

Figure 1: **a** Raw data of dot blot analysis of biotinylated RNAs (input) and supernatant of biochemical separation with Streptavidin-HRP. **b** RT-qPCR analysis of MYC, PDLIM5 and GAPDH mRNAs in supernatant fractions of the biochemical separation, normalized to the respective input. **c** 1% agarose gel analysis of 4sU-labeled MCF-7 RNAs subjected to chemical conversion as indicated. **d** qPCR analysis of samples derived from chemical conversion for MYC, PDLIM5 and GAPDH mRNAs. ΔC_t values are shown relative to the respective untreated samples. **e** Quantification of the relative fraction of full length band and the two cleavage products derived from mock digestion (-NotI) of the restriction enzyme digestion assay in three independent experiments. untr. = untreated.

Concentration vs. mean read count across all samples

Used counts (Input and Supernatant adjusted, concentration in 1:5)

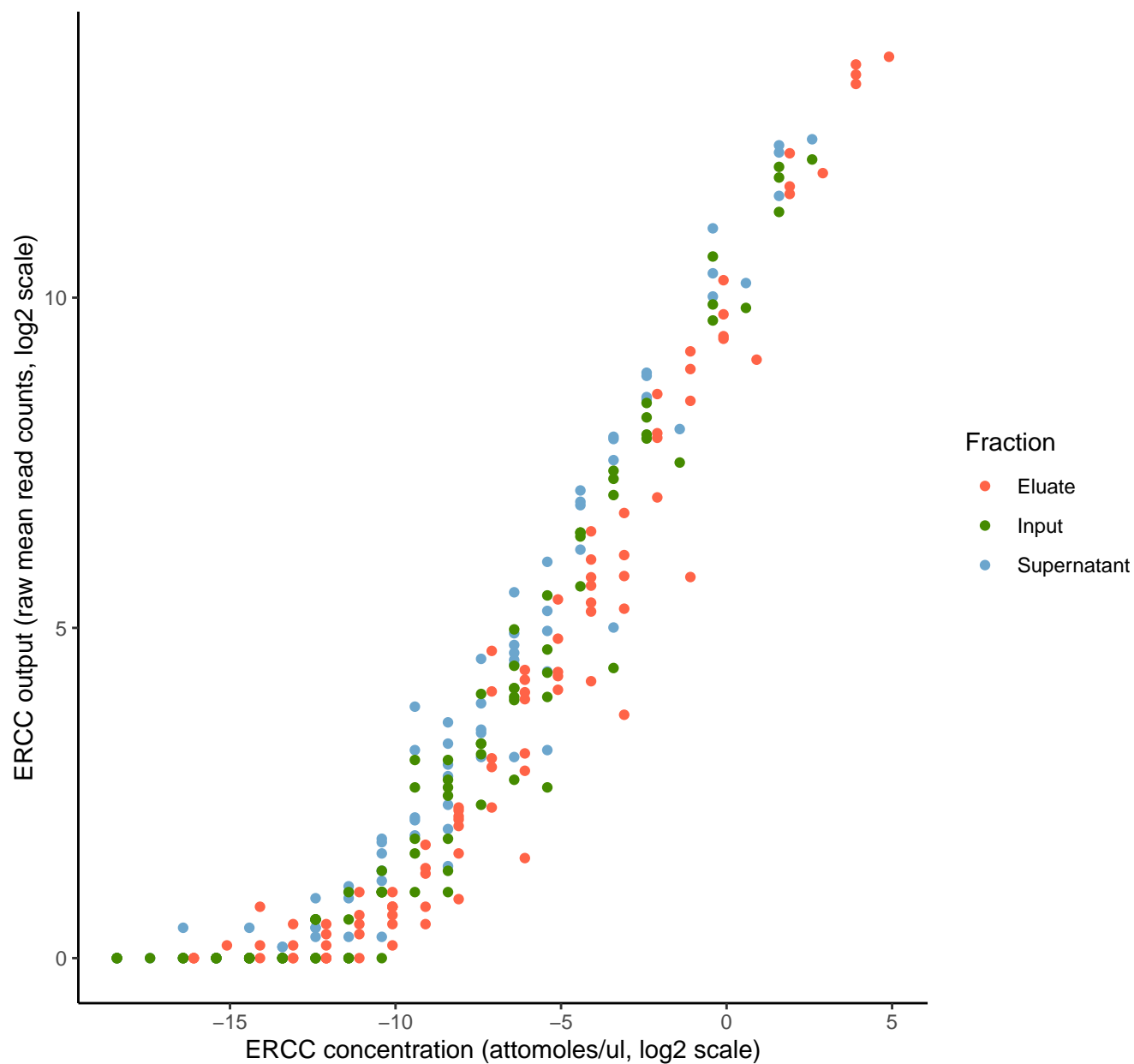


Figure 2: ERCC quantification and coverage. Scatter plots of read counts as a function of input concentration per ERCC. The input and supernatant ERCC concentration is diluted in 1:5, see Methods.

