

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 23h* 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	0.98 <sub>94.39</sub>	0.60 <sub>70.05</sub>	1.00 <sub>95.49</sub>	0.96 <sub>93.1</sub>	0.95 <sub>92.43</sub>	0.78 <sub>81.57</sub>	0.27 <sub>48.37</sub>	0.00 <sub>31.29</sub>	0.93 <sub>91.15</sub>	0.96 <sub>93.12</sub>
<b>rnaQUAST</b>										
2 Transcripts $\geq 1000$ nt	0.23 <sub>27785</sub>	1.00 <sub>83530</sub>	0.30 <sub>32866</sub>	0.04 <sub>13418</sub>	0.13 <sub>20560</sub>	0.00 <sub>10862</sub>	0.05 <sub>14196</sub>	0.06 <sub>15007</sub>	0.03 <sub>13245</sub>	0.11 <sub>18629</sub>
3 Misassemblies	0.91 <sub>1066</sub>	0.00 <sub>11194</sub>	0.72 <sub>3253</sub>	1.00 <sub>94</sub>	0.84 <sub>1844</sub>	0.88 <sub>1436</sub>	1.00 <sub>134</sub>	0.97 <sub>381</sub>	0.90 <sub>1206</sub>	0.93 <sub>908</sub>
4 Mismatches per transcript	0.63 <sub>1.68</sub>	0.44 <sub>2.31</sub>	0.91 <sub>0.77</sub>	1.00 <sub>0.47</sub>	0.71 <sub>1.43</sub>	0.00 <sub>3.76</sub>	0.88 <sub>0.87</sub>	0.97 <sub>0.58</sub>	0.85 <sub>0.95</sub>	0.79 <sub>1.15</sub>
5 Average alignment length	0.26 <sub>1143.49</sub>	0.39 <sub>1500.78</sub>	0.04 <sub>578.88</sub>	0.00 <sub>482.26</sub>	0.17 <sub>929.86</sub>	1.00 <sub>3072.9</sub>	0.10 <sub>725.58</sub>	0.09 <sub>703.75</sub>	0.07 <sub>649.42</sub>	0.00 <sub>476.18</sub>
6 95 %-assembled isoforms	0.93 <sub>4065</sub>	1.00 <sub>4330</sub>	0.79 <sub>3575</sub>	0.56 <sub>2749</sub>	0.56 <sub>2736</sub>	0.22 <sub>1523</sub>	0.00 <sub>714</sub>	0.25 <sub>1631</sub>	0.54 <sub>2654</sub>	0.78 <sub>3535</sub>
7 Duplication ratio	0.73 <sub>1.806</sub>	0.00 <sub>3.97</sub>	0.78 <sub>1.644</sub>	0.98 <sub>1.049</sub>	0.85 <sub>1.459</sub>	0.63 <sub>2.09</sub>	1.00 <sub>1.006</sub>	0.85 <sub>1.435</sub>	1.00 <sub>1.004</sub>	0.95 <sub>1.147</sub>
8 Ex90N50	0.55 <sub>2958</sub>	0.62 <sub>3115</sub>	0.21 <sub>2178</sub>	0.34 <sub>2489</sub>	0.50 <sub>2841</sub>	1.00 <sub>3965</sub>	0.15 <sub>2048</sub>	0.00 <sub>1714</sub>	0.60 <sub>3071</sub>	0.22 <sub>2203</sub>
9 # full-length transcripts	1.00 <sub>7935</sub>	0.00 <sub>2868</sub>	0.99 <sub>7872</sub>	0.84 <sub>7122</sub>	0.86 <sub>7222</sub>	0.08 <sub>3262</sub>	0.05 <sub>3126</sub>	0.49 <sub>5349</sub>	0.84 <sub>7101</sub>	0.81 <sub>6960</sub>
<b>TransRate</b>										
10 Reference coverage	0.51 <sub>0.07</sub>	0.89 <sub>0.11</sub>	1.00 <sub>0.12</sub>	0.46 <sub>0.07</sub>	0.47 <sub>0.07</sub>	0.00 <sub>0.03</sub>	0.47 <sub>0.07</sub>	0.56 <sub>0.08</sub>	0.46 <sub>0.07</sub>	0.61 <sub>0.08</sub>
