

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 *Arabidopsis thaliana* 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	0.96 _{95.71}	0.88 _{91.93}	1.00 _{97.55}	0.73 _{85.31}	0.85 _{90.64}	0.34 _{67.15}	0.82 _{89.04}	0.00 _{51.53}	0.93 _{94.29}	0.92 _{93.67}
rnaQUAST										
2 Transcripts ≥ 1000 nt	0.33 ₁₃₃₅₇	1.00 ₃₂₆₄₇	0.55 ₁₉₇₁₄	0.17 ₈₇₀₉	0.32 ₁₂₈₈₂	0.00 ₃₇₇₅	0.20 ₉₅₇₇	0.29 ₁₂₀₁₈	0.26 ₁₁₁₇₁	0.26 ₁₁₁₆₀
3 Misassemblies	0.84 ₁₂₁₂	0.06 ₆₈₀₄	0.00 ₇₂₂₇	1.00 ₅₁	0.73 ₁₉₉₅	0.84 ₁₂₃₃	0.98 ₂₀₁	0.90 ₇₆₉	0.84 ₁₁₈₉	0.94 ₅₀₅
4 Mismatches per transcript	0.79 _{1.32}	0.42 _{3.47}	0.91 _{0.61}	1.00 _{0.1}	0.60 _{2.43}	0.00 _{5.9}	0.99 _{0.14}	0.89 _{0.72}	0.94 _{0.43}	0.96 _{0.34}
5 Average alignment length	0.55 _{978.21}	0.89 _{1232.69}	0.24 _{745.09}	0.00 _{561.75}	0.57 _{990.35}	1.00 _{1312.17}	0.38 _{848.36}	0.64 _{1044.9}	0.51 _{944.39}	0.33 _{807.42}
6 95 %-assembled isoforms	0.80 ₂₃₀₂	1.00 ₂₇₅₃	0.65 ₁₉₆₀	0.32 ₁₂₁₃	0.63 ₁₉₀₉	0.00 ₄₈₉	0.30 ₁₁₇₅	0.67 ₂₀₀₄	0.81 ₂₃₁₈	0.77 ₂₂₃₇
7 Duplication ratio	0.88 _{1.128}	0.00 _{1.976}	0.66 _{1.341}	1.00 _{1.008}	0.94 _{1.067}	0.85 _{1.149}	1.00 _{1.01}	0.82 _{1.185}	1.00 _{1.007}	0.98 _{1.027}
8 Ex90N50	0.83 ₁₄₅₁	0.71 ₁₃₀₅	0.41 ₉₄₀	0.00 ₄₄₉	0.99 ₁₆₃₆	0.83 ₁₄₅₂	0.30 ₈₀₅	1.00 ₁₆₅₄	0.48 ₁₀₃₀	0.66 ₁₂₃₉
9 # full-length transcripts	0.81 ₇₂₁₉	1.00 ₈₃₆₁	0.96 ₈₁₃₃	0.52 ₅₄₁₁	0.80 ₇₁₆₃	0.00 ₂₂₂₇	0.63 ₆₀₈₇	0.70 ₆₅₄₁	0.91 ₇₈₀₀	0.86 ₇₅₀₁
TransRate										
10 Reference coverage	0.69 _{0.16}	0.90 _{0.19}	1.00 _{0.21}	0.65 _{0.15}	0.57 _{0.13}	0.00 _{0.03}	0.64 _{0.15}	0.65 _{0.15}	0.67 _{0.15}	0.69 _{0.15}
11 Mean ORF percentage	0.57 _{74.79}	0.38 _{72.07}	0.41 _{72.5}	1.00 _{80.78}	0.56 _{74.66}	0.00 _{66.84}	0.99 _{80.71}	0.66 _{76.08}	0.55 _{74.47}	0.63 _{75.67}
12 Optimal score ^a	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13 Percentage bases uncovered ^a	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14 Number of ambiguous bases	0.61 ₃₅₉₆₄	0.00 ₇₈₇₆₂	0.37 ₅₃₁₈₀	0.72 ₂₈₆₃₂	0.66 ₃₃₀₀₈	1.00 ₉₀₈₁	0.73 ₂₇₆₆₆	0.70 ₃₀₁₇₈	0.71 ₂₉₅₆₉	0.69 ₃₀₆₂₁
DETONATE										
15 Nucleotide F1	0.85 _{0.71}	0.31 _{0.42}	0.62 _{0.59}	0.95 _{0.77}	0.86 _{0.72}	0.00 _{0.26}	0.95 _{0.76}	0.70 _{0.63}	1.00 _{0.79}	0.99 _{0.79}
16 Contig F1	0.73 _{0.05}	0.43 _{0.03}	0.59 _{0.04}	0.59 _{0.04}	0.58 _{0.04}	0.00 ₀	0.44 _{0.03}	0.57 _{0.04}	0.87 _{0.06}	1.00 _{0.07}
17 KC score	0.90 _{0.71}	0.77 _{0.66}	1.00 _{0.74}	0.66 _{0.62}	0.79 _{0.66}	0.29 _{0.48}	0.77 _{0.66}	0.00 _{0.37}	0.92 _{0.71}	0.94 _{0.72}
18 RSEM EVAL	0.99 _{-0.46}	0.83 _{-0.6}	1.00 _{-0.45}	0.69 _{-0.73}	0.90 _{-0.54}	0.43 _{-0.96}	0.75 _{-0.67}	0.00 _{-1.34}	0.84 _{-0.59}	0.87 _{-0.57}
BUSCO										
19 Complete BUSCOs	1.00 ₁₁₁₇	0.99 ₁₁₀₈	1.00 ₁₁₁₉	0.93 ₁₀₅₈	0.98 ₁₁₀₃	0.00 ₂₆₂	0.78 ₉₃₀	0.92 ₁₀₄₉	0.95 ₁₀₇₄	0.88 ₁₀₁₂
20 Missing BUSCOs	1.00 ₂₁₉	0.97 ₂₄₈	0.99 ₂₂₄	0.97 ₂₄₈	0.99 ₂₂₉	0.00 ₁₁₆₂	0.95 ₂₆₉	0.92 ₂₉₆	0.99 ₂₂₅	0.95 ₂₆₄
Summed up metric (0,1)-score	14.15	11.53	12.36	11.9	13.31	5.57	12.6	11.03	14.18	14.3

^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.