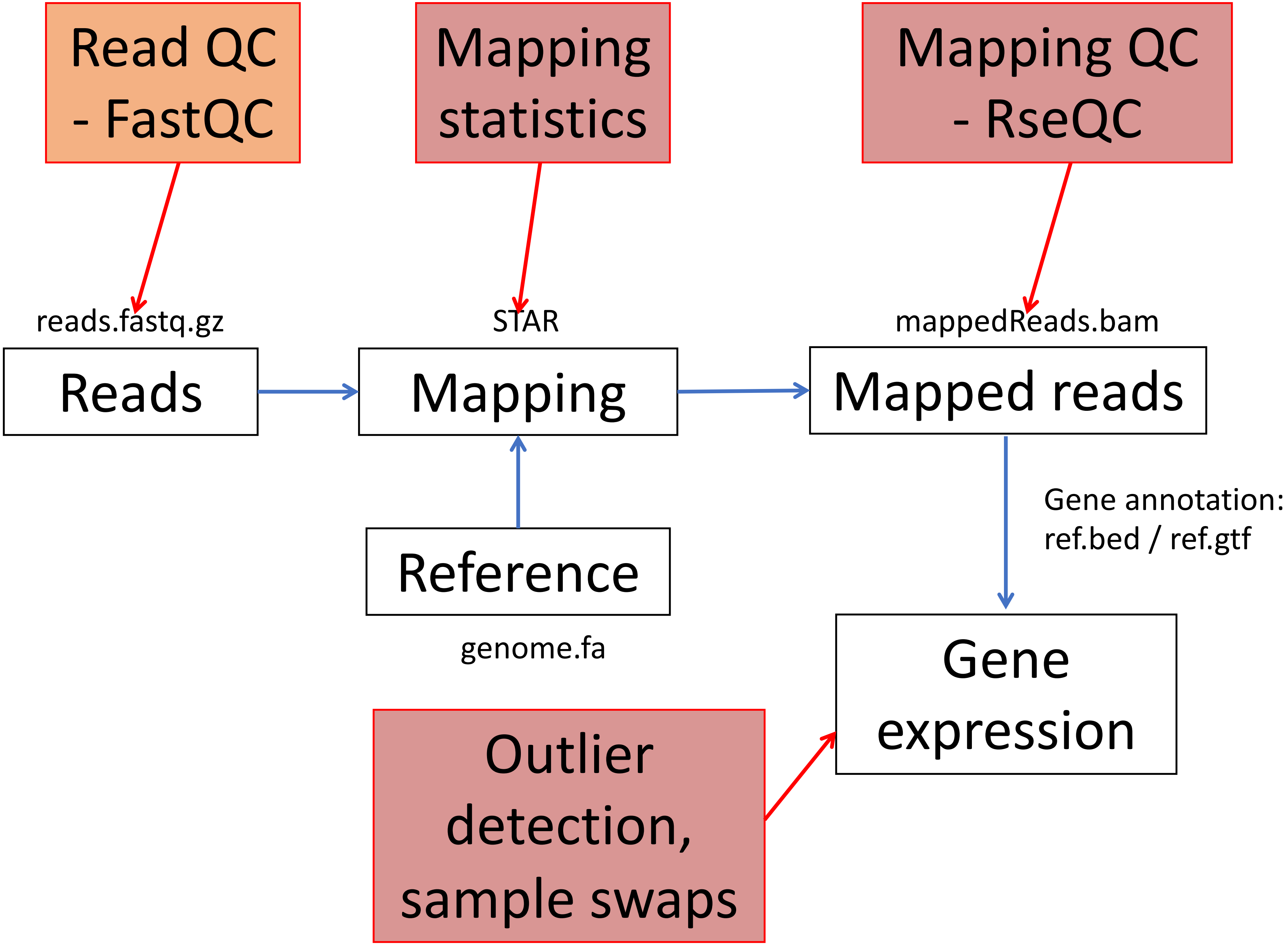
After mapping QC

RNA-seq data analysis

Johan Reimegård | 13-May-2019



Read QC - FastQC

Mapping statistics

Mapping QC - RseQC

reads.fastq.gz

STAR

mappedReads.bam

Reads

Mapping

Mapped reads

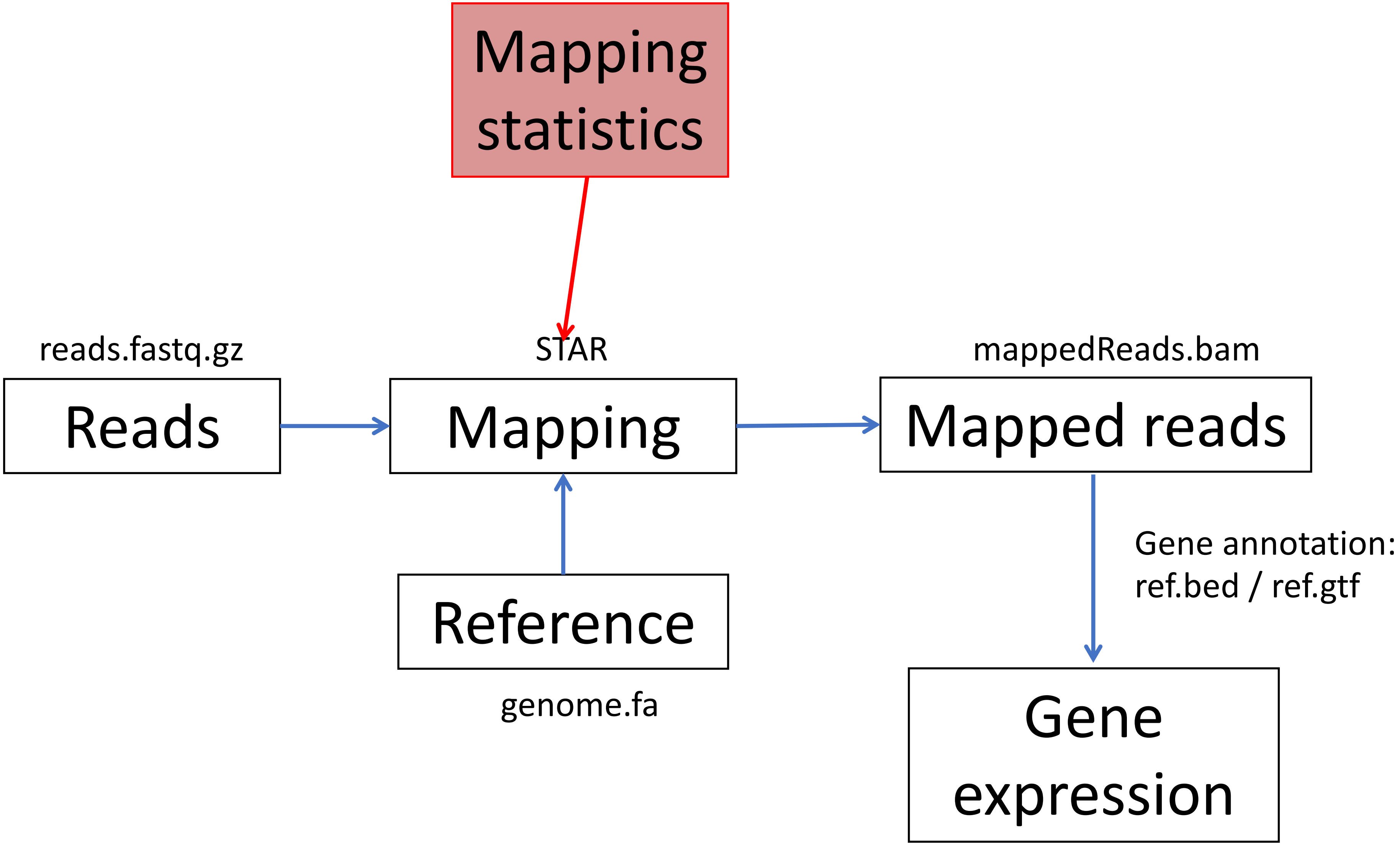
Gene annotation:
ref.bed / ref.gtf

Reference

genome.fa

Gene expression

Outlier detection, sample swaps



Mapping statistics

reads.fastq.gz

Reads

STAR

Mapping

mappedReads.bam

Mapped reads

Reference

genome.fa

Gene annotation:
ref.bed / ref.gtf

Gene expression

Mapping logs – mapping efficiency

- Program specific how the output will be (STAR, Bowtie, BWA, Tophat…)
- Always gives:
    - % uniquely mapping – ideally around 90% for 100 bp reads
    - % multi-mapping – will depend on read length
    - % unmapped – could indicate contaminations, adaptors
- Also statistics on:
    - Mismatches / indels
    - Splice junctions

Star log example

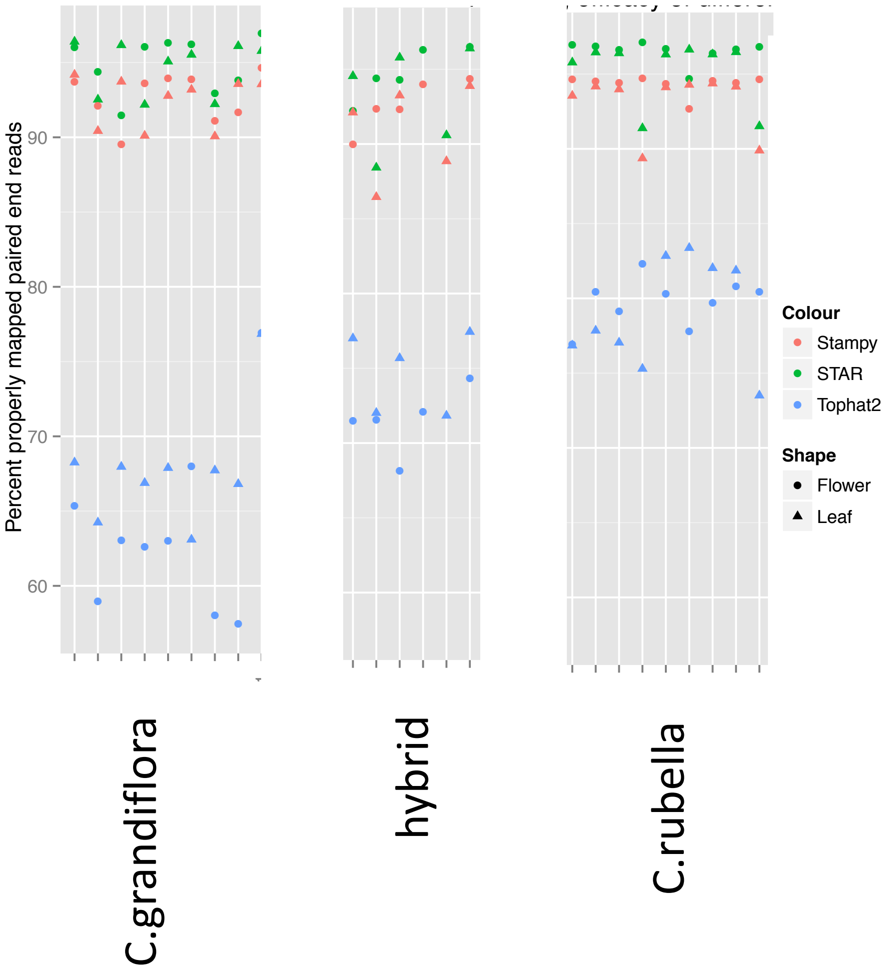
```
[johanr@rackham3 star]$ more sample12_Log.final.out
                                 Started job on |	May 11 20:01:21
                             Started mapping on |	May 11 20:02:59
                                    Finished on |	May 11 20:10:30
       Mapping speed, Million of reads per hour |	211.40

                          Number of input reads |	26483380
                      Average input read length |	202
                                    UNIQUE READS:
                   Uniquely mapped reads number |	23584867
                        Uniquely mapped reads % |	89.06%
                          Average mapped length |	198.57
                       Number of splices: Total |	15591437
            Number of splices: Annotated (sjdb) |	15442151
                       Number of splices: GT/AG |	15453389
                       Number of splices: GC/AG |	110331
                       Number of splices: AT/AC |	13452
               Number of splices: Non-canonical |	14265
                      Mismatch rate per base, % |	0.33%
                         Deletion rate per base |	0.01%
                        Deletion average length |	1.97
                        Insertion rate per base |	0.01%
                       Insertion average length |	1.36
                             MULTI-MAPPING READS:
        Number of reads mapped to multiple loci |	838432
             % of reads mapped to multiple loci |	3.17%
        Number of reads mapped to too many loci |	5600
             % of reads mapped to too many loci |	0.02%
                                  UNMAPPED READS:
       % of reads unmapped: too many mismatches |	0.00%
                 % of reads unmapped: too short |	7.73%
                     % of reads unmapped: other |	0.03%
```

