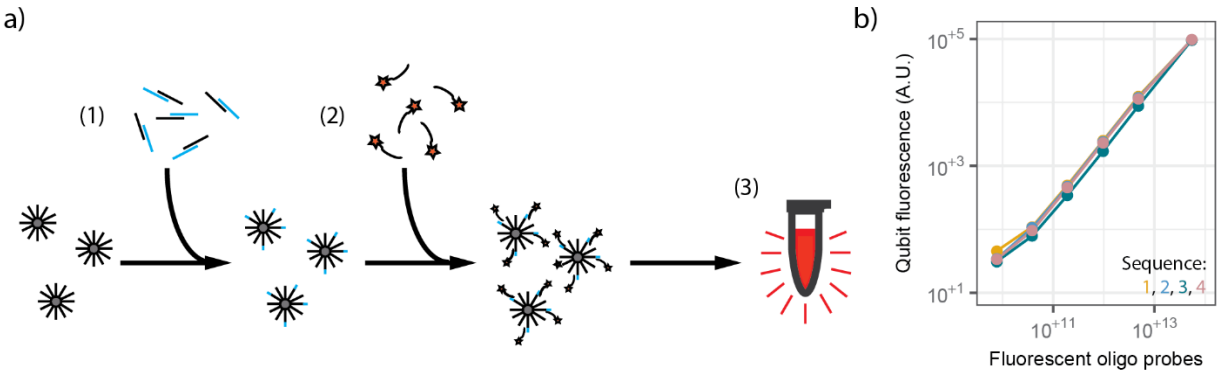
Supplemental Materials for:

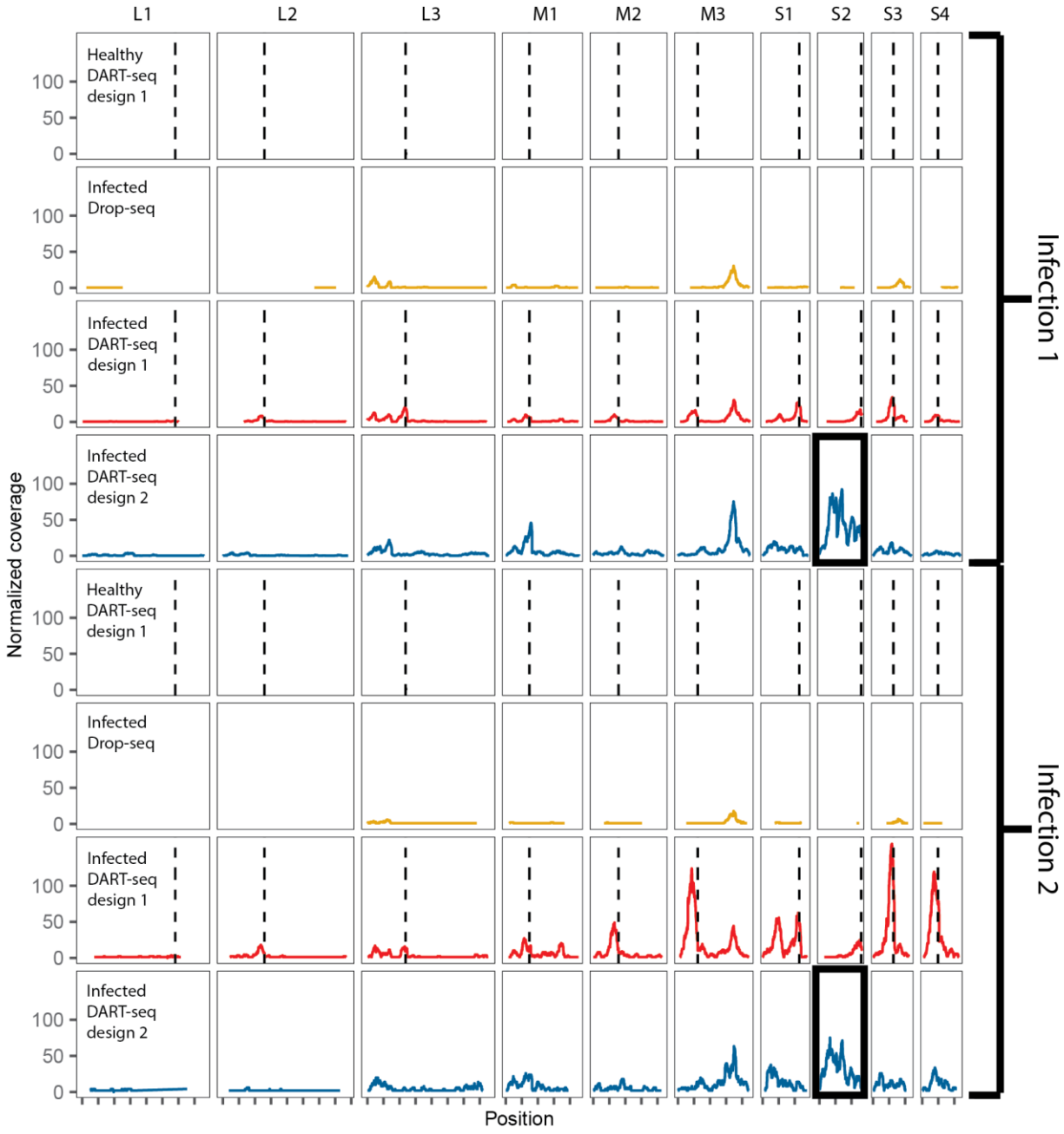
Simultaneous multiplexed amplicon sequencing and transcriptome profiling in single cells

