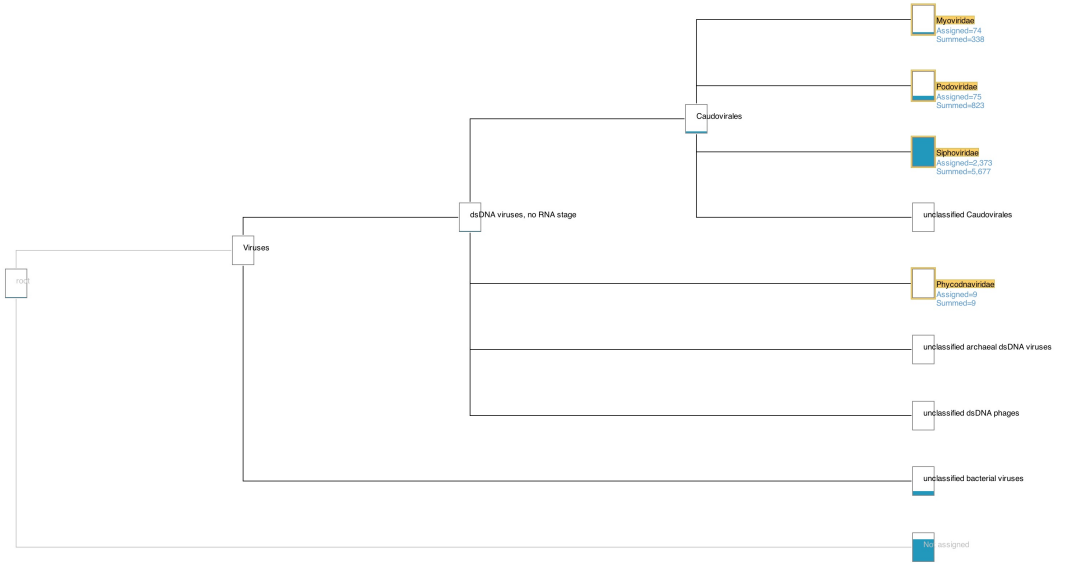
Vir_R1 graphical word cloud



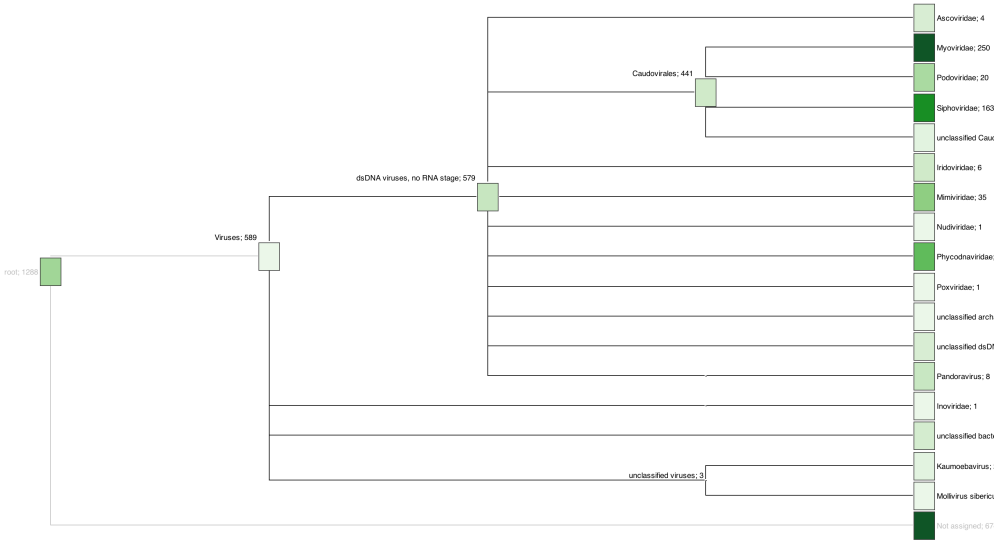
Bact_R1 graphical word cloud



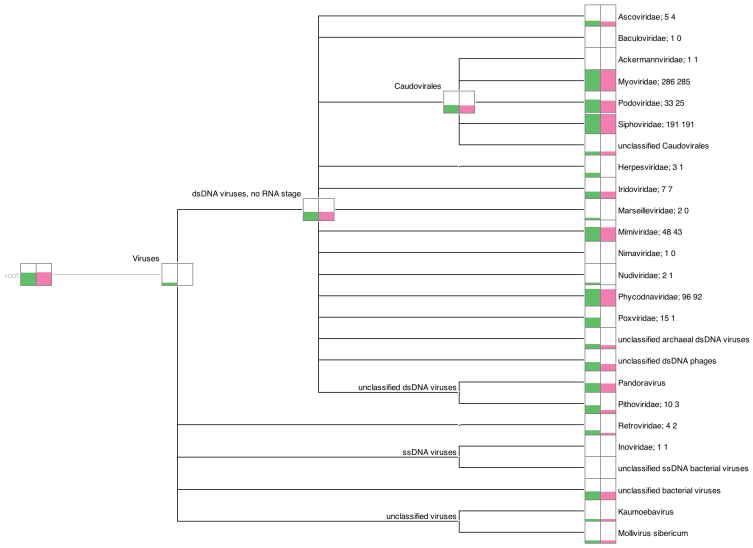
Bact_R1 MEGAN



Vir_R1 MEGAN



Bact_R1 paramater set 2 MEGAN



Comparison of MEGAN executions for Bact

1.2 Abstract

1.2.1 Pipeline comments

Sistema de ejecución

Hemos empleado la misma estrategia de la anterior práctica: lanzar los comandos bash desde un jupyter notebook, de forma que tenemos almacenadas las ejecuciones y las salidas en el mismo sitio donde documentamos. Además nos facilita las reejecuciones.

Hemos probado a obtener la estadísticas de los proceso automáticamente para generar desde la propia pipeline todos los datos solicitados, ya que las salidas de las ejecuciones pueden ser leidas y procesadas desde python y lo hemos conseguido en parte: los programas sin interfaz línea de comandos FASTQ y MEGAN6 no facilitan estas automatizaciones. *No obstante no hemos podido investigar con más atención este punto (no descartamos su inviabilidad).*

Parameters

At *kneaddata* we employed the trimming sliding window trimmomatic option: starts scanning at the 5-end and clips the read once the average quality within the window falls below a threshold. And also we adjust the *MINLEN* parameter: drop the read if it is below a specified length. We use a conservative length of 200 bp.

After decontamination and alignments only are retained the 4% of sequences of Bact_R1 and the 20% of Vir_R1 as we show in the reduction of the counters across the different steps:

Sample	Vir_R1	Bact_R1	Vir_R1(%)	Bact_R1(%)
raw single	100000	200000	100	100
trimmed single	63602	127652	63,60	63,83
decontaminaded Human and PhiX	63331	32358	63,33	16,18
final single	63331	32358	63,33	16,18
diamond vs viral protein db	12725	1288	20,09	3,98

1.2.2 Results comments

1.3 Pipeline

Script 1.3.1 (python)

```python
import warnings
warnings.filterwarnings('ignore')
import pandas as pd
```

1.3.1 Check files

Check the number of reads of our files and look at the header with grep and head commands

Script 1.3.2 (bash)

```
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
cd Documentos/Tema_4a
head -n 1 *fq
grep -c "@M02255" *fq
EOT
```

