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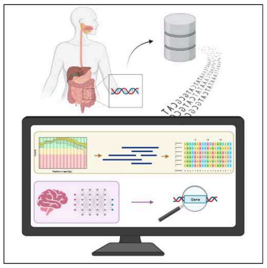
Biomarker's detection for diseases associated with metabolic disorder syndrome



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We use this protocol and it's working

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Abstract

The metabolic syndrome (MetS) is known to substantially reduce the quality of life. MetS is associated with a high incidence of non-communicable diseases such as type 2 diabetes mellitus, cardiovascular diseases, cancer, among others. Multiple investigations focus the early diagnosis of MetS and its possible evolution in the patient on the basis of gene expression and clinical parameters.

However, we are interested in supporting the clinical diagnosis and prognosis of MetS-associated diseases based on the gut microbiota. Which means that we will take into account the set of microorganisms (bacteria, fungi, archaea, viruses and parasites) that reside in the intestine, given their relationship with diseases such as obesity, type 2 diabetes, as well as its influence on control glycemic.

Beyond of traditional diagnostic methods, Machine Learning (ML) can learn non-linear interactions iteratively from large amounts of data. This is possible using computer algorithms, which are already being applied in various fields, including the evaluation and prediction of disease risk.

The genes analysis belonging to the intestinal microbiota would allow the identification of excretory proteins with biomarker potential for the diagnosis and prognosis of diabetes and metabolic syndrome using supervised Machine Learning algorithms. For this reason, this project seeks to create a “pipeline” of classification algorithms (set of concatenated software) for data mining and analysis that allows predicting the appearance of type 2 diabetes and the progression of complications based on in the gut microbiota.

Guidelines

Main objectives:

1. Analyze the intestinal microbiota of a patient.
   ~ Assembly of the intestinal genome.
   ~ Identification of the bacteria to which each genome belongs.
1. Identify if the biomarkers that allow diagnosis and prognosis of diabetes and metabolic syndrome are present.
2. Create a map that allows the identification of genes associated with microorganisms that may be involved in susceptibility or resistance to MetS-produced diseases.
3. Learn automatically from the identified biomarkers to improve the diagnosis and prognosis of type 2 diabetes and obesity.

Experimental design:

Milestone 1: Access and download of the public databases in Amazon S3, of experiments of the intestinal microbiota associated with patients with type 2 diabetes. (1192 databases of between 4 and 12 Giga Bytes each).

- Download the SRA Toolkit
  ~ SRA official website: https://www.ncbi.nlm.nih.gov/sra
  ~ Anaconda: https://youtu.be/Q4jbA2UEP44
  ~ Github: https://github.com/ncbi/sra-tools
- Extract the FASTQ type files from the SRA access
  ~ “prefetch”: download the SRA files
  ~ “fasterq-dump”: extracts the FASTQ file

Milestone 2: Genome assembly from single-cell and multi-cell bacterial data. Being double read (forward & reverse), the assembly can generate up to four times the size of the original databases.

- Prepare the environment for experimentation.
- There are two methods for reading
  ~ Simple ending
  ~ Double ended
- Read the sequences.
- Extract the FASTQ file.

Milestone 3: Comparative analysis of the sequences obtained in milestone 2 with the sequences of each bacterium (BLAST).

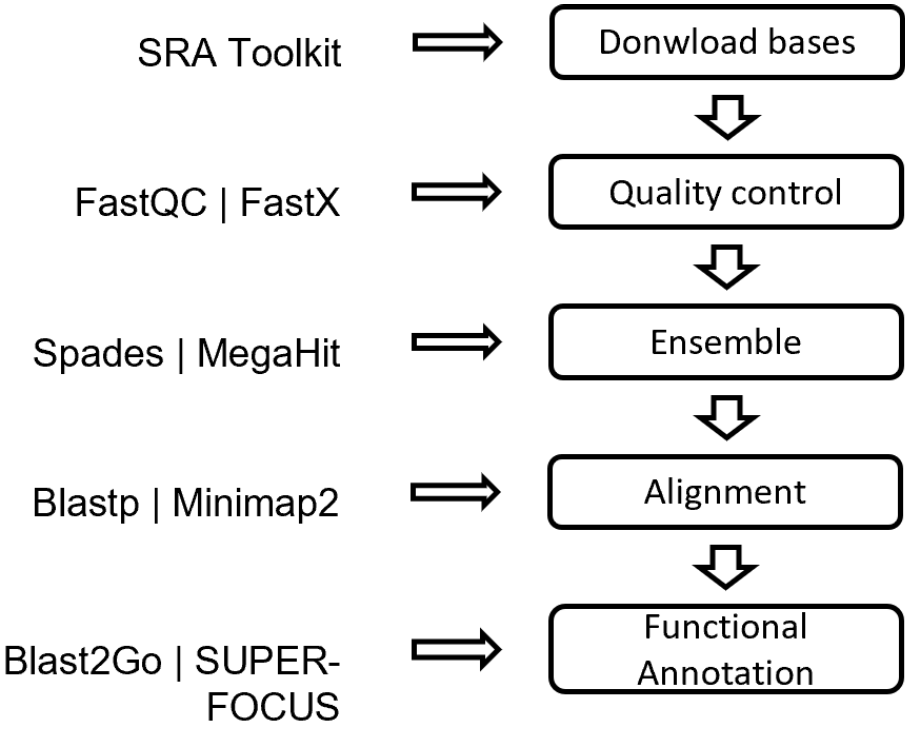
- Download and configure BLAST+
- Download the BLAST BDs from:
  a) **https://blast.ncbi.nlm.nih.gov/**
  b) https://ftp.ncbi.nlm.nih.gov/blast/db/.
- Perform the search with the filtered database

Milestone 4: Identify and segment genes with known functions and create a map that identifies them.

