Measuring DNA methylation using high-throughput sequencing

Mackenzie Gavery
11/10/16

DNA methylation – how to we measure it?

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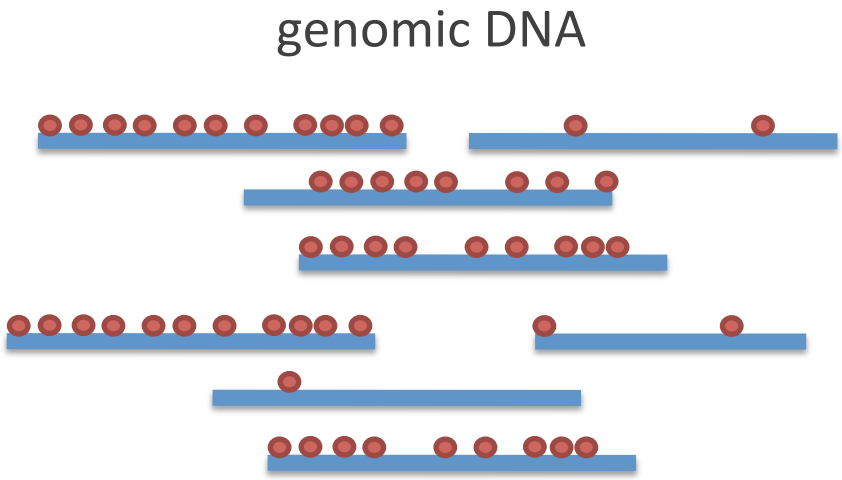
Reduced Representation Bisulfite Sequencing (RRBS)



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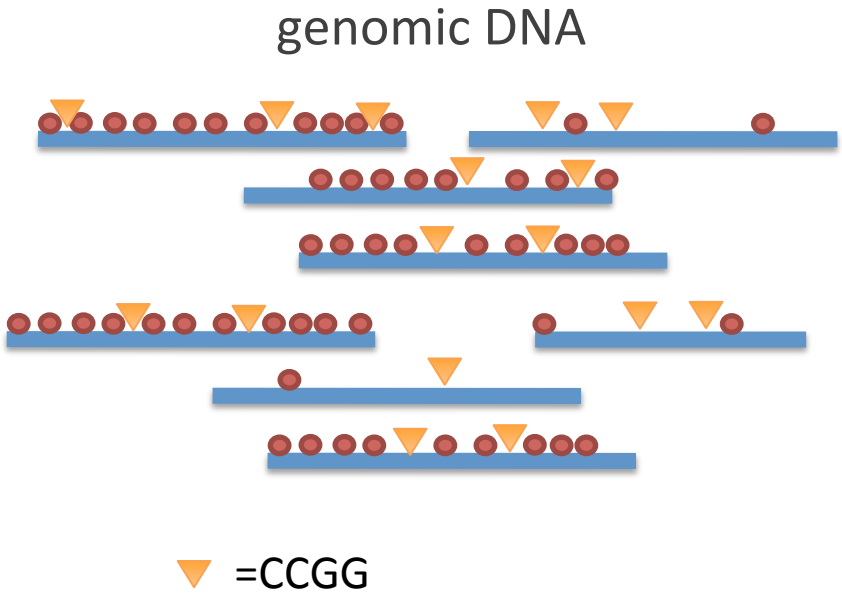

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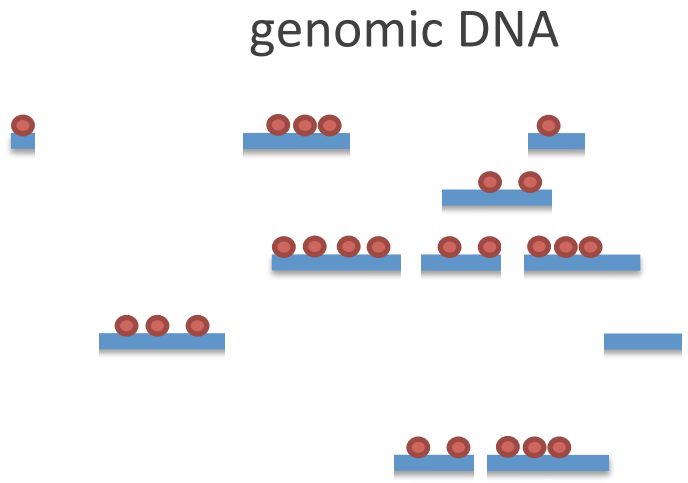

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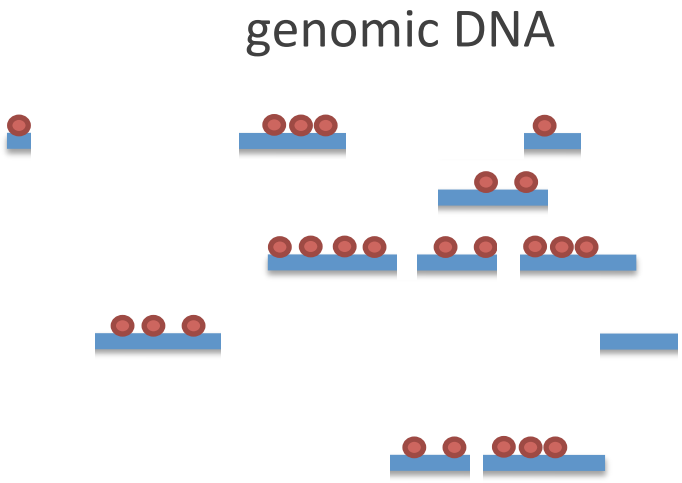
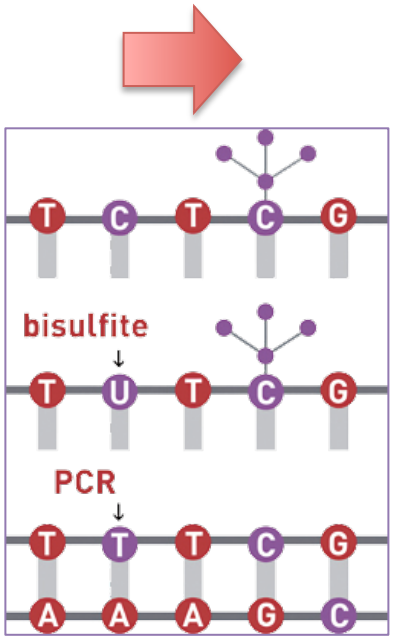
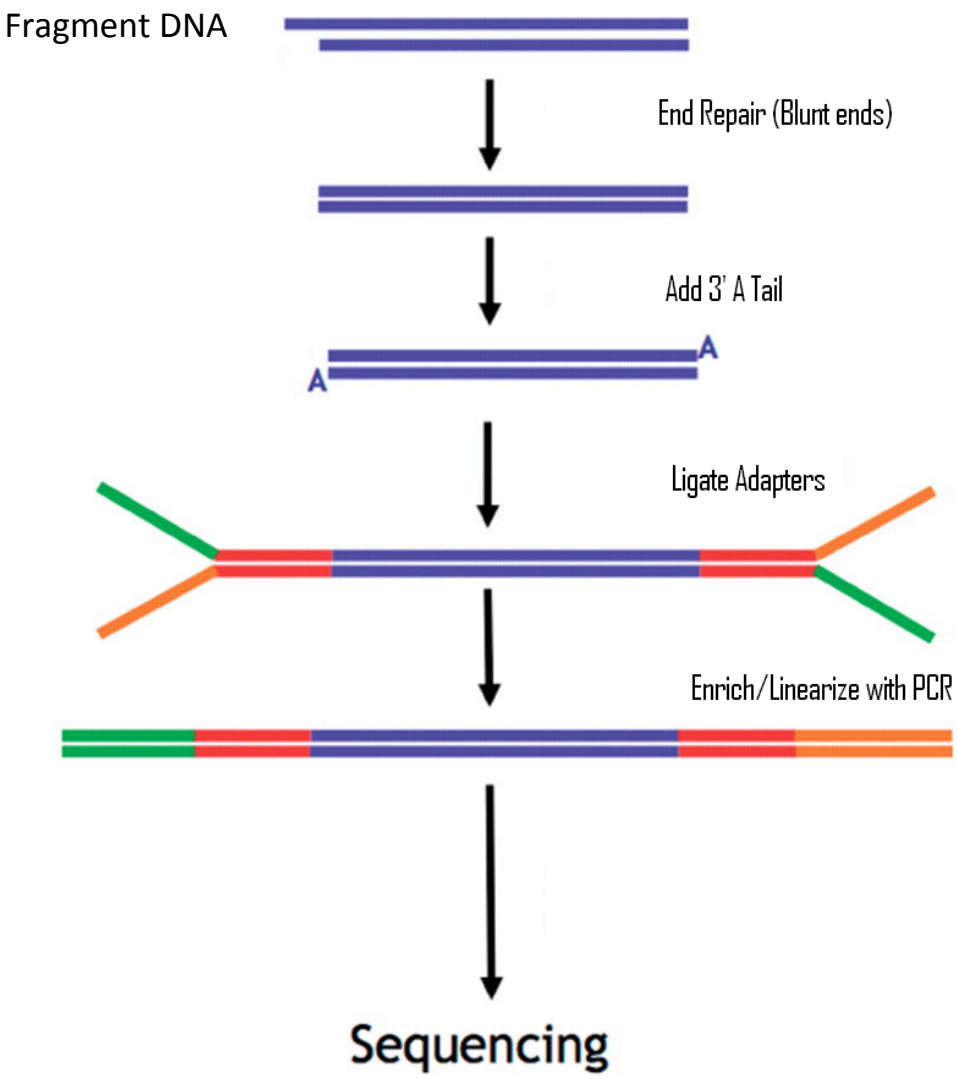




DNA methylation analysis

- Library prep
- Sequencing
- Bioinformatics (DNA methylation)
  - QC/Trimming
  - Mapping
  - Extracting Methylation Data
  - Interpretation (e.g. differential methylation)

Library Prep



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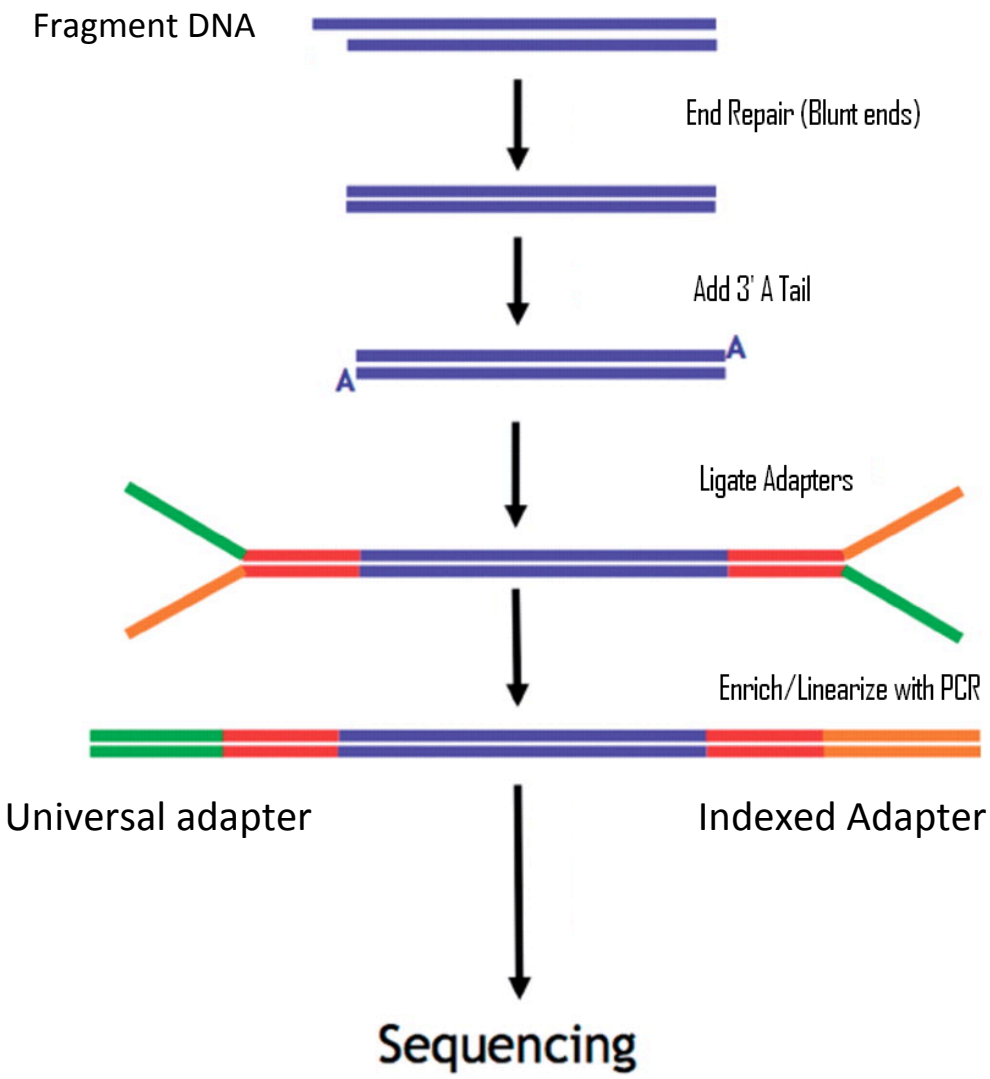

