

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 *Candida albicans* 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 _{97.32}	0.60 _{93.61}	1.00 _{98.45}	0.73 _{95.12}	0.86 _{96.72}	0.85 _{96.66}	0.00 _{86.34}	0.84 _{96.51}	0.92 _{97.51}	0.92 _{97.43}
rnaQUAST										
2 Transcripts ≥ 1000 nt	0.09 ₃₄₁₇	1.00 ₉₈₁₃	0.74 ₇₉₇₉	0.00 ₂₇₆₉	0.18 ₄₀₃₈	0.21 ₄₂₆₇	0.03 ₂₉₄₉	0.19 ₄₀₇₇	0.10 ₃₅₀₄	0.17 ₃₉₃₉
3 Misassemblies	0.89 ₇₂	0.00 ₆₁₃	0.27 ₄₄₉	1.00 ₉	0.62 ₂₃₆	0.59 ₂₅₉	1.00 ₈	0.41 ₃₆₂	0.91 ₆₀	0.88 ₈₂
4 Mismatches per transcript	0.27 _{1.82}	0.09 _{2.12}	0.58 _{1.29}	1.00 _{0.57}	0.17 ₂	0.00 _{2.28}	0.86 _{0.81}	0.75 ₁	0.28 _{1.8}	0.19 _{1.96}
5 Average alignment length	0.56 _{969.33}	0.53 _{941.6}	0.29 _{773.58}	0.00 _{567.17}	0.79 _{1131.33}	0.87 _{1189.55}	0.50 _{922.1}	0.16 _{678.95}	1.00 _{1279.41}	0.85 _{1171.71}
6 95%-assembled isoforms	0.46 ₄₁₄₆	0.79 ₄₈₀₈	1.00 ₅₂₁₄	0.03 ₃₂₉₀	0.39 ₄₀₀₅	0.40 ₄₀₃₃	0.00 ₃₂₃₈	0.25 ₃₇₃₆	0.61 ₄₄₃₇	0.66 ₄₅₃₃
7 Duplication ratio	0.97 _{1.039}	0.00 _{2.25}	0.27 _{1.916}	1.00 _{1.007}	0.92 _{1.105}	0.89 _{1.136}	1.00 _{1.003}	0.67 _{1.412}	1.00 _{1.001}	0.95 _{1.059}
8 Ex90N50	0.73 ₁₈₆₂	0.40 ₁₅₈₈	0.49 ₁₆₆₄	0.14 ₁₃₇₄	0.80 ₁₉₂₃	0.74 ₁₈₇₆	0.00 ₁₂₅₇	0.08 ₁₃₂₁	1.00 ₂₀₉₀	0.93 ₂₀₃₄
9 # full-length transcripts	0.76 ₁₇₃₂	0.91 ₁₈₃₆	1.00 ₁₈₉₅	0.08 ₁₂₇₄	0.71 ₁₆₉₈	0.71 ₁₇₀₁	0.00 ₁₂₂₃	0.38 ₁₄₈₀	0.93 ₁₈₄₇	0.91 ₁₈₃₇
TransRate										
10 Reference coverage	0.06 _{0.18}	0.31 _{0.24}	1.00 _{0.38}	0.13 _{0.2}	0.00 _{0.17}	0.00 _{0.17}	0.12 _{0.2}	0.62 _{0.3}	0.02 _{0.17}	0.13 _{0.2}
11 Mean ORF percentage	0.51 _{81.69}	0.33 _{79.54}	0.49 _{81.47}	0.71 _{84.15}	0.32 _{79.4}	0.26 _{78.65}	0.65 _{83.37}	1.00 _{87.69}	0.13 _{77.02}	0.00 _{75.46}
12 Optimal score ^a	0.83 _{0.46}	0.05 _{0.06}	0.00 _{0.03}	0.88 _{0.48}	0.65 _{0.36}	0.66 _{0.37}	0.75 _{0.41}	0.04 _{0.05}	1.00 _{0.54}	0.73 _{0.41}
13 Percentage bases uncovered ^a	0.85 _{0.13}	0.00 _{0.87}	0.05 _{0.82}	0.98 _{0.02}	0.64 _{0.31}	0.59 _{0.36}	1.00 ₀	0.44 _{0.48}	1.00 ₀	0.82 _{0.16}
14 Number of ambiguous bases	0.96 ₉₃₉₁	0.00 ₂₆₀₁₀	0.20 ₂₂₅₈₁	0.98 ₈₉₄₇	0.88 ₁₀₆₅₁	0.86 ₁₁₀₇₂	1.00 ₈₆₅₂	0.76 ₁₂₇₉₃	0.97 ₉₁₇₃	0.89 ₁₀₅₆₇
DETONATE										
15 Nucleotide F1	0.92 _{0.73}	0.00 _{0.51}	0.37 _{0.59}	0.94 _{0.73}	0.99 _{0.74}	0.92 _{0.73}	0.93 _{0.73}	0.54 _{0.64}	1.00 _{0.74}	0.97 _{0.74}
16 Contig F1	0.81 _{0.08}	0.78 _{0.08}	1.00 _{0.08}	0.44 _{0.06}	0.49 _{0.06}	0.50 _{0.07}	0.00 _{0.05}	0.60 _{0.07}	0.42 _{0.06}	0.25 _{0.06}
17 KC score	0.70 _{0.68}	0.36 _{0.62}	1.00 _{0.73}	0.28 _{0.6}	0.71 _{0.68}	0.71 _{0.68}	0.00 _{0.55}	0.92 _{0.72}	0.85 _{0.71}	0.77 _{0.69}
18 RSEM EVAL	1.00 _{-0.33}	0.68 _{-0.42}	0.96 _{-0.34}	0.57 _{-0.45}	0.93 _{-0.35}	0.89 _{-0.36}	0.00 _{-0.61}	0.89 _{-0.36}	0.86 _{-0.37}	0.93 _{-0.35}
BUSCO										
19 Complete BUSCOs	0.65 ₁₃₅₅	0.71 ₁₃₈₂	0.87 ₁₄₆₁	0.00 ₁₀₄₂	0.78 ₁₄₁₆	0.78 ₁₄₁₆	0.06 ₁₀₇₀	0.10 ₁₀₈₈	1.00 ₁₅₂₃	0.95 ₁₄₉₉
20 Missing BUSCOs	0.70 ₁₆₄	0.79 ₁₄₀	0.90 ₁₀₉	0.40 ₂₄₈	0.81 ₁₃₃	0.81 ₁₃₅	0.43 ₂₄₀	0.00 ₃₅₉	1.00 ₈₁	0.97 ₈₈
Summed up metric (0,1)-score	13.63	8.33	12.49	10.28	12.64	12.24	8.31	9.63	15.0	13.86

^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.