Output

```
==> Bact1_R1_200000.fq <==
@M02255:131:000000000-AJC6R:1:2102:25217:13392_1:N:0:ACAGTG

==> Bact1_R2_200000.fq <==
@M02255:131:000000000-AJC6R:1:2102:25217:13392_2:N:0:ACAGTG

==> Vir1_R1_100000.fq <==
@M02255:131:000000000-AJC6R:1:1105:23249:10170_1:N:0:AGTCAA

==> Vir1_R2_100000.fq <==
```

```
@M02255:131:000000000-AJC6R:1:1105:23249:10170_2:N:0:AGTCAA
Bact1_R1_200000.fq:200000
Bact1_R2_200000.fq:200000
Vir1_R1_100000.fq:100000
Vir1_R2_100000.fq:100000
```

1.3.2 Quality (fastqc)

Script 1.3.3 (bash)

```
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
export PATH=$PATH:/home/microbioinf/miniconda3/bin
cd Documentos/Tema_4a
fastqc Vir1_R1_100000.fq -o fastQC_output/
fastqc Vir1_R2_100000.fq -o fastQC_output/
fastqc Bact1_R1_200000.fq -o fastQC_output/
fastqc Bact1_R2_200000.fq -o fastQC_output/
EOT
```

Output

```
Analysis complete for Vir1_R1_100000.fq
Analysis complete for Vir1_R2_100000.fq
Analysis complete for Bact1_R1_200000.fq
Analysis complete for Bact1_R2_200000.fq
```

1.3.3 Trimming and decontaminating

Trimming poor quality ends and short sequences (**Trimmomatic**) and removal of reads aligning to the human and phiX174 genomes (***bowtie2**). The later one is a contaminant used as spike by Illumina kits to control quality of the sequencing process.

We are only filtering only R1 files because forward reads have usually better quality than reverse reads.

Processing

Script 1.3.4 (bash)

```
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
export PATH=$PATH:/home/microbioinf/miniconda3/bin
cd Documentos/Tema_4a
kneaddata -i Bact1_R1_200000.fq -o kneaddata_out \
-db /home/shared/bowtiedb/GRCh38_PhiX \
--trimmomatic /home/microbioinf/miniconda3/pkgs/trimmomatic-0.38-1/share/trimmomatic-0.38-1/
\
-t 2 --trimmomatic-options "SLIDINGWINDOW:4:20 MINLEN:200" \
--bowtie2-options " --sensitive" --remove-intermediate-output
```

```
EOT
```

Output

```
Initial number of reads ( /home/microbioinf/Documentos/Tema_4a/Bact1_R1_200000.fq ): 200000
Running Trimmomatic ...
Total reads after trimming ( /home/microbioinf/Documentos/Tema_4a/kneaddata_out/Bact1_R1_2000
↪ 00_kneaddata.trimmed.fastq ):
↪ 127652
Decontaminating ...
Running bowtie2 ...
Total reads after removing those found in reference database ( /home/microbioinf/Documentos/T
↪ ema_4a/kneaddata_out/Bact1_R1_200000_kneaddata_GRCh38_PhiX_bowtie2_clean.fastq ):
↪ 32358
Total reads after merging results from multiple databases (
↪ /home/microbioinf/Documentos/Tema_4a/kneaddata_out/Bact1_R1_200000_kneaddata.fastq ):
↪ 32358

Final output file created:
/home/microbioinf/Documentos/Tema_4a/kneaddata_out/Bact1_R1_200000_kneaddata.fastq
```

Script 1.3.5 (bash)

```
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
export PATH=$PATH:/home/microbioinf/miniconda3/bin
cd Documentos/Tema_4a
kneaddata -i Vir1_R1_100000.fq -o kneaddata_out \
-db /home/shared/bowtiedb/GRCh38_PhiX \
--trimmomatic /home/microbioinf/miniconda3/pkgs/trimmomatic-0.38-1/share/trimmomatic-0.38-1/
\
-t 2 --trimmomatic-options "SLIDINGWINDOW:4:20 MINLEN:200" \
--bowtie2-options " --sensitive" --remove-intermediate-output
EOT
```