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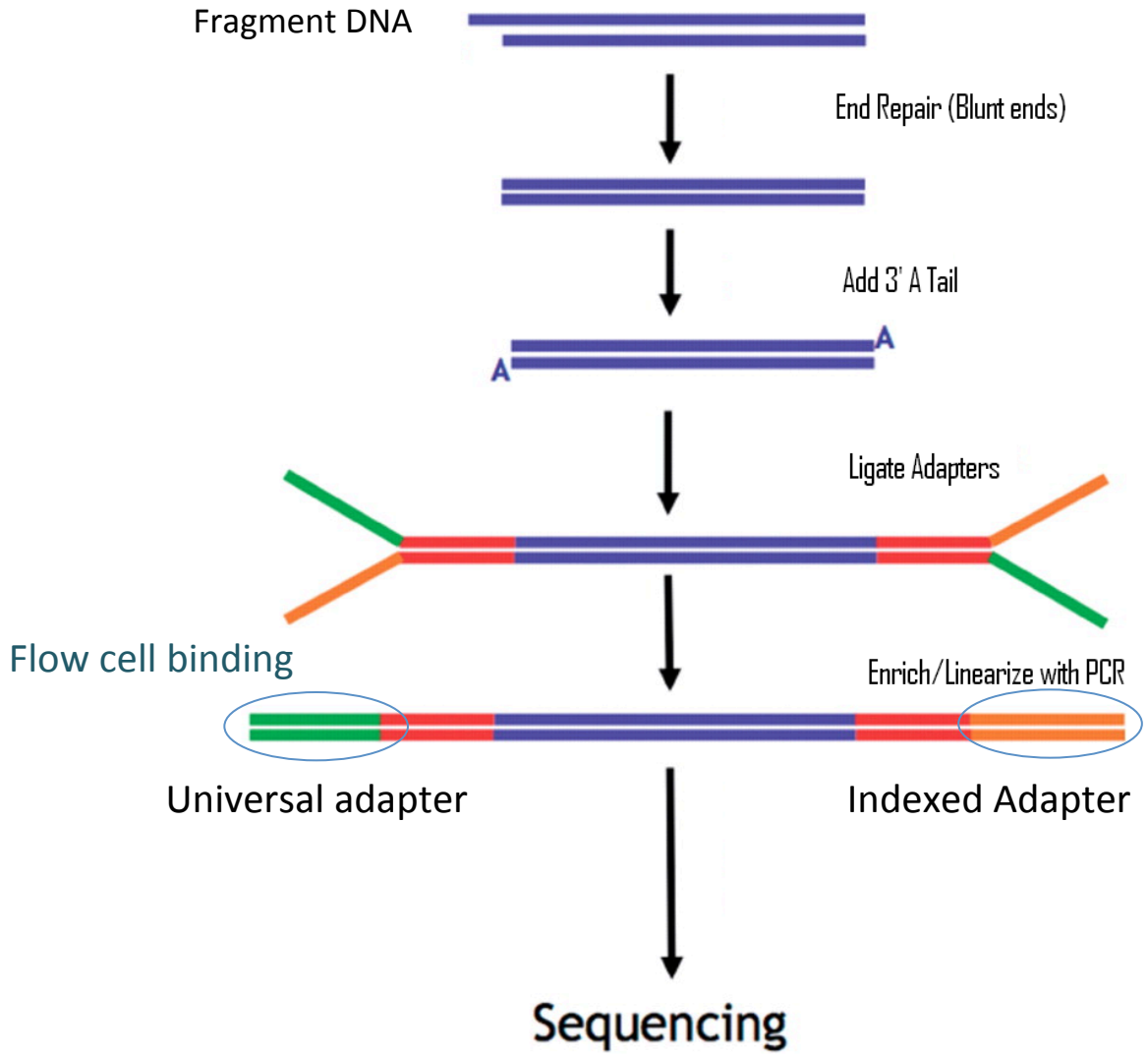

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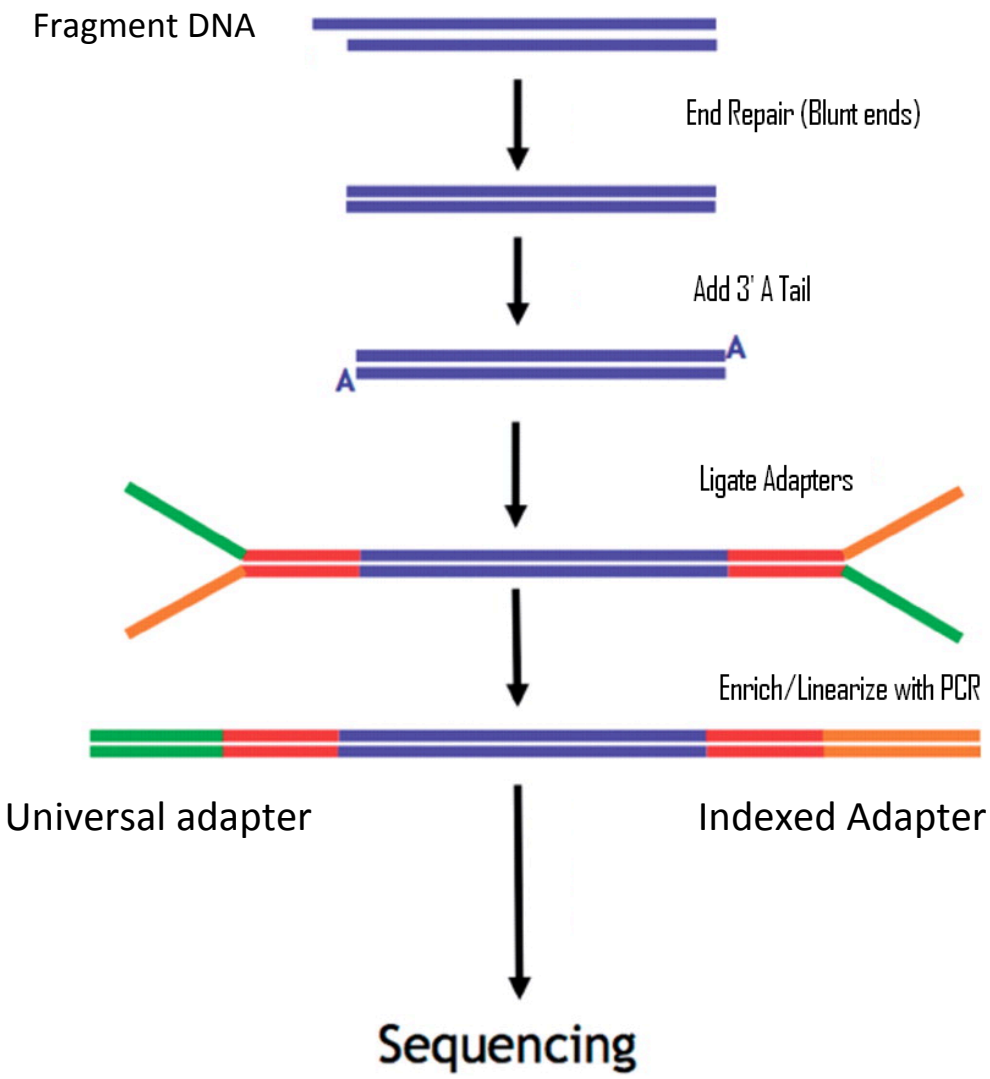

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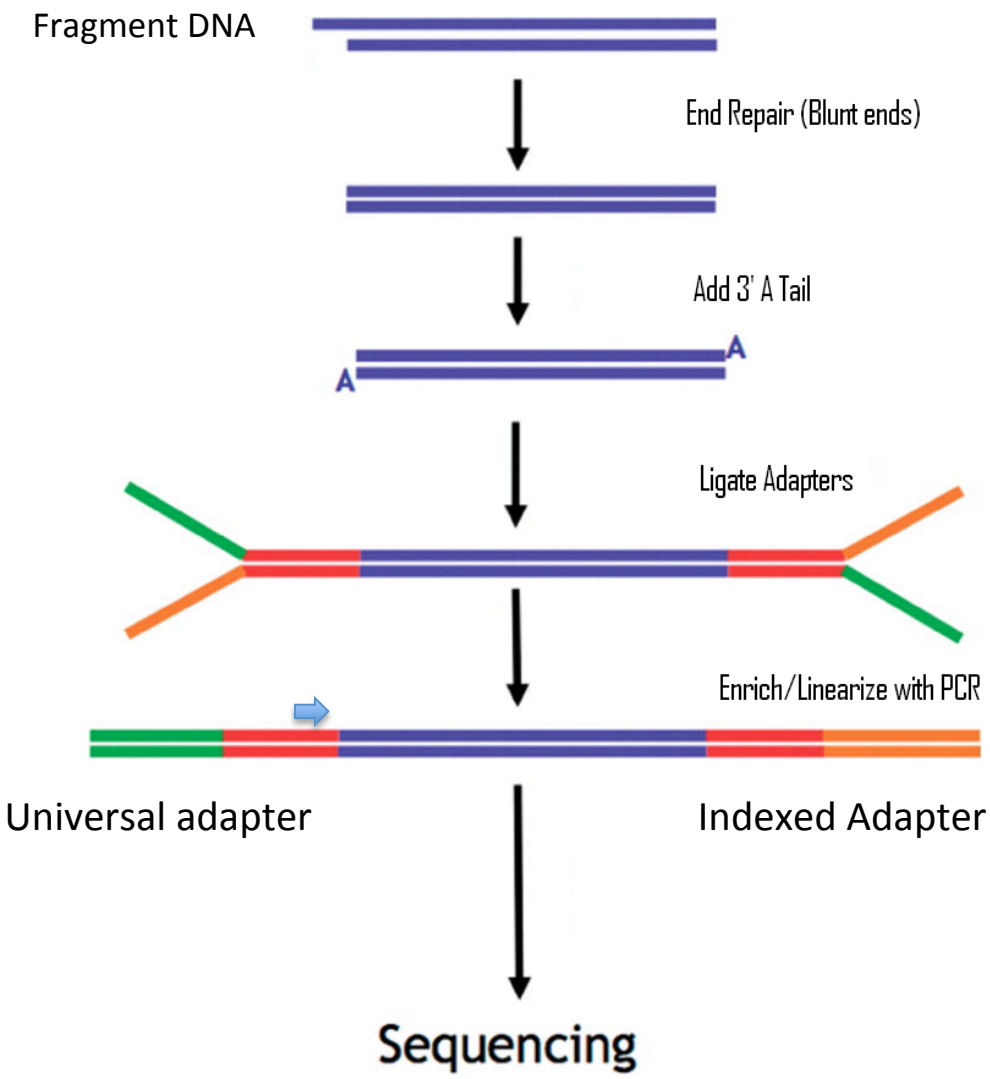

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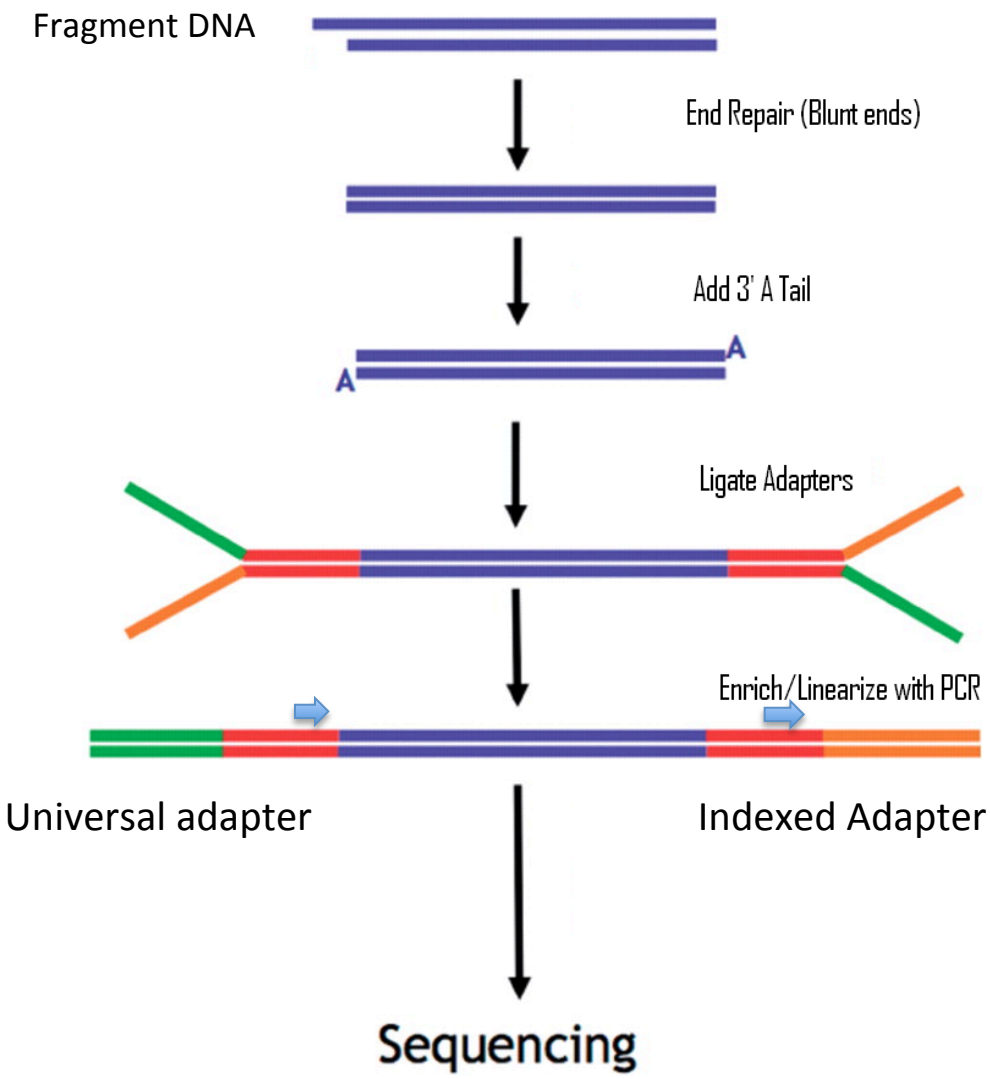

