

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* 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
<b>Evaluation metrics 1–20</b>										
<b>HISAT2</b>										
1 Overall mapping rate	0.81 <sub>91.9</sub>	0.69 <sub>88.04</sub>	1.00 <sub>98.34</sub>	0.75 <sub>89.93</sub>	0.66 <sub>86.83</sub>	0.24 <sub>72.6</sub>	0.00 <sub>64.61</sub>	0.58 <sub>84.27</sub>	0.81 <sub>92.04</sub>	0.93 <sub>95.95</sub>
<b>rnaQUAST</b>										
2 Transcripts $\geq 1000$ nt	0.22 <sub>64061</sub>	1.00 <sub>207474</sub>	0.20 <sub>59779</sub>	0.03 <sub>27529</sub>	0.11 <sub>43201</sub>	0.00 <sub>22611</sub>	0.00 <sub>23516</sub>	0.05 <sub>31328</sub>	0.05 <sub>31039</sub>	0.15 <sub>49860</sub>
3 Misassemblies	0.99 <sub>3378</sub>	0.00 <sub>216127</sub>	0.99 <sub>2743</sub>	1.00 <sub>279</sub>	0.97 <sub>7329</sub>	0.98 <sub>5603</sub>	1.00 <sub>302</sub>	0.99 <sub>2837</sub>	0.99 <sub>2022</sub>	0.98 <sub>5126</sub>
4 Mismatches per transcript	0.74 <sub>1.38</sub>	0.77 <sub>1.25</sub>	0.93 <sub>0.57</sub>	1.00 <sub>0.27</sub>	0.73 <sub>1.44</sub>	0.00 <sub>4.63</sub>	0.91 <sub>0.67</sub>	0.77 <sub>1.26</sub>	0.88 <sub>0.8</sub>	0.78 <sub>1.25</sub>
5 Average alignment length	0.27 <sub>795.23</sub>	0.06 <sub>343.48</sub>	0.01 <sub>246.85</sub>	0.00 <sub>218</sub>	0.21 <sub>654.41</sub>	1.00 <sub>2335.73</sub>	0.13 <sub>487.11</sub>	0.23 <sub>711.83</sub>	0.09 <sub>410.22</sub>	0.09 <sub>412.24</sub>
6 95 %-assembled isoforms	0.99 <sub>6788</sub>	0.10 <sub>868</sub>	1.00 <sub>6824</sub>	0.31 <sub>2264</sub>	0.28 <sub>2105</sub>	0.39 <sub>2824</sub>	0.07 <sub>709</sub>	0.00 <sub>242</sub>	0.23 <sub>1755</sub>	0.46 <sub>3253</sub>
7 Duplication ratio	0.00 <sub>2.396</sub>	0.03 <sub>2.355</sub>	0.47 <sub>1.743</sub>	0.87 <sub>1.187</sub>	0.50 <sub>1.708</sub>	0.01 <sub>2.389</sub>	1.00 <sub>1.012</sub>	0.63 <sub>1.53</sub>	1.00 <sub>1.015</sub>	0.87 <sub>1.192</sub>
8 Ex90N50	0.00 <sub>326</sub>	0.17 <sub>666</sub>	0.06 <sub>441</sub>	0.19 <sub>711</sub>	0.51 <sub>1370</sub>	1.00 <sub>2381</sub>	0.19 <sub>708</sub>	0.49 <sub>1324</sub>	0.42 <sub>1186</sub>	0.22 <sub>782</sub>
9 # full-length transcripts	0.97 <sub>8930</sub>	0.83 <sub>8024</sub>	1.00 <sub>9110</sub>	0.64 <sub>6806</sub>	0.89 <sub>8440</sub>	0.26 <sub>4456</sub>	0.00 <sub>2783</sub>	0.63 <sub>6758</sub>	0.46 <sub>5676</sub>	0.69 <sub>7155</sub>
<b>TransRate</b>										
10 Reference coverage	0.87 <sub>0.23</sub>	0.33 <sub>0.09</sub>	1.00 <sub>0.26</sub>	0.34 <sub>0.09</sub>	0.31 <sub>0.09</sub>	0.27 <sub>0.07</sub>	0.31 <sub>0.08</sub>	0.00 <sub>0</sub>	0.30 <sub>0.08</sub>	0.42 <sub>0.11</sub>
