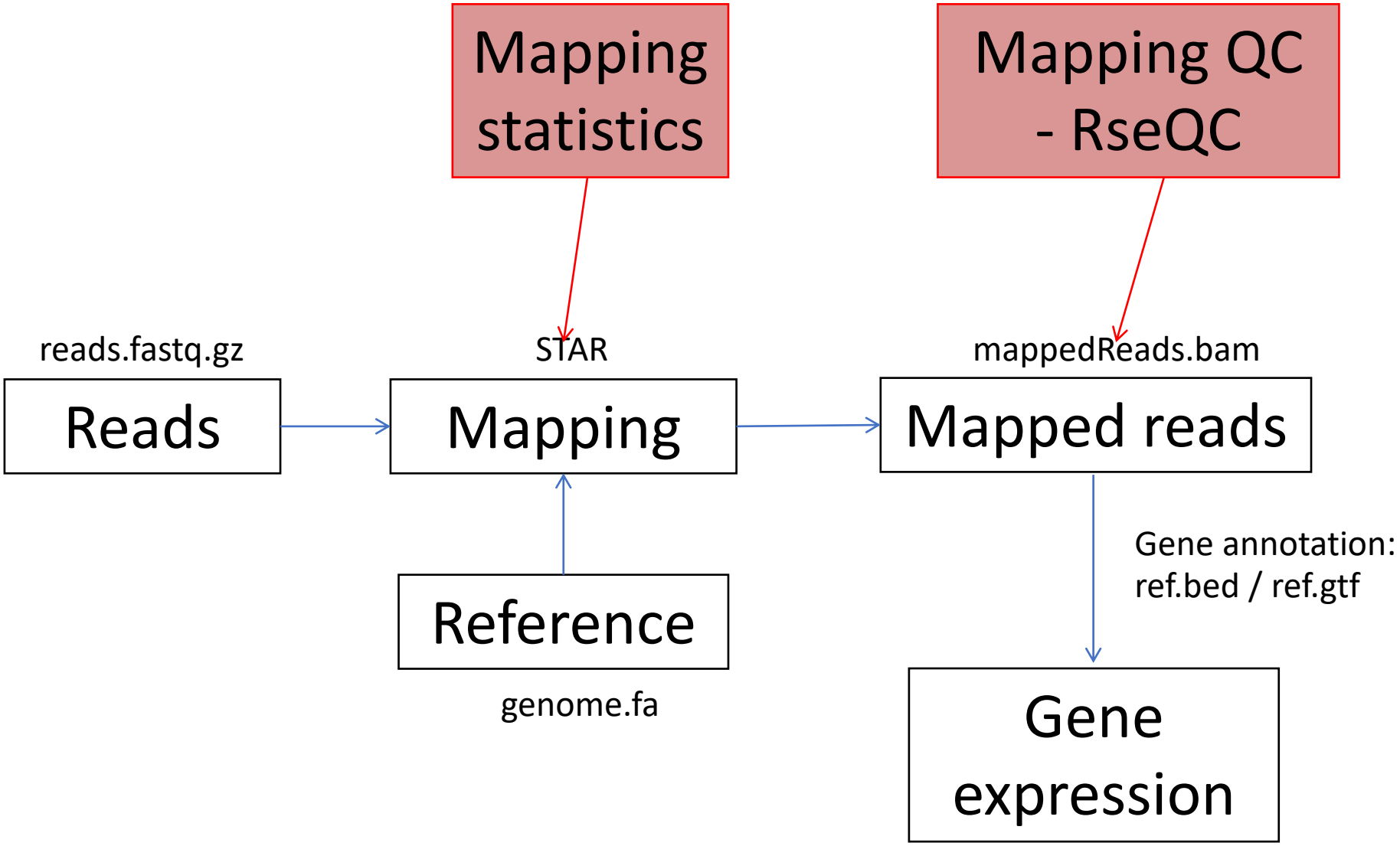
