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

Florian V. De Rop1,2, Joy N. Ismail1,2, Carmen Bravo González-Blas1,2, Gert J. Hulselmans1,2, Christopher C. Flerin1,2,4, Jasper Janssens1,2, Koen Theunis1,2,4, Valerie M. Christiaens1,2, Jasper Wouters1,2, Gabriele Marcassa1,3, Joris de Wit1,3, Suresh Poovathingal1,#, and Stein Aerts1,2,#

1 VIB-KU Leuven Center for Brain & Disease Research

2 Laboratory of Computational Biology, Department of Human Genetics, KU Leuven

3 Laboratory of Synapse Biology, Department of Neurosciences, KU Leuven

4 Aligning Science Across Parkinson’s (ASAP) Collaborative Research Network, Chevy Chase, MD,= 20815.

# Shared last author; correspondence to suresh.poovathingal@kuleuven.be and stein.aerts@kuleuven.be.

 **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