

Supplementary Table 1. Alignment precision computed over all uniquely mapped reads

			GSNAP		TopHat2		STAR		OLego		SOAPSplICE	
8M	50 <sub>bp</sub>	SE	PPV	0.993 ± 0.000	0.992 ± 0.001	0.985 ± 0.000	0.845 ± 0.004	0.788 ± 0.005				
			Unique (%)	79.633 ± 0.609	80.776 ± 0.545	80.447 ± 0.604	90.665 ± 0.361	95.226 ± 0.101				
			Unmapped (%)	15.533 ± 0.299	15.167 ± 0.291	14.595 ± 0.318	4.422 ± 0.093	4.588 ± 0.099				
	76 <sub>bp</sub>	SE	PPV	0.974 ± 0.001	0.995 ± 0.001	0.981 ± 0.001	0.862 ± 0.003	0.806 ± 0.004				
			Unique (%)	83.453 ± 0.507	80.342 ± 0.506	81.703 ± 0.549	87.974 ± 0.452	92.409 ± 0.148				
			Unmapped (%)	12.599 ± 0.247	16.523 ± 0.294	14.612 ± 0.285	6.667 ± 0.105	7.165 ± 0.123				
	50 <sub>bp</sub>	PE	PPV	0.988 ± 0.000	0.991 ± 0.005	0.985 ± 0.000	0.848 ± 0.003	0.929 ± 0.002				
			Unique (%)	82.969 ± 0.543	82.028 ± 0.447	78.199 ± 0.557	90.822 ± 0.544	75.068 ± 0.537				
			Unmapped (%)	13.782 ± 0.297	14.721 ± 0.317	18.853 ± 0.434	4.328 ± 0.076	24.716 ± 0.532				
	76 <sub>bp</sub>	PE	PPV	0.974 ± 0.001	0.995 ± 0.001	0.977 ± 0.000	0.86 ± 0.005	0.848 ± 0.003				
			Unique (%)	84.731 ± 0.296	80.888 ± 0.337	79.212 ± 0.425	88.404 ± 1.679	84.337 ± 0.356				
			Unmapped (%)	12.546 ± 0.338	16.612 ± 0.440	18.272 ± 0.494	6.692 ± 0.161	15.233 ± 0.376				
20M	50 <sub>bp</sub>	SE	PPV	0.993 ± 0.000	0.993 ± 0.001	0.985 ± 0.000	0.843 ± 0.004	0.786 ± 0.005				
			Unique (%)	79.381 ± 0.532	80.56 ± 0.556	80.19 ± 0.519	90.482 ± 0.414	95.221 ± 0.067				
			Unmapped (%)	15.7 ± 0.413	15.319 ± 0.403	14.721 ± 0.408	4.447 ± 0.081	4.596 ± 0.061				
	76 <sub>bp</sub>	SE	PPV	0.973 ± 0.001	0.995 ± 0.000	0.98 ± 0.001	0.861 ± 0.003	0.805 ± 0.004				
			Unique (%)	83.227 ± 0.595	80.088 ± 0.645	81.464 ± 0.603	87.769 ± 0.487	92.402 ± 0.111				
			Unmapped (%)	12.685 ± 0.206	16.637 ± 0.263	14.716 ± 0.249	6.712 ± 0.099	7.175 ± 0.072				
	50 <sub>bp</sub>	PE	PPV	0.988 ± 0.000	0.992 ± 0.000	0.984 ± 0.000	0.846 ± 0.004	0.928 ± 0.002				
			Unique (%)	82.916 ± 0.571	81.878 ± 0.541	78.027 ± 0.620	90.979 ± 0.277	74.944 ± 0.631				
			Unmapped (%)	14.12 ± 0.330	15.074 ± 0.341	19.21 ± 0.439	4.406 ± 0.058	24.847 ± 0.608				
	76 <sub>bp</sub>	PE	PPV	0.974 ± 0.001	0.994 ± 0.001	0.976 ± 0.001	0.861 ± 0.004	0.848 ± 0.004				
			Unique (%)	84.73 ± 0.513	80.973 ± 0.575	79.229 ± 0.643	87.643 ± 0.323	84.171 ± 0.406				
			Unmapped (%)	12.502 ± 0.353	16.567 ± 0.462	18.199 ± 0.552	6.679 ± 0.156	15.39 ± 0.395				

**Supplementary Table 2.** Alignment precision computed over all uniquely mapped reads spanning known junctions

			GSNAP		TopHat2		STAR		OLego	
8M	50 <sub>bp</sub>	SE	PPV	0.974 ± 0.000	0.999 ± 0.000	0.954 ± 0.001	0.908 ± 0.002			
			Unique (%)	94.425 ± 0.294	94.795 ± 0.343	93.496 ± 0.335	84.319 ± 0.387			
			Unmapped (%)	1.121 ± 0.097	0.724 ± 0.051	0.277 ± 0.032	5.815 ± 0.236			
	76 <sub>bp</sub>	PE	PPV	0.98 ± 0.001	0.998 ± 0.000	0.951 ± 0.000	0.906 ± 0.001			
			Unique (%)	96.769 ± 0.376	95.504 ± 0.308	92.088 ± 0.367	85.447 ± 0.361			
			Unmapped (%)	0.49 ± 0.090	0.775 ± 0.118	4.972 ± 0.262	5.777 ± 0.156			
	50 <sub>bp</sub>	SE	PPV	0.974 ± 0.001	0.999 ± 0.000	0.967 ± 0.001	0.94 ± 0.001			
			Unique (%)	95.995 ± 0.388	94.761 ± 0.320	95.776 ± 0.355	85.688 ± 0.630			
			Unmapped (%)	0.088 ± 0.016	1.587 ± 0.156	0.48 ± 0.083	3.288 ± 0.141			
	76 <sub>bp</sub>	PE	PPV	0.976 ± 0.001	0.998 ± 0.000	0.95 ± 0.001	0.94 ± 0.001			
			Unique (%)	97.273 ± 0.278	95.453 ± 0.258	93.324 ± 0.262	86.539 ± 0.558			
			Unmapped (%)	0.065 ± 0.015	1.566 ± 0.081	3.914 ± 0.174	3.302 ± 0.123			
20M	50 <sub>bp</sub>	SE	PPV	0.974 ± 0.000	0.999 ± 0.000	0.955 ± 0.001	0.908 ± 0.003			
			Unique (%)	94.454 ± 0.300	94.891 ± 0.280	93.467 ± 0.357	84.285 ± 0.530			
			Unmapped (%)	1.177 ± 0.083	0.764 ± 0.065	0.318 ± 0.076	5.733 ± 0.114			
	76 <sub>bp</sub>	PE	PPV	0.98 ± 0.000	0.998 ± 0.000	0.951 ± 0.000	0.907 ± 0.001			
			Unique (%)	96.628 ± 0.357	95.301 ± 0.334	92.141 ± 0.436	85.202 ± 0.400			
			Unmapped (%)	0.4 ± 0.039	0.699 ± 0.055	4.806 ± 0.298	5.772 ± 0.118			
	50 <sub>bp</sub>	SE	PPV	0.974 ± 0.001	0.998 ± 0.000	0.968 ± 0.001	0.941 ± 0.001			
			Unique (%)	95.774 ± 0.919	94.413 ± 0.899	95.463 ± 0.928	85.535 ± 1.064			
			Unmapped (%)	0.125 ± 0.081	1.687 ± 0.188	0.598 ± 0.173	3.445 ± 0.164			
	76 <sub>bp</sub>	PE	PPV	0.976 ± 0.001	0.998 ± 0.000	0.95 ± 0.000	0.938 ± 0.007			
			Unique (%)	97.109 ± 0.445	95.214 ± 0.410	93.079 ± 0.450	87.679 ± 3.101			
			Unmapped (%)	0.077 ± 0.020	1.595 ± 0.115	3.936 ± 0.184	3.293 ± 0.118			
40M	50 <sub>bp</sub>	SE	PPV	0.974 ± 0.000	0.999 ± 0.000	0.955 ± 0.001	0.908 ± 0.001			
			Unique (%)	94.307 ± 0.335	94.903 ± 0.357	93.375 ± 0.295	84.111 ± 0.575			
			Unmapped (%)	1.189 ± 0.137	0.732 ± 0.095	0.325 ± 0.098	5.815 ± 0.097			
	76 <sub>bp</sub>	PE	PPV	0.98 ± 0.000	0.998 ± 0.000	0.951 ± 0.001	0.905 ± 0.004			
			Unique (%)	96.454 ± 0.908	95.208 ± 0.818	91.853 ± 0.917	85.069 ± 0.667			
			Unmapped (%)	0.434 ± 0.031	0.734 ± 0.041	4.876 ± 0.286	5.82 ± 0.111			
	50 <sub>bp</sub>	SE	PPV	0.974 ± 0.001	0.998 ± 0.000	0.967 ± 0.001	0.942 ± 0.001			
			Unique (%)	96.005 ± 0.651	94.571 ± 0.558	95.706 ± 0.633	85.629 ± 0.902			
			Unmapped (%)	0.095 ± 0.028	1.63 ± 0.093	0.524 ± 0.078	3.375 ± 0.087			
	76 <sub>bp</sub>	PE	PPV	0.975 ± 0.001	0.998 ± 0.000	0.95 ± 0.001	0.94 ± 0.001			
			Unique (%)	97.216 ± 0.436	95.229 ± 0.425	93.195 ± 0.506	86.25 ± 0.555			
			Unmapped (%)	0.089 ± 0.041	1.67 ± 0.155	3.91 ± 0.253	3.366 ± 0.185			

Supplementary Table 3. Alignment precision computed over all uniquely mapped reads spanning novel junctions

			GSNAP		TopHat2		STAR		OLego		SOAPSplICE	
8M	50 <sub>bp</sub>	SE	PPV	0.892 ± 0.010	0.955 ± 0.012	0.779 ± 0.011	0.885 ± 0.007	0.709 ± 0.003				
			Unique (%)	57.148 ± 4.497	81.237 ± 5.222	90.915 ± 1.833	66.846 ± 5.322	81.498 ± 0.308				
			Unmapped (%)	39.407 ± 4.140	11.86 ± 5.264	0.979 ± 0.725	24.786 ± 5.312	17.436 ± 0.253				
	76 <sub>bp</sub>	PE	PPV	0.851 ± 0.026	0.942 ± 0.053	0.769 ± 0.031	0.886 ± 0.014	0.762 ± 0.002				
			Unique (%)	91.111 ± 1.856	85.545 ± 2.632	88.082 ± 2.239	68.43 ± 3.945	76.631 ± 0.266				
			Unmapped (%)	5.382 ± 0.969	8.411 ± 1.859	8.071 ± 1.649	22.986 ± 2.733	22.243 ± 0.190				
	50 <sub>bp</sub>	SE	PPV	0.88 ± 0.015	0.952 ± 0.016	0.839 ± 0.016	0.929 ± 0.007	0.815 ± 0.005				
			Unique (%)	95.079 ± 1.709	82.698 ± 4.804	93.725 ± 3.222	72.628 ± 4.936	85.755 ± 0.319				
			Unmapped (%)	0.208 ± 0.300	12.953 ± 4.484	1.161 ± 0.993	18.496 ± 3.857	12.646 ± 0.262				
	76 <sub>bp</sub>	PE	PPV	0.882 ± 0.020	0.97 ± 0.008	0.84 ± 0.011	0.93 ± 0.004	0.841 ± 0.002				
			Unique (%)	96.645 ± 1.722	83.056 ± 3.900	90.177 ± 4.212	74.37 ± 2.844	83.917 ± 0.387				
			Unmapped (%)	0.023 ± 0.019	12.453 ± 2.431	5.973 ± 2.560	17.859 ± 1.973	14.477 ± 0.225				
20M	50 <sub>bp</sub>	SE	PPV	0.874 ± 0.041	0.948 ± 0.015	0.759 ± 0.033	0.881 ± 0.011	0.708 ± 0.006				
			Unique (%)	56.927 ± 2.271	82.368 ± 4.640	91.044 ± 2.726	68.583 ± 4.685	81.604 ± 0.175				
			Unmapped (%)	38.182 ± 2.672	8.973 ± 1.783	0.827 ± 0.608	21.561 ± 2.151	17.347 ± 0.144				
	76 <sub>bp</sub>	PE	PPV	0.85 ± 0.014	0.94 ± 0.032	0.776 ± 0.011	0.886 ± 0.011	0.763 ± 0.002				
			Unique (%)	90.198 ± 2.474	84.705 ± 3.947	88.285 ± 2.559	70.165 ± 3.292	76.572 ± 0.383				
			Unmapped (%)	6.207 ± 1.295	9.101 ± 2.281	8.254 ± 1.885	23.08 ± 2.750	22.225 ± 0.282				
	50 <sub>bp</sub>	SE	PPV	0.892 ± 0.007	0.963 ± 0.010	0.851 ± 0.008	0.929 ± 0.004	0.816 ± 0.004				
			Unique (%)	94.786 ± 2.508	82.333 ± 2.764	93.445 ± 2.607	73.506 ± 1.949	85.489 ± 0.753				
			Unmapped (%)	0.162 ± 0.120	12.545 ± 2.595	0.647 ± 0.229	17.825 ± 2.173	12.771 ± 0.296				
	76 <sub>bp</sub>	PE	PPV	0.88 ± 0.014	0.957 ± 0.015	0.829 ± 0.009	0.919 ± 0.022	0.844 ± 0.002				
			Unique (%)	96.874 ± 1.669	84.125 ± 3.757	91.53 ± 2.139	75.385 ± 3.111	84.106 ± 0.323				
			Unmapped (%)	0.039 ± 0.041	12.01 ± 3.939	5.464 ± 1.453	17.469 ± 3.102	14.296 ± 0.190				
40M	50 <sub>bp</sub>	SE	PPV	0.896 ± 0.012	0.964 ± 0.009	0.78 ± 0.016	0.889 ± 0.008	0.707 ± 0.005				
			Unique (%)	57.425 ± 3.548	84.291 ± 3.224	91.6 ± 2.276	69.378 ± 4.214	81.582 ± 0.218				
			Unmapped (%)	38.395 ± 4.281	8.204 ± 1.888	0.709 ± 0.340	22.495 ± 3.795	17.373 ± 0.172				
	76 <sub>bp</sub>	PE	PPV	0.851 ± 0.014	0.936 ± 0.029	0.775 ± 0.011	0.884 ± 0.010	0.76 ± 0.003				
			Unique (%)	91.965 ± 1.302	85.946 ± 2.551	89.424 ± 2.518	70.052 ± 2.104	76.391 ± 0.707				
			Unmapped (%)	5.479 ± 1.276	8.638 ± 3.036	7.123 ± 1.932	22.773 ± 2.821	22.441 ± 0.552				
	50 <sub>bp</sub>	SE	PPV	0.894 ± 0.015	0.962 ± 0.016	0.85 ± 0.013	0.931 ± 0.007	0.816 ± 0.005				
			Unique (%)	95.806 ± 1.935	83.378 ± 3.146	94.702 ± 2.198	75.558 ± 3.717	85.746 ± 0.246				
			Unmapped (%)	0.072 ± 0.073	11.958 ± 2.522	0.521 ± 0.234	15.717 ± 1.739	12.663 ± 0.125				
	76 <sub>bp</sub>	PE	PPV	0.872 ± 0.017	0.943 ± 0.028	0.833 ± 0.013	0.924 ± 0.006	0.843 ± 0.002				
			Unique (%)	96.792 ± 1.688	83.07 ± 3.084	91.606 ± 2.653	71.622 ± 4.171	83.972 ± 0.280				
			Unmapped (%)	0.015 ± 0.009	12.836 ± 2.883	4.687 ± 0.946	17.885 ± 1.924	14.404 ± 0.145				