11 Mean ORF percentage	0.64 <sub>50.82</sub>	0.00 <sub>42.09</sub>	0.72 <sub>51.92</sub>	0.44 <sub>48.02</sub>	0.22 <sub>45.1</sub>	0.04 <sub>42.57</sub>	0.76 <sub>52.46</sub>	1.00 <sub>55.7</sub>	0.30 <sub>46.13</sub>	0.31 <sub>46.25</sub>
12 Optimal score <sup>a</sup>	0.30 <sub>0.13</sub>	0.00 <sub>0.02</sub>	0.23 <sub>0.11</sub>	0.66 <sub>0.27</sub>	0.32 <sub>0.14</sub>	0.14 <sub>0.07</sub>	0.61 <sub>0.25</sub>	0.13 <sub>0.07</sub>	1.00 <sub>0.4</sub>	0.57 <sub>0.23</sub>
13 Percentage bases uncovered <sup>a</sup>	0.38 <sub>0.59</sub>	0.00 <sub>0.94</sub>	0.33 <sub>0.63</sub>	0.67 <sub>0.33</sub>	0.57 <sub>0.42</sub>	0.11 <sub>0.84</sub>	1.00 <sub>0.02</sub>	0.48 <sub>0.5</sub>	0.99 <sub>0.03</sub>	0.79 <sub>0.21</sub>
14 Number of ambiguous bases	0.72 <sub>286479</sub>	0.00 <sub>843235</sub>	0.53 <sub>437845</sub>	0.78 <sub>241236</sub>	0.83 <sub>206635</sub>	1.00 <sub>72918</sub>	0.91 <sub>138699</sub>	0.94 <sub>117068</sub>	0.86 <sub>177477</sub>	0.71 <sub>294083</sub>
<b>DETONATE</b>										
15 Nucleotide F1	0.59 <sub>0.43</sub>	0.08 <sub>0.18</sub>	0.77 <sub>0.51</sub>	0.89 <sub>0.57</sub>	0.71 <sub>0.48</sub>	0.00 <sub>0.15</sub>	0.86 <sub>0.55</sub>	0.42 <sub>0.35</sub>	0.97 <sub>0.61</sub>	1.00 <sub>0.62</sub>
16 Contig F1	0.08 <sub>0.02</sub>	0.09 <sub>0.02</sub>	0.99 <sub>0.2</sub>	1.00 <sub>0.21</sub>	0.05 <sub>0.01</sub>	0.00 <sub>0</sub>	0.08 <sub>0.02</sub>	0.11 <sub>0.02</sub>	0.07 <sub>0.01</sub>	0.06 <sub>0.01</sub>
17 KC score	0.87 <sub>0.51</sub>	0.00 <sub>0.24</sub>	1.00 <sub>0.55</sub>	0.42 <sub>0.37</sub>	0.51 <sub>0.4</sub>	0.40 <sub>0.37</sub>	0.14 <sub>0.29</sub>	0.58 <sub>0.42</sub>	0.47 <sub>0.39</sub>	0.60 <sub>0.43</sub>
18 RSEM EVAL	0.98 <sub>-6.51</sub>	0.45 <sub>-11.82</sub>	1.00 <sub>-6.26</sub>	0.72 <sub>-9.03</sub>	0.85 <sub>-7.72</sub>	0.62 <sub>-10.03</sub>	0.00 <sub>-16.3</sub>	0.73 <sub>-8.96</sub>	0.42 <sub>-12.12</sub>	0.91 <sub>-7.16</sub>
<b>BUSCO</b>										
19 Complete BUSCOs	0.96 <sub>4004</sub>	0.79 <sub>3588</sub>	1.00 <sub>4106</sub>	0.39 <sub>2625</sub>	0.92 <sub>3909</sub>	0.13 <sub>2009</sub>	0.00 <sub>1682</sub>	0.70 <sub>3385</sub>	0.39 <sub>2625</sub>	0.58 <sub>3089</sub>
20 Missing BUSCOs	0.99 <sub>1804</sub>	0.93 <sub>1922</sub>	1.00 <sub>1770</sub>	0.83 <sub>2164</sub>	0.98 <sub>1812</sub>	0.00 <sub>4078</sub>	0.63 <sub>2615</sub>	0.84 <sub>2133</sub>	0.78 <sub>2268</sub>	0.92 <sub>1949</sub>
<b>Summed up metric (0,1)-score</b>	12.38	6.31	14.24	11.92	11.13	6.59	8.61	10.3	11.47	12.03

<sup>a</sup>Not 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.