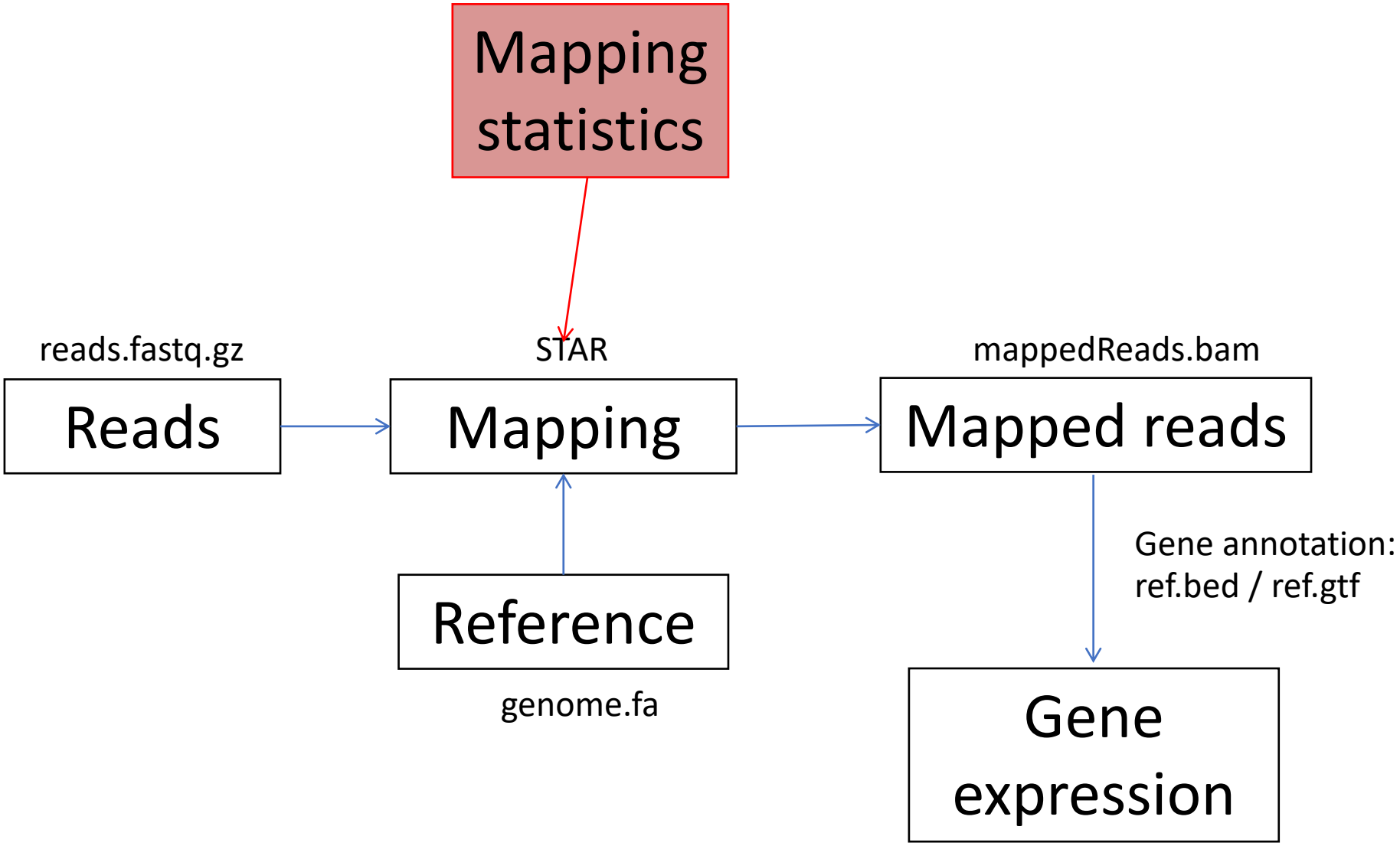
After mapping QC

