Analysis of RNA-seq data : answers to questions

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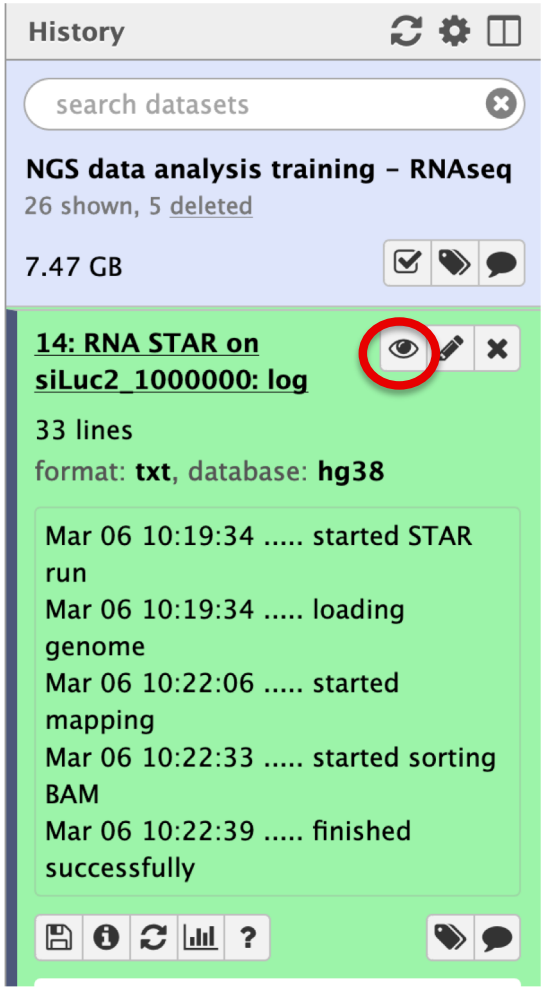
Question 1

- Number of uniquely mapped reads

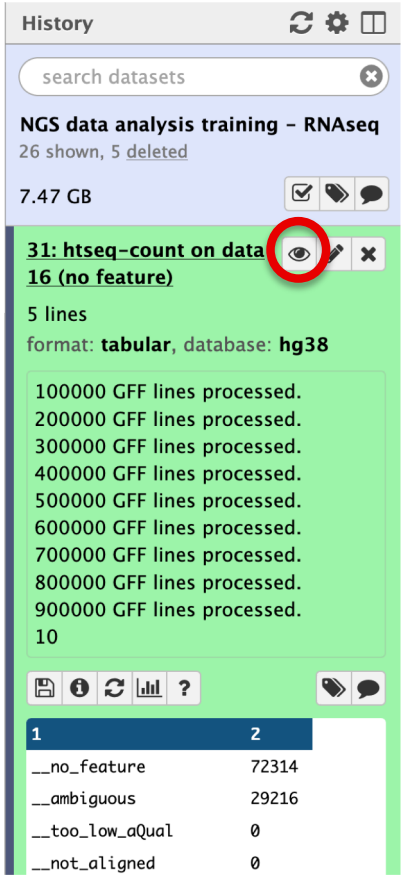
Started job on	Mar 06 10:19:34
Started mapping on	Mar 06 10:22:06
Finished on	Mar 06 10:22:39
Mapping speed, Million of reads per hour	109.09
Number of input reads	1000000
Average input read length	50
UNIQUE READS:	
Uniquely mapped reads number	852838
Uniquely mapped reads %	85.28%
Average mapped length	49.83
Number of splices: Total	137420
Number of splices: Annotated (sjdb)	136195
Number of splices: GT/AG	136013
Number of splices: GC/AG	1157
Number of splices: AT/AC	111
Number of splices: Non-canonical	139
Mismatch rate per base, %	0.15%
Deletion rate per base	0.01%
Deletion average length	1.60
Insertion rate per base	0.00%
Insertion average length	1.29
MULTI-MAPPING READS:	
Number of reads mapped to multiple loci	133764
% of reads mapped to multiple loci	13.38%
Number of reads mapped to too many loci	3843
% of reads mapped to too many loci	0.38%
UNMAPPED READS:	
% of reads unmapped: too many mismatches	0.00%
% of reads unmapped: too short	0.73%
% of reads unmapped: other	0.22%
CHIMERIC READS:	
Number of chimeric reads	0
% of chimeric reads	0.00%



