Methpipe Manual

November 3, 2010

The methpipe software package is a comprehensive tool chain for analyzing whole genome bisulfite sequencing data (BS-Seq). This documentation will guide you step by step to learn how to perform the data analysis in a BS-Seq project with methpipe. To facilitate understanding of the work flow, we divide the analysis procedure into four steps: (1) Pre-mapping processing: this step include assessing read quality and pre-processing reads, such as trimming adapters; (2) Mapping: this step maps sequence reads to a reference genome; (3) Analyzing methylation status at single site: in this step, we will estimate methylation frequency at single site, either CpG sites or non-CpG sites. We will also obtain information about sequencing depth and bisulfite conversion rate; (4) Higher level analysis: this step includes higher level more biologically interesting analysis, such as identifying hypo-methylated regions and/or differentially methylated regions. Later we will describe in detail the usage of tools in each step.

In the guide we will use a small project as example. Suppose that in this project you would like to study the methylation pattern in two cell types, B cells and neutrophiles. The B cell methylome is profiled with sing-end sequencing and your university's sequencing center sends back to you these sequence files:

`bcell/s-1.fq bcell/s-2.fq bcell/s-3.fq bcell/s-4.fq.`

The neutrophile methylome is profiled with pair-end sequencing and you have the following sequences files:

`neut/s-1-1.fq neut/s-1-2.fq neut/s-2-1.fq neut/s-2-2.fq,`

Now we are ready to uncover the interesting biology about DNA methylation from this dataset.

1 Pre-mapping processing

Before mapping sequenced reads to a reference genome, we need to pre-process the raw read sequences, in particular we need to trim possible adapter sequences retained in the 3' end of raw reads. Further we may be interested in examining the quality of reads in our library and visualizing raw reads in UCSC Genome Browser.

1.1 Trim adapters

As the length of reads that sequencing technology is able to produce has been increasing all the time, it is possible there are adapter sequences in the 3' end of some reads. These retained adapter sequences affects the mappability of such reads. Even if they are somehow mapped to the reference genome, the adapter sequences may introduce bias to our estimate of methylation frequency. Therefore it is necessary to trim these retained adapter sequences.

The program **trim-adapter** is used to trim adapter sequences, if any, from the 3' end of raw reads. Suppose the adapter sequences is *GATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCTCGTATGCCG*, we trim the adapter sequences from raw reads in the file bcell/s-1.fq with the following command:

```
$ ./trim-adapter  s-1.fq -o s-1-clipped.fq
-a GATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCTCGTATGCCG
```

If you would like to check the effective read length after trimming adapter sequences and Ns from 3' end, you can add **-l** options to specify the output file for effective read length, for example,

```
$ ./trim-adapter  s-1.fq -o s-1-clipped.fq  -l s-1-length.txt
-a GATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCTCGTATGCCG
```

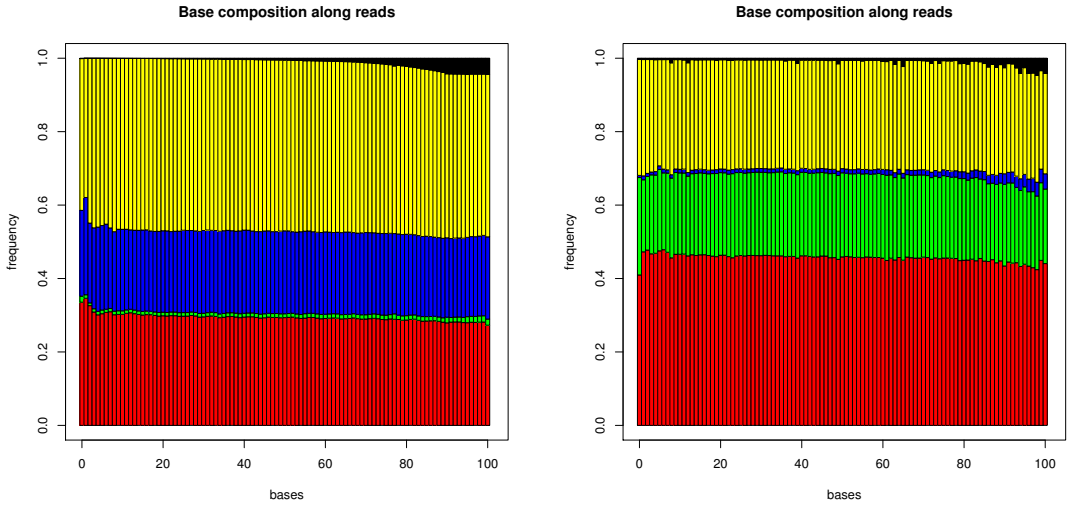
The file s-1-length.txt lists the effective read length of each read after trimming adapter sequences and Ns, which can be used to compute the statistics of effective reads lengths with any general purpose statistical package, such as R.

