Installation of the programs BWA 0.7.17 and Samtools 1.9 and Mapping, extraction and analysis of the results.

\* The symbol ## stands for: Important comments

\*\* The symbol> means that there is a command line to be executed.

## The genomes will be downloaded through the online platform, SRA EXPLORER (https://sra-explorer.info/#).

## The download will generate two files in the .fastq.gz format. that corresponds to reads F (Foward) and R (Reverse).

**Board** **1: Commands to download and install BWA 0.7.17 (Burrows-Wheeler Aligner)**

* wget https://sourceforge.net/projects/bio-bwa/files/bwa-0.7.17.tar.bz2

## *Extract BWA files*

* tar -xjvf bwa-0.7.17.tar.bz2

## *Install the software*

* make

**Board**  **2: Download and install Samtools 1.9 (Sequence Alignment / Map)**

* wget https://sourceforge.net/projects/samtools/files/samtools/1.9/samtools-1.9.tar.bz2

*## Unzip file*

* tar -xjvf samtools-1.9.tar.bz2
* configure
* make
* make install

**Board 3: Sequence mapping, using BWA 0.7.17 (Burrows-Wheeler Aligner)**

##*Construction of the index (sequences / genomes of interest for the search) in the format.fasta***.**

* bwa seqs\_index.fasta

*##* *Sequence mapping*

* bwa mem seqs\_index.fasta SRA\_1.fastq SRA\_2.fastq > Anuran\_especies\_mapping.sam

\*\*\* The “seqs\_index.fasta” file can be renamed, as well as the “Anuran\_especies” file

**Board 4: Conversion, manipulation and data extraction, through Samtools 1.9**

*## Convert the .sam (heavier) file to .bam (lighter)*

* samtools view -bS Anuran\_especies\_mapping.sam> Anuran\_especies\_mapping.bam

*## Sort the alignment file*

* samtools sort Anuran\_especies\_mapping.bam > Anuran\_especies\_mapping\_sorted.bam

*##* *Extract reads that have been mapped*

* samtools view -b -F 4 Anuran\_especies\_mapping\_sorted.bam > Anuran\_especies \_mapping\_sorted\_mapped.bam

*##* *To view the results, it is necessary to create a file for comparison*

* samtools index Anuran\_especies\_mapping\_sorted\_mapped.bam > Anuran\_especies \_mapping\_sorted\_mapped.bam.bai

**Board 5: Collect files in .CSV format through Samtools 1.9**

* samtools stats Anuran\_especies\_mapping.bam > Anuran\_especies\_stats.csv
* samtools flagstats Anuran\_especies\_mapping.bam > Anuran\_especies\_flagstats.csv