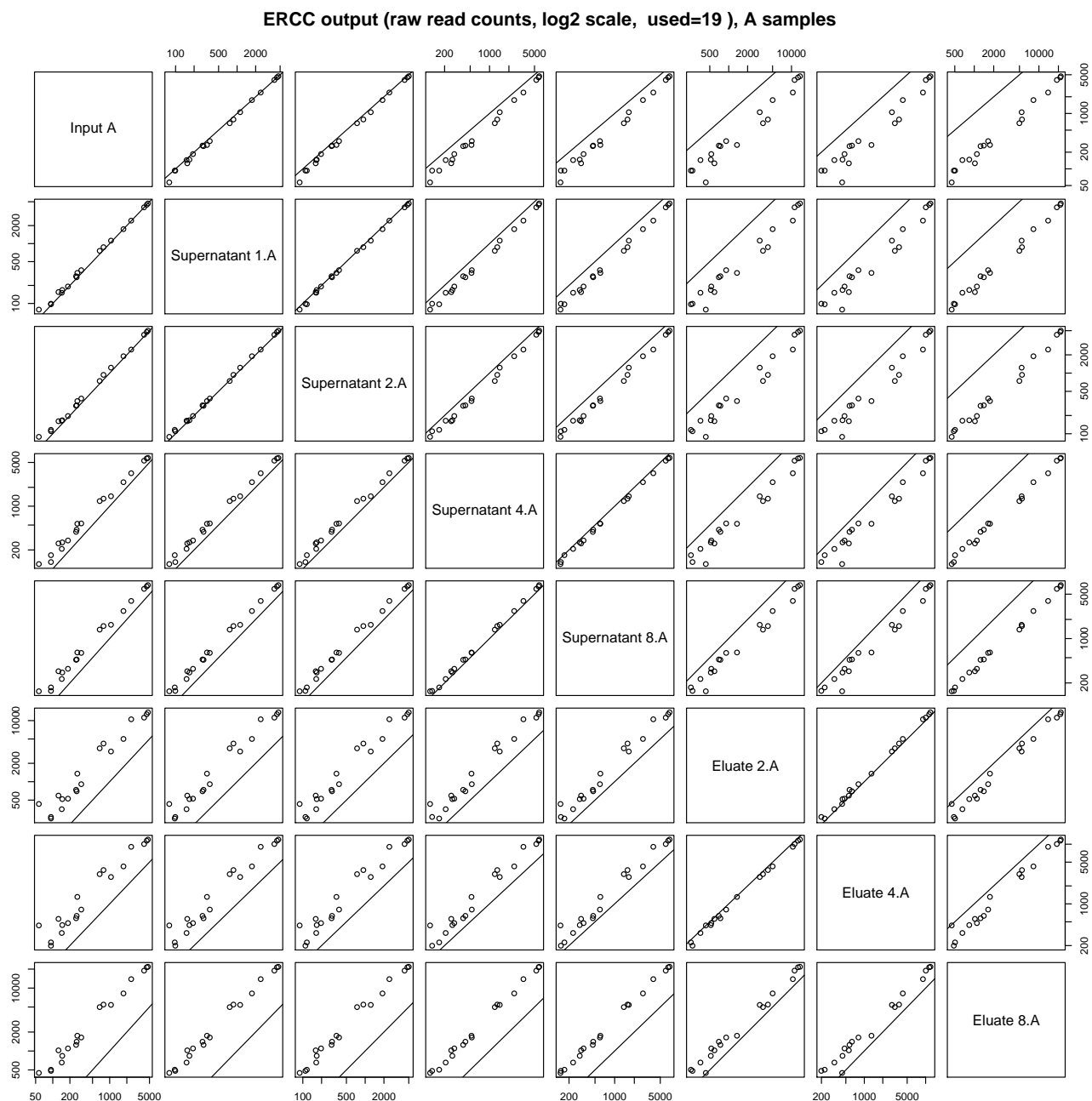


Figure 3: ERCC quantification and coverage. Scatter plot of read counts for each ERCC transcript for all pairs of replicates (A replicates).

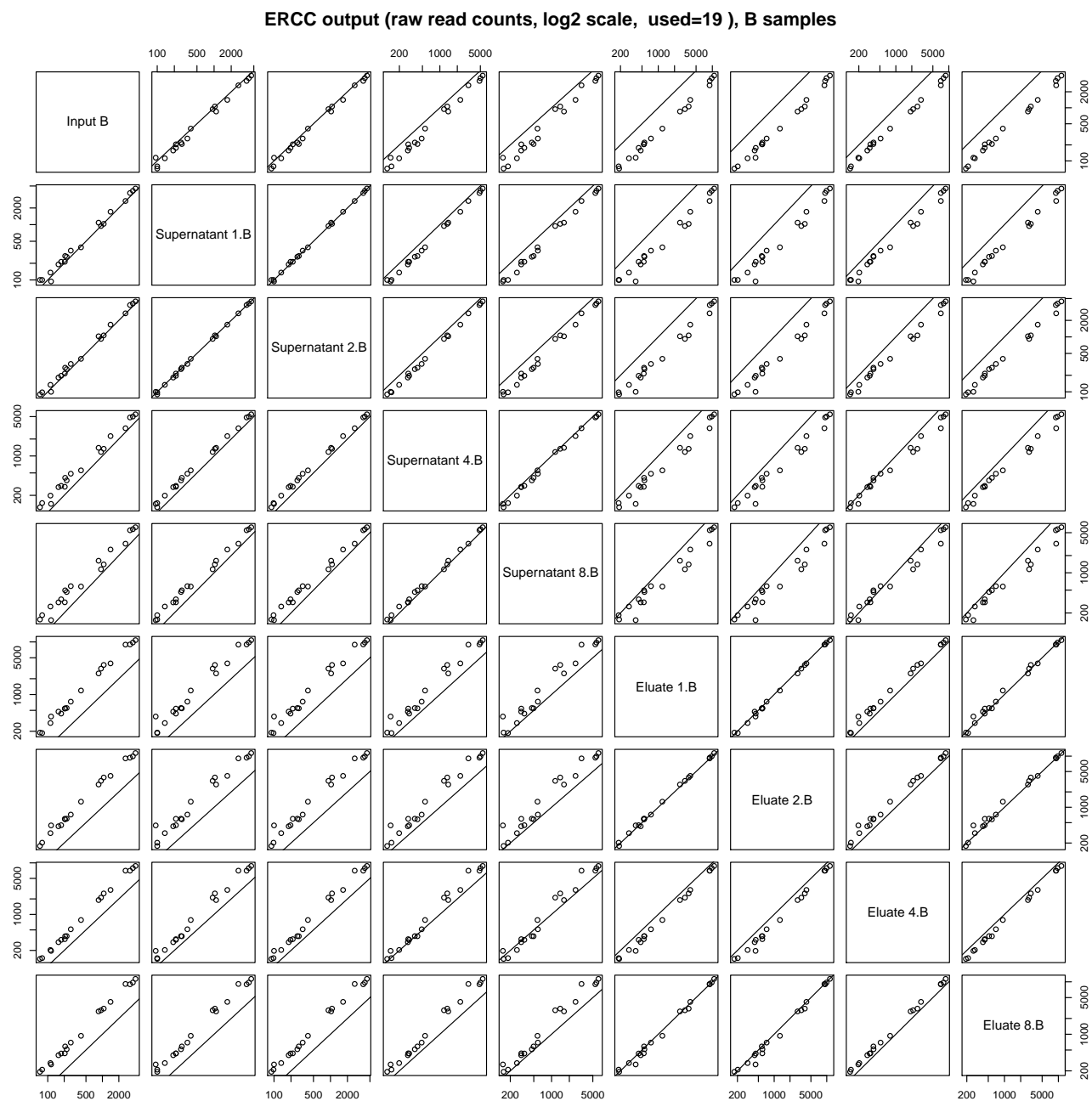


Figure 4: ERCC quantification and coverage. Scatter plot of read counts for each ERCC transcript for all pairs of replicates (B replicates).