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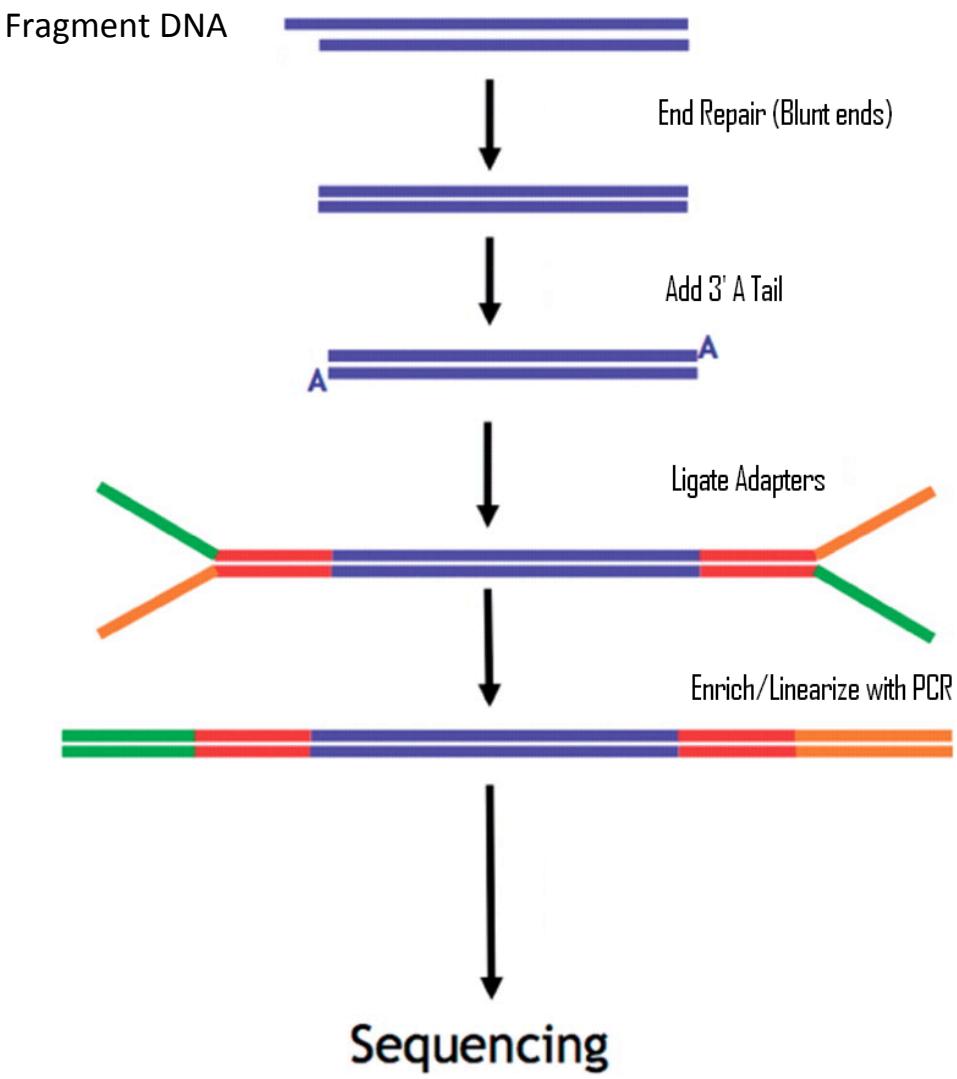

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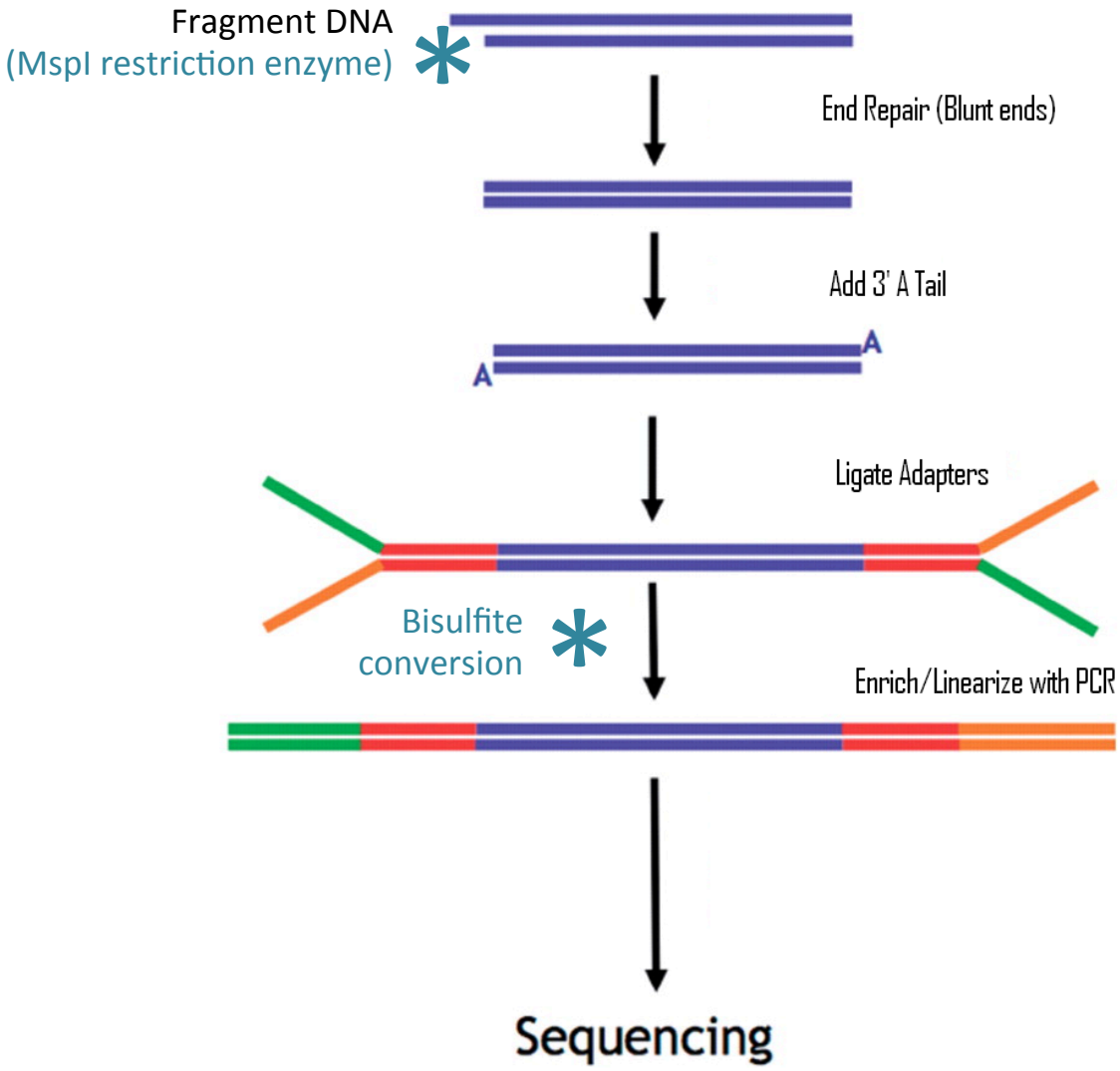
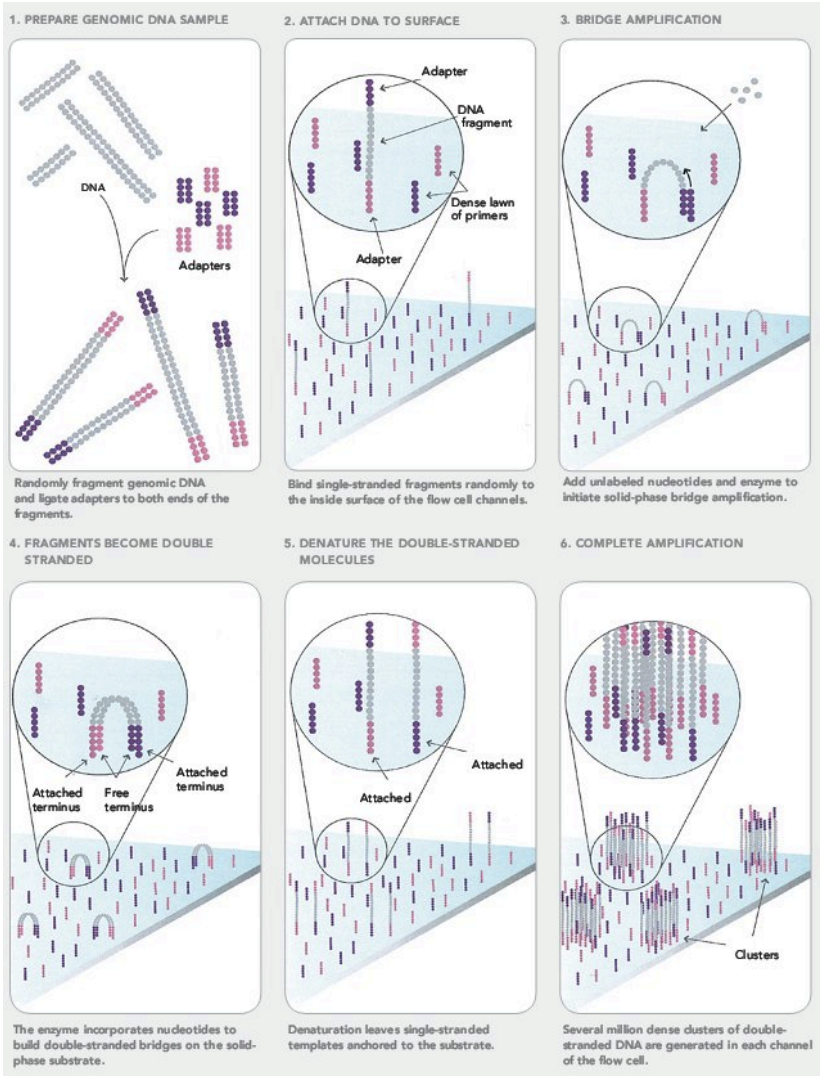


Sequencing

- Indexed libraries are pooled together
- Illumina HiSeq

  (single read 100bp)
- ~200 million reads



Fastq Files

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+
@@C?A@?4CFFFFG@EEGG@AGHA?CAD=FHDFB;;FHF=FEBHFD?C;>CAC==CAB8<<ACCDDCEDCDD:A@09<9<@B?B>@<8@:(087&+2<@A<
```

Fastq Files