Authors: Mridusmita Saikia[1,2,*], Philip Burnham[1,*], Sara H. Keshavjee[1], Michael F. Z. Wang[1], Michael Heyang[1], Pablo Moral-Lopez[2], Meleana M. Hinchman[2], Charles G. Danko[2], John S. L. Parker[2], Iwijn De Vlaminck[1]
*These authors contributed equally
To whom correspondence should be addressed: vlaminck@cornell.edu

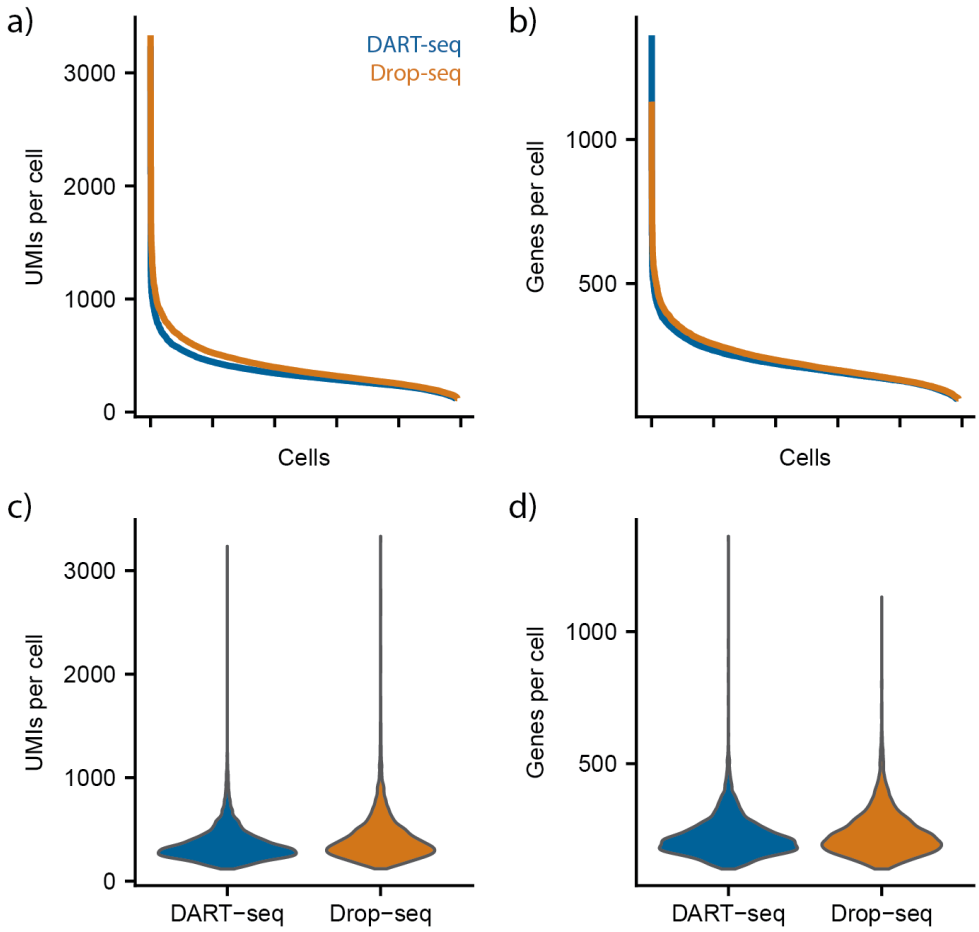
Affiliations:
[1]Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY 14853, USA
[2]Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA



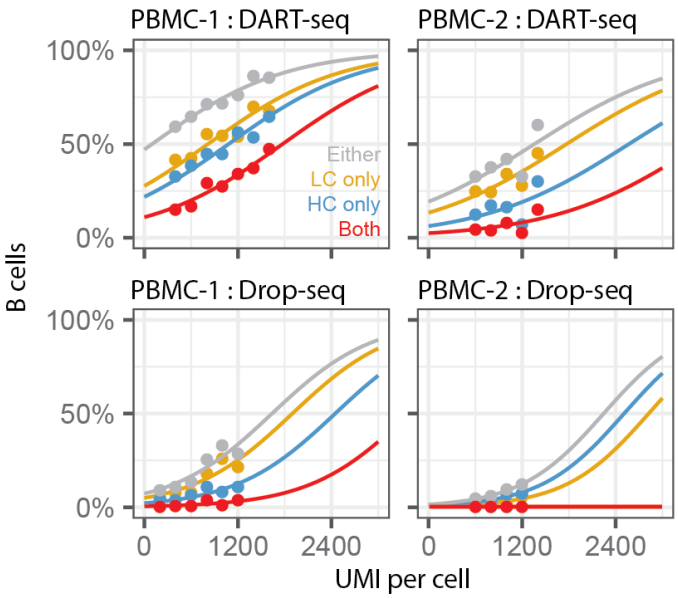
Supplementary Figure 1: a) Assay design to test the efficiency of custom primer ligation tp Drop-seq beads: (1) DART-seq beads are created with addition of custom primers at various concentrations, (2) Fluorescent oligo probes (Cy5), complementary to the custom primer, are hybridized to the DART-seq beads, (3) Fluorescence signal of 3000 beads is measured with a Qubit 3.0 fluorometer. **b)** Calibration of the fluorescence signal per oligo detected by Qubit.



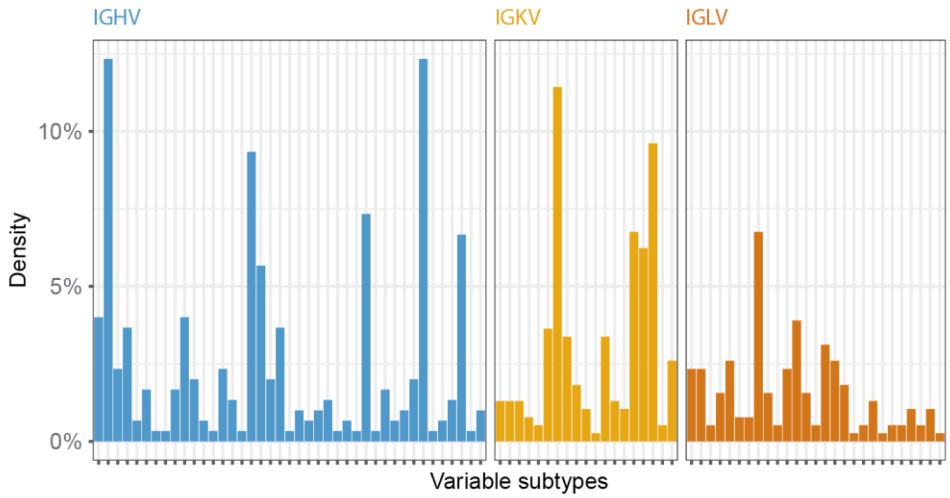
Supplementary Figure 2: DART-seq is reproducible between biological replicates. DART-seq was performed on T3D reovirus infection of L-cells in two separate experiments with same conditions (cells infected at 10 MOI and collected at 15 hours post infection). Coverage of viral genome segments is shown relative to the amount of host transcriptome captured. Dotted lines represent the custom primer loci corresponding to DART-seq design 1, and DART-seq design 2 (see main text). No viral sequences were detected in a non-infected cell line.



Supplementary Figure 3: (a-b) Number of UMIs (a) and unique genes detected as function of cell rank for DART-seq and Drop-seq for the same sample (CD19+ B cells)**. (c-d)** Violin plots of the number of UMI and gene detected in DART-seq and Drop-seq assays for the same sample (CD19+ B cells). To make these comparisons, both datasets were sampled to the same number of raw sequences ($167x10^6$).



Supplementary Figure 4: DART-seq outperforms Drop-seq in the detection of heavy and light chain sequences from B cells in PBMCs. The plot shows the percentage of B cells for which heavy and/or light chain transcripts were detected as function of the UMI count per cell. Cells were binned by the number of UMI detected (bin width 200 UMI, 0-2400 UMI per cell, bins with fewer than 20 cells omitted, 26 - 2396 cells per bin). Distributions were fit with a sigmoid curve.



Supplementary Figure 5: Comparison of variable isoforms detected with DART-seq by representation within Ig heavy and Ig light variable regions.