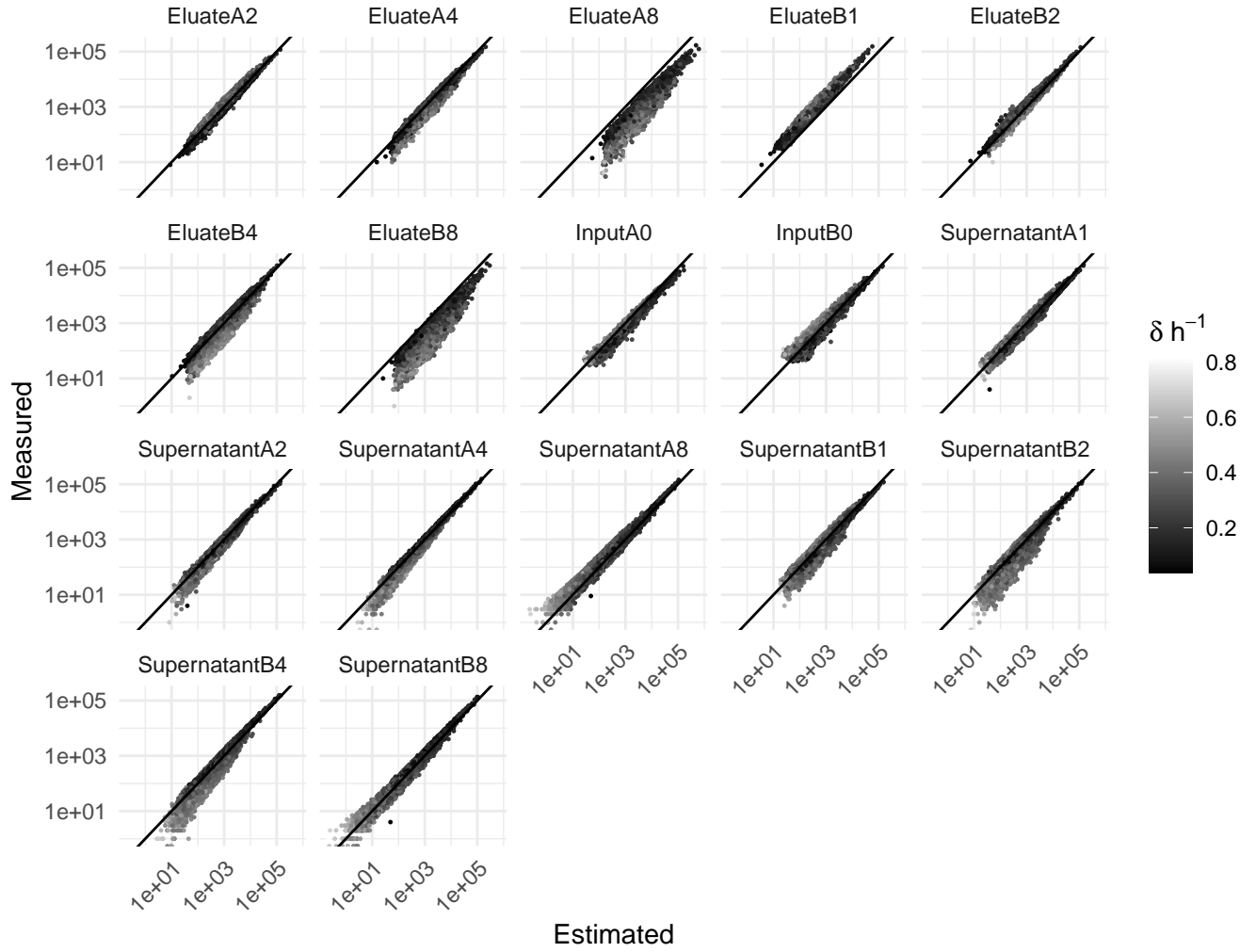


Figure 5: The ‘ERCC model’ (see Methods for the model description). Scatter plot of raw read counts for all genes versus predicted mean abundances for all pairs of replicates, using pulseR and ERCC spike-ins for normalization. Estimated decay rates δ are shown in grey scale.

aDecay rate δ h⁻¹ (n=11603)

pulseR _{ERCC}	0.9	0.9	0.89	0.82	0.8	0.76	1	1
pulseR _{BSA}	0.9	0.9	0.89	0.81	0.79	0.75	1	1
pulseR _{SLAM}	0.77	0.76	0.77	0.77	0.74	1	0.75	0.76
pulseR _{TLS}	0.8	0.81	0.79	0.81	1	0.74	0.79	0.8
pulseR _{TUC}	0.84	0.82	0.81	1	0.81	0.77	0.81	0.82
GS _{SLAM}	0.98	0.98	1	0.81	0.79	0.77	0.89	0.89
GS _{TLS}	0.98	1	0.98	0.82	0.81	0.76	0.9	0.9
GS _{TUC}	1	0.98	0.98	0.84	0.8	0.77	0.9	0.9
	GS _{TUC}	GS _{TLS}	GS _{SLAM}	pulseR _{TUC}	pulseR _{TLS}	pulseR _{SLAM}	pulseR _{BSA}	pulseR _{ERCC}

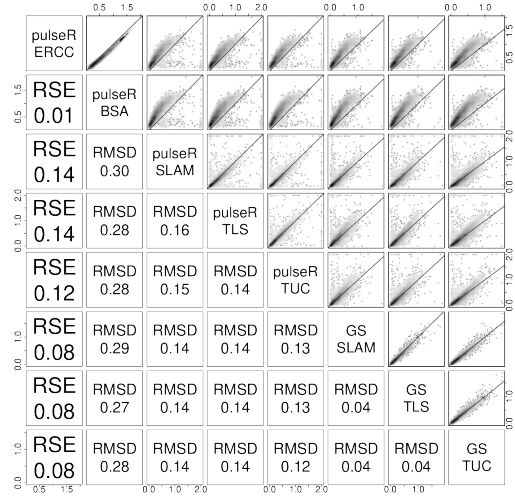
b

Figure 6: **a** Pearson's correlation coefficient for RNA decay rate estimates δ between any pair of methods for 11,603 common genes. **b** Scatter matrix of decay rate estimates δ between any pair of methods for 11,603 common genes with, on the lower diagonal elements, residual standard error (RSE) from a fitted regression model using the ERCC results, and root-mean-square deviation (RMSD) for all other comparisons. Early time points (0, 1, and 2 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator for δ in GRAND-SLAM was computed on the 1 h and 2 h samples.

aDecay rate δ h⁻¹ (n=11603)

pulseR _{ERCC}	0.93	0.93	0.92	0.86	0.86	0.85	0.99	1
pulseR _{BSA}	0.93	0.93	0.92	0.84	0.85	0.83	1	0.99
pulseR _{SLAM}	0.85	0.85	0.86	0.93	0.93	1	0.83	0.85
pulseR _{TLS}	0.87	0.88	0.87	0.94	1	0.93	0.85	0.86
pulseR _{TUC}	0.88	0.86	0.86	1	0.94	0.93	0.84	0.86
GS _{SLAM}	0.98	0.98	1	0.86	0.87	0.86	0.92	0.92
GS _{TLS}	0.98	1	0.98	0.86	0.88	0.85	0.93	0.93
GS _{TUC}	1	0.98	0.98	0.88	0.87	0.85	0.93	0.93
	GS _{TUC}	GS _{TLS}	GS _{SLAM}	pulseR _{TUC}	pulseR _{TLS}	pulseR _{SLAM}	pulseR _{BSA}	pulseR _{ERCC}

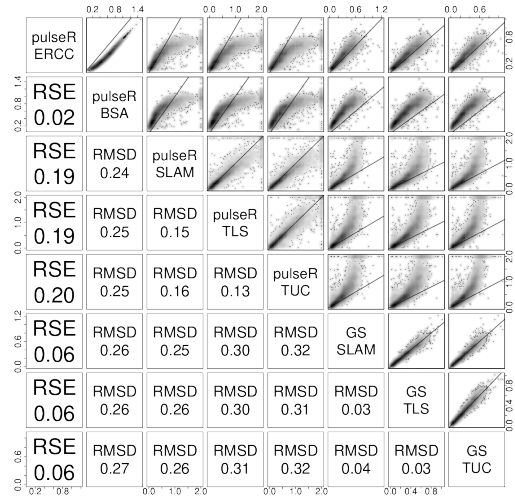
b

Figure 7: **a** Pearson's correlation coefficient for RNA decay rate estimates δ between any pair of methods for 11,603 common genes. **b** Scatter matrix of decay rate estimates δ between any pair of methods for 11,603 common genes with, on the lower diagonal elements, residual standard error (RSE) from a fitted regression model using the ERCC results, and root-mean-square deviation (RMSD) for all other comparisons. Intermediate time points (0, 2, and 4 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator for δ in GRAND-SLAM was computed on the 2 h and 4 h samples.

