Electronic Supplementary Material (ESI) for Lab on a Chip.

Cilo-seq: Highly Sensitive Cell-in-library-out Single-cell Transcriptome Sequencing with Digital Microfluidics

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Supplement Figure and Table.

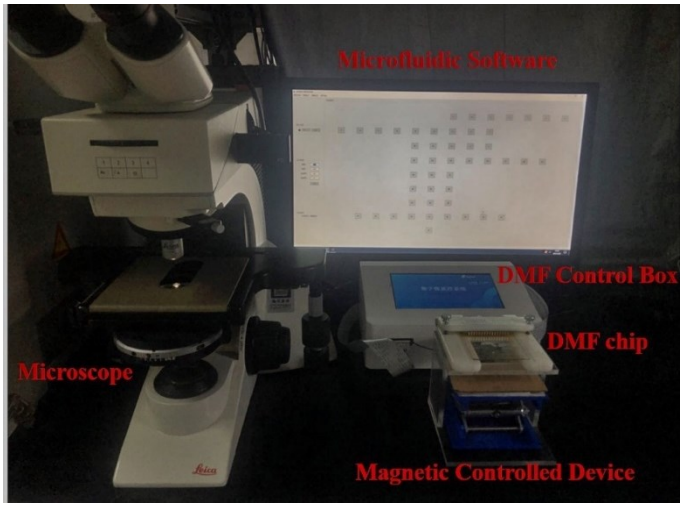

Figure S1. Integrated DMF system containing DMF chip, DMF control box, microfluidic software and magnetically controlled device.

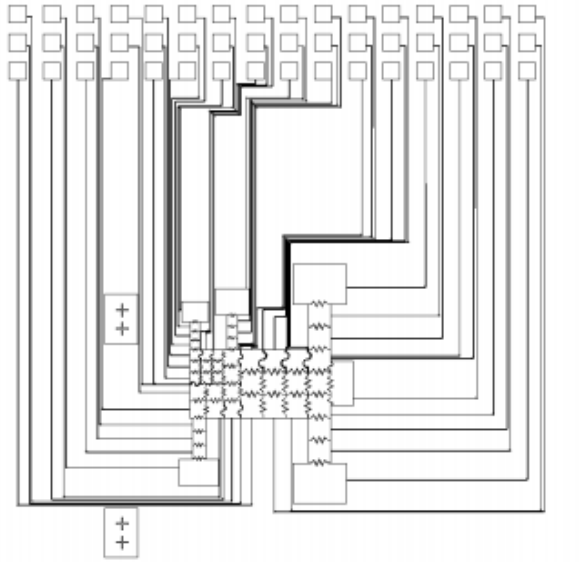

Figure S2. Schematic diagram of Cilo-seq chip fabrication.

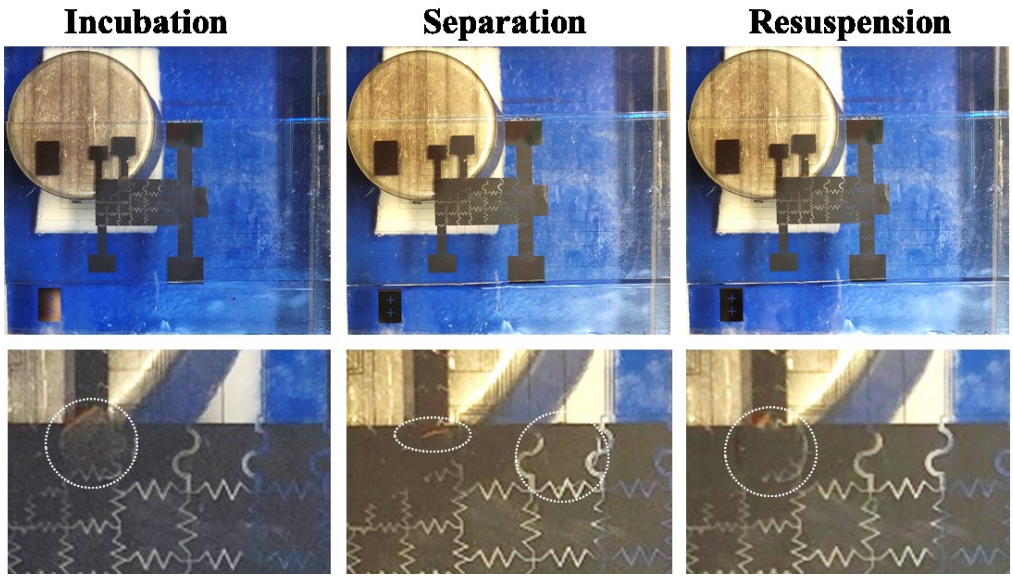

Figure S3. The droplet movement during magnetic separation.

