Tn5 Loading Protocol

Quick Guide

Catalog No. 81286, 81284
vA1

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Overview

Protein Details

Recombinant Tn5 Transposase protein was expressed in E. coli as the full-length protein (Accession No. ADY68344.1) without any tags or redundant amino acids. This Tn5 mutant is called 'hyperactive Tn5 transposase' since it can be used to randomly insert Tn5 Transposon into any target DNA *in vitro*. The molecular weight of this protein is 53.3 kDa.

Diagram

Legend

Mosaic End Reverse Sequence

Oligo A

Oligo B

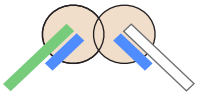
Genomic (sample) DNA

Recombinant Tn5

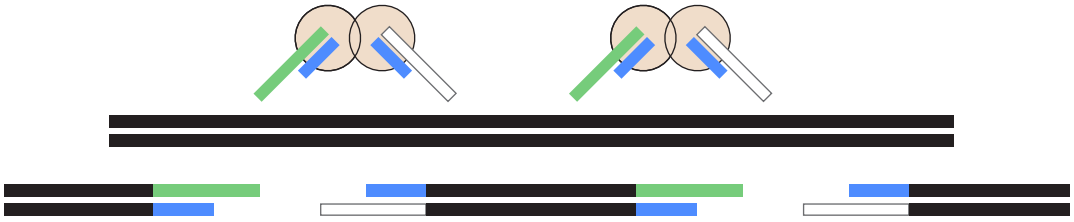
1. Mixing and Annealing Oligo A and Oligo B with the reverse sequence (R) of the Mosaic End Sequence (ME), in two separate reactions.

MER- Oligo A MER- Oligo B

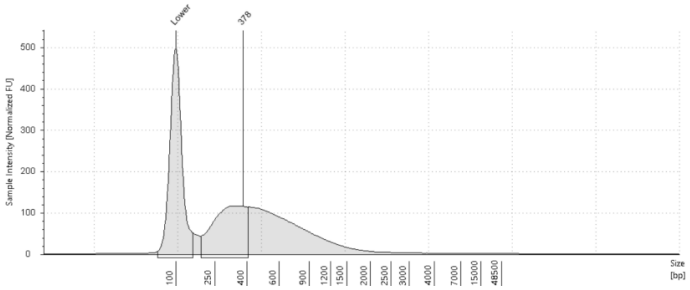
2. Loading the transposomes with the annealed oligos.



3. Tagmentation reaction



4. Loading efficiency test



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Before Starting

1. Order the oligos needed to load the transposomes.
   - Oligo A: 5’ TCG TCG GCA GCG TC**A GAT GTG TAT AAG AGA CAG**
   - Oligo B: 5’ G TCT CGT GGG CTC GG**A GAT GTG TAT AAG AGA CAG**
   - Mosaic End Reverse Oligo: 5’ PO4-CTG TCT CTT ATA CAC ATC T

Note: The sequence in bold will anneal with the Mosaic End Reverse Oligo. The underlined sequence of the Oligo A is the Nextera Read 1 Sequencing Primer (Rd1 SP) and Oligo B is the Nextera Read 2 Sequencing Primer (Rd2 SP).

Resuspend the Oligo A, Oligo B and Mosaic End Reserve Oligo with 10 mM Tris pH8 to a final concentration of 100 µM.

2. Prepare the 1X Tn5 Transposome Storage Solution. Store it at room temperature.

Chemical	CAS Number	Final Concentration
Glycerol (Molecular Biology), density 1.28 g/mL		50% (v/v)
Sodium chloride (NaCl) 5 M	CAS 7647-14-5	200 mM
HEPES, 1 M, pH 7.5	CAS 7365-45-9	100 mM
Dithiothreitol (DTT)	CAS 3483-12-3	2.0 mM
Triton X-100	CAS 9002-93-1	0.1%
EDTA 0.5 M, pH 8	CAS 6381-92-6	0.2 mM
Potassium Hydroxide	CAS 1310-58-3	pH to 7.2
Water, Molecular Biology Grade		

Tn5 Loading Protocol

Make Mosaic End Adapter Mixes A and B:

1. Calculate the molarity of the transposomes. The concentration will be on the vial or on the Technical Data Sheet (TDS) provided, and is typically 0.3-0.5 mg/mL, while the molecular weight is 53.3 kDa.