aDecay rate δ h^{-1} (n=11603)

pulseR _{ERCC}	0.89	0.89	0.87	0.8	0.8	0.78	0.99	1
pulseR _{BSA}	0.9	0.89	0.88	0.78	0.78	0.75	1	0.99
pulseR _{SLAM}	0.77	0.78	0.78	0.87	0.88	1	0.75	0.78
pulseR _{TLS}	0.8	0.81	0.8	0.91	1	0.88	0.78	0.8
pulseR _{TUC}	0.81	0.81	0.8	1	0.91	0.87	0.78	0.8
GS _{SLAM}	0.96	0.96	1	0.8	0.8	0.78	0.88	0.87
GS _{TLS}	0.96	1	0.96	0.81	0.81	0.78	0.89	0.89
GS _{TUC}	1	0.96	0.96	0.81	0.8	0.77	0.9	0.89
	GS _{TUC}	GS _{TLS}	GS _{SLAM}	pulseR _{TUC}	pulseR _{TLS}	pulseR _{SLAM}	pulseR _{BSA}	pulseR _{ERCC}

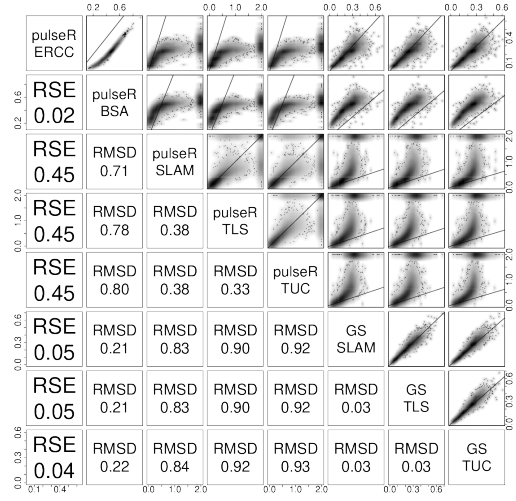
b

Figure 8: **a** Pearson's correlation coefficient for RNA decay rate estimates δ between any pair of methods for 11,603 common genes. **b** Scatter matrix of decay rate estimates δ between any pair of methods for 11,603 common genes with, on the lower diagonal elements, residual standard error (RSE) from a fitted regression model using the ERCC results, and root-mean-square deviation (RMSD) for all other comparisons. Later time points (0, 4, and 8 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator for δ in GRAND-SLAM was computed on the 4 h and 8 h samples.