11 Mean ORF percentage	0.46 <sub>53.73</sub>	0.02 <sub>47.19</sub>	0.46 <sub>53.73</sub>	0.30 <sub>51.42</sub>	0.13 <sub>48.88</sub>	0.00 <sub>46.97</sub>	0.37 <sub>52.33</sub>	1.00 <sub>61.58</sub>	0.18 <sub>49.53</sub>	0.04 <sub>47.54</sub>
12 Optimal score <sup>a</sup>	0.30 <sub>0.14</sub>	0.00 <sub>0.03</sub>	0.05 <sub>0.05</sub>	1.00 <sub>0.4</sub>	0.45 <sub>0.2</sub>	0.10 <sub>0.07</sub>	0.58 <sub>0.24</sub>	0.05 <sub>0.05</sub>	0.98 <sub>0.39</sub>	0.51 <sub>0.22</sub>
13 Percentage bases uncovered <sup>a</sup>	0.36 <sub>0.61</sub>	0.00 <sub>0.94</sub>	0.35 <sub>0.61</sub>	0.88 <sub>0.13</sub>	0.49 <sub>0.48</sub>	0.13 <sub>0.82</sub>	1.00 <sub>0.01</sub>	0.51 <sub>0.47</sub>	0.99 <sub>0.02</sub>	0.80 <sub>0.19</sub>
14 Number of ambiguous bases	0.72 <sub>115578</sub>	0.00 <sub>301780</sub>	0.66 <sub>131712</sub>	0.92 <sub>64547</sub>	0.83 <sub>87621</sub>	1.00 <sub>43913</sub>	0.95 <sub>57271</sub>	0.93 <sub>60817</sub>	0.92 <sub>63872</sub>	0.77 <sub>102095</sub>
<b>DETONATE</b>										
15 Nucleotide F1	0.60 <sub>0.44</sub>	0.04 <sub>0.22</sub>	0.61 <sub>0.45</sub>	0.86 <sub>0.55</sub>	0.69 <sub>0.48</sub>	0.00 <sub>0.2</sub>	0.87 <sub>0.55</sub>	0.44 <sub>0.38</sub>	0.91 <sub>0.56</sub>	1.00 <sub>0.6</sub>
16 Contig F1	0.27 <sub>0.01</sub>	0.27 <sub>0.01</sub>	1.00 <sub>0.04</sub>	0.79 <sub>0.04</sub>	0.24 <sub>0.01</sub>	0.00 <sub>0</sub>	0.25 <sub>0.01</sub>	0.63 <sub>0.03</sub>	0.23 <sub>0.01</sub>	0.44 <sub>0.02</sub>
17 KC score	0.98 <sub>0.77</sub>	0.17 <sub>0.21</sub>	1.00 <sub>0.78</sub>	0.87 <sub>0.69</sub>	0.94 <sub>0.74</sub>	0.89 <sub>0.71</sub>	0.10 <sub>0.16</sub>	0.00 <sub>0.1</sub>	0.22 <sub>0.25</sub>	0.72 <sub>0.59</sub>
18 RSEM EVAL	1.00 <sub>-1.43</sub>	0.31 <sub>-3.87</sub>	0.99 <sub>-1.45</sub>	0.95 <sub>-1.6</sub>	0.97 <sub>-1.54</sub>	0.83 <sub>-2.02</sub>	0.18 <sub>-4.31</sub>	0.00 <sub>-4.95</sub>	0.38 <sub>-3.6</sub>	0.93 <sub>-1.67</sub>
<b>BUSCO</b>										
19 Complete BUSCOs	0.99 <sub>3885</sub>	0.97 <sub>3832</sub>	1.00 <sub>3907</sub>	0.79 <sub>3435</sub>	0.94 <sub>3784</sub>	0.00 <sub>1695</sub>	0.16 <sub>2042</sub>	0.52 <sub>2852</sub>	0.91 <sub>3710</sub>	0.77 <sub>3405</sub>
20 Missing BUSCOs	1.00 <sub>1944</sub>	0.97 <sub>2014</sub>	1.00 <sub>1954</sub>	0.96 <sub>2042</sub>	0.99 <sub>1978</sub>	0.00 <sub>4392</sub>	0.81 <sub>2419</sub>	0.67 <sub>2764</sub>	1.00 <sub>1952</sub>	0.98 <sub>1992</sub>
<b>Summed up metric (0,1)-score</b>	<b>13.43</b>	<b>7.68</b>	<b>13.87</b>	<b>14.53</b>	<b>12.72</b>	<b>7.55</b>	<b>9.22</b>	<b>8.99</b>	<b>12.94</b>	<b>13.13</b>

<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.