Apr 03, 2025

HyDrop-v2-ATAC

Nature Communications

DOI
https://dx.doi.org/10.17504/protocols.io.x54v97mmpg3e/v1

Suresh Poovathingal[1], Florian De Rop[2,3,4,5], Valerie Christiaens[2,3,4,5], Koen Theunis[2,3,4,5], Stein Aerts[2,3,4,5]

[1]VIB-KU Leuven Center for Brain & Disease Research, CBD Technologies, Single Cell & Microfluidics Expertise Unit, 3000 Leuven, Belgium.;
[2]Laboratory of Computational Biology, VIB Center for AI & Computational Biology, Leuven, Belgium;
[3]VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium;
[4]Department of Human Genetics, KU Leuven, Leuven, Belgium;
[5]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, United States

Aertslab

Koen Theunis
Laboratory of Computational Biology, VIB Center for AI & Com...

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.x54v97mmpg3e/v1

External link: https://doi.org/10.1038/s41467-026-68742-4

Protocol Citation: Suresh Poovathingal, Florian De Rop, Valerie Christiaens, Koen Theunis, Stein Aerts 2025. HyDrop-v2-ATAC. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v97mmpg3e/v1

Manuscript citation:
Dickmänken H, Wojno M, Mahieu L, Theunis K, Ekşi EC, Christiaens V, Kempynck N, Rop FVD, Roels N, Spanier KI, Vandepoel R, Hulselmans G, Poovathingal S, Aerts S (2026) Evaluating single-cell ATAC-seq atlasing technologies using sequence-to-function modeling. Nature Communications 17(). doi: 10.1038/s41467-026-68742-4

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: March 27, 2025

Last Modified: April 03, 2025

Protocol Integer ID: 125622

Keywords: ASAPCRN, scATAC, scATAC-seq, atac-seq, atac, tn5, hydrogel, hydrop, hydrop-atac, droplet, microfluidic, microfluidics, transposase, assay for transposase accessible chromatin, single-cell, atac this protocol, hydrop v2, bulk atac tagmentation, preparation of nuclei, pcr1 cleanup, hydrop run, purification, library purification, nuclei, protocol, preparation, protocol details the preparation, protocol detail

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000430
Aligning Science Across Parkinson's
Grant ID: ASAP-025179

Abstract

This protocol details the preparation of nuclei, bulk ATAC tagmentation, microfluidics setup, HyDrop run, PCR1 cleanup, library purification, and quality control.

Attachments

XLSX

HyDrop-v2-ATAC.xlsx
29KB

Materials

Preparations:

Experimental parameters:

	A	B	C	D	E
	Parameter	**Value**			
	Occupancy	**0.6**			
	total nuclei to recover	7000	nuclei	desired recovery	
	total nuclei to load	11666.67		correction for occupancy	
	total nuclei to load	12833.34	nuclei	includes losses	
	PCR volume	110	µL	this includes 25% extra	
	bead volume	45.8	µL	this includes 25% extra	
	Total emulsion vol	155.8			
	nuclei in PCR mix	116.67	nuc/µL		
	nuclei in droplets	82.36	nuc/µL	Concentration impacts doublet rate!	
	cellflow	600	µL/hr		inDrop: 40 nuclei/uL
	oilflow	700	µL/hr		10x: 100 nuclei/uL
	beadflow	250	µL/hr		
	c_ratio	0.70588235		Ratio impacts PCR concentrations!	
	pcr1_cycles	12	cycles		
	pcr2_cycles	12	cycles	May be adjusted	
	Tagmentation time	60	min.		
	Tagmentation temp	37	C		

Reagents to be thawed: µ

	A	B
	Thaw	**Location**

	A	B
	2% Digitonin	-20
	30% BSA	-20
	PBS	4
	5X HF Buffer	-20

Bulk ATAC tagmentation (1:30)

ATAC TD Buffer (ATD)