pulseR ^{0.1,2,4,8h} _{ERCC}	0.89	0.89	0.87	0.83	0.83	0.81	0.97	0.98	0.86	0.85	0.83	0.77	0.78	0.75	0.93	0.91	0.71	0.71	0.69	0.67	0.66	0.59	0.82	0.81	0.87	0.85	0.83	0.82	0.83	0.8	1	1
pulseR ^{0.1,2,4,8h} _{BSA}	0.9	0.9	0.89	0.83	0.83	0.8	0.99	0.99	0.86	0.85	0.84	0.77	0.77	0.75	0.95	0.92	0.71	0.71	0.69	0.66	0.65	0.58	0.82	0.81	0.87	0.85	0.84	0.81	0.83	0.79	1	1
pulseR ^{0.1,2,4,8h} _{SLAM}	0.74	0.75	0.75	0.79	0.78	0.8	0.73	0.74	0.88	0.88	0.89	0.93	0.93	0.94	0.86	0.87	0.9	0.89	0.9	0.86	0.84	0.83	0.9	0.91	0.87	0.87	0.89	0.97	0.97	1	0.79	0.8
pulseR ^{0.1,2,4,8h} _{TLS}	0.77	0.78	0.77	0.81	0.82	0.81	0.76	0.77	0.9	0.9	0.89	0.93	0.95	0.92	0.87	0.88	0.89	0.9	0.88	0.86	0.86	0.79	0.9	0.91	0.89	0.9	0.89	0.98	1	0.97	0.83	0.83
pulseR ^{0.1,2,4,8h} _{TUC}	0.77	0.77	0.76	0.81	0.8	0.8	0.75	0.76	0.9	0.89	0.88	0.95	0.94	0.92	0.87	0.87	0.91	0.89	0.88	0.88	0.84	0.8	0.9	0.91	0.89	0.89	0.89	1	0.98	0.97	0.81	0.82
GS ^{1,2,4,8h} _{SLAM}	0.88	0.9	0.92	0.8	0.79	0.8	0.8	0.79	0.97	0.97	0.98	0.85	0.85	0.84	0.9	0.89	0.92	0.92	0.92	0.76	0.75	0.7	0.88	0.87	0.97	0.98	1	0.89	0.89	0.89	0.84	0.83
GS ^{1,2,4,8h} _{TLS}	0.89	0.93	0.9	0.81	0.81	0.8	0.81	0.81	0.97	0.98	0.97	0.85	0.86	0.83	0.9	0.9	0.91	0.93	0.9	0.76	0.75	0.69	0.88	0.87	0.98	1	0.98	0.89	0.9	0.87	0.85	0.85
GS ^{1,2,4,8h} _{TUC}	0.93	0.92	0.91	0.82	0.81	0.81	0.83	0.83	0.97	0.96	0.96	0.85	0.85	0.82	0.91	0.9	0.91	0.9	0.88	0.76	0.74	0.68	0.87	0.86	1	0.98	0.97	0.89	0.89	0.87	0.87	0.87
pulseR ^{0.1,2h} _{ERCC}	0.71	0.72	0.72	0.76	0.75	0.76	0.72	0.73	0.91	0.91	0.91	0.88	0.89	0.88	0.92	0.94	0.9	0.9	0.89	0.82	0.8	0.76	1	1	0.86	0.87	0.87	0.91	0.91	0.91	0.81	0.81
pulseR ^{0.1,2h} _{BSA}	0.73	0.74	0.74	0.77	0.75	0.77	0.74	0.75	0.92	0.92	0.92	0.88	0.88	0.88	0.93	0.94	0.9	0.9	0.89	0.81	0.79	0.75	1	1	0.87	0.88	0.88	0.9	0.9	0.9	0.82	0.82
pulseR ^{0.1,2h} _{SLAM}	0.53	0.54	0.54	0.6	0.6	0.62	0.51	0.52	0.71	0.71	0.71	0.76	0.77	0.77	0.67	0.68	0.77	0.76	0.77	0.77	0.74	1	0.75	0.76	0.68	0.69	0.7	0.8	0.79	0.83	0.58	0.59
pulseR ^{0.1,2h} _{TLS}	0.59	0.6	0.59	0.65	0.65	0.66	0.58	0.59	0.76	0.76	0.75	0.81	0.82	0.81	0.72	0.74	0.8	0.81	0.79	0.81	1	0.74	0.79	0.8	0.74	0.75	0.75	0.84	0.86	0.84	0.65	0.66
pulseR ^{0.1,2h} _{TUC}	0.6	0.61	0.6	0.66	0.66	0.67	0.58	0.6	0.78	0.78	0.77	0.84	0.84	0.83	0.73	0.75	0.84	0.82	0.81	1	0.81	0.77	0.81	0.82	0.76	0.76	0.76	0.88	0.86	0.86	0.66	0.67
GS ^{1,2h} _{SLAM}	0.7	0.72	0.71	0.7	0.68	0.7	0.61	0.62	0.9	0.91	0.92	0.84	0.84	0.86	0.8	0.81	0.98	0.98	1	0.81	0.79	0.77	0.89	0.89	0.88	0.9	0.92	0.88	0.88	0.9	0.69	0.69
GS ^{1,2h} _{TLS}	0.72	0.74	0.73	0.72	0.71	0.72	0.64	0.65	0.92	0.93	0.92	0.85	0.87	0.84	0.82	0.83	0.98	1	0.98	0.82	0.81	0.76	0.9	0.9	0.9	0.93	0.92	0.89	0.9	0.89	0.71	0.71
GS ^{1,2h} _{TUC}	0.71	0.73	0.72	0.71	0.7	0.72	0.63	0.64	0.92	0.92	0.92	0.87	0.86	0.85	0.81	0.83	1	0.98	0.98	0.84	0.8	0.77	0.9	0.9	0.91	0.91	0.92	0.91	0.89	0.9	0.71	0.71
pulseR ^{0.2,4h} _{ERCC}	0.83	0.84	0.84	0.84	0.83	0.84	0.86	0.88	0.93	0.93	0.92	0.86	0.86	0.85	0.99	1	0.83	0.83	0.81	0.75	0.74	0.68	0.94	0.94	0.9	0.9	0.89	0.87	0.88	0.87	0.92	0.91
pulseR ^{0.2,4h} _{BSA}	0.86	0.87	0.87	0.85	0.84	0.83	0.9	0.91	0.93	0.93	0.92	0.84	0.85	0.83	1	0.99	0.81	0.82	0.8	0.73	0.72	0.67	0.93	0.92	0.91	0.9	0.9	0.87	0.87	0.86	0.95	0.93
pulseR ^{0.2,4h} _{SLAM}	0.69	0.7	0.69	0.75	0.74	0.76	0.68	0.69	0.85	0.85	0.86	0.93	0.93	1	0.83	0.85	0.85	0.84	0.86	0.83	0.81	0.77	0.88	0.88	0.82	0.83	0.84	0.92	0.92	0.94	0.75	0.75
pulseR ^{0.2,4h} _{TLS}	0.72	0.73	0.72	0.77	0.77	0.79	0.7	0.73	0.87	0.88	0.87	0.94	1	0.93	0.85	0.86	0.86	0.87	0.84	0.84	0.82	0.77	0.88	0.89	0.85	0.86	0.85	0.94	0.95	0.93	0.77	0.78
pulseR ^{0.2,4h} _{TUC}	0.71	0.72	0.71	0.77	0.77	0.79	0.7	0.72	0.88	0.86	0.86	1	0.94	0.93	0.84	0.86	0.87	0.85	0.84	0.84	0.81	0.76	0.88	0.88	0.85	0.85	0.85	0.95	0.93	0.93	0.77	0.77
GS ^{2,4h} _{SLAM}	0.85	0.87	0.88	0.81	0.8	0.82	0.78	0.79	0.98	0.98	1	0.86	0.87	0.86	0.92	0.92	0.92	0.92	0.92	0.77	0.75	0.71	0.92	0.91	0.96	0.97	0.98	0.88	0.89	0.89	0.84	0.83
GS ^{2,4h} _{TLS}	0.86	0.89	0.88	0.82	0.82	0.81	0.79	0.8	0.98	1	0.98	0.86	0.88	0.85	0.93	0.93	0.92	0.93	0.91	0.78	0.76	0.71	0.92	0.91	0.96	0.98	0.97	0.89	0.9	0.88	0.85	0.85
GS ^{2,4h} _{TUC}	0.88	0.88	0.88	0.84	0.82	0.82	0.81	0.81	1	0.98	0.98	0.88	0.87	0.85	0.93	0.93	0.92	0.92	0.9	0.78	0.76	0.71	0.92	0.91	0.97	0.97	0.97	0.9	0.9	0.88	0.86	0.86
pulseR ^{0.4,8h} _{ERCC}	0.89	0.89	0.87	0.8	0.8	0.78	0.99	1	0.81	0.8	0.79	0.72	0.73	0.69	0.91	0.88	0.64	0.65	0.62	0.6	0.59	0.52	0.75	0.73	0.83	0.81	0.79	0.76	0.77	0.74	0.99	0.98
pulseR ^{0.4,8h} _{BSA}	0.9	0.89	0.88	0.78	0.78	0.75	1	0.99	0.81	0.79	0.78	0.7	0.7	0.68	0.9	0.86	0.63	0.64	0.61	0.58	0.58	0.51	0.74	0.72	0.83	0.81	0.8	0.75	0.76	0.73	0.99	0.97
pulseR ^{0.4,8h} _{SLAM}	0.77	0.78	0.78	0.87	0.88	1	0.75	0.78	0.82	0.81	0.82	0.79	0.79	0.76	0.83	0.84	0.72	0.72	0.7	0.67	0.66	0.62	0.77	0.76	0.81	0.8	0.8	0.8	0.81	0.8	0.8	0.81
pulseR ^{0.4,8h} _{TLS}	0.8	0.81	0.8	0.91	1	0.88	0.78	0.8	0.82	0.82	0.8	0.77	0.77	0.74	0.84	0.83	0.7	0.71	0.68	0.66	0.65	0.6	0.75	0.75	0.81	0.81	0.79	0.8	0.82	0.78	0.83	0.83
pulseR ^{0.4,8h} _{TUC}	0.81	0.81	0.8	1	0.91	0.87	0.78	0.8	0.84	0.82	0.81	0.77	0.77	0.75	0.85	0.84	0.71	0.72	0.7	0.66	0.65	0.6	0.77	0.76	0.82	0.81	0.8	0.81	0.81	0.79	0.83	0.83
GS ^{4,8h} _{SLAM}	0.96	0.96	1	0.8	0.8	0.78	0.88	0.87	0.88	0.88	0.88	0.71	0.72	0.69	0.87	0.84	0.72	0.73	0.71	0.6	0.59	0.54	0.74	0.72	0.91	0.9	0.92	0.76	0.77	0.75	0.89	0.87
GS ^{4,8h} _{TLS}	0.96	1	0.96	0.81	0.81	0.78	0.89	0.89	0.88	0.89	0.87	0.72	0.73	0.7	0.87	0.84	0.73	0.74	0.72	0.61	0.6	0.54	0.74	0.72	0.92	0.93	0.9	0.77	0.78	0.75	0.9	0.89
GS ^{4,8h} _{TUC}	1	0.96	0.96	0.81	0.8	0.77	0.9	0.89	0.88	0.86	0.85	0.71	0.72	0.69	0.86	0.83	0.71	0.72	0.7	0.6	0.59	0.53	0.73	0.71	0.93	0.89	0.88	0.77	0.77	0.74	0.9	0.89

Figure 9: **a** Pearson's correlation coefficient for RNA decay rate estimates δ between any pair of methods for 11,603 common genes using all subset of time points.