```
@SN747:551:C99B9ACXX:7:1110:1855:1151 1:N:0:CAGATC
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+
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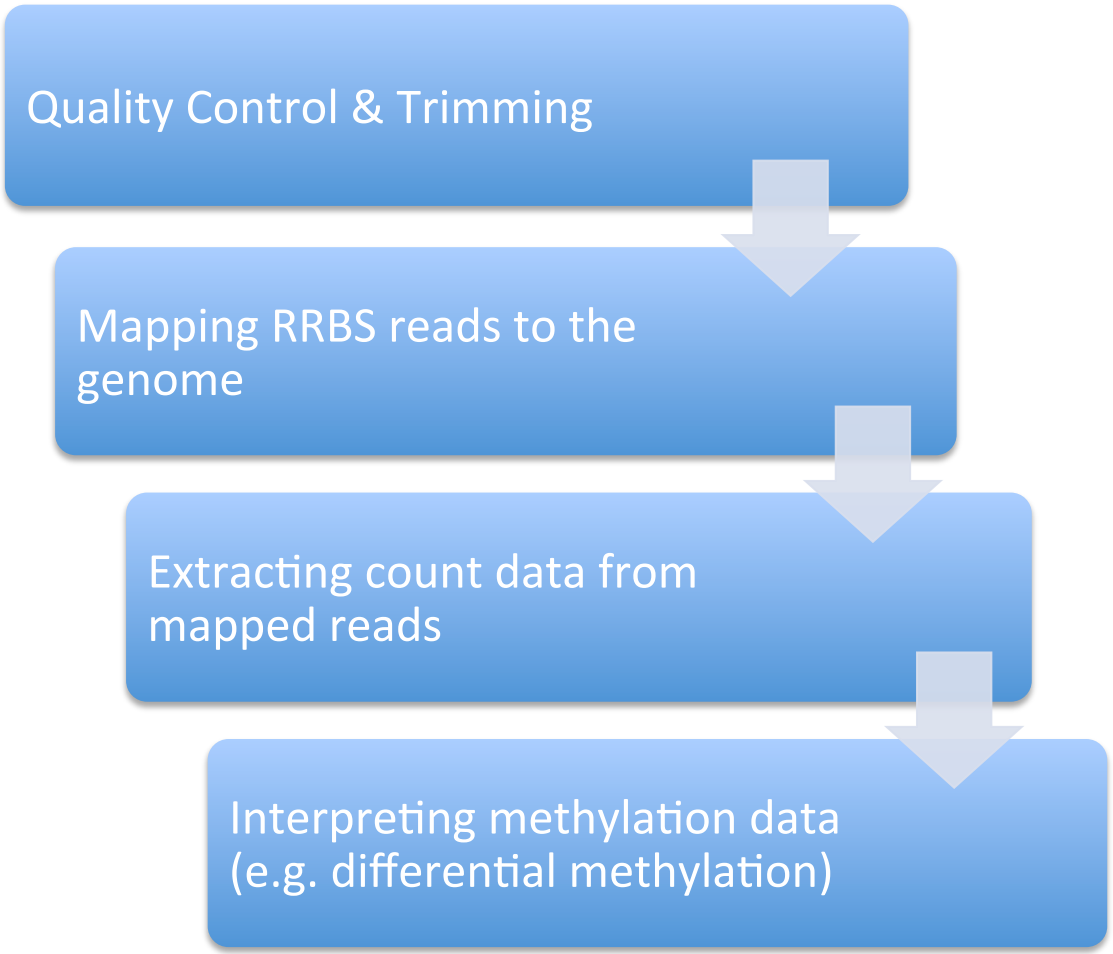
```

How do you analyze 200 million reads!?

Bioinformatics (DNA methylation)



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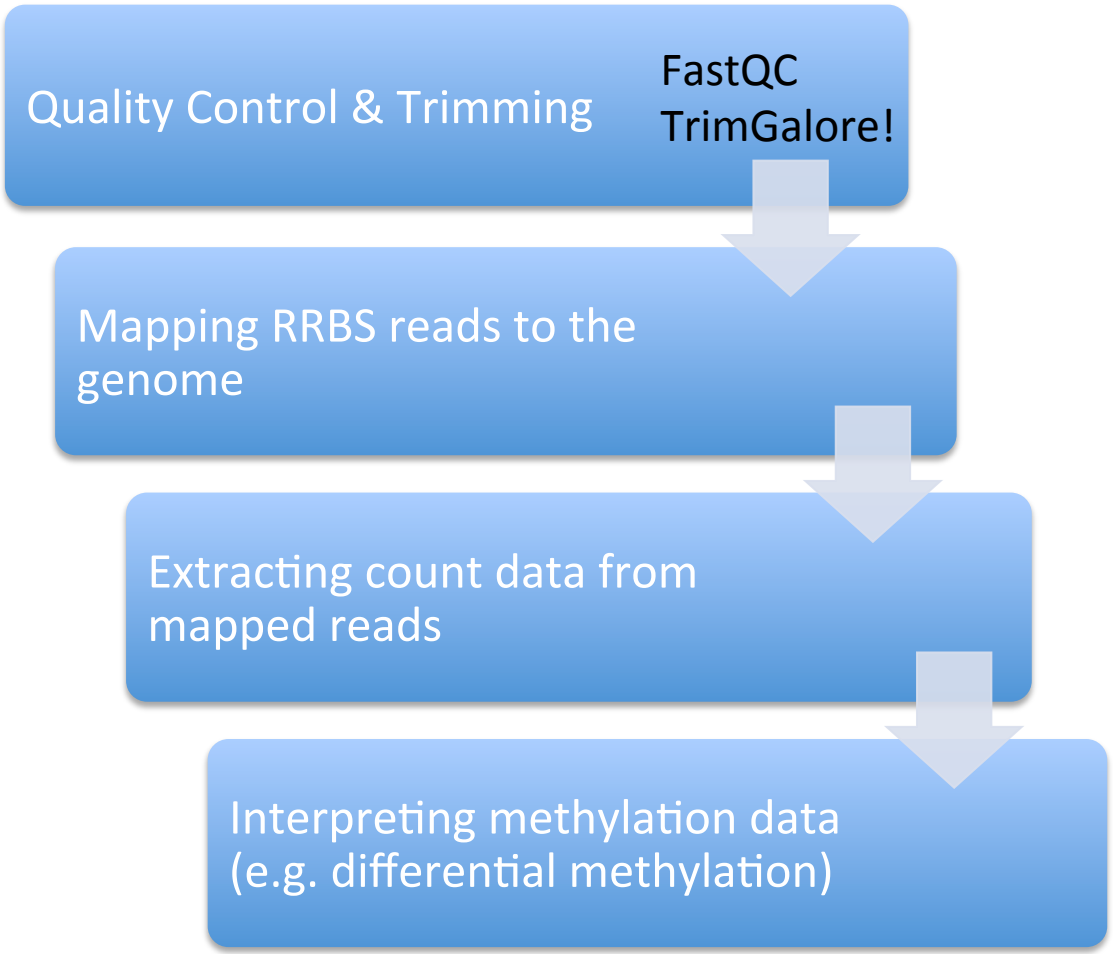
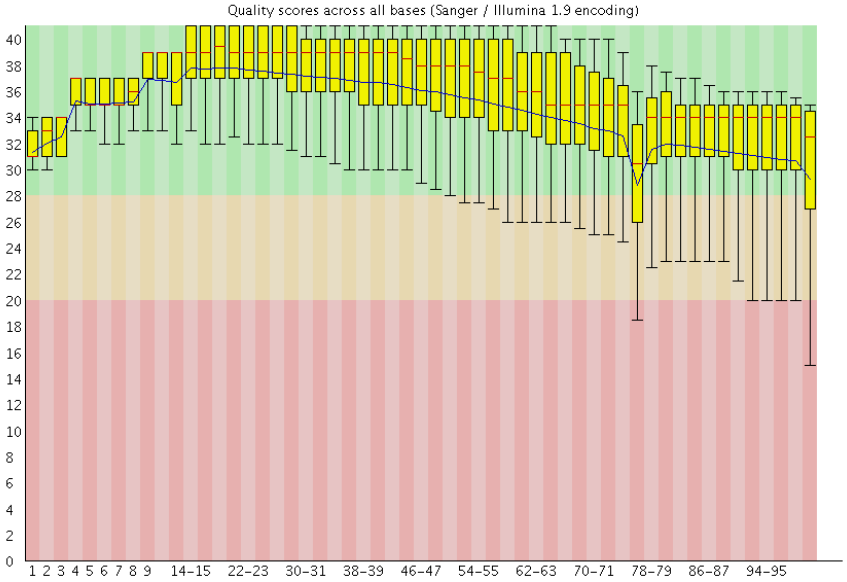
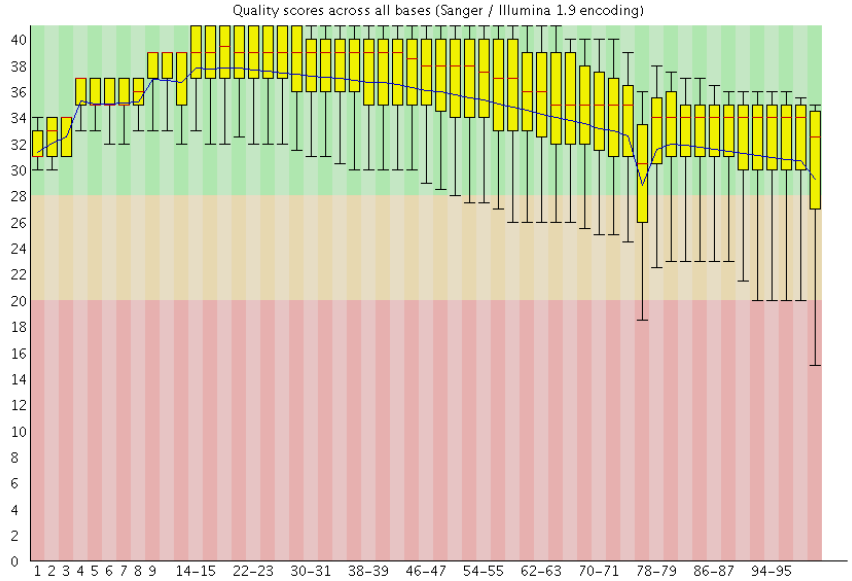


Per base sequence quality



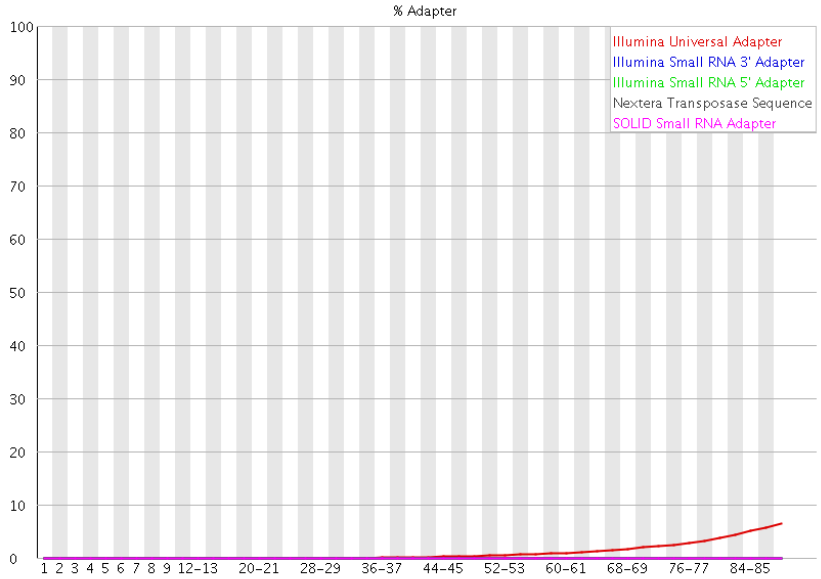
Position in read (bp)

Per base sequence quality



Position in read (bp)

Adapter Content



Position in read (bp)

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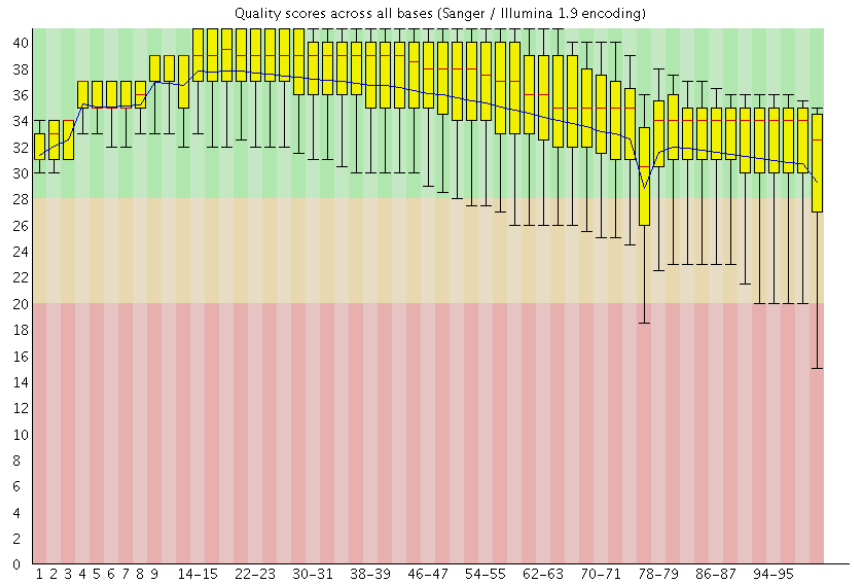
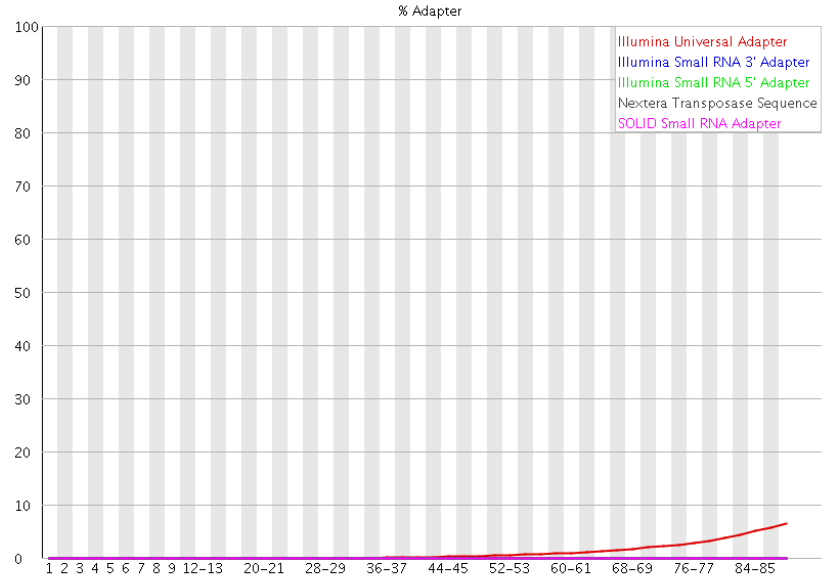


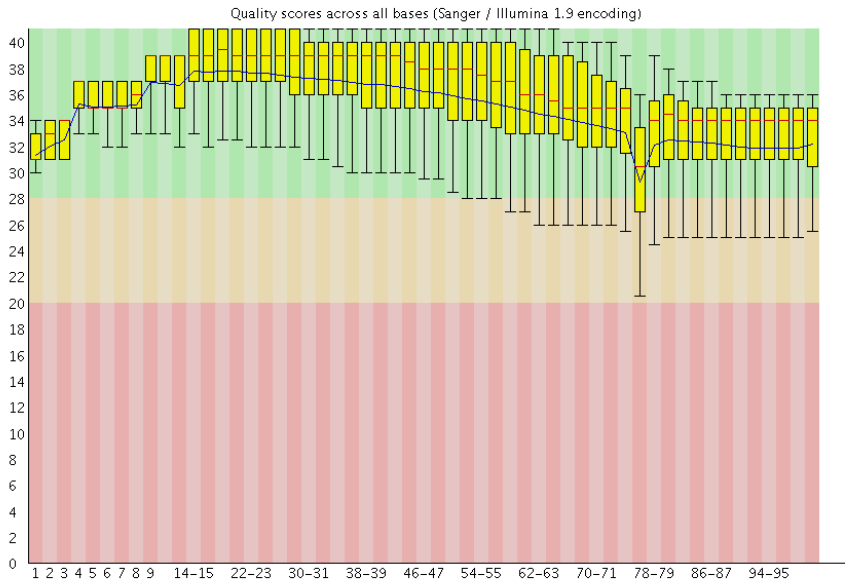
Position in read (bp)

Adapter Content



Position in read (bp)

Per base sequence quality



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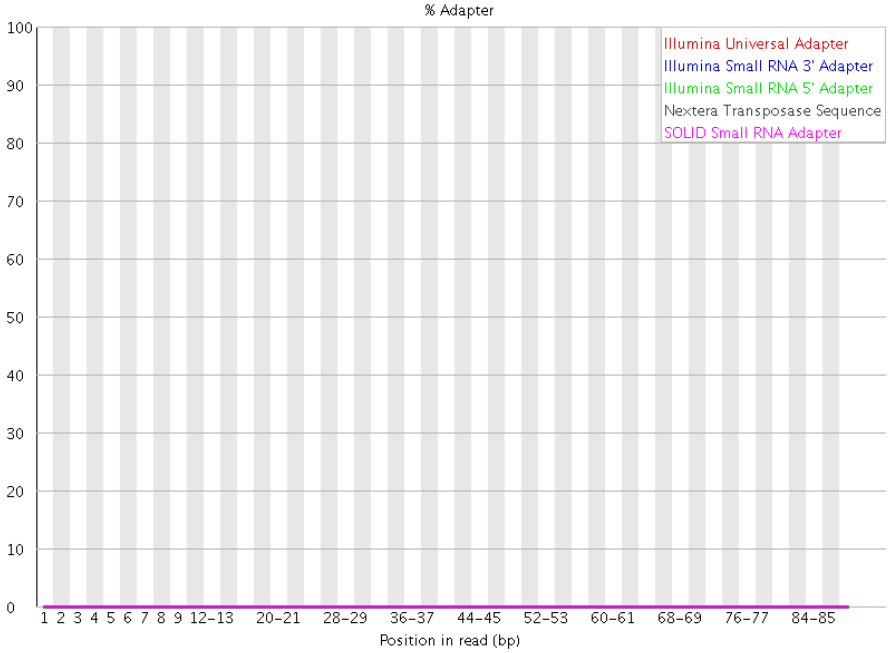
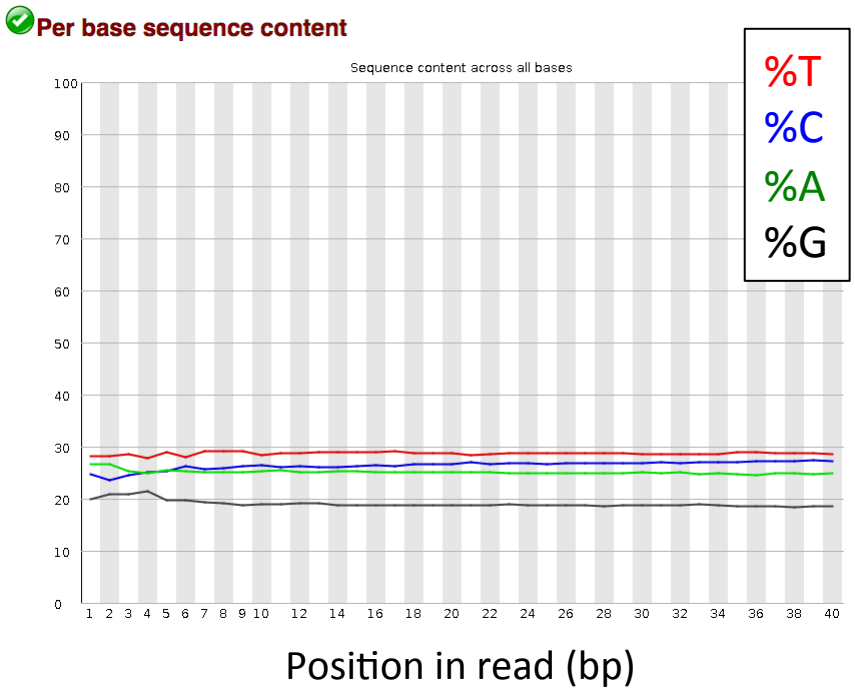