RNA-seq data analysis

Johan Reimegård | 15-November-2021


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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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

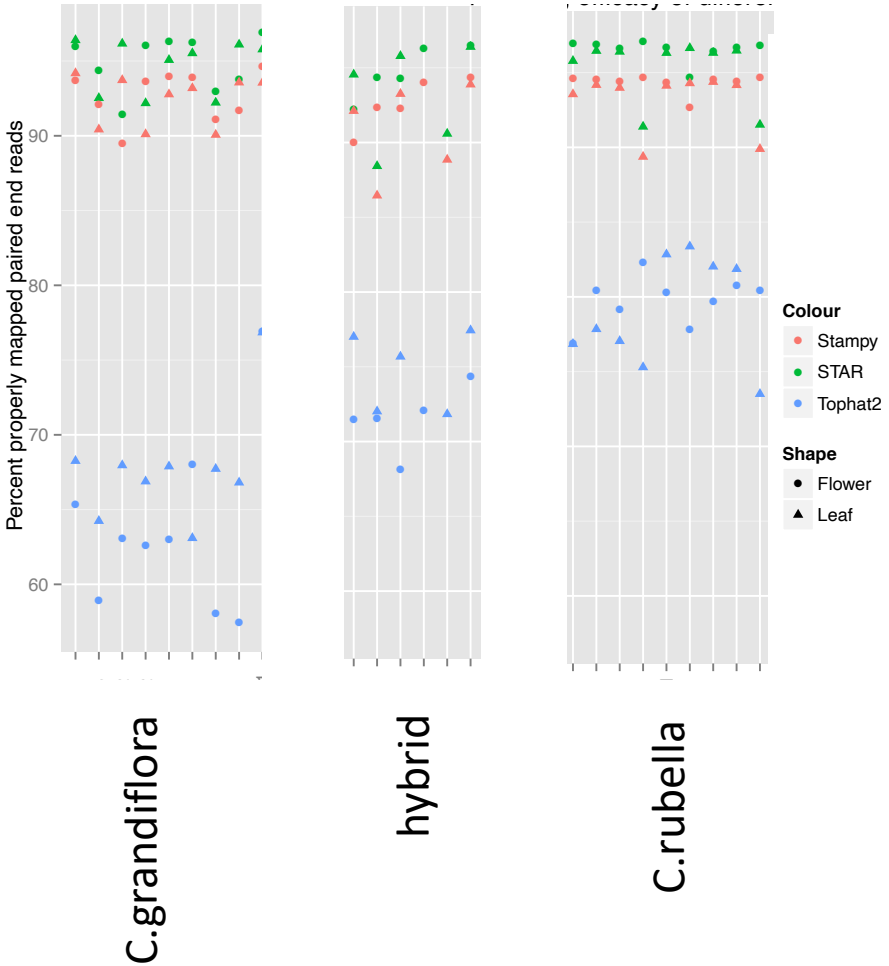
```
[johanr@rackham3 star]$ more sample12_Log.final.out
                          Started job on | May 11 20:01:21
                      Started mapping on | May 11 20:02:59
                             Finished on | May 11 20:10:30
Mapping speed, Million of reads per hour | 211.40


                    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%
```

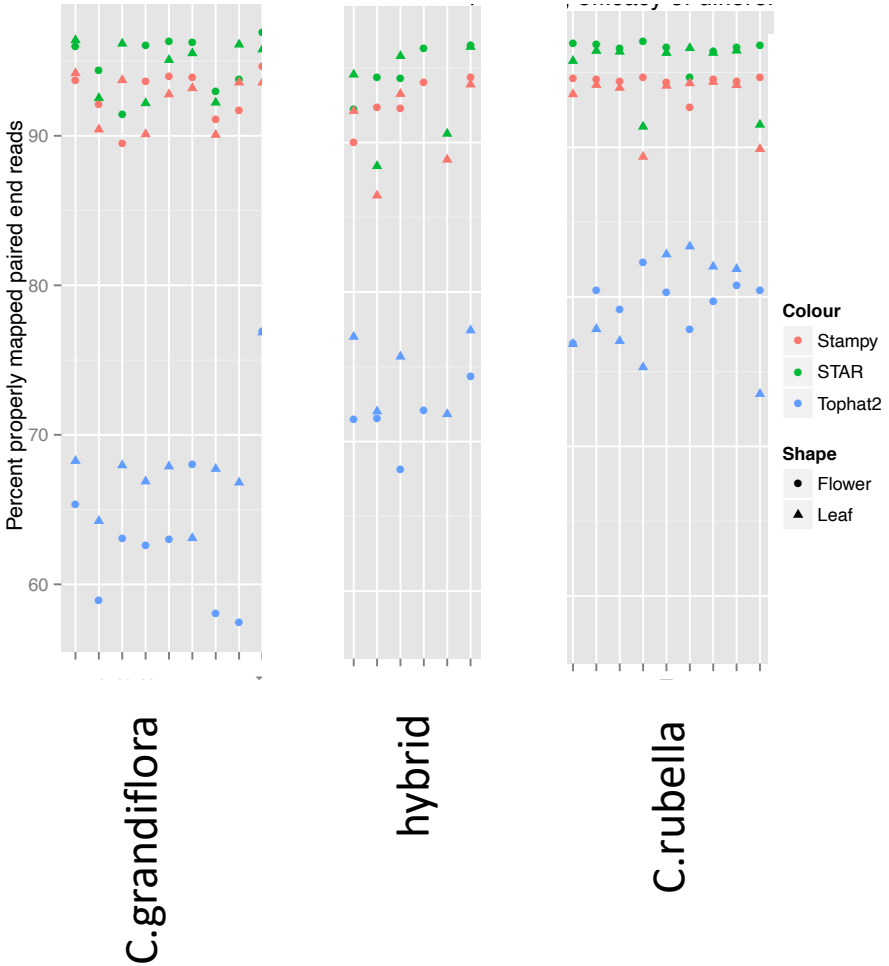
Hisat2 log example

```
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
```