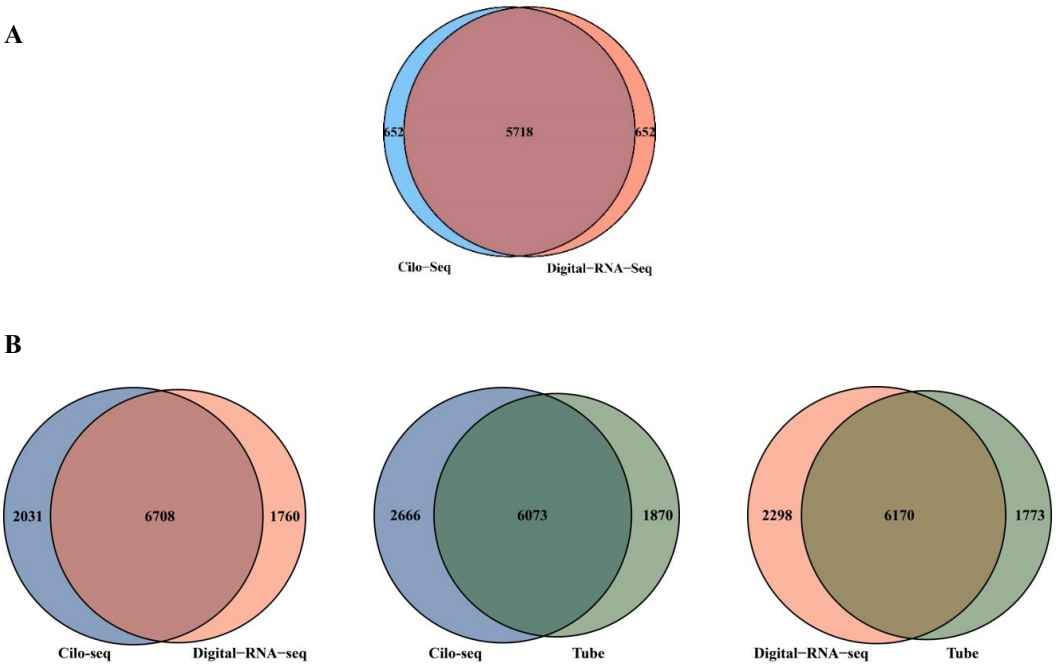

Figure S4. A） The Venn diagrams of overlapped 6370 genes in Cilo-seq and Digital-RNA-seq. B) Venn diagrams indicating the overlap of genes in three different methods including Cilo-seq, Digital-RNA-seq and tube method.

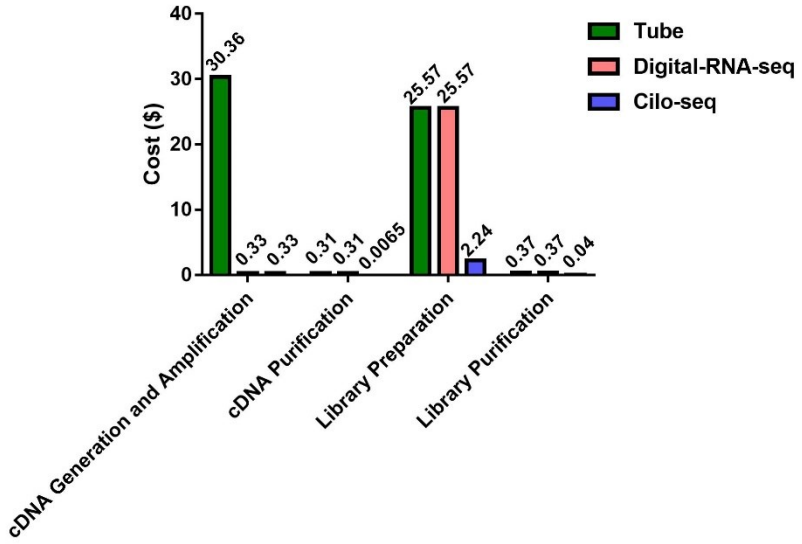

Figure S5. Reagent cost comparison of Cilo-seq, Digital-RNA-seq and tube method.

Table S1. Primer sequences used for Cilo-seq.

Primer Name	Sequence
Oligo-dT	5’-AAGCAGTGGTATCAACGCAGAGTACT30VN-3′
TSO	5′-AAGCAGTGGTATCAACGCAGAGTACATrGrG+G-3′
PCR primer	5′-AAGCAGTGGTATCAACGCAGAGT-3′

Table S2. The whole steps including droplet volumes, reagent concentrations, reaction temperature, incubation time.

Step	Droplet Volume	Regent Concentration	Incubation Temperature and Time
Cell Isolation	76.8 nL	Cell Suspension with the cell concentration below 10^5 cell mL^{-1}	Room Temperature Less than 30s
Cell Lysis	76.8 nL	Lysis buffer (0.1% Triton X-100 solution, 1.25 U μL^{-1} RNase Inhibitor, 3.25 μM oligo-dT primer, and 1.67 mM dNTP, 0.05% Pluronic F68)	72°C 3min
cDNA Generation	120 nL	Reverse transcription mixes (1× first strand buffer, 40 U μL^{-1} Superscript II reverse transcriptase, 0.87 mM DTT, 1.31 M betaine, 0.87 U μL^{-1} RNase Inhibitor, 5.3 μM TSO primer, 17.5 mM $MgCl_2$ and 0.05% Pluronic F68)	42°C 90min; 75°C 15min
cDNA Amplification	176.8 nL	PCR mixes (1× fidelity buffer, 0.5 μM PCR primer, 0.5 mM dNTP, 0.17 U μL^{-1} HiFi Hot Start DNA Polymerase and 0.05% Pluronic F68	98°C 3min; 30 cycles (98°C 30s, 67°C 25s, 72°C 6min); 72°C 5min
cDNA Purification	518 nL	1×VAHTS DNA Clean Beads,	Room Temperature 10min

