VERSION 1

JUL 28, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bmbik2ke

Protocol Citation: Sai Ma, Aviv Regev, Jason Buenrostro 2021. SHARE-seq V1. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bmbik2ke>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Sep 13, 2020

Last Modified: Jul 28, 2021

PROTOCOL integer ID:
 42058

SHARE-seq V1 V.1

Sai Ma^{1,2,3}, Aviv Regev^{1,3,4,5}, Jason Buenrostro^{1,3}

¹Broad Institute of MIT and Harvard; ²Harvard; ³MIT; ⁴HHMI; ⁵Genentech

Human Cell Atlas Method Development Community

SHAREseq

Sai Ma

ABSTRACT

SHARE-seq for joint profiling of chromatin accessibility and gene expression at scale.

ATTACHMENTS

[Table S1. Oligo design.xlsx](#)

GUIDELINES

1. This protocol can be used to multiplex multiple samples in one run. Keep the samples in separate tubes during fixation, transposition and RT, until loaded into Round 1 barcoding plate.
2. This protocol can be slightly modified for ATAC-only and RNA-only library preparation.

MATERIALS

Oligos (see attached Table)
 PBS (Thermo Fisher Scientific, 10010049)
 Enzymatic RNase Inhibitor (Qiagen Enzymatics, Y9240L)
 SUPERase In RNase Inhibitor (Thermo Fisher Scientific, AM2696)
 NxGen RNase Inhibitor (Lucigen, 30281-2)
 16% Formaldehyde (w/v) (Thermo Fisher Scientific, 28906)
 Glycine (Sigma-Aldrich, 50046)
 1M Tris HCl pH 7.5 (Thermo Fisher Scientific, 15567027)
 1M Tris HCl pH 8.0 (Thermo Fisher Scientific, 15568025)
 5M NaCl (Thermo Fisher Scientific, AM9760G)
 1M MgCl₂ (Sigma-Aldrich, 63069)
 10% NP40 (Thermo Fisher Scientific, 28324)
 Buffer EB (Qiagen, 19086)
 DNA Clean & Concentrator-5 (Zymo, D4014)
 PEG 6000 (Sigma-Aldrich, 528877-100GM)
 Maxima H Minus Reverse Transcriptase with buffer (Thermo Fisher Scientific,

EP0753)
 Deoxynucleotide (dNTP) Solution Mix (NEB, N0447L)
 T4 DNA ligase (NEB, M0202L)
 10× T4 ligase buffer New England Biolabs, B0202S)
 Proteinase K from Tritirachium album (20mg/ml in H₂O) (Sigma-Aldrich, P2308-100MG)
 Sodium Dodecyl Sulfate 20% (SDS) Solution (VWR, 97062-440)
 Phenylmethanesulfonyl fluoride (PMSF) (Sigma-Aldrich, P7626)
 2-Propanol (IPA) (Sigma-Aldrich, I9516)
 0.5M EDTA (Thermo Fisher Scientific, AM9260G)
 Tween 20 (Sigma-Aldrich, P9416-100ML)
 Ficoll PM-400 (20%) (Sigma-Aldrich, F5415-25ML)
 KAPA HiFi HotStart ReadyMix (Fisher Scientific, NC0295239)
 AMPure XP (Beckman Coulter, A63880)
 100% Ethanol (Sigma-Aldrich, 8.18760.2500)
 Qubit dsDNA HS Assay Kit, (Thermo Fisher Scientific, Q32854)
 Gel (E-gel/Flash gels)
 FlashGel DNA Cassettes (Lonza, 57031)
 Dithiothreitol (DTT), 0.1M Solution (Thermo Fisher Scientific, 707265ML)
 NEBNext High-Fidelity 2X PCR Master Mix (New England Biolabs, M0541L)
 Glycerol (Thermo Fisher Scientific, 15514011)
 Unloaded Tn5
 TD buffer from Nextera kit
 10,000× SYBRgreen (Thermo Fisher Scientific, S7563)
 TRIS-acetate buffer 0.2M, pH 7.8 (Bioworld, 40120265-2)
 Potassium acetate (Sigma-Aldrich, 95843-100ML-F)
 Magnesium acetate (Sigma-Aldrich, 63052-100ML)
 Dimethylformamide (DMF) (Thermo Fisher Scientific, 20673A)
 Protease Inhibitor Cocktail (Sigma-Aldrich, P8340)
 Quick-Load® Purple 100 bp DNA Ladder (NEB, N0551S)
 Gel Loading Dye, Purple (6X) (NEB, B7024S)

BEFORE START INSTRUCTIONS

1. By default, spin down cells at 500g for 3 min at RT using a swing bucket centrifuge. For primary samples, may use 1000g instead.
2. For each sample, start with 1 million-100k cells for fixation.
3. For primary samples, if cell recovery is low after spinning, pre-rinse tubes with 7.5% BSA before spinning.
4. Resuspend All oligos should be resuspended to 100µM or 1mM concentration in the IDTE buffer.
5. Add RNase inhibitor freshly.

