Bioinformatics Lessons Schedule

Date	Subject
01-07	Process RNA-seq
01-14	Process RNA-seq, continued
01-21	Process RNA-seq, continued
01-28	Analyze RNA-seq
02-04	Analyze RNA-seq, continued
02-11	Analyze RNA-seq, continued
02-18	skipped
02-25	Process RRBS
03-03	Process RRBS, continued
03-10	Process RRBS, continued
03-17	Analyze RRBS
03-24	Analyze RRBS, continued
03-31	Presentations / Practice Starts

Process RRBS, continued

2020-03-03

Step 2: Trimming

```
for i in *1.fq.gz;
     do trim_galore
          --rrbs
          --fastqc
          --paired
          -q 30
          --illumina
          --output ../01_trim/
          $i
          ${i/1.fq.gz/2.fq.gz};
done
```

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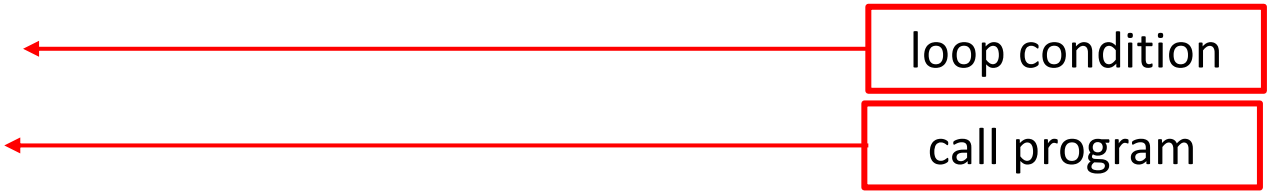

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done
```

loop condition

call program

trimming RRBS

RRBS flag lets trim_galore know to trim bases that were filled in by end repair

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run FastQC again after trimming

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reads are paired-end

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Minimum quality = 30

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loop condition

call program

trimming RRBS

run FastQC again after trimming

reads are paired-end

Minimum quality = 30

trim Illumina adapters

output goes here

reads

Step 3: Aligning

```
for i in *1_val_1.fq.gz; do
     bismark
          --bowtie2
          /mnt/data/data_jj/jj4/rrbs/tools \
          /genomes/hg19lambda
          --output ../02_align
          -1 $i
          -2 ${i/1_val_1.fq.gz/ \
               2_val_2.fq.gz};
done
```

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for i in *1_val_1.fq.gz; do
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loop condition

call program

Aligns to a normal genome and a C-to-T converted genome

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call program

aligner

Aligns to a normal genome and a C-to-T converted genome

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            --output ../02_align
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            -2 ${i/1_val_1.fq.gz/ \
                  2_val_2.fq.gz};
done
```

loop condition

call program

aligner

path to genome

Aligns to a normal genome and a C-to-T converted genome

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path to genome

output here

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call program

aligner

path to genome

output here

Read 1

Read 2

Aligns to a normal genome and a C-to-T converted genome

Step 3: Call Methylation

```
for i in *.bam; do
     bismark_methylation_extractor
          --paired-end
          --include_overlap
          --bedGraph
          --output ../03_extract_meth
          $i;
done
```

Step 3: Call Methylation

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for i in *.bam; do
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loop condition

call program

Counts number of Cs and Ts at each position

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     bismark_methylation_extractor
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          --bedGraph
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          $i;
done
```

loop condition

call program

reads paired

Counts number of Cs and Ts at each position

Step 3: Call Methylation

```
for i in *.bam; do
     bismark_methylation_extractor
          --paired-end
          --include_overlap
          --bedGraph
          --output ../03_extract_meth
          $i;
done
```

loop condition

call program

reads paired

Include CpGs where read 1 and read 2 overlap

Counts number of Cs and Ts at each position

Step 3: Call Methylation

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for i in *.bam; do
     bismark_methylation_extractor
          --paired-end
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          --bedGraph
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          $i;
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```

loop condition

call program

reads paired

Include CpGs where read 1 and read 2 overlap

output results for each CpG

Counts number of Cs and Ts at each position

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Counts number of Cs and Ts at each position

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loop condition

call program

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Include CpGs where read 1 and read 2 overlap

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Output here

Bam file

Counts number of Cs and Ts at each position

(Very) General Bioinformatics Workflow

1. Check quality
2. Trim
3. Align
4. Count
5. Statistics