1.2 Assessing read quality

It is optional to assess read quality before mapping, however such assessment may help us to spot potential problems during library preparation and/or bisulfite sequencing. The program **read-quality-prof** is used to generate an average summary of base composition and quality scores from 5' to 3' along all reads. We run **read-quality-prof** as following

```
$ ./read-quality-prof  < s-1.fq > s-1-qual.txt
```

The output file s-1-qual.txt can be visualized with the R software. Fig. 1 shows the base composition profile in the pair-end sequencing sample, where the left panel shows T-rich reads and the right panel A-rich reads. Note the small proportion of C and G reflects the effect of bisulfite conversion.



(a) T-rich reads

(b) A-rich reads

Figure 1: Base composition

2 Mapping

rmapbs.cpp This program takes fastq file as input and output mapped read file

3 Post-mapping processing

merge.cpp merge sorted MappedRead file, with option to control whether remove redundant file

mask-overlap.cpp mask overlapping region of paired-reads, also generate summary of fragment length, number of unpaired reads

unique.cpp filter program to remove duplicate reads

sort.cpp sort MappedRead file: either by genomic location or by name

revcomp.cpp do reverse complement operation on MappedRead

4 Analysis

methcount.cpp This program reads mapped read file and output methylation frequency for each CpG site.

bsrate.cpp This program reads mapped read file and estimate bisulfite conversion rate by checking methylation status of non-CpG C's

5 Visualization

6 A sample work flow

This part shows how these tools are connected to get the methylation profile. Suppose we have the following BS-Seq library

```
reads/s_1_1.txt reads/s_1_2.txt reads/s_2_1.txt reads/s_2_2.txt
```

The final result can be obtained as following. For clarity, we show all the intermediate result. In real application, some intermediate files can be avoided by using pipes.

```
# Pre-mapping processing #

## trim adapter ##
$ ./trim-adapter reads/s_1_1.txt preprocessed/s_1_1.txt
$ ./trim-adapter reads/s_1_2.txt preprocessed/s_1_2.txt
$ ./trim-adapter reads/s_2_1.txt preprocessed/s_2_1.txt
$ ./trim-adapter reads/s_2_2.txt preprocessed/s_2_2.txt

# Mapping #
$ ./rmapbs -c genome_seq_dir -o mapped/s_1_1.mr preprocessed/s_1_1.txt
$ ./rmapbs -A -c genome_seq_dir -o mapped/s_1_2.mr preprocessed/s_1_2.txt
$ ./rmapbs -c genome_seq_dir -o mapped/s_2_1.mr preprocessed/s_2_1.txt
$ ./rmapbs -A -c genome_seq_dir -o mapped/s_2_2.mr preprocessed/s_2_2.txt

# post-mapping processing

## reverse complement A-rich strand ##
$ ./revcomp mapped/s_1_2.mr > tmpfile && mv tmpfile mapped/s_1_2.mr
$ ./revcomp mapped/s_2_2.mr > tmpfile && mv tmpfile mapped/s_2_2.mr

## mask overlapping ##
#### first sort by name ####
$ ./sort -N mapped/s_1_1.mr -o tmpfile && mv tmpfile  mapped/s_1_1.mr
$ ./sort -N mapped/s_1_2.mr -o tmpfile && mv tmpfile  mapped/s_1_2.mr
$ ./sort -N mapped/s_2_1.mr -o tmpfile && mv tmpfile  mapped/s_2_1.mr
$ ./sort -N mapped/s_2_2.mr -o tmpfile && mv tmpfile  mapped/s_2_2.mr

#### masking ####
$ ./mask-overlap mapped/s_1_1.mr mapped/s_1_2.mr masked/s_1_1.mr masked/s_1_2.mr
```

```
$ ./mask-overlap mapped/s_2_1.mr mapped/s_2_2.mr masked/s_2_1.mr masked/s_2_2.mr

#### sort by genomic location ####
$ ./sort masked/s_1_1.mr -o tmpfile && mv tmpfile  masked/s_1_1.mr
$ ./sort masked/s_1_2.mr -o tmpfile && mv tmpfile  masked/s_1_2.mr
$ ./sort masked/s_2_1.mr -o tmpfile && mv tmpfile  masked/s_2_1.mr
$ ./sort masked/s_2_2.mr -o tmpfile && mv tmpfile  masked/s_2_2.mr

## combine all result ##
#### merge ####
$ ./merge -o all.mr masked/s_1_1.mr masked/s_1_2.mr masked/s_2_1.mr masked/s_2_2.mr

#### jackpot removal #####
$ ./unique all.mr -o tmpfile && mv tmpfile all.mr

# analysis #
## methcounts ##
$ ./methcounts -c genome_sequence_file -o all-methcounts.bed all.mr
```