Buffer preparation

1 Prepare following buffer and store at 4°C

Nuclei Isolation Buffer (NIB)	Volume (ml)	Final conc.
1M Tris HCl pH 7.5	0.5	10mM
5M NaCl	0.1	10mM
1M MgCl ₂	0.15	3mM
10% NP40	0.5	0.1%
H ₂ O	48.75	
Total	50	

2× BW	Volume (ml)	2× conc.
1M Tris 8.0	0.5	10mM
5M NaCl	20	2M
0.5M EDTA	0.1	1mM
H ₂ O	29.4	
Total	50	

1x B&W-T	Volume (ml)	1x conc.
1M Tris 8.0	0.25	5mM
5M NaCl	10	1M
0.5M EDTA	0.05	0.5mM
100% Tween 20	0.025	0.05%
H ₂ O	39.675	
Total	50	

Oligo resuspension buffer (IDTE)	Volume (ml)	1x conc.
1M Tris 8.0	0.5	10mM
0.5M EDTA	0.01	0.1mM
H ₂ O	49.5	

Total	50	
-------	----	--

Oligo annealing buffer (STE)	Volume (ml)	1× conc.
1M Tris 8.0	0.5	10mM
5M NaCl	0.5	50mM
0.5M EDTA	0.1	1mM
H ₂ O	48.9	
Total	50	

Dilution Buffer	Volume (μl)	Final conc.
100% glycerol (use wide bore tips)	500	50%
1M Tris pH 7.5	50	50mM
5M NaCl	20	100mM
5mM EDTA	20	0.1mM
100mM DTT	10	1mM
10% NP-40	10	0.1%
H ₂ O	390	
Total	1000	

2 Prepare following buffer and store at room temperature.

2× RCB	Volume (ml)	2× conc.
1M Tris 8.0	1	100mM
5M NaCl	0.2	100mM
20% SDS	0.2	0.40%
H ₂ O	8.58	
Total	10	

PEG 6000 50%: mix equal mass of PEG6000 and H₂O, dissolve PEG by heating to 65°C for 4min

and cooling down to RT.

Anneal oligo plates

- 3 Dilute 120µl Round 1 linker oligo (1mM) with 11880µl STE buffer. Mix 90µl diluted Round 1 linker oligo with 10µl Round 1 oligo (100µM) in a 96 well PCR plate.
- 4 Dilute 120µl Round 2 linker oligo (1mM) with 9480µl STE buffer. Mix 88µl diluted Round 2 linker oligo with 12µl Round 2 oligo (100µM) in a 96 well PCR plate.
- 5 Dilute 144µl Round 3 linker oligo (1mM) with 9360µl STE buffer. Mix 86µl diluted Round 3 linker oligo with 14µl Round 3 oligo (100µM) in a 96 well PCR plate.
- 6 Anneal Round 1, Round 2, and Round 3 plates.

	95°C	2 min
		slow ramp, - 1°C/c ycle, 1 min per cycle
	20°C	2 min
	4°C	Forev er
	Total	~1 h 26 min

- 7 After annealing, check if there is significant water evaporation for the wells at the corners. If there is, add water to compensate.
- 8 Aliquot 10µl of annealed oligo to a new plate. Should be enough for 9×3 plates. Store the plates at -20°C.

Anneal adapter oligo

- 9 Dilute Phosphorylated_Read2, Read1, Blocked_ME_Comp to 100µM with the IDTE buffer. Prepare transposition adapter and tagmentation adapter mix in PCR tubes.

Transposition adapter	Volume (µl)
100µM Phosphorylated_Read2	6.5
100µM Read1	6.5
100µM Blocked_ME_Comp	13
1M Tris pH 8.0	0.26
5M NaCl	0.26
Total	26.5

Tagmentation adapter	Volume (µl)
100µM Read1	13
100µM Blocked_ME_Comp	13
1M Tris pH 8.0	0.26
5M NaCl	0.26
Total	26.5

- 10 Anneal oligos in a thermal cycler.

