A diffusion-based microfluidic device for single-cell RNA-seq

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Supplementary Figures

Figure S1. The various steps involved in single cell trapping. Black arrows show the directions of the flow. In step1, valves B and C open to allow a diluted cell suspension in PBS to pass through the cell-trapping chamber. In step 2, once a single cell is selected, valves B and C are closed and valve A is opened to allow excessive cells to flow to the waste outlet. The path is then rinsed to remove residual cells in the channel connected to the inlet. After rinsing, valves B and D are opened and valves A and C are closed in order for lysis buffer to push the trapped single cell into the reaction chamber. The entire process is carried out under the microscope to ensure healthy and intact single cells are trapped.

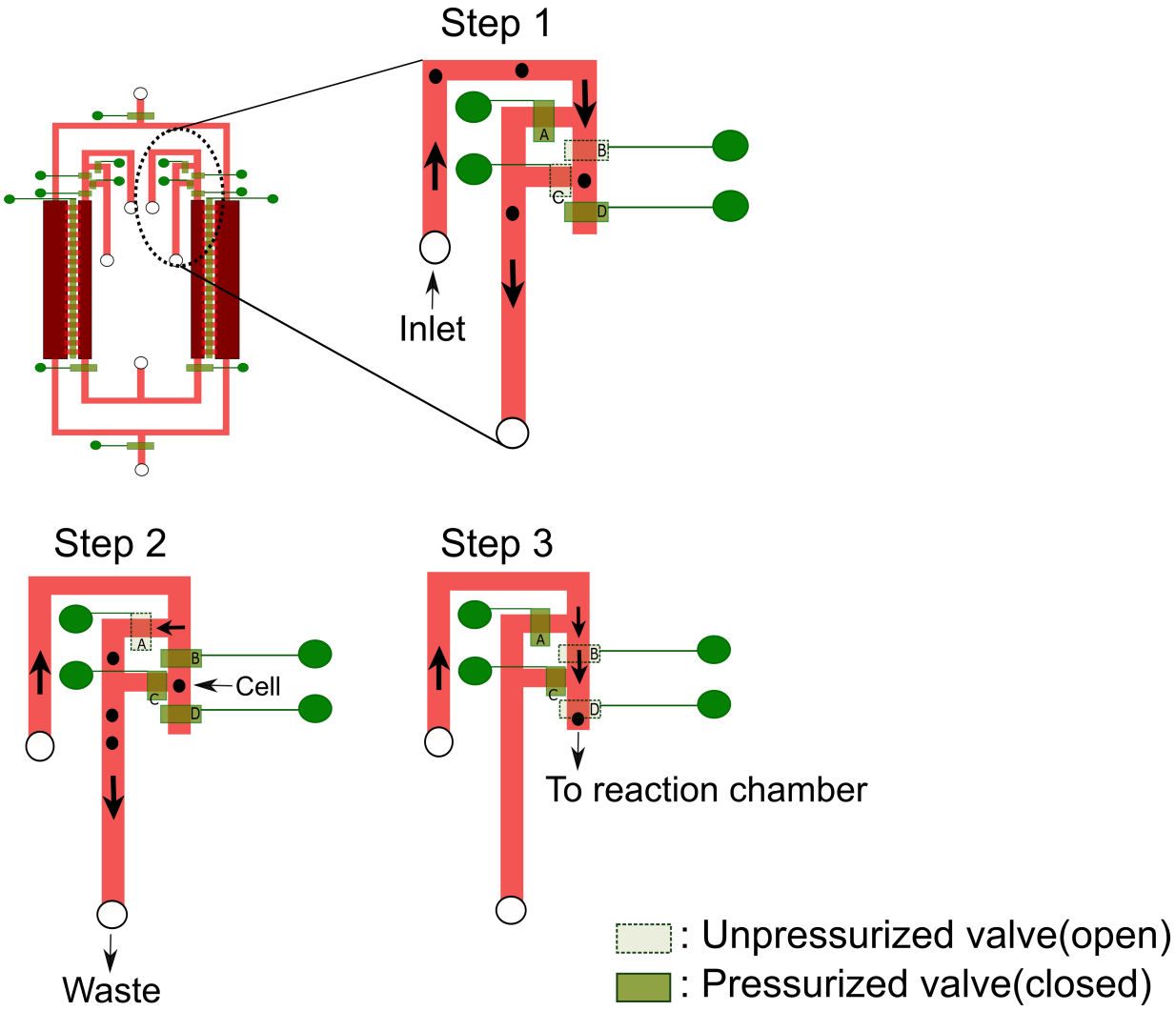


