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Scale-scATAC

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We use this protocol and it's working

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Abstract

This protocol allows you to scale your droplet based snATAC-seq by pre-indexing with barcoded Tn5.

Attachments

XLSX

Scale-scATAC.xlsx

19KB

Materials

	20X Diluted Nuclei Buffer	Stock	Final	Volume			
	Tris-HCl pH 7.4	1000	mM	200	mM	200	µl
	$MgCl_2$	1000	mM	100	mM	100	µl
	Nuclease-free Water					700	µl
	Total Volume					1000	µl

	Diluted Nuclei Buffer	Stock	Final	Volume			
	20X Nuclei Buffer (10x Genomics)	20	X	1	X	50	µl
	Nuclease-free Water					950	µl
	Total Volume					1000	µl

	Diluted Nuclei Buffer	Stock	Final	Volume			
	Tris-HCl pH 7.4	1000	mM	10	mM	10	µl
	$MgCl_2$	1000	mM	5	mM	5	µl
	Nuclease-free Water					985	µl
	Total Volume					1000	µl

	Tagmentation Buffer V1	Stock	Final	Volume			
	Tris-HCl pH 7.4	1000	mM	10	mM	0,1	µl
	DMF	100	%	15	%	1,5	µl
	$MgCl_2$	1000	mM	5	mM	0,05	µl
	Nuclease-free Water					5,35	µl

	Total Volume					7	µl

	Tagmentation Buffer	Stock	Final (in 15uL)	Volume			
	Tris-HCl pH 7.4 (or Tris acetate 7.6)	1000	mM	10	mM	0,10	µl
	DMF	100	%	10	%	1,50	µl
	Pitstop	1000	uM	70	uM	1,05	
	$MgCl_2$ (or Mg acetate)	1000	mM	5	mM	0,05	µl
	Nuclease-free Water					4,30	µl
	Total Volume					7	µl

	Loading buffer w/o SBS	Stock conc		Final conc		V(µl)
	Tris-HCl pH 7.4	1000	mM	10	mM	5
	DMF	100	%	10	%	50
	MgCl2	10000	mM	5	mM	2,5
	NaCl	50000	mM	20	mM	2
	Glycerol (filtered)	50	%	10	%	100

	Nuclease-free Water					340,5
	Total Volume					500

	Final Loading buffer	Stock conc		Final conc		V(µl)
	Loading buff w/o SBS					95
	short SBS oligo	100	uM	5	uM	5
	Total Volume					100

	Name	Oligo for loading buffer
	Short SBS	CGTGTGCTCTTCCGATCT

	Name	i7 index primer		Reverse complement index
	s3_i7_S701	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		TAAGGCGA
	s3_i7_S702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		CGTACTAG
	s3_i7_S703	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		AGGCAGAA

	s3_i7_S706	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		TAGGCATG
	s3_i7_S707	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		CTCTCTAC
	s3_i7_S708	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		CAGAGAGG
	s3_i7_T267	CAAGCAGAAGACGGCATACGAGATTACTCTTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GAAGAGTA
	s3_i7_T268	CAAGCAGAAGACGGCATACGAGATAACTAACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GGTTAGTT
	s3_i7_T269	CAAGCAGAAGACGGCATACGAGATTCCTCAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		ATTGAGGA
	s3_i7_T271	CAAGCAGAAGACGGCATACGAGATGCTGCTTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GAAGCAGC
	s3_i7_T272	CAAGCAGAAGACGGCATACGAGATACCGAATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GATTCGGT
	s3_i7_T273	CAAGCAGAAGACGGCATACGAGATCTCCAGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		CTCTGGAG
	s3_i7_T274	CAAGCAGAAGACGGCATACGAGATTGCTAGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GACTAGCA
	s3_i7_T275	CAAGCAGAAGACGGCATACGAGATCGACCTGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GCAGGTCG
	s3_i7_T276	CAAGCAGAAGACGGCATACGAGATAGCCAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GGCTGGCT

	s3_i7_T278	CAAGCAGAAGACGGCATACGAGATGGTATGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		TCCATACC

Before start

Prepare barcoded Tn5 with: **dx.doi.org/10.17504/protocols.io.j8nlk1w1wg5r/v1**

Prepare nuclei

1 **Count and dilute nuclei**

1.1 Resuspend nuclei in 1X DNB

1.2 Count nuclei

1.3 Dilute nuclei to desired concentration in 1X DNB

	A	B	C
	Number of Nuclei per Well	**Required Nuclei Concentration (Nuclei/µl)**	**Approx. Final Output of Well**
	20,000	4,000	5,000
	25,000	5,000	6,250
	30,000	6,000	7,500
	35,000	7,000	8,750
	40,000	8,000	10,000
	45,000	9,000	11,250
	50,000	10,000	12,500

Tagmentation

1h 6m

2 **Mix diluted nuclei with tagmentation buffer**

2.1 For 24 wells: mix 150 µL diluted nuclei with 210 µL Tagmentation Buffer

2.2 Add 12 uL nuclei/TB-mix to each well while keeping plate on ice.

2.3 Use a multichannel-channel pipet set to 12 µL to mix nuclei 5 times (no bubbles please)

2.4 Seal plate. DO NOT SPIN PLATE. Immediately proceed to tagmentation

3 Tagmentation

3.1 Tagment for 01:00:00 @ 37 °C in a thermocycler block with heated lid (47°C) 1h

4 Stop tagmentation

4.1 Take plate from thermocycler and place 00:05:00 on ice.

4.2 Take multi-channel pipet, pipet gently 3x, and combine samples to same row.

4.3 Pool all wells in 1.5mL DNA LoBind tube

4.4 Centrifuge 400 x g, 4°C, 00:06:00 6m

4.5 Remove supernatant without disturbing nuclei pellet

4.6 Add 30 uL of loading buffer

4.7 Count nuclei

5 Prepare for HyDrop-ATAC or 10X ATAC

5.1 Prepare PCR buffer for HyDrop-ATAC or Barcoding reagent for 10x Genomics ATAC.

5.2 Dilute nuclei to desired concentration with loading buffer and take 15 µL

5.3 Add PCR mix

6 **Run sample on HyDrop or 10x Genomics**

7 **PCR**

7.1 First PCR: limit cycles to 4

7.2 Second (index) PCR: use 2.5 µL of the i7 index primer (see Oligo's) instead of the 10x Index primers. P5 can be used from the kit.
#Cycles: 8 to 10

8 **Sequencing**

	A	B	C	D
	R1	R2	i7	i5
	40	74	8	16

Depending on the sequencing kit, R1 and R2 can be extended.

Protocol references

Mulqueen, R.M., Pokholok, D., O'Connell, B.L. *et al.* High-content single-cell combinatorial indexing. *Nat Biotechnol* **39**, 1574–1580 (2021). https://doi.org/10.1038/s41587-021-00962-z

https://scale.bio/wp-content/uploads/2023/05/ScaleBio_scATAC_pre-indexing_protocol_v1.6_24Nov22.pdf