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

Plate Scale Tn5 based tagmentation library prep protocol

Forked from Tn5 based tagmentation library prep protocol, high throughput

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ABSTRACT

This protocol is a fork of (dx.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.

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.

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 $MgCl_2$, 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 INSTRUCTIONS

- 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 (uL) x 100rxns	Volume (uL) 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 °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: 5m

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

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: 45m

	A	B	C
	Temp (°C)	Time	Cycles
	72	5:00	-
	95	3:00	-
	98	0:20	12
	65	0:15	
	72	0:30	
	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 µL from each well of PCR plate, total 240 µL crude pooled library. 5m
Prepare ZYMO Select-A-Size DNA Clean and Concentrator Kit.

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 µL crude pooled library
2. 500 µL 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 µL of DNA Wash Buffer to the column. Centrifuge at 10,000 x g for 00:00:30 30s
Discard the flow-through.

19.3 Add 200 µL of DNA Wash Buffer to the column. Centrifuge at 10,000 x g for 00:01:00 1m
. Discard the Collection Tube.

19.4 Transfer the column to a new 1.5 mL tube, add 22 µL (This number can be varied 1m 30s
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

19.5 Check and record concentration of purified pool replicate on Qubit. 5m

20 Purified pools can be stored at -20 °C .

Right-side Size Selection via Gel Extraction and Final QC 2h 20m

21 40m

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.

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 1h

1. Load each pool and ladder on agarose gel
2. Run gel at 85 V for approx. 01:00:00

25 Prepare 4 1.5 mL microcentrifuge tubes. Label each, and record their weights. 5m

26 10m

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.

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. 1m
2. Spin at 16,000 x g for 00:01:00
3. Discard the flow-through

28.4 1. Re-insert column into collection tube. 1m
2. Add 200 µL DNA Wash Buffer
3. Spin at 16,000 x g 00:01:00
4. Discard the flow-through.

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. 2m

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

3. Spin at 16,000 x g for 00:01:00 to elute DNA.

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.