Troubleshooting

Before start

To facilitate bioinformatics processing in each step of the process, a group of specific tools are necessary:



Pipeline of bioinformatics tools

Download public databases

1 The SRA (Sequence Read Archive) is the standard format in which all NGS data is uploades into NCBI. To download and convert SRA files into FASTQ, download SRA Toolkit

Software

SRA-Toolkit	NAME
NCBIA	DEVELOPER
Github	SOURCE LINK

2 Prepare the SRA-Toolkit workspace. For this step it is necessary to be located in the destination folder.

Command

Set the default folder to download sequences (Linux)

Download space

```
vdb-config --prefetch-to-cwd
```

Command

new command name

```
vdb-config --interactive
```

3 **Access and download public databases. In this case, a database from the human gut metagenome in Amazon S3 was used. "The gut microbiome related effect of Berberine and probiotics in treating Type 2 Diabetes" (NCBI Accession number PRJNA643353) is a database with 1192 datasets (4-12 GBs each) in consecutive order from SAMN15421765 to SAMN1522956 of experiments. The data were obtained from a randomized, double-blind, placebo-controlled trial on newly diagnosed type 2 diabetes patients from 20 centers in China where 409 patients were randomly assigned to receive BBR, probiotic with BBR, probiotics, or placebo for 3 months.**

Dataset

Human gut metagenome database NAME

https://www.ncbi.nlm.nih.gov/bioproject/PRJNA643353 LINK

3.1 Use prefetch command followed by the number of the run from the desired experiment to download and create a folder with the archive in .sra format through SRA Toolkit. In this case, the BioSample SAMN15421765 is being used, which Run number is SRR12234739

Command

Downloads and creates a folder with the archive in .sra

prefetch

```
prefetch SRR12234739
```

4 Extract FASTQ files from SRA access with fasterq-dump.

Command

Extract FASTQ files from SRA access

fasterq-dump

```
fasterq-dump SRR12234739 --split-files --skip-technical
```

Quality control

5

NGS data can be affected by multiple reasons during the library preparations or the sequencing process, which can negatively impact the quality of the raw data. To perform quality control of the raw data download, download FASTX-Toolkit

Software

FASTX-Toolkit	NAME
Hannon Lab	DEVELOPER
Hannon Lab	SOURCE LINK

6 Clean the sequences based on quality and size. Since there is no established consensus on the value these parameters should have, a value = >30 is assumed to determine good sequences.

Command

Removes sequences of low quality

fastq_quality_trimmer

```
fastq_quality_trimmer -t 30 -l 30 -v -i "$SRR12234739_1.fastq" -o
"$SRR12234739 _1_trimmed.fastq"
```

Command

Removes sequences of low quality

fastq_quality_trimmer

```
fastq_quality_trimmer -t 30 -l 30 -v -i "$SRR12234739_2.fastq" -o
"$SRR12234739 _2_trimmed.fastq"
```

Genome Assembly

7 Sequence reads from NGS consist of small genetic sequences much shorter than genomes and even genes. Thus, the assembly of these short sequences into larger sequences (contigs) is necessary. To perform the genome assembly of the reads, download Spades.

Software

Spades	NAME
Center for Algorithmic Biotechnology	DEVELOPER
CAB	SOURCE LINK

8 Read files with forward and reverse reads using -1 and -2 respectively

Command

Assembly

Assembly

```
spades.py -t 40 -m 160 -1 "$SRR12234739_1.fastq" -2
"$SRR12234739_2.fastq" --only-assembler -o ensemble
```

Genome alignment

9 After the assembly, a reference genome is used to further piece together the sequenced data. Install BLAST setup for Unix to perform the Genome alignment

Software

BLAST	NAME
NCBI	DEVELOPER
NCBI	SOURCE LINK

10 Download the reference databases (if necessary)

Command

Database download

Database download

```
$ perl ../bin/update_blastdb.pl --passive --decompress
16S_ribosomal_RNA
```

11 Execute BLAST for nucleotides alignment

Command

blastn

blastn

```
blastn -query "$SRR12234739_contigs.fasta" -db "${DB}" -out
"$SRR12234739_result.out" -outfmt 6 -num_threads 40 &
```

Functional annotation

12 Now it is necessary to determine the biological function of the sequenced data. Install Prokka to perform functional annotation of the data.

Software

Prokka	NAME
Torsten Seemann	DEVELOPER
Github	SOURCE LINK

13 Perform functional annotation

Command

prokka

prokka

```
prokka contigs.fasta --addgenes --mincontiglen 200 --centre Prokka --
mincontiglen 200 --kingdom Bacteria --gcode 10 --evalue 1e-06 --cpus 0
```