Output

```
Initial number of reads ( /home/microbioinf/Documentos/Tema_4a/Vir1_R1_100000.fq ): 100000
Running Trimmomatic ...
Total reads after trimming (
↪ /home/microbioinf/Documentos/Tema_4a/kneaddata_out/Vir1_R1_100000_kneaddata.trimmed.fastq
↪ ): 63602
Decontaminating ...
Running bowtie2 ...
Total reads after removing those found in reference database ( /home/microbioinf/Documentos/T
↪ ema_4a/kneaddata_out/Vir1_R1_100000_kneaddata_GRCh38_PhiX_bowtie2_clean.fastq ):
↪ 63331
Total reads after merging results from multiple databases (
↪ /home/microbioinf/Documentos/Tema_4a/kneaddata_out/Vir1_R1_100000_kneaddata.fastq ): 63331
```

```
Final output file created:
/home/microbioinf/Documentos/Tema_4a/kneaddata_out/Vir1_R1_100000_kneaddata.fastq
```

Process statistics

Script 1.3.6 (bash)

```bash
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
export PATH=$PATH:/home/microbioinf/miniconda3/bin
cd Documentos/Tema_4a/kneaddata_out
kneaddata_read_count_table --input ./ --output kneaddata_read_counts.txt
EOT
```

Output

```
Reading log: ./Bact1_R1_200000_kneaddata.log
Reading log: ./Vir1_R1_100000_kneaddata.log
Read count table written: kneaddata_read_counts.txt
```

Script 1.3.7 (python)

```python
data = """
cat Documentos/Tema_4a/kneaddata_out/kneaddata_read_counts.txt
EOT
"""
output = !ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT' {data}

data = []
# To list of lists
for row in output:
    data.append(row.split('\t'))
# To dataframe
df = pd.DataFrame(data[1:], columns=data[0])
df.style.hide_index().set_properties(**{'text-align': 'right', 'font-family' : 'courier',
 ↪  'color' : 'darkgreen', "font-size" : "11pt"}).\
set_properties(**{'text-align': 'right', 'font-family' : 'courier', 'color' : 'darkblue',
 ↪  "font-size" : "12pt"}, subset=['Sample'])
```

Display output

```
<pandas.io.formats.style.Styler at 0x11bc43e48>
```

With grep we can identify the non-contaminated high-quality files

Script 1.3.8 (bash)

```bash
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
```

```
cd Documentos/Tema_4a/kneaddata_out
grep -c "@M02255:" *fastq
EOT
```

Output

```
Bact1_R1_200000_kneaddata.fastq:32358
Bact1_R1_200000_kneaddata_GRCh38_PhiX_bowtie2_contam.fastq:95294
Vir1_R1_100000_kneaddata.fastq:63331
Vir1_R1_100000_kneaddata_GRCh38_PhiX_bowtie2_contam.fastq:271
```

Check quality

Script 1.3.9 (bash)

```
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
cd Documentos/Tema_4a/kneaddata_out
mkdir fastQC_HighQuality
export PATH=$PATH:/home/microbioinf/miniconda3/bin
fastqc Bact1_R1_200000_kneaddata.fastq -o fastQC_HighQuality/
fastqc Vir1_R1_100000_kneaddata.fastq -o fastQC_HighQuality/
EOT
```

Output

```
Analysis complete for Bact1_R1_200000_kneaddata.fastq
Analysis complete for Vir1_R1_100000_kneaddata.fastq
```

1.3.4 Blastx comparison with a viral protein database

We are going to use a Refseq database of viral proteins (around 100Mb) from ncbi (ftp://ftp.ncbi.nlm.nih.gov/refseq/release/viral/), and you have to download it in two separated files that can be joined into one with cat.