   For example:

   - Concentration: 0.5mg/ml or changing the units to µg/µL: 0.5 µg/µL
   - MW: 53.3 kDa or 53300 daltons

   Molarity (µM) = (Concentration (µg/µL) / MW (kDa)) * 1000

   Molarity (µM) = (0.5 /53.3) * 1000= 9.38 µM

   In this example, the molarity will be 9.38 µM

2. Combine equal volumes of Mosaic End Reverse Oligo (100 µM) and Oligo A (100 µM). The total volume of this reaction is arbitrary and is dependent on the final quantity of loaded transposase needed. Refer to Step 8 below to see the volume required in the example scenario from Step 1.
3. Combine equal volumes of Mosaic End Reverse Oligo (100 µM) and Oligo B (100 µM).

Note: The resulting molarity of this annealing reaction is 50 µM. The annealing of the Mosaic End Reverse Oligo with the Oligo A or Oligo B will reduce the number of molecules by half, bringing the final molarity to 50 µM.

4. Place tubes in a 95°C thermal cycler with heated lid (105°C) for 3 minutes.
5. Stop the program and allow the mixed oligos to cool down to room temperature in the heat block without opening the lid for 30 minutes.
6. Remove samples from the heat-block and incubate at room temperature for an additional 30 minutes.

Prepare Assembled Transposomes

7. In one tube, combine Tn5 in a 1:0.5:0.5 molar ratio with the annealed oligos. The annealed oligos are at a concentration of 50 µM Oligo A-mosaic and 50 µM Oligo B-mosaic.

   Example for loading the entire **10 µg** Tn5 vial:

   Starting Material:

   Tn5 Molarity: **9.38 µM**

   Each annealed oligos Molarity: 50 µM

   To achieve 1:0.5:0.5 molar ratio, each oligo is added at a molarity of 4.69 µM (half of **9.38 µM**).

Note: The final volume (Vf) of this reaction is arbitrary. If all 10 µg of the vial is to be loaded, 20 µL of the Tn5 will be used in this reaction example (10 µg format for a concentration of 0.5 µg/µL):

$$\mathbf{Ci * Vi = Cf * Vf}$$

$$\mathbf{50\ \mu M * Vi = 4.69\ \mu M * 20\ \mu L}$$

$$\mathbf{Vi = 1.87\ \mu L}$$

In this example, add 1.87 µL Oligo A- mosaic and 1.87 µL Oligo B- mosaic to 20 µL Tn5 enzyme. Mix by gently vortexing or by pipette.

Caution: This step is critical for proper transposomes loading. The Tn5 buffer contains 50% glycerol and is viscous. To mix the transposomes properly, flick the tube gently, or lightly vortex.

8. Incubate for 1 hour at room temperature. At this step, the loaded transposomes have a molarity of ~8 µM in this example.

Note: A loading efficiency test can be performed now. Further dilution of the loaded Transposomes is optional.

9. Dilute to 1 µM in 1x Transposome Storage Buffer. Add 166.18 µL of 1x Tn5 Transposome Storage Solution to the 23.74 µL from the previous step to bring the loaded Tn5 to 1 µM. The final volume of this dilution is 189.92 µL.
10. Mix gently by flicking the tube or pipetting up and down.
11. Store at -20°C
12. Proceed with the loading efficiency test.

Note: A loading efficiency test to assess the proper loading of the transposomes is useful to determine the amount of loaded Tn5 needed to tagment 150 ng of genomic DNA.

Final formulation of diluted Tn5 in the loaded Assembled Transposomes

Initial Concentrations	Final loaded and diluted concentrations
Recombinant Tn5 Transposase, bulk	1 µM
Oligo A, 100 µM in 10mM Tris-pH8	0.5 µM
Oligo B, 100 µM in 10mM Tris-pH8	0.5 µM
Mosaic End Reverse Oligo, 100 µM in 10mM Tris-pH8	1 µM

Efficiency Test Loaded Tn5

You will need:

- Assembled Tn5 Transposomes
- Tagmentation Reaction Buffer
- Genomic DNA, 50 ng/ µL
- Freshly prepared 0.5% SDS
- Thermal cycler
- Columns or beads for purification
- Agilent TapeStation or similar

Note: Prepare Assembled Tn5 Transposomes and Tagmentation Reaction Buffer before starting.