Map log can be used to compare how well different programs work on different samples

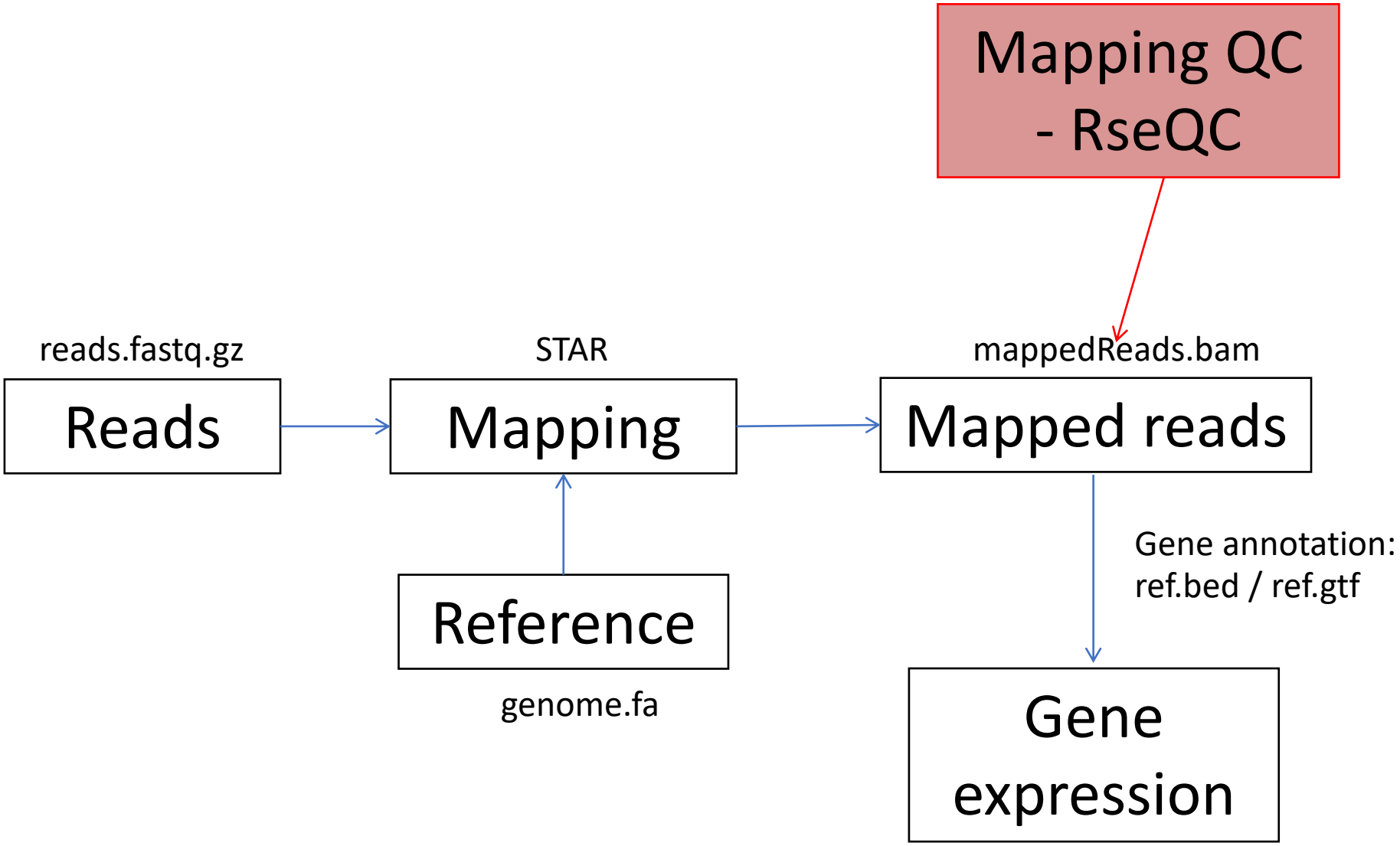


More variation when using Tophat2 with default settings than when using STAR or Stampy with default setting