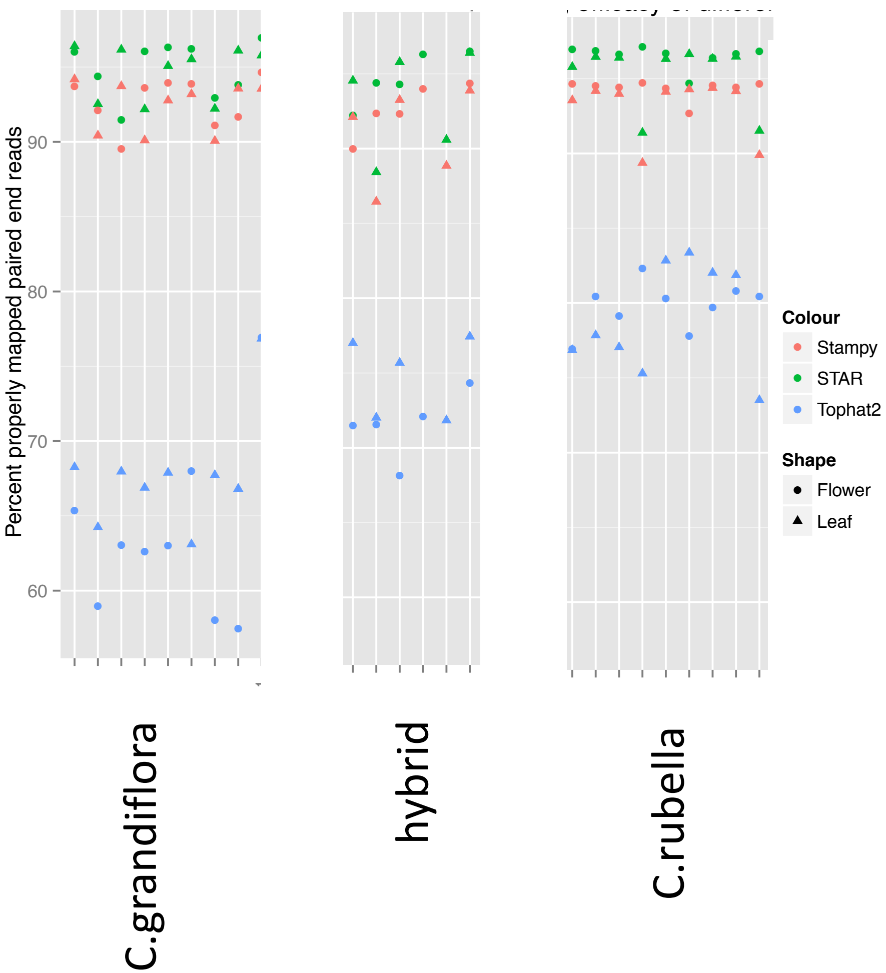
Hisat 2 log example

```
• 13229276 reads; of these:
•   13229276 (100.00%) were paired; of these:
•     2258930 (17.08%) aligned concordantly 0 times
•     10385753 (78.51%) aligned concordantly exactly 1 time
•     584593 (4.42%) aligned concordantly >1 times
•     ----
•     2258930 pairs aligned concordantly 0 times; of these:
•       271241 (12.01%) aligned discordantly 1 time
•     ----
•     1987689 pairs aligned 0 times concordantly or
  discordantly; of these:
•       3975378 mates make up the pairs; of these:
•         2915792 (73.35%) aligned 0 times
•         963693 (24.24%) aligned exactly 1 time
•         95893 (2.41%) aligned >1 times
• 88.98% overall alignment rate
```

Means that you can compare how well different programs behaves on different samples