Set up the reference database for diamond This will create a binary DIAMOND database file with the name: *viralprotein.dmnd*

Script 1.3.10 (bash)

```
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
cd Documentos/Tema_4a
export PATH=$PATH:/home/microbioinf/miniconda3/bin
diamond makedb --in viral.protein.faa -d viralprotein
EOT
```

Output

```
diamond v0.8.36.98 | by Benjamin Buchfink <buchfink@gmail.com>
Check http://github.com/bbuchfink/diamond for updates.

#CPU threads: 3
Database file: viral.protein.faa
Opening the database file...  [0.015813s]
Loading sequence data (0 sequences processed)...  [0.149662s]
Loading sequence data (100000 sequences processed)...  [0.142183s]
Loading sequence data (200000 sequences processed)...  [0.158145s]
Loading sequence data (300000 sequences processed)...  [0.040807s]
Writing trailer...  [0.003161s]
Closing the input file...  [4e-05s]
Closing the database file...  [0.061918s]
Processed 323029 sequences, 82978170 letters.
Total time = 0.572598s
```

Blastx alignments

Script 1.3.11 (bash)

```
%%bash
ssh microbioinf@192.168.56.101 2>/dev/null /bin/bash <<'EOT'
cd Documentos/Tema_4a
export PATH=$PATH:/home/microbioinf/miniconda3/bin
diamond blastx -d viralprotein.dmnd  -q kneaddata_out/Vir1_R1_100000_kneaddata.fastq -o
↪  Vir1HQ_Vs_viralprotein.m8
diamond blastx -d viralprotein.dmnd  -q kneaddata_out/Bact1_R1_200000_kneaddata.fastq -o
↪  Bact1HQ_Vs_viralprotein.m8
EOT
```