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?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After mapping - RseQC package

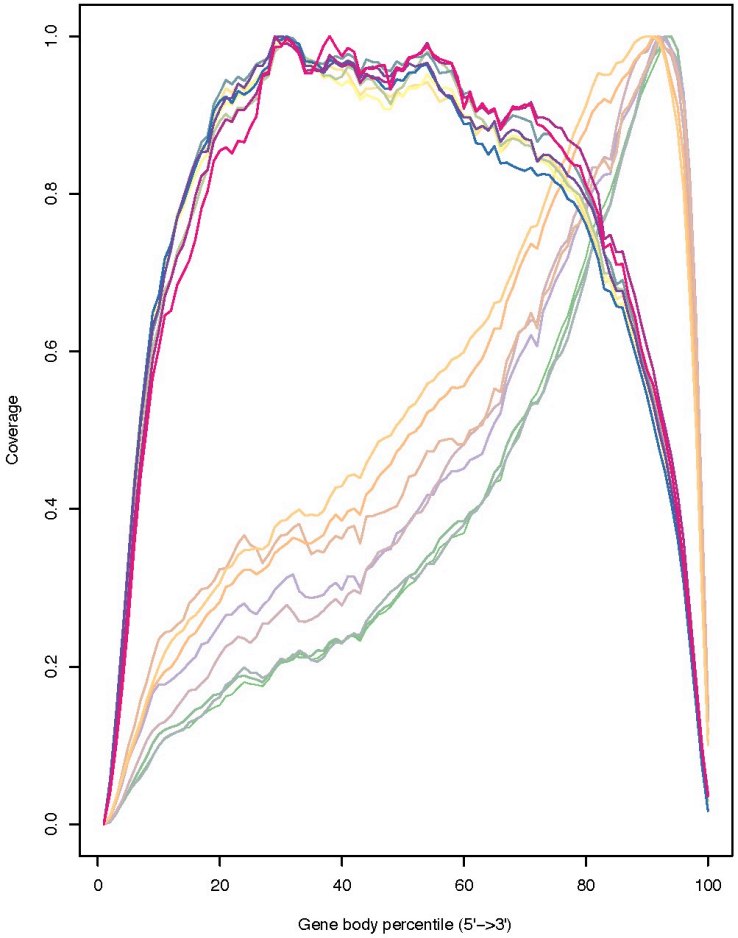
- General sequence QC:
  - sequence quality
  - nucleotide composition bias
  - PCR bias and
  - GC bias
- RNA-seq specific QC:
  - evaluate sequencing saturation
  - mapped reads distribution
  - coverage uniformity
  - strand specificity
  - Etc..
- Some tools for file manipulations