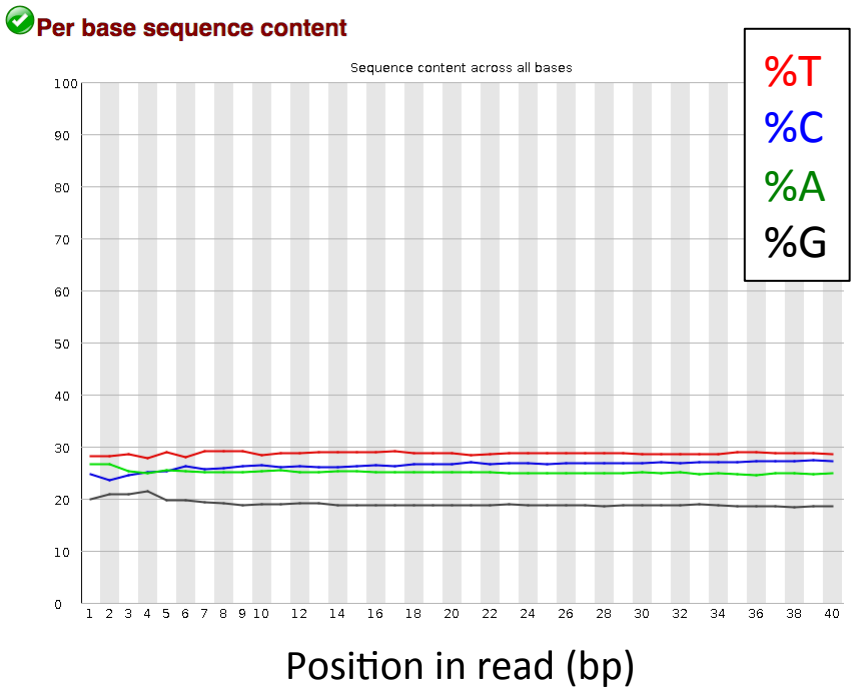


QC


Per base sequence content
Sequence content across all bases
%T
%C
%A
%G
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Position in read (bp)

QC


Per base sequence content
Sequence content across all bases
%T
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40
30
20
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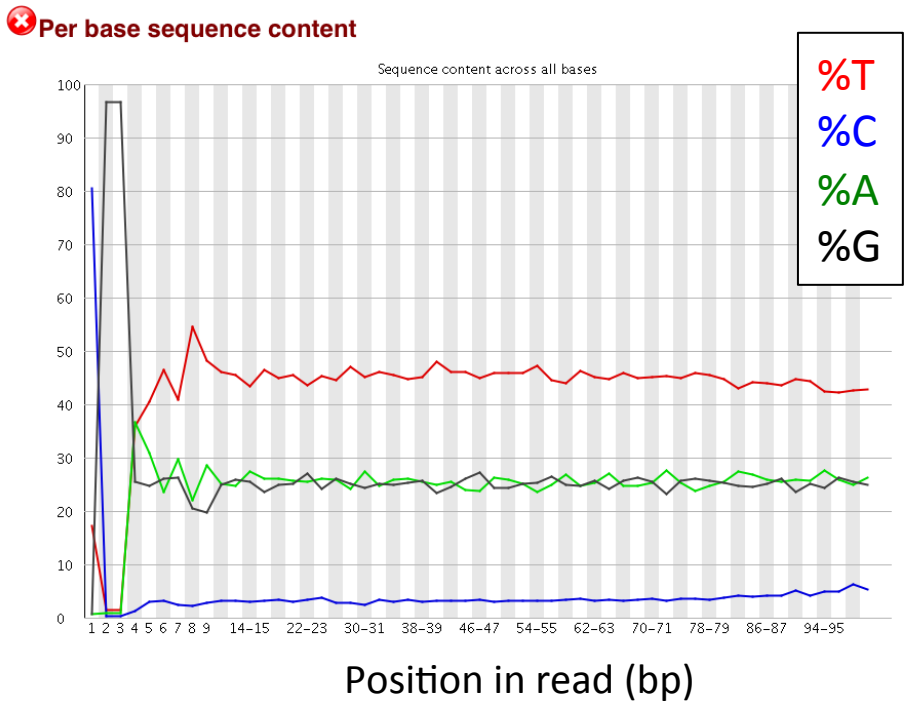
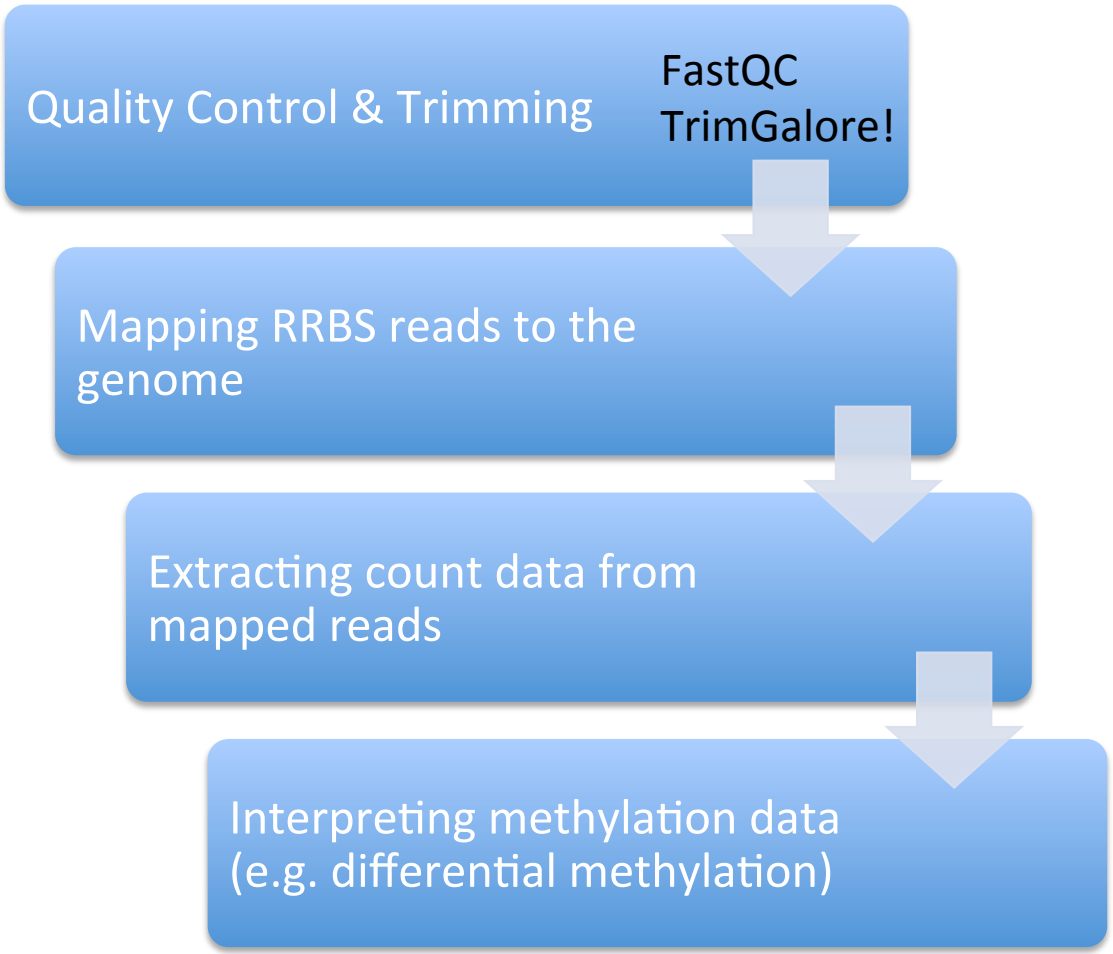
Position in read (bp)


Per base sequence content
Sequence content across all bases
%T
%C
%A
%G
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 14-15 22-23 30-31 38-39 46-47 54-55 62-63 70-71 78-79 86-87 94-95
Position in read (bp)

Bioinformatics (DNA methylation)



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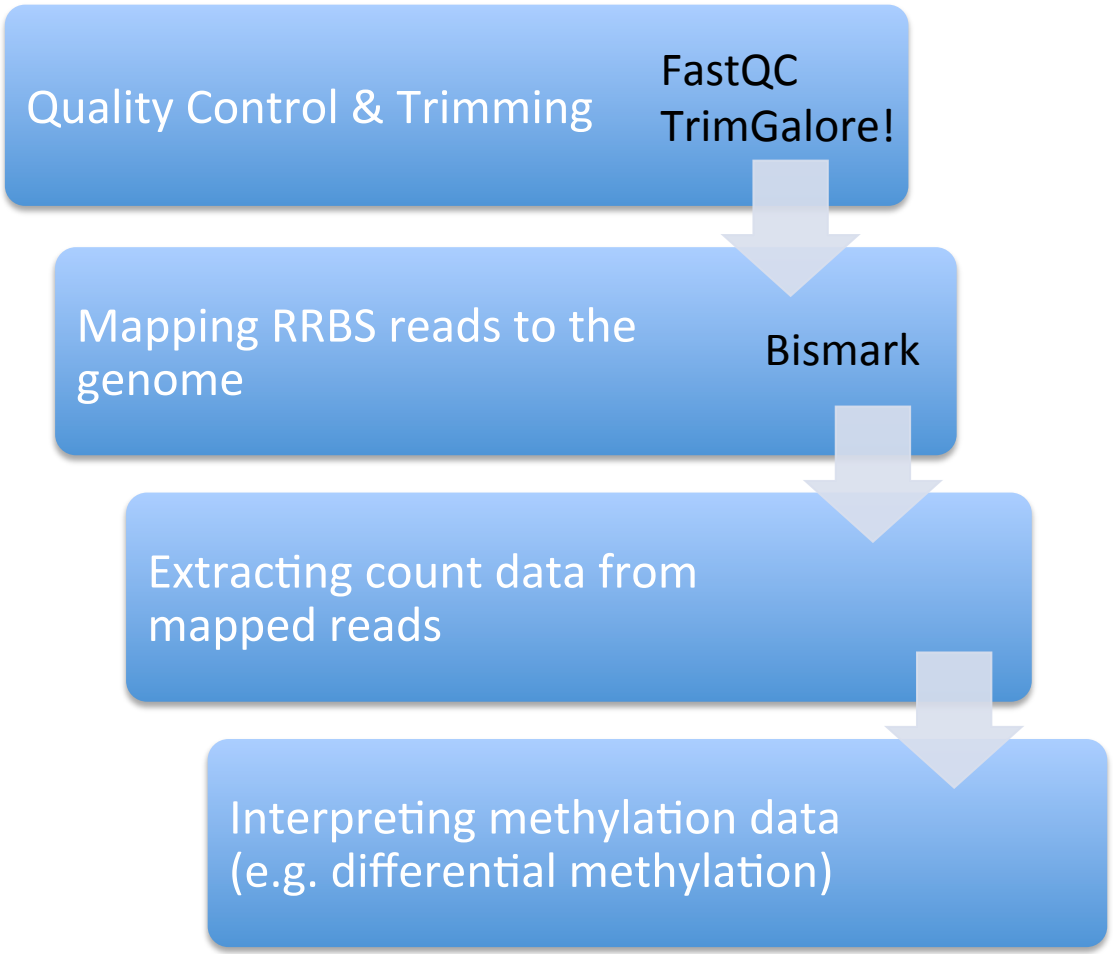

O. mykiss scaffold 13

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0G0G4G1G12G0G2G3G0G1G4G1G8G7G0G4G2G0G6 XM:Z:x.....x.h.Z.Z.....hh..h...xh.h...Zx.h.....z.....hh.Z.Zx..xh.....Z XR:Z:CT