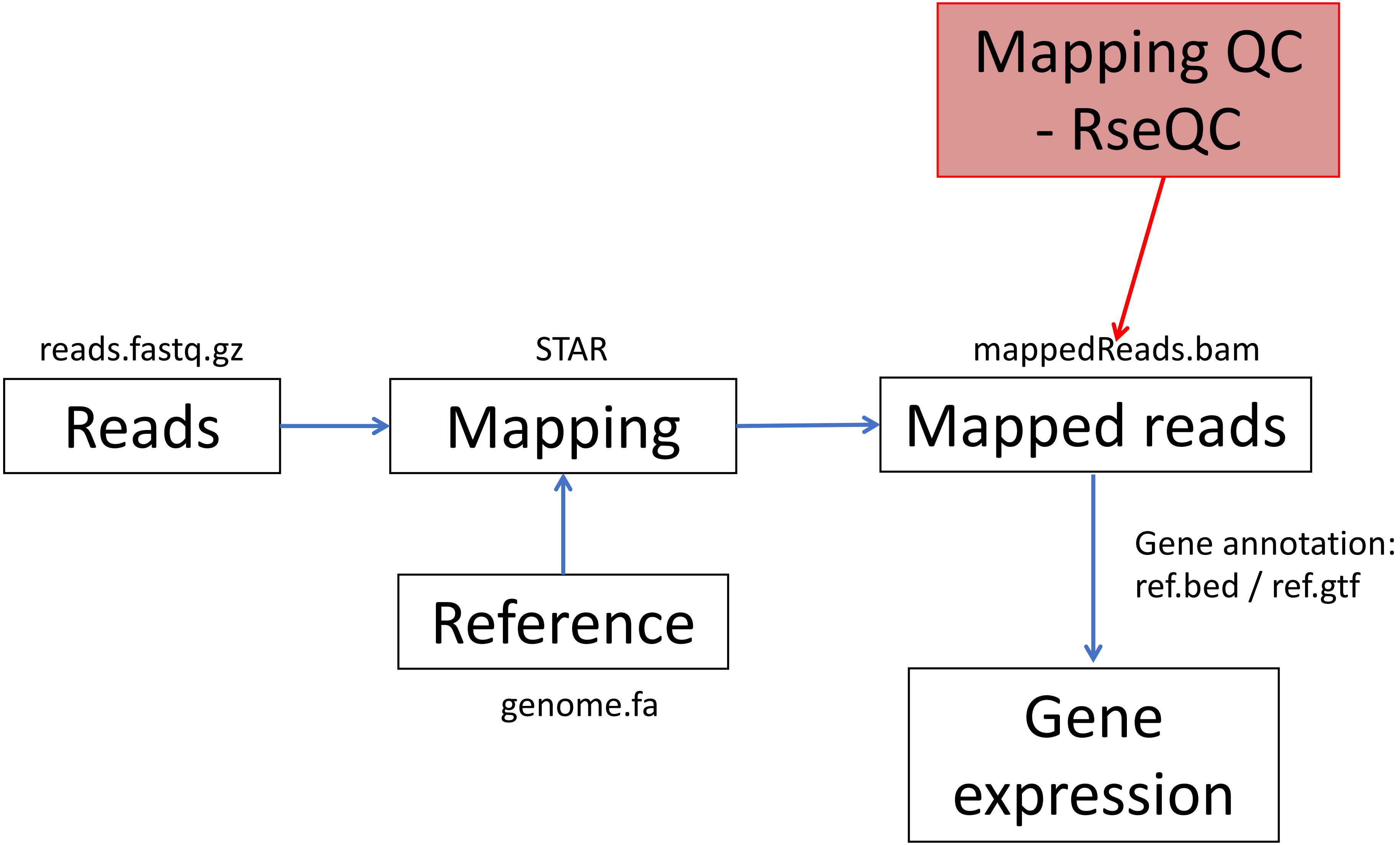


More variation when using top hat 2 with default settings than when using STAR or Stampy with default setting



Bad mapping – what to do?

- First step – try to figure out why it failed. With the use of FastQC/RseQC/Mapping logs.
    - Perhaps also look for contaminant species
    - Redo library prep controlling for possible errors
- Low mapping, but not completely failed.
    - Figure out why!
    - Is it equal for all samples?
    - Could it introduce any bias in the data?



Mapping QC - RseQC

reads.fastq.gz

Reads

STAR

Mapping

mappedReads.bam

Mapped reads

Reference

genome.fa

Gene annotation:
ref.bed / ref.gtf

Gene expression

After mapping - RseQC package

- General sequence QC:
  - sequence quality
  - nucleotide composition bias
  - PCR bias and
  - GC bias
- RNA-seq specific QC:
  - evaluate sequencing saturation
  - mapped reads distribution
  - coverage uniformity
  - strand specificity
  - Etc..
- Some tools for file manipulations

