A high throughput, cost-efficient library preparation protocol for large scale next generation sequencing Version 2

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Abstract

Previously, *Picelli et al* (Picelli et al., 2014) reported a Tn5 transposase-based library construction procedure for Illumina sequencing. Here, we describe an optimised procedure for high throughput library preparation to facilitate large-scale sequencing that does not rely on advanced lab equipment. The Tn-5 transposase used can be purified using a publicly available construct (Picelli et al., 2014). Reaction buffers and primers can be prepared using standard chemicals available from common suppliers.

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Guidelines

1. Introduction

Previously, *Picelli et al* (Picelli et al., 2014) reported a Tn5 transposase-based library construction procedure for Illumina sequencing. Here, we describe an optimised procedure for high throughput library preparation to facilitate large-scale sequencing that does not rely on advanced lab equipment. The Tn-5 transposase used can be purified using a publicly available construct (Picelli et al., 2014). Reaction buffers and primers can be prepared using standard chemicals available from common suppliers.

2. DNA input recommendations

2.1. Sensitivity to DNA preparation protocol

We have tested this protocol with DNA prepared using several different methods (QIAGEN Maxi Blood kits, QIAGEN DNeasy Blood & Tissue Kits, QIAGEN Gentra Puregene Blood Kit) with DNA eluded in water and TE. We did not find the protocol to be sensitive to these factors.

2.2. DNA quality and quantity

The quality of the input DNA is not a major concern for preparation of sequencing libraries using this protocol. We have prepared libraries using DNA stored in -20°C for up to ~20 years from chicken and foxes with good result.

This protocol is optimised for DNA input of 10 ng. Lower DNA input, 1-5 ng, will yield sufficient amount of library for sequencing by increasing the number of PCR cycles. If possible, we would recommend using 10 ng in order to reduce the amount of PCR duplicates.

Protocol

3. Enzyme purification

Step 1.

The enzyme was produced from a plasmid constructed by *Picelli et al* (Picelli et al., 2014), which has been deposited to AddGene (http://www.addgene.org/, pTXB1-Tn5; plasmid # 60240). A protocol describing enzyme purification from this is available in *Picelli et al* (Picelli et al., 2014).

4. Buffer preparation

Step 2.

4.1. 2xTn5 dialysis buffer (DF):	***1L*** *(H_20 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

4.2. 5X TAPS-MgCl2

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

All chemicals are ordered from Sigma (https://www.sigmaaldrich.com/).

5. Workflow

Step 3.

The workflow includes the following steps (numbers in parentheses refer to sections below): Primer annealing (6) - indexing primer preparation (7) - Assemble transposon (8) - Tagmentation and deactivating the Tn5 (9) - PCR amplification (10) - Double size selection (11) - Quantification and Pooling (12).

6. Primer annealing

Step 4.

Three oligos, Tn5-rev, Tn5-A and Tn5 B were ordered from IDT (eu.idtdna.com) with standard desalting. Their sequences are:
Tn5-rev,
5′-[phos]CTGTCTCTTATACACATCT-3′;
Tn5-A (Illumina FC-121-1030),
5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′;
Tn5-B (Illumina FC-121-1031),
5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′

Two double-stranded primers, Primer A and Primer B are obtained by mixing equal molar of the corresponding oligos.
Primer A = mix equal molar Tn5-rev with Tn5-A

Primer B = mix equal molar Tn5-rev with Tn5-B

Prepare the two primers (A & B) separately using two 1.5 ml Eppendorf tubes. First denature them by incubating at 70 °C for 1 min and then anneal them by chilling on ice afterwards. If performed in a PCR tube, the incubation time should be reduced to 30s.

7. Indexing primer preparation

Step 5.

PCR indexing primers can be synthesised using, for example, the Illumina adapter sequences described on page 14 in the Illumina Nextera XT Index Kit v2 (Index 2 i5/i7 adapters; https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/experiment-design/illumina-adapter-sequences-1000000002694-06.pdf). We ordered the indexing primers from IDT with standard desalting. The primers were diluted to 10 uM and arranged in a 96 well plate. As 24 and 16 primers are available at the i5/i7 sides, 384 unique indexes are available. We recommend users to be careful in this step to avoid contamination (Figure 1).

Figure 1. Layout of the indexing primer plate. In this case, well A1 will contain index primer N702 and S502 with concentration both at 10 uM.

8. Transposon assembly in solution and assay of transposase activity

Step 6.

8a. Transposon assembly

The transposon need to be assembled every time before tagmentation as the assembled solution cannot be stored in -20°C. We assume that the Tn5 is stored in 1X DF buffer as described in *Picelli et al* (Picelli et al., 2014).

