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MultiQuas (*Multi*ple reference *quasi*species reconstruction protocol) V.2

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ABSTRACT

The following protocol summarizes the major steps to run the MultiQuas pipeline to evaluate viral variability and reconstruct the viral quasispecies from NGS data (particularly Miseq reads). It is based on the assumption that 1 o more known references are available. These references could be obtained using other haplotype reconstruction softwares. Nonetheless, it is recommended that only a few trusted references are used.

PROTOCOL CITATION

Marco Cacciabue 2021. MultiQuas (Multiple reference quasispecies reconstruction protocol). **protocols.io** https://protocols.io/view/multiquas-multiple-reference-quasispecies-reconstr-bxprpmm6
Version created by Marco Cacciabue

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MATERIALS TEXT

QuRe

source by Mattia C. F. Prosperi

FastQC 0.11.9

by Simon Andrews

Align_to_references.sh

```
#!/bin/bash


start=`date +%s`


bbduk.sh in1=$2 out1=reads_1.fq in2=$3 out2=reads_2.fq ref=
[path/to/bbmap/instalation]/bbmap/resources/adapters.fa ktrim=r k=23 mink=11
hdist=1 tpe tbo qtrim=rl trimq=20 minlen=50 maq=20


bowtie2-build $1 VFAref

bowtie2 --no-discordant --no-mixed -p $4 -x VFAref -1 reads_1.fq -2 reads_2.fq |
samtools view -@ 4 -bT $1 - > SAMPLE.bam


samtools sort -@ 4 -m 2G SAMPLE.bam > SAMPLE_sorted.bam

samtools view -@ 4 -h -F 4 -b SAMPLE_sorted.bam > SAMPLE_map.bam

samtools index -@ 4 SAMPLE_map.bam SAMPLE_map.bai

samtools depth -d10000000 SAMPLE_map.bam > coverage.txt




lofreq viterbi -f $1 -o SAMPLE_map_viterbi.bam SAMPLE_map.bam



samtools sort  -@ 4 -m 2G SAMPLE_map_viterbi.bam >
SAMPLE_map_viterbi_sorted.bam

samtools index -@ 4 SAMPLE_map_viterbi_sorted.bam
SAMPLE_map_viterbi_sorted.bai


lofreq indelqual --dindel -f $1 -o SAMPLE_map_viterbi_sorted_indels.bam
SAMPLE_map_viterbi_sorted.bam

samtools index -@ 4 SAMPLE_map_viterbi_sorted_indels.bam
SAMPLE_map_viterbi_sorted_indels.bai

lofreq call-parallel --pp-threads $4 --call-indels --use-orphan  -f $1
SAMPLE_map_viterbi_sorted_indels.bam -o variants.vcf


end=`date +%s`
echo Execution time was `expr $end - $start` seconds.
```

bbduk
source by Brian Bushnell

samtools 1.12
source

bcftools 1.12
source

Bowtie2 2.4.4
source

DISCLAIMER:

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Brief pipeline description

1 Reads are trimmed and filtered using

bbduk
source by Brian Bushnell

2 Filtered and trimmed reads are aligned to a set of user-defined references (multifasta format) with

Bowtie2 2.4.4
source

3 Reads are then split into different classes (one for each reference and one for the unmapped reads) using

SAMtools 1.8
Linux
source by Wellcome Trust Sanger Institute

4 For each class, reads are merged using

PEAR - Paired-End reAd mergeR
source by Alexandros Stamatakis

and then haplotypes are reconstructed with

QuRe
source by Mattia C. F. Prosperi

Important note: If the time limit is reached this step is repeated with a subset of the reads in order to reduce computation time and resources required by QuRe. Steps include 0.99, 0.9, 0.8, 0.7, 0.6 and 0.5 proportions of the toal reads for each class in that order.

5 Next, the proportions of each haplotype class (predicted by QuRe) are adjusted to reflect the number of reads of the corresponding class.

6 All reconstructed haplotypes are aligned to the first reference in the multifasta file using

mafft 7.487
source by Kazutaka Katoh

7 Additionally, reads are then aligned to the first reference in the multifasta file. Single Nucleotide Variants (SNVs) are called using

Lofreq 2
source by Andreas Wilm

8 Finally, concordance between the SNVs (expected minor allele frequency) from the predicted quasispecies and the Lofreq variants using an in-house R script. Higher value of R-squared indicates a better quasispecies reconstruction. Important: this step does not mean validation ot the obtained results, but it allows the user to choose between different haplotypes reconstructions.

Installing docker

9 In order to run the pipeline, a wrapper file is available (bash) which automatically perfoms all the above numbered steps. A docker image is available that includes all the necessary dependencies. If you do not yet have docker installed, do so at this time, and ensure that is in your PATH. For more information please visit https://www.docker.com/get-started

10 The docker image ("rm") is available at Docker hub
To pull the image, use the command below:

docker pull

```
docker pull cacciabue/multiquas:latest
```

This will download and install the corresponding docker image. Only has to be run the first time (it may take several minutes depending on your internet connection)

10.1 Alternatively, If you don't want to use Docker, you can install all dependencies by yourself (only for linux users). The dependencies are:

- BCFtools v1.8 (or later version) http://www.htslib.org/download/
- Samtools v1.8 (or later version) http://www.htslib.org/download/
- Bowtie2 v2.2.4 http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
- PEAR https://cme.h-its.org/exelixis/web/software/pear/doc.html
- seqtk https://github.com/lh3/seqtk
- bbmap https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/
- Lofreq v2 https://csb5.github.io/lofreq/
- mafft https://mafft.cbrc.jp/alignment/software/
- R v4.1 (or later version) https://www.r-project.org/
- R package seqinr https://cran.r-project.org/web/packages/seqinr/index.html
- R package ape https://cran.r-project.org/web/packages/ape/
- R package VariantAnnotation https://bioconductor.org/packages/release/bioc/html/VariantAnnotation.html

- R package Biostrings https://bioconductor.org/packages/release/bioc/html/Biostrings.html
- R package ggplot2 https://ggplot2.tidyverse.org/
- R package ggrepel https://cran.r-project.org/web/packages/ggrepel/vignettes/ggrepel.html

and download the following file (bash script)

AGREGAR ARCHIVO O QUITAR!!!!!!

and the two following R script should also be downloaded

11 Depending on your operating system follow these steps

11.1 On windows: open a windows terminal (WIN + R), type "cmd" and press enter. A Windows terminal should be up and running.

11.2 Navigate to the folder containing the fastq files and the reference file.