85°C	2 min
	slow ramp, 1°C/cycle, 1 min per cycle
20°C	2 min
4°C	Forever

Total	~1 h 14 min
-------	-------------------

- 11** Aliquot 25µl Glycerol (pre-heated to 65°C) to a new tube and then equilibrated to RT. 25µl of annealed oligo is mixed with glycerol. The annealed adapters can be immediately used for and stored at -20°C.

Fixation

40m

- 12** Spin down cells, wash cells with 0.5ml PBS-2RI, count alive cells with Trypan blue, resuspend cells with cold PBS-2RI at concentration of 1 million cells/ml.

	PBS-2RI	Volume (µl)
	1× PBS	2000
	7.5% BSA	10.7
	Enzymatic RI	5
	SUPERase RI	2.5

- 13** For each ml of cells in PBS-2RI, add 66.7µl of 1.6% or 3.2% FA (final concentration 0.1-0.2% FA, 0.1% for cell line, 0.2% for tissue) at RT, mix. Keep at RT for 5 min.
- 14** For each ml of cells, quench fixation by adding 56.1µl of 2.5M Glycine, 50µl of 1M Tris pH 8.0, 13.3µl of 7.5% BSA, pipette mix. Keep on ice for 5 min.
- 15** Spin down, remove supernatant, add 0.5ml PBS-2RI without disturbing the cell pellet.
- 16** Spin down, remove supernatant, add 0.5ml PBS-2RI without disturbing the cell pellet.

17 Resuspend cells to 50µl PBS-2RI.

	NIB-RI	Volume (µl)
	NIB	8000
	Enzymatic RI	20
	SUPERase RI	20
	7.5% BSA	42.7

Note

If starting with nuclei, replace PBS-2RI in this section with NIB-RI

ATAC

50m

18 Assemble transposome by mixing Tn5, dilution buffer and annealed transposition adapter oligo. Incubate at RT for 30min. The assembled transposome could be stored at -20°C for up to 2 weeks.

19 Calculate the number of required ATAC reactions. No. samples (N)=no. cells/10,000. For example, for 100k cells, $N=100k/10k=10$. Prepare 2× TB and 1× TB accordingly. Critical: The 2× TB should be prepared freshly.

	2× TB buffer	Volume (µl)
	0.2M Tris-acetate	330
	5M K-acetate	26.4
	1M Mg-acetate	20
	10% NP-40	20
	100% DMF	320
	H2O	283.6
	Total	1000

1× TB	No. samples (N)
2× TB	25×N
H ₂ O	16.45×N
PIC	0.2×N
Enzymatic In	0.85×N
Total	42.5×N

PBS-RI	Volume (μl)
1× PBS	400
Enzymatic RI	1

20 Dilute cells with PBS-RI to 2 million/ml, aliquot 5×N μl of diluted sample to a new tube. For example, for 100k cells, N=100k/10k =10, aliquot 5×10μl=50μl cells to a new tube.

21 Add 42.5×N of 1× TB to sample, incubate at RT for 10min.

22 Add 2.5×N μl of assembled Tn5 to sample, mix well by pipetting.

Transposome	Volume (μl)
1× home-made Tn5	0.625×N
Dilution Buffer	0.625×N
annealed transposition adapter with glycerol	1.25×N
Total	2.5×N

23 Aliquot sample into 50μl per well in 96 well-plate, seal plate, shake at 500rpm for 30min, 37C

24 Pool sample, spin down, add 0.5ml NIB-RI, spin down

25 Resuspend cells in 60µl NIB-RI

RT

1h

26 Add 240µl RT mix to 60µl cells/NIB, aliquot 50µl to 6 PCR wells. Run RT thermal cycles. At the same time, start thawing the oligo plate at RT.

	Reverse transcription (RT) mix	Volume (µl)
	5× RT buffer	70
	Enzymatics RNase Inhibitor	2.19
	SUPERase RI	4.38
	dNTPs	17.5
	100µM RT Primer	35
	H ₂ O	10.94
	50% PEG	105
	Maxima H Minus Reverse Transcriptase (add right before RT reaction)	35
	Total	280

50°C	10min	3
8°C	12 s	
15°C	45 s	
20°C	45 s	

30°C	30 s	cycles
42°C	2 min	
50°C	3 min	
50°C	5 min	
Total	37 min	

27 Pool samples, spin down.

Note

It is normal that the pellet sometimes looks bigger than before.

