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Tn5 based tagmentation library prep protocol, high throughput

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ABSTRACT

A very cost effective library preparation for whole-genome Illumina-based sequencing was previously demonstrated by by Picelli et al (Picelli et al., 2014). In this protocol, we optimized the Picelli et al protocol for very high throughput and is capable of generating libraries in 2x 96 well plates every 2 days. This protocol is adapted slightly from a protocol by Zan and Carlborg for high throughput library preparation (https://dx.doi.org/10.17504/protocols.io.rt8d6rw), but optimized for a project focused on high throughput of Darwin's finch samples (https://www.biorxiv.org/content/10.1101/2021.01.19.426595v1).

ATTACHMENTS

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Appendix 1

1)2xTn5 dialysis Bf(DF):	**1L** (H20 added to vol.)
100 mM Hepes, pH 7.2	100 mL 1M or 23,83 g
200 mM NaCl	11.69 g NaCl
0.2 mM EDTA	**400 uL 500 mM**
2 mM DTT	2 mL 1M
0.2% Triton X-100	2 mL Triton X-100
20% Glycerol	252 g 100% Glycerol

2) 5X TAPS-MgCl2

50 mM TAPS-NaOH at pH 8.5, 25 mM $MgCl_2$

Appendix 2

Tn5MErev, 5'-[phos]CTGTCTCTTATACACATCT-3';
Tn5ME-A (Illumina FC-121-1030), 5'-TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAG**-3';
Tn5ME-B (Illumina FC-121-1031), 5'-GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG**-3'

Primer A = mix equal molar Tn5MErev with Tn5ME-A
Primer B = mix equal molar Tn5MErev with Tn5ME-B

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 illumine or synthesis it your self. I was using orders from IDT with standard desalting and it worked fine.

MATERIALS TEXT

2xTn5 dialysis Bf(DF):

A	B
Hepes, pH 7.2	100 mM
NaCl	200 mM
EDTA	0.2 mM
DTT	2 mM
Triton X-100	0.20%
Glycerol	20%

5X TAPS-MgCl2:

50 mM TAPS-NaOH at pH 8.5, 25 mM $MgCl_2$

Transposon Assembly 2h 1m

1

Remove primers Tn5ME-A, Tn5ME-B, Tn5ME-Arev, Tn5ME-Brev, DF buffer, and Tn5 from freezer. Melt primers and buffer, mix by vortexing and spin. Leave Tn5 to melt on bench. Turn on **70 °C** incubator.

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2 Prepare primers A and B in **1.5 mL** tube:

27.5 µl primer Tn5ME-A + **27.5 µl** primer Tn5ME-rev = primer A (**55 µl**)

27.5 µl primer Tn5ME-B + **27.5 µl** primer Tn5ME-rev = primer B (**55 µl**)

3 1m

Incubate at **70 °C** for **00:01:00** then place **On ice** .

4

In a new **1.5 mL** tube combine the following:

85 µl Tn5 (**64 Micromolar (µM)**)

55 µl primer A

55 µl primer B

110 µl 2x DF buffer

Total **305 µl**

Mix by pipetting.

5 2h

Incubate at **Room temperature** for **02:00:00** .

Tagmentation 2h 17m

6 2h

Start thermocyclers so they are up to temp. Program: **55 °C** **01:00:00** , **55 °C** **01:00:00** for **15 µl** volume.

7

In new **2 mL** tube add: (1x)

A	B
900uL H2O	4.5uL
400uL 5x TAPS buffer	2uL
400uL 40% PEG	2uL
200uL Tn5 mix	1uL
DNA (10ng/uL)	1uL
Total 1900uL mix	

Mix by inverting. Aliquot **225 µl** into a strip of 8, and use strip to aliquot **9 µl** into 2x 96-well plates using multichannel. If DNA is of questionable quality the volume can be increased to 1.5 or more, just scale water accordingly.

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8

Move to DNA bench. Spin thawed DNA dilution plates. Add **1 µl** DNA to prepared plates of mix. Seal with film and spin.

9 10m

Incubate in thermocycler **00:10:00** at **55 °C** .

10 7m

Remove from thermocycler. Carefully remove film and add **2.5 µl** 0.2% SDS (prepared in a strip tube). Seal with new film and return to thermocyclers to incubate for **00:07:00** .

PCR Enrichment 7m

11

Remove KAPA HiFi dNTPs and GC buffer from freezer and thaw, vortex, and spin. Index plates should be thawed and spun down.

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

12

In a **2 mL** tube mix the following: (1x)

A	B
576uL H2O	3uL
960uL 5x PCR buffer	5uL
57.6uL dNTPs (10mM)	0.3uL
38.4uL HiFi PCR enzyme	0.2uL
Total 1632uL	

Mix by inverting and spin.