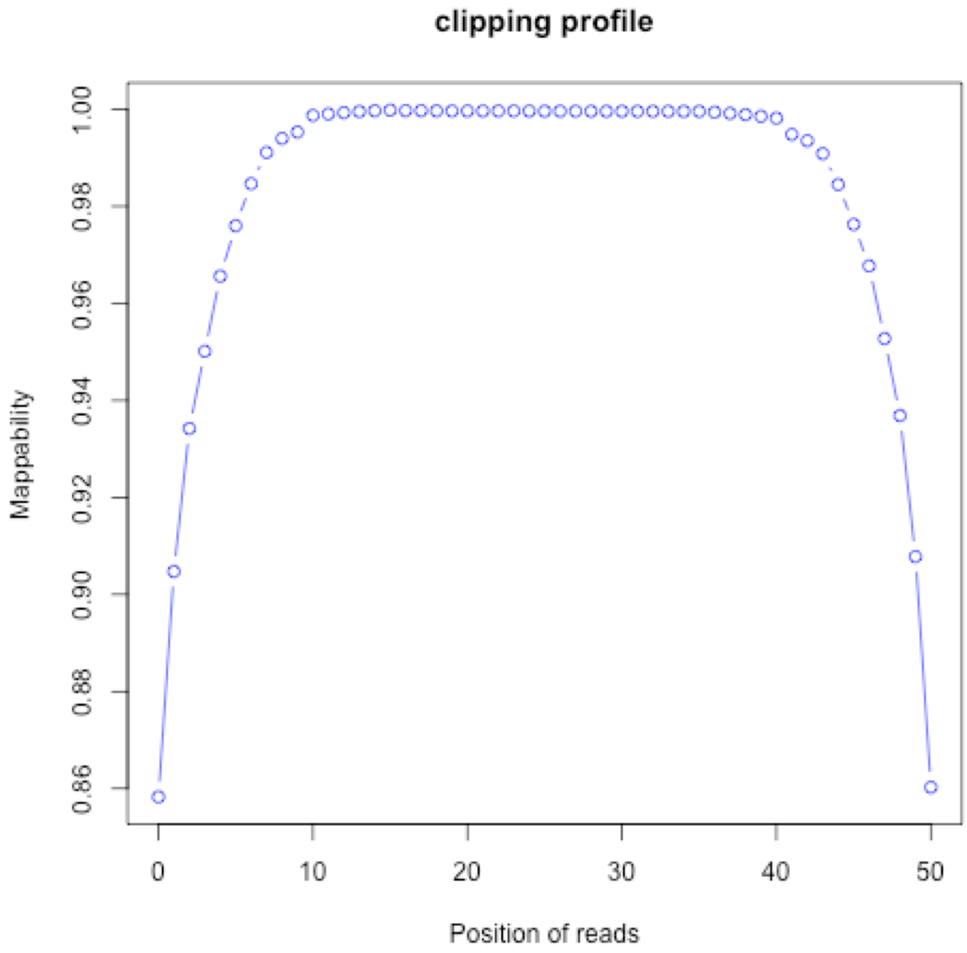
Code

```
$ module load bioinfo-tools
$ module load rseqc/2.4

$ geneBody_coverage.py –r
ref.bed12 –i mappedReads.bam -o
genecoverage
```