XG:Z:GA
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SN747:551:C99B9ACXX:8:2315:20190:99881_1:N:0:ACAGTG 16 scaffold_614 418590 0 101M * 0 0
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.....x..z..z XR:Z:CT XG:Z:GA
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NM:i:15 MD:Z:4G1G5G0G2G14G5G0G3G0G1G3G2T7T33G3
XM:Z:....h.h.Z..Zxh..h.....Z.....Z.h...Z.hh...hh.h...z.Z.....Z..H.....Z.....x..Z XR:Z:CT XG:Z:GA

“Looking” at data using IGV

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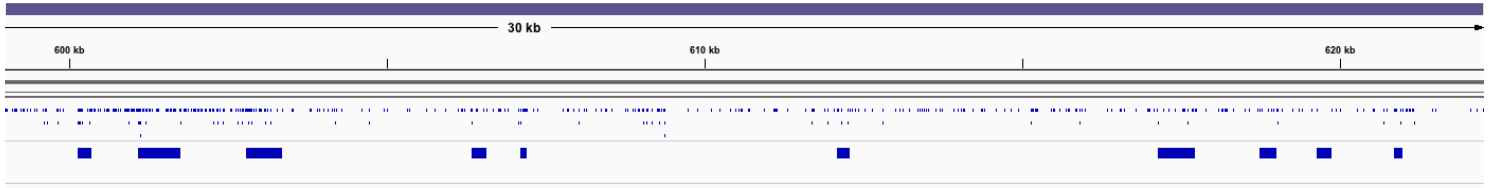
O. mykiss scaffold 13



CG motif

RRBS fragments

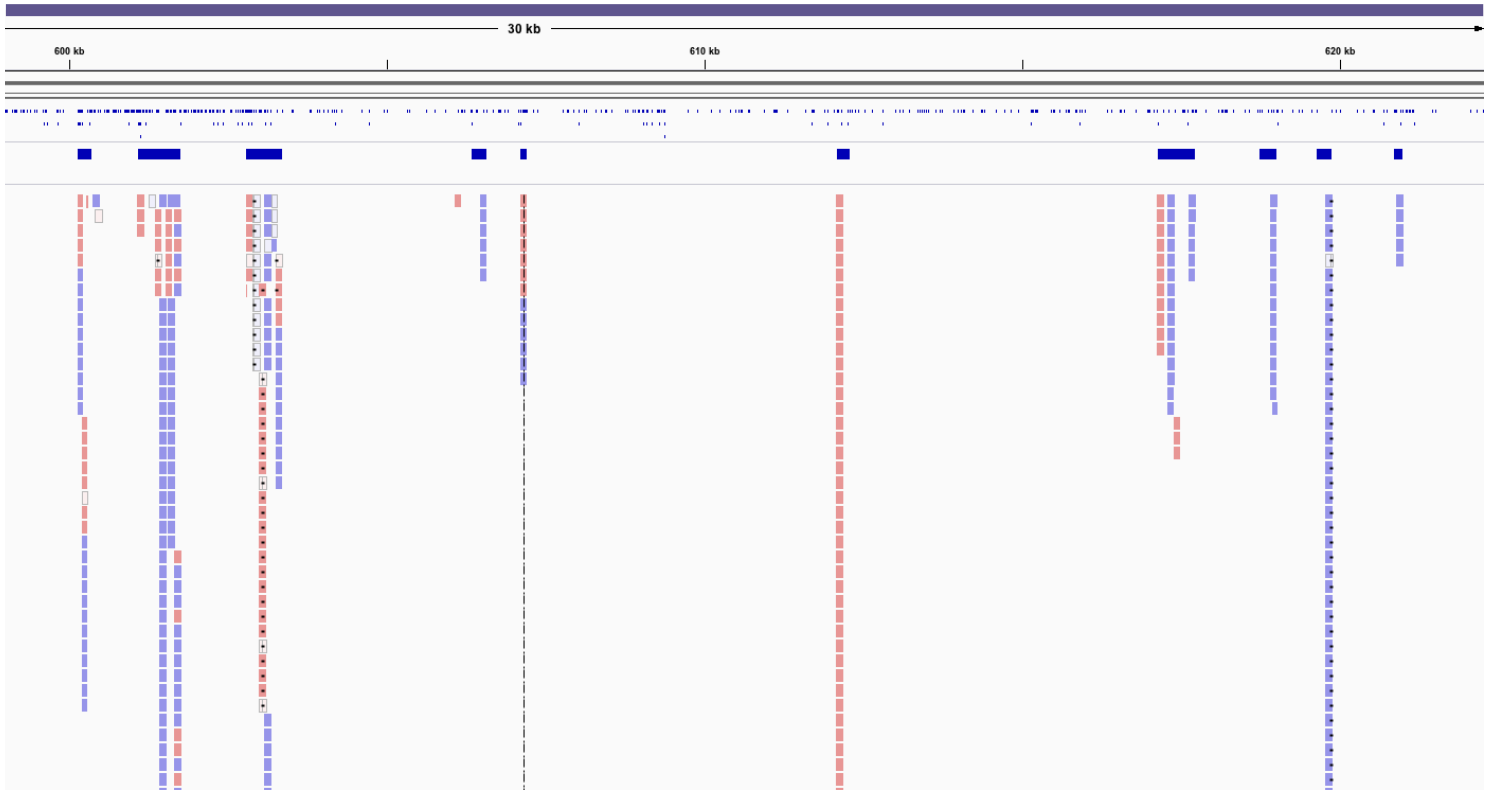
“Looking” at data using IGV

O. mykiss scaffold 13

CG motif

RRBS fragments

mapped reads

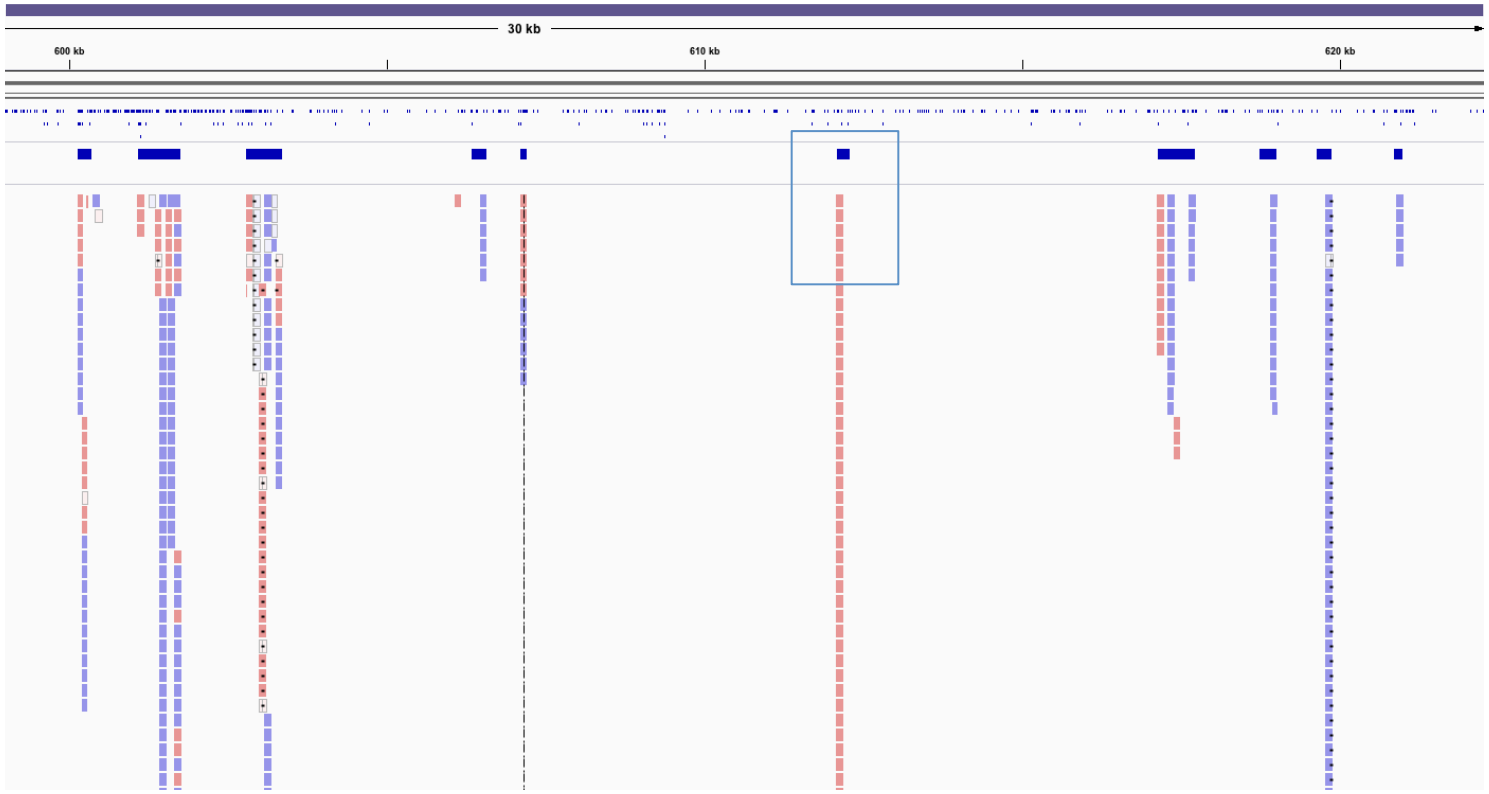


“Looking” at data using IGV

O. mykiss scaffold 13

CG motif
RRBS fragments

mapped reads



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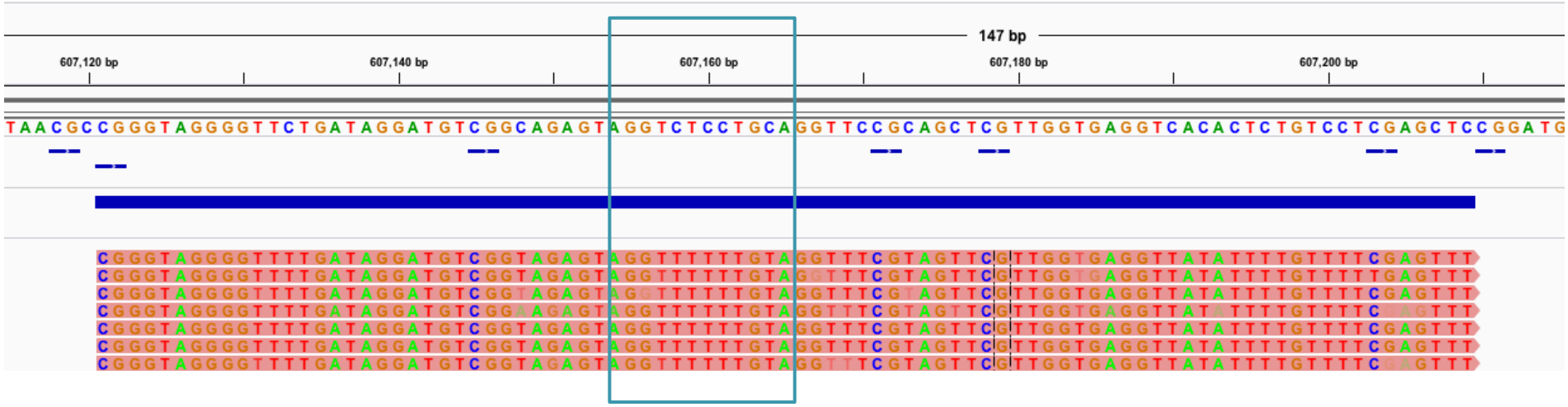

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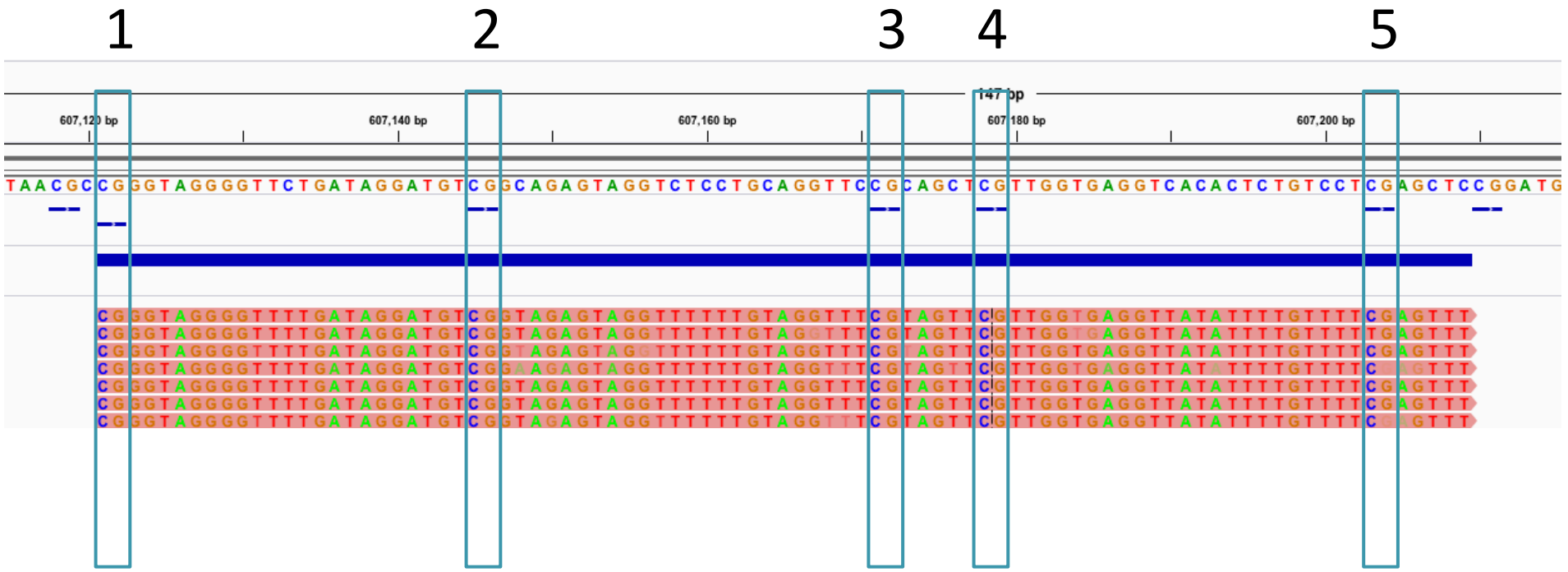