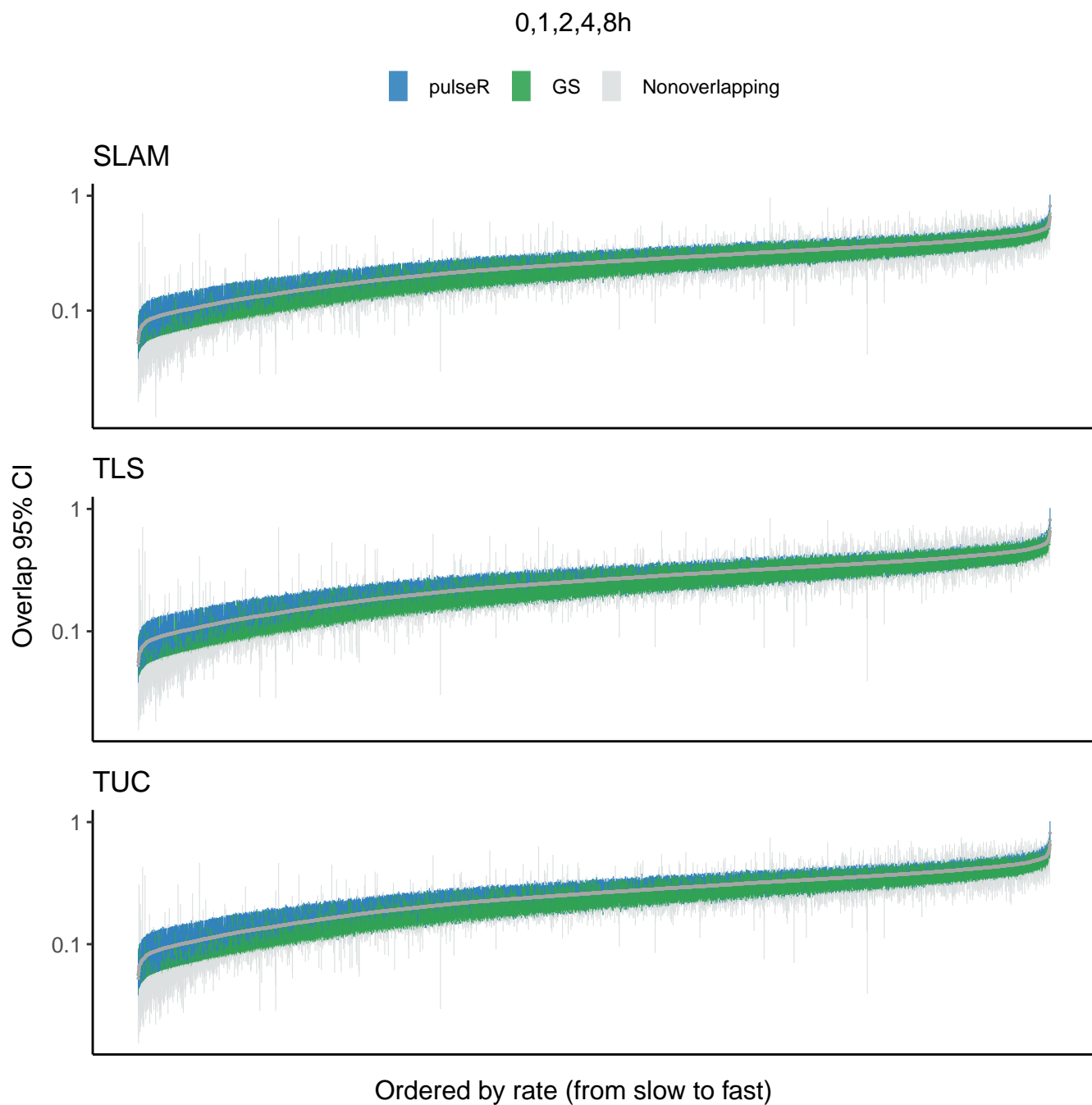


Figure 10: Approximate confidence intervals calculated for pulseR and GRAND-SLAM for the nucleotide conversion protocols and their overlap with those obtained for the BSA purification method with ERCC spike-ins (ERCC model). A grey line indicates where there is no overlap and corresponds to the difference between the maximum lower bound and the minimum upper bound for that estimate. All time points (0, 1, 2, 4, and 8 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator for δ in GRAND-SLAM was computed on the 1, 2, 4, and 8 h samples. Genes are ordered by rates as estimated with the ERCC model.

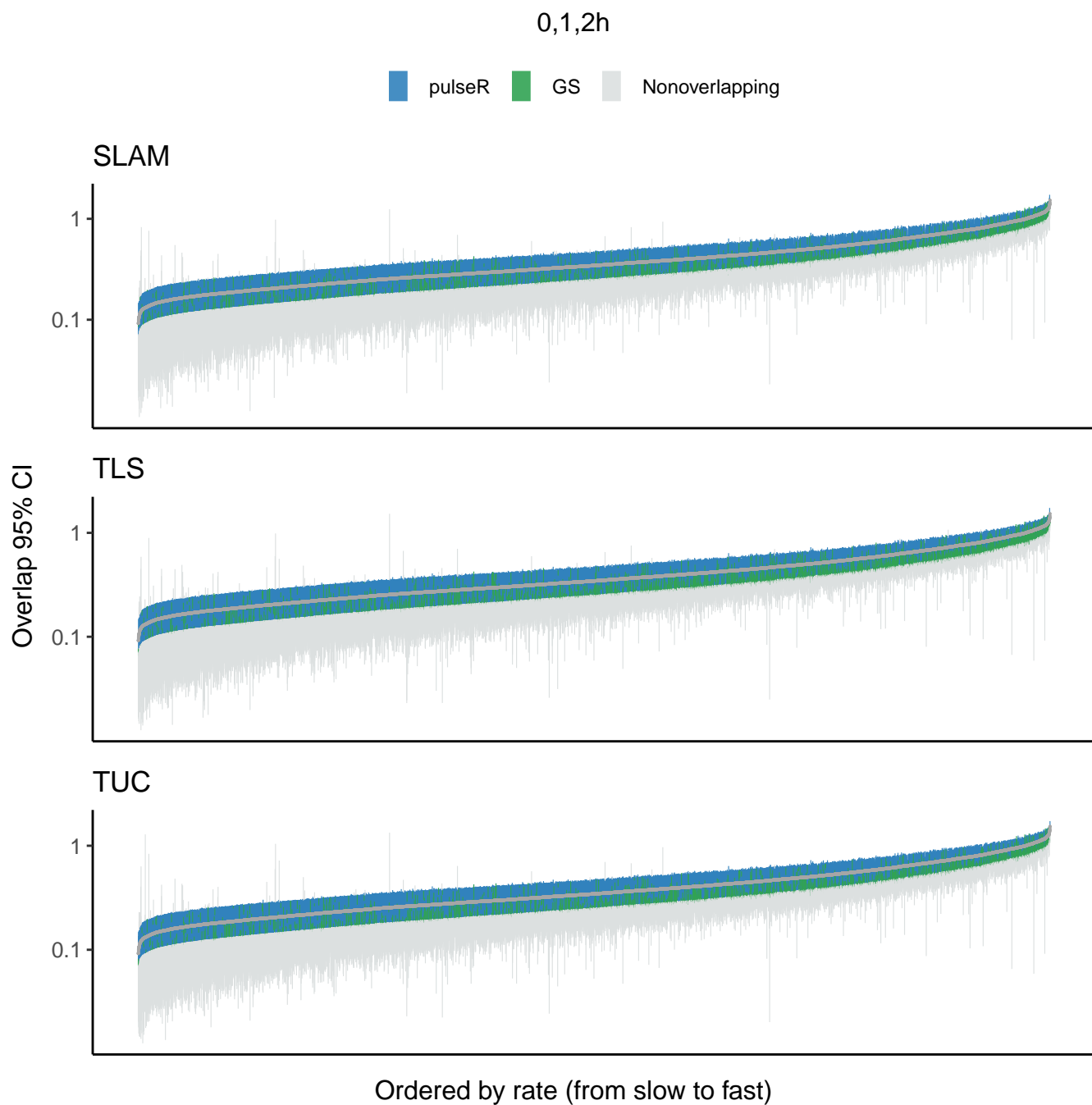


Figure 11: Approximate confidence intervals calculated for pulseR and GRAND-SLAM for the nucleotide conversion protocols and their overlap with those obtained for the BSA purification method with ERCC spike-ins (ERCC model). A grey line indicates where there is no overlap and corresponds to the difference between the maximum lower bound and the minimum upper bound for that estimate. Early time points (0, 1, and 2 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator for δ in GRAND-SLAM was computed on the 1 and 2 h samples. Genes are ordered by rates as estimated with the ERCC model.