Figure S2. The number of genes detected at FPKM > 0 is plotted against various diffusion durations (time for which the diffusion valve was open) of the reverse transcription (R) and PCR (P) steps in a 2-unit MID-RNA-Seq device. a) The diffusion duration for reverse transcription step (R) was varied while the PCR diffusion time (P) was kept constant at 20 min. b) The diffusion duration for the PCR step was varied while the reverse transcription diffusion duration was kept at 40 min. Error bars indicate standard deviation between 2 replicates of the same 2-unit device.

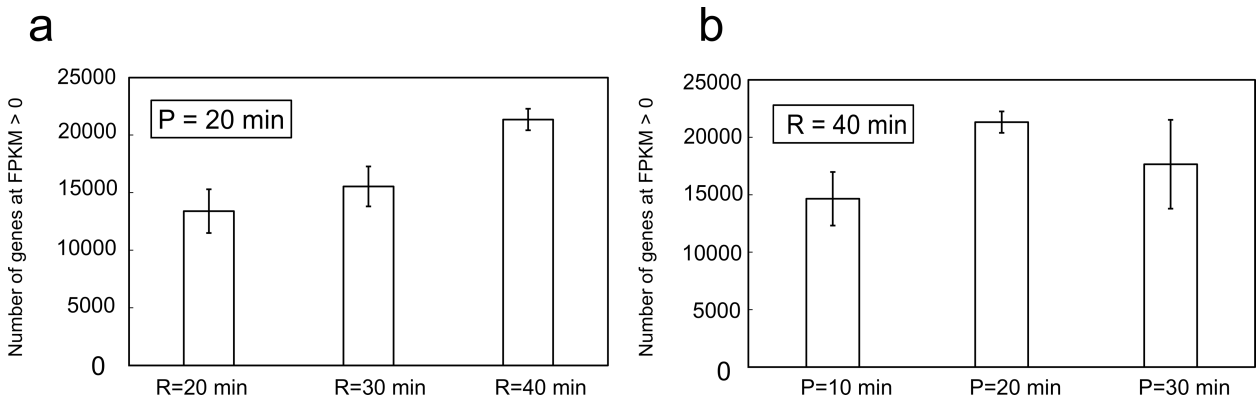


Figure S3. 4-unit (a) and 6-unit (b) MID-RNA-Seq devices with single cell-trapping ability. The 4-unit device shows the possibility of multiplexing in the horizontal direction and the 6-unit device shows multiplexing capability in the vertical direction thus demonstrating the various possibilities for device design to increase throughput.