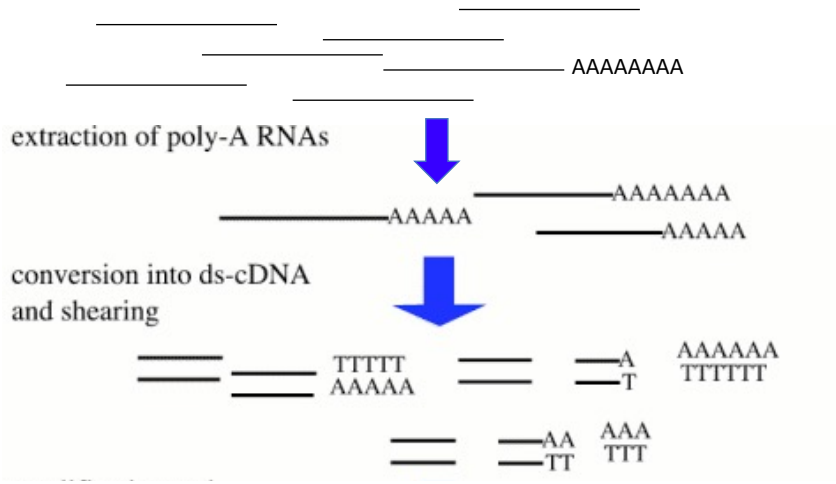
Code

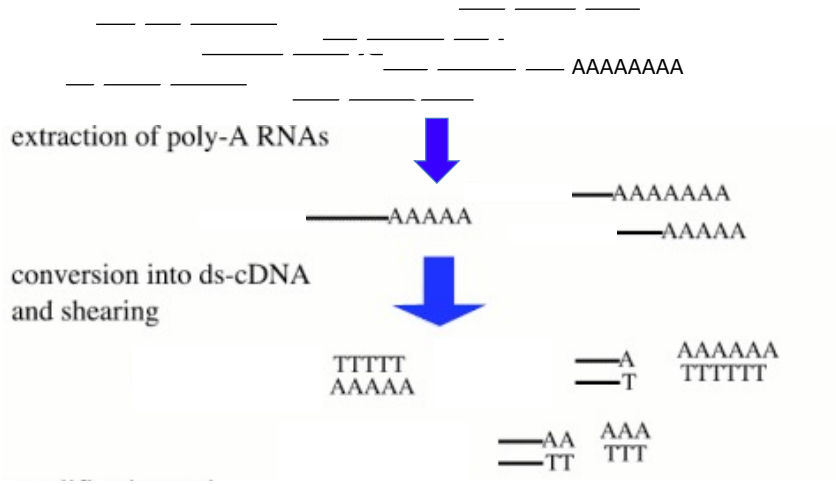
```
$ geneBody_coverage.py –r
ref.bed12 –i mappedReads.bam -o
genecoverage
```