Output

```
diamond v0.8.36.98 | by Benjamin Buchfink <buchfink@gmail.com>
Check http://github.com/bbuchfink/diamond for updates.

#CPU threads: 3
Scoring parameters: (Matrix=BLOSUM62 Lambda=0.267 K=0.041 Penalties=11/1)
#Target sequences to report alignments for: 25
Temporary directory:
Opening the database...  [1.5e-05s]
Opening the input file...  [2.8e-05s]
Opening the output file...  [4.1e-05s]
Loading query sequences...  [0.235952s]
Running complexity filter...  [3.78898s]
Building query histograms...  [0.318931s]
Allocating buffers...  [6.2e-05s]
Loading reference sequences...  [0.107758s]
Building reference histograms...  [1.18488s]
Allocating buffers...  [6.1e-05s]
```

```
Initializing temporary storage...  [0.000619s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 0.
Building reference index...  [0.969958s]
Building query index...  [0.217957s]
Building seed filter...  [0.091635s]
Searching alignments...  [0.137262s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 1.
Building reference index...  [1.11468s]
Building query index...  [0.246178s]
Building seed filter...  [0.090143s]
Searching alignments...  [0.138724s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 2.
Building reference index...  [1.127s]
Building query index...  [0.249563s]
Building seed filter...  [0.109712s]
Searching alignments...  [0.183329s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 3.
Building reference index...  [1.26957s]
Building query index...  [0.370472s]
Building seed filter...  [0.150778s]
Searching alignments...  [0.22253s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 0.
Building reference index...  [1.34065s]
Building query index...  [0.265761s]
Building seed filter...  [0.113665s]
Searching alignments...  [0.429611s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 1.
Building reference index...  [1.4586s]
Building query index...  [0.295433s]
Building seed filter...  [0.120444s]
Searching alignments...  [0.16581s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 2.
Building reference index...  [1.45692s]
Building query index...  [0.302227s]
Building seed filter...  [0.110777s]
Searching alignments...  [0.159347s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 3.
Building reference index...  [1.3176s]
Building query index...  [0.278914s]
Building seed filter...  [0.115424s]
Searching alignments...  [0.165368s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 0.
Building reference index...  [1.33535s]
Building query index...  [0.246887s]
Building seed filter...  [0.117666s]
Searching alignments...  [0.147365s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 1.
Building reference index...  [1.31552s]
Building query index...  [0.204659s]
Building seed filter...  [0.087173s]
Searching alignments...  [0.110421s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 2.
```

```
Building reference index...  [1.10052s]
Building query index...  [0.21389s]
Building seed filter...  [0.086331s]
Searching alignments...  [0.109901s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 3.
Building reference index...  [0.938978s]
Building query index...  [0.179356s]
Building seed filter...  [0.085301s]
Searching alignments...  [0.109947s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 0.
Building reference index...  [0.920597s]
Building query index...  [0.171162s]
Building seed filter...  [0.085914s]
Searching alignments...  [0.101702s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 1.
Building reference index...  [1.04422s]
Building query index...  [0.199129s]
Building seed filter...  [0.084341s]
Searching alignments...  [0.106007s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 2.
Building reference index...  [1.0729s]
Building query index...  [0.204229s]
Building seed filter...  [0.085288s]
Searching alignments...  [0.127639s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 3.
Building reference index...  [0.903671s]
Building query index...  [0.167927s]
Building seed filter...  [0.089396s]
Searching alignments...  [0.105627s]
Deallocating buffers...  [0.009547s]
Computing alignments...  [1.84423s]
Deallocating reference...  [0.00306s]
Loading reference sequences...  [2.2e-05s]
Deallocating buffers...  [0.002337s]
Deallocating queries...  [0.002664s]
Loading query sequences...  [2.2e-05s]
Closing the input file...  [7e-06s]
Closing the output file...  [0.005769s]
Closing the database file...  [1.7e-05s]
Total time = 34.1638s
Reported 75459 pairwise alignments, 75459 HSSPs.
12725 queries aligned.