13

Aliquot **200 µl** into an 8-strip. Move to DNA bench. Add **7.5 µl** of mix to plates from tagmentation (which have **12.5 µl** of product, so will end with **20 µl**).

14

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Add index. Index plates are pre-prepared with equal amounts of both indices, so add **5 µl** of the mix (=2.5uL each index) at **[M]10 Micromolar (µM)** each.

Be careful that plate positions match!

15

Cover PCR plates with lids (not film), spin, and place in thermocyclers for the following program:

72°	3min	
98°	30sec	
98°	30sec	x9
63°	30sec	
72°	3min	
8°	hold	

16 7m

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

Bead Cleanup 1h 34m

17 30m

Remove beads from cold room and let sit at **Room temperature** for **00:30:00** . Mix by inversion, light shaking before use. Pour about **2.6 mL** into boat.

18 10m

Add **7.5 µl** (with filter tips) to each well of PCR product. Cover with film and shake by hand until completely homogenous. Incubate at **Room temperature** **00:10:00** . Spin briefly.

19 5m

Place on magnet stands for **00:05:00** until all beads are on the sides.

20

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Pipette **28 µl** to new plates. Pipette opposite the beads so as not to disturb them.

21 

10m

Add **4.5 µl** beads (stir beads with pipette tip first). Cover with film, shake, and incubate **00:10:00** at **Room temperature** . Spin briefly.

22 Place on magnet stand for **00:05:00** .

5m

23 While still on magnet, pipette out and throw away **22.5 µl** of liquid-don't touch beads. About **10 µl** remains.

24 

1m

Make fresh 80% EtOH and add **70 µl** to each. Place large stack of paper towels over the top and invert (towels, plate, and magnet) and shake. Leave upside down on towels for about a **00:01:00** .

25 

6m

Repeat #24, but before dumping lift plate from magnet and allow all beads to drop to the bottoms of the tubes. Place back on magnets and leave for **00:03:00** before dumping. Shake vigorously and leave plate and magnet on its side on the bench to dry for about **00:03:00** . Let EtOH evaporate but do not leave too long, ensure no cracking in bead dot.

26 

Add **22 µl** sterile H_2O to each.

27 

22m

Cover with film, remove from magnets and shake by hand on trays vigorously. Spin briefly and put on shaker **00:02:00** @ 2000. Incubate at **Room temperature** **00:20:00** . Spin.

28 

5m

Place on magnets for **00:05:00** . Prepare new plates during incubation.

29 

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Remove film and pipette **19 µl** into new plates. Seal with lids. Proceed to QC or place in cold room.

QC & Pool 3m 30s

30 Use a fluorescence plate reader to quantify the amount of DNA in the two plates.

We used a Tecan (Switzerland) plate reader and calibrated using a Qubit.

31 Pool accordingly for equimolar libraries. Store pools in cold room or freeze at **-20 °C** if not doing final wash within a day.

Pool according to the required concentration needed for the sequencing platform. We usually made dilution plates to ~1.5ng/uL for Illumina NovaSeq applications, but changed slightly depending on the lowest concentration samples in the plates. Plan to lose ~half measurement from post-pooling wash.

Post-pooling wash 1h 15m

32  30m

Take beads out to bring to **Room temperature** **00:30:00** before starting. Mix thoroughly.

33 Calculate how much of each pool you need to start with **1000 ng** or as needed for your sequencing. Calculate how much beads you need for each pool (.45x and .3x).

Adjust for how much you need to send for sequencing. You'll end up with less than half of this starting concentration.

34 Transfer calculated amount of each pool into **2 mL** tubes.

35 

Add 0.45x AmpPure beads. Mix by gentle pipetting until homogenous.

36  10m

Incubate **00:10:00** **Room temperature** . Put remaining pools back in cold room.

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37 Place tubes on tube magnet block for **00:05:00** . Label new **2 mL** tubes. 5m

38 

Transfer supernatant to new tubes. Pipette away from beads.

39 

Add 0.3x beads. Mix until homogenous.

40  10m

Incubate **00:10:00** at **Room temperature** .

41 Place on magnet **00:05:00** . 5m

42 

Pipette off supernatant. Leave tubes open on magnet.

43 

Wash beads with **500 µl** fresh 80% EtOH. Leave a couple seconds and pipette off EtOH into waste.

44 Let dry **00:05:00** at **Room temperature** . Aliquot some sterile H_2O into a **2 mL** tube. 5m

45 

Suspend beads in **100 µl** H_2O, vortex well.

46 Place on magnet **00:10:00** . 10m

47 Move supernatant to new tubes.

48 Measure product on Qubit and Tapestation as desired. Adjust volumes to needed concentration via speedvac if needed. If too low can repeat this section with higher starting amount.

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