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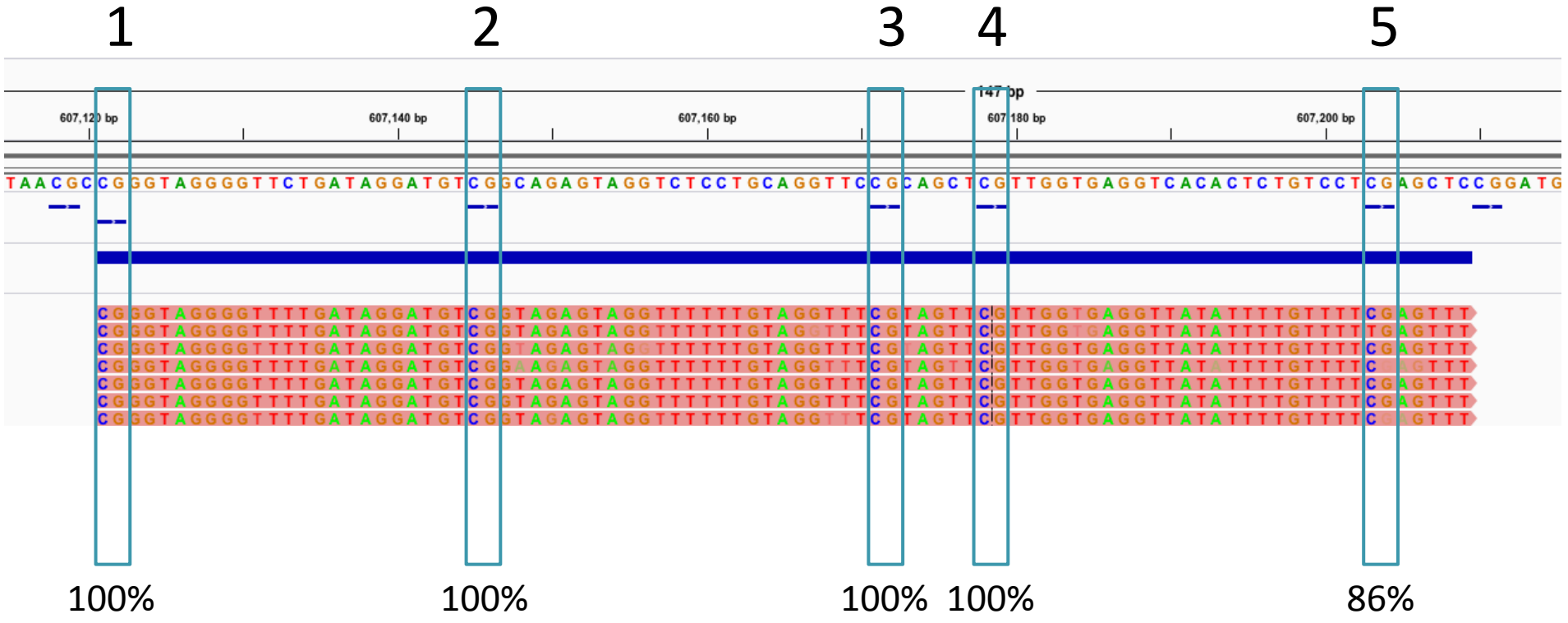
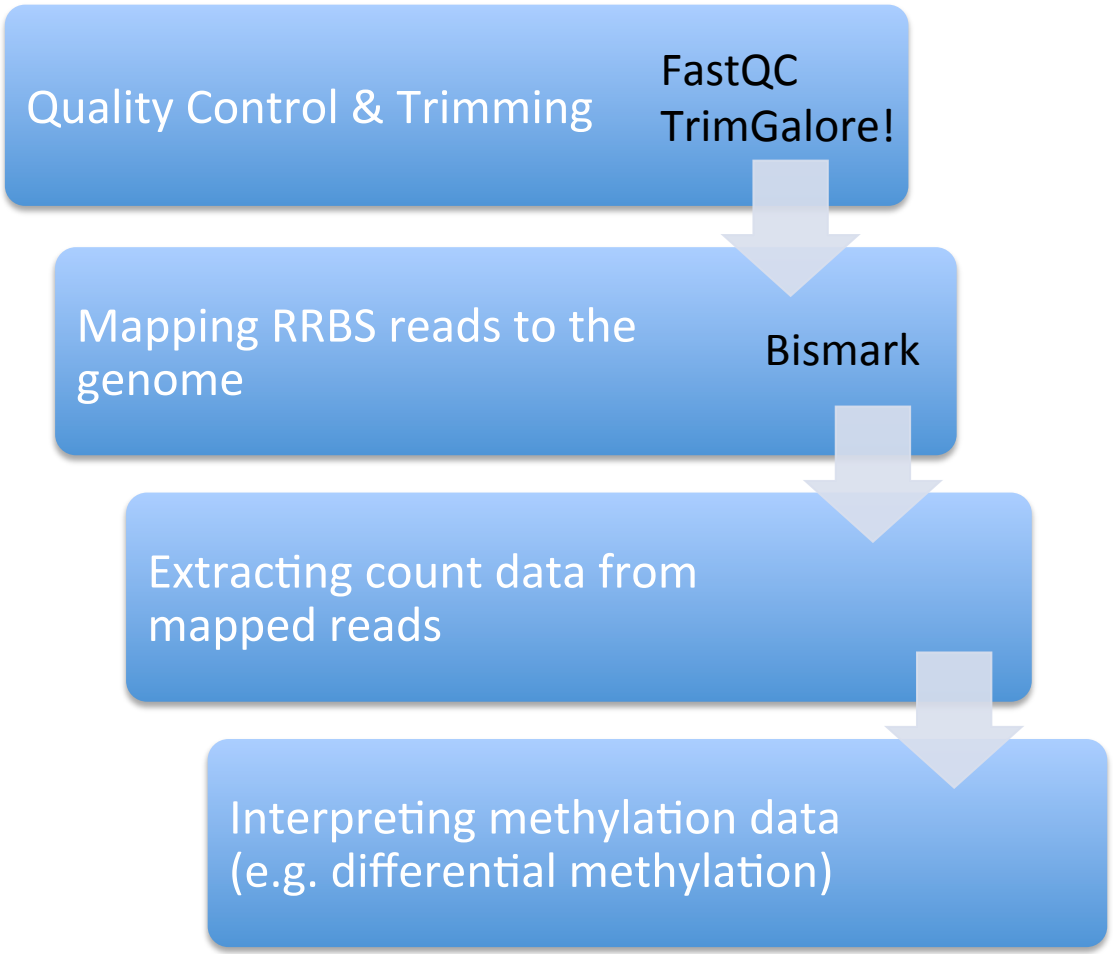


Bioinformatics (DNA methylation)



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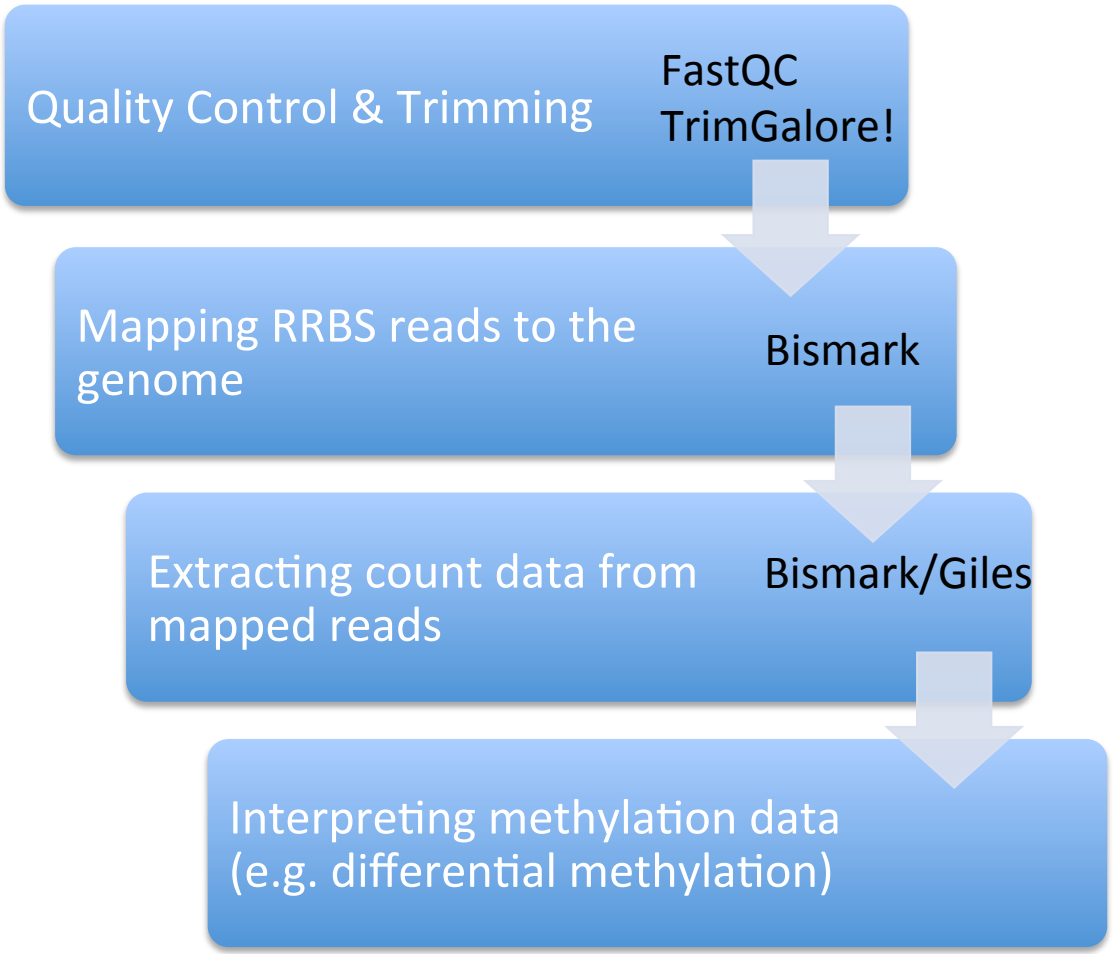


Bioinformatics (DNA methylation)

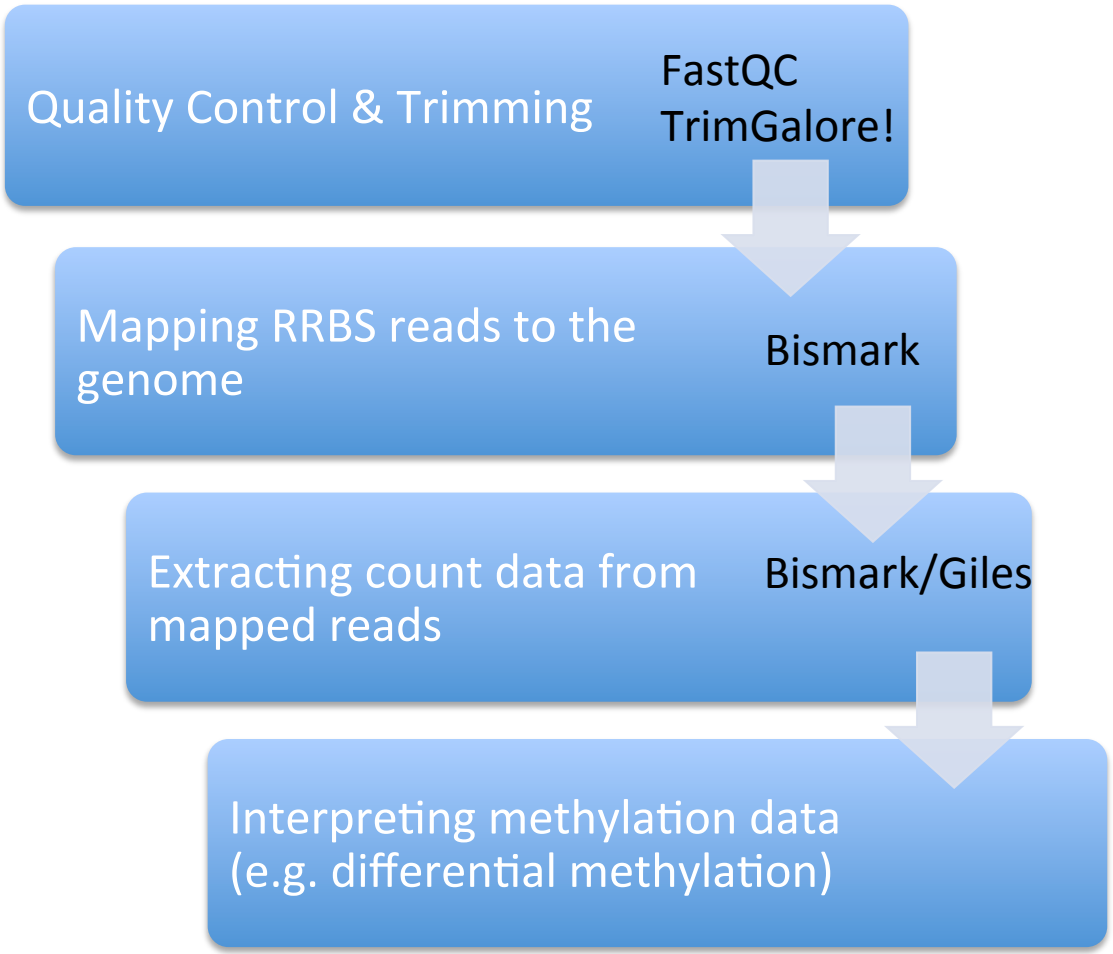
- Quality Control & Trimming — FastQC, TrimGalore!
- Mapping RRBS reads to the genome — Bismark
- Extracting count data from mapped reads — Bismark/Giles
- Interpreting methylation data (e.g. differential methylation)

Bioinformatics (DNA methylation)

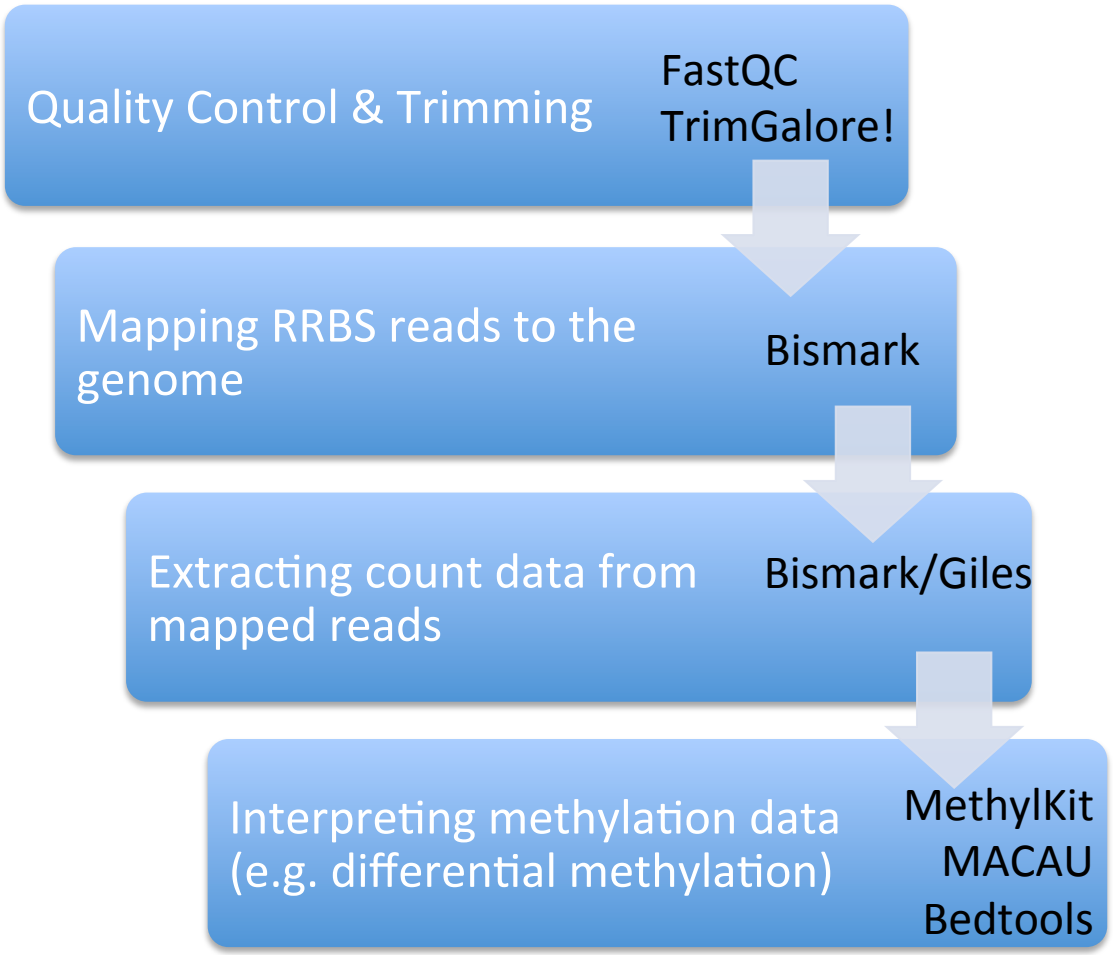
- Quality Control & Trimming — FastQC, TrimGalore!
- Mapping RRBS reads to the genome — Bismark
- Extracting count data from mapped reads — Bismark/Giles
- Interpreting methylation data (e.g. differential methylation)

chr	base	coverage	freqC	freqT
scaffold_1	5941	20	1.00	0.00
scaffold_1	5973	20	0.95	0.05
scaffold_1	5982	20	0.95	0.05
scaffold_1	5994	20	0.95	0.05
