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Plate Scale Tn5 based tagmentation library prep protocol



Forked from [Tn5 based tagmentation library prep protocol, high throughput](#)

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Cade Mirchandani^{1,2}, max genetti^{1,2}, Pingting Wang¹, Evan Pepper-Tunick^{3,4}, Shelbi Russell^{1,2},
Russell Corbett-Detig^{1,2}

¹Department of Biomolecular Engineering, University of California, Santa Cruz;

²Genomics Institute, University of California Santa Cruz, Santa Cruz, CA, United States;

³Institute for Systems Biology, Seattle, Washington, USA;

⁴Molecular Engineering and Sciences Institute, University of Washington, Seattle, Washington, USA



Cade Mirchandani

Department of Biomolecular Engineering, University of Califo...

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We use this protocol and it's working

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Abstract

This protocol is a fork of ([dx.doi.org/10.17504/protocols.io.bv5gn83w](https://doi.org/10.17504/protocols.io.bv5gn83w)), which a demonstrated efficient and high-throughput tagmentation sequencing library prep protocol based on Picelli et al 2014. In this version, the tagmentation and amplification steps remain largely the same - based on the Tn5 transposase and KAPA HiFi kit, respectively. Here, significant modifications have been made to the library pooling and cleanup processes. Specifically, this protocol involves pooling the crude PCR products, followed by the removal of small fragments via a spin column kit, and depletion of large fragments through agarose gel extraction. These modifications enable faster processing times while still producing high quality sequencing libraries.

This protocol has been optimized for sequencing Drosophila cell culture samples, from which high-quality genomic DNA (gDNA) can be readily obtained. The accessibility to high-quality gDNA simplifies the normalization of gDNA input amounts prior to tagmentation, thereby streamlining the pooling and cleanup steps.

Guidelines

Appendix 1

Oligo Sequences:

	A	B	C
	Name	Sequence	Concentration
	Tn5ME-R	5'-[phos]CTGTCTCTTATACACATCT-3'	100uM
	Tn5ME-A	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'	100uM
	Tn5ME-B	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'	100uM

Index Primers:

PCR index primer are following Nextera XT Index Kit v2 - Index 2 (i5/i7) Adapters, sequences can be found on page 14 of Illumina adapter sequences. You can either order the whole kit from Illumina or synthesize it your self. I was using orders from IDT with standard desalting and it worked fine.

Materials

Tagmentation:

Tn5 Enzyme Mix:

	A	B
	Tn5	0.2-0.4 mg/mL
	Tris-HCl, pH 7.5	25 mM
	NaCl	800 mM
	EDTA	0.1 mM
	DTT	1 mM
	Glycerol	50%

5X TAPS-PEG 8000:

50 mM TAPS-NaOH at pH 8.5, 25 mM MgCl₂, PEG 8000 40%

PCR Amplification:

- KAPA HiFi PCR Kit (KAPA code KK2101; Roche Catalog #07958838001)

Size Selection:

- ZYMO Select-A-Size DNA Clean and Concentrator Kit (Catalog #D4080)
- NEB Monarch DNA Gel Extraction Kit (Catalog #T1020S)
- NEB 6X gel loading dye (Catalog #B7024S)
- NEB 100bp DNA ladder (Catalog #N3231S)
- Invitrogen SYBR Gold (Catalog #S11494)

QC

- Thermofisher Qubit dsDNA Quantitation, High Sensitivity (Catalog #Q32851)
- Agilent D1000 ScreenTape (Catalog #5067-5582)
- Agilent D1000 Reagents (Catalog #5067-5583)

Before start

- AR and BR oligo aliquots can be prepared in advance by combining equal volumes of Tn5ME-A/B and Tn5ME-R. Store at -20C. To use, thaw on bench, vortex and quick spin, then proceed to step 3. This is recommended to minimize freeze/thaw cycles on oligo stocks.
- A normalized gDNA plate should be prepared before starting. 10-20ng/ul is sufficient.



