Jan 12, 2026

Tn5 loading scalebarcodes

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlk1w1wg5r/v1

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Protocol Citation: Koen Theunis 2026. Tn5 loading scalebarcodes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk1w1wg5r/v1

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

Created: January 09, 2026

Last Modified: January 12, 2026

Protocol Integer ID: 238301

Keywords: ASAPCRN, Tn5, scaleATAC, scATAC-seq, tn5 transposase, scalebarcodes this protocol, barcoded adapter complex, loading scalebarcode, tn5, adapter complex

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

Abstract

This protocol describes how to prepare barcoded adapter complexes that can be loaded onto Tn5 transposase.

Attachments

XLSX

Tn5_loading_scalebar...

13KB

Guidelines

All mixes contain glycerol so make sure to pipet slowly and sufficient for proper mixing.

Materials

	A	B	C	D	E	F
	Oligo anneal buffer B	**Stock conc**		**Final conc**		**V (µl)**
	Tris-HCl (pH 8)	1000	mM	40	mM	40,0
	NaCl	5000	mM	50	mM	10,0
	UltraPure Water					950,0
	Total Volume					1000,0

	A	B	C	D
	Name	**Truseq_R2_SBS12_partial**	**Tn5_96plx_idx**	**ME**
	R2_18_ME_scl_4	CGTGTGCTCTTCCGATCT	CTGCTTCC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_5	CGTGTGCTCTTCCGATCT	GGTCACGA	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_6	CGTGTGCTCTTCCGATCT	AACTGTAG	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_16	CGTGTGCTCTTCCGATCT	GCGGCACA	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_17	CGTGTGCTCTTCCGATCT	CTAGCGCT	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_18	CGTGTGCTCTTCCGATCT	TCGATATC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_28	CGTGTGCTCTTCCGATCT	CGCCGATC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_29	CGTGTGCTCTTCCGATCT	TCTCTACT	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_30	CGTGTGCTCTTCCGATCT	CTCTCGTC	AGATGTGTATAAGAGACAG

	A	B	C	D
	R2_18_ME_scl_40	CGTGTGCTCTTCCGATCT	AACGTTCC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_41	CGTGTGCTCTTCCGATCT	GCAGAATT	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_42	CGTGTGCTCTTCCGATCT	ATGAGGCC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_52	CGTGTGCTCTTCCGATCT	TGGATCGA	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_53	CGTGTGCTCTTCCGATCT	AGTTCAGG	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_54	CGTGTGCTCTTCCGATCT	GACCTGAA	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_64	CGTGTGCTCTTCCGATCT	GCGCAAGC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_65	CGTGTGCTCTTCCGATCT	AAGATACT	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_66	CGTGTGCTCTTCCGATCT	GGAGCGTC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_76	CGTGTGCTCTTCCGATCT	TCCAACGC	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_77	CGTGTGCTCTTCCGATCT	CCGTGAAG	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_78	CGTGTGCTCTTCCGATCT	TTACAGGA	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_88	CGTGTGCTCTTCCGATCT	TAAGGTCA	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_89	CGTGTGCTCTTCCGATCT	TTGCCTAG	AGATGTGTATAAGAGACAG
	R2_18_ME_scl_90	CGTGTGCTCTTCCGATCT	CCATTCGA	AGATGTGTATAAGAGACAG
	Name	**Nextera_R1_A14**		**ME**
	R1-A-oligo	TCGTCGGCAGCGTC		AGATGTGTATAAGAGACAG
	Name			**ME'**

	A	B	C	D
	Nextera_Mosaic_End_REV COMP			/5Phos/CTGTCTCTTATAC ACATCT

Order HPLC purified oligo's

	A	B	C	D	E	F
	Tn5 dilution buffer	**Stock conc**		**Final conc**		**V (µl)**
	Tris-HCl (pH 7.4)	1000	mM	50	mM	250,0
	NaCl	5000	mM	100	mM	100,0
	EDTA	50	mM	0,1	mM	10,0
	DTT	1000	mM	1	mM	5,0
	Glycerol	100	%	50	%	2500,0
	Water					2135,0
	Total Volume					5000,0

Add DTT fresh

Prepare oligo's

1 Dissolve oligo's in Oligo annealing buffer (40mM Tris-HCl (pH 8), 50mM NaCl) to a stock of [M] 100 micromolar (µM) .
Work in 96-well plates for easy pipetting.

Anneal oligo's

2 Mix oligo's equimolar for each barcode:

	A	B	C	D
		µL	**Stock**	
	A=R1	3	100	µM
	B=R2-barcodedoligo1-24	3	100	µM
	ME'	6	100	µM

3 5min 95°C, ramp (-1°C/s) to 65°C, 5min 65°C, ramp (-1°C/s) to 4°C.

	A	B
	Temperature (°C)	**Time**
	95 °C	5min
	ramp (-1°C/s)	
	65 °C	5min
	ramp (-1°C/s)	
	4 °C	Hold

Adapters are now [M] 50 micromolar (µM) .

Dilute oligo's and Tn5

4

	A	B	C	D	E	F
		Stock conc		**Final conc**		**V (µl)**
	oligo mix	50	µM	24	mM	12,0
	Tn5 dil buffer					13,0
	Total Volume					25,0

5

	A	B	C	D	E
		Storage	**Vol (µL)**	**Conc (ng/µL)**	**Molarity (µM)**
	EMBL Tn5	-80	20	1000	18

	A	B	C	D	E	F
	Tn5 (diluted in fresh dilution buffer)	**Stock conc**		**Final conc**		**V (µl)**
	Tn5	18	µM	6	µM	20,0
	Tn5 dil buffer					40,0
	Total Volume					60,0

Tn5 is now 333,33 ng/µL; 6 µM

Load Tn5

6

	A	B	C	D	E	F
	Tn5 loading on ice	**Stock conc**		**Final conc**		**V (µl)**
	Tn5 (diluted in fresh dilution buffer)	6	µM	5	µM	5,0
	adapter1-24	24	µM	4	µM	1,0
	Total Volume					6,0

Repeat this for every barcoded adapter mix.
Adapter loaded Tn5 is now max at 4 µM = 222,22ng/µL functional Tn5.

7 Incubate for 00:45:00 @ 23 °C on a heater/shaker (300 RPM).

Dilute Tn5 to stock concentration and store

8 Dilute to 62,5 ng/µL for 50 µL reactions
Dilute to 25 ng/µL for 15 µL tagmentation reactions (like 10x Genomics ATAC)

9 Store at -20 °C.

Protocol references

Our barcoded oligo's are modified versions of the oligonucleotide list that was published in: 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

DIY Tn5 protocols would not have been possible without the work of: Simone Picelli, Åsa K. Björklund, Björn Reinius, Sven Sagasser, Gösta Winberg and Rickard Sandberg. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. http://www.genome.org/cgi/doi/10.1101/gr.177881.114.

The source of our Tn5 is EMBL and they describe it in the following paper:
Hennig BP, Velten L, Racke I, Tu CS, Thoms M, Rybin V, Besir H, Remans K, Steinmetz LM. Large-Scale Low-Cost NGS Library Preparation Using a Robust Tn5 Purification and Tagmentation Protocol. G3 (Bethesda). 2018 Jan 4;8(1):79-89. doi: 10.1534/g3.117.300257. PMID: 29118030; PMCID: PMC5765368.