

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 *Homo sapiens simulated* 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	0.95 _{96.39}	0.62 _{73.26}	1.00 _{99.56}	0.89 _{91.68}	0.92 _{94.02}	0.91 _{93.03}	0.79 _{85.34}	0.00 _{30.77}	0.96 _{97.02}	0.96 _{96.72}
rnaQUAST										
2 Transcripts ≥ 1000 nt	0.24 ₈₄₅₁	1.00 ₂₈₁₄₃	0.17 ₆₇₀₃	0.07 ₄₂₆₃	0.11 ₅₁₆₇	0.20 ₇₄₂₄	0.02 ₂₇₄₀	0.00 ₂₃₄₁	0.01 ₂₆₂₃	0.14 ₆₀₅₃
3 Misassemblies	0.97 ₁₃₉	0.00 ₄₀₉₄	0.97 ₁₁₇	0.99 ₄₉	0.87 ₅₃₃	0.81 ₇₈₅	1.00 ₈	0.99 ₆₆	0.99 ₅₀	0.92 ₃₅₁
4 Mismatches per transcript	0.44 _{1.23}	0.00 _{2.11}	0.82 _{0.46}	0.89 _{0.31}	0.23 _{1.65}	0.13 _{1.85}	1.00 _{0.1}	0.85 _{0.39}	0.94 _{0.22}	0.14 _{1.82}
5 Average alignment length	1.00 _{2261.33}	0.88 _{2090.55}	0.06 _{939.31}	0.11 _{1009.08}	0.51 _{1581.3}	0.64 _{1755.16}	0.00 _{859.97}	0.14 _{1061.01}	0.09 _{979.98}	0.56 _{1649.15}
6 95%-assembled isoforms	0.96 ₂₈₉₈	1.00 ₂₉₉₅	0.95 ₂₈₇₇	0.46 ₁₆₇₉	0.41 ₁₅₇₀	0.45 ₁₆₆₀	0.00 ₅₇₇	0.20 ₁₀₅₆	0.43 ₁₆₂₅	0.68 ₂₂₂₀
7 Duplication ratio	0.70 _{2.154}	0.00 _{4.854}	0.85 _{1.572}	0.93 _{1.274}	0.87 _{1.507}	0.76 _{1.949}	1.00 _{1.013}	0.89 _{1.437}	1.00 _{1.012}	0.86 _{1.535}
8 Ex90N50	0.96 ₃₂₃₄	0.99 ₃₃₀₀	0.78 ₂₉₀₂	0.75 ₂₈₃₆	0.97 ₃₂₅₂	0.79 ₂₉₀₉	0.00 ₁₃₉₇	0.51 ₂₃₆₈	1.00 ₃₃₁₅	0.60 ₂₅₄₉
9 # full-length transcripts	0.85 ₁₄₉₂	1.00 ₁₆₇₂	0.71 ₁₃₂₃	0.45 ₁₀₀₆	0.62 ₁₂₁₀	0.65 ₁₂₄₆	0.00 ₄₅₀	0.18 ₆₆₆	0.46 ₁₀₀₇	0.57 ₁₁₄₁
TransRate										
10 Reference coverage	0.34 _{0.24}	0.74 _{0.38}	1.00 _{0.46}	0.16 _{0.18}	0.17 _{0.19}	0.22 _{0.2}	0.14 _{0.18}	0.00 _{0.13}	0.09 _{0.16}	0.35 _{0.25}
11 Mean ORF percentage	0.63 _{43.38}	0.10 _{37.43}	0.81 _{45.49}	0.00 _{36.28}	0.52 _{42.22}	0.64 _{43.57}	1.00 _{47.62}	0.98 _{47.37}	0.29 _{39.62}	0.36 _{40.38}
12 Optimal score ^a	0.22 _{0.11}	0.00 _{0.01}	0.47 _{0.22}	0.43 _{0.21}	0.29 _{0.14}	0.26 _{0.13}	0.74 _{0.35}	0.10 _{0.06}	1.00 _{0.47}	0.40 _{0.19}
13 Percentage bases uncovered ^a	0.40 _{0.5}	0.00 _{0.83}	0.63 _{0.32}	0.81 _{0.17}	0.74 _{0.22}	0.49 _{0.43}	1.00 _{0.01}	0.80 _{0.17}	1.00 _{0.01}	0.71 _{0.25}
14 Number of ambiguous bases	0.77 ₃₀₉₉₀	0.00 ₁₁₀₇₂₃	0.84 ₂₄₂₈₂	0.93 ₁₅₂₅₉	0.90 ₁₈₀₂₅	0.82 ₂₅₈₅₁	0.97 ₁₀₄₈₉	1.00 ₇₈₀₁	0.97 ₁₀₆₇₄	0.88 ₂₀₃₀₂
DETONATE										
15 Nucleotide F1	0.61 _{0.57}	0.00 _{0.22}	0.81 _{0.68}	0.90 _{0.73}	0.86 _{0.71}	0.67 _{0.6}	0.99 _{0.78}	0.41 _{0.45}	1.00 _{0.79}	0.83 _{0.7}
16 Contig F1	0.31 _{0.06}	0.03 _{0.05}	1.00 _{0.1}	0.54 _{0.08}	0.01 _{0.05}	0.00 _{0.05}	0.06 _{0.05}	0.43 _{0.07}	0.10 _{0.05}	0.13 _{0.05}
17 KC score	0.94 _{0.88}	0.72 _{0.74}	1.00 _{0.92}	0.50 _{0.6}	0.84 _{0.82}	0.85 _{0.82}	0.51 _{0.6}	0.00 _{0.26}	0.77 _{0.77}	0.79 _{0.79}
18 RSEM EVAL	0.96 _{-2.79}	0.75 _{-4.62}	1.00 _{-2.38}	0.68 _{-5.27}	0.86 _{-3.66}	0.86 _{-3.65}	0.44 _{-7.53}	0.00 _{-11.51}	0.74 _{-4.78}	0.83 _{-3.91}
BUSCO										
19 Complete BUSCOs	0.94 ₅₈₈	1.00 ₆₁₃	0.80 ₅₃₇	0.16 ₂₈₉	0.77 ₅₂₅	0.78 ₅₂₇	0.00 ₂₂₆	0.04 ₂₄₂	0.47 ₄₀₇	0.61 ₄₆₂
20 Missing BUSCOs	0.99 ₂₆	1.00 ₂₂	1.00 ₂₁	0.74 ₁₀₉	0.98 ₂₈	0.98 ₂₉	0.65 ₁₄₂	0.00 ₃₆₄	0.90 ₅₅	0.99 ₂₆
Summed up metric (0,1)-score	14.16	9.83	15.68	11.4	12.47	11.88	10.31	7.51	13.22	12.32

^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.