Step-by-step protocol for TransTag library preparation

Preparing Reagents/Buffers:

- **In-house Tn5 assembly with adapter ME-B/ME-Rev only**:
  Purification of Tn5 transposase was performed using the pTXB1-Tn5 plasmid (Addgene, #60240) according to the protocol described previously (Picelli et al., 2014). Purified Tn5 can be stored in aliquots in -20 °C. The concentration of our purified unloaded Tn5 stock is 36 μM, and assembled Tn5 concentration is 12 μM. Alternatively, unloaded Tn5 (concentration of 37 μM) is commercially available to purchase (Diagenode, C01070010-10).

  In contrast to original paper using both annealed mosaic end (ME) adapter ME-A /ME-Rev and ME-B/ME-Rev to assemble Tn5, we only used annealed adapter ME-B/ME-Rev to load into Tn5 during the assembly step. Assembled Tn5 can be stored in -20 °C.

- **Alkaline lysis buffer**: 25 mM NaOH, pH=12.

- **Neutralization buffer**: 100 mM Tris-HCl, pH=8.

- **2× Tagmentation buffer** (100 μL):

Reagent	**Amount**
Tris-HCl, pH 7.5 (1 M)	2 μL
$MgCl_2$ (1M)	1 μL
N,N-Dimethylformamide	20 μL
1×PBS	66 μL
10% Tween 20	2 μL
ddH_2O	9 μL

Note: 2× Tagmentation buffer is freshly made prior to performing tagmentation.

- **10% SDS**

- **80% ethanol**: freshly made

Step-By-Step Method Details:

Genomic DNA extraction and cleanup

1. Genomic DNA extraction

   a. Zebrafish genomic DNA can be extracted from adult zebrafish (fin clip), embryos and larvae. Transfer a small piece of adult fin clip or whole embryo/larvae to a clean PCR tube.

   b. Add 50 μL Alkaline lysis buffer to the PCR tube and incubate the tube at 95 °C for 30 minutes in a PCR machine.

   c. Briefly vortex the tube and add equal volume (50 μL) of Neutralization buffer. Spin down briefly on a bench-top microcentrifuge, and transfer supernatant to a new tube to keep

the genomic DNA. Genomic DNA can be stored at 4 °C.

2. DNA clean-up

a. Extracted genomic DNA can be further cleaned up using a DNA Clean & Concentrator kit (Zymo Research, D4064) or other similar kits according to manufacturer's standard protocol. For genomic DNA, it is recommended to use a volume ratio of 2:1 for DNA binding buffer: sample using the Zymo Research DNA Clean & Concentrator kit.

b. Elute DNA in 25 µL double-distilled water (ddH_2O), and quantify DNA concentration using a NanoDrop spectrophotometer. Cleaned up DNA can be stored at 4 °C.

Note: Typical concentration of DNA is > 20 ng/µL.

Tagmentation

1. In a new PCR tube, set up a 50 µL tagmentation reaction for each sample as stated in the following table.

Reagent	**Amount**
2× Tagmentation buffer	25 µL
Genomic DNA (100 ng)	Variable volume
ddH_2O	Adjust total volume to 50 µL
Assembled Tn5 with ME-B/Rev	1 µL

Note: 100 ng genomic DNA per sample is sufficient to generate a TransTag library.

2. Pipet gently to mix the sample. Incubate the tube at 37°C for 30 minutes in a PCR machine.

3. Add 1 µL of 10% SDS to the tube, and mix well by pipetting. Incubate for 5 minutes at room temperature.

4. Perform clean-up using AMPure beads.

a. Add 40 µL (0.8 volumes of sample) AMPure beads, mixing by pipetting up and down for at least 10 times.
b. Incubate at room temperature for 5 minutes.
c. Place on magnet and allow to clear, then withdraw liquid carefully. Keep the tube on magnet.
d. Add 200 µL freshly made 80% ethanol without disturbing the beads. Incubate for 30 seconds.
e. Withdraw liquid carefully and add 200 µL freshly made 80% ethanol to incubate for 30 seconds.
f. Withdraw liquid and after a quick spin remove the remaining liquid with a 20 µL pipette.
g. Air-dry the beads for 5 minutes. Remove the tube from magnet stand, add 25 µL ddH_2O and vortex on full for 30 seconds.
h. Incubate on the bench for 5 minutes, then place it back to magnet stand and allow it to clear.
i. Transfer liquid to a new PCR tube with a pipette.

Note: Tagmentation by Tn5 transposase is dependent on magnesium (Mg^{2+}), and therefore it is important to avoid any chelators, such as EDTA/EGTA, in reaction buffers. Typical concentration of genomic DNA is 10 -100 ng/μL.

Library preparation by two-steps PCRs

1. Perform Step 1 PCR using NEB Next 2× HiFi Master Mix using the following protocol.

Reagent	**Amount**
NEB Next 2× HiFi Master Mix	12.5 μL
i7 primer (10 μM)	1 μL
ME-A-Tol2 primer (10 μM)	1 μL
Tagmented DNA	10.5 μL

PCR condition as follows:
72 °C for 5 minutes;
98 °C for 30 seconcs;
20 cycles of 98 °C for 10 seconds, 63 °C for 15 seconds;
72°C for 1 minute.

2. Perform Step 2 PCR with NEB Next 2× HiFi Master Mix using the following protocol.

Reagent	**Amount**
NEB Next 2× HiFi Master Mix	25 μL
i7 primer (10 μM)	2 μL
i5 primer (10 μM)	2 μL
Step 1 PCR product	12.5 μL
ddH_2O	8.5 μL

PCR condition as follows:
98 °C for 30 seconds;
20 cycles of 98 °C for 10 seconds, 63 °C for 15 seconds;
72°C for 1 minute.

Note: If you work with multiple samples, you can order a list of uniquely barcoded i5 and i7 primers. In this step-2 PCR, sample library can be multiplexed using a unique i5/i7 primer pair combination for each sample. Keep good note of primers used for each sample.

Important note: use the same i7 primer for both step 1 and step 2 PCR reactions.

3. Post-PCR library clean-up

a. Perform library clean-up by adding 55 μL (1.1× volumes of sample) of AMPure beads to each PCR sample. Mix by pipetting up and down for at least 10 times.
b. Incubate at room temperature for 5 minutes.
c. Place on magnet and allow to clear, then withdraw liquid carefully. Keep the tube on magnet.

d. Add 200 µL freshly made 80% ethanol without disturbing the beads. Incubate for 30 seconds.
e. Withdraw liquid carefully and add 200 µL freshly made 80% ethanol to incubate for 30 seconds.
f. Withdraw liquid and after a quick spin remove the remaining liquid with a 20 µL pipette.
g. Air-dry the beads for 5 minutes. Remove the tube from magnet stand, add 15 µL ddH_2O and vortex on full for 30 seconds.
h. Incubate on the bench for 5 minutes, then place it back to magnet stand and allow it to clear.
i. Transfer liquid to a new PCR tube with a pipette. This is the final TransTag library that is ready for multiplexing and DNA sequencing.

Note: Typical concentration of TransTag library after clean-up is > 20 ng/µL. TransTag libraries can be pooled together for an individual sequencing run. Alternatively, they can be pooled together with other genomic libraries as spike-in samples. We recommend sequencing for 1 – 5 million reads using paired-end 150bp as the longer reads would contain longer sequences of flanking genomic regions, increasing chances of confidently identifying insertion sites of transgenes.