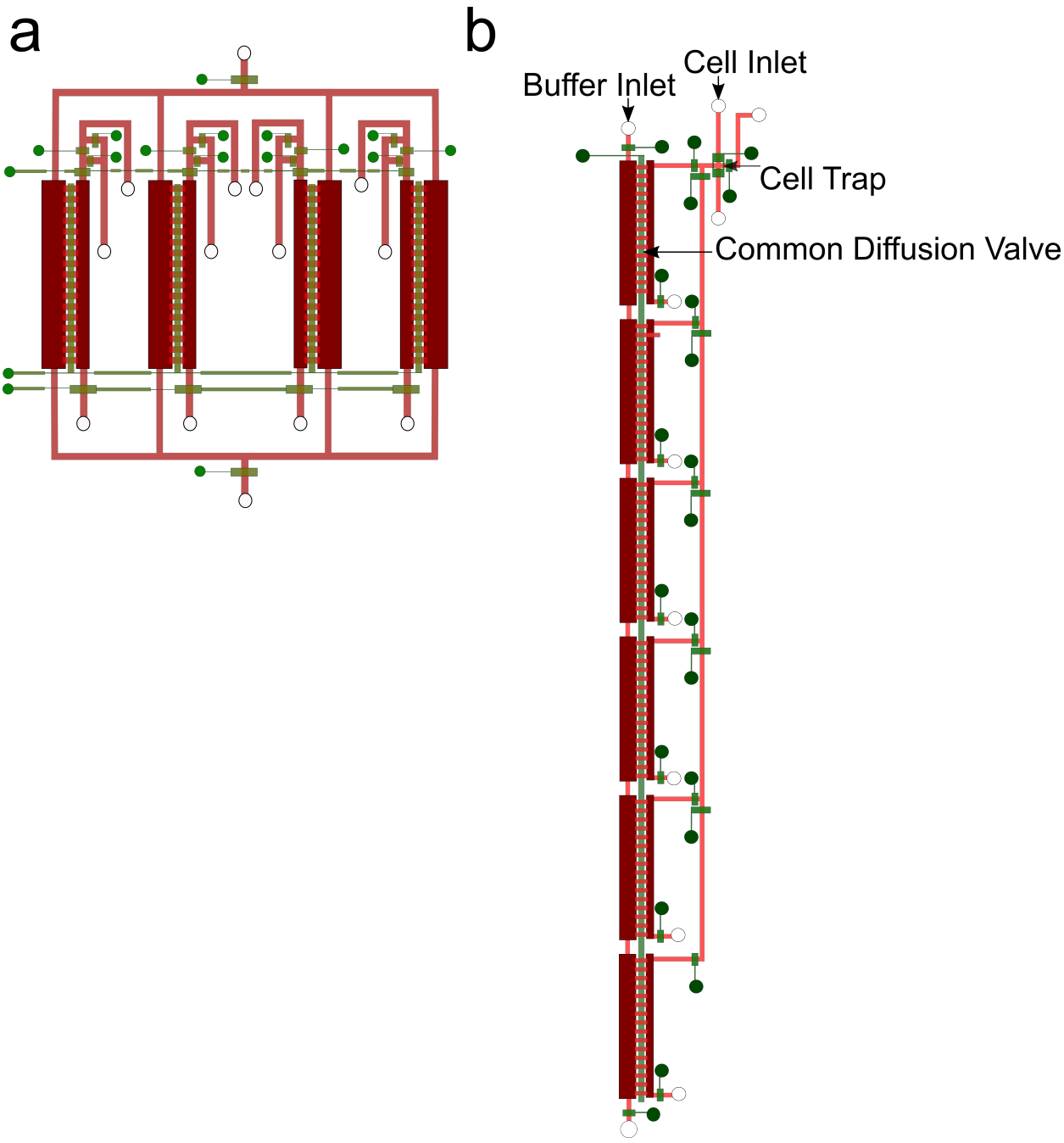


Figure S4. Genes with FPKM>1 detected in the four units of the 4-unit MID-RNA-seq device using RNA from GM12878 cells. The numbers indicate how many genes overlap between each unit.

