

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 + EBOV 7h* 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	0.91 _{92.58}	0.53 _{85.78}	1.00 _{94.22}	0.79 _{90.52}	0.72 _{89.14}	0.46 _{84.63}	0.00 _{76.39}	0.82 _{90.99}	0.87 _{91.94}	0.95 _{93.4}
rnaQUAST										
2 Transcripts ≥ 1000 nt	0.16 ₃₁₃₁₉	1.00 ₁₁₅₅₉₆	0.22 ₃₇₄₁₀	0.00 ₁₅₂₅₁	0.08 ₂₃₄₈₇	0.08 ₂₂₉₂₈	0.01 ₁₆₁₈₁	0.20 ₃₅₇₈₅	0.00 ₁₅₅₉₈	0.07 ₂₁₈₆₇
3 Misassemblies	0.94 ₁₂₉₄	0.00 ₁₈₈₉₃	0.75 ₄₇₆₉	1.00 ₁₂₅	0.87 ₂₅₀₉	0.87 ₂₆₄₉	1.00 ₂₀₃	0.87 ₂₆₀₅	0.90 ₂₀₄₂	0.94 ₁₂₁₉
4 Mismatches per transcript	0.60 _{1.75}	0.36 _{2.52}	0.90 _{0.75}	1.00 _{0.44}	0.68 _{1.49}	0.00 _{3.67}	0.88 _{0.83}	0.70 _{1.43}	0.91 _{0.72}	0.81 _{1.07}
5 Average alignment length	0.29 _{1040.88}	0.44 _{1364.38}	0.04 _{534.4}	0.00 _{455.96}	0.20 _{866.61}	1.00 _{2505.43}	0.12 _{697.75}	0.23 _{918.85}	0.02 _{489.2}	0.01 _{470.16}
6 95%-assembled isoforms	0.90 ₄₆₉₆	1.00 ₅₁₁₅	0.74 ₄₀₀₄	0.56 ₃₂₂₅	0.50 ₂₉₆₅	0.48 ₂₈₅₁	0.00 ₇₈₀	0.50 ₂₉₆₄	0.46 ₂₇₉₅	0.71 ₃₈₅₀
7 Duplication ratio	0.78 _{1.757}	0.00 _{4.347}	0.80 _{1.663}	0.98 _{1.06}	0.87 _{1.451}	0.74 _{1.884}	1.00 _{1.007}	0.72 _{1.954}	1.00 _{1.013}	0.95 _{1.161}
8 Ex90N50	0.56 ₂₈₃₉	0.64 ₃₀₀₃	0.19 ₂₀₃₉	0.35 ₂₃₈₅	0.52 ₂₇₄₅	1.00 ₃₇₆₄	0.15 ₁₉₆₇	0.32 ₂₃₂₈	0.53 ₂₇₆₆	0.00 ₁₆₄₇
9 # full-length transcripts	0.93 ₈₄₅₈	1.00 ₈₈₆₁	0.92 ₈₄₀₅	0.77 ₇₅₆₉	0.77 ₇₅₄₀	0.64 ₆₈₄₄	0.00 ₃₁₉₇	0.76 ₇₅₂₄	0.67 ₇₀₀₄	0.71 ₇₂₂₆
TransRate										
10 Reference coverage	0.37 _{0.08}	0.90 _{0.12}	1.00 _{0.13}	0.27 _{0.08}	0.26 _{0.08}	0.00 _{0.06}	0.27 _{0.08}	0.92 _{0.12}	0.28 _{0.08}	0.48 _{0.09}
11 Mean ORF percentage	0.77 _{52.15}	0.00 _{43.82}	0.75 _{51.97}	0.51 _{49.38}	0.35 _{47.64}	0.35 _{47.66}	0.61 _{50.41}	1.00 _{54.69}	0.39 _{48.01}	0.20 _{45.99}
12 Optimal score ^a	0.35 _{0.19}	0.00 _{0.02}	0.12 _{0.07}	0.64 _{0.33}	0.33 _{0.18}	0.20 _{0.11}	0.76 _{0.39}	0.04 _{0.04}	1.00 _{0.51}	0.57 _{0.3}
13 Percentage bases uncovered ^a	0.39 _{0.58}	0.00 _{0.95}	0.35 _{0.62}	0.87 _{0.13}	0.51 _{0.47}	0.20 _{0.76}	1.00 _{0.01}	0.25 _{0.71}	0.98 _{0.03}	0.80 _{0.2}
14 Number of ambiguous bases	0.82 ₁₃₁₂₈₄	0.00 ₄₁₃₄₁₈	0.74 ₁₅₉₀₉₂	0.97 ₇₇₁₇₁	0.90 ₁₀₁₇₈₈	0.96 ₈₂₂₃₉	1.00 ₆₇₅₆₄	0.81 ₁₃₄₁₀₈	0.97 ₇₆₆₄₆	0.85 ₁₂₀₅₉₇
DETONATE										
15 Nucleotide F1	0.64 _{0.46}	0.00 _{0.2}	0.63 _{0.46}	0.86 _{0.55}	0.71 _{0.49}	0.25 _{0.3}	0.87 _{0.56}	0.42 _{0.37}	0.91 _{0.57}	1.00 _{0.61}
16 Contig F1	0.27 _{0.01}	0.28 _{0.01}	1.00 _{0.05}	0.80 _{0.04}	0.23 _{0.01}	0.00 ₀	0.24 _{0.01}	0.63 _{0.03}	0.28 _{0.01}	0.43 _{0.02}
17 KC score	0.87 _{0.55}	0.10 _{0.45}	1.00 _{0.57}	0.43 _{0.49}	0.58 _{0.51}	0.50 _{0.5}	0.00 _{0.43}	0.90 _{0.55}	0.36 _{0.48}	0.93 _{0.56}
18 RSEM EVAL	1.00 _{-1.62}	0.38 _{-2.35}	0.98 _{-1.64}	0.81 _{-1.85}	0.85 _{-1.8}	0.72 _{-1.95}	0.00 _{-2.8}	0.90 _{-1.74}	0.52 _{-2.19}	0.92 _{-1.71}
BUSCO										
19 Complete BUSCOs	1.00 ₄₁₁₉	0.97 ₄₀₅₅	1.00 ₄₁₂₁	0.77 ₃₆₆₇	0.93 ₃₉₇₉	0.73 ₃₅₉₀	0.00 ₂₁₃₄	0.83 ₃₇₇₇	0.76 ₃₆₄₄	0.71 ₃₅₄₂
20 Missing BUSCOs	1.00 ₁₇₅₉	0.91 ₁₈₁₇	0.98 ₁₇₇₁	0.85 ₁₈₅₅	0.96 ₁₇₈₅	0.00 ₂₃₉₂	0.28 ₂₂₁₇	0.69 ₁₉₅₅	0.88 ₁₈₃₅	0.97 ₁₇₈₀
Summed up metric (0,1)-score	13.54	8.5	14.11	13.24	11.81	9.17	8.18	12.5	12.7	13.0

^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.