	A	B	C	D	E
	4X ATAC TD	**Vol (uL)**	**Stock**	**Final**	
	dH2O	135			
	DMF	100	100%	40%	
	Tris-HCl (pH 7.4)	10	1000	40	mM
	MgCl2	5	1000	20	mM
		250			

ATAC Reaction Mix (ARM)

	A	B	C	D	E
	ATAC Reaction Mix	**Vol (uL)**	**Stock**	**Final**	
	4X ATAC TD	3.75	400%	100 %	
	PBS	**5.7**			
	Nuclei in PBS/BSA				
	Tn5	3	62.5	12.5	ng/ µL
	Pitstop	1.05	1000	70	µM

	A	B	C	D	E
	Tween 20	0.75	**2.00%**	0.10 %	
	Digitonin	0.75	**0.20%**	0.01 %	
		15			

Nuclei loading

PCR mix:

	A	B	C	D	E	F
	PCR mix	**Vol (uL)**	**Stock**	**In PCR mix**	**In droplet**	
	Phusion HF Buffer	31.17	500%	142%	100%	
	dH2O	**27.28**				
	Optiprep	16.5	100%	15%		
	dNTPs	6.25	25	1.42	1	mM
	Nuclei	**15**				
	DTT	3.9	2400	85	60	mM
	Phusion HF Polymerase	3.85	2	0.07	0.05	U/ µL
	Deep Vent Polymerase	3.85	2	0.07	0.05	U/ µL
	ET SSB	2.2	0.5	0.01	0.01	U/ µL
		110				

HyDrop run

HyDrop run with flow rates:

	A	B	C	D
	Flow rates	**Prior**	**Stable**	
	Cells	250	600	µL/hr
	Oil	250	750	µL/hr
	Beads	250	250	µL/hr

PCR program:

	A	B	C
	Temp	**Time**	sample A
	72C	20 mins	
	98C	3 mins	
	98C	10 s	|
	55C	30 s	x 12
	72C	1 min	|
	72C	5min	
	4C	inf	

PCR1 cleanup and library purification

GITC Buffer:

	A	B	C	D	E
	GITC Buffer	**Vol (uL)**	**Stock**	**Final**	
	GITC	833.33	6000	5000	mM
	dH2O	66.67			
	EDTA	50	500	25	mM

	A	B	C	D	E
	Tris-HCl (pH 7.4)	50	1000	50	mM
		1000			

Clean up mix:

	A	B	C	D	E
	Dyna GITC Mix	**Vol (uL)**	**Stock**	**Final**	
	GITC buffer	180			
	Dynabeads	10			
	DTT	10	2400	120	mM
		200			

EB-DTT-TW Buffer:

	A	B	C	D	E
	EB-DTT-Tween	**Vol (uL)**	**Stock**	**Final**	
	EB	490			
	DTT	5	2400	24	mM
	Tween 20	5	10%	0.10 %	
		500			

Final PCR amplification and library purification

PCR Mix:

	A	B	C	D
	PCR 2 mix	**Vol (µL)**	**Stock**	**Final**

	A	B	C	D
	KAPA	50	200%	100 %
	Library	40		
	i7	5	20	1
	Universal P5 partial	5	20	1
		100		

PCR amplification:

	A	B	C
	Temp	**Time**	
	95	03:00	
	98	00:10	|
	63	00:30	x 12
	72	01:00	|
	72	01:00	
	4	Hold	

Fresh 80% EtOH:

	A	B	C	D
	80% EtOH	**Vol (µL)**	**Stock**	**Final**
	EtOH	8000	100%	80%
	dH2O	2000		
		10000		