Transposon Assembly

2h 1m

1



Remove oligos Tn5ME-A, Tn5ME-B, Tn5ME-R, and Tn5 enzyme mix from freezer. Thaw primers, mix by vortexing and spin. Keep Tn5 enzyme On ice . Turn on 95 °C thermocycler.

2 In separate PCR tubes combine:

7 µL Tn5ME-A + 7 µL Tn5ME-R = AR oligo (14 µL)

7 µL Tn5ME-B + 7 µL Tn5ME-R = BR oligo (14 µL)

3 Place oligos in thermocycler on the following program:

5m



1. 95 °C x 00:05:00

2. 25 °C x -0.1 °C /sec

3. 4 °C x ∞

4 In separate 1.5 mL tubes combine the following:

5m

13 µL AR oligo + 91 µL Tn5 = Tn5-AR (104 µL)

13 µL BR oligo + 91 µL Tn5 = Tn5-BR (104 µL)



Mix by pipetting gently.

5 Incubate at Room temperature for 01:00:00 .

1h



Tagmentation

2h 17m

6 Preheat thermocycler to 55 °C



- 7 Prepare tagmentation plate by aliquoting  2 μL ( 10-20 ng) gDNA to each well of new 96 well plate.

5m



- 8 In new  2 mL tube prepare Tn5 Reaction Master Mix:

5m



	A	B	C
Reagent		Volume (μL) x 100rxns	Volume (μL) x 1 rxn
Molecular grade water		1200	12
5x TAPS-PEG 8000		400	4
Tn5-AR		100	1
Tn5-BR		100	1
Total		1800	18

Mix by inverting.

Aliquot  18 μL of master mix to each well of tagmentation plate. Seal with foil and spin.

- 9 1. Incubate in thermocycler  00:08:00 at  55 $^{\circ}\text{C}$.

8m

2. Remove plate from thermocycler and **immediately** place  On ice .



- 10 1. Carefully remove film and add  5 μL 0.2% SDS (prepared in a strip tube).

10m

2. Seal with new film and incubate for  00:10:00 at  Room temperature



PCR Enrichment

7m

- 11 Remove KAPA HiFi dNTPs and Fidelity 5X buffer from freezer and thaw, vortex, and spin. Index primer plates should be thawed and spun down.



Note

KAPA HiFi PCR enzyme should only be out of the freezer briefly.

- 12 In a  2 mL tube prepare PCR Master Mix

5m



	A	B	C
	Reagent	Volume (uL) x 100rxns	Volume (uL) x 1 rxn
	Molecular grade water	1175	11.75
	5X Fidelity Buffer	500	5
	10 mM dNTPs	75	0.75
	HiFi	50	0.5
	Total	1800	18

Mix by inverting and spin. Store  On ice .

13 Prepare PCR plate by adding the following to each well in new 96 well plate:

1.  2 μ L combined index primers ( 1 μ L 5uM forward &  1 μ L 5uM reverse).
2.  5 μ L tagmentation product from tagmentation plate.
3.  18 μ L PCR Master Mix

5m



Note

Be careful that plate positions match. Make sure to record index primers used.

14 Seal PCR plate with foil, spin, and place in thermocyclers for the following program:

A	B	C
Temp (°C)	Time	Cycles
72	5:00	-
95	3:00	-
98	0:20	12
65	0:15	
72	0:30	

45m





	A	B	C
	72	5:00	-
	4	∞	-

15 Check concentration of 8-10 randomly selected wells. Desired concentration on Qubit is 15-75ng/uL.

10m



16 When complete freeze at -20 °C Overnight or proceed to cleanup.

7m



Pooling and Left-side Size Selection

1h 34m

17 In a 1.5 mL tube, add 2.5 μ L from each well of PCR plate, total 240 μ L crude pooled library. Prepare ZYMO Select-A-Size DNA Clean and Concentrator Kit.

5m



18 In order to not exceed the capacity of the Zymo-Spin IC-S column, split the pool into two replicates:

2m



In 2 new 1.5 mL microcentrifuge tubes add the following:

1. 100 μ L crude pooled library
2. 500 μ L Select-a-Size DNA Binding Buffer

Mix thoroughly by pipetting the entire volume up and down 5 times

19 Steps 19.1 - 19.4 are to be done for each prepared binding mixture.

19.1 Transfer the binding mixture to a Zymo-Spin IC-S Column in a Collection Tube. Centrifuge at 10,000 x g for 00:00:30 . Discard the flow-through.

