**Supplementary file to “HyDrop enables droplet based single-cell ATAC-seq and single-cell RNA-seq using dissolvable hydrogel beads”**

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**HyDrop-RNA**

**Reaction in Emulsion**

**Lysis, mRNA anneals to poly-T** barcodes

5-TTTTTTTTAATACGACTCACTATAGGGAAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT

3-AAAAAAAAAAAAAAAAAA-[mRNA]

**RT with barcoded RT primer (First strand synthesis)**

5-TTTTTTTTAATACGACTCACTATAGGGAAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-CCC

3-AAAAAAAAAAAAAAAAAA-[mRNA]

**And template switching with TSO**

5-TTTTTTTTAATACGACTCACTATAGGGAAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-CCCATTCACTCTGCGTTGATACCACTGCTT-3

AAAAAAAAAAAAAAAAAAAAAAAAA-[mRNA]-GGGTAAGTGAGACGCAACTATGGTGACGAA-5

**cDNA amplification in bulk (post emulsion breakage)**

**Amplification with TSO-Primer**

TSO-P

5-AAGCAGTGGTATCAACGCAGAGT**-3** -->

5-TTTTTTTTAATACGACTCACTATAGGGAAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-CCCATTCACTCTGCGTTGATACCACTGCTT-3

3-AAAAAAAATTATGCTGAGTGATATCCCTTCGTCACCATAGTTGCGTCTCATG-[BC1]-GTCGATGACG-[BC2]-GCTCATGGGA-[BC3]-NNNNNNNNNNAAAAAAAAAAAAAAAAAAAAAAAAA-[cDNA]-GGGTAAGTGAGACGCAACTATGGTGACGAA-5

<--3-TGAGACGCAACTATGGTGACGAA-5

TSO-P

5-AAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-CCCATTCACTCTGCGTTGATACCACTGCTT-3

3-TTCGTCACCATAGTTGCGTCTCATG-[BC1]-GTCGATGACG-[BC2]-GCTCATGGGA-[BC3]-NNNNNNNNNNAAAAAAAAAAAAAAAAAAAAAAAAA-[cDNA]-GGGTAAGTGAGACGCAACTATGGTGACGAA-5

**Sequencing library preperation**

**Then, perform NEBNext fragmentation, and dA-tailing**

5-AAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-A-3

3-TTCGTCACCATAGTTGCGTCTCATG-[BC1]-GTCGATGACG-[BC2]-GCTCATGGGA-[BC3]-NNNNNNNNNNAAAAAAAAAAAAAAAAAAAAAAAAA-[cDNA]-5

**Then, NEBNext Ligation with Illumina adapter**

5-AAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-AGATCGGAAGAGCACACGTCTGAACTCCAGTC\

||||||||||||||||||||||||| ||| |||||||||| ||| |||||||||| ||| |||||||||| |||||||||||||||||||||||| ||||| ||||||||||||| U

3-TTCGTCACCATAGTTGCGTCTCATG-[BC1]-GTCGATGACG-[BC2]-GCTCATGGGA-[BC3]-NNNNNNNNNNAAAAAAAAAAAAAAAAAAAAAAAAA-[cDNA]-TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA/

**And NEB USER enzyme treatment, followed by PCR with P5 index**

I7 index

5-CAAGCAGAAGACGGCATACGAGAT-[I7]-CTGTCCGCGGAAGCAGTGGTATCAACGCAGAGTAC-3

5-AAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-AGATCGGAAGAGCACACGTCTGAACTCCAGTC-3

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3-TTCGTCACCATAGTTGCGTCTCATG-[BC1]-GTCGATGACG-[BC2]-GCTCATGGGA-[BC3]-NNNNNNNNNNAAAAAAAAAAAAAAAAAAAAAAAAA-[cDNA]-TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-5

<-TCGCAGCACATCCCTTTCTCACA-[i5]-CACATCTAGAGCCACCAGCGGCATAGTAA**-**5

i5 index

5-CAAGCAGAAGACGGCATACGAGAT-[i7]-CTGTCCGCGGAAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-[i5]-GTGTAGATCTCGGTGGTCGCCGTATCATT-3

3-GTTCGTCTTCTGCCGTATGCTCTA-[i7]-GACAGGCGCCTTCGTCACCATAGTTGCGTCTCATG-[BC1]-GTCGATGACG-[BC2]-GCTCATGGGA-[BC3]-NNNNNNNNNNAAAAAAAAAAAAAAAAAAAAAAAAA-[cDNA]-TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-[i5]-CACATCTAGAGCCACCAGCGGCATAGTAA-5

**Sequencing**

HyDrop\_CustSeq\_Short TruSeq Read 1

<--3-GACAGGCGCCTTCGTCACCATAGTTGCGTCTCATG-5 <--3-TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-5

5-CAAGCAGAAGACGGCATACGAGAT-[i7]-CTGTCCGCGGAAGCAGTGGTATCAACGCAGAGTAC-[BC1]-CAGCTACTGC-[BC2]-CGAGTACCCT-[BC3]-NNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTT-[cDNA]-AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-[i5]-GTGTAGATCTCGGTGGTCGCCGTATCATT-3

3-GTTCGTCTTCTGCCGTATGCTCTA-[i7]-GACAGGCGCCTTCGTCACCATAGTTGCGTCTCATG-[BC1]-GTCGATGACG-[BC2]-GCTCATGGGA-[BC3]-NNNNNNNNNNAAAAAAAAAAAAAAAAAAAAAAAAA-[cDNA]-TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-[i5]-CACATCTAGAGCCACCAGCGGCATAGTAA-5

5-CTGTCCGCGGAAGCAGTGGTATCAACGCAGAGTAC-3--> 5-AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3-->

HyDrop\_CustSeq\_R2 TruSeq Index 2