http://rseqc.sourceforge.net/

Gene coverage - geneBody_coverage.py



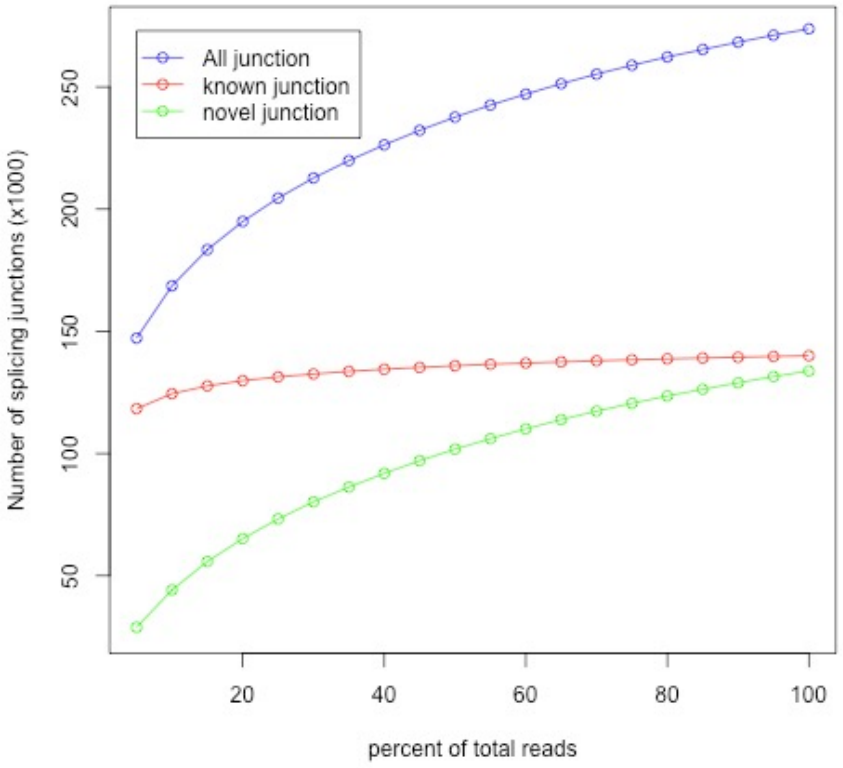


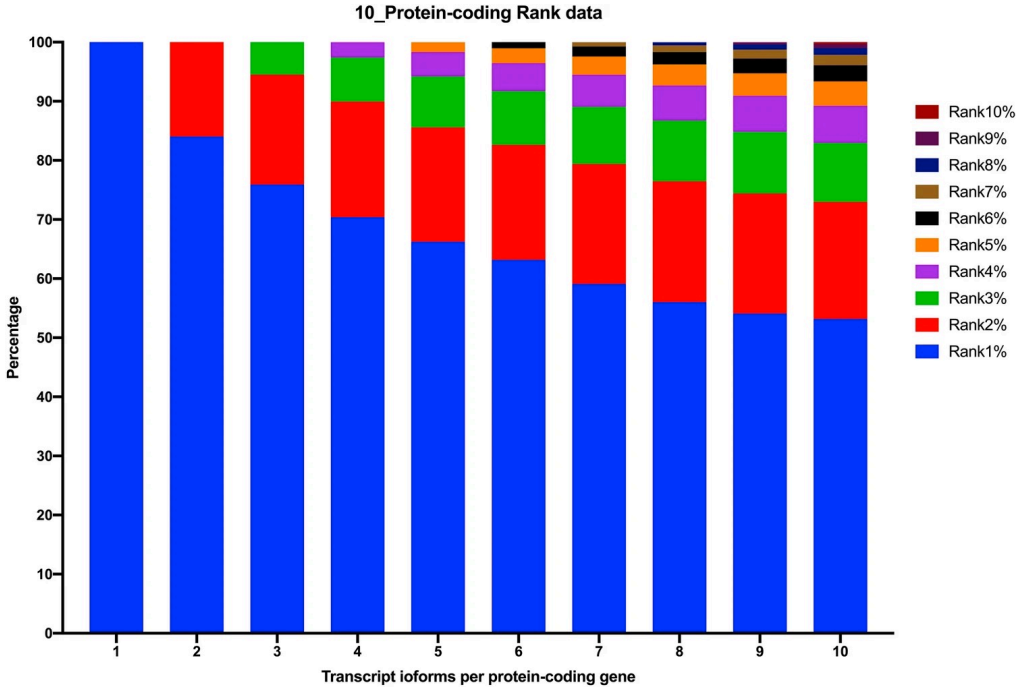


Where in the genome do your reads map? - read_distribution.py

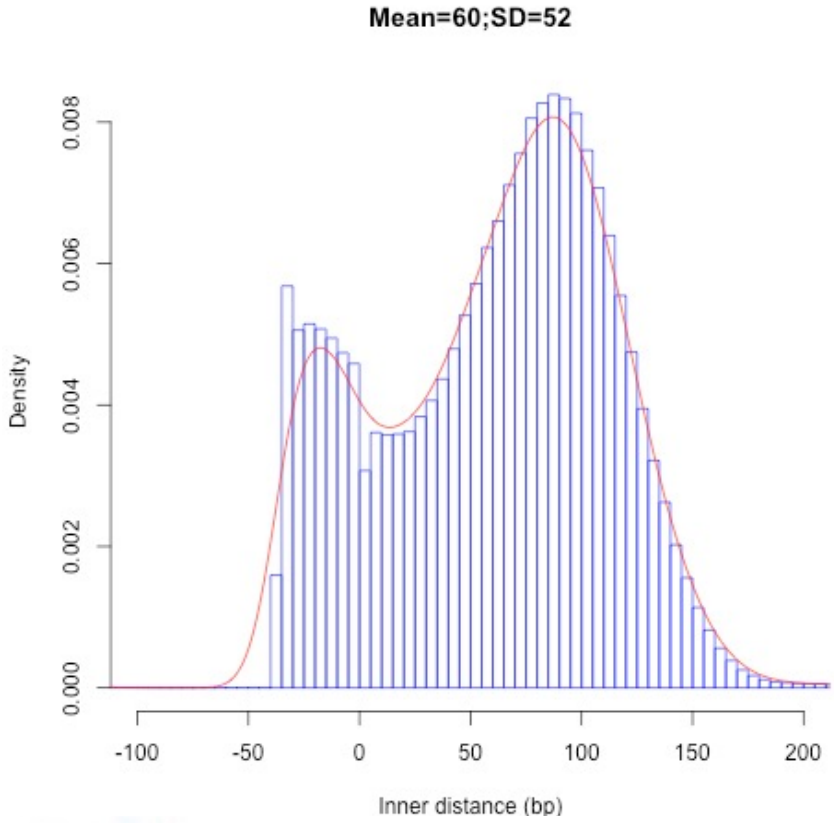
Group	Total_bases	Tag_count	Tags/Kb
CDS_Exons	33302033	20002271	600.63
5'UTR_Exons	21717577	4408991	203.01
3'UTR_Exons	15347845	3643326	237.38
Introns	1132597354	6325392	5.58
TSS_up_1kb	17957047	215331	11.99
TSS_up_5kb	81621382	392296	4.81
TSS_up_10kb	149730983	769231	5.14
TES_down_1kb	18298543	266161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





