

Table 1: Selected metrics based on the output of **rnaQUAST**, **HISAT2**, **DETONATE**, **TransRate** and **BUSCO** for the transcripts assembled by all ten tools on the *Mus musculus* RNA-Seq data set. Details and much more statistics, complementing this evaluation, can be found in the electronic supplement, content S4–S10. The RSEM-EVAL score is divided by 10^9 . Number of ambiguous bases is given in thousand. F1 score – an F1 score of 1 would mean that all nucleotides/contigs in the estimated true assembly were recovered with at least 90% identity. KC score – *k*-mer compression score reflecting the similarity of each assembly to **DETONATE**'s estimated “true” assembly. The Ex90N50 statistic is computed as usual N50 but limited to the top most highly expressed transcripts that represent 90% of the total normalized expression data. Normalized scores in the range between 0 and 1 are displayed. Raw values for each metric and assembly tool are given in subscript next to the normalized values. In the last row, the summarized *metric score (MS)* of (0,1)-normalized scores is given for each assembly tool.

	Trinity	Oases	Trans-ABYSS	SOAP-Trans	Bridger	BinPacker	IDBA-Tran	Shannon	SPAdes-sc	SPAdes-rna
<i>k</i> -mer size	default	25,35,45,55,65	25,35,45,55,65	default	default	default	25,35,45,55,65	default	default	default
Evaluation metrics 1–20										
HISAT2										
1 Overall mapping rate	0.89 _{92.14}	0.82 _{89.29}	0.93 _{93.96}	0.86 _{90.98}	0.88 _{91.66}	0.00 _{54.31}	0.38 _{70.64}	0.76 _{86.6}	0.93 _{93.83}	1.00 _{96.86}
rnaQUAST										
2 Transcripts ≥ 1000 nt	0.40 ₂₁₈₀₄	1.00 ₅₁₈₃₂	0.56 ₂₉₇₁₉	0.21 ₁₂₆₀₃	0.33 ₁₈₂₆₆	0.00 ₂₀₃₇	0.21 ₁₂₂₉₄	0.30 ₁₆₉₁₅	0.22 ₁₃₀₉₉	0.35 ₁₉₃₆₇
3 Misassemblies	0.99 ₅₆₄	0.00 ₅₂₆₆₈	0.99 ₆₁₂	1.00 ₆₁	0.95 ₂₆₄₃	0.99 ₆₂₈	1.00 ₄₁	0.96 ₁₉₂₇	0.99 ₅₇₀	0.98 ₁₀₈₀
4 Mismatches per transcript	0.85 _{0.84}	0.90 _{0.63}	0.97 _{0.32}	1.00 _{0.16}	0.83 _{0.91}	0.00 _{4.61}	0.99 _{0.19}	0.86 _{0.8}	0.88 _{0.71}	0.86 _{0.8}
5 Average alignment length	0.52 _{1216.86}	0.00 _{406.08}	0.22 _{752.81}	0.07 _{519.96}	0.43 _{1073.4}	1.00 _{1972.81}	0.26 _{811.99}	0.30 _{877.71}	0.44 _{1089.26}	0.32 _{910.47}
6 95%-assembled isoforms	0.96 ₆₀₃₂	0.02 ₁₈₃	1.00 ₆₂₈₁	0.35 ₂₂₄₄	0.35 ₂₂₃₆	0.08 ₅₅₄	0.14 ₉₆₇	0.00 ₇₁	0.41 ₂₆₃₃	0.51 ₃₂₆₉