diamond v0.8.36.98 | by Benjamin Buchfink <buchfink@gmail.com>
Check http://github.com/bbuchfink/diamond for updates.

#CPU threads: 3
Scoring parameters: (Matrix=BLOSUM62 Lambda=0.267 K=0.041 Penalties=11/1)
#Target sequences to report alignments for: 25
Temporary directory:
Opening the database...  [1.5e-05s]
Opening the input file...  [2.8e-05s]
Opening the output file...  [7.2e-05s]
```

```
Loading query sequences...  [0.128431s]
Running complexity filter...  [1.89243s]
Building query histograms...  [0.160397s]
Allocating buffers...  [7.4e-05s]
Loading reference sequences...  [0.112703s]
Building reference histograms...  [1.18714s]
Allocating buffers...  [0.000131s]
Initializing temporary storage...  [0.000738s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 0.
Building reference index...  [0.973812s]
Building query index...  [0.118915s]
Building seed filter...  [0.085079s]
Searching alignments...  [0.088929s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 1.
Building reference index...  [1.2453s]
Building query index...  [0.13635s]
Building seed filter...  [0.087573s]
Searching alignments...  [0.096991s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 2.
Building reference index...  [1.17932s]
Building query index...  [0.136485s]
Building seed filter...  [0.08577s]
Searching alignments...  [0.087322s]
Processing query chunk 0, reference chunk 0, shape 0, index chunk 3.
Building reference index...  [0.960882s]
Building query index...  [0.116979s]
Building seed filter...  [0.08987s]
Searching alignments...  [0.086879s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 0.
Building reference index...  [0.947816s]
Building query index...  [0.125525s]
Building seed filter...  [0.089527s]
Searching alignments...  [0.081632s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 1.
Building reference index...  [1.09524s]
Building query index...  [0.129516s]
Building seed filter...  [0.088017s]
Searching alignments...  [0.080841s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 2.
Building reference index...  [1.11805s]
Building query index...  [0.12736s]
Building seed filter...  [0.086305s]
Searching alignments...  [0.083204s]
Processing query chunk 0, reference chunk 0, shape 1, index chunk 3.
Building reference index...  [0.932964s]
Building query index...  [0.109149s]
Building seed filter...  [0.082084s]
Searching alignments...  [0.080282s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 0.
Building reference index...  [0.93305s]
Building query index...  [0.101445s]
Building seed filter...  [0.081855s]
```

```
Searching alignments...  [0.07451s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 1.
Building reference index...  [1.04542s]
Building query index...  [0.114735s]
Building seed filter...  [0.081284s]
Searching alignments...  [0.074126s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 2.
Building reference index...  [1.08343s]
Building query index...  [0.124619s]
Building seed filter...  [0.082408s]
Searching alignments...  [0.069044s]
Processing query chunk 0, reference chunk 0, shape 2, index chunk 3.
Building reference index...  [0.901484s]
Building query index...  [0.104237s]
Building seed filter...  [0.081542s]
Searching alignments...  [0.070414s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 0.
Building reference index...  [0.907908s]
Building query index...  [0.094342s]
Building seed filter...  [0.079934s]
Searching alignments...  [0.070951s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 1.
Building reference index...  [1.05012s]
Building query index...  [0.109936s]
Building seed filter...  [0.082596s]
Searching alignments...  [0.07083s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 2.
Building reference index...  [1.09294s]
Building query index...  [0.117459s]
Building seed filter...  [0.083417s]
Searching alignments...  [0.073389s]
Processing query chunk 0, reference chunk 0, shape 3, index chunk 3.
Building reference index...  [0.929055s]
Building query index...  [0.099267s]
Building seed filter...  [0.082223s]
Searching alignments...  [0.069398s]
Deallocating buffers...  [0.010661s]
Computing alignments...  [0.215504s]
Deallocating reference...  [0.004664s]
Loading reference sequences...  [5.2e-05s]
Deallocating buffers...  [0.000984s]
Deallocating queries...  [0.001405s]
Loading query sequences...  [2.7e-05s]
Closing the input file...  [1.9e-05s]
Closing the output file...  [0.002279s]
Closing the database file...  [1.7e-05s]
Total time = 24.599s
Reported 6449 pairwise alignments, 6449 HSSPs.
1288 queries aligned.
```