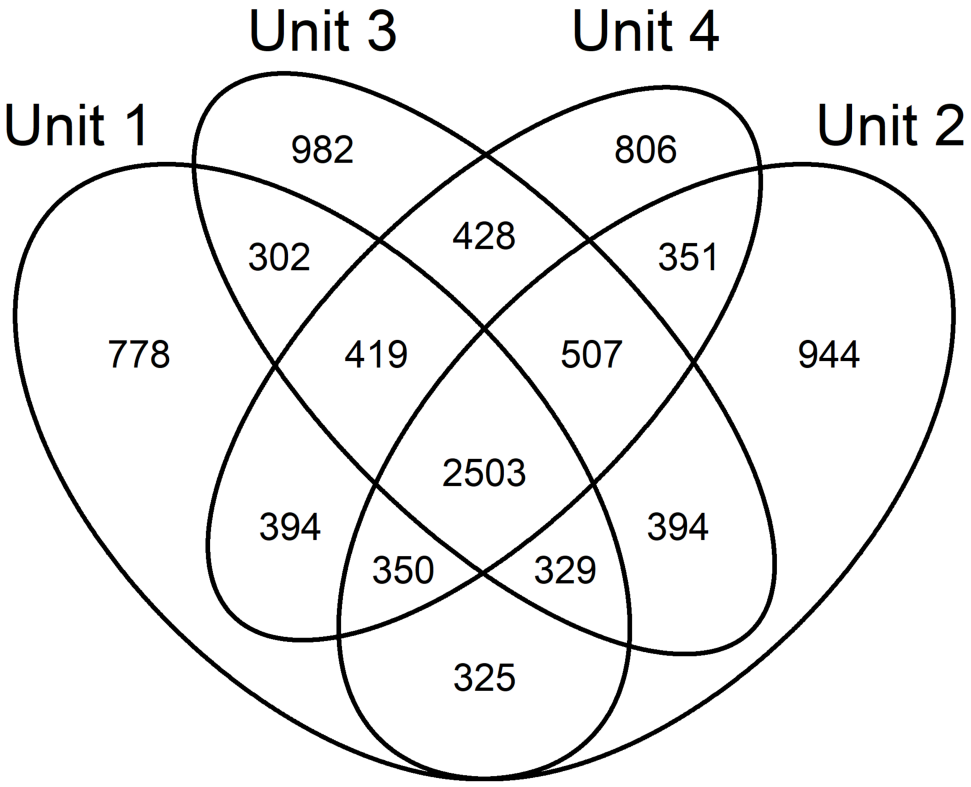
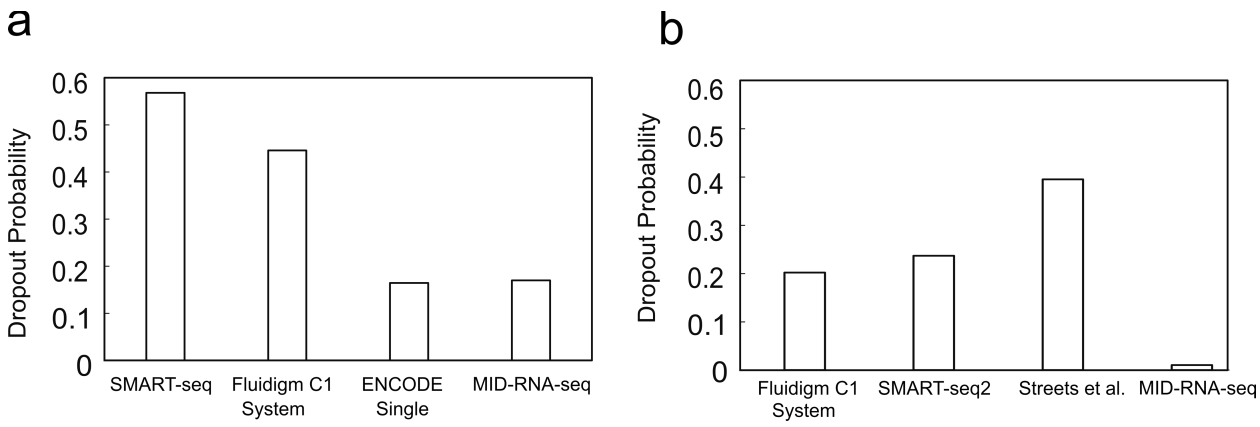


Figure S5. Dropout probability for a) human single-cell datasets b) mouse single-cell datasets taken using MID-RNA-seq. A set of 18842 human genes and another set of 13529 mouse genes are used in (a) and (b) respectively to determine dropout probability which is defined as the percentage of genes of the set that are not covered by the single-cell data (i.e. FPKM = 0). Human datasets include ENCODE datasets(GSM2343071/2), SMART-seq from Ref. 1, Fluidigm C1 System from Ref. 16. Mouse datasets include SMART-seq2 from Ref. 11, Streets et al. from Ref. 5 and Fluidigm C1 System from Ref. 7. All datasets have been depth-matched before comparison. Cuffdiff average replicate profiling has been used.



Supplementary information:

R scripts to calculate loess regression curve and R^2 value

c <- lowess (Data$column1FPKM, Data$column2FPKM, f=1)

ss.dist <- sum(scale(Data$ column1FPKM, scale=FALSE)^2)

ss.resid <- sum(resid(c)^2)

1-ss.resid/ss.dist #Gives R^2 value