28 Wash with 500µl NIB, spin down, and resuspend with 1152µl NIB-RI.

29 Optional pause point, after moving supernatant, store the nuclei pellet at -80°C

*

||

Hybridization-ligation

4h

30 Thaw oligo plates to RT before hybridization.

31 Pipette mix 1152µl sample with 3456µl hybridization buffer, keep sample at RT, aliquot 40µl of mixture to Round 1 plate, pipette mix, and shake at 300rpm for 30min, RT

Hybridization buffer	Stock conc.	Final conc.	Volume (µl)
Water	N/A	N/A	2761.9

T4 ligase buffer	10×	1×	576
SUPERase RI	20U/μl	0.05U/μl	14.4
Enzymatics RI	40U/μl	0.32U/μl	46.08
10% NP40	10%	0.1%	57.60
Total			3456

- 32** Add 10μl Blocking oligo 1 to each well, changing tips between rows. Set a multichannel to 40 μL and pipette each well up and down 4-5 times., and shake at 300rpm for 30min, RT

Blocking oligo 1	Stock conc.	Volume (μl)
Round_1_blocking	100μM	253
T4 ligase buffer	10×	211
Water		687
Total		1152

- 33** Pool samples, pipette mix in the reservoir. Aliquot 50μl of mixture to Round 2 plate, pipette mix, and shake at 300rpm for 30min, RT

- 34** Add 10μl Blocking oligo 2 to each well, changing tips between rows. Set a multichannel to 40 μL and pipette each well up and down 4-5 times., and shake at 300rpm for 30min, RT.

Blocking oligo 2	Stock conc.	Volume (μl)
Round_2_blocking	100μM	304
T4 ligase buffer	10×	211
Water		637
Total		1152

- 35** Pool samples, pipette mix in the reservoir. Aliquot 60μl of mixture to Round 3 plate, pipette mix, shake at 300rpm for 30min, RT.

- 36** Add 10µl Blocking oligo 3 to each well, changing tips between rows. Set a multichannel to 40 µL and pipette each well up and down 4-5 times., and shake at 300rpm for 30min, RT.

Blocking oligo 3	Stock conc.	Volume (µl)
Round_3_blocking	100µM	265
Triton X-100	5%	23
Water		864
Total		1152

- 37** Pool samples, spin down, remove supernatant, wash with 1ml NIB-RI.

- 38** Spin down, remove supernatant, resuspend and wash with 1ml NIB-RI.

- 39** Spin down, remove supernatant, resuspend to 80µl NIB-RI.

- 40** Mix sample with 320µl Ligation mix, aliquot 50µl to 8 PCR tubes, shake at 300rpm for 30min, RT.

Ligation mix	Volume (µl)
Enzymatics RI	3.2
SUPERase RI	1
10× ligation buffer	40
T4 Ligase 400U/µl	20
H2O	251.8
10% NP40	4
Total	320

41 Gently resuspend with 1 mL NIB-RI and filter through 40 µm strainer (Flowmi) into a new 1.5 mL tube.

42 Spin, discard supernatant and resuspend to 50µl in NIB-RI.

43 Count cells with Trypan blue. Should now be nuclei that will stain with Trypan. Expect ~10-20K if started from 100K cells. Decide number of cells to be sequenced in each sub-library (generally 1-2K for troubleshooting/QC or 10K-20K for a full run).

Note

optional pause point, may store product at -80°C for at least a few days. We haven't extensively tested this. Freezing extra nuclei at this stage at -80°C will usually result in good RNA quality but reduced ATAC yield.

Reverse crosslinking-pull down

2h 30m

44 Assuming N of 25µl sub-libraries are prepared. For each tube add 25µl 2× RCB, 2µl proteinase K (20mg/ml stock), 1µl SUPERase RI, 55°C, 1h on thermal cycler.

Note

optional stopping point, may store product at -80°C for at least a few days. We haven't extensively tested this. Freezing extra nuclei at this stage at -80°C will usually result in good RNA quality but reduced ATAC yield.

45 Mix with 2.5µl of 100mM PMSF (dissolved in IPA) and incubate at RT for 10min. At the same time, prepare following buffers.