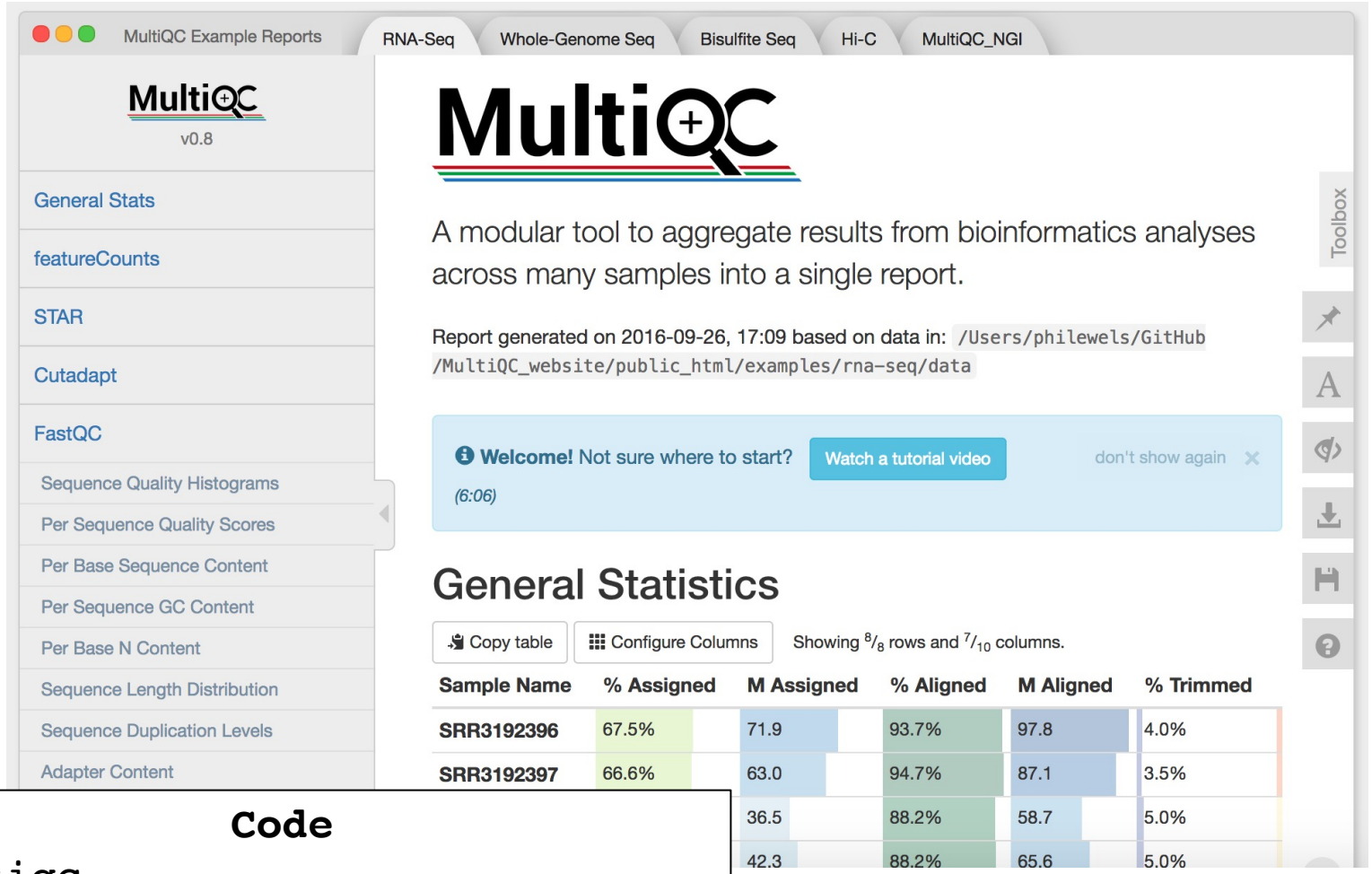
Distance between PE-reads - inner_distance.py



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



Code

```
$ multiqc .
```

(http://multiqc.info/)

Thank you.

Johan Reimegård | 30-November-2020