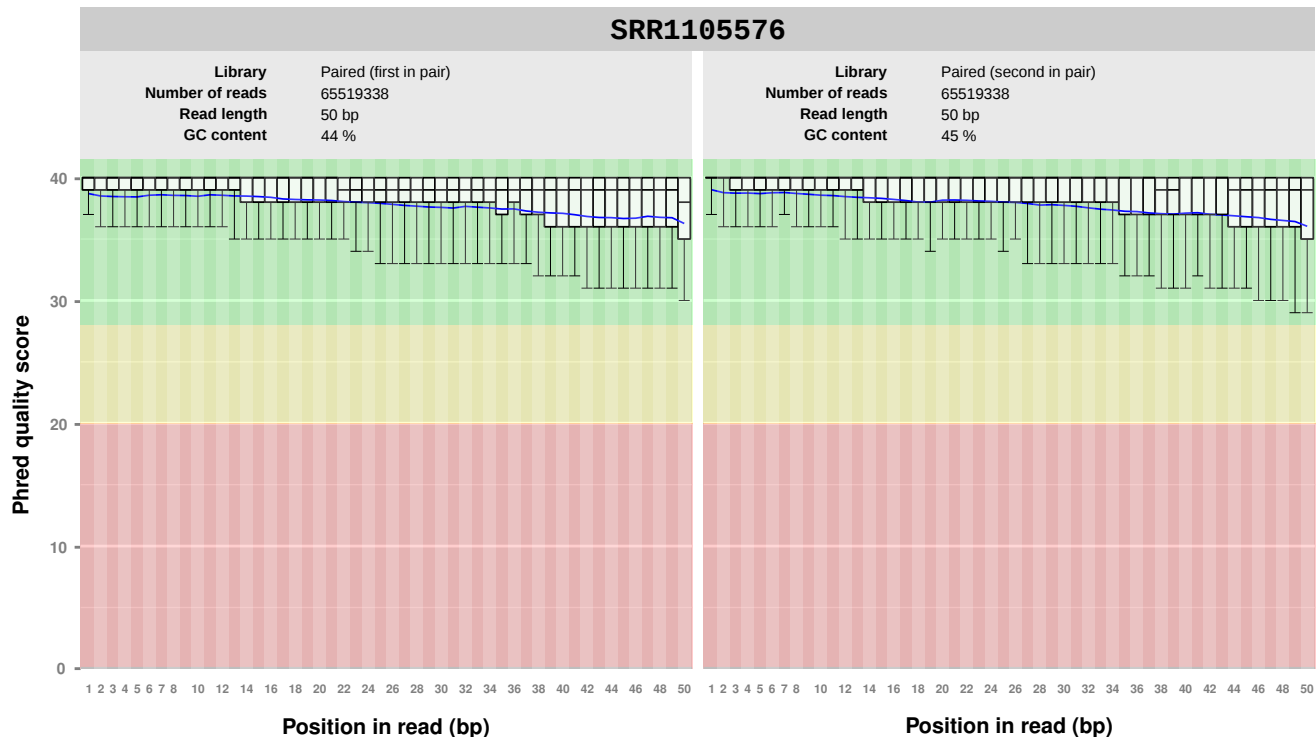
Supplementary Table 4. Splice junction detection performance of all alignment methods

			GSNAP		TopHat2		STAR		OLego		SOAPSsplice	
8M	50 <sub>bp</sub>	SE	PPV	0.96 ± 0.001	0.919 ± 0.002	0.997 ± 0.000	0.997 ± 0.000	0.979 ± 0.001	0.976 ± 0.001			
			Sensitivity	0.946 ± 0.001	0.963 ± 0.001	0.939 ± 0.002	0.89 ± 0.002	0.767 ± 0.002				
			F <sub>1</sub> score	0.953 ± 0.001	0.94 ± 0.001	0.967 ± 0.001	0.932 ± 0.001	0.859 ± 0.002				
	50 <sub>bp</sub>	PE	PPV	0.862 ± 0.002	0.917 ± 0.001	0.986 ± 0.001	0.973 ± 0.001	0.967 ± 0.001				
			Sensitivity	0.971 ± 0.002	0.974 ± 0.001	0.953 ± 0.002	0.906 ± 0.002	0.797 ± 0.003				
			F <sub>1</sub> score	0.913 ± 0.001	0.945 ± 0.001	0.969 ± 0.001	0.938 ± 0.001	0.874 ± 0.002				
	76 <sub>bp</sub>	SE	PPV	0.917 ± 0.002	0.93 ± 0.002	0.99 ± 0.001	0.98 ± 0.000	0.958 ± 0.002				
			Sensitivity	0.96 ± 0.001	0.969 ± 0.001	0.96 ± 0.001	0.91 ± 0.001	0.86 ± 0.001				
			F <sub>1</sub> score	0.938 ± 0.001	0.949 ± 0.001	0.975 ± 0.001	0.944 ± 0.001	0.906 ± 0.001				
	76 <sub>bp</sub>	PE	PPV	0.806 ± 0.002	0.929 ± 0.001	0.984 ± 0.001	0.975 ± 0.001	0.952 ± 0.002				
			Sensitivity	0.975 ± 0.001	0.977 ± 0.001	0.966 ± 0.001	0.927 ± 0.002	0.878 ± 0.002				
			F <sub>1</sub> score	0.882 ± 0.001	0.953 ± 0.001	0.975 ± 0.001	0.95 ± 0.001	0.913 ± 0.001				
20M	50 <sub>bp</sub>	SE	PPV	0.951 ± 0.001	0.908 ± 0.002	0.997 ± 0.000	0.973 ± 0.001	0.972 ± 0.001				
			Sensitivity	0.949 ± 0.002	0.965 ± 0.001	0.943 ± 0.002	0.9 ± 0.002	0.786 ± 0.003				
			F <sub>1</sub> score	0.95 ± 0.001	0.936 ± 0.001	0.969 ± 0.001	0.935 ± 0.001	0.869 ± 0.002				
	50 <sub>bp</sub>	PE	PPV	0.814 ± 0.003	0.903 ± 0.002	0.983 ± 0.001	0.965 ± 0.001	0.959 ± 0.002				
			Sensitivity	0.973 ± 0.001	0.976 ± 0.001	0.958 ± 0.001	0.915 ± 0.002	0.817 ± 0.003				
			F <sub>1</sub> score	0.887 ± 0.002	0.938 ± 0.001	0.97 ± 0.001	0.94 ± 0.001	0.882 ± 0.002				
	76 <sub>bp</sub>	SE	PPV	0.894 ± 0.001	0.92 ± 0.001	0.988 ± 0.001	0.974 ± 0.001	0.949 ± 0.003				
			Sensitivity	0.962 ± 0.001	0.97 ± 0.001	0.962 ± 0.002	0.918 ± 0.002	0.872 ± 0.002				
			F <sub>1</sub> score	0.927 ± 0.001	0.944 ± 0.001	0.975 ± 0.001	0.945 ± 0.001	0.909 ± 0.002				
	76 <sub>bp</sub>	PE	PPV	0.741 ± 0.003	0.917 ± 0.002	0.981 ± 0.001	0.968 ± 0.001	0.942 ± 0.002				
			Sensitivity	0.977 ± 0.001	0.979 ± 0.001	0.97 ± 0.001	0.934 ± 0.002	0.89 ± 0.002				
			F <sub>1</sub> score	0.843 ± 0.002	0.947 ± 0.001	0.976 ± 0.001	0.95 ± 0.001	0.915 ± 0.001				
40M	50 <sub>bp</sub>	SE	PPV	0.946 ± 0.001	0.903 ± 0.001	0.996 ± 0.000	0.968 ± 0.001	0.97 ± 0.001				
			Sensitivity	0.95 ± 0.001	0.965 ± 0.001	0.944 ± 0.001	0.905 ± 0.001	0.795 ± 0.002				
			F <sub>1</sub> score	0.948 ± 0.000	0.933 ± 0.001	0.97 ± 0.000	0.936 ± 0.001	0.874 ± 0.001				
	50 <sub>bp</sub>	PE	PPV	0.78 ± 0.003	0.896 ± 0.001	0.982 ± 0.001	0.959 ± 0.001	0.955 ± 0.001				
			Sensitivity	0.974 ± 0.001	0.977 ± 0.001	0.96 ± 0.001	0.92 ± 0.001	0.825 ± 0.003				
			F <sub>1</sub> score	0.866 ± 0.002	0.935 ± 0.001	0.971 ± 0.001	0.939 ± 0.001	0.885 ± 0.002				
	76 <sub>bp</sub>	SE	PPV	0.879 ± 0.002	0.915 ± 0.001	0.987 ± 0.000	0.969 ± 0.001	0.942 ± 0.002				
			Sensitivity	0.961 ± 0.001	0.969 ± 0.001	0.962 ± 0.001	0.922 ± 0.001	0.878 ± 0.002				
			F <sub>1</sub> score	0.919 ± 0.001	0.941 ± 0.001	0.974 ± 0.001	0.945 ± 0.001	0.909 ± 0.001				
	76 <sub>bp</sub>	PE	PPV	0.693 ± 0.004	0.91 ± 0.002	0.979 ± 0.001	0.961 ± 0.001	0.933 ± 0.003				
			Sensitivity	0.978 ± 0.001	0.98 ± 0.001	0.971 ± 0.001	0.937 ± 0.001	0.895 ± 0.002				
			F <sub>1</sub> score	0.811 ± 0.003	0.944 ± 0.001	0.975 ± 0.001	0.949 ± 0.001	0.914 ± 0.001				