	345.6 nL	0.05% Pluronic F68 ddH_2O, 0.05% Pluronic F68	Room Temperature 10min
	1.5 μL	1×TTBL, 33% TTE, 0.05% Pluronic F68	55°C 10min
Library Preparation	375 nL	5×TS, 0.05% Pluronic F68	Room Temperature 5min
	2.5 μL	2×TAB, 4%TAE, 4%N7XX, 4%N5XX, 0.05% Pluronic F68	72°C 3min; 98°C 30s; 30 cycles (98°C 15s, 60°C 30s, 72°C 3min); 72°C 5min
Library Purification	3 μL	1×VAHTS DNA Clean Beads, 0.05% Pluronic F68	Room Temperature 10min
	5 μL	ddH_2O, 0.05% Pluronic F68	Room Temperature 10min

Table S3. FPKM values of 3 ERCC samples.

Name	ERCC1	ERCC2	ERCC3
ERCC-00002	953869	538287	613295
ERCC-00003	145784	33054	49307
ERCC-00004	763373	173879	342051
ERCC-00009	1007413	95740	114708
ERCC-00012	42	1	0
ERCC-00013	124	0	31
ERCC-00014	25	59	17
ERCC-00016	0	102	35
ERCC-00017	0	0	0
ERCC-00019	10507	658	1143
ERCC-00022	9834	9729	15038
ERCC-00024	9	15	0
ERCC-00025	1580	968	633
ERCC-00028	122	103	28
ERCC-00031	109	29	68
ERCC-00033	97	8	10
ERCC-00034	2341	280	319
ERCC-00035	9535	3360	3283
ERCC-00039	912	98	99
ERCC-00040	176	55	26
ERCC-00041	9	18	0
ERCC-00042	53023	23923	18402
ERCC-00043	35888	14384	17780
ERCC-00044	4781	2943	2494
ERCC-00046	793185	143950	294647
ERCC-00048	0	0	0
ERCC-00051	6423	575	2320
ERCC-00053	1327	377	507
ERCC-00054	382	70	174
ERCC-00057	0	0	0
ERCC-00058	183	23	17
ERCC-00059	3131	415	592
ERCC-00060	36253	10986	24088
ERCC-00061	0	0	0
ERCC-00062	3177	817	1047
ERCC-00067	422	100	60
ERCC-00069	236	6	30
ERCC-00071	6962	1606	4532
ERCC-00073	170	77	26

ERCC-00074	3632780	703152	1638272
ERCC-00075	0	2	1
ERCC-00076	20932	14933	23613
ERCC-00077	50	0	114
ERCC-00078	8304	861	809
ERCC-00079	11461	1497	2971
ERCC-00081	6	1	0
ERCC-00083	0	0	0
ERCC-00084	9453	1148	1053
ERCC-00085	1107	213	277
ERCC-00086	14	4	9
ERCC-00092	22711	13530	12788
ERCC-00095	88889	3598	8768
ERCC-00096	1515985	470179	605499
ERCC-00097	24	98	88
ERCC-00098	2	9	15
ERCC-00099	532	306	178
ERCC-00104	12	2	0
ERCC-00108	290301	91321	114646
ERCC-00109	9	0	6
ERCC-00111	106565	30604	32820
ERCC-00112	11122	4254	5795
ERCC-00113	223699	178358	280320
ERCC-00116	7384	8168	4660
ERCC-00117	10	2	0
ERCC-00120	61	0	10
ERCC-00123	21	19	5
ERCC-00126	561	144	148
ERCC-00130	669364	424383	501014
ERCC-00131	7410	5425	6222
ERCC-00134	3	19	22
ERCC-00136	392197	69380	79943
ERCC-00137	40	30	10
ERCC-00138	4	1	2
ERCC-00142	2	1	3
ERCC-00143	1015	200	148
ERCC-00144	522	737	935
ERCC-00145	88623	22078	22687
ERCC-00147	40	11	18
ERCC-00148	1090	56	229
ERCC-00150	74	74	77
ERCC-00154	832	88	315

ERCC-00156	29	16	5
ERCC-00157	2275	210	96
ERCC-00158	84	10	17
ERCC-00160	593	489	521
ERCC-00162	6004	1644	3855
ERCC-00163	6698	661	1012
ERCC-00164	39	0	7
ERCC-00165	29693	2482	2831
ERCC-00168	54	3	9
ERCC-00170	1707	202	240
ERCC-00171	442267	129736	194301