scaffold_1	5998	8	0.88	0.12
scaffold_1	6012	20	1.00	0.00
scaffold_1	6101	2	1.00	0.00
scaffold_1	6103	2	1.00	0.00
scaffold_1	6278	9	0.89	0.11
scaffold_1	6285	42	0.98	0.02

Bioinformatics (DNA methylation)



Bioinformatics (DNA methylation)

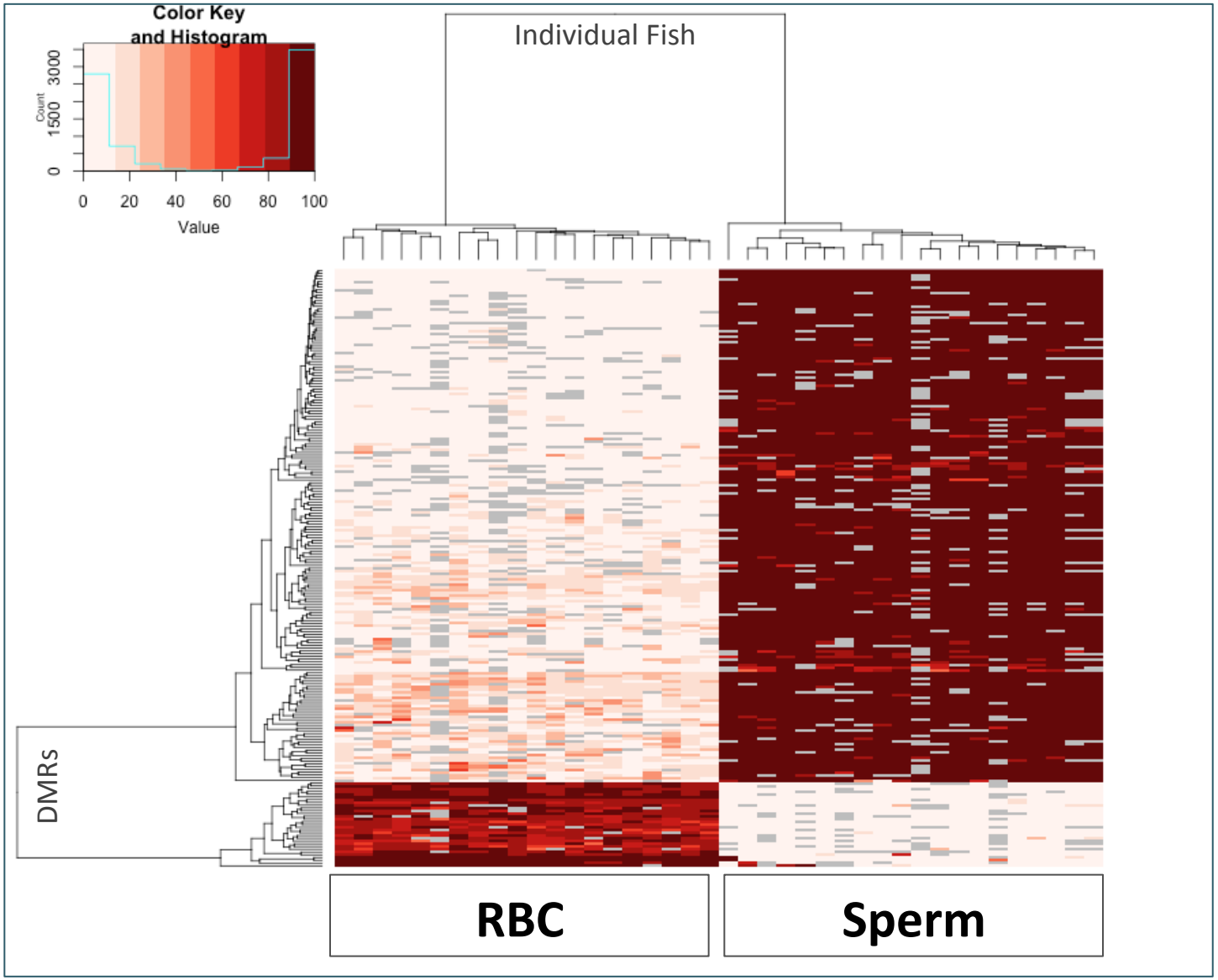


Differential Methylation Analysis

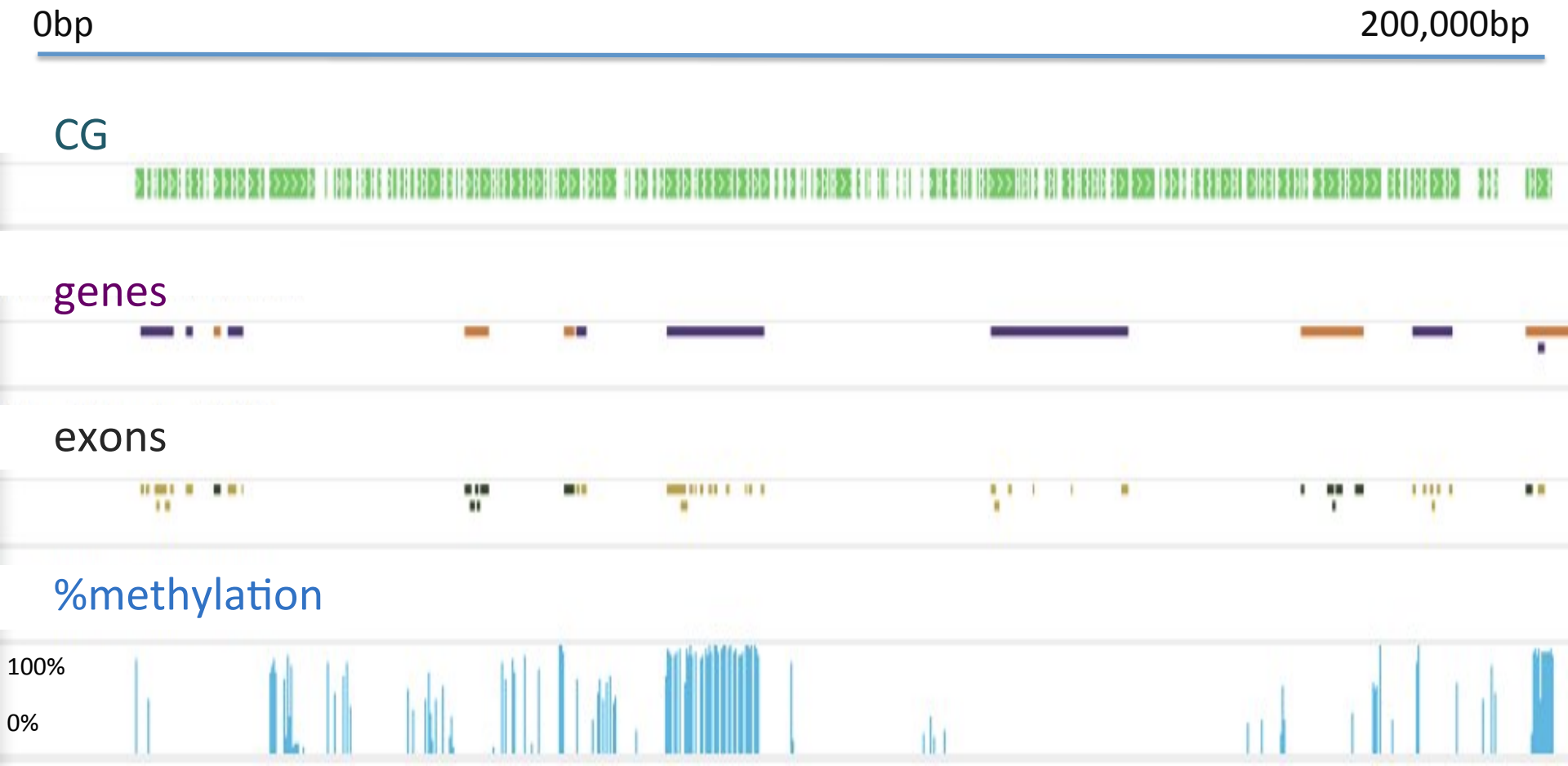
Differential Methylation Analysis

- MethylKit
  - R package
  - Logistic regression
  - Single variable (e.g. treatment v. control)
- MACAU
  - Beta-binomial mixed model
  - Multiple variables including relatedness

Differential Methylation Analysis

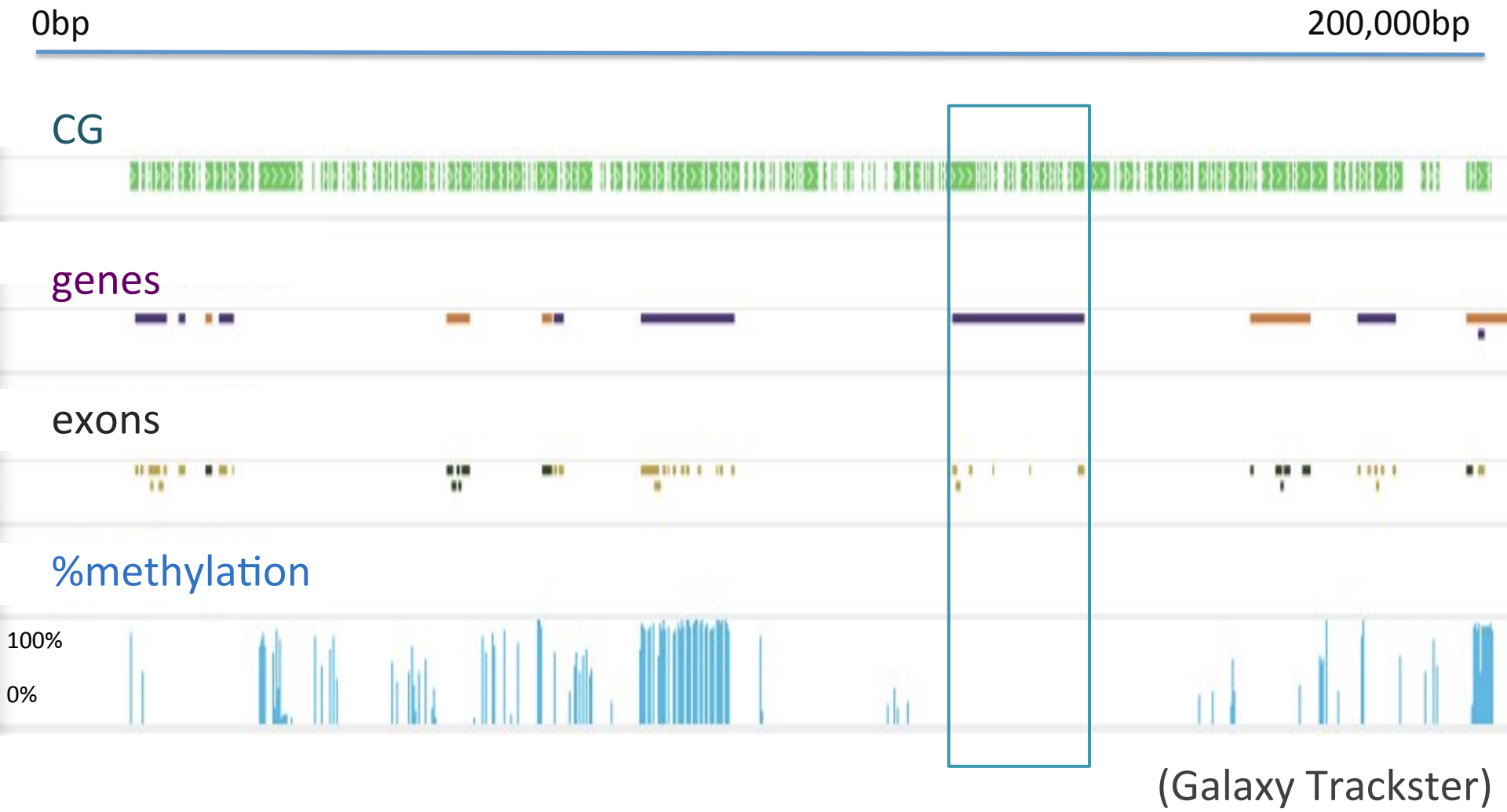


Descriptive Methylome Data



(Galaxy Trackster)

Descriptive Methylome Data



(Galaxy Trackster)

Summary

- It can be hard to work with data that you can't **see**
  - Moving data takes a lot of time and space
  - Formatting data is a constant challenge
  - Software packages are great, but it's always important to understand what they are doing
  - Bisulfite sequencing has unique challenges