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Low-cost Tagmentation Library Prep for low-coverage Illumina sequencing

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

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Disclaimer

Abstract

Tagmentation is a popular approach for preparing Illumina sequencing libraries, but kits can be pricey. Using a bulk-prepped and pre-loaded Tn5 tagmentation enzyme, this protocol is a simple, low-cost and high-throughput procedure for prepping libraries. It is designed for multiplexed low-coverage whole genome sequencing, a useful method for genotyping of many samples at low per-sample cost. Samples are prepped in 96-well PCR plates and combined into multiplexed batches. We have successfully used this protocol with Mimulus guttatus and Mimulus nasutus wild-collected and greenhouse-grown samples.

Attachments

XLS

Tn5_libraries_i7_ind...

46KB

XLSX

i5_indexes_withbarco...

10KB

Before you start

1 Obtain Tn5 tagmentation enzyme, pre-loaded with universal adapter sequences. Enzyme purification and loading guidelines are not provided here: see reference Lu et al. (2017).

2 Quantify input DNA. We recommend 96-well fluorescence quantification with a plate reader and a kit such as the Invitrogen Quant-iT dsDNA Assay kit, Catalog #**Q33120.**

3 Dilute DNA to approximately 1 ng/uL per sample in molecular-grade H_2O. The required input is 1 uL of diluted DNA; we have had success with less than 1 ng on some samples, but final read coverage is correlated with initial sample concentration. If sample concentration is already relatively low (<5 ng/uL), we typically do not dilute. We dilute samples in batches of similar concentration, erring on the side of higher final concentrations. For example, all samples at initial concentration of 5-15 ng/uL might be diluted by 1/5.

4 Before starting tagmentation, pull out the following to thaw:
- Tagmentation (TD) buffer
- Taq Master Mix (for PCR step)
- i5 and i7 indices (for PCR step) -- prepare working dilutions if necessary

i5 indices are in individual tubes at 100 uM -- they do not need to be diluted (optionally, dilute to 10uM). Use a separate i5 index for each plate of 96 samples, one index per plate.
i7 indices are in a 96-well plate, the stock plate is at 100uM. Make a working dilution plate of 10uM.
Use this same i7 plate for all sample plates, one well per sample.

NOTE: our i5 and i7 index adapter sequences are included in attached files to this protocol.

Tagmentation

5 Set up tagmentation reaction

5.1 Prepare a 96-well PCR plate with 1 uL diluted DNA (~1 ng/uL) per sample well

5.2 Create reagent Master Mix:

	A	B	C
	Reagent	Amount per s ample	Amount per 9 6-well plate

	A	B	C
	H2O	3.497 uL	349.7 uL
	TD Buffer	0.42 uL	42 uL
	Tn5 enzyme	0.083 uL	8.3 uL

5.3 Add 4 uL Master Mix to each well of diluted DNA in 96-well PCR plate (final volume 5 uL)

6 Run tagmentation reaction in thermocycler:

	A	B	C
	Step	Duration	Temperature
	1	15 mins	55C
	2	Hold	10C

Once temperature has reached 10C, quickly move to the next step. Keep the reaction on ice!

PCR

7 Set up PCR Reaction
Samples and PCR reagents should remain on ice as much as possible during PCR setup

7.1 Prepare PCR Master Mix (one mix per plate):

	A	B	C
	Reagent	Amount per sample	Amount per 96-well plate
	OneTaq Hot Start 2X Master Mix*	12.5 uL	1350 uL
	H2O	6.95 uL	750.6 uL
	i5 index adapter, 100uM stock**	0.05 uL	5.4 uL

*OneTaq Hot Start 2X Master Mix, New England Biolabs, Catalog #M0484L
**Optionally, dilute stock to 10uM and add 10X listed volume (adjust H2O amounts accordingly)

7.2 Add 0.5 uL i7 adapters, 10uM, (96-well plate format) to each tagmentation sample well using multichannel pipet

7.3 Add 19.5 uL PCR Master Mix to each tagmentation sample well.
To allow for multichannel pipetting, either move PCR Master Mix to a thin trough, or divide evenly into 8-count strip tubes, 263uL per row of 12 samples.