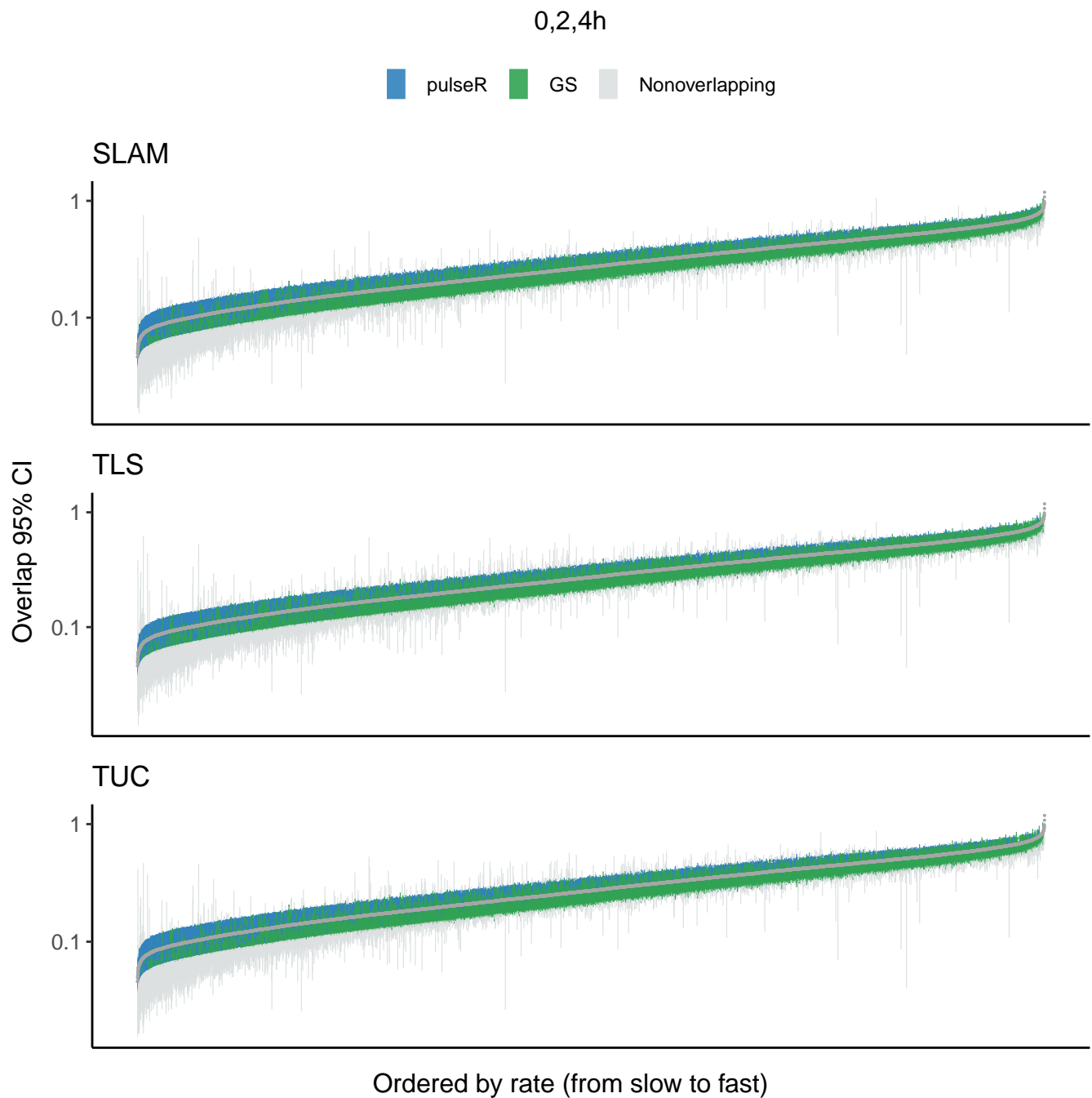


Figure 12: Approximate confidence intervals calculated for pulseR and GRAND-SLAM for the nucleotide conversion protocols and their overlap with those obtained for the BSA purification method with ERCC spike-ins (ERCC model). A grey line indicates where there is no overlap and corresponds to the difference between the maximum lower bound and the minimum upper bound for that estimate. Intermediate time points (0, 2, and 4 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator for δ in GRAND-SLAM was computed on the 2 and 4 h samples. Genes are ordered by rates as estimated with the ERCC model.