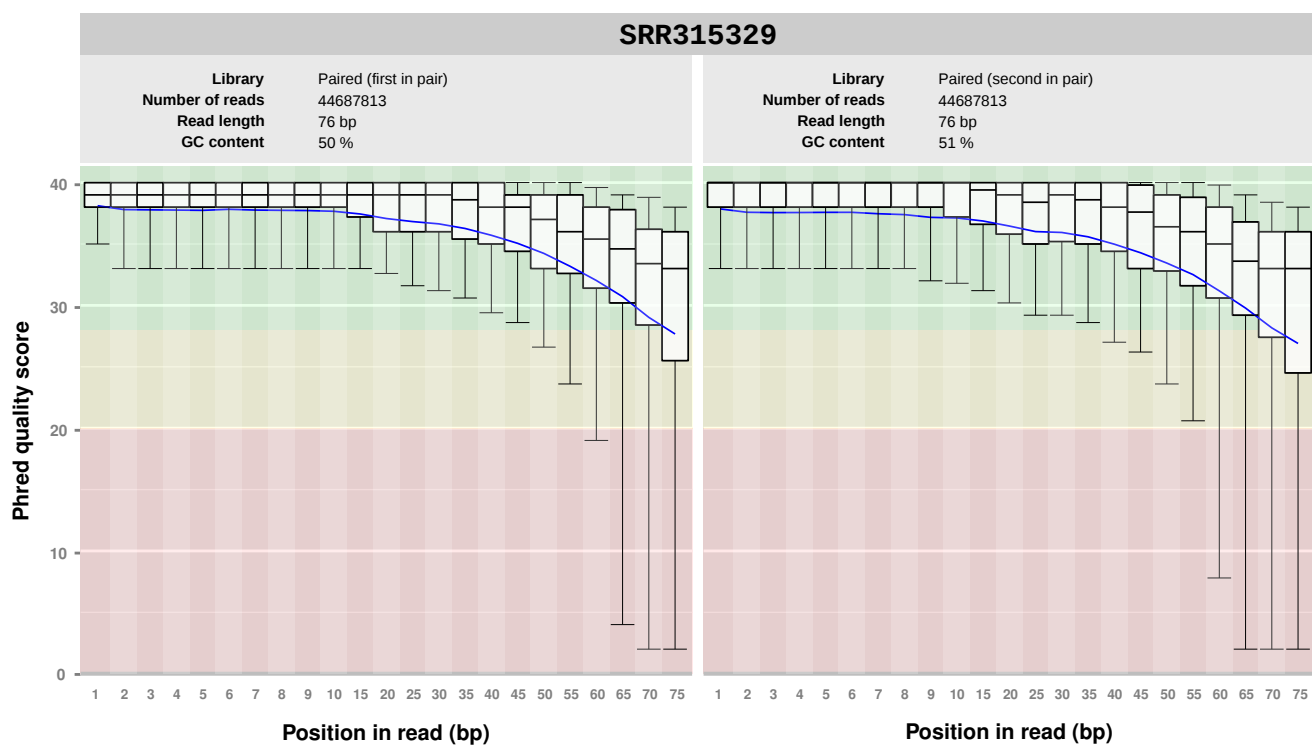
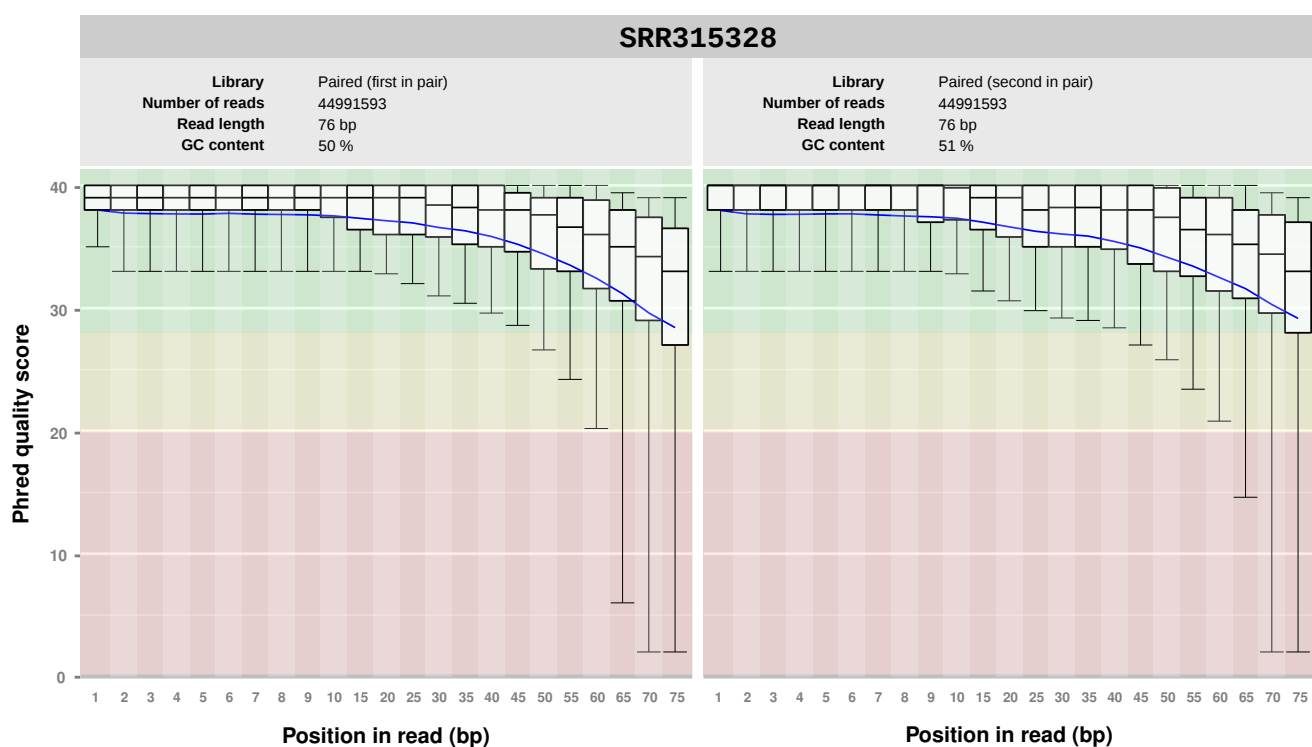
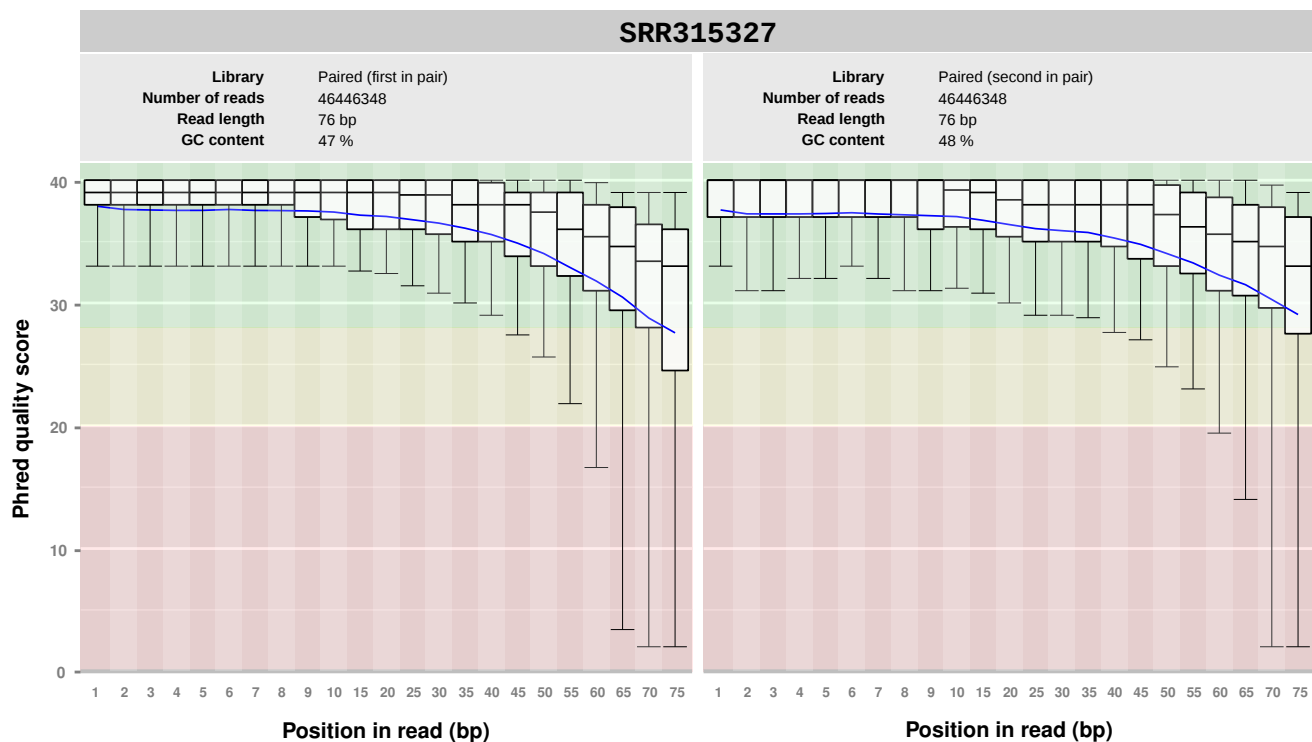
Supplementary Table 5. Splice junction detection performance of TopHat2 with and without FineSplice

		Sensitivity		PPV		F <sub>1</sub> score		Realign w/ segment mismatches	
		TopHat2	FineSplice	TopHat2	FineSplice	TopHat2	FineSplice		
8M	50 <sub>bp</sub>	SE	0.963	0.939	0.920	0.982	0.941	0.960	-
			0.963	0.930	0.929	0.977	0.946	0.953	1
			0.963	0.922	0.929	0.977	0.946	0.948	2
		PE	0.973	0.954	0.919	0.985	0.945	0.969	-
			0.972	0.955	0.921	0.983	0.946	0.969	1
			0.972	0.955	0.921	0.983	0.946	0.969	2
	76 <sub>bp</sub>	SE	0.968	0.943	0.930	0.994	0.949	0.968	-
			0.967	0.927	0.939	0.995	0.953	0.960	1
			0.967	0.927	0.939	0.995	0.953	0.960	2
		PE	0.977	0.965	0.929	0.989	0.952	0.977	-
			0.976	0.966	0.925	0.987	0.950	0.976	1
			0.976	0.966	0.925	0.987	0.950	0.976	2
20M	50 <sub>bp</sub>	SE	0.966	0.941	0.908	0.989	0.936	0.964	-
			0.966	0.922	0.919	0.987	0.942	0.953	1
			0.966	0.922	0.918	0.986	0.941	0.953	2
		PE	0.978	0.960	0.906	0.984	0.940	0.972	-
			0.977	0.966	0.902	0.973	0.938	0.969	1
			0.978	0.966	0.902	0.973	0.938	0.969	2
	76 <sub>bp</sub>	SE	0.967	0.950	0.919	0.993	0.943	0.971	-
			0.967	0.911	0.929	0.990	0.947	0.949	1
			0.967	0.911	0.929	0.990	0.947	0.949	2
		PE	0.978	0.961	0.917	0.991	0.947	0.976	-
			0.978	0.971	0.901	0.974	0.938	0.973	1
			0.978	0.971	0.901	0.974	0.938	0.973	2
40M	50 <sub>bp</sub>	SE	0.966	0.941	0.905	0.989	0.934	0.964	-
			0.966	0.922	0.916	0.991	0.940	0.955	1
			0.966	0.925	0.915	0.991	0.940	0.956	2
		PE	0.977	0.955	0.897	0.984	0.936	0.970	-
			0.977	0.960	0.892	0.977	0.933	0.968	1
			0.977	0.960	0.892	0.977	0.932	0.968	2
	76 <sub>bp</sub>	SE	0.969	0.943	0.916	0.994	0.942	0.968	-
			0.969	0.935	0.925	0.994	0.947	0.963	1
			0.969	0.935	0.925	0.994	0.947	0.963	2
		PE	0.981	0.965	0.913	0.990	0.946	0.977	-
			0.981	0.970	0.887	0.982	0.932	0.976	1
			0.981	0.970	0.887	0.982	0.932	0.976	2

