

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 3h* 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	0.94 _{90.73}	0.88 _{86.92}	1.00 _{93.9}	0.93 _{89.8}	0.93 _{89.95}	0.00 _{36.6}	0.73 _{78.41}	0.94 _{90.23}	0.96 _{91.41}	1.00 _{93.83}
rnaQUAST										
2 Transcripts ≥ 1000 nt	0.26 ₂₇₁₅₄	1.00 ₉₈₁₉₆	0.30 ₃₀₇₆₇	0.12 ₁₃₁₁₀	0.19 ₂₀₄₃₄	0.00 ₁₆₄₆	0.12 ₁₃₅₀₈	0.28 ₂₈₂₄₈	0.12 ₁₂₇₇₅	0.16 ₁₇₁₉₅
3 Misassemblies	0.94 ₁₅₈₀	0.00 ₂₄₈₄₅	0.68 ₇₉₅₆	1.00 ₁₄₈	0.93 ₁₈₅₃	0.99 ₂₇₉	1.00 ₁₇₈	0.92 ₂₁₆₁	0.95 ₁₂₆₂	0.97 ₉₄₃
4 Mismatches per transcript	0.77 _{1.73}	0.66 _{2.38}	0.95 _{0.7}	1.00 _{0.43}	0.83 _{1.43}	0.00 _{6.18}	0.94 _{0.8}	0.85 _{1.31}	0.91 _{0.94}	0.90 ₁
5 Average alignment length	0.25 _{1083.56}	0.35 _{1308.22}	0.02 _{517.92}	0.00 _{464.37}	0.19 _{925.62}	1.00 _{2899.39}	0.11 _{718.4}	0.19 _{921.05}	0.08 _{661.32}	0.00 _{462.09}
6 95%-assembled isoforms	0.97 ₃₈₂₆	1.00 ₃₉₄₈	0.78 ₃₁₅₃	0.62 ₂₅₅₃	0.68 ₂₇₈₁	0.00 ₂₅₅	0.09 ₅₆₉	0.55 ₂₃₀₃	0.65 ₂₆₅₃	0.83 ₃₃₃₅
7 Duplication ratio	0.76 _{1.697}	0.00 _{3.91}	0.81 _{1.554}	0.99 _{1.042}	0.85 _{1.434}	0.65 _{2.014}	1.00 _{1.005}	0.72 _{1.808}	1.00 _{1.003}	0.96 _{1.117}
8 Ex90N50	0.60 ₂₇₅₃	0.45 ₂₄₆₁	0.18 ₁₉₄₇	0.40 ₂₃₇₂	0.56 ₂₆₇₆	1.00 ₃₅₃₀	0.17 ₁₉₂₄	0.33 ₂₂₃₆	0.59 ₂₇₃₅	0.00 ₁₆₀₃
9 # full-length transcripts	0.98 ₈₁₀₁	1.00 ₈₂₈₈	0.97 ₈₀₇₂	0.86 ₇₂₁₉	0.90 ₇₅₁₉	0.00 ₄₉₆	0.34 ₃₁₂₁	0.84 ₇₀₄₀	0.90 ₇₅₃₇	0.89 ₇₄₁₀
TransRate										
10 Reference coverage	0.67 _{0.08}	1.00 _{0.12}	0.94 _{0.12}	0.57 _{0.07}	0.56 _{0.07}	0.00 ₀	0.55 _{0.07}	0.90 _{0.11}	0.62 _{0.08}	0.68 _{0.08}
11 Mean ORF percentage	0.74 _{54.51}	0.32 _{48.7}	0.82 _{55.63}	0.60 _{52.67}	0.54 _{51.85}	0.00 _{44.28}	0.73 _{54.47}	1.00 _{58.17}	0.52 _{51.51}	0.43 _{50.27}
12 Optimal score ^a	0.30 _{0.19}	0.00 _{0.02}	0.17 _{0.11}	0.61 _{0.36}	0.32 _{0.2}	0.05 _{0.05}	0.73 _{0.43}	0.05 _{0.05}	1.00 _{0.57}	0.60 _{0.35}
13 Percentage bases uncovered ^a	0.37 _{0.6}	0.00 _{0.94}	0.38 _{0.58}	0.89 _{0.11}	0.50 _{0.48}	0.11 _{0.83}	1.00 _{0.01}	0.26 _{0.69}	1.00 _{0.01}	0.84 _{0.16}
14 Number of ambiguous bases	0.67 ₁₀₉₄₉₆	0.00 ₃₁₈₉₃₆	0.61 ₁₂₇₄₄₈	0.82 ₆₂₇₅₃	0.76 ₈₃₁₈₇	1.00 ₆₇₅₂	0.85 ₅₄₄₉₄	0.69 ₁₀₄₁₂₆	0.83 ₆₀₉₀₀	0.72 ₉₄₂₇₄
DETONATE										
15 Nucleotide F1	0.72 _{0.46}	0.29 _{0.21}	0.73 _{0.46}	0.91 _{0.56}	0.79 _{0.49}	0.00 _{0.05}	0.90 _{0.55}	0.60 _{0.39}	0.93 _{0.57}	1.00 _{0.61}
16 Contig F1	0.32 _{0.01}	0.32 _{0.01}	1.00 _{0.05}	0.82 _{0.04}	0.29 _{0.01}	0.00 ₀	0.29 _{0.01}	0.60 _{0.03}	0.28 _{0.01}	0.48 _{0.02}
17 KC score	0.93 _{0.52}	0.56 _{0.4}	1.00 _{0.54}	0.72 _{0.45}	0.89 _{0.51}	0.00 _{0.22}	0.67 _{0.44}	0.92 _{0.52}	0.85 _{0.49}	1.00 _{0.54}
18 RSEM EVAL	0.98 _{-1.24}	0.70 _{-1.73}	1.00 _{-1.2}	0.91 _{-1.35}	0.97 _{-1.25}	0.00 _{-2.96}	0.64 _{-1.84}	0.97 _{-1.26}	0.93 _{-1.32}	1.00 _{-1.2}
BUSCO										
19 Complete BUSCOs	1.00 ₃₉₇₉	0.95 ₃₈₀₃	1.00 ₃₉₈₂	0.88 ₃₅₂₈	0.97 ₃₈₇₉	0.00 ₂₀₅	0.52 ₂₁₆₂	0.89 ₃₅₇₅	0.98 ₃₈₉₀	0.91 ₃₆₅₅
20 Missing BUSCOs	1.00 ₁₈₂₉	0.98 ₁₈₉₂	0.99 ₁₈₃₈	0.97 ₁₉₃₄	0.99 ₁₈₆₂	0.00 ₅₉₇₆	0.90 ₂₂₁₆	0.93 ₂₀₉₆	1.00 ₁₈₁₃	0.99 ₁₈₅₁
Summed up metric (0,1)-score	14.17	10.45	14.35	14.63	13.64	4.81	12.25	13.42	15.08	14.35

^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.