30s



19.2 Add 700 μ L of DNA Wash Buffer to the column. Centrifuge at 10,000 x g for 00:00:30 . Discard the flow-through.

30s



19.3 Add 200 μ L of DNA Wash Buffer to the column. Centrifuge at 10,000 x g for 00:01:00 . Discard the Collection Tube.

1m





19.4 Transfer the column to a new  1.5 mL tube, add  22 μL (This number can be varied based on the concentration of the pooled library) of DNA Elution Buffer directly to the column matrix, and incubate for  00:01:00 at  Room temperature . Centrifuge at 10,000 x g for  00:00:30

1m 30s



19.5 Check and record concentration of purified pool replicate on Qubit.

5m



20 Purified pools can be stored at  -20 $^{\circ}\text{C}$.



Right-side Size Selection via Gel Extraction and Final QC

2h 20m

- 21
1. Cast a 1.2% agarose gel (TBE; 0.5cm thick).
 2. While gel is solidifying, prepare:
 - NEB 6X gel loading dye
 - NEB 100bp DNA ladder
 - NEB Monarch DNA Gel Extraction Kit
 3. Thaw purified pools on bench and quick spin.

40m



Note

Ensure you add the appropriate amount of nucleic acid gel stain via pre-loading, precasting, or post-staining.

22 To prevent overloading gel lanes and minimize gel thickness, divide each pool replicate between two PCR tubes.

2m

1. Aliquot  10 μL from each pool replicate into two new PCR tubes, resulting in a total of 4 PCR tubes.
2. To each tube, add  2 μL 6X gel loading dye.



23 In a new PCR tube, prepare DNA ladder by combining:

2m

1.  4 μL Molecular biology grade water
2.  1 μL 100bp DNA Ladder
3.  1 μL 6X gel loading dye





- 24 1. Load each pool and ladder on agarose gel
2. Run gel at  85 V for approx.  01:00:00 1h
- 25 Prepare 4  1.5 mL microcentrifuge tubes. Label each, and record their weights. 5m
- 26 1. Extract gel slices from lanes the pools were run on. Desired size range is between ~450-800bp.
2. Place extracted slice into new, labeled  1.5 mL microcentrifuge tube. 10m 
- 27 Re-weigh microcentrifuge tubes with gel slices and record difference to find gel slice weight. 5m 
- 28 Steps 28.1 - 28.6 are to be done for each extracted gel slice.
- 28.1 If the gel slice is greater than  150 mg , add 3 volumes of Gel Dissolving Buffer to the gel slice. Else, add 4 volumes. 2m 
- 28.2 Incubate at  50 °C for approx.  00:10:00 , inverting periodically until the gel slice is completely dissolved. 10m 
- 28.3 1. Insert the column into the collection tube and load the sample onto the column.
2. Spin at 16,000 x g for  00:01:00
3. Discard the flow-through 1m 
- 28.4 1. Re-insert column into collection tube.
2. Add  200 µL DNA Wash Buffer
3. Spin at 16,000 x g  00:01:00
4. Discard the flow-through. 1m  
Repeat once.
- 28.5 Transfer column to a new  1.5 mL microcentrifuge tube.



- 28.6
1. Add  16 μL of DNA Elution Buffer to the center of the matrix.
 2. Incubate for  00:01:00 at  Room temperature
 3. Spin at 16,000 x g for  00:01:00 to elute DNA.

2m



- 29
- Pool all 4 elutions in to a new  1.5 mL tube. This is the final pool.



- 30
- Check final pool on Qubit and Agilent Tapestation.

Protocol references

Picelli S, Björklund AK, Reinius B, Sagasser S, Winberg G, Sandberg R. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Res.* 2014 Dec;24(12):2033-40. doi: 10.1101/gr.177881.114. Epub 2014 Jul 30. PMID: 25079858; PMCID: PMC4248319.