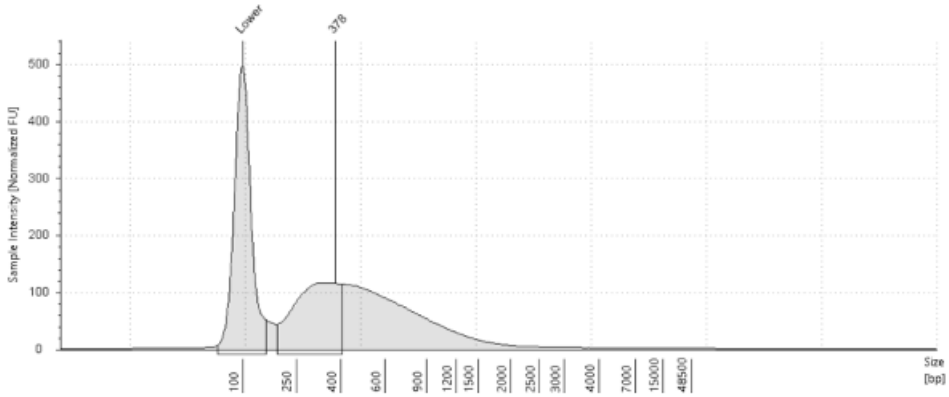
1. Prepare a master mix with Tagmentation Reaction Buffer and genomic DNA (keep genomic DNA constant at 150 ng and titrate volume of Assembled Tn5 Transposomes).
2. Aliquot each sample and add remaining water to bring to volume (25 µL minus the Assembled Tn5 Transposomes volume).
3. Add Assembled Tn5 Transposomes (for example, titrate 1 µL, 2 µL, 4 µL).

	2x Tagmentation buffer	150 ng genomic DNA	Loaded Tn5	H_2O	Total
Reaction 1	10	3	1	6	20
Reaction 2	10	3	2	5	20
Reaction 3	10	3	3	4	20

4. Mix the reaction by pipetting up and down and place at 55°C for 7 minutes.
5. Add 2.5 µL of 0.5% SDS and incubate for another 3 minutes at 55°C.
6. Purify the tagmented DNA. This can be achieved by using any DNA column purification kit or a 1.2X beads purification. For a 1.2X beads purification, add 27 µL of SPRI beads to the 22.5 µL final reaction volume after step 5. Elute in 10 µL 10 mM Tris, pH 8.0.
7. QC: Run 1 or 2 µL on Agilent TapeStation.

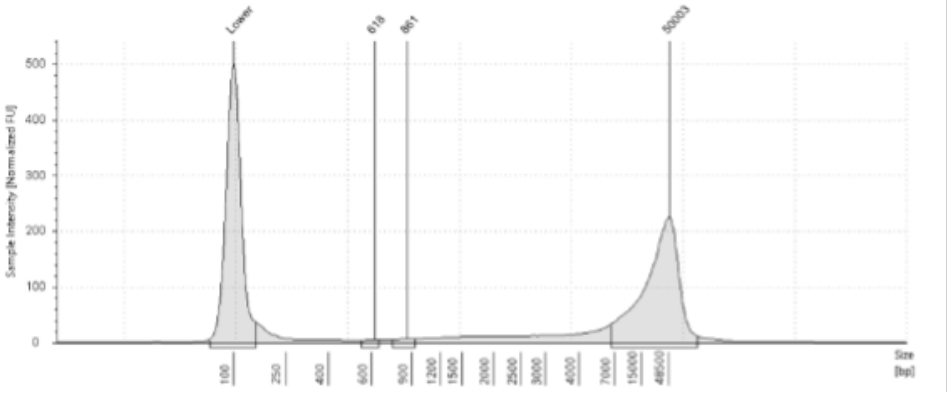
Tn5 Tagmentation Results

Successful tagmentation



Unsuccessful tagmentation

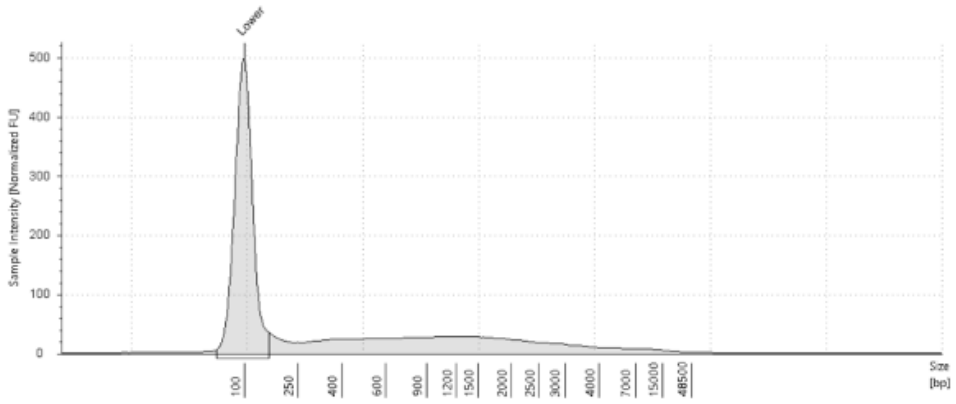
Because there is no fragmentation of the genomic input DNA after tagmentation, this reaction is considered unsuccessful