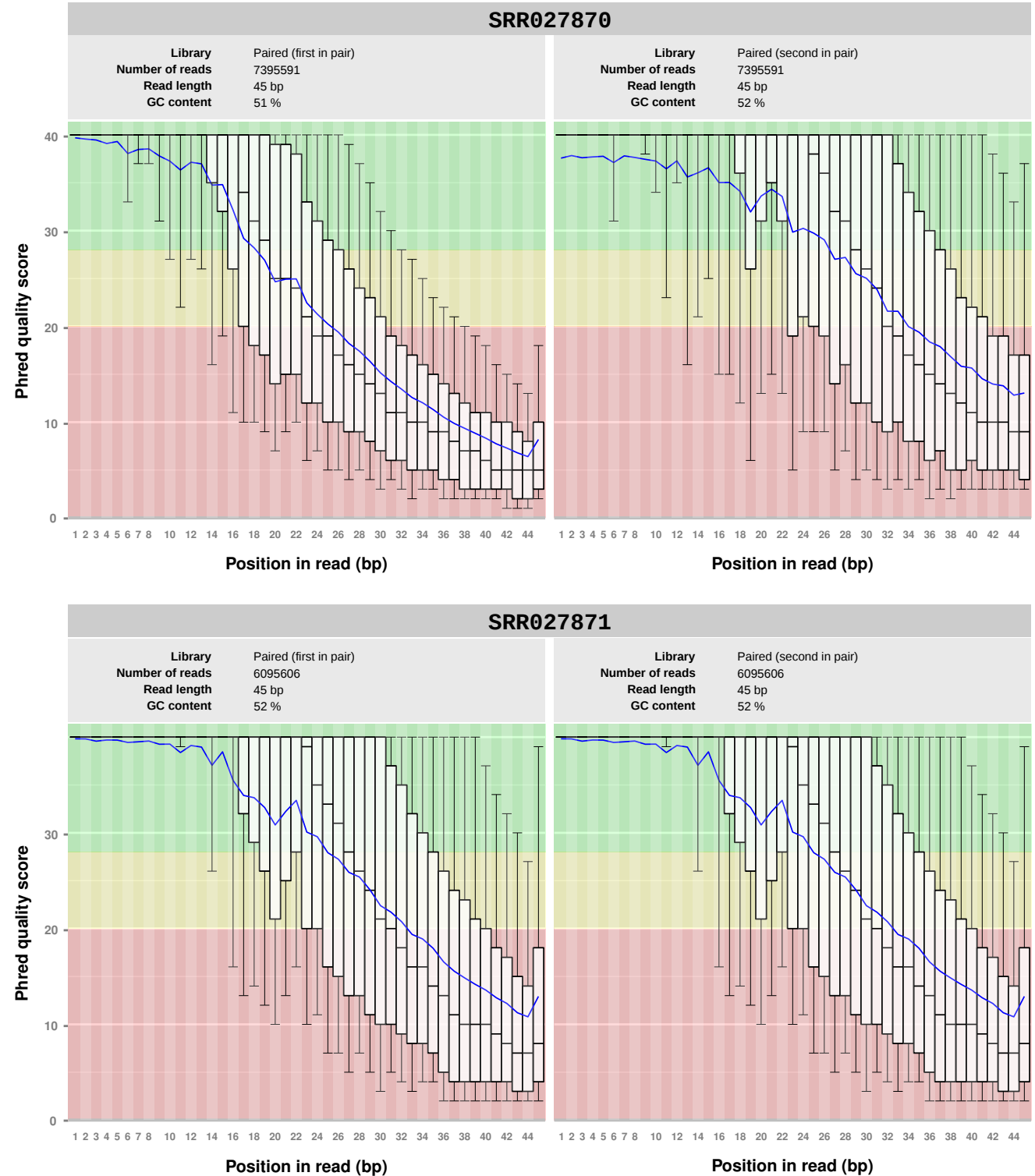
**Supplementary Figure 1.** Summary table and quality plots for the experimental data used for estimating the 50 bp error profile (SRA Experiment SRX424347, run accession number on top panel)



**Supplementary Figure 2.** Summary tables and quality plots for the human high-quality dataset (SRA Experiment SRX084679, run accession number on top panel)

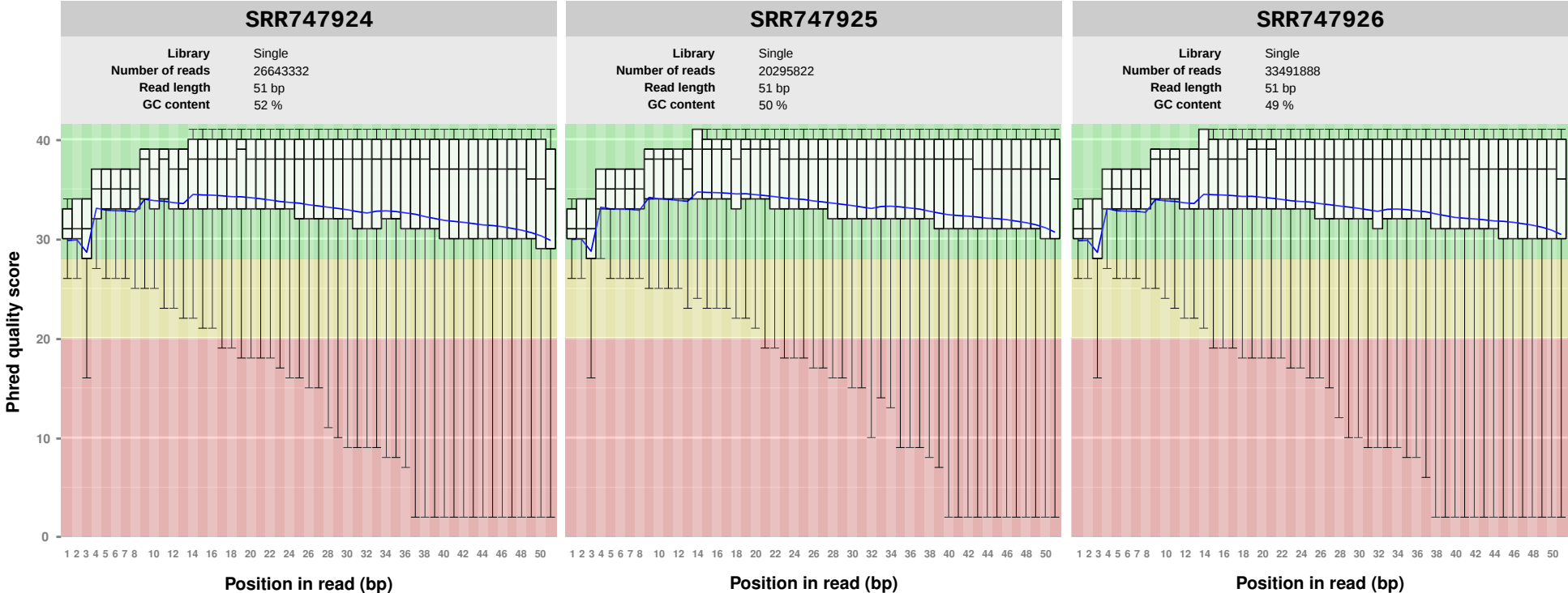


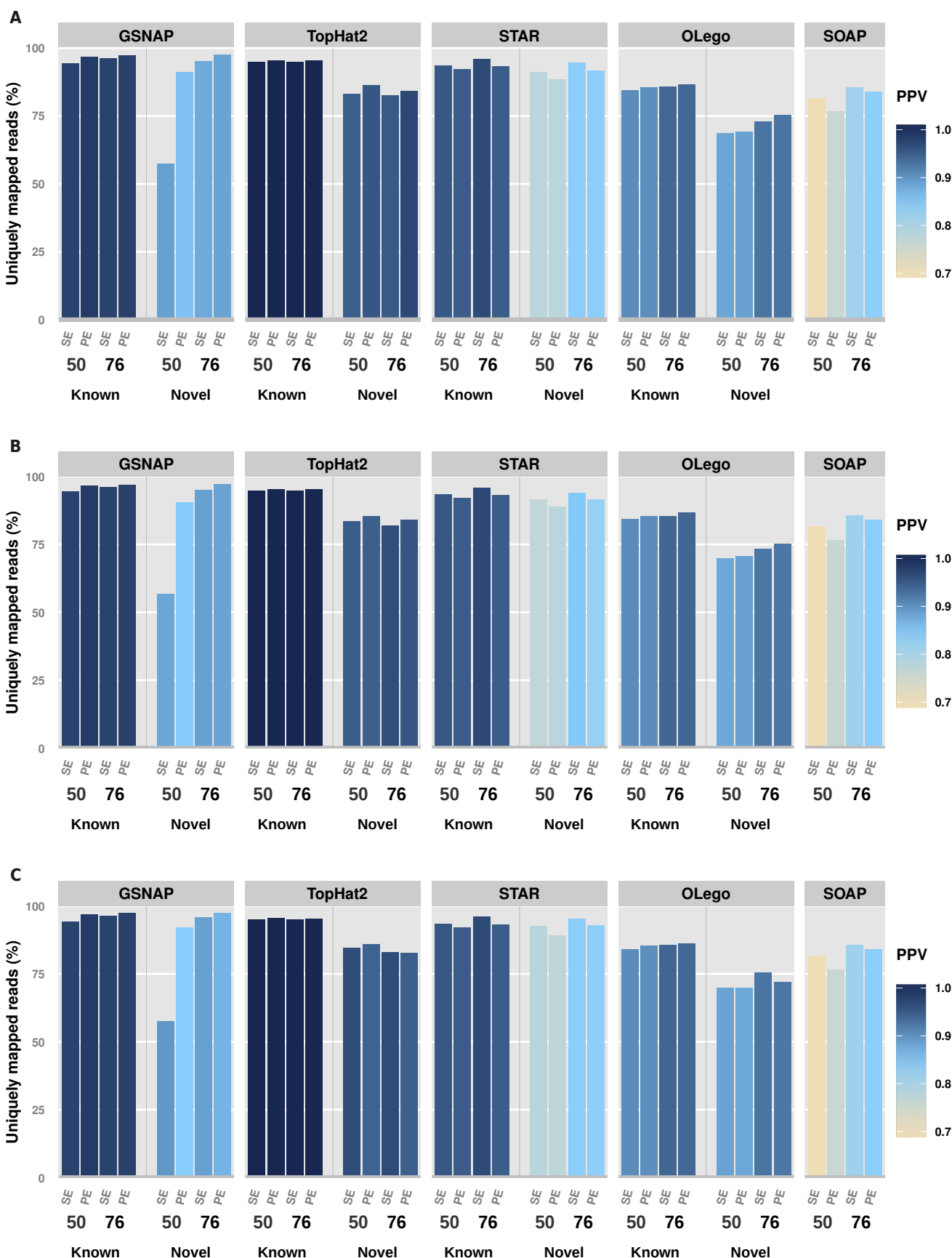
**Supplementary Figure 3.** Summary tables and quality plots for the human low-quality dataset (SRA Experiment SRX011546, run accession number on top panel)



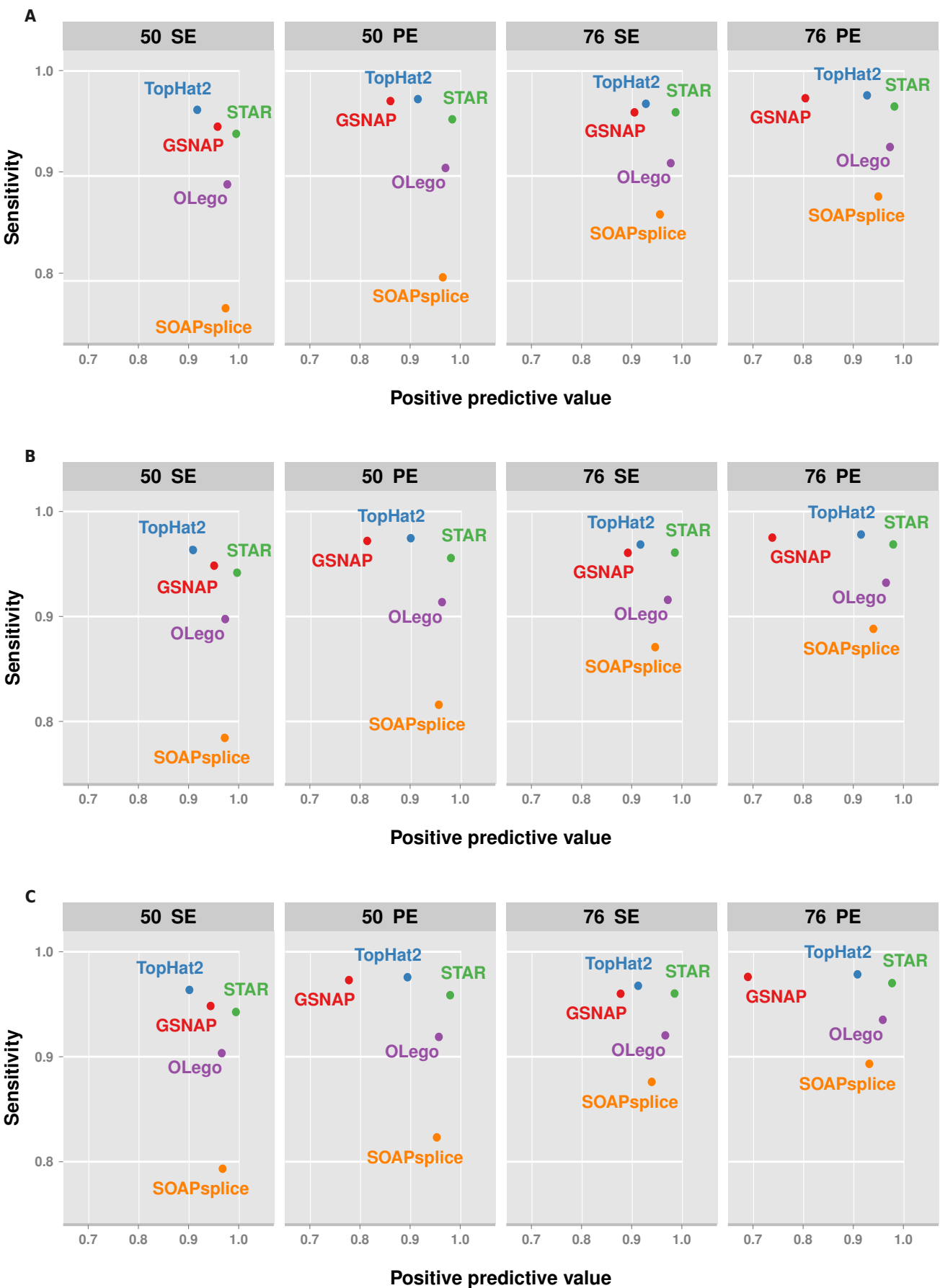


**Supplementary Figure 4.** Summary tables and quality plots for the pig dataset (SRA Experiments SRX242929, SRX242930 and SRX242931, run accession number on top panel)