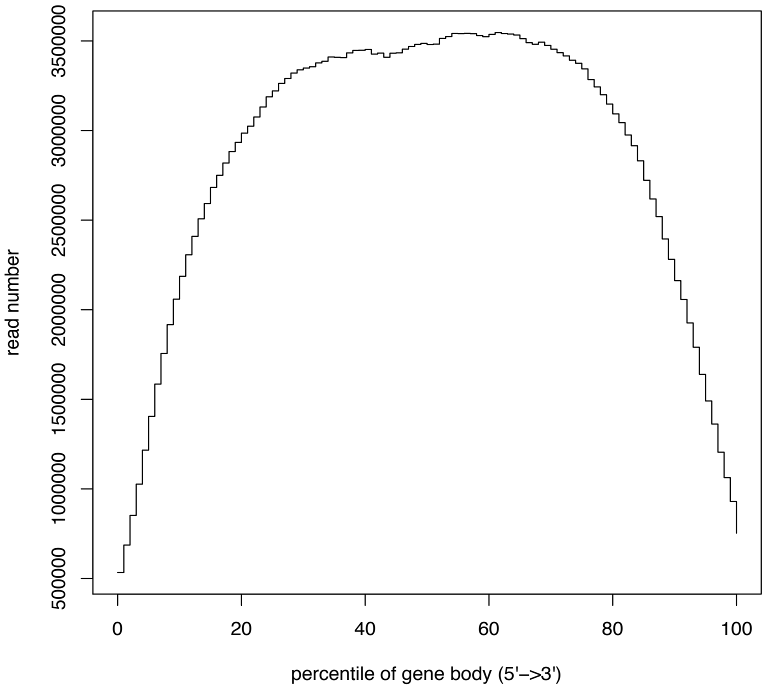
http://rseqc.sourceforge.net/

Soft clipping - clipping_profile.py

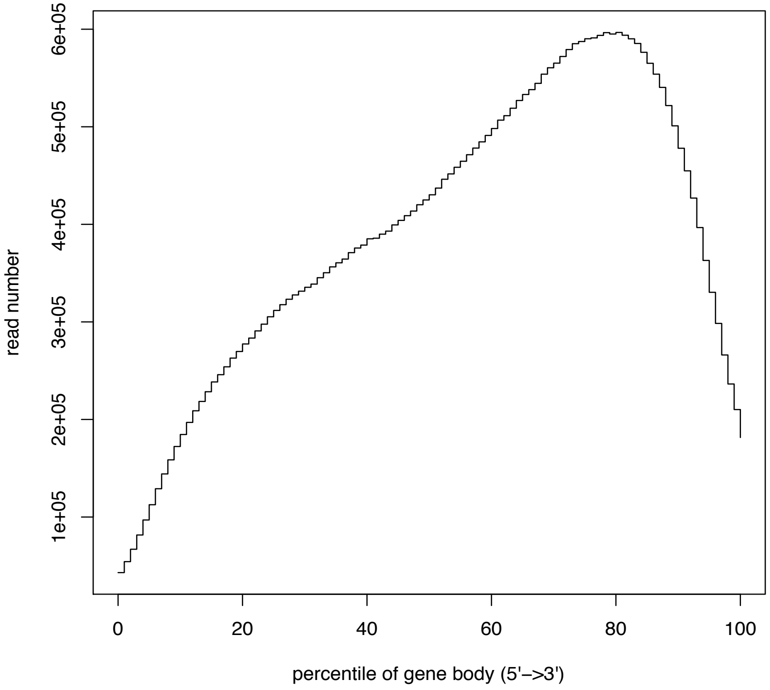


Gene coverage - geneBody_coverage.py

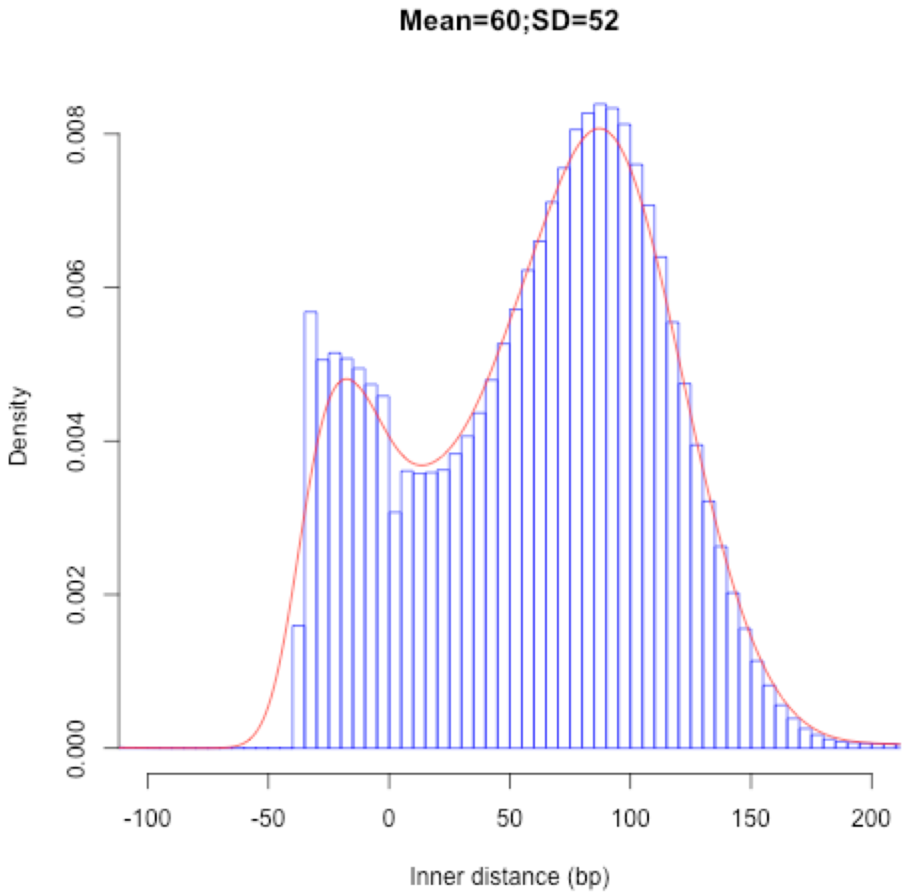
Not degraded



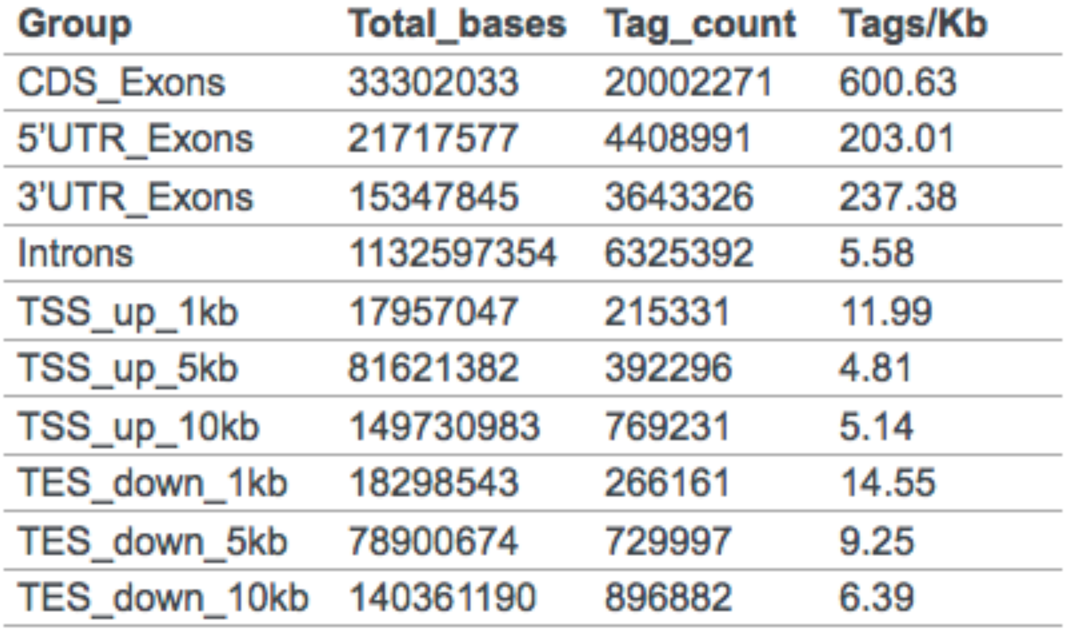
Degraded



Distance between PE-reads - inner_distance.py

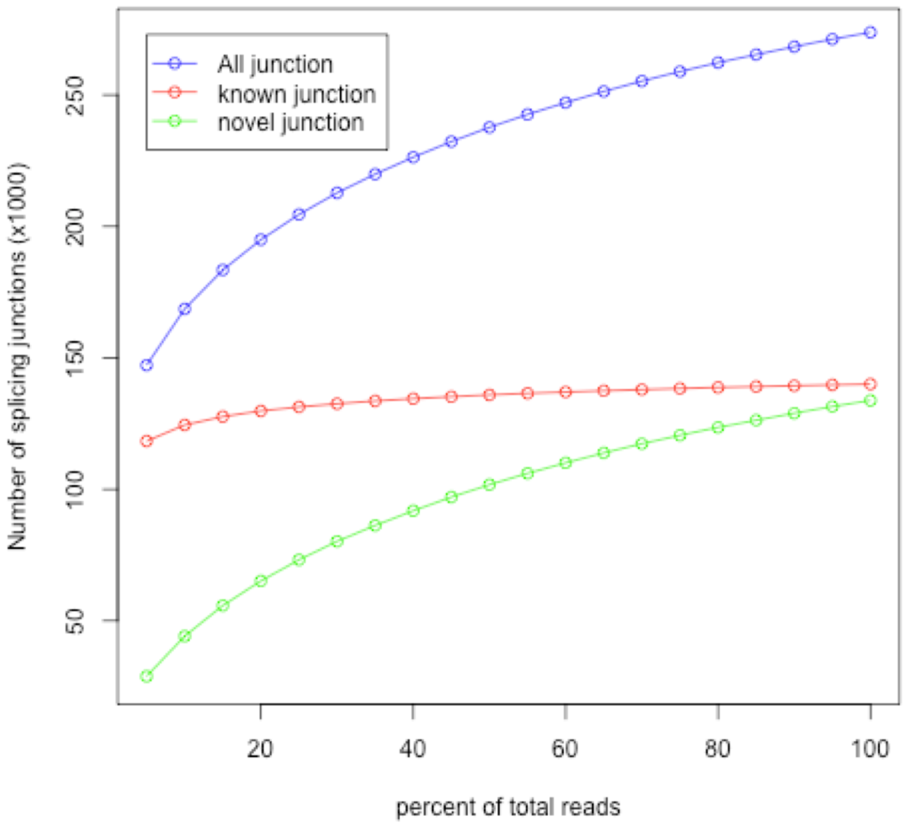


Where in the genome do your reads map? - read_distribution.py