Preparations

1 Evaluate the experimental parameters below

	A	B	C	D	E
	Parameter	**Value**			
	Occupancy	**0.6**			
	total nuclei to recover	7000	nuclei	desired recovery	
	total nuclei to load	11666.67		correction for occupancy	
	total nuclei to load	12833.34	nuclei	includes losses	
	PCR volume	110	uL	this includes 25% extra	
	bead volume	45.8	uL	this includes 25% extra	
	Total emulsion vol	155.8			
	nuclei in PCR mix	116.67	nuc/uL		
	nuclei in droplets	82.36	nuc/uL	Concentration impacts doublet rate!	
	cellflow	600	uL/hr		inDrop: 40 nuclei/uL
	oilflow	700	uL/hr		10x: 100 nuclei/uL
	beadflow	250	uL/hr		
	c_ratio	0.70588235		Ratio impacts PCR concentrations!	
	pcr1_cycles	12	cycles		
	pcr2_cycles	12	cycles	May be adjusted	
	Tagmentation time	60	min.		
	Tagmentation temp	37	C		

2 Thaw the following reagents and keep On ice .

	A	B
	Thaw	**Location**
	2% Digitonin	-20
	30% BSA	-20
	PBS	4
	5X HF Buffer	-20

3 Put centrifuges at 4 °C and heat block at 65 °C .

4 Reserve deep well PCR block.

Nuclei preparation

5 Prepare nuclei with your nuclei extraction protocol of choice

6 Resuspend nuclei in PBS/BSA (0,04% BSA).

Bulk ATAC tagmentation (1:30)

1h

7 Pre-heat block at 37 °C .

8 Perform nuclei count.

9 Meanwhile, prepare ATAC TD Buffer (ATD) and ATAC Reaction Mix (ARM).

	A	B	C	D
	4X ATAC TD	**Vol (uL)**	**Stock**	**Final**
	dH2O	135		
	DMF	100	100%	40%
	Tris-HCl (pH 7.4)	10	1000	40 mM
	MgCl2	5	1000	20 mM
		250		

	A	B	C	D
	ATAC Reaction Mix	**Vol (uL)**	**Stock**	**Final**
	4X ATAC TD	3.75	400%	100%
	PBS	**5.7**		
	Nuclei in PBS/BSA			
	Tn5	3	62.5	12.5 ng/uL
	Pitstop	1.05	1000	70 uM
	Tween 20	0.75	**2.00%**	0.10%
	Digitonin	0.75	**0.20%**	0.01%
		15		

10 Pippette the ARM mix gently to mix all the content.

11 Incubate the ATAC Reaction Mix for 01:00:00 at 37 °C without shaking. 1h

Microfluidics Setup

12 Turn on pumps and microscope lamp.

13 Create new directory for pictures of the run and change picture output path.

14 Take 1× 3 mL syringe and fill with droplet oil. Connect tubing, put in oil pump and prime.

15 Take 1× 3 mL syringe and fill with mineral oil. Connect tubing and pipet tip and prime.

16 Take 1× 3 mL syringe and fill with silicone oil. Connect tubing and pipet tip and prime.

17 Load the beads and put in the bead slot.

18 Place chip and connect outlet tubing.

19 Place cold block or ice box and PCR tubes in position.

20 Prepare program on PCR block.

Nuclei loading

21 Prepare the PCR mix as below (add PCR enzymes and nuclei only after other ingredients are mixed).

	A	B	C	D	E
	PCR mix	**Vol (uL)**	**Stock**	**In PCR mix**	**In droplet**
	Phusion HF Buffer	31.17	500%	142%	100%
	dH2O	**27.28**			
	Optiprep	16.5	100%	15%	
	dNTPs	6.25	25	1.42	1 mM

	A	B	C	D	E
	Nuclei	**15**			
	DTT	3.9	2400	85	60 mM
	Phusion HF Polymerase	3.85	2	0.07	0.05 U/uL
	Deep Vent Polymerase	3.85	2	0.07	0.05 U/uL
	ET SSB	2.2	0.5	0.01	0.01 U/uL
		110			

HyDrop Run

22 Perform HyDrop run with following flow rates:

	A	B	C
	Flow rates	**Prior**	**Stable**
	Cells	250	600 uL/hr
	Oil	250	750 uL/hr
	Beads	250	250 uL/hr

23 After the run, perform the linear amplification using the PCR program below:

if emulsion comes above border of PCR, take away oil from below

	A	B	C
	Temp	**Time**	sample A
	72C	20 mins	
	98C	3 mins	
	98C	10 s	|
	55C	30 s	x 12
	72C	1 min	|

	A	B	C
	72C	5min	
	4C	inf	

PCR1 cleanup and library purification

24m 30s

24 Thaw GITC Buffer at 65 °C for 00:10:00 and bring to Room temperature, check if precipitate is gone. 10m

If none is available in freezer, prepare GITC Buffer as below.