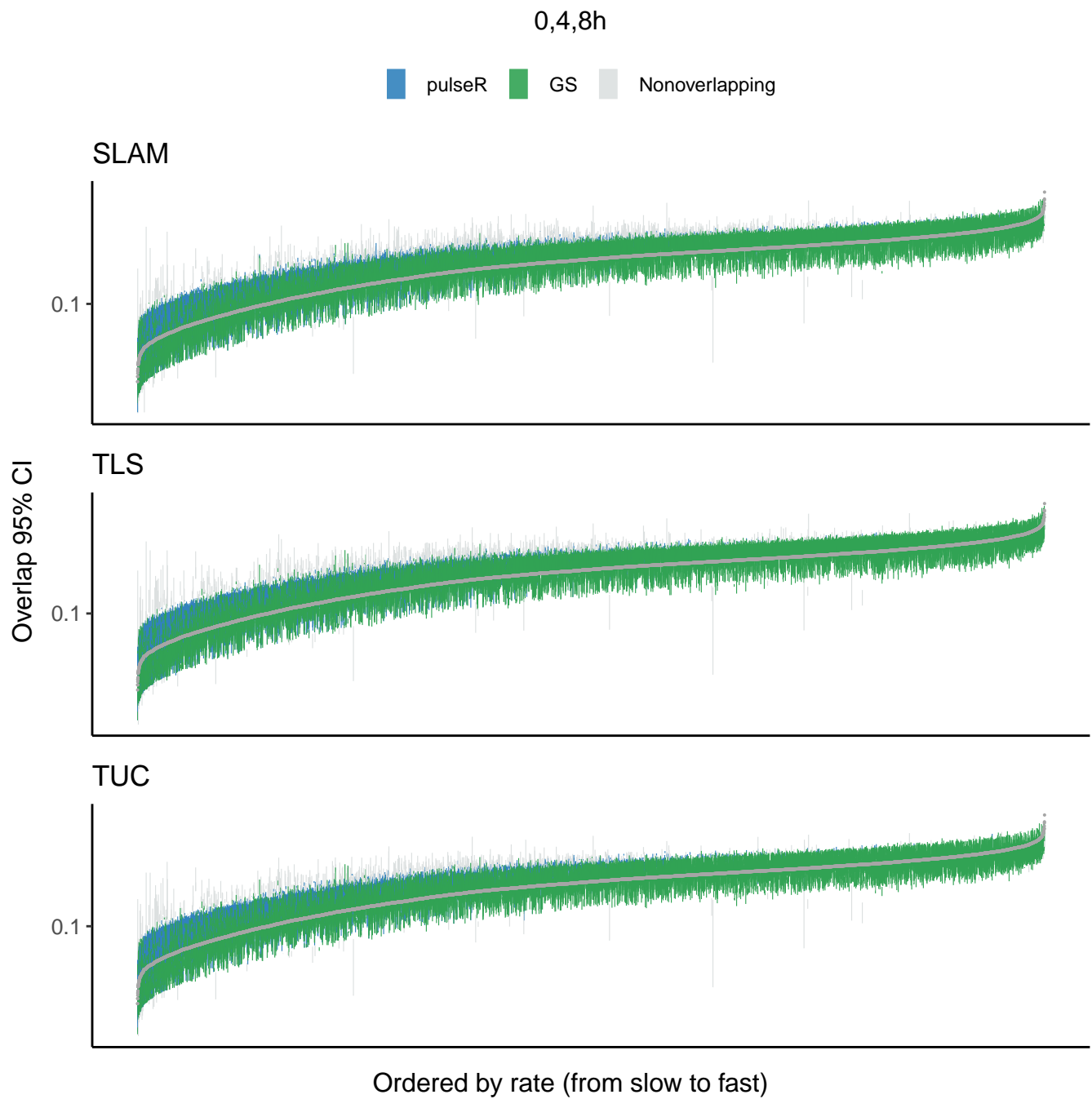


Figure 13: Approximate confidence intervals calculated for pulseR and GRAND-SLAM for the nucleotide conversion protocols and their overlap with those obtained for the BSA purification method with ERCC spike-ins (ERCC model). A grey line indicates where there is no overlap and corresponds to the difference between the maximum lower bound and the minimum upper bound for that estimate. Later time points (0, 4, and 8 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator for δ in GRAND-SLAM was computed on the 4 and 8 h samples. Genes are ordered by rates as estimated with the ERCC model.

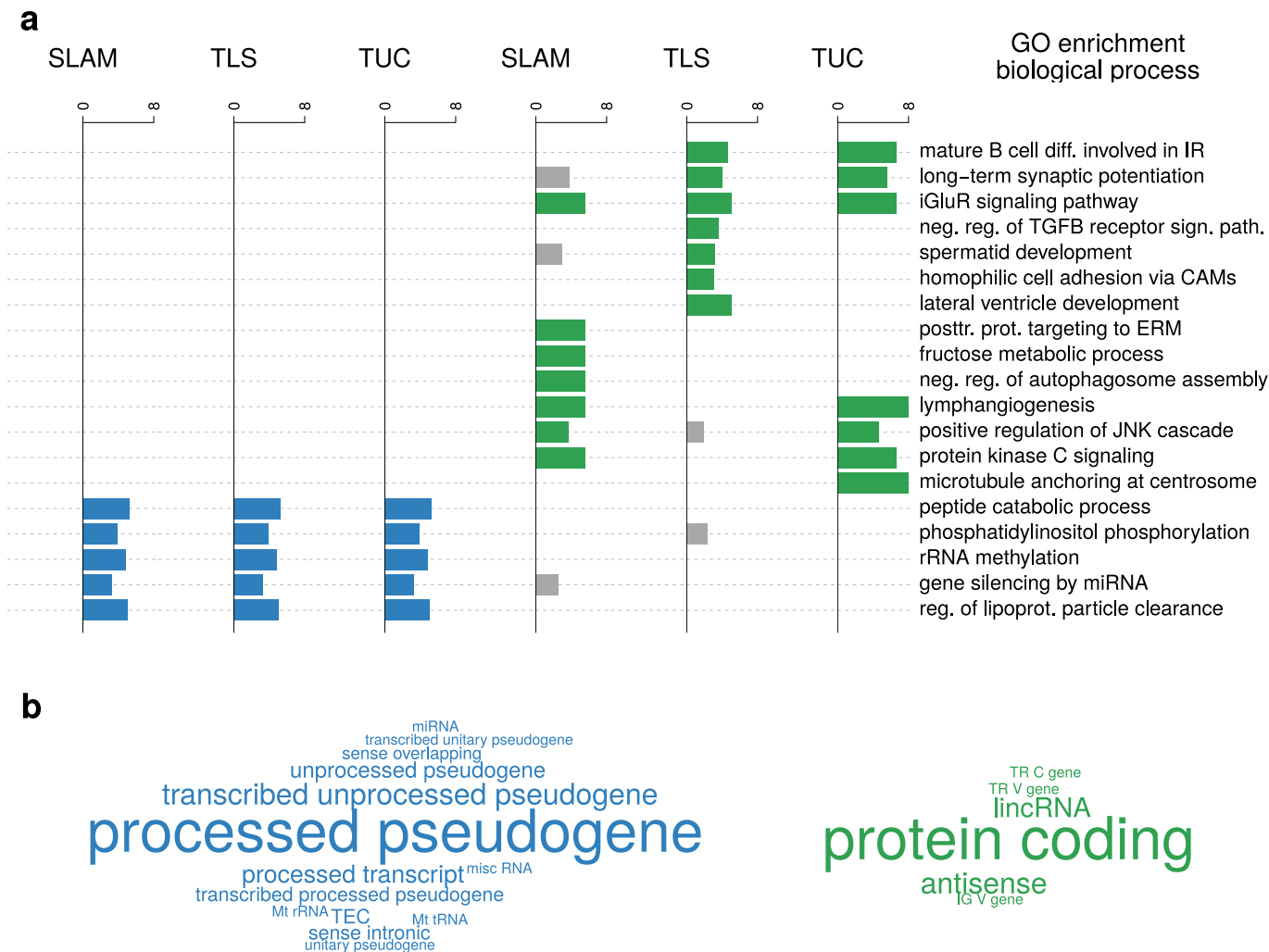


Figure 14: **a** Gene Ontology (GO) enrichment analysis (BP or Biological Process) comparing the genes for which valid decay rates were estimated, and found in either pulseR (blue) or GRAND-SLAM (green) specific subsets associated with the SLAM, the TLS, or the TUC data sets (see Figure 4c, the pulseR and GRAND-SLAM only sets). The diagram indicates the log enrichment on the x-axis. **b** Word cloud of gene biotypes for exclusive pulseR (left) and GRAND-SLAM (right) gene sets across all methods (SLAM, TLS, and TUC). By default, GRAND-SLAM considers certain biotypes only (protein coding, lincRNA, and antisense). All time points (0, 1, 2, 4, and 8 h samples) were used to estimate parameters in pulseR. The maximum a posteriori estimator (MAP) for δ in GRAND-SLAM was computed on the 1, 2, 4, and 8 h samples. See Figure 4 in the manuscript for description and legend.