8a.1. Reaction (sufficient for 280 DNA samples):

Tn5	100 µl (340 µg)
Pre-annealed primer A (100uM)	64 ul
Pre-annealed primer B (100uM)	64 ul
2X DF buffer	128 ul
Volume	356 ul

Incubate at room temperature for at least 60 min, we have extended it to several hours without problems. Note: This reaction uses100 ul Tn5, assuming the concentration of Tn5 is 3.4 µg/µl. The volume of Tn5 should be adjusted according to the actual concentration of the prepared enzyme to ensure 340 µg Tn5 is added

8b. Assay of transposase activity

Every time a new batch of enzyme is purified, an assay of its activity should be performed. This can be skipped if an old batch with known activity is used (proceed to step 9).

8b.1. Reaction

H_2O	13.5 µl
5X TAPS-$MgCl_2$-PEG 8000	4 µl
Target DNA at 50 ng/µl	1 µl
Tn5	1.5 µl
Volume	20 µl

Incubate the reaction for 10 min at 55°C. Then add 2.5 ul 0.2% SDS and incubate another 7 min at 55°C. Load sample on an agar gel, where a successfully assembled transposase should produce a smear ranging in size from 100-1000 bp.

9. Tagmentation

Step 7.

9.1. Reaction:

5X TAPS buffer	2 µl
40% PEG	2 µl
Tn5	1.2 µl
DNA(10 ng/µl)	1µl
Water	3.8 µl
Volume	10 µl

Incubate reaction at 55°C for 10 min. Add 2.5 ul 0.2% SDS and incubate at 55°C for another 7 min to deactivate the Tn5.

10. PCR enrichment

Step 8.

10.1. Reaction:

Tagmentation product from from above (step 9)	12.5 µl
5X PCR buffer	5 µl
HiFi PCR Enzyme	0.2 µl
dNTP (10mM)	0.3 µl
Index1 (10 uM)	2.5 µl
Index2 (10 uM)	2.5 µl
Water	2 µl
Volume	25 µl

10.2. PCR program:

72°C 3min (Gap filling)
10 cycles of:
-98°C 30s
-98°C 30s
-63°C 30s
72 °C 3min

11. Double size selection using AMPure beads

Step 9.

Check if there is evaporation, especially for wells located around the border of the 96 well plate. If so, fill them to the correct volume with water before bead purification is initiated.

We use Ambion Magnetic Stand-96,(P/N: AM10027). However, any magnetic stand for 96-well plate will work equally well. Double-size selection is performed to cut under-tormented fragments and remove primer/primer dimers. The resulting insert size should be around 350 bp.

11.1 Procedure

1. Warm up AMPure beads to room temperature (30 min in room temperature - RT).
2. Add 7.5 ul beads to each sample, mix it evenly using a vortex and then incubate it for 10 min at RT. Leave it on the magnetic stand until the solution is clear to remove large fragments (> 1kb).
3. Pipette the supernatant into a new tube and add 4.8 ul beads, incubate for 10 min at RT and put it on the magnetic stands until the solution is clear (5min) to remove small fragments (<200bp).
4. Add 70 ul 80% ethanol, quickly flip the plates (with the magnets stand on) and pour out the liquid. Wipe the plate clean using a paper towel.
5. Repeat step 4 one more time and dry the samples by leaving them at RT for 5 min.
6. Add 20 ul water to the sample and suspend the beads by vortex. Incubate for 5-10 min at RT and then put the plate on magnetic stands until the solution is clear (5min).
7. The sequencing library is obtained by carefully pipetting off the supernatant to a new tube. We recommend discarding the last 3-4 ul to avoid contamination.

12. Quality control and pooling

Step 10.

The concentration of the generated library can be measured using Qubit™ dsDNA HS Assay Kit. For example, Qubit for individual samples or a TECAN Infinite® 200 PRO for high-through measurements.

To save time and costs, checking of insert sizes are performed after pooling of the libraries. Equal amount of DNA (20 ng) from each sample are mixed in a pool. Insert sizes for the libraries in the pooled sample are then checked using a TapeStation (Agilent High Sensitivity D1000 ScreenTape). If primer contamination is observed, additional bead selection can be performed (adding 0.75X beads to the pool and repeat 11.1 step 4 to 7 as described above)

13. Sequencing

Step 11.

We have obtained high-quality sequences from libraries prepared using this protocol of Illumina HiSeq 4000 and HiSeq X10. In principle, however, the libraries should be useful for sequencing on any Illumina sequencing platform supporting the Nextera protocol.

14. Troubleshooting

Step 12.

For troubleshooting, we refer the reader to the full publication describing the development of the original

protocol (Picelli et al., 2014).

15. Acknowledgements

Step 13.

We thank Simone Picelli for helpful advice with the initial library preparation and the Protein Science Facility (PSF) at the Karolinska Institute, Stockholm for help with production of the Tn5 enzyme. pTXB1-Tn5 was a gift from Rickard Sandberg (Addgene plasmid # 60240).

16. References

Step 14.

Picelli, S., Björklund, A. K., Reinius, B., Sagasser, S., Winberg, G., & Sandberg, R. (2014). Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Research*, *24*(12), 2033–2040. http://doi.org/10.1101/gr.177881.114