	A	B	C	D
	GITC Buffer	**Vol (uL)**	**Stock**	**Final**
	GITC	833.33	6000	5000 mM
	dH2O	66.67		
	EDTA	50	500	25 mM
	Tris-HCl (pH 7.4)	50	1000	50 mM
		1000		

25 Make clean up mix:

	A	B	C	D
	Dyna GITC Mix	**Vol (uL)**	**Stock**	**Final**
	GITC buffer	180		
	Dynabeads	10		
	DTT	10	2400	120 mM
		200		

26 Prepare EB-DTT-TW buffer:

	A	B	C	D
	EB-DTT-Tween	**Vol (uL)**	**Stock**	**Final**
	EB	490		
	DTT	5	2400	24 mM
	Tween 20	5	10%	0.10%
		500		

27 Add 125 µL of recovery agent, gently invert 10 times.

28 Incubate for 00:01:00 and spin down the tubes. 1m

29 Discard recovery agent from bottom of tubes.

30 Add 200 µL of the cleanup mix using P200 pippette.

31 Incubate the mix for 00:10:00 with mixing every 5 minutes. 10m

32 Pull beads to the sides on magnetic rack and remove supernatant.

33 Add 300 µL of 80% EtOH for 00:00:30 without disturbing pellet. 30s

34 Remove supernatant and repeat the above 80% EtOH wash one additional time.

35 Air-dry dry magnetic pellet for no more than 00:01:00 . 1m

36 Spin down pellet and resuspend in 50.5 µL of the EB-DTT-Tween.

37 Incubate for 00:02:00 . 2m

38 Pull beads to the sides on magnetic rack, transfer 50 µL of the elute to a new PCR tube.

39 Add 1.2X volume of Ampure beads (60 µL).

40 Perform the purification as recommended by the manufacturer.

41 Resuspend in 40.5 µL of EB-DTT and take 40 µL eluate to the next step.

Final PCR amplification and library purification 10m 30s

42 Prepare the following PCR mix

	A	B	C	D
	PCR 2 mix	**Vol (uL)**	**Stock**	**Final**
	KAPA	50	200%	100 %
	Library	40		
	i7	5	20	1
	Universal P5 partial	5	20	1
		100		

43 Mix the contents well and proceed to amplification:

	A	B	C
	Temp	**Time**	
	95	03:00	
	98	00:10	|
	63	00:30	x 12
	72	01:00	|
	72	01:00	
	4	Hold	

44 After the PCR, add 40 µL of Ampure XP beads (0.4x purification to remove big fragments).

45 Magnetise and transfer all clear supernatant to a new PCR tube.

46 Add 80 µL of Ampure beads to the clear supernatant and mix well.

47 Incubate the mix for 00:05:00 .

5m

48 Prepare fresh 80% EtOH.

	A	B	C	D
	80% EtOH	**Vol (uL)**	**Stock**	**Final**
	EtOH	8000	100%	80%
	dH2O	2000		
		10000		

49 Pull beads to the sides on magnetic rack.

50 Add 200 µL of 80% EtOH for 00:00:30 without disturbing pellet. 30s

51 Remove supernatant and repeat 80% EtOH wash.

52 Air-dry dry magnetic pellet for no more than 2 minutes.

53 Spin down pellet and resuspend in 20.5 µL of the EB.

54 Incubate for 00:05:00 . 5m

55 Pull beads to the sides on magnetic rack and transfer 20 µL to a new PCR tube.

Quality control

56 Perform Qbit and Bioanalyzer.