Question 1

- No feature reads
  - Number
    - 72314
  - Proportion :
    - 72314*100/852838 = 8.48
- Ambiguous reads
  - Number
    - 29216
  - Proportion
    - 29216*100/852838 = 3.43

1	2
__no_feature	72314
__ambiguous	29216
__too_low_aQual	0
__not_aligned	0
__alignment_not_unique	408248



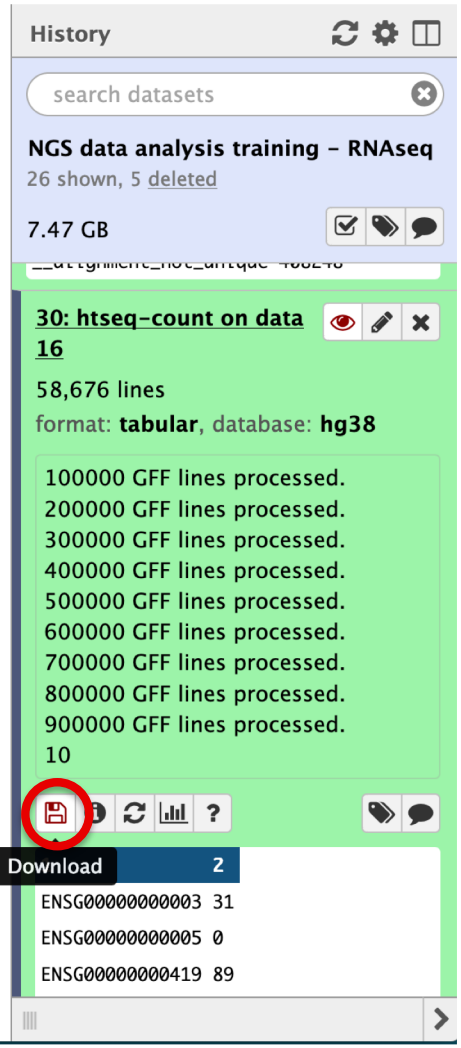
Question 1

- Proportion of reads among uniquely aligned reads
  - Assigned : 100-8.48-3.43= 88.09 %
  - No feature : 8.48 %
  - Ambiguous : 3.43 %