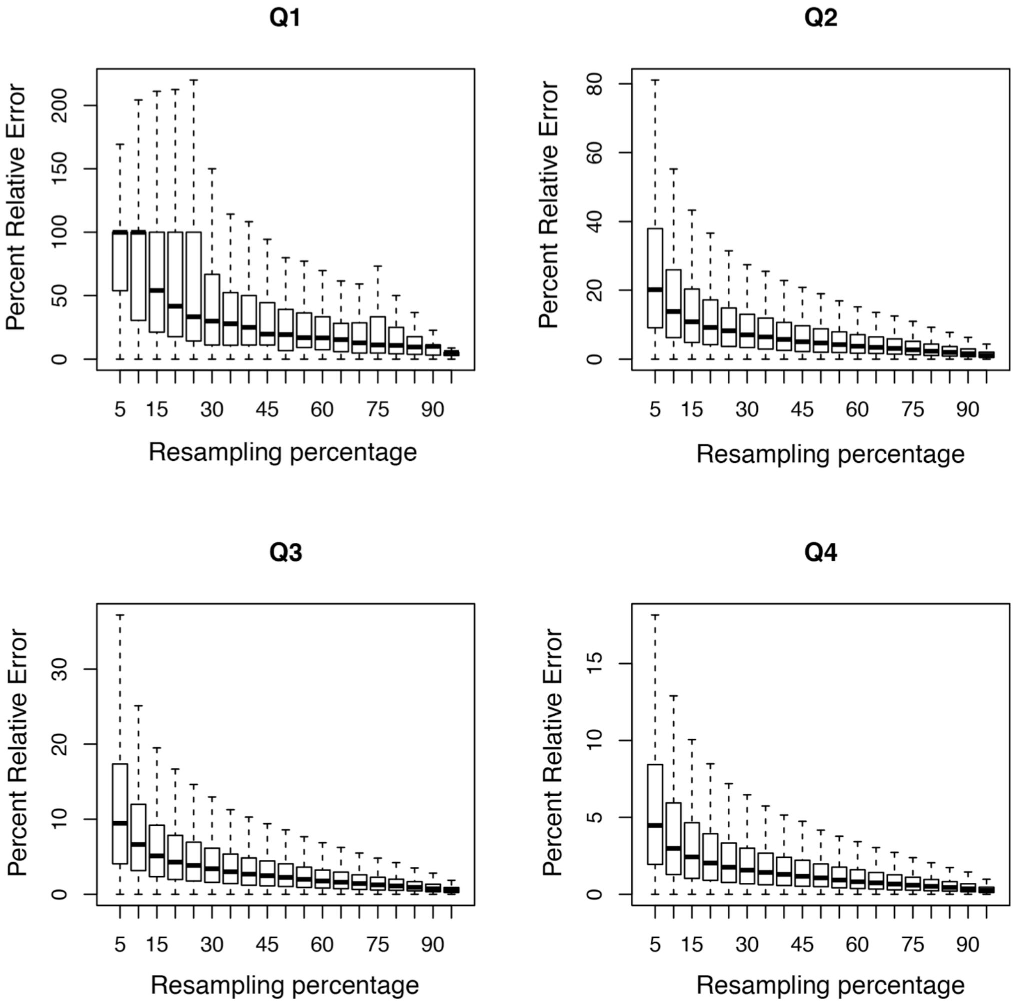


Group	Total_bases	Tag_count	Tags/Kb
CDS_Exons	33302033	20002271	600.63
5'UTR_Exons	21717577	4408991	203.01
3'UTR_Exons	15347845	3643326	237.38
Introns	1132597354	6325392	5.58
TSS_up_1kb	17957047	215331	11.99
TSS_up_5kb	81621382	392296	4.81
TSS_up_10kb	149730983	769231	5.14
TES_down_1kb	18298543	266161	14.55
TES_down_5kb	78900674	729997	9.25
TES_down_10kb	140361190	896882	6.39