8 Run PCR reaction in thermocycler:

	A	B	C
	Step	Duration	Temperature
	1	3 mins	72 C
	2	1 min	95 C
	3:	**Repeat x18**	
	3a	**10 sec**	**95 C**
	3b	**20 sec**	**55 C**
	3c	**3 mins**	**72 C**
	4	5 mins	72 C
	5	Hold	4 C

After PCR, plates can be held at 4C overnight if necessary. If leaving overnight, do NOT combine samples yet! Once combined, proceed immediately to bead cleanup.

Pooling and bead cleanup

9 Bring SPRI magnetic beads* to room temperature for cleanup, and resuspend fully using a vortexer.
*We have used "CleanNGS DNA & RNA Cleanup Magnetic Beads" from Bulldawg Bio, Catalog #CNGS050

10 Pool 48 samples (cols 1-6, or cols 7-12 from one plate) into a single batch for cleanup.

10.1 Pipette 20 uL from each sample well into a single microcentrifuge tube (~960 uL total)

10.2 Mix well with a P1000 pipet and transfer half (~480 uL) to a second tube. Proceed with cleanup of both tubes. You will have two tubes per 48-sample batch (4 tubes per 96-well plate).

11 Use SPRI magnetic beads and magnetic tube rack to clean up PCR products.

11.1 Add 1.5 times sample volume of SPRI beads to each tube (720 uL SPRI beads per 480 uL sample tube).
Gently pipet up and down 5-10 times to mix thoroughly.

11.2 Incubate 5 mins at room temperature on tabletop.

11.3 Place tubes on magnetic tube rack. Wait 5 mins. Beads should collect on side of tube, leaving a clear supernatant. Your DNA will be bound to the magnetic beads at this point.

11.4 **WHILE TUBES REMAIN ON MAGNET:
Discard clear supernatant with a pipet, taking care not to disturb beads

11.5 **WHILE TUBES REMAIN ON MAGNET:
Pipet 500 uL fresh 70% Ethanol directly onto beads. Let sit for 1 min. (Wash #1)

11.6 **WHILE TUBES REMAIN ON MAGNET:
Discard clear supernatant with a pipet, taking care not to disturb beads

11.7 **WHILE TUBES REMAIN ON MAGNET:
Pipet 500 uL fresh 70% Ethanol directly onto beads. Let sit for 1 min. (Wash #2)

11.8 **WHILE TUBES REMAIN ON MAGNET:
Discard clear supernatant with a pipet, taking care not to disturb beads

11.9 **WHILE TUBES REMAIN ON MAGNET:
Let beads air-dry until they have a matte appearance, with slight cracking. If necessary, use a P20 or P200 to remove excess liquid drops without disturbing beads.

11.10 Once dry, remove tubes from magnet.
Pipet 17 uL molecular-grade H2O directly onto beads. Gently pipet liquid back onto beads repeatedly, massaging the beads until they resuspend. You should end up with a single aliquot of uniform brown liquid at the bottom of the tube, with no beads stuck to the side.

11.11 Incubate 3 mins at room temperature on tabletop.

11.12 Place tubes back onto the magnetic tube rack. Wait 5 minutes for beads to collect on the side of the tube, leaving a clear supernatant. YOUR DNA IS NOW IN THE CLEAR SUPERNATANT.

11.13 **WHILE TUBES REMAIN ON MAGNET:

Carefully pipet ~15 uL of the clear supernatant, which contains your DNA, into a fresh labelled tube. Do not disturb or pipet up any of the brown beads. You may need to leave a small amount (1-2 uL) behind with the beads. If the beads are accidentally disturbed, return the liquid to the tube and let the beads settle onto the magnet again before re-trying.
At this point, the two replicate tubes from each 48-sample batch can be combined, for a final volume of 30 uL per batch of 48 samples.

12 The library prep is now complete. We use a Qubit fluorometer to measure the concentration of each 48-sample batch, then combine equal ng amounts into a single tube to submit for sequencing.

Protocol references

Lu, Z., Hofmeister, B. T., Vollmers, C., DuBois, R. M., & Schmitz, R. J. (2017). Combining ATAC-seq with nuclei sorting for discovery of cis-regulatory regions in plant genomes. *Nucleic Acids Research*, *45*(6), e41. https://doi.org/10.1093/nar/gkw1179