Question 1

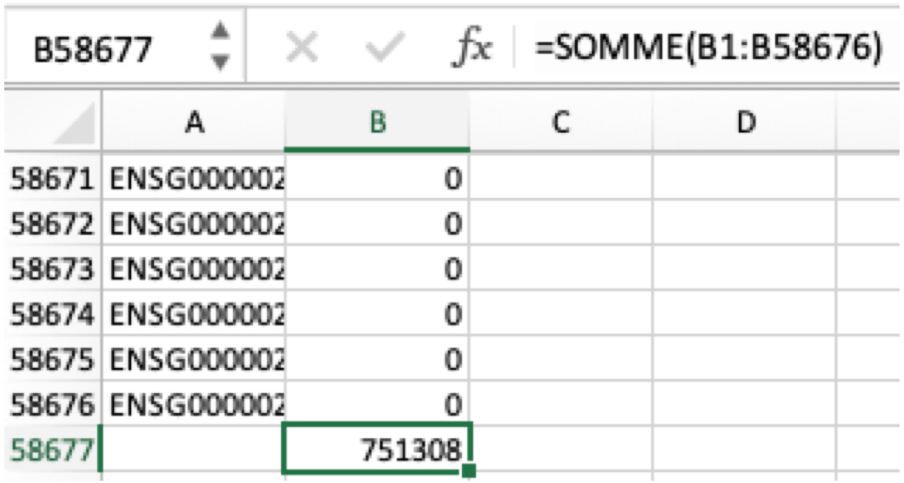
- Number of assigned reads

1	2
ENSG00000000003	31
ENSG00000000005	0
ENSG00000000419	89
ENSG00000000457	18
ENSG00000000460	55
ENSG00000000938	0
ENSG00000000971	3
ENSG00000001036	66
ENSG00000001084	50
ENSG00000001167	38
ENSG00000001460	6
ENSG00000001461	18
ENSG00000001497	69
ENSG00000001561	2
ENSG00000001617	2
ENSG00000001626	0
ENSG00000001629	53
ENSG00000001630	5
ENSG00000001631	3
ENSG00000002016	6
ENSG00000002079	0
ENSG00000002330	27
ENSG00000002549	68
ENSG00000002586	123
ENSG00000002587	1
ext=tabular 0000002726	0



Question 1

- Number of assigned reads
  - Open the downloaded file with excel
  - Calculate the total number of reads in the second column



→ Number of assigned reads = 751308

→ Proportion of assigned reads = 751308 *100/852838 = 88.09

Number of assigned reads
= number of uniquely aligned reads – number of no feature reads – number of ambiguous reads
= 852838 – 72314 – 29216 = 751308

Question 2

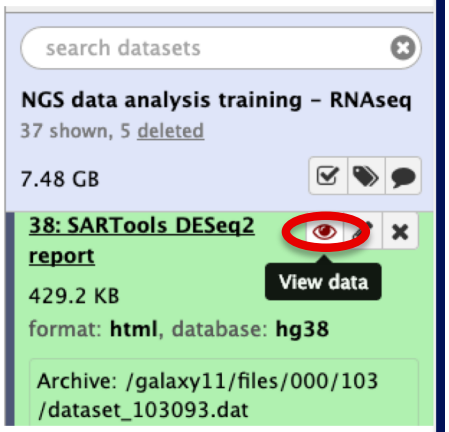
- Values of normalization factors for Mitf dataset

4 Normalization

Normalization aims at correcting systematic technical biases in the data, in order to make read counts comparable across samples. The normalization proposed by DESeq2 relies on the hypothesis that most features are not differentially expressed. It computes a scaling factor for each sample. Normalized read counts are obtained by dividing raw read counts by the scaling factor associated with the sample they belong to. Scaling factors around 1 mean (almost) no normalization is performed. Scaling factors lower than 1 will produce normalized counts higher than raw ones, and the other way around. Two options are available to compute scaling factors: locfunc="median" (default) or locfunc="shorth". Here, the normalization was performed with locfunc="median".

	siL[illegible]	siLuc3	siMitf3	siMitf4
Size factor	0.95	1.02	0.95	1.10

Table 5: Normalization factors.



Question 3

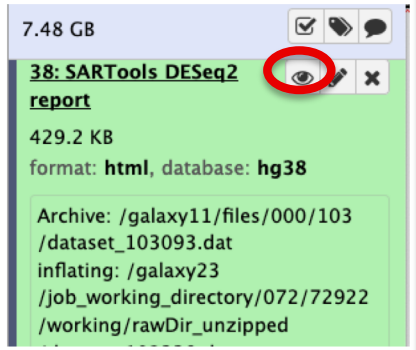
- Number of significantly differentially expressed genes between siMitf and siLuc (FDR<0.05)

5.6 Final results

A p-value adjustment is performed to take into account multiple testing and control the false positive rate to a chosen level \(\alpha\). For this analysis, a BH p-value adjustment was performed [Benjamini, 1995 and 2001] and the level of controlled false positive rate was set to 0.05.

Test vs Ref	# down	# up	# total
siMitf vs siLuc	3335	3663	6998

Table 7: Number of up-, down- and total number of differentially expressed features for each comparison.



→ 6998 significantly differentially expressed genes
  → 3335 genes significantly under-exressed in siMitf vs siLuc
  → 3663 genes significantly over-expressed in siMitf vs siLuc