Supplemental Method S1

Single-Cell DNA BAG-seq Laboratory Protocol

Equipment (similar to Drop-seq/DroNc-seq set-up):

Name	Company	Catalog Number
Inverted Microscope	Motic	AE31
Legato 100 Syringe Pumps (3)	KD Scientific	788100
3 mL Syringes	Becton Dickinson	309657
10 mL Syringes	Becton Dickinson	302995
PE-2 Tubing	Scientific Commodities	BB31695-PE/2
26 Gauge Needles	Becton Dickinson	305111
22 Gauge Needles	Becton Dickinson	305155
16 Gauge Needles	Becton Dickinson	305197
PDMS co-flow microfluidic droplet generation device “DroNc-seq device”	Nanoshift, LLC	
40 µm cell strainers	Corning	352340
70 µm cell strainers	Corning	431751
100 µm cell strainers	Corning	352360

Primers:

Name	Sequence
Linker TG	/5ACryd//iSp18/TGTGTTGGGTGTGTTTGGKKKKKKKGKKKKKKKKNN
Adapter1-BC1-vt-#DNAx	ACGCAGAGTCCGCTC <barcode1> NNNN CA+T+G (detailed sequences at bottom)
Adapter2-BC2-Adapter1-#DNAx	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG <barcode2> ACGCAGAGTCCGCTC (detailed sequences at bottom)
TG primer	TGTGTTGGGTGTGTTT*G*G
P5-TG	AATGATACGGCGACCACCGAGATCTACAC GGAGATGTG TGTGTTGGGTGTGTTT*G*G
Read1-TG	GGAGATGTG TGTGTTGGGTGTGTTTGG

Chemical Reagents:

Name	Company	Catalog Number
Nuclei Preparation		
Nuclei PURE lysis buffer (only for nuclei prep from tumor sample)	Sigma-Aldrich	NUC201-1KT
Nuclei EZ lysis buffer	Sigma-Aldrich	NUC101-1KT
10X PBS	Thermo Fisher Scientific	AM9624
UltraPure BSA	Thermo Fisher Scientific	AM2618
Trypsin-EDTA(0.25%, phenol red)	Thermo Fisher Scientific	25200056
Aqueous Phase 2		
Acrylamide/bis-acrylamide, 40% solution	Sigma-Aldrich	A9926
Acrylamide solution, 40%	Sigma-Aldrich	A4058
0.5 M EDTA	Thermo Fisher Scientific	AM9260G
20% Sarkosyl	Sigma-Aldrich	L7414

0.1M DTT	Thermo Fisher Scientific	707265ML
Ammonium persulfate	Sigma-Aldrich	09913-100G
10% NP-40	Thermo Fisher Scientific	28324
Proteinase K	Sigma-Aldrich	P4850
Oil Phase		
HFE-7500	Oakwood Chemical	051243
FC-40	Sigma-Aldrich	F9755
008-Fluorosurfactant	Ran Technologies	008-FluoroSurfactant-5G
TEMED	Bio-Rad	1610801
Post-Droplet Generation		
Mineral Oil	Sigma-Aldrich	69794-500ML
20X SSC	Thermo Fisher Scientific	15557044
Perfluorooctanol (PFO)	Sigma-Aldrich	370533
10 mM dNTP	Sigma-Aldrich	11814362001
DNA Polymerase I, Large (Klenow) Fragment	New England BioLabs	M0210M
Exonuclease I	New England BioLabs	M0293
NlaIII	New England BioLabs	R0125L
Klenow Fragment (3-5 exo minus)	New England BioLabs	M0212L
Quick Ligase	New England BioLabs	M2200
KAPA HiFi HotStart ReadyMix	Roche	KK2602
Agencourt AMPure XP beads	Beckman Coulter	A63880
Nextera XT DNA Library Prep Kit	Illumina	FC-131-1024

Chemical Solutions to Prepare

PBS-BSA (1X PBS, 0.05% BSA)
20% Surfactant in HFE-7500
20% Surfactant in FC-40
10% Ammonium persulfate
6X SSC
TE-SDS (10 mM Tris pH 8.0, 1 mM EDTA, 0.5% SDS)
STOP-25 (10 mM Tris pH 8.0, 25 mM EDTA, 0.1% Tween-20, 0.1 M KCl)
STOP-10 (10 mM Tris pH 8.0, 10 mM EDTA, 0.1% Tween-20, 0.1 M KCl)
STOP-1 (10 mM Tris pH 8.0, 1 mM EDTA, 0.1% Tween-20, 0.1 M KCl)
HBW (10 mM Tris pH 8.0, 1 mM EDTA, 0.1% Tween-20)

scDNA BAG-seq Experiment:

Nuclei Preparation from Tumor Sample

1. Keep reagents on ice and tumor sample on dry ice. Set centrifuge temperature to 4°C.
2. Use scalpels to mince sample on a sterile plate. Add 500 µL of Nuclei PURE lysis buffer onto the sample.
3. Transfer sample using pipette from plate to a glass tissue grinder. Use pestles to further mince the sample while keeping the grinder on ice.
4. Add 3.5 mL of EZ lysis buffer, then transfer all to a 15 mL conical. Pipet up and down.
5. Once in 15 mL conical tube, keep on ice for 5 minutes.
6. Centrifuge the conical tube at 500 rcf for 5 minutes at 4°C, then discard supernatant. Re-suspend the pellet in 700 µL of EZ lysis buffer. Pipet up and down, and gently vortex.
7. Add 3.3 ml of EZ buffer and pipet up and down. Wait 5 minutes. Gently vortex. Spin down using the same settings, then discard supernatant.
8. Add 600 µL of PBS-BSA, pipet up and down, pass through a 70 µm filter, and count nuclei.
9. Use PBS-BSA to create a final dilution of 2.5E5 nuclei in 1 mL volume.

Nuclei Preparation from Cell Culture

1. Follow standard cell harvest protocol to harvest cells from plates. Collect cell solution in a 15 mL conical and spin down at 500 rcf for 5 minutes.
2. Discard supernatant, then re-suspend pellet in 8 mL of 1x PBS. Spin once more using same settings, and aspirate supernatant.
3. Add 4 ml of EZ lysis buffer into pellet, pipet up and down, and wait 5 minutes. Lightly vortex, spin down again, and discard supernatant
4. Add 700 µL of EZ lysis buffer to break pellet, pipet up and down, lightly vortex. Then add 3.3 mL of EZ lysis buffer, pipet up and down, and keep on ice for 5 minutes. Lightly vortex, spin down, and discard supernatant.
5. Add 1.5 mL of PBS-BSA. Pass through a 40 µm filter, and count.
6. If preparing a 1:1 mix of the two cell types, use PBS-BSA to create a final dilution of 2.5E5 cells in 1 mL.

Oil Phase Preparation

1. Combine the following; vortex to mix (*enough for 2 samples*):

600 µL	20% surfactant
9.6 µL	TEMED
1.8 mL	HFE7500

Aqueous Phase 2 Preparation

1. Combine the following; pipet to mix (*enough for 2 samples*):

Aqueous Phase 2 Solution	**Volume (µL)**	**Final Concentration**
AA/bis-acrylamide, 40%	180	
Acrylamide solution, 40%	129	
H_2O	271	
500 µM Linker TG primer	160	40 µM
0.5M EDTA	50	12.5 mM
1M Tris pH 7.5	100	50 mM
20 % Sarkosyl	10	0.1%

Proteinase K	20	
0.1M DTT	20	1 mM
10% APS	60	0.3%
Total	**1 mL**	

Microfluidics Setup & Oil Droplet Collection

1. Turn on microscope. Place microfluidic device on microscope stage.
2. Insert a piece of PE/2 tubing into the output well and place the other end into a waste container (15 mL conical tube).
3. Use the following syringes to collect the three solutions.
    a. **Oil Phase:** Use a 10 mL syringe with a 16 G needle to aspirate solution into syringe.
    b. **Cell Solution or Aqueous Phase 2**: Use a 3 mL syringe and a 22 G needle to aspirate solution into syringe.
4. For each syringe, change the needle to a 26 G needle and insert into PE/2 tubing.
5. Place syringes into their designated syringe pumps and insert tubing into the corresponding wells of the microfluidics chamber
    a. From left to right: Oil – Aqueous 2 – Cell Solution (Aqueous 1) – Output
6. Once all syringes and tubing are in place, set the following flow rates:
    a. **Oil Phase:** 3,000 µL/hr
    b. **Cell Solution:** 750 µL/hr
    c. **Aqueous Phase 2:** 750 µL/hr
7. Begin by first turning on the cell solution pump.
8. Once cell solution is flowing, turn on the aqueous phase 2 pump.
9. Once the interphase between cell solution and aqueous phase two has stabilized, turn on the oil phase pump and wait for the droplet formation to stabilize.
10. Once the flow has stabilized, place the output tubing on a glass slide under the microscope to assess the size uniformity of oil droplets.
11. Once droplet size is uniform, collect droplets in 300 µL of mineral oil in 1.5 mL tubes.
12. After collection, incubate BAGs at 50°C overnight.

Oil Change and Incubation

1. Discard bottom layer of the tube using 22 G needle. Add 300 µL of FC-40 oil with 5% surfactant into the bottom of the tube.
2. Incubate at 95°C for 12 minutes to denature genomic DNA.
3. Incubate at 55°C for 1 hour, then incubate at room temperature for at least 10 minutes.

Droplet Breakage

1. Remove mineral oil in top layer using pipet. Remove mineral oil in bottom layer using a 3 mL syringe and 22G needle.
2. Add 600 µL 6X SSC buffer and 150 µL perfluorooctanol (PFO) in a fume hood. Shake the tube by hand to break the droplets and release balls of acrylamide gel (BAGs).
3. Centrifuge 1000 rcf for 1.5 minutes.
4. Discard the top and bottom layer, leaving the translucent BAG layer in the tube.
5. Add 600 µL 6x SSC and shake tube multiple times. Centrifuge 1000 rcf for 1.5 minutes. Discard the top layer and any visible PFO from the bottom of the tube.

