Bismark methylation call

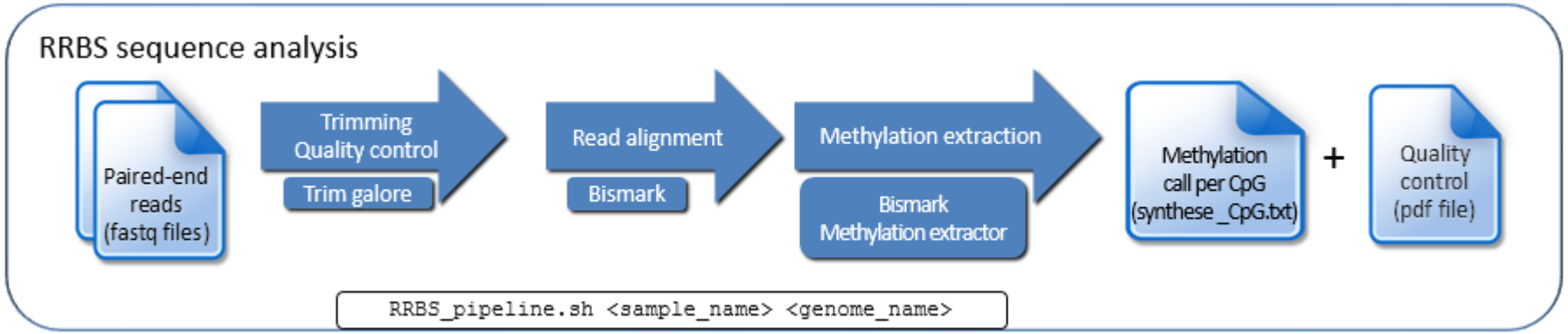
This process is the first step of RRBS analysis.
It takes one or two fastq files per sample depending if sequencing process has been done single end or paired end.

Sequences are first trimmed and filtered using trim_galore.

Then mapping is performed by Bismark and bowtie v1.

Methylation percentage and coverage are extracted from alignement files.

Finally, a quality control files is produced.



Prerequisites

Before to analyze any biological samples, you first need to prepare the genome for Bismark mappig process.

For this purpose :

1. In a dedicated directory, download from ensembl web site the genome of your choice
   (by instance : ftp://ftp.ensembl.org/pub/release-86/fasta/bos_taurus/dna/Bos_taurus.UMD3.1.dna.toplevel.fa.gz)
2. Uncompress the files you have downloaded (`gunzip *gz`)
3. Launch genome preparation :

   ```
   RRBS_HOME/Bismark_methylation_call/bismark_genome_preparation_bowtie1.sh <genome directory>
   ```

   `<genome directory>` is '.' if you are located in the directory where genome fasta files are stored.

   This preparation takes a while ! Be patient …

This preparation needs to be done once. You can use its results to analyze as many biological samples of the same species as you want.

Launch analysis

To launch an analysis, you need to create a directory `<sample directory>/fastq`
(where `<sample directory>` is some label identifying your biological sample.

In this directory, place the fastq file (or the pair of fastq files if you have performed a paired end sequencing).

Then, launch Bismark methylation call :

```
RRBS_HOME/Bismark_methylation_call/RRBS_pipeline <sample directory> <genome directory>
```