## **Supporting Information for**

## Performance of computational algorithms to deconvolve heterogeneous bulk tumor tissue depends on experimental factors

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Figs S1 to S7  $\,$ 



Fig. S1. Relaxed probability thresholds for hash demultiplexing increase number of assigned cells. A) Assignments for Batch A where any cell with greater than 85% probability of originating from a sample is assigned to that sample. B) Assignments for Batch B at the 85% probability threshold. C) Assignments for Batch A at a threshold of greater than 80% probability of originating from a sample. D) Assignments for Batch B at the 80% probability threshold.



Fig. S2. Hash demultiplexing demonstrates cell type bias. A) Proportion of cell types in Batch A across all cells and in unassigned cells at various probability thresholds. Epithelial cells and fibroblasts are proportionally greater and T cells proportionally lesser in unassigned cells than in all cells. B) Proportion of cell types in Batch B.



Fig. S3. Genetic demultiplexing is concordant across source of bulk reference genotypes. A) Confusion matrix of genetic demultiplexing assignments for Batch A when using reference genotypes from rRNA<sup>-</sup> Chunk samples vs rRNA<sup>-</sup> Dissociated samples. B) Genetic demultiplexing assignments for Batch B using reference genotypes from rRNA<sup>-</sup> Chunk samples vs rRNA<sup>-</sup> Dissociated samples. C) Confusion matrix of genetic demultiplexing assignments for Batch A when using reference genotypes from rRNA<sup>-</sup> Chunk samples vs rRNA<sup>-</sup> Dissociated samples. C) Confusion matrix of genetic demultiplexing assignments for Batch A when using reference genotypes from rRNA<sup>-</sup> Dissociated samples. D) Genetic demultiplexing assignments for Batch B using reference genotypes from rRNA<sup>-</sup> Dissociated samples. D) Genetic demultiplexing assignments for Batch B using reference genotypes from rRNA<sup>-</sup> Dissociated samples. D) Genetic demultiplexing assignments for Batch B using reference genotypes from rRNA<sup>-</sup> Dissociated samples. D) Genetic demultiplexing assignments for Batch B using reference genotypes from rRNA<sup>-</sup> Dissociated samples. D) Genetic demultiplexing assignments for Batch B using reference genotypes from rRNA<sup>-</sup> Dissociated samples.

FDR ≤ 0.05 FDR > 0.05



Fig. S4. Stromal cell types are more abundant in dissociated bulk samples. Results from Gene Set Enrichment Analysis of rRNA<sup>-</sup> Chunk samples vs rRNA<sup>-</sup> Dissociated samples. Gene signatures associated with endothelial cells, fibroblasts, macrophages, and other immune cells (blue) are more abundant in rRNA<sup>-</sup> Dissociated samples, whereas red blood cell gene signatures (orange) are more abundant in rRNA<sup>-</sup> Chunk samples.

А	rRNA– Chunk												
nnl	s0.26	-0.02	-0.01	-0.03	0.09	0.03	0.08	0	0.2	-0.04	0.13	-0.01	-0.02
musi	-0.23	-0.04	-0.01	-0.03	-0.02	0	0.05	0	0.15	-0.1	0.42	-0.01	-0.02
po epi	-0.12			-0.03			-0.05		-0.03	0.45	0.04		-0.01
Cibersort	-0.23	-0.05	-0.01	-0.03	-0.02	0	0.02	0	0.16	0.14	0.1	0.03	0.04
bisqu	-0.17	0.02	0.07	0.05	0.04	0.06	0	0.04	-0.03	0	0.03	0.02	0.01
bayesprisr	-0.18	-0.05	-0.01	-0.03	-0.01	0	-0.03	0	0.03	0.36	0.07	-0.01	-0.01
	4 Cells	ena calis	₹D <sup>C</sup>	AYCONS N	ionocites h	Mast cells Mar	Johnages	<sup>₩<sup>C</sup></sup>	problasts Epit	elial cells	elial cells	¢ <sup>c</sup>	B cells