46 Remove supernatant, wash twice with 100×N µl B&W-T without RI.

- 47** Remove supernatant, wash once with $100\times N$ μl B&W-T/RI, and resuspend beads in $50\times N$ μl 2 $\times$ B&W/RI.

1 $\times$ B&W-T/RI	Volume/ μl
1 $\times$ B&W-T	$400\times(N+1)$
SUPERase RI	$4\times(N+1)$

2 $\times$ B&W/RI	Volume/ μl
2 $\times$ BW	$100\times(N+1)$
SUPERase RI	$2\times(N+1)$

- 48** Add 50 μl of beads to each sample, rotate at 10rpm, RT, 60min.

- 49** Put on a magnetic rack, transfer supernatant containing chromatin fragments to a new tube for library preparation later! The supernatant is stable at RT for a few hours.

- 50** Wash cDNA/RNA-bound beads with 100 μl 1 $\times$ B&W-T/RI, RT, three times.

- 51** Put the sample on the magnetic rack, wash with 100 μl 1 $\times$ STE/RI, without resuspending beads.

1 $\times$ STE/RI	Volume/ μl
STE	$200\times(N+1)$
SUPERase RI	$N+1$

ATAC library preparation

2h

52

Note

ATAC library and RNA library are usually performed in parallel.

- 53** Clean sample by Qiagen Minelute column with 500µl DNA binding buffer (Zymo) or PB buffer (Qiagen), elute to 11µl EB, elute again with additional 11µl EB (22µl in total).

Note

Zymo DNA clean up column may result in inconsistent results between samples.

- 54** Mix ~20µl sample with 29µl PCR mix, 1µl of 25µM Ad1.xx primer and H₂O, run PCR for 5 cycles.

ATAC PCR mix	Volume (µl)
NEBNext 2× mix	25×N
P7 primer 25µM	N
H ₂ O	3×N
Total	29×N

ATAC PCR		
72°C	5 min	1 cycle
98°C	30 s	1 cycle
98°C	10 s	5 cycles
65°C	30 s	
72°C	60 s	

- 55** qPCR: 5µl sample + 10µl qPCR mix, calculate the number of cycles needed to reach $\frac{1}{3}$ of the plateau fluorescence (0.33 Ct).

qPCR		
98°C	30 s	1 cycle
98°C	10 s	

65°C	30 s	20 cycles
72°C	60 s	

ATAC qPCR mix	Volume (μl)
NEBNext 2× mix	5×N
Ad1.1 25μM	0.2×N
P7 25μM	0.2×N
10× SYBRgreen	0.9×N
H2O	3.7×N
Total	10×N

- 56** check qPCR product size on gel, expect smear with average size ~400bp. Nucleosome pattern may or may not show up

Note

10,000× SYBRgreen was diluted by water to 100×, and stored in black tube at -20°C. 100× SYBR was further diluted by water to 10× freshly.

- 57** Run additional PCR cycles for the rest of sample (~45μl), N=0.33 Ct cycles.

Additional PCR		
98°C	30s	
98°C	10s	0.33 Ct cycles
65°C	30s	
72°C	60s	

- 58** Expect ~11-13 additional cycles for 2K cells or ~8-10 for 10K cells.

- 59** Clean sample by Zymo DNA clean up kit (225µl binding buffer or 250µl if skipped qPCR), elute to 6µl EB, elute again with additional 6µl EB (~10µl in total). Store the libraries at -20°C until quantification.

RNA library preparation (Template switching)

2h

- 60** Remove all supernatant, resuspend beads in 50µl Template switch mix, be careful to avoid beads to dry.
- 61** Rotate samples at RT for 30min at 10rpm.
- 62** Incubate at 42°C for 90min, shaking at 300rpm, resuspend beads every 30min by pipetting mix up and down.

	Template switch mix	Volume (µl)	Final conc.
	H2O	1.25×(N+1)	
	50% PEG 6000	15×(N+1)	15%
	5× Maxima RT buffer	10×(N+1)	1x
	Ficoll PM-400 (20%)	10×(N+1)	4%
	10mM dNTPs, each	5×(N+1)	1mM
	NxGen RNase Inhibitor	5×(N+1)	0.1x
	100µM TSO (BC_0127)	1.25×(N+1)	2.5µM
	Maxima RT Rnase H Minus (add last)	2.53×(N+1)	
	Total	50×(N+1)	

cDNA amplification

2h

- 63** Mix each sample with 100µl H₂O, magnetize and discard supernatant, wash with 200µl STE, without resuspend beads.