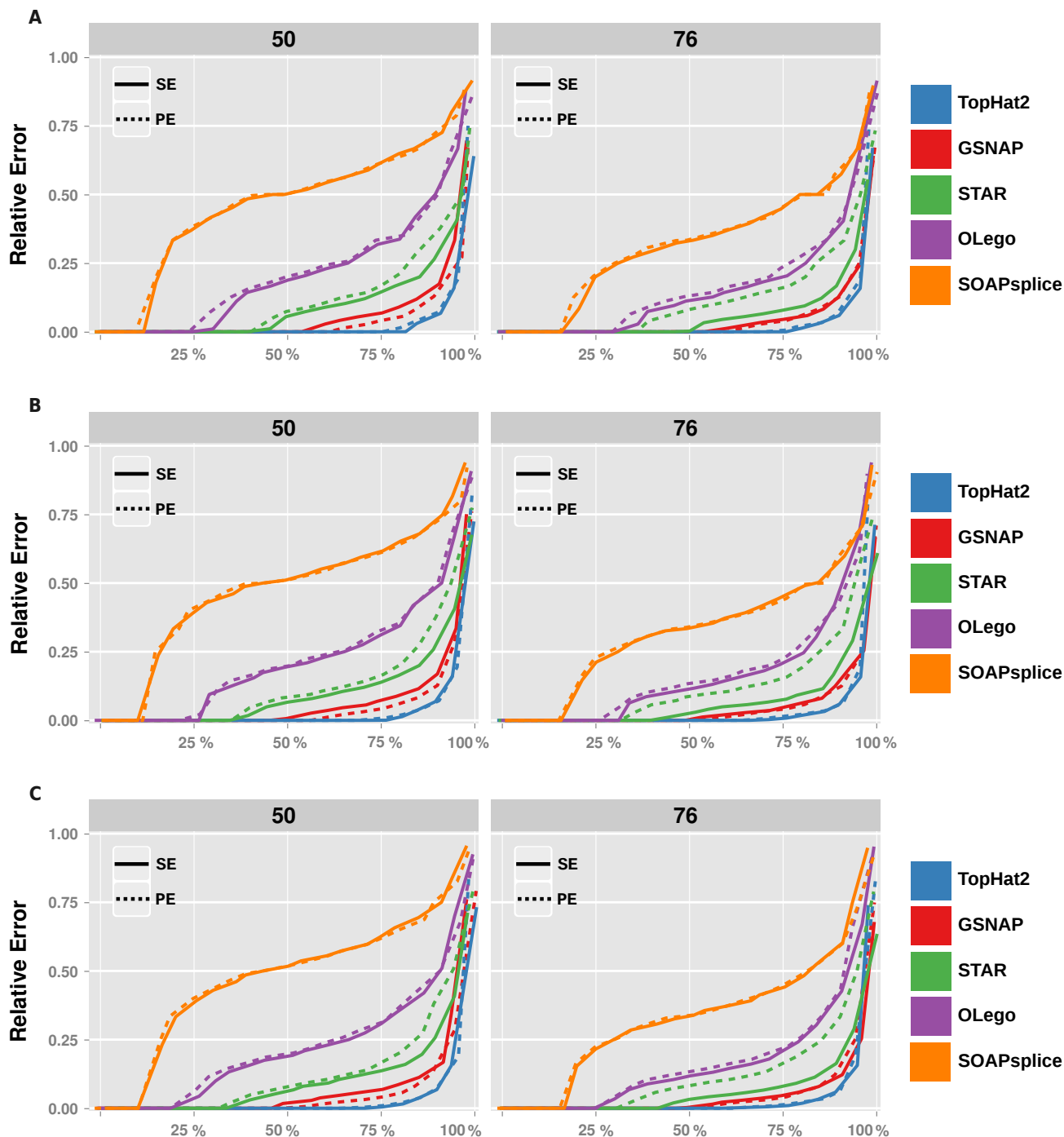




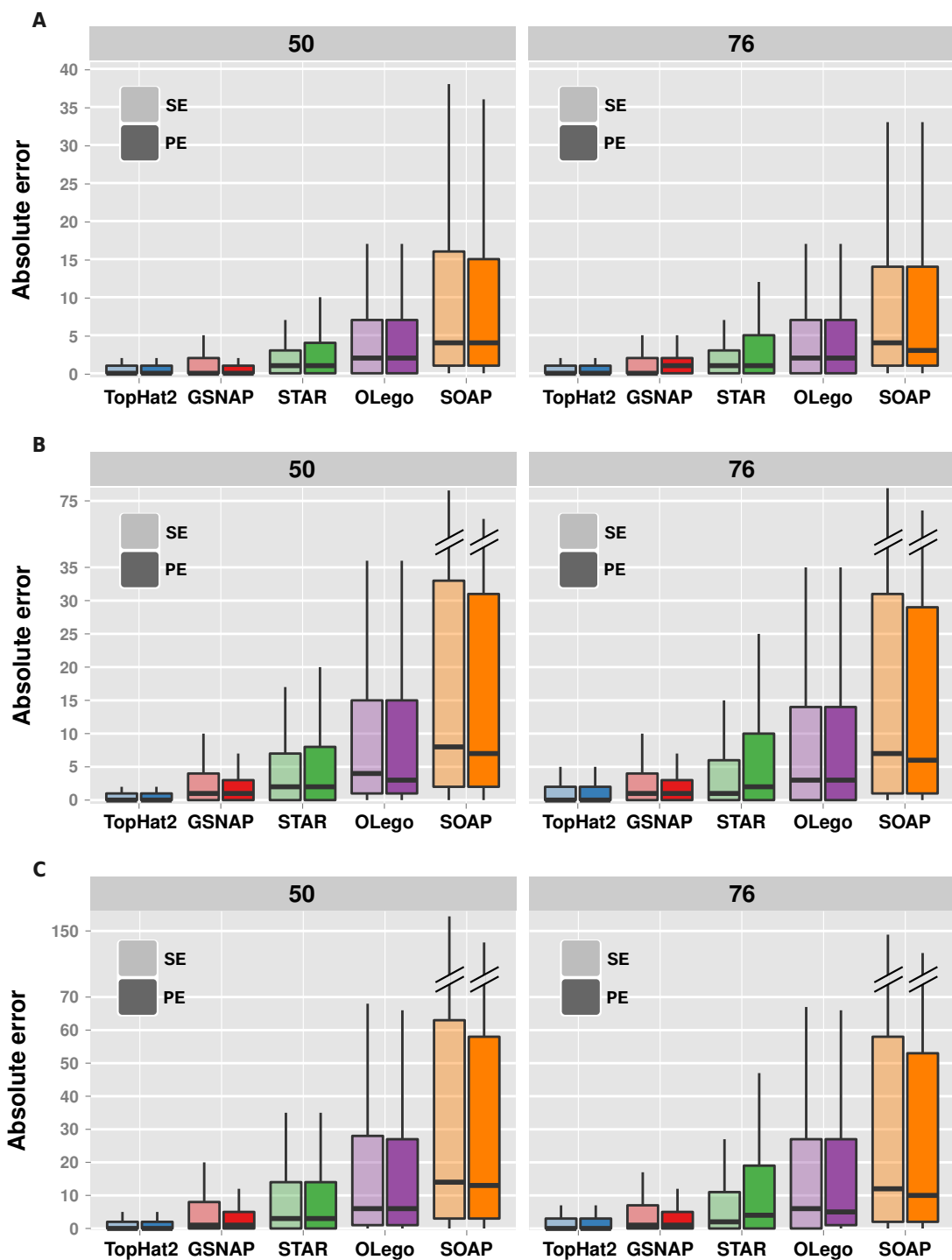
**Supplementary Figure 5.** Percentage of uniquely mapped reads (bar chart, y-axis) and positive predictive value (ratio of correctly aligned nucleotides, color coded), averaged over 10 simulated data sets per experimental condition, for each alignment method (on a separate panel). Left and right sides of each panel correspond to reads spanning known and novel junctions (except for SOAPsplice, which aligns exclusively *ab initio*) Each bar corresponds to a different simulation setup: 50 or 76 bp read length, single-end (SE) or paired-end (PE) library, at 8M (A), 20M (B) and 40M (C) sequencing depth.



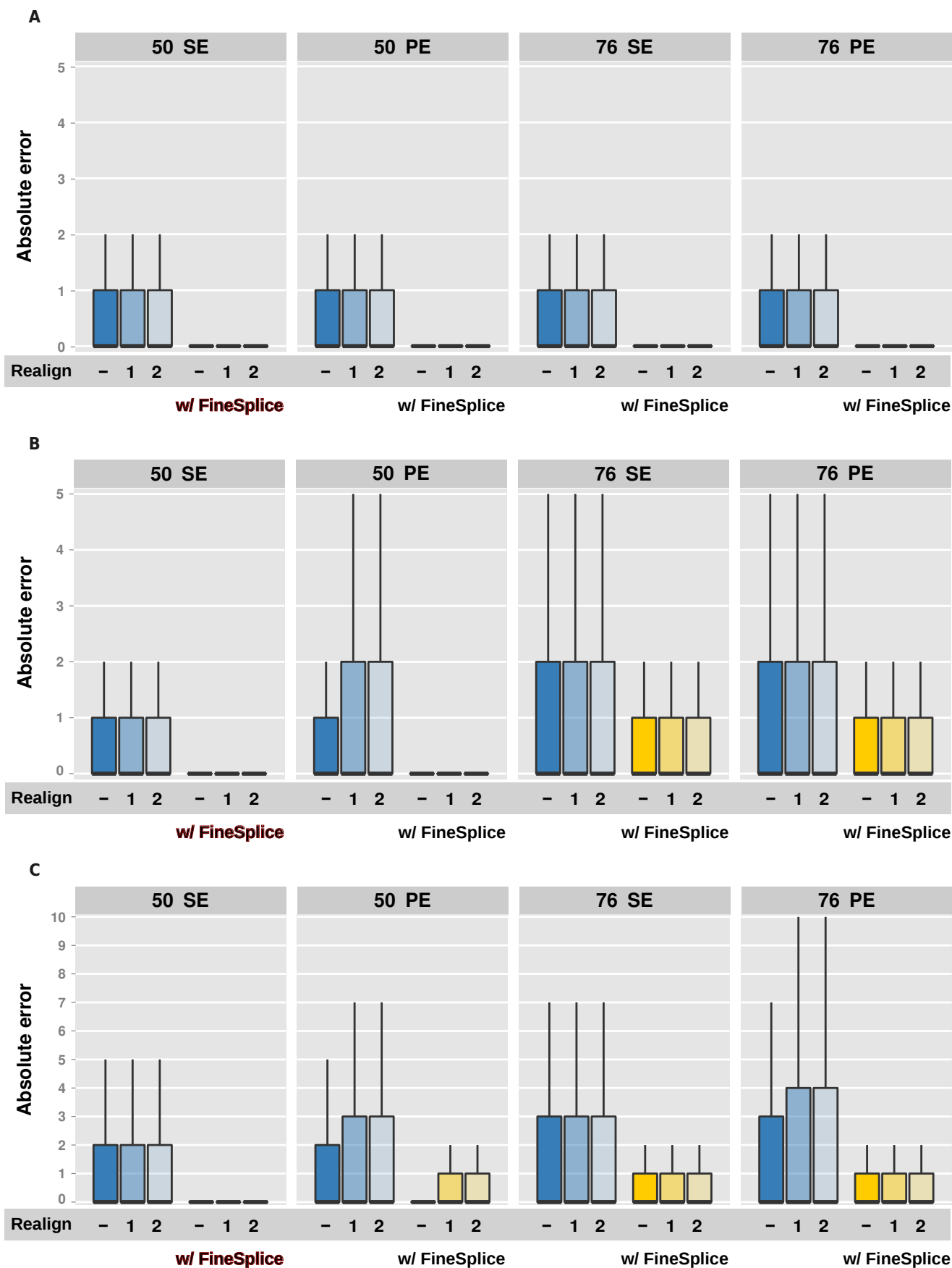
**Supplementary Figure 6.** Junction detection sensitivity (y-axis) and positive predictive value (x-axis), averaged over 10 simulated data sets per experimental condition, at 8M (A), 20M (B) and 40M (C) reads sequencing depth. Panels correspond to different simulation setups: 50 or 76 bp read length, single-end (SE) or paired-end (PE) library.