Script 1.3.12 (bash)

```
%%bash --out output
ssh microbioinf@192.168.56.101 2>/dev/null  /bin/bash <<'EOT'
cd Documentos/Tema_4a
head -n 1 *fq
grep -c "@M02255" *fq
EOT
```

Script 1.3.13 (python)

```
print(output)
```

Output

```
==> Bact1_R1_200000.fq <==
@M02255:131:000000000-AJC6R:1:2102:25217:13392_1:N:0:ACAGTG

==> Bact1_R2_200000.fq <==
@M02255:131:000000000-AJC6R:1:2102:25217:13392_2:N:0:ACAGTG

==> Vir1_R1_100000.fq <==
@M02255:131:000000000-AJC6R:1:1105:23249:10170_1:N:0:AGTCAA

==> Vir1_R2_100000.fq <==
@M02255:131:000000000-AJC6R:1:1105:23249:10170_2:N:0:AGTCAA
Bact1_R1_200000.fq:200000
Bact1_R2_200000.fq:200000
Vir1_R1_100000.fq:100000
Vir1_R2_100000.fq:100000
```

Script 1.3.14 (python)

```
alist = output.split('\n')
alist
```

Display output

```
['==> Bact1_R1_200000.fq <==',
 '@M02255:131:000000000-AJC6R:1:2102:25217:13392_1:N:0:ACAGTG',
 '',
 '==> Bact1_R2_200000.fq <==',
 '@M02255:131:000000000-AJC6R:1:2102:25217:13392_2:N:0:ACAGTG',
 '',
 '==> Vir1_R1_100000.fq <==',
 '@M02255:131:000000000-AJC6R:1:1105:23249:10170_1:N:0:AGTCAA',
 '',
 '==> Vir1_R2_100000.fq <==',
 '@M02255:131:000000000-AJC6R:1:1105:23249:10170_2:N:0:AGTCAA',
 'Bact1_R1_200000.fq:200000',
```

```
'Bact1_R2_200000.fq:200000',
'Vir1_R1_100000.fq:100000',
'Vir1_R2_100000.fq:100000',
'']
```