7 Duplication ratio	0.39 _{1.547}	0.00 _{1.9}	0.17 _{1.75}	0.97 _{1.031}	0.62 _{1.348}	0.62 _{1.346}	1.00 _{1.005}	0.75 _{1.23}	1.00 _{1.003}	0.85 _{1.135}
8 Ex90N50	0.00 ₄₂₀	0.48 ₂₀₄₄	0.03 ₅₃₀	0.70 ₂₇₉₆	0.79 ₃₁₀₀	0.68 ₂₇₁₅	0.37 ₁₆₇₂	0.49 ₂₀₆₇	1.00 ₃₈₁₄	0.88 ₃₄₁₈
9 # full-length transcripts	0.97 ₈₂₁₀	0.71 ₆₂₉₃	1.00 ₈₄₀₁	0.86 ₇₃₇₅	0.90 ₇₆₃₉	0.00 ₁₀₁₄	0.43 ₄₂₀₈	0.57 ₅₂₄₈	0.91 ₇₇₃₆	0.93 ₇₈₅₉
TransRate										
10 Reference coverage	0.88 _{0.22}	0.09 _{0.02}	1.00 _{0.25}	0.38 _{0.1}	0.40 _{0.1}	0.06 _{0.02}	0.37 _{0.09}	0.00 ₀	0.34 _{0.08}	0.43 _{0.11}
11 Mean ORF percentage	0.82 _{52.21}	0.40 _{45.36}	0.92 _{53.79}	0.78 _{51.45}	0.66 _{49.56}	0.00 _{38.86}	0.95 _{54.28}	1.00 _{55.04}	0.37 _{44.85}	0.29 _{43.58}
12 Optimal score ^a	0.53 _{0.22}	0.00 _{0.02}	0.25 _{0.12}	1.00 _{0.4}	0.42 _{0.18}	0.29 _{0.13}	0.72 _{0.29}	0.43 _{0.18}	0.94 _{0.38}	0.41 _{0.18}
13 Percentage bases uncovered ^a	0.43 _{0.52}	0.00 _{0.91}	0.31 _{0.63}	0.88 _{0.12}	0.55 _{0.41}	0.34 _{0.6}	1.00 _{0.02}	0.54 _{0.43}	1.00 _{0.01}	0.64 _{0.33}
14 Number of ambiguous bases	0.60 ₇₈₇₂₆	0.00 ₁₈₈₉₄₇	0.46 ₁₀₅₁₅₃	0.74 ₅₃₆₁₁	0.67 ₆₆₉₅₄	1.00 ₆₅₁₄	0.79 ₄₅₅₂₆	0.75 ₅₂₉₄₈	0.75 ₅₁₅₇₅	0.63 ₇₃₄₆₁
DETONATE										
15 Nucleotide F1	0.78 _{0.42}	0.26 _{0.19}	0.73 _{0.4}	0.97 _{0.51}	0.84 _{0.45}	0.00 _{0.07}	0.94 _{0.5}	0.69 _{0.38}	1.00 _{0.52}	0.95 _{0.5}
16 Contig F1	0.37 _{0.01}	0.07 ₀	1.00 _{0.02}	0.95 _{0.02}	0.27 _{0.01}	0.00 ₀	0.44 _{0.01}	0.30 _{0.01}	0.21 _{0.01}	0.58 _{0.01}
17 KC score	0.88 _{0.66}	0.42 _{0.47}	0.90 _{0.67}	0.62 _{0.55}	0.74 _{0.6}	0.00 _{0.28}	0.57 _{0.53}	0.64 _{0.56}	0.94 _{0.69}	1.00 _{0.71}
18 RSEM EVAL	0.97 _{-2.23}	0.59 _{-3.34}	1.00 _{-2.15}	0.83 _{-2.64}	0.92 _{-2.38}	0.05 _{-4.89}	0.00 _{-5.03}	0.73 _{-2.94}	0.70 _{-3.01}	0.90 _{-2.45}
BUSCO										
19 Complete BUSCOs	0.97 ₃₈₉₅	0.75 ₃₀₆₆	1.00 ₃₉₉₂	0.90 ₃₆₁₆	0.98 ₃₉₁₄	0.00 ₃₁₀	0.64 ₂₆₇₉	0.68 ₂₈₂₉	0.95 ₃₈₂₃	0.94 ₃₇₇₃
20 Missing BUSCOs	0.98 ₁₉₅₉	0.85 ₂₄₇₆	0.99 ₁₉₂₆	0.97 ₁₉₈₇	0.99 ₁₉₂₆	0.00 ₅₈₇₁	0.92 ₂₁₈₈	0.84 ₂₅₃₄	1.00 ₁₈₇₇	1.00 ₁₈₈₀
Summed up metric (0,1)-score	14.21	7.36	14.43	15.05	13.5	5.1	12.13	11.58	14.98	14.46

^aNot available for the *E. coli* and *A. thaliana* data set because this metric is only calculated by **TransRate** in the case of paired-end data.