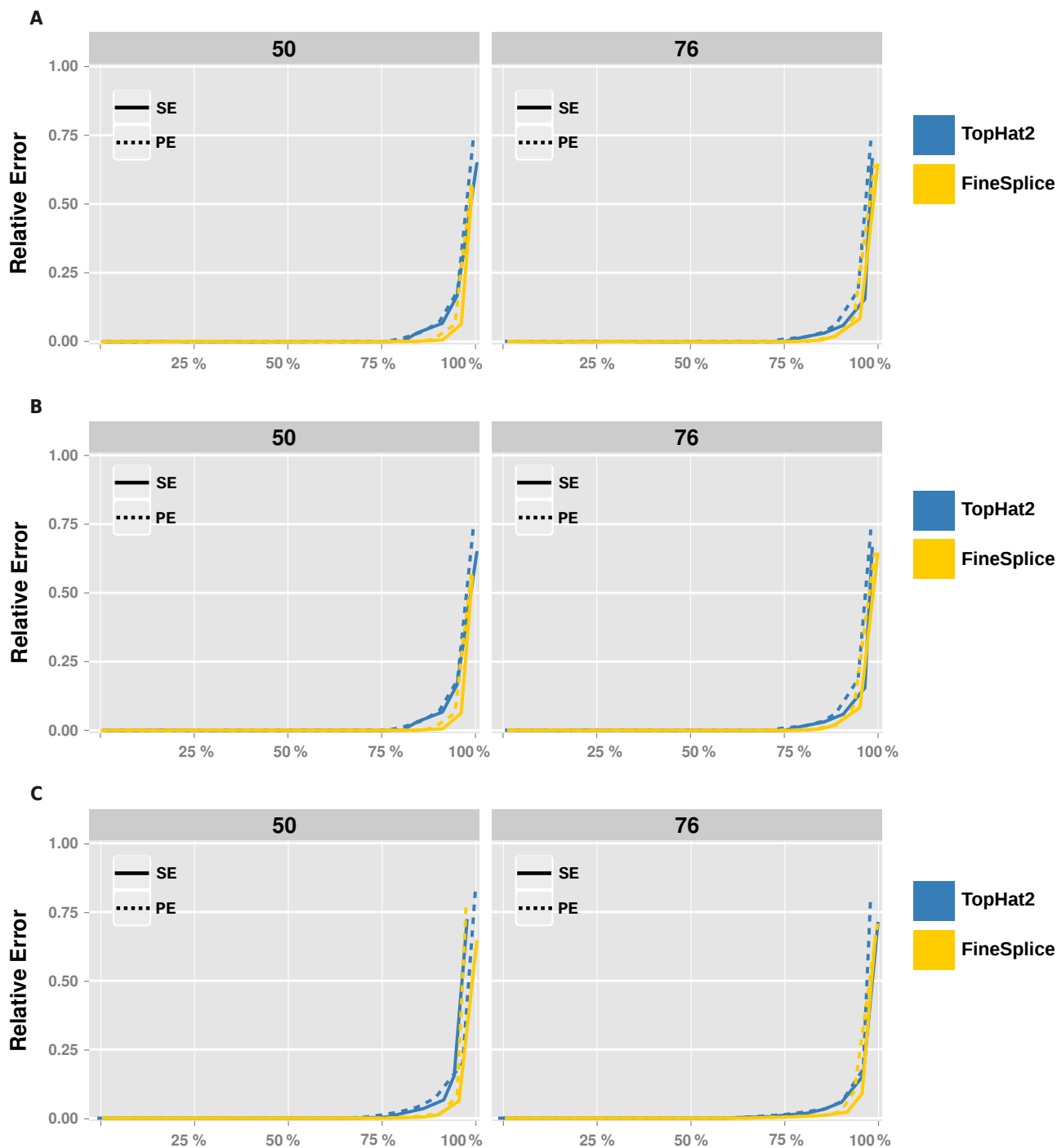
**Supplementary Figure 7.** Relative quantification error (absolute difference between alignment counts and true read count relative to the true value, y-axis) at increasing percentiles (x-axis) for each aligner at 8M (**A**), 20M (**B**) and 40M (**C**) reads sequencing depth, all values being averaged over 10 simulated data sets per experimental condition. 50 and 76 bp reads are represented on separate panels, single-end (SE) and paired-end (PE) reads with, respectively, continuous and dashed lines.



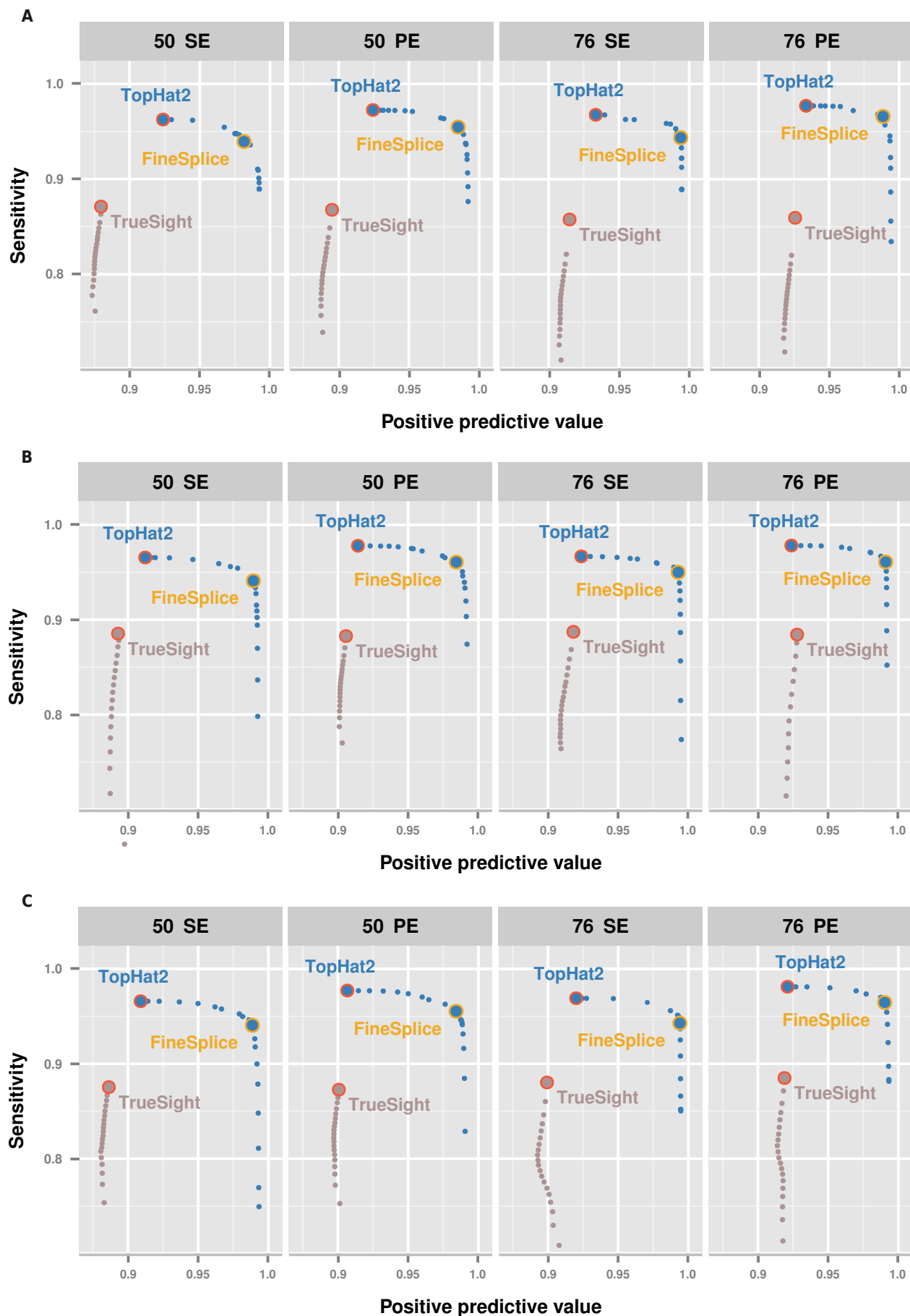
**Supplementary Figure 8.** Absolute quantification error (absolute difference between alignment counts and true read counts, y-axis) in a box plot representation encompassing all 10 simulated data sets, at 8M (**A**), 20M (**B**) and 40M (**C**) reads sequencing depth. 50 and 76 bp reads represented on separate panels, single-end (SE) and paired-end (PE) with distinct fill gradients.



**Supplementary Figure 9.** TopHat2 absolute quantification error before (blue bars) and after filtering with FineSplice (yellow bars) in a box plot representation (cf. Supplementary Figure 4). Different simulation settings are shown on separate panels: 50 and 76 bp read length, single-end (SE) and paired-end (PE) library, at 8M (A), 20M (B) and 40M (C) reads sequencing depth. Each bar corresponds to different TopHat2 alignment options, either default (-), or with realignment of ambiguously mapping multi-exon reads, allowing up to 1 or 2 mismatches in read segments alignment.



**Supplementary Figure 10.** TopHat2 relative quantification error percentiles (cf. Supplementary Figure 3), with and without FineSplice at 8M (**A**), 20M (**B**) and 40M (**C**) reads sequencing depth. Different simulation settings on separate panels: 50 and 76 bp read length, single-end (SE) and paired-end (PE) library.



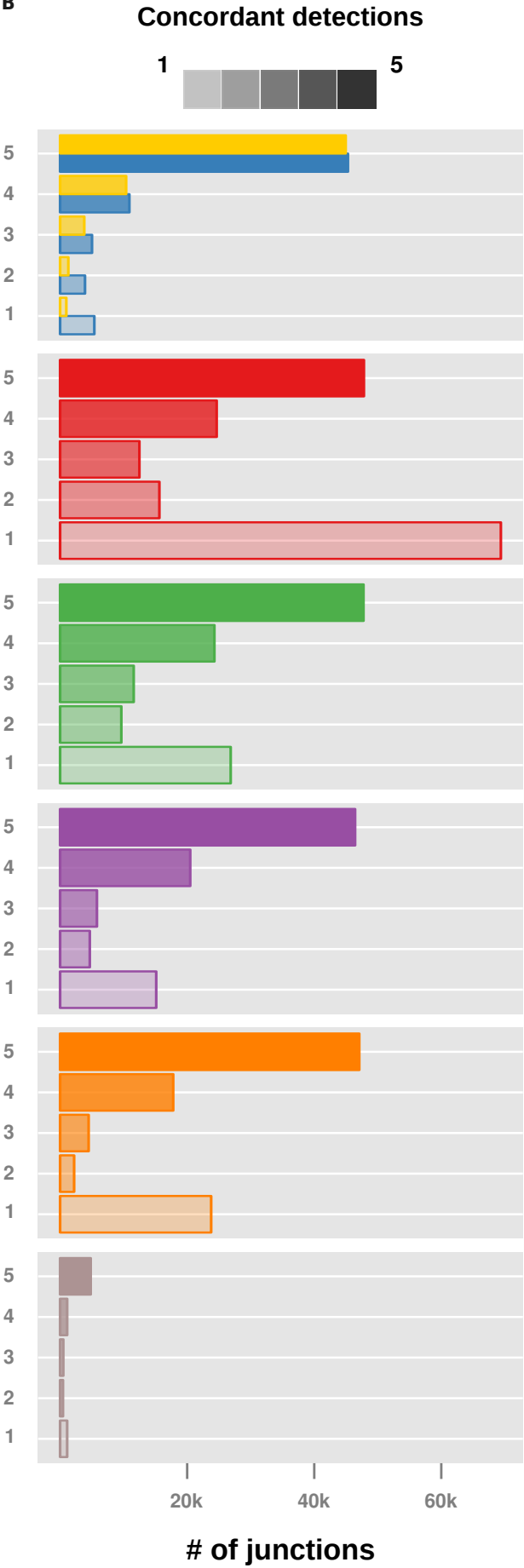
**Supplementary Figure 11.** Comparison of FineSplice and TrueSight detection performance in simulated data. Sensitivity (y-axis) and positive predictive value (x-axis) of inferred junctions evaluated at increasing thresholds for the respective score (posterior probability), with larger dots (outlined) corresponding to the default behaviour of each algorithm (no threshold for TrueSight and TopHat2 alone, 0.5 for TopHat2 w/ FineSplice). Panels correspond to different simulation setups, at 8M (**A**), 20M (**B**) and 40M (**C**) reads sequencing depth.