(Estimated bulk	proportion – sinale	cell proportion)
	proportion onigio	

							rRNA	– Disso	ciated					
	nnls	-0.26	-0.05	-0.01	-0.03	0.15	0	0.07	0	0.26	-0.09	0.13	-0.01	-0.02
pou	music	-0.24	-0.05	-0.01	-0.03	-0.02	0	0.07	0	0.16	-0.12	0.41	-0.01	-0.02
	epic	-0.16			-0.03			-0.05		0.02	0.38	0.09		-0.01
Met	cibersortx	-0.22	-0.05	0	-0.03	-0.02	0	0.04	0	0.24	0	0.15	0.03	0.02
	bisque ·	-0.13	0.02	0.05	0.01	0.02	0.08	0.02	0.03	0.01	0.06	-0.05	0	0
	bayesprism ·	-0.14	-0.05	-0.01	-0.03	-0.01	0	-0.01	0	0.08	0.19	0.15	-0.01	-0.02
		T Cells	sma cells	2DC	NH COLLE N	onocytes	Mast cells	tophage5	<sup>,</sup> € ∜	problasts colt	elial cells	elialcells	0 <sup>C</sup>	BCBIE

(Estimated bulk proportion – single cell proportion)

;							polyA	+ Disso	ciated					
	nnls-	-0.26	-0.04	-0.01	-0.03	0.14	0	-0.08	0	-0.19	0.69	-0.05	-0.01	-0.02
Method	music-	-0.25	-0.05	-0.01	-0.03	-0.01	0	0.05	0	0.18	-0.11	0.4	-0.01	-0.02
	epic-	-0.17			-0.03			-0.05		-0.03	0.46	0.06		-0.01
	cibersortx -	-0.24	-0.05	-0.01	-0.03	-0.02	0	0.03	0	0.27	0	0.13	0.04	0.01
	bisque -	-0.18	0.02	0.06	0.06	0.02	0.09	0.01	0.06	-0.05	0.05	-0.02	0.01	0
I	bayesprism -	-0.16	-0.05	-0.01	-0.03	0	0	0	0	0.1	0.19	0.13	-0.01	-0.02
		T CEILE DIS	ena cells	80 <sup>0</sup>	NY Cells N	ionocytes	Mast cells Mac	Jophages	₩ <sup>C</sup>	problasts Epit	elial cells	lelial cells	¢ <sup>c</sup>	& cells

(Estimated bulk proportion - single cell proportion)

Fig. S5. Deconvolution estimates vary based on input bulk type. A) The average difference between estimated cell type proportion in rRNA<sup>-</sup> Chunk samples minus the corresponding cell type proportion in scRNA-seq Individual samples. Gray boxes represent cell types not estimated by a given method. B) The difference in cell type proportion based on rRNA<sup>-</sup> Dissociated sample deconvolution estimates and scRNA-seq Individual samples. C) The difference in cell type proportion based on polyA<sup>+</sup> Dissociated sample deconvolution estimates and scRNA-seq Individual samples.

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Fig. S6. Robustness to very small reference profiles A) The variance of deconvolution proportion estimates, stratified by cell type and method, when using our default reference profile (genetic), the reference profile of cells assigned to a sample by hash demultiplexing (hashing), and a simulated sample of approximately 2000 cells (Sim2000). B) Variance of proportion estimates across the same reference profiles as in A but also including results from Sim1000. C) Same as B but also including results from Sim500. D) Same as C but also including results from Sim200.



Fig. S7. Alternate deconvolution methods that return cell type scores do not match single cell proportions. A-G) Correlation between the cell type score returned by the deconvolution method and the corresponding proportion of cells in the scRNA-seq Individual sample. The name of the deconvolution method and the Pearson correlation (r value) is shown at the top of each panel.