Linear Extension

1. Wash BAG layer using 1X NEB2 buffer. Pipet to mix. Centrifuge 1000 rcf for 1.5 minutes, discard the supernatant.
2. Prepare the following linear extension solution; pipet up and down to mix:

Linear Extension Solution	**Volume (µL)**
H_2O	830
NEB2 buffer	100
dNTP	60
DNA Polymerase I, Large (Klenow) Fragment	10
Total	**1 mL**

3. Transfer linear extension solution to BAG layer. Pipet up and down ~10x to mix.
4. Incubate at room temperature for 1.5 hours with rotation.
5. Incubate at 37°C for 30 minutes with rotation.
6. Stop the reaction with the following steps:
    a. Add 1:1 ratio of STOP-25 buffer. Mix by pipetting.
    b. Centrifuge at 1000 rcf for 1 minute. Discard supernatant.
    c. Add ~500 µL of STOP-25 buffer to each tube. Combine contents into one tube.
    d. Centrifuge at 1000 rcf for 1 minute. Discard supernatant.
    e. Add 1 mL of STOP-10 buffer, mix, and pass solution through 100 µm cell strainer, and transfer the solution to a new 1.5 mL Eppendorf tube.
    f. Continue to exonuclease treatment or re-suspend in 800 µL STOP-10 and store at 4°C.

----------Safe Stopping Point: Store BAGs in STOP-10 at 4°C overnight----------

Exonuclease Treatment

1. If stored in STOP-10, centrifuge 1000 rcf for 1.5 minutes and discard the supernatant.
2. Wash once with STOP-1.
3. Wash once with 1x Exo I buffer. Discard supernatant.
4. Prepare the following exonuclease treatment solution and vortex.

Exonuclease Treatment Solution	**Volume (µL)**
H_2O	680
10x Exo I buffer	80
Exo I enzyme	40
Total	**800 µL**

5. Transfer 800 µL Exonuclease Treatment Solution to the tube of BAGs; pipet to mix.
6. Incubate 37°C with rotation for 1 hour.
7. Centrifuge at 1000 rcf for 1 minute. Discard supernatant.
8. Stop the reaction with the following washes:
    a. STOP-25 twice
    b. STOP-10 once.
9. Continue to NlaIII cutting or re-suspend in 800 µL STOP-10 and store at 4°C.

----------Safe Stopping Point: Store BAGs in STOP-10 at 4°C overnight----------

NlaIII cutting

1. If stored in STOP-10, centrifuge 1000 rcf for 1.5 minutes and discard the supernatant.
2. Wash once with STOP-1.

3. Wash once with 1x CutSmart buffer.
4. Prepare the following NlaIII solution 1 and vortex.

NlaIII Solution 1	**Volume (µL)**
H_2O	840
10x CutSmart buffer	100
NlaIII enzyme	60
Total	**1 mL**

5. Transfer NlaIII Solution 1 to the tube of BAGs; pipet to mix.
6. Incubate 37°C with rotation for 1.5 hours.
7. Centrifuge at 1000 rcf for 1.5 minutes. Discard supernatant
8. Prepare the following NlaIII solution 2 and vortex.

NlaIII Solution 2	**Volume (µL)**
H_2O	880
CutSmart buffer	100
NlaIII enzyme	20
Total	**1 mL**

9. Transfer NlaIII Solution 2 to the tube of BAGs; pipet to mix.
10. Incubate 37°C with rotation for 1 hour.
11. Centrifuge at 1000 rcf for 1.5 minutes. Discard supernatant.
12. Stop the reaction with the following washes:
    a. STOP-25 twice
    b. STOP-10 once.
13. Continue to First split-pool or re-suspend in 800 µL STOP-10 and store at 4°C.

----------Safe Stopping Point: Store BAGs in STOP-10 at 4°C overnight----------

First Split-Pool

1. Preparation:
    a. If stored in STOP-10 overnight, centrifuge 1000 rcf for 1.5 minutes. Discard supernatant.
    b. Wash once with HBW buffer. Discard supernatant.
2. Prepare the following hybridization master mix and vortex.

Hybridization Master Mix	**Volume (µL)**
H_2O	400
2x Quick Ligase Buffer	700
dNTP	100
Total	**1200 µL**

    a. Transfer Hybridization Master Mix into BAG tube (assuming there is 100 µL of BAG pellet in HBW buffer). Pipet to mix.
    b. Quickly add 162.5 µL into each tube of one PCR strip. Use multipipette to transfer 13 µl of this solution to each tube in a 96-well PCR plate.
    c. Add 1 µL of 100 uM “Adapter1-BC1-vt-#DNAx” primers into each corresponding well. Each well should have a unique well-specific primer. Briefly spin down, and invert plate.
    d. Incubate at room temperature for 4 minutes in inverted position.
    e. Incubate at 50°C for 5 minutes in thermocycler with heated lid.

f. Transfer plates directly to cold racks in 4°C.
g. Keep inverted at 4°C for 10 minutes, then another 10 minutes with rotation. Follow these steps for hybridization:

3. On ice, prepare the following Extension Master Mix; vortex to mix.

Extension Master Mix	**Volume (µL)**
H_2O	200
2X Quick Ligase Buffer	250
Klenow 3-5 exo minus	75
Quick Ligase enzyme	75
Total	**600**

h. Transfer 75 µL of Extension Master Mix into each tube of one PCR strip
i. Put the plate on cold rack, and using multipipette add 6 µL of Extension Master Mix to each tube in BAG plate. Pipet up and down ~10x
j. Transfer plates to thermocycler with the following conditions:

Gel Extension Program:
10°C 30 minutes
22°C 10 minutes (ramp rate 0.2°C / second)

k. Incubate at room temperature with rotation for 40 minutes.
l. Incubate 37°C with rotation for 40 minutes.

4. Stop the reaction with the following:
    a. Add 100 µL STOP-25 to each well of the plate. Centrifuge plates 1000 rcf for 2 minutes at room temperature.
    b. Without disturbing the BAG pellet, discard 85 µL from **the top** of each tube.
    a. Add 5 mL STOP-25 into solution basin. Transfer the remaining BAG solutions from the plate into the solution basin (35 µL per tube).
    b. Transfer BAG solution from solution basin into a 15 mL conical tube. Centrifuge 200 rcf for 4 minutes. Discard supernatant.
    c. Add 800 µL STOP-25, pipet up and down, and transfer BAG solution into a 1.5 mL tube. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant. Re-suspend in 800 uL STOP-10 buffer.

----------Safe Stopping Point: Store BAGs in STOP-10 at 4°C overnight----------

Second Split-Pool

1. Preparation:
    a. Thaw “TG primer” and “Adapter2-BC2-Adapter1-#DNAx” barcode primers.
    b. Take out BAGs from 4°C, discard supernatant, and perform one STOP-10 wash. Discard supernatant. Re-suspend BAGs in 500 µL HBW.
2. Use a microscope to count the number of BAGs per µL.
3. Create the following TG primer/BAG mix:

TG primer/BAG mix	**Volume (µL)**
10 µM “TG primer”	100
BAGs in HBW	x
H_2O	$764 - x$
Total	**864 µL**

    a. Transfer 108 µL of TG primer/BAG mix into each tube of one PCR strip. Use multipipette to transfer 9 µL of mix into each tube of 96-well plate.

4. Add 1 µL of 96 different 10 µM "Adapter2-BC2-Adapter1-#DNAx" primers into each corresponding well. Each well should have a unique primer. Briefly spin.
5. On ice, add 10 µL of Ultra II Q5 Master Mix to each tube of 96-well plate. Pipet to mix.
6. Proceed to PCR:

Q5 Gel PCR program:

95°C 2 min
98°C 15 s
12 cycles of:
98°C 15 s
63°C 1 min
65°C 1 min
Then:
65°C 5 min
4°C infinite

7. Purify sample twice with AMPure XP beads using the following guidelines:
   a. First Pool: Pool 10 ul from each of the eight tubes in each column into a new PCR tube.

(ex. 1A-1H) into a new PCR tube, as shown.

1A	2	3	4	5	6	7	8	9	10	11	12
B											
C											
D											
E											
F											
G											
H											

1	2	3	4	5	6	7	8	9	10	11	12

(After pool: 80 µL each)

   b. First Purification: Purify these 12 samples with 1.0x ratio (80 µL) of AMPure XP beads, and elute with 25 µL of H_2O using AMPure XP protocol.
   c. Second Pool:

Pool 23 ul from three tubes into one tube.

1	2	3	4	5	6	7	8	9	10	11	12

1	2

(After pool: 69 µL each)

   d. Second Purification: Purify these four samples with another 0.8x ratio (56 µL) of AMPure XP beads, and elute with 21 µL H_2O using AMPure XP protocol.
8. Run a High Sensitivity DNA Chip on an Agilent Bioanalyzer.

Making Final Sequencing Library

1. Prepare following reaction mix for each of the samples

Final PCR Reaction Mix	**Volume (µL)**
Q5 Ultra Master Mix	20

10 µM “P5-TG” primer	1
10 µM N70x Nextera primer	1
DNA sample	18
Total	**40 µL**

2. Proceed to PCR

Final PCR:

95°C 20s
4 cycles of:
98°C 15 s
62°C 40 s
72°C 40 s
3 cycles of:
98°C 10 s
67°C 20 s
72°C 40 s
Then:
72°C 4 min
4°C infinite

3. Post-PCR purification: Purify sample with 0.8x ratio of Ampure XP Beads, and elute with 10 µL H_2O using AMPure XP protocol.
4. Run a High Sensitivity DNA Chip on an Agilent Bioanalyzer to check the final concentration of the sample.
5. Sequence your sample using Illumina platform with custom Read1 sequencing primer “Read1-TG”.

Barcode Sequences

First Split-Pool Barcodes

Oligo name	Sequence
Adapter1-BC1-vt-#DNA1	ACGCAGAGTCCGCTCCTAAGNNNNCA+T+G
Adapter1-BC1-vt-#DNA2	ACGCAGAGTCCGCTCACAATNNNNCA+T+G
Adapter1-BC1-vt-#DNA3	ACGCAGAGTCCGCTCTAATGNNNNCA+T+G
Adapter1-BC1-vt-#DNA4	ACGCAGAGTCCGCTCCTTCGNNNNCA+T+G
Adapter1-BC1-vt-#DNA5	ACGCAGAGTCCGCTCTATAGNNNNCA+T+G
Adapter1-BC1-vt-#DNA6	ACGCAGAGTCCGCTCGGTCANNNNCA+T+G
Adapter1-BC1-vt-#DNA7	ACGCAGAGTCCGCTCTGATANNNNCA+T+G
Adapter1-BC1-vt-#DNA8	ACGCAGAGTCCGCTCCGACANNNNCA+T+G
Adapter1-BC1-vt-#DNA9	ACGCAGAGTCCGCTCTGAGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA10	ACGCAGAGTCCGCTCATTCCNNNNCA+T+G
Adapter1-BC1-vt-#DNA11	ACGCAGAGTCCGCTCGATCGNNNNCA+T+G
Adapter1-BC1-vt-#DNA12	ACGCAGAGTCCGCTCTCAGANNNNCA+T+G
Adapter1-BC1-vt-#DNA13	ACGCAGAGTCCGCTCCATGGNNNNCA+T+G
Adapter1-BC1-vt-#DNA14	ACGCAGAGTCCGCTCTCTGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA15	ACGCAGAGTCCGCTCACTGANNNNCA+T+G
Adapter1-BC1-vt-#DNA16	ACGCAGAGTCCGCTCACACANNNNCA+T+G
Adapter1-BC1-vt-#DNA17	ACGCAGAGTCCGCTCAATATNNNNCA+T+G
Adapter1-BC1-vt-#DNA18	ACGCAGAGTCCGCTCTCAAGNNNNCA+T+G
Adapter1-BC1-vt-#DNA19	ACGCAGAGTCCGCTCTTAGGNNNNCA+T+G
Adapter1-BC1-vt-#DNA20	ACGCAGAGTCCGCTCAATGCNNNNCA+T+G
Adapter1-BC1-vt-#DNA21	ACGCAGAGTCCGCTCAGTACNNNNCA+T+G
Adapter1-BC1-vt-#DNA22	ACGCAGAGTCCGCTCCGAATNNNNCA+T+G
Adapter1-BC1-vt-#DNA23	ACGCAGAGTCCGCTCCAACGNNNNCA+T+G
Adapter1-BC1-vt-#DNA24	ACGCAGAGTCCGCTCTAACANNNNCA+T+G
Adapter1-BC1-vt-#DNA25	ACGCAGAGTCCGCTCGAACTNNNNCA+T+G
Adapter1-BC1-vt-#DNA26	ACGCAGAGTCCGCTCATTGGNNNNCA+T+G
Adapter1-BC1-vt-#DNA27	ACGCAGAGTCCGCTCCATCCNNNNCA+T+G
Adapter1-BC1-vt-#DNA28	ACGCAGAGTCCGCTCATAGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA29	ACGCAGAGTCCGCTCTATCTNNNNCA+T+G
Adapter1-BC1-vt-#DNA30	ACGCAGAGTCCGCTCTCTTANNNNCA+T+G
Adapter1-BC1-vt-#DNA31	ACGCAGAGTCCGCTCGTACANNNNCA+T+G
Adapter1-BC1-vt-#DNA32	ACGCAGAGTCCGCTCGTAGCNNNNCA+T+G
Adapter1-BC1-vt-#DNA33	ACGCAGAGTCCGCTCTAAGCNNNNCA+T+G
Adapter1-BC1-vt-#DNA34	ACGCAGAGTCCGCTCATAACNNNNCA+T+G
Adapter1-BC1-vt-#DNA35	ACGCAGAGTCCGCTCCGTAGNNNNCA+T+G
Adapter1-BC1-vt-#DNA36	ACGCAGAGTCCGCTCGATACNNNNCA+T+G
Adapter1-BC1-vt-#DNA37	ACGCAGAGTCCGCTCTGACGNNNNCA+T+G
Adapter1-BC1-vt-#DNA38	ACGCAGAGTCCGCTCGATGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA39	ACGCAGAGTCCGCTCAGTGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA40	ACGCAGAGTCCGCTCAGTCGNNNNCA+T+G
Adapter1-BC1-vt-#DNA41	ACGCAGAGTCCGCTCAATCANNNNCA+T+G
Adapter1-BC1-vt-#DNA42	ACGCAGAGTCCGCTCACTCTNNNNCA+T+G
Adapter1-BC1-vt-#DNA43	ACGCAGAGTCCGCTCAATTGNNNNCA+T+G
Adapter1-BC1-vt-#DNA44	ACGCAGAGTCCGCTCCTATCNNNNCA+T+G
Adapter1-BC1-vt-#DNA45	ACGCAGAGTCCGCTCCGTGANNNNCA+T+G
Adapter1-BC1-vt-#DNA46	ACGCAGAGTCCGCTCTCTACNNNNCA+T+G
Adapter1-BC1-vt-#DNA47	ACGCAGAGTCCGCTCTGTCCNNNNCA+T+G
Adapter1-BC1-vt-#DNA48	ACGCAGAGTCCGCTCGCATANNNNCA+T+G
Adapter1-BC1-vt-#DNA49	ACGCAGAGTCCGCTCACTAGNNNNCA+T+G
Adapter1-BC1-vt-#DNA50	ACGCAGAGTCCGCTCGCACGNNNNCA+T+G

Adapter1-BC1-vt-#DNA51	ACGCAGAGTCCGCTCAGAAGNNNNCA+T+G
Adapter1-BC1-vt-#DNA52	ACGCAGAGTCCGCTCACTTCNNNNCA+T+G
Adapter1-BC1-vt-#DNA53	ACGCAGAGTCCGCTCGATTANNNNCA+T+G
Adapter1-BC1-vt-#DNA54	ACGCAGAGTCCGCTCCGTCTNNNNCA+T+G
Adapter1-BC1-vt-#DNA55	ACGCAGAGTCCGCTCTATTCNNNNCA+T+G
Adapter1-BC1-vt-#DNA56	ACGCAGAGTCCGCTCGTAATNNNNCA+T+G
Adapter1-BC1-vt-#DNA57	ACGCAGAGTCCGCTCATATANNNNCA+T+G
Adapter1-BC1-vt-#DNA58	ACGCAGAGTCCGCTCAGACTNNNNCA+T+G
Adapter1-BC1-vt-#DNA59	ACGCAGAGTCCGCTCATTAANNNNCA+T+G
Adapter1-BC1-vt-#DNA60	ACGCAGAGTCCGCTCTCTCGNNNNCA+T+G
Adapter1-BC1-vt-#DNA61	ACGCAGAGTCCGCTCCGATGNNNNCA+T+G
Adapter1-BC1-vt-#DNA62	ACGCAGAGTCCGCTCGCTAANNNNCA+T+G
Adapter1-BC1-vt-#DNA63	ACGCAGAGTCCGCTCTGTGGNNNNCA+T+G
Adapter1-BC1-vt-#DNA64	ACGCAGAGTCCGCTCGTATGNNNNCA+T+G
Adapter1-BC1-vt-#DNA65	ACGCAGAGTCCGCTCACATGNNNNCA+T+G
Adapter1-BC1-vt-#DNA66	ACGCAGAGTCCGCTCGGATTNNNNCA+T+G
Adapter1-BC1-vt-#DNA67	ACGCAGAGTCCGCTCCAATANNNNCA+T+G
Adapter1-BC1-vt-#DNA68	ACGCAGAGTCCGCTCGCAGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA69	ACGCAGAGTCCGCTCTTACCNNNNCA+T+G
Adapter1-BC1-vt-#DNA70	ACGCAGAGTCCGCTCTCATCNNNNCA+T+G
Adapter1-BC1-vt-#DNA71	ACGCAGAGTCCGCTCAGATCNNNNCA+T+G
Adapter1-BC1-vt-#DNA72	ACGCAGAGTCCGCTCGTTAGNNNNCA+T+G
Adapter1-BC1-vt-#DNA73	ACGCAGAGTCCGCTCGTTGANNNNCA+T+G
Adapter1-BC1-vt-#DNA74	ACGCAGAGTCCGCTCCAAGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA75	ACGCAGAGTCCGCTCCTTGTNNNNCA+T+G
Adapter1-BC1-vt-#DNA76	ACGCAGAGTCCGCTCGAAGANNNNCA+T+G
Adapter1-BC1-vt-#DNA77	ACGCAGAGTCCGCTCTGTAANNNNCA+T+G
Adapter1-BC1-vt-#DNA78	ACGCAGAGTCCGCTCTCACTNNNNCA+T+G
Adapter1-BC1-vt-#DNA79	ACGCAGAGTCCGCTCGCAACNNNNCA+T+G
Adapter1-BC1-vt-#DNA80	ACGCAGAGTCCGCTCACAGCNNNNCA+T+G
Adapter1-BC1-vt-#DNA81	ACGCAGAGTCCGCTCCTACTNNNNCA+T+G
Adapter1-BC1-vt-#DNA82	ACGCAGAGTCCGCTCGAATCNNNNCA+T+G
Adapter1-BC1-vt-#DNA83	ACGCAGAGTCCGCTCAGAGANNNNCA+T+G
Adapter1-BC1-vt-#DNA84	ACGCAGAGTCCGCTCAGTTANNNNCA+T+G
Adapter1-BC1-vt-#DNA85	ACGCAGAGTCCGCTCCATAANNNNCA+T+G
Adapter1-BC1-vt-#DNA86	ACGCAGAGTCCGCTCGGTTGNNNNCA+T+G
Adapter1-BC1-vt-#DNA87	ACGCAGAGTCCGCTCGGTATNNNNCA+T+G
Adapter1-BC1-vt-#DNA88	ACGCAGAGTCCGCTCCTAGANNNNCA+T+G
Adapter1-BC1-vt-#DNA89	ACGCAGAGTCCGCTCATACGNNNNCA+T+G
Adapter1-BC1-vt-#DNA90	ACGCAGAGTCCGCTCCGTTCNNNNCA+T+G
Adapter1-BC1-vt-#DNA91	ACGCAGAGTCCGCTCTGAACNNNNCA+T+G
Adapter1-BC1-vt-#DNA92	ACGCAGAGTCCGCTCGTTCTNNNNCA+T+G
Adapter1-BC1-vt-#DNA93	ACGCAGAGTCCGCTCCTTACNNNNCA+T+G
Adapter1-BC1-vt-#DNA94	ACGCAGAGTCCGCTCTTATTNNNNCA+T+G
Adapter1-BC1-vt-#DNA95	ACGCAGAGTCCGCTCTATGANNNNCA+T+G
Adapter1-BC1-vt-#DNA96	ACGCAGAGTCCGCTCCTAAGNNNNCA+T+G

Second Split-Pool Barcodes

Oligo name	Sequence
Adapter2-BC2-Adapter1-#DNA1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGAAT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATATTA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AATCGA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA4	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CACATA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA5	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTGATG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA6	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGTTT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA7	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATAACG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA8	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACGTA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA9	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAAGCA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA10	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGTAC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA11	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATGGA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA12	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAGGAA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA13	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGATT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA14	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTAATC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA15	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATTCCT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA16	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTCTA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA17	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTACTA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA18	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTTCAC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA19	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAACAC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA20	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATAGTT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA21	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACTAT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA22	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGGAAA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA23	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTTAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA24	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTACA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA25	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGGAA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA26	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTGTG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA27	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTTTC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA28	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCAA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA29	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCGTG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA30	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCATAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA31	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAACTG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA32	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATTGTA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA33	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGAGG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA34	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTAAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA35	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCAGT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA36	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCAAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA37	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATTGAC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA38	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAGTCA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA39	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTAGT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA40	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAATA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA41	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAAAGT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA42	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAGCAT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA43	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTCTCT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA44	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCAGT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA45	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGGCT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA46	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAACCA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA47	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAGATG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA48	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATAAT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA49	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCTAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA50	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTTAAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA51	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTGAA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA52	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTTCCA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA53	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTCAAT ACGCAGAGTCCGCTC

Adapter2-BC2-Adapter1-#DNA54	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTTTCG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA55	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGACA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA56	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAAGGG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA57	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATTAGT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA58	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTAACA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA59	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTTTC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA60	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTATT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA61	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAACTT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA62	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAGCA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA63	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTAGTG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA64	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGTAA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA65	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACCTC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA66	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AATTAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA67	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATTTG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA68	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATTAT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA69	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTGTCT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA70	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTTCT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA71	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTTAGG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA72	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATATT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA73	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTATTG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA74	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCCT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA75	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAGTTC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA76	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AATGTC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA77	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTTCA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA78	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATACG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA79	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATATAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA80	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAAACG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA81	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAAGG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA82	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAGGTT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA83	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCGCA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA84	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCAGC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA85	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTAAT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA86	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCAAC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA87	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCACA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA88	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGTGA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA89	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATAGA ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA90	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACTTG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA91	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGAAG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA92	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGAACT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA93	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTTCG ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA94	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAAGGT ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA95	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAGTC ACGCAGAGTCCGCTC
Adapter2-BC2-Adapter1-#DNA96	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATTCAG ACGCAGAGTCCGCTC

Supplemental Method S2

Single-Cell RNA BAG-seq Laboratory Protocol

Equipment (similar to Drop-seq/DroNc-seq set-up):

Name	Company	Catalog Number
Inverted Microscope	Motic	AE31
Legato 100 Syringe Pumps (3)	KD Scientific	788100
3 mL Syringes	Becton Dickinson	309657
10 mL Syringes	Becton Dickinson	302995
PE-2 Tubing	Scientific Commodities	BB31695-PE/2
26 Gauge Needles	Becton Dickinson	305111
22 Gauge Needles	Becton Dickinson	305155
16 Gauge Needles	Becton Dickinson	305197
PDMS co-flow microfluidic droplet generation device “DroNc-seq device”	Nanoshift, LLC	
40 µm cell strainers	Corning	352340
70 µm cell strainers	Corning	431751

Primers:

Name	Sequence
Linker A2	/5ACryd//iSp18/AAGCAGTGGTATCAACGCAGAGTNNWNNNSTTTTT TTTTTTTTTTTTTTTTTTTTTTTT
Adapter1-BC1-vt-#RNAx	AGTGGAAAAGGAAGGTGGT <barcode1> NNNNNN rGrGrG (detailed sequences at bottom)
Adapter2-BC2-Adapter1-#RNAx	GACCGACTCGCATTACCCTAT <barcode2> AGTGGAAAAGGAAGGTGG/3InvdT/ (detailed sequences at bottom)
Adapter3-BC3-Adapter2-#RNAx	AAGGCTCTTGACACGAAGGATAG <barcode3>NN GACCGACTCGCATTACCCT*A*T (detailed sequences at bottom)
IS-short primer	AAGCAGTGGTATCAACGCAG
P5-SAM	AATGATACGGCGACCACCGAGATCTACAC CGCTGTCC AAGGCTCTTGACACGAAGGAT*A*G
Read1-SAM	CGCTGTCC AAGGCTCTTGACACGAAGGATAG
Adapter3-BC2a-Adapter1-#RNAx	AAGGCTCTTGACACGAAGGATAG <barcode2a> AGTGGAAAAGGAAGGTG*G*T (detailed sequences at bottom)

Chemical Reagents:

Name	Company	Catalog Number
Cell Preparation		
10X PBS	Thermo Fisher Scientific	AM9624
UltraPure BSA	Thermo Fisher Scientific	AM2618
Trypsin-EDTA(0.25%, phenol red)	Thermo Fisher Scientific	25200056
Aqueous Phase 2		
Acrylamide/bis-acrylamide, 40% solution	Sigma-Aldrich	A9926
Acrylamide solution, 40%	Sigma-Aldrich	A4058

0.5 M EDTA	Thermo Fisher Scientific	AM9260G
20% Sarkosyl	Sigma-Aldrich	L7414
SUPERase In RNase Inhibitor	Thermo Fisher Scientific	AM2696
0.1M DTT	Thermo Fisher Scientific	707265ML
Ammonium persulfate	Sigma-Aldrich	09913-100G
10% NP-40	Thermo Fisher Scientific	28324
Oil Phase		
HFE-7500	Oakwood Chemical	051243
008-Fluorosurfactant	Ran Biotechnologies	008-FluoroSurfactant-5G
TEMED	Bio-Rad	1610801
Post-Droplet Generation		
Mineral Oil	Sigma-Aldrich	69794-500ML
20X SSC	Thermo Fisher Scientific	15557044
Perfluorooctanol (PFO)	Sigma-Aldrich	370533
Maxima H Reverse Transcriptase	Thermos Fisher Scientific	EP0753
10 mM dNTP	Sigma-Aldrich	11814362001
Exonuclease I	New England Bio Labs	M0293
Ribonuclease H	Thermo Fisher Scientific	18021-071
Bst 2.0 DNA Polymerase	New England Bio Labs	M0537L
KAPA HiFi HotStart ReadyMix	Roche	KK2602
Agencourt AMPure XP beads	Beckman Coulter	A63880
Nextera XT DNA Library Prep Kit	Illumina	FC-131-1024

Chemical Solutions to Prepare

- PBS-BSA (1X PBS, 0.05% BSA)
- 20% Surfactant in HFE-7500
- 10% Ammonium persulfate
- 6X SSC
- TE-SDS (10 mM Tris pH 8.0, 1 mM EDTA, 0.5% SDS)
- STOP-25 (10 mM Tris pH 8.0, 25 mM EDTA, 0.1% Tween-20, 0.1 M KCl)
- STOP-10 (10 mM Tris pH 8.0, 10 mM EDTA, 0.1% Tween-20, 0.1 M KCl)
- STOP-1 (10 mM Tris pH 8.0, 1 mM EDTA, 0.1% Tween-20, 0.1 M KCl)
- 1X Exonuclease Reaction Buffer
- HBW (10 mM Tris pH 8.0, 1 mM EDTA, 0.1% Tween-20)

scRNA BAG-seq Experiment

Cell Solution Preparation

1. Warm trypsin, 1X PBS and tissue culture media in a 37°C water bath.
2. Prepare PBS-BSA solution on ice.
3. Retrieve adherent cells from incubator and discard tissue culture media from plates.
4. Add 8 mL 1X PBS to each plate. Tilt plates back and forth to wash adherent cells. Then, discard PBS.
5. Trypsinize cells for 5 minutes with 1 mL warm Trypsin-EDTA. Then, add 6 mL tissue culture media to plate and pipet up and down multiple times. Collect solution in a 15 mL conical tube.
6. Centrifuge cells 300 rcf for 5 minutes. Discard supernatant.
7. Resuspend pellet with 8 mL 1X PBS. Centrifuge cells 300 rcf for 5 minutes. Discard supernatant.
8. Resuspend pellet with 1.5 mL PBS-BSA and filter cell solution through a 40 µm strainer.
9. Count cells.
10. Prepare a 1 mL solution of cells with a concentration of 2.5E5 cells/mL.

Oil Phase Preparation

1. Combine the following; vortex to mix (*enough for 2 samples*):

600 µL	20% surfactant
9.6 µL	TEMED
1.8 mL	HFE7500

Aqueous Phase 2 Preparation

1. Combine the following; pipet to mix (*enough for 2 samples*):

Aqueous Phase 2 Solution	Volume (µL)	Final Concentration
AA/bis-acrylamide, 40%	180	
Acrylamide solution, 40%	129	
H_2O	191	
500 µM LinkerA2 primer	80	20 µM
0.5 M EDTA	50	12.5 mM
1 M Tris pH 7.5	70	35 mM
20 % Sarkosyl	10	0.1%
SUPERase In RNase Inhibitor	100	1 U/µL
0.1 M DTT	30	1.5 mM
10% NP-40	100	0.5%
10% Ammonium persulfate (APS)	60	0.3%
Total	**1 mL**	

Microfluidics Setup & Oil Droplet Collection

1. Turn on microscope. Place microfluidic device on microscope stage.
2. Insert a piece of PE/2 tubing into the output well and place the other end into a waste container (15 mL conical tube).
3. Use the following syringes to collect the three solutions.

a. **Oil Phase:** Use a 10 mL syringe with a 16 G needle to aspirate solution into the syringe.
b. **Cell Solution or Aqueous Phase 2**: Use a 3 mL syringe and a 22 G needle to aspirate solution into the syringe.

4. For each syringe, change the needle to a 26 G needle and insert into PE/2 tubing.
5. Place syringes into their designated syringe pumps and insert tubing into the corresponding wells of the microfluidics chamber
    a. From left to right: Oil – Aqueous 2 – Cell Solution (Aqueous 1) – Output
6. Once all syringes and tubing are in place, set the following flow rates:
    a. **Oil Phase:** 3,000 µL/hr
    b. **Cell Solution:** 750 µL/hr
    c. **Aqueous Phase 2:** 750 µL/hr
7. Begin by first turning on the cell solution pump.
8. Once cell solution is flowing, turn on the aqueous phase 2 pump.
9. Once the interphase between cell solution and aqueous phase two has stabilized, turn on the oil phase pump and wait for the droplet formation to stabilize.
10. Once the flow has stabilized, place the output tubing on a glass slide under the microscope to assess the size uniformity of oil droplets.
11. Once droplet size is uniform, collect droplets in 300 µL of mineral oil in 1.5 mL tubes.
12. After collection, incubate BAGs at room temperature for 2.5 hours.
13. Incubate BAGs at 50°C for 5 minutes, then incubate at room temperature for 10 minutes.

Droplet Breakage

1. Remove mineral oil in the top layer using pipet. Remove oil in the bottom layer using a 3 mL syringe and 22G needle.
2. Add 600 µL 6X SSC buffer and 150 µL perfluorooctanol (PFO) in a fume hood. Shake the tube by hand to break the droplets and release balls of acrylamide gel (BAGs).
3. Centrifuge 1000 rcf for 1.5 minutes.
4. Discard the top and bottom layer, leaving the translucent BAG layer in the tube.
5. Add 600 µL 6x SSC and shake tube multiple times. Centrifuge 1000 rcf for 1.5 minutes. Discard the top layer and any visible PFO from the bottom of the tube.

Reverse Transcription / First Split-Pool

1. Wash the BAG layer from previous step with the following RT wash solution.
    a. **RT wash solution**: 850 µL 5x Reverse Transcriptase Buffer, 25 µL 0.1M DTT, and 25 µL SUPERase In RNase inhibitor
    b. Pipet up and down, centrifuge 1000 rcf for 1.5 minutes, discard the supernatant.
2. Prepare the following BAG solution; pipet up and down to mix:

BAG Solution	**Volume (µL)**
5x RT Buffer	250
SUPERase In RNase Inhibitor	15
0.1 M DTT	15
BAGs	200
Total	**480**

    a. Transfer 60 µL BAG solution to each tube of 8-well PCR strip.
    b. Quickly transfer 5 µL into each tube of a 96-well plate using a multipipette.
3. Prepare the following reverse transcription master mix in a 5 ml tube; vortex to mix:

Reverse Transcription Master Mix	Volume (µL)
H_2O	1048
10 mM dNTP	200
0.1 M DTT	100
SUPERase In RNase Inhibitor	52
Maxima H- Reverse Transcriptase	100
Total	**1.5 mL**

   a. Transfer 185 µL Reverse Transcription Master Mix into each well of an 8-well PCR strip.
   b. Transfer 13.5 µL into each well of the 96-well plate.
4. Add 2 µL of well-specific 50 µM Template-Switch Oligos ("Adapter1-BC1-vt-#RNAx") into each corresponding well. Each well should have a unique well-specific primer.
5. Incubate with rotation at room temperature for 30 minutes.
6. Incubate with rotation at 42°C for 1 hour, followed by 50°C for 1 hour.
7. Stop the reaction with the following steps:
   a. Add 100 µL TE-SDS to each reaction tube of the plate. Centrifuge plates 1000 rcf for 2 minutes at room temperature.
   b. Without disturbing the BAG pellet, discard 85 µL from **the top** of each tube.
   c. Add 5 ml TE-SDS into solution basin. Transfer the remaining BAG solutions from the plate into the solution basin (~35 µL per tube).
   d. Transfer BAG solution from solution basin into a 15 mL conical tube. Centrifuge 300 rcf for 4 minutes. Discard supernatant.
   e. Add 800 µL STOP-10, pipet up and down, and transfer BAG solution into a 1.5 mL tube. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant.
   f. Continue to exonuclease treatment or re-suspend in 800 µL STOP-10 and store at 4°C.

----------Safe Stopping Point: Store BAGs in STOP-10 at 4°C overnight----------

Exonuclease Treatment

1. If stored in STOP-10, centrifuge 1000 rcf for 1.5 minutes and discard supernatant.
2. Resuspend in STOP-1 for a wash. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant.
3. Resuspend in 1X Exonuclease I Reaction Buffer for a wash. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant.
4. Prepare the following exonuclease treatment solution; vortex to mix:

Exonuclease Treatment Solution	Volume (µL)
H_2O	665
10X Exonuclease I Reaction buffer	80
Exonuclease I enzyme	40
Ribonuclease H	15
Total	**800 µL**

5. Transfer 800 µL Exonuclease Treatment Solution to the tube of BAGs; pipet to mix.
6. Incubate tubes with rotation at 37°C for 1 hour.
7. Centrifuge 1000 rcf for 1 minute. Discard supernatant.
8. Stop the reaction with the following washes:

a. Resuspend in 800 µL STOP-25. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant. Repeat for a total of two STOP-25 washes.
b. Use the above wash method for one STOP-10 wash.

----------Safe Stopping Point: Store BAGs in STOP-10 at 4°C overnight----------

Second Split-Pool

0. Note: If one desires to do 2 cycles of split-pool in total, skip this section and go ahead to the next section "Third Split-Pool" and use primers "Adapter3-BC2a-Adapter1-#RNAx"
1. Preparation:
    a. If stored in STOP-10 overnight, centrifuge 1000 rcf for 1.5 minutes. Discard supernatant.
    b. Resuspend in 800 µL HBW for a wash. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant. Resuspend BAGs in 200 µL HBW.
2. Denaturation
    a. Prepare fresh Denaturation Solution: (Adapted from (Zilionis et al. 2017))
        i. 9.7 mL H_2O + 150 µL 10 M NaOH + 150 µL 30% (wt/wt) Brij-35
    b. Add 1 mL Denaturation Solution to BAGs. Pipet up and down 5 times. Incubate 10 minutes at room temperature on rotator. Centrifuge 400 rcf for 2 minutes at room temperature. Discard supernatant.
    c. Wash BAGs twice with Denaturation Solution
        i. Add 1 mL Denaturation Solution to BAGs. Pipet up and down 5 times. Incubate 3 minutes at room temperature on rotator. Centrifuge 400 rcf for 2 minutes at room temperature. Discard supernatant.
3. Neutralization
    a. Prepare Neutralization Solution
        i. 7.7 mL H_2O + 1 mL 1 M Tris (pH 8.0) + 1 mL 1 M NaCl + 200 µL 0.5 M EDTA + 100 µL 10% (vol/vol) Tween-20
    b. Wash BAGs twice with Neutralization Buffer
        i. Add 1 mL Neutralization Solution to BAGs. Pipet up and down 5 times. Incubate 3 minutes at room temperature on rotator. Centrifuge 400 rcf for 2 minutes at room temperature. Discard supernatant.
    c. Wash BAGs once with HBW
        i. Add 1 mL HBW to BAGs. Pipet up and down 5 times. Centrifuge 1000 rcf for 2 minutes. Discard supernatant.
    d. Re-suspend BAGs in HBW (400 µL total)
4. Preset thermocycler to 75°C and incubator to 57°C.
5. Prepare the following Isothermal Amplification Buffer (IAB) Mix; vortex to mix:

IAB Mix	**Volume (µL)** for one 96-well plate	*Volume (µL) per well*
H_2O	1150.4	*~12*
10x IABuffer	201.6	*2.1*
10 mM dNTP	144	*1.5*
BAGs in HBW	400	*~4.2*
Total:	**1896 µL**	*19.75 µL*

    a. Transfer 237 µL IAB mix to each tube of 8-well PCR strip.

b. Quickly transfer 19.75 µL into each tube of a 96-well plate using a multipipette.

6. Add 1.25 µL of the 96 different 100 µM “Adapter2-BC2-Adapter1-#RNAx” primers into each corresponding well. Each well should have a unique primer.
7. Invert plate for 2 minutes. Then incubate the plate at 75°C for 5 minutes.
8. Incubate plate at 57°C with rotation for 20 minutes.
9. Prepare the following BST Master Mix on ice; pipet to mix:

BST Master Mix	**Volume (µL)** for one 96-well plate	*Volume (µL) per well*
H_2O	310	*3.1*
10x IABuffer	40	*0.4*
Bst2.0	50	*0.5*
Total:	**400 µL**	*4 µL*

a. Transfer 50 µL Bst Mix to each tube of 8-well PCR strip.
b. Transfer 4 µL into each tube of a 96-well plate using a multipipette. Pipet to mix.

10. Incubate at 57°C with rotation for 1 hr.
11. Stop the reaction with the following washes:
    a. Add 100 µL STOP-25 to each well of the plate. Centrifuge plates 1000 rcf for 2 minutes at room temperature.
    b. Without disturbing the BAG pellet, discard 85 µL from the top of each tube.
    a. Add 5 mL STOP-25 into solution basin. Transfer the remaining BAG solutions from the plate into the solution basin (40 µL per tube).
    b. Transfer BAG solution from solution basin into a 15 mL conical. Centrifuge 300 rcf for 4 minutes. Discard supernatant.
    c. Add 800 µL STOP-10, pipet up and down, and transfer BAG solution into a 1.5 mL tube. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant.
12. Continue to third split-pool or resuspend BAGs in 800 µL STOP-10 and store at 4°C.

----------Safe Stopping Point: Store BAGs in STOP-10 at 4°C overnight----------

Third Split-Pool

1. If stored in STOP-10 overnight, centrifuge 1000 rcf for 1.5 minutes. Discard supernatant.
2. Resuspend in 800 µL HBW for a wash. Centrifuge 1000 rcf for 1.5 minutes. Discard supernatant. Resuspend BAGs in 500 µL HBW.
3. Use a microscope to count the number of BAGs per µL.
4. Prepare the following BAG mix; pipet to mix:

BAG Mix	**Volume (µL)**
BAGs in HBW	x
H_2O	720 - x
Total	720 µL

a. Transfer 90 µL BAG mix to each tube of 8-well PCR strip.
b. Quickly transfer 7.5 µL into each tube of a 96-well plate using a multipipette.

5. Add 1.5 µL of 96 different 10 µM “Adapter3-BC3-Adapter2-#RNAx” primers into each corresponding well. Each well should have a unique primer.
6. On ice, add 10 µL KAPA HiFi HotStart ReadyMix to each tube of 96-well plate. Pipet to mix.
7. Place on thermal cycler for the following program:

RNA: Linear Extension
95°C 20 s
8 cycles of:
98°C 30 s
60°C 1 min
72°C 3 min
Then:
72°C 3 min
4°C infinite

8. On ice, add 1 µL of 10 µM "IS-short" primer. Use another set of tips to pipet to mix.
9. Place on thermal cycler for the following program:

RNA: Exponential amplification
95°C 2 minutes
4 cycles of:
98°C 20 s
60°C 1 min
72°C 3 min
6 cycles of:
98°C 20 s
62°C 20 s
72°C 3 min
Then:
72°C 5 min
4°C infinite

10. Purify sample twice with AMPure XP beads using the following guidelines:
    a. First Pool: Pool 10 ul from each of the eight tubes in each column into a new PCR tube.

(ex. 1A-1H) into a new PCR tube, as shown.

1A	2	3	4	5	6	7	8	9	10	11	12
B											
C											
D											
E											
F											
G											
H											

1	2	3	4	5	6	7	8	9	10	11	12

(After pool: 80 µL each)

    b. First Purification: Purify these 12 samples with 0.7x ratio (56 µL) of AMPure XP beads, and elute with 15 µL of H_2O using AMPure XP protocol.
    c. Second Pool

Pool 14 ul from six tubes into one tube.

1	2	3	4	5	6	7	8	9	10	11	12

1	2

(After pool: 84 µL each)

d. Second Purification: Purify these two samples with another 0.7x ratio (59 µL) of AMPure XP beads, and elute with 15 µL H_2O using AMPure XP protocol.

11. Run a High Sensitivity DNA Chip on an Agilent Bioanalyzer.

Tagmentation of cDNA and Making Final Sequencing Library

1. Using Bioanalyzer data, combine 1.5 ng of cDNA with H_2O in a total volume of 5 µL. Add 10 µL of Nextera TD buffer
2. On ice, add 5 µL of Amplicon Tagment enzyme. Pipet to mix.
3. Incubate sample at 55°C for 4 minutes.
4. Quickly add 5 µL of Neutralization Buffer. Pipet to mix.
5. Incubate at room temperature for 5 minutes
6. On ice, combine the following; pipet to mix:

Final PCR Reaction Mix	**Volume (in µL)**
H_2O	8
10 µM Nextera N70x oligo	1
10 µM "P5-SAM" primer	1
Nextera PCR mix	15
Total	**25 µL**

12. Place on thermal cycler for the following program:

Final PCR gel
95°C 30s
12 cycles of:
95°C 10 s
55°C 30 s
72°C 30 s
Then:
72°C 5 min
4°C infinite

13. Purify sample with 0.8x ratio (40 µL) of Ampure XP Beads, and elute with 15 µL H_2O using AMPure XP protocol.
14. Run a High Sensitivity DNA Chip on an Agilent Bioanalyzer to check final concentration of sample.
15. The library should have an average bp size of 400-500bp.
16. Sequence your sample using Illumina platform with custom Read1 sequencing primer "Read1-SAM".

Barcode Sequences

First Split-Pool Barcodes

Oligo name	Sequence
Adapter1-BC1-vt-#RNA1	AGTGGAAAAGGAAGGTGGT TATTAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA2	AGTGGAAAAGGAAGGTGGT CTATGG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA3	AGTGGAAAAGGAAGGTGGT AAAACG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA4	AGTGGAAAAGGAAGGTGGT CAAACA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA5	AGTGGAAAAGGAAGGTGGT TATCGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA6	AGTGGAAAAGGAAGGTGGT ACTAAG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA7	AGTGGAAAAGGAAGGTGGT GCATCT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA8	AGTGGAAAAGGAAGGTGGT AGTTTG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA9	AGTGGAAAAGGAAGGTGGT CGATTT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA10	AGTGGAAAAGGAAGGTGGT ATCAGC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA11	AGTGGAAAAGGAAGGTGGT AACAAG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA12	AGTGGAAAAGGAAGGTGGT GAAGTT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA13	AGTGGAAAAGGAAGGTGGT GGTCTA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA14	AGTGGAAAAGGAAGGTGGT GACTAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA15	AGTGGAAAAGGAAGGTGGT GTATCA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA16	AGTGGAAAAGGAAGGTGGT TGCATA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA17	AGTGGAAAAGGAAGGTGGT TAATCA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA18	AGTGGAAAAGGAAGGTGGT TGGTAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA19	AGTGGAAAAGGAAGGTGGT ATGTGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA20	AGTGGAAAAGGAAGGTGGT TACTGG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA21	AGTGGAAAAGGAAGGTGGT AGCTGA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA22	AGTGGAAAAGGAAGGTGGT TAAATG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA23	AGTGGAAAAGGAAGGTGGT CTGAAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA24	AGTGGAAAAGGAAGGTGGT TAATGC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA25	AGTGGAAAAGGAAGGTGGT TAACTA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA26	AGTGGAAAAGGAAGGTGGT CGAAAA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA27	AGTGGAAAAGGAAGGTGGT ACGAAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA28	AGTGGAAAAGGAAGGTGGT ATGACG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA29	AGTGGAAAAGGAAGGTGGT AGGAGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA30	AGTGGAAAAGGAAGGTGGT CTAGAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA31	AGTGGAAAAGGAAGGTGGT TAGATT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA32	AGTGGAAAAGGAAGGTGGT TATGTC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA33	AGTGGAAAAGGAAGGTGGT CGGATA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA34	AGTGGAAAAGGAAGGTGGT ACGTTT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA35	AGTGGAAAAGGAAGGTGGT ACTTGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA36	AGTGGAAAAGGAAGGTGGT GAGTAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA37	AGTGGAAAAGGAAGGTGGT ATCTAA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA38	AGTGGAAAAGGAAGGTGGT GTTTGC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA39	AGTGGAAAAGGAAGGTGGT AGGATG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA40	AGTGGAAAAGGAAGGTGGT CTAGCA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA41	AGTGGAAAAGGAAGGTGGT AAACGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA42	AGTGGAAAAGGAAGGTGGT GGTCAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA43	AGTGGAAAAGGAAGGTGGT TCAAAG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA44	AGTGGAAAAGGAAGGTGGT AAAGCA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA45	AGTGGAAAAGGAAGGTGGT AGTGAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA46	AGTGGAAAAGGAAGGTGGT GTAGTC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA47	AGTGGAAAAGGAAGGTGGT CATGAG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA48	AGTGGAAAAGGAAGGTGGT GACTTG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA49	AGTGGAAAAGGAAGGTGGT ACAATC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA50	AGTGGAAAAGGAAGGTGGT ATTATG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA51	AGTGGAAAAGGAAGGTGGT GGATTG NNNNNN rGrGrG

Adapter1-BC1-vt-#RNA52	AGTGGAAAAGGAAGGTGGT TAGGCA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA53	AGTGGAAAAGGAAGGTGGT TACGTG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA54	AGTGGAAAAGGAAGGTGGT ATAGGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA55	AGTGGAAAAGGAAGGTGGT TTAGAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA56	AGTGGAAAAGGAAGGTGGT TCGGAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA57	AGTGGAAAAGGAAGGTGGT TCTAGC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA58	AGTGGAAAAGGAAGGTGGT TCTGAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA59	AGTGGAAAAGGAAGGTGGT GGAAAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA60	AGTGGAAAAGGAAGGTGGT GAGGAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA61	AGTGGAAAAGGAAGGTGGT CAATTC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA62	AGTGGAAAAGGAAGGTGGT TCGTTA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA63	AGTGGAAAAGGAAGGTGGT AGGACA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA64	AGTGGAAAAGGAAGGTGGT TACAGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA65	AGTGGAAAAGGAAGGTGGT TTACGG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA66	AGTGGAAAAGGAAGGTGGT TACGGA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA67	AGTGGAAAAGGAAGGTGGT TATACT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA68	AGTGGAAAAGGAAGGTGGT GTAAGG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA69	AGTGGAAAAGGAAGGTGGT AGTAGA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA70	AGTGGAAAAGGAAGGTGGT TTCGTA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA71	AGTGGAAAAGGAAGGTGGT AATAGG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA72	AGTGGAAAAGGAAGGTGGT CTAGTT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA73	AGTGGAAAAGGAAGGTGGT AAGTCG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA74	AGTGGAAAAGGAAGGTGGT CTAAGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA75	AGTGGAAAAGGAAGGTGGT CAGAGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA76	AGTGGAAAAGGAAGGTGGT TAGGGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA77	AGTGGAAAAGGAAGGTGGT GAACAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA78	AGTGGAAAAGGAAGGTGGT CTTGGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA79	AGTGGAAAAGGAAGGTGGT ATTCGT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA80	AGTGGAAAAGGAAGGTGGT AGGTTC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA81	AGTGGAAAAGGAAGGTGGT GAATGG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA82	AGTGGAAAAGGAAGGTGGT CAAAAG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA83	AGTGGAAAAGGAAGGTGGT AATGCG NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA84	AGTGGAAAAGGAAGGTGGT TTCAAT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA85	AGTGGAAAAGGAAGGTGGT GTTAAA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA86	AGTGGAAAAGGAAGGTGGT TAGTAA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA87	AGTGGAAAAGGAAGGTGGT ATTGTT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA88	AGTGGAAAAGGAAGGTGGT TCATTC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA89	AGTGGAAAAGGAAGGTGGT ATATAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA90	AGTGGAAAAGGAAGGTGGT CAACGA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA91	AGTGGAAAAGGAAGGTGGT AATTCT NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA92	AGTGGAAAAGGAAGGTGGT AAGAAC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA93	AGTGGAAAAGGAAGGTGGT TGTTGC NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA94	AGTGGAAAAGGAAGGTGGT AACAGA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA95	AGTGGAAAAGGAAGGTGGT GGAATA NNNNNN rGrGrG
Adapter1-BC1-vt-#RNA96	AGTGGAAAAGGAAGGTGGT ATATTA NNNNNN rGrGrG

Second Split-Pool Barcodes

Oligo name	Sequence
Adapter2-BC2-Adapter1-#RNA1	GACCGACTCGCATTACCCTATTCACCAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA2	GACCGACTCGCATTACCCTATCCAGTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA3	GACCGACTCGCATTACCCTATTCCTAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA4	GACCGACTCGCATTACCCTATCCATTCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA5	GACCGACTCGCATTACCCTATTCAACCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA6	GACCGACTCGCATTACCCTATCTGCAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA7	GACCGACTCGCATTACCCTATGAAATAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA8	GACCGACTCGCATTACCCTATAGGTATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA9	GACCGACTCGCATTACCCTATACTCTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA10	GACCGACTCGCATTACCCTATTCCCATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA11	GACCGACTCGCATTACCCTATGGTTTTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA12	GACCGACTCGCATTACCCTATTCTGTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA13	GACCGACTCGCATTACCCTATAGTCACAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA14	GACCGACTCGCATTACCCTATAACACAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA15	GACCGACTCGCATTACCCTATTGTATTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA16	GACCGACTCGCATTACCCTATTTACAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA17	GACCGACTCGCATTACCCTATATATTCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA18	GACCGACTCGCATTACCCTATGCCTATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA19	GACCGACTCGCATTACCCTATAGCGAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA20	GACCGACTCGCATTACCCTATAAACCTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA21	GACCGACTCGCATTACCCTATAACAACAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA22	GACCGACTCGCATTACCCTATCTTATTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA23	GACCGACTCGCATTACCCTATGACTGAAGTGGAAAAGGAAGGTGG/3InvdT/
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Adapter2-BC2-Adapter1-#RNA25	GACCGACTCGCATTACCCTATTTTCTCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA26	GACCGACTCGCATTACCCTATGAGAATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA27	GACCGACTCGCATTACCCTATATGTAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA28	GACCGACTCGCATTACCCTATATTTATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA29	GACCGACTCGCATTACCCTATGCGATAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA30	GACCGACTCGCATTACCCTATATGAACAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA31	GACCGACTCGCATTACCCTATAATGTCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA32	GACCGACTCGCATTACCCTATTCGCTTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA33	GACCGACTCGCATTACCCTATAACGGTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA34	GACCGACTCGCATTACCCTATATAGACAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA35	GACCGACTCGCATTACCCTATAATACTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA36	GACCGACTCGCATTACCCTATGAACCAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA37	GACCGACTCGCATTACCCTATGGACATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA38	GACCGACTCGCATTACCCTATGCAAATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA39	GACCGACTCGCATTACCCTATATTTGCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA40	GACCGACTCGCATTACCCTATCGCATTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA41	GACCGACTCGCATTACCCTATAGCTCAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA42	GACCGACTCGCATTACCCTATTAAGCTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA43	GACCGACTCGCATTACCCTATCGTACAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA44	GACCGACTCGCATTACCCTATCTGAATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA45	GACCGACTCGCATTACCCTATCCCAATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA46	GACCGACTCGCATTACCCTATCCTCAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA47	GACCGACTCGCATTACCCTATGCAATCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA48	GACCGACTCGCATTACCCTATGAATCCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA49	GACCGACTCGCATTACCCTATAAGCCAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA50	GACCGACTCGCATTACCCTATGCATTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA51	GACCGACTCGCATTACCCTATGAACTTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA52	GACCGACTCGCATTACCCTATACTTACAGTGGAAAAGGAAGGTGG/3InvdT/

Adapter2-BC2-Adapter1-#RNA53	GACCGACTCGCATTACCCTATAAGTGAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA54	GACCGACTCGCATTACCCTATAAACTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA55	GACCGACTCGCATTACCCTATGAGTTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA56	GACCGACTCGCATTACCCTATAATCAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA57	GACCGACTCGCATTACCCTATCTGTGAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA58	GACCGACTCGCATTACCCTATAAAGTTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA59	GACCGACTCGCATTACCCTATAACTAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA60	GACCGACTCGCATTACCCTATTTACCTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA61	GACCGACTCGCATTACCCTATGGATTCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA62	GACCGACTCGCATTACCCTATAGAATTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA63	GACCGACTCGCATTACCCTATTTTGGTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA64	GACCGACTCGCATTACCCTATAACTCCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA65	GACCGACTCGCATTACCCTATAACCATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA66	GACCGACTCGCATTACCCTATATAGTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA67	GACCGACTCGCATTACCCTATAAGTTCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA68	GACCGACTCGCATTACCCTATGAATAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA69	GACCGACTCGCATTACCCTATAATCCCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA70	GACCGACTCGCATTACCCTATCCATGAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA71	GACCGACTCGCATTACCCTATACAATAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA72	GACCGACTCGCATTACCCTATTCAGACAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA73	GACCGACTCGCATTACCCTATATTTTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA74	GACCGACTCGCATTACCCTATGACTTTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA75	GACCGACTCGCATTACCCTATATGTTTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA76	GACCGACTCGCATTACCCTATAGACAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA77	GACCGACTCGCATTACCCTATAAACGCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA78	GACCGACTCGCATTACCCTATATTGAAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA79	GACCGACTCGCATTACCCTATTTACGCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA80	GACCGACTCGCATTACCCTATCCTACTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA81	GACCGACTCGCATTACCCTATGCAGTTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA82	GACCGACTCGCATTACCCTATCTTTCAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA83	GACCGACTCGCATTACCCTATCGTTACAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA84	GACCGACTCGCATTACCCTATAGTTTCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA85	GACCGACTCGCATTACCCTATAGAAACAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA86	GACCGACTCGCATTACCCTATAAATATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA87	GACCGACTCGCATTACCCTATTATCGCAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA88	GACCGACTCGCATTACCCTATCGATTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA89	GACCGACTCGCATTACCCTATAGGACTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA90	GACCGACTCGCATTACCCTATCCAAGTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA91	GACCGACTCGCATTACCCTATATGGGTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA92	GACCGACTCGCATTACCCTATTTTAATAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA93	GACCGACTCGCATTACCCTATAACGTAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA94	GACCGACTCGCATTACCCTATTCAGGTAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA95	GACCGACTCGCATTACCCTATAATTCAAGTGGAAAAGGAAGGTGG/3InvdT/
Adapter2-BC2-Adapter1-#RNA96	GACCGACTCGCATTACCCTATACATGCAGTGGAAAAGGAAGGTGG/3InvdT/

Third Split-Pool Barcodes

Oligo name	Sequence
Adapter3-BC3-Adapter2-#RNA1	AAGGCTCTTGACACGAAGGATAG TACCGTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA2	AAGGCTCTTGACACGAAGGATAG CCATTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA3	AAGGCTCTTGACACGAAGGATAG TCTTTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA4	AAGGCTCTTGACACGAAGGATAG AAACATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA5	AAGGCTCTTGACACGAAGGATAG GTTTGGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA6	AAGGCTCTTGACACGAAGGATAG CAAACTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA7	AAGGCTCTTGACACGAAGGATAG ACCTATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA8	AAGGCTCTTGACACGAAGGATAG TCACTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA9	AAGGCTCTTGACACGAAGGATAG AGTTGTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA10	AAGGCTCTTGACACGAAGGATAG CTTGGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA11	AAGGCTCTTGACACGAAGGATAG CTGGATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA12	AAGGCTCTTGACACGAAGGATAG TTCACGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA13	AAGGCTCTTGACACGAAGGATAG CTCTATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA14	AAGGCTCTTGACACGAAGGATAG AGAATGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA15	AAGGCTCTTGACACGAAGGATAG CATCCANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA16	AAGGCTCTTGACACGAAGGATAG GTTTCTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA17	AAGGCTCTTGACACGAAGGATAG TCAGATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA18	AAGGCTCTTGACACGAAGGATAG CAATGGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA19	AAGGCTCTTGACACGAAGGATAG TGCTCANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA20	AAGGCTCTTGACACGAAGGATAG TCTGGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA21	AAGGCTCTTGACACGAAGGATAG CTTTTGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA22	AAGGCTCTTGACACGAAGGATAG ACTCAGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA23	AAGGCTCTTGACACGAAGGATAG AACTAGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA24	AAGGCTCTTGACACGAAGGATAG CCCTTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA25	AAGGCTCTTGACACGAAGGATAG CTGATGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA26	AAGGCTCTTGACACGAAGGATAG CATCAGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA27	AAGGCTCTTGACACGAAGGATAG AACCTGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA28	AAGGCTCTTGACACGAAGGATAG CAAATGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA29	AAGGCTCTTGACACGAAGGATAG TCCCTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA30	AAGGCTCTTGACACGAAGGATAG TTTATGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA31	AAGGCTCTTGACACGAAGGATAG CTAAGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA32	AAGGCTCTTGACACGAAGGATAG AATACANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA33	AAGGCTCTTGACACGAAGGATAG CTAACGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA34	AAGGCTCTTGACACGAAGGATAG TTCTTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA35	AAGGCTCTTGACACGAAGGATAG GATGGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA36	AAGGCTCTTGACACGAAGGATAG GGGATTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA37	AAGGCTCTTGACACGAAGGATAG GATATTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA38	AAGGCTCTTGACACGAAGGATAG AACTTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA39	AAGGCTCTTGACACGAAGGATAG GCAACANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA40	AAGGCTCTTGACACGAAGGATAG AGAGAGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA41	AAGGCTCTTGACACGAAGGATAG TTTGGGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA42	AAGGCTCTTGACACGAAGGATAG GAGAGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA43	AAGGCTCTTGACACGAAGGATAG TCCTCTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA44	AAGGCTCTTGACACGAAGGATAG CCTCTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA45	AAGGCTCTTGACACGAAGGATAG CTTAGGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA46	AAGGCTCTTGACACGAAGGATAG TTTTATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA47	AAGGCTCTTGACACGAAGGATAG TAGCTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA48	AAGGCTCTTGACACGAAGGATAG TCAACTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA49	AAGGCTCTTGACACGAAGGATAG AGTATTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA50	AAGGCTCTTGACACGAAGGATAG TGCTATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA51	AAGGCTCTTGACACGAAGGATAG GTTCCANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA52	AAGGCTCTTGACACGAAGGATAG TGAACGNNGACCGACTCGCATTACCCT*A*T

Adapter3-BC3-Adapter2-#RNA53	AAGGCTCTTGACACGAAGGATAG TGATGGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA54	AAGGCTCTTGACACGAAGGATAG GTATTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA55	AAGGCTCTTGACACGAAGGATAG GTACAGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA56	AAGGCTCTTGACACGAAGGATAG AAACCGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA57	AAGGCTCTTGACACGAAGGATAG CATTTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA58	AAGGCTCTTGACACGAAGGATAG AGGGATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA59	AAGGCTCTTGACACGAAGGATAG AAATCANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA60	AAGGCTCTTGACACGAAGGATAG GGAACTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA61	AAGGCTCTTGACACGAAGGATAG CTCATANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA62	AAGGCTCTTGACACGAAGGATAG TAATCGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA63	AAGGCTCTTGACACGAAGGATAG TAGTTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA64	AAGGCTCTTGACACGAAGGATAG AATATGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA65	AAGGCTCTTGACACGAAGGATAG TAGTCTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA66	AAGGCTCTTGACACGAAGGATAG CATTCTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA67	AAGGCTCTTGACACGAAGGATAG TTATGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA68	AAGGCTCTTGACACGAAGGATAG CCTCATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA69	AAGGCTCTTGACACGAAGGATAG GACTTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA70	AAGGCTCTTGACACGAAGGATAG ACAACGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA71	AAGGCTCTTGACACGAAGGATAG GATTTGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA72	AAGGCTCTTGACACGAAGGATAG GACCTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA73	AAGGCTCTTGACACGAAGGATAG CGAATTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA74	AAGGCTCTTGACACGAAGGATAG TTATTGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA75	AAGGCTCTTGACACGAAGGATAG ACTTTGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA76	AAGGCTCTTGACACGAAGGATAG AACTGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA77	AAGGCTCTTGACACGAAGGATAG GCTACTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA78	AAGGCTCTTGACACGAAGGATAG AAATTGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA79	AAGGCTCTTGACACGAAGGATAG ACCTCANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA80	AAGGCTCTTGACACGAAGGATAG GGATGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA81	AAGGCTCTTGACACGAAGGATAG GACATGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA82	AAGGCTCTTGACACGAAGGATAG AATCTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA83	AAGGCTCTTGACACGAAGGATAG TAATTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA84	AAGGCTCTTGACACGAAGGATAG TCCTGANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA85	AAGGCTCTTGACACGAAGGATAG GTTCTTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA86	AAGGCTCTTGACACGAAGGATAG GCTTCANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA87	AAGGCTCTTGACACGAAGGATAG TTAGAGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA88	AAGGCTCTTGACACGAAGGATAG TAACTGNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA89	AAGGCTCTTGACACGAAGGATAG CTGTTANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA90	AAGGCTCTTGACACGAAGGATAG CACCATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA91	AAGGCTCTTGACACGAAGGATAG AAATGTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA92	AAGGCTCTTGACACGAAGGATAG CTCTCANNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA93	AAGGCTCTTGACACGAAGGATAG TTCCCTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA94	AAGGCTCTTGACACGAAGGATAG TTCGATNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA95	AAGGCTCTTGACACGAAGGATAG CTATGTNNGACCGACTCGCATTACCCT*A*T
Adapter3-BC3-Adapter2-#RNA96	AAGGCTCTTGACACGAAGGATAG TCATCANNGACCGACTCGCATTACCCT*A*T

Second Split-Pool Barcodes for 2 cycles Split-Pool

Oligo name	Sequence
Adapter3-BC2a-Adapter1-#RNA1	AAGGCTCTTGACACGAAGGATAG CACAGAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA2	AAGGCTCTTGACACGAAGGATAG TAGTAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA3	AAGGCTCTTGACACGAAGGATAG TACGCTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA4	AAGGCTCTTGACACGAAGGATAG TTTAGCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA5	AAGGCTCTTGACACGAAGGATAG GGAAGAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA6	AAGGCTCTTGACACGAAGGATAG TACCTGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA7	AAGGCTCTTGACACGAAGGATAG AAAGTCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA8	AAGGCTCTTGACACGAAGGATAG GGTACAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA9	AAGGCTCTTGACACGAAGGATAG AATGAGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA10	AAGGCTCTTGACACGAAGGATAG GGATTCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA11	AAGGCTCTTGACACGAAGGATAG TTAGCCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA12	AAGGCTCTTGACACGAAGGATAG TCATCGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA13	AAGGCTCTTGACACGAAGGATAG CAGGTTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA14	AAGGCTCTTGACACGAAGGATAG AACGGAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA15	AAGGCTCTTGACACGAAGGATAG ACTGTGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA16	AAGGCTCTTGACACGAAGGATAG CCAATAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA17	AAGGCTCTTGACACGAAGGATAG TTCAGGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA18	AAGGCTCTTGACACGAAGGATAG CTTAATAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA19	AAGGCTCTTGACACGAAGGATAG GTTCAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA20	AAGGCTCTTGACACGAAGGATAG TTTGTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA21	AAGGCTCTTGACACGAAGGATAG CTAAAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA22	AAGGCTCTTGACACGAAGGATAG TGGAATAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA23	AAGGCTCTTGACACGAAGGATAG GAAACAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA24	AAGGCTCTTGACACGAAGGATAG AGTTTTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA25	AAGGCTCTTGACACGAAGGATAG GCGAAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA26	AAGGCTCTTGACACGAAGGATAG TCTAAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA27	AAGGCTCTTGACACGAAGGATAG CGAGTTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA28	AAGGCTCTTGACACGAAGGATAG AAATGGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA29	AAGGCTCTTGACACGAAGGATAG CTAATGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA30	AAGGCTCTTGACACGAAGGATAG CTAAGTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA31	AAGGCTCTTGACACGAAGGATAG TTTGAGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA32	AAGGCTCTTGACACGAAGGATAG TCTGGAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA33	AAGGCTCTTGACACGAAGGATAG GGATAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA34	AAGGCTCTTGACACGAAGGATAG TAGGATAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA35	AAGGCTCTTGACACGAAGGATAG TAAGTGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA36	AAGGCTCTTGACACGAAGGATAG CTTCACAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA37	AAGGCTCTTGACACGAAGGATAG GTCATAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA38	AAGGCTCTTGACACGAAGGATAG AGGTGAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA39	AAGGCTCTTGACACGAAGGATAG ACGATCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA40	AAGGCTCTTGACACGAAGGATAG GAGTTTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA41	AAGGCTCTTGACACGAAGGATAG GTATTGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA42	AAGGCTCTTGACACGAAGGATAG GCAATCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA43	AAGGCTCTTGACACGAAGGATAG GATATTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA44	AAGGCTCTTGACACGAAGGATAG TTACAGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA45	AAGGCTCTTGACACGAAGGATAG TCGTTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA46	AAGGCTCTTGACACGAAGGATAG TAAGAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA47	AAGGCTCTTGACACGAAGGATAG TCCTAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA48	AAGGCTCTTGACACGAAGGATAG AAACTTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA49	AAGGCTCTTGACACGAAGGATAG CAGTGAAGTGGAAAAGGAAGGTG*G*T

Adapter3-BC2a-Adapter1-#RNA50	AAGGCTCTTGACACGAAGGATAG CCAGAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA51	AAGGCTCTTGACACGAAGGATAG ACAACAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA52	AAGGCTCTTGACACGAAGGATAG CAGGAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA53	AAGGCTCTTGACACGAAGGATAG TGAGTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA54	AAGGCTCTTGACACGAAGGATAG GATTTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA55	AAGGCTCTTGACACGAAGGATAG AGAGAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA56	AAGGCTCTTGACACGAAGGATAG CTGAACAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA57	AAGGCTCTTGACACGAAGGATAG AAGCATAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA58	AAGGCTCTTGACACGAAGGATAG TTGTGAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA59	AAGGCTCTTGACACGAAGGATAG AAACGAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA60	AAGGCTCTTGACACGAAGGATAG TCCCTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA61	AAGGCTCTTGACACGAAGGATAG GAATATAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA62	AAGGCTCTTGACACGAAGGATAG TTGTTCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA63	AAGGCTCTTGACACGAAGGATAG AACGTGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA64	AAGGCTCTTGACACGAAGGATAG CATGTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA65	AAGGCTCTTGACACGAAGGATAG CATAGCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA66	AAGGCTCTTGACACGAAGGATAG CAGTTCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA67	AAGGCTCTTGACACGAAGGATAG TAAAACAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA68	AAGGCTCTTGACACGAAGGATAG AGGATTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA69	AAGGCTCTTGACACGAAGGATAG GATGAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA70	AAGGCTCTTGACACGAAGGATAG CTGCAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA71	AAGGCTCTTGACACGAAGGATAG GAAGTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA72	AAGGCTCTTGACACGAAGGATAG TCAGGTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA73	AAGGCTCTTGACACGAAGGATAG GAGCAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA74	AAGGCTCTTGACACGAAGGATAG CTCACAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA75	AAGGCTCTTGACACGAAGGATAG ACGGTTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA76	AAGGCTCTTGACACGAAGGATAG CATAAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA77	AAGGCTCTTGACACGAAGGATAG AAGCTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA78	AAGGCTCTTGACACGAAGGATAG GAAGGTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA79	AAGGCTCTTGACACGAAGGATAG CATTTGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA80	AAGGCTCTTGACACGAAGGATAG CAATAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA81	AAGGCTCTTGACACGAAGGATAG TAATTCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA82	AAGGCTCTTGACACGAAGGATAG TCCATGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA83	AAGGCTCTTGACACGAAGGATAG TGCTATAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA84	AAGGCTCTTGACACGAAGGATAG AGGCAAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA85	AAGGCTCTTGACACGAAGGATAG AACAGGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA86	AAGGCTCTTGACACGAAGGATAG CAACATAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA87	AAGGCTCTTGACACGAAGGATAG TCTCTCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA88	AAGGCTCTTGACACGAAGGATAG AGTTCCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA89	AAGGCTCTTGACACGAAGGATAG TCCAACAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA90	AAGGCTCTTGACACGAAGGATAG AGGAACAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA91	AAGGCTCTTGACACGAAGGATAG GCTTTCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA92	AAGGCTCTTGACACGAAGGATAG AATGGCAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA93	AAGGCTCTTGACACGAAGGATAG ACACTAAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA94	AAGGCTCTTGACACGAAGGATAG TTGCTGAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA95	AAGGCTCTTGACACGAAGGATAG GGTTCTAGTGGAAAAGGAAGGTG*G*T
Adapter3-BC2a-Adapter1-#RNA96	AAGGCTCTTGACACGAAGGATAG CAATTTAGTGGAAAAGGAAGGTG*G*T