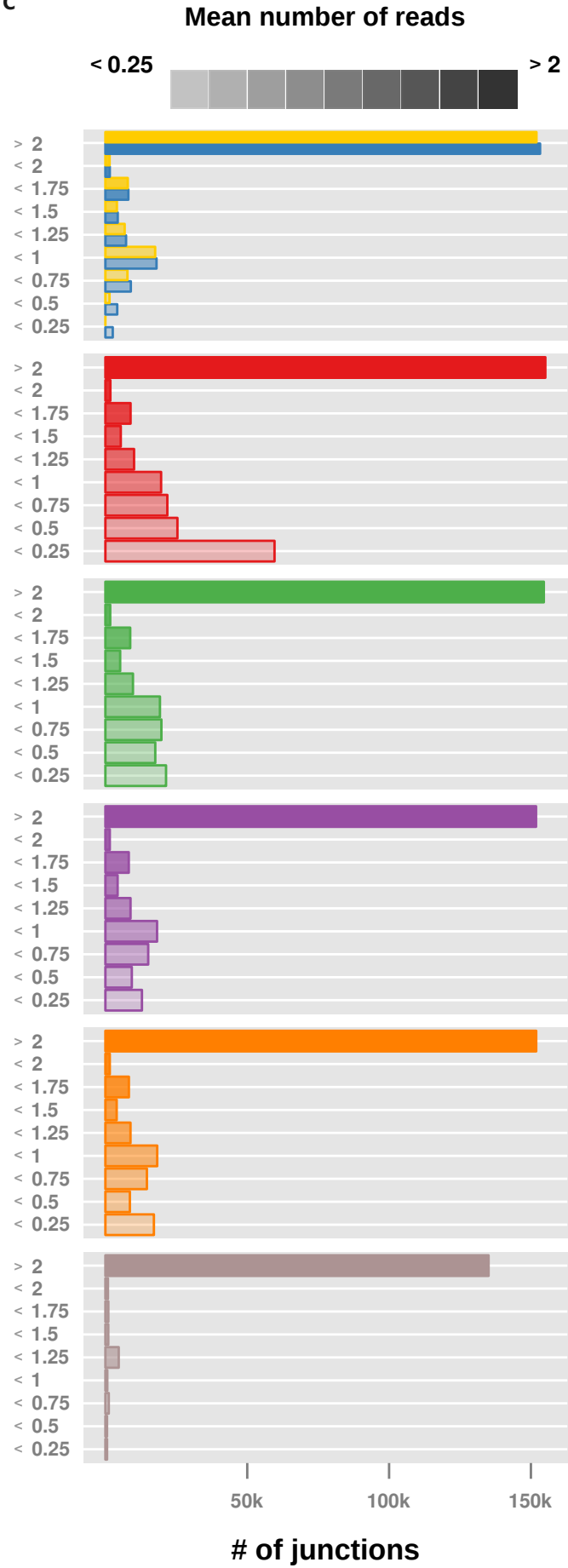
A

		PPV (mean ± SD)		Sn (mean ± SD)		F <sub>1</sub> (mean ± SD)	
<div></div>	TopHat2	0.949	± 0.003	0.923	± 0.002	0.936	± 0.002
<div></div>	FineSplice	0.989	± 0.001	0.918	± 0.003	0.952	± 0.003
<div></div>	GSNAP	0.694	± 0.004	0.998	± 0.001	0.819	± 0.005
<div></div>	STAR	0.825	± 0.020	0.992	± 0.001	0.901	± 0.008
<div></div>	OLego	0.891	± 0.003	0.956	± 0.012	0.922	± 0.009
<div></div>	SOAPSsplice	0.873	± 0.002	0.949	± 0.015	0.909	± 0.012
<div></div>	TrueSight	0.986	± 0.001	0.671	± 0.004	0.799	± 0.003

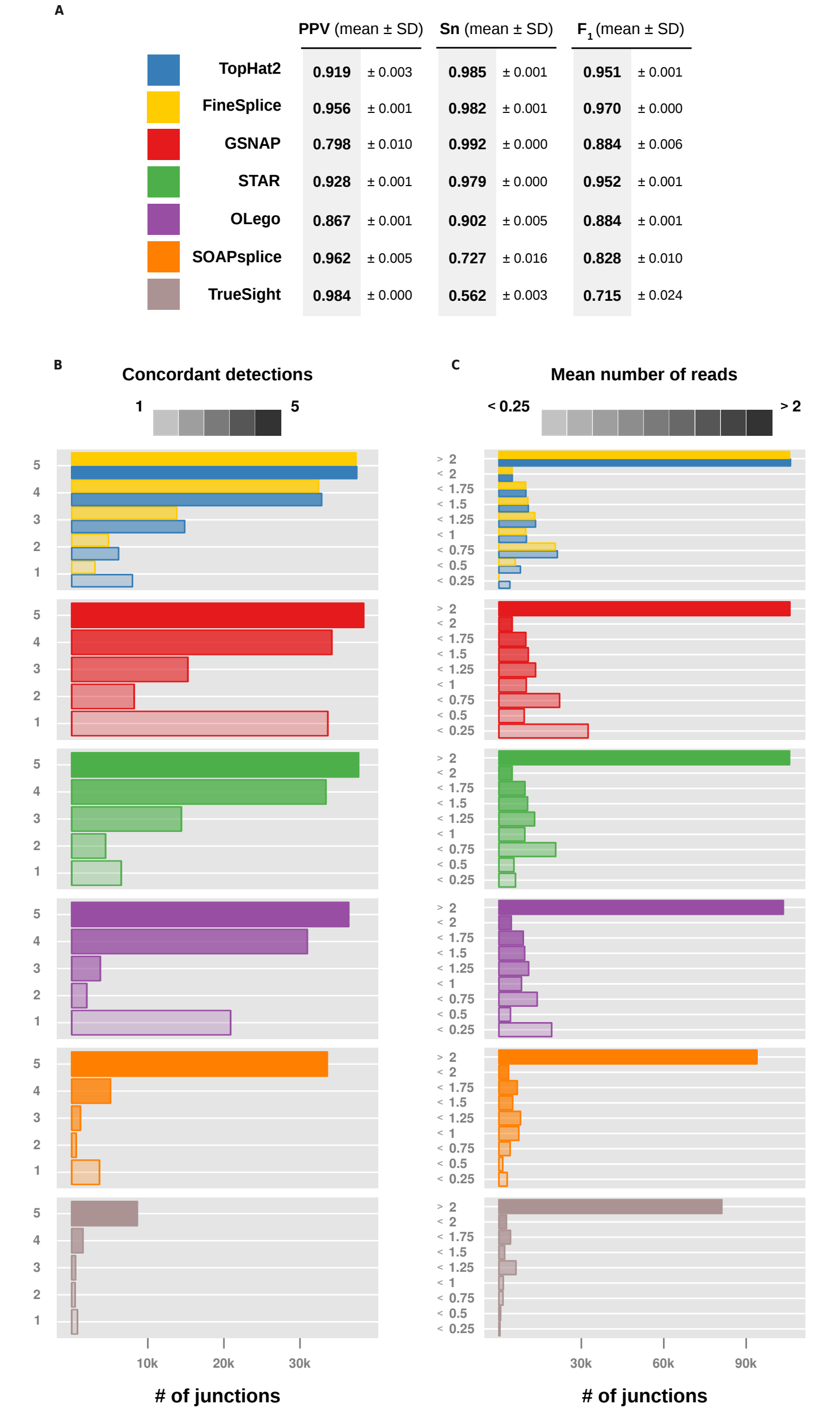
B



C



**Supplementary Figure 12.** Splice junction detection performance in high-quality experimental data (human dataset comprising three high-depth, paired-end sequencing runs at 76 bp read length). Pseudo sensitivity and pseudo precision metrics, along with the corresponding F1 scores, have been evaluated by regarding as true those junctions with a median number of overlapping reads over all alignments greater than 0 (table A, mean ± standard deviation). The bar plots show the number of concordant detections (B) and the mean read count across all alignments (C) for all junctions detected by each method (on separate panels) or accepted by FineSplice (top panel).



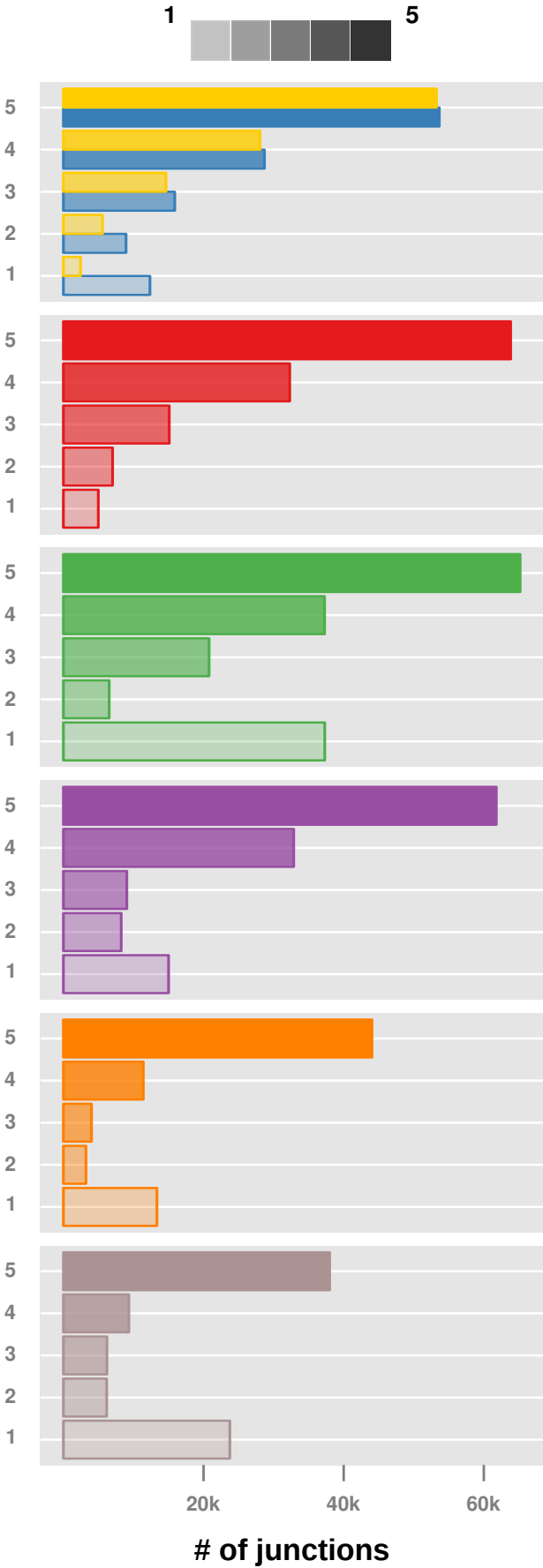
**Supplementary Figure 13.** Splice junction detection performance in low-quality experimental data (human dataset comprising two low-depth, paired-end sequencing runs at 45 bp read length). Pseudo sensitivity and pseudo precision metrics, along with the corresponding F1 scores, have been computed by regarding as true those junctions with a median number of overlapping reads over all alignments greater than 0 (table A, mean ± standard deviation). The bar plots show the number of concordant detections (B) and the mean read count across all alignments (C) for all junctions detected by each method (on separate panels) or accepted by FineSplice (top panel).

A

		PPV (mean ± SD)		Sn (mean ± SD)		F <sub>1</sub> (mean ± SD)	
<div></div>	TopHat2	0.939	± 0.004	0.944	± 0.005	0.942	± 0.002
<div></div>	FineSplice	0.978	± 0.002	0.941	± 0.005	0.960	± 0.004
<div></div>	GSNAP	0.958	± 0.002	0.972	± 0.002	0.965	± 0.002
<div></div>	STAR	0.878	± 0.011	0.994	± 0.000	0.932	± 0.006
<div></div>	OLego	0.921	± 0.005	0.942	± 0.064	0.931	± 0.004
<div></div>	SOAPSplICE	0.940	± 0.002	0.831	± 0.018	0.882	± 0.010
<div></div>	TrueSight	0.897	± 0.003	0.811	± 0.016	0.852	± 0.008