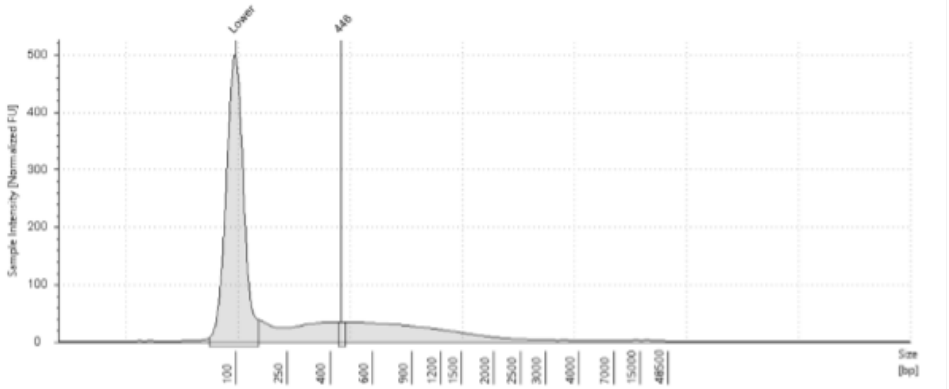


More assembled Tn5 is needed.

Both profiles correspond to successfully loaded Tn5 (no input DNA visible on the high molecular size end of the TapeStation) with slightly lower activity. More Tn5 should be used in subsequent reactions.

Performing a titration of the loaded Tn5 is helpful to assess tagmentation activity.





Problem/Question	Recommendation
Why are the Oligos and the Mosaic End Reverse Oligo reconstituted in 10mM Tris pH8?	They could also be resuspended in low EDTA TE (10 mM Tris-HCl pH 8.0 0.1 mM EDTA) or molecular grade water. A high concentration of EDTA may impact the performance of loaded Tn5 transposase.
If my trace doesn't display tagmentation, what should I do next?	Try a higher volume of your loaded Tn5 per reaction. Or consider loading again if there is Tn5 remaining. Even if there is little activity you will still see a smear, but the fragments will be larger. If the unsuccessful tagmentation trace is observed, it is likely that loading didn't work.
Why didn't my loading work?	The oligos and Tn5 likely were not mixed thoroughly enough. The Tn5 contains 50% glycerol, making mixing more difficult. Make sure to gently vortex or mix thoroughly by slowly pipetting the solution up and down.
Can I use concentrated loaded transposase, or do I need to dilute it to 1 µM?	Test the concentrated loaded transposase and determine the amount of loaded transposase needed for the specific experimental plan. Then, dilute the assembled Tn5 transposomes, or store them in the concentrated form.
Why is the final concentration of the annealed oligos 0.58 µM in the 1 µM loaded transposase solution?	4.69 µM of each annealed oligo were added, and the final volume of the loading reaction is 23.74 µL. After dilution to 1 µM, it is a final volume of 189.92 µL, and using the following equation, the molarity of each annealed oligo can be calculated: $Ci * Vi = Cf * Vf$ $4.7\ \mu M * 23.74\ \mu L = Cf * 189.92\ \mu L$ $Cf = 0.58\ \mu M$
Why is the final concentration of the loaded Transposomes ~8µM?	In the loading reaction, we add 20 µL of the recombinant Tn5 Transposase at a molarity of 9.38 µM. After the addition of each Oligo, the final volume of the reaction is 23.6 µL. The following formula can be used to calculate the molarity of the loaded transposase: $Ci * Vi = Cf * Vf$ $9.38\ \mu M * 20\ \mu L = Cf * 23.74\ \mu L$ $Cf = 7.90\ \mu M \sim 8\ \mu M$
What is Oligo A, Oligo B and Mosaic End Repair?	The "Oligo A" has a part of the Mosaic End Reverse Oligo sequence that will allow the annealing with the Mosaic End Reverse Oligo followed by the Read 1 Sequencing Primer sequence (Rd1 SP). The "Oligo B" also has a part of the Mosaic End Reverse Oligo sequence and the Read 2 Sequencing Primer (Rd2 SP). The reverse Mosaic End Sequence is the complementary sequence to the Mosaic End Sequence on Oligo A and B. It is a 19 basepair sequence that anneals to the 19 base pair ME region of Oligo A and Oligo B during the annealing step, making that region double stranded. Tn5 monomers bind this double stranded sequence and subsequently dimerize, resulting in a "loaded" Tn5 transposome dimer.

Problem/Question	Recommendation
Can I use my own adapter sequences? If so, will the protocol need to be adjusted?	Yes. Adapter sequences will need to contain part of the Mosaic End Reverse sequence (the bolded sequence in step one) to be annealed to the Mosaic End Reverse Oligo. Longer adapters (up to 70 bp) result in less efficient tagmentation. More transposomes may be required for the reaction.