Known and novel splice junctions – junction_saturation.py or junction_annotation.py



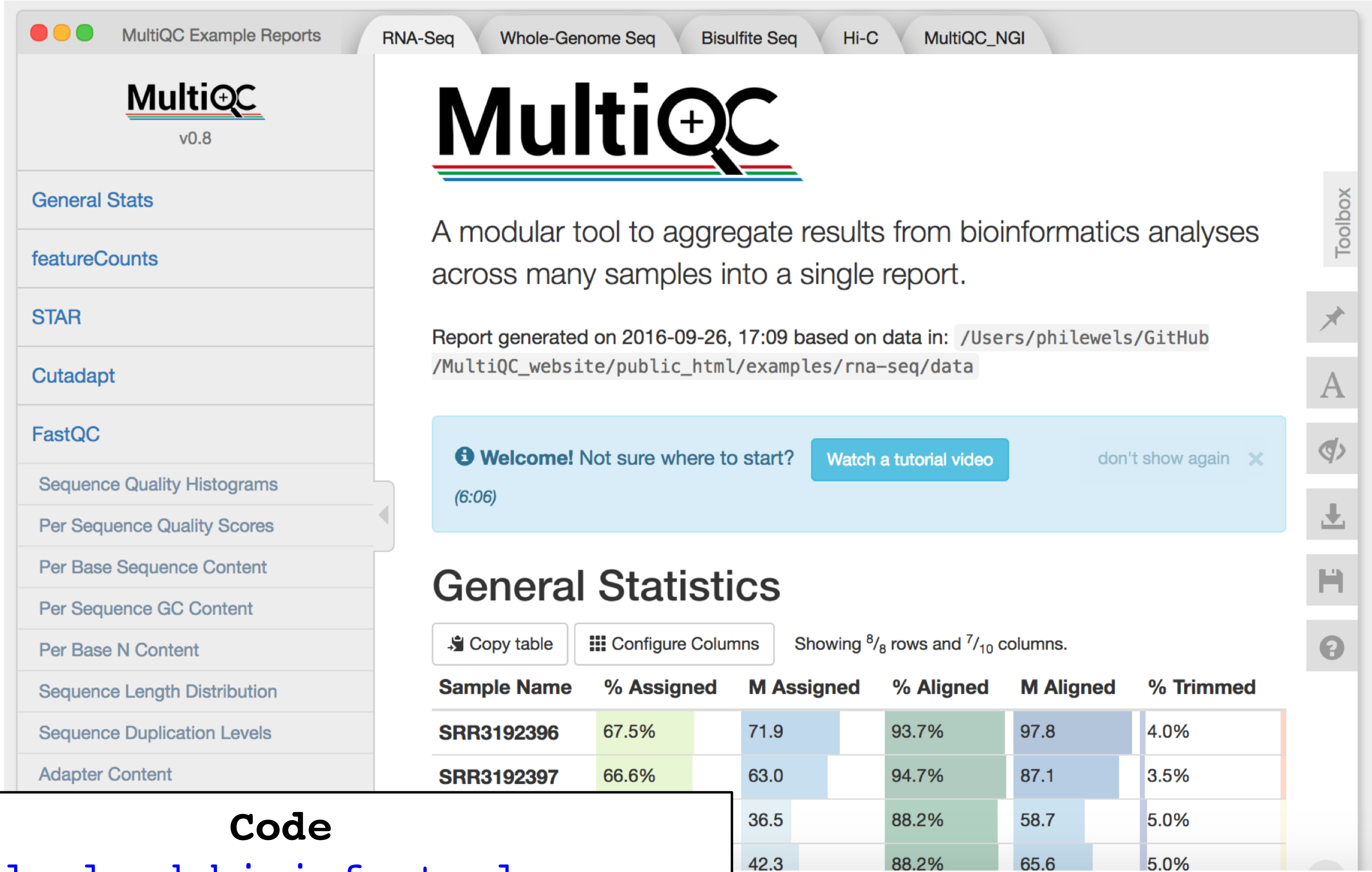
Gene detection subsampling - RPKM_saturation.py How deep do you need to sequence?



Bad RseQC output – what to do?

- Try to figure out what went wrong.
  - Redo library prep controlling for possible errors
  - Is it equal for all samples?
  - Could it introduce any bias in the data?
- RNA-degradation in some samples
  - Possible to use a region at 3’ end for expression estimates.

MultiQC – summary of QC stats



A modular tool to aggregate results from bioinformatics analyses across many samples into a single report.

Report generated on 2016-09-26, 17:09 based on data in: `/Users/philewels/GitHub/MultiQC_website/public_html/examples/rna-seq/data`

Welcome! Not sure where to start? (6:06)

General Statistics

Showing $^8/_8$ rows and $^7/_{10}$ columns.

Sample Name	% Assigned	M Assigned	% Aligned	M Aligned	% Trimmed
SRR3192396	67.5%	71.9	93.7%	97.8	4.0%
SRR3192397	66.6%	63.0	94.7%	87.1	3.5%
		36.5	88.2%	58.7	5.0%
		42.3	88.2%	65.6	5.0%

Code

```
$ module load bioinfo-tools
$ module load MultiQC
$ multiqc .
```

(http://multiqc.info/)

Thank you. Questions?

Johan Reimegård | 13-May-2019