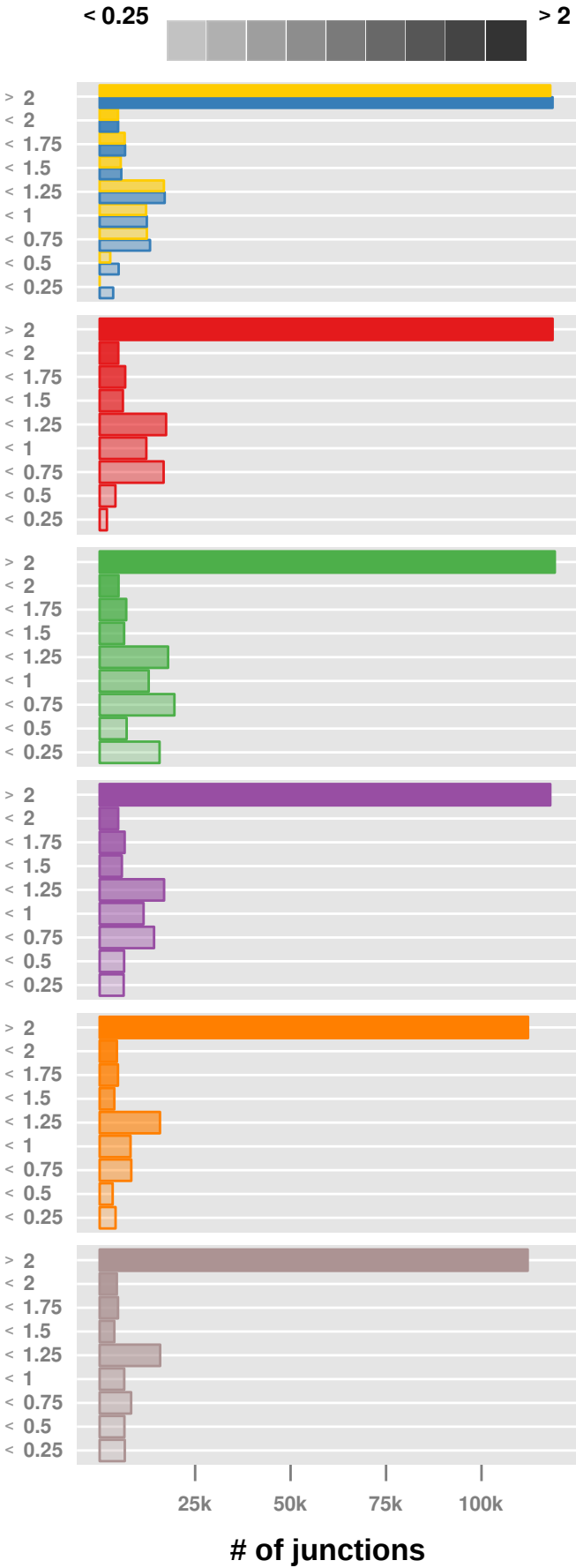
B

Concordant detections

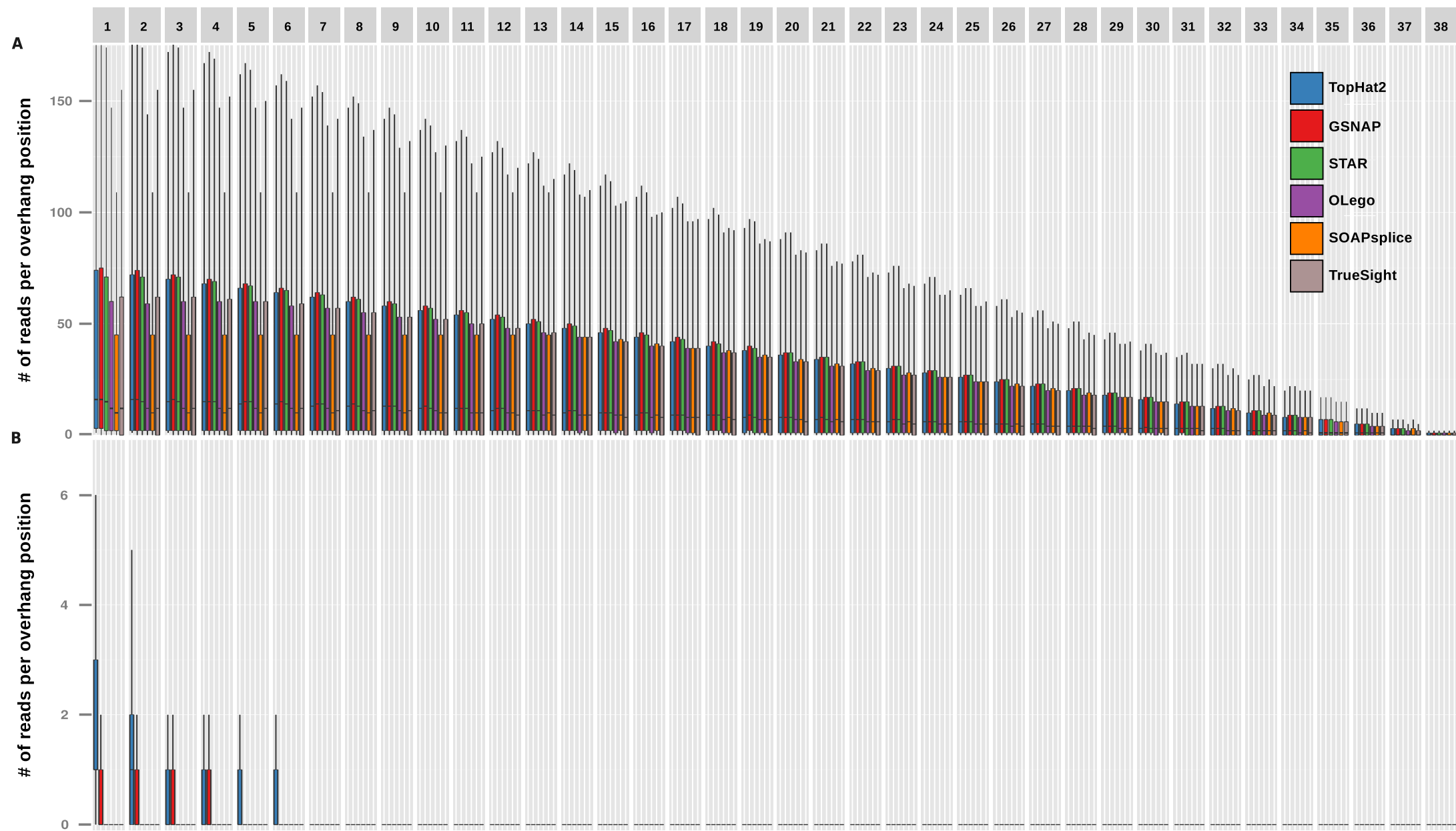


C

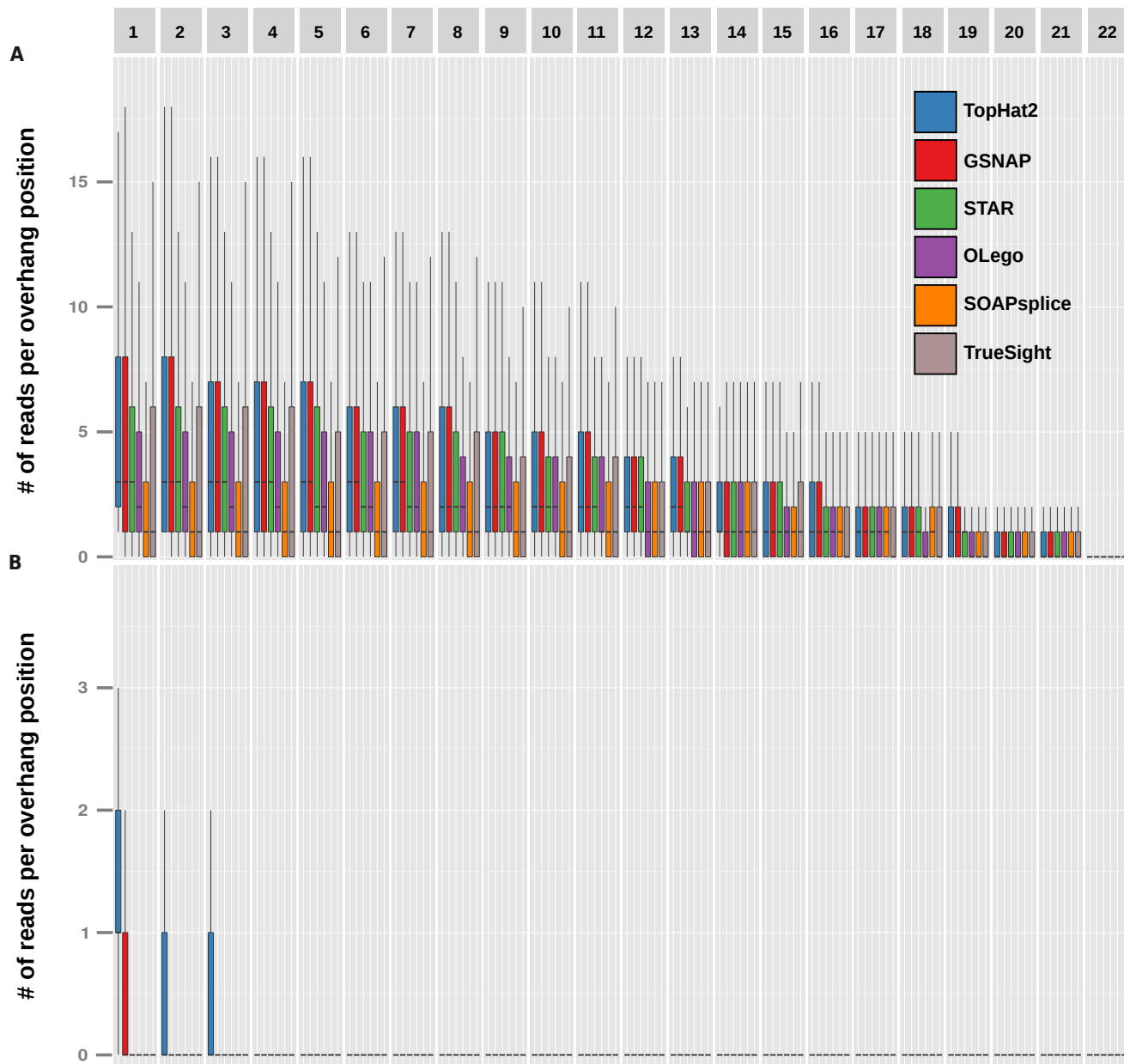
Mean number of reads



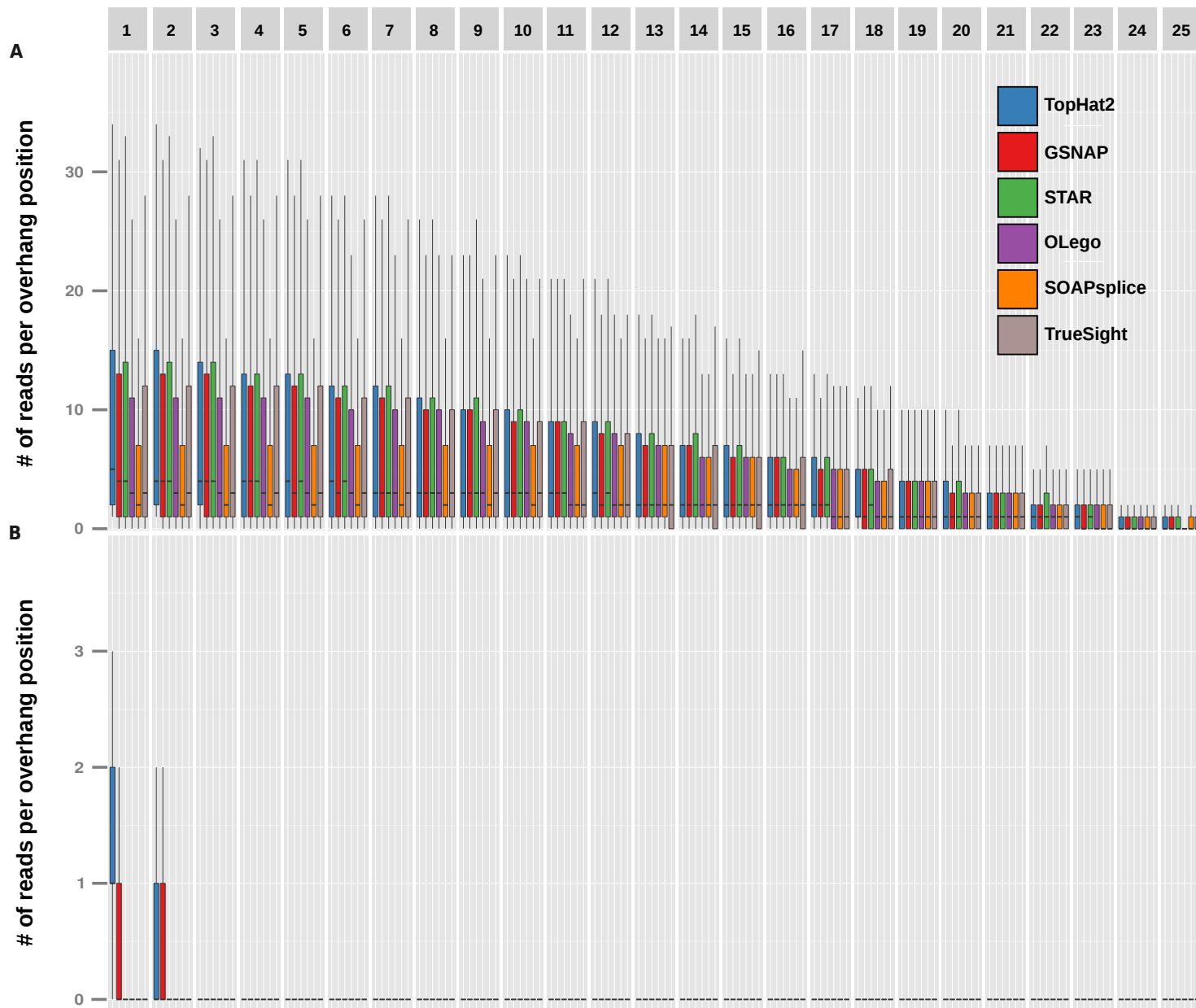
**Supplementary Figure 14.** Splice junction detection performance in experimental data with poor transcript annotation (pig dataset, comprising three single-end sequencing runs at 51 bp read length). Pseudo sensitivity and pseudo precision metrics, along with the corresponding F1 scores, have been evaluated by regarding as true those junctions with a median number of overlapping reads greater than 0 (table A, mean ± standard deviation). The bar plots show the number of concordant detections (B) and the mean read count across all alignments (C) for all junctions detected by each method (on separate panels) or accepted by FineSplice (top panel).



**Supplementary Figure 15.** Distribution of read overhangs across all alignment methods in experimental high-quality data (human dataset comprising three high-depth, paired-end sequencing runs at 76 bp read length). Read counts per overhang position for splice junctions accepted (**A**) or discarded (**B**) by FineSplice (all sequencing runs) are shown in a box plot representation for each alignment method.



**Supplementary Figure 16.** Distribution of read overhangs across all alignment methods in experimental low-quality data (human dataset comprising two low-depth, paired-end sequencing runs at 45 bp read length). Read counts per overhang position for splice junctions accepted (A) or discarded (B) by FineSplice (all sequencing runs) are shown in a box plot representation for each alignment method.



**Supplementary Figure 17.** Distribution of read overhangs across all alignment methods in experimental data with poor transcript annotation (pig dataset, comprising three single-end sequencing runs at 51 bp read length). Read counts per overhang position for splice junctions accepted (A) or discarded (B) by FineSplice (all sequencing runs) are shown in a box plot representation for each alignment method.