64 Mix 55µl PCR mix with beads, transfer sample to PCR plate.

65 Run PCR for 5 cycles, with sample against magnetic rack, transfer supernatant to new PCR wells.

cDNA PCR mix	Volume (µl)
Kapa Hifi 2× mix	$27.5 \times (N+1)$
RNA PCR primer 25µM	$0.88 \times (N+1)$
P7 primer 25µM	$0.88 \times (N+1)$
H2O	$23.24 \times (N+1)$
Total	$55 \times (N+1)$

PCR	
95°C	3min
98°C	20s
65°C	45s
72°C	3min

66 qPCR: For each sublibrary, mix 2.5µl sample with 7.5µl qPCR mix, run qPCR protocol and calculate 0.33 Ct.

RNA qPCR mix	Volume (µl)
Kapa Hifi 2× mix	$3.75 \times (N+1)$
RNA PCR primer 25µM	$0.12 \times (N+1)$
P7 25µM	$0.12 \times (N+1)$
EVAgreen 20×	$0.5 \times (N+1)$

H2O	$3.01 \times (N+1)$
Total	$7.5 \times (N+1)$

qPCR		
95°C	3min	
98°C	20s	20 cycles
65°C	20s	
72°C	3min	

- 67** check qPCR product size on gel, expect smear with average size > 1kb.

- 68** Run additional PCR cycles for the rest of the sample (~50µl), N=0.33 Ct cycles.

Additional cycles		
95°C	3min	
98°C	20s	0.33 Ct cycles
65°C	20s	
72°C	3min	

Note

For cell line, expect 6-7 additional cycles for 2k cells, ~4 additional cycles for 10k cells. More cycles are expected for primary cells or nuclei.

- 69** Purify each sample with 42µl AMPure beads (0.8×) for cell line sample or 31.5µl AMPure beads (0.6×) for primary samples, elute cDNA to 10µl EB. Store the libraries at -20°C.

- 70** Quantify cDNA concentration by Qubit using 1µl cDNA. Ideally, dilute 50ng cDNA to 5ng/µl in H₂O.

Note

Use nanodrop may result in inaccurate quantification
Expect > 50ng cDNA. If cDNA amount is low, it can get away with tagmenting 20ng cDNA; in this case, adjust the volume of H₂O and cDNA accordingly.

- 71** Assemble in-house produced Tn5, prepare transposome by mixing Tn5, dilution buffer and annealed oligo, and incubate the mix at RT for 30min.

Assemble transposome	Volume (µl)
1× Tn5	1.25× (N+1)
Dilution Buffer	1.25× (N+1)
annealed tagmentation adapter with glycerol	2.5× (N+1)
Total	5× (N+1)

- 72** Mix 10µl of 5ng/µl cDNA with 10µl H₂O, 25µl TD buffer (Illumina) and 5µl assembled Tn5. Incubate at 55°C for 5min on a thermal cycler.

- 73** Purify tagmented library with QIAGEN minelute column using 250µl PB buffer, elute twice with 11µl EB each time (22µl in total).

- 74** Add 29µl of post-tagmentation PCR mix with 1µl tube specific Ad 1.xx primer, amp 7 cycles.

Post-tagmentation PCR mix	Volume (µl)
Sample in EB	20 each
NEBNext 2× mix	25× (N+1)

25μM P7	$1 \times (N+1)$
25μM Ad 1.xx	$1 \times (N+1)$
H2O	$3 \times (N+1)$
Total	$50 \times (N+1)$

Tagmentation PCR		
72°C	5min	
98°C	30s	
98°C	10s	7 cycles
65°C	30s	
72°C	60s	

- 75** Purify each sample with 35μl AMPure beads (0.7×), elute library to 10μl EB. Store the libraries at -20°C until quantification.

Library quantification and sequencing

1 h 30m

- 76** Recommend quantifying with KAPA qPCR kit and pool to 4nM. Expect > 30nM, average library size ~350bp with minimal adapter dimer.

- 77** We typically sequence libraries with a Next-seq High output 150 cycle kit. The sequencing read configuration is 50×99×8×10 for the RNA-seq library; 30×99×8×30 for the pooled ATAC/RNA or ATAC-seq library. In general, ~25k reads per cell per library type for ~50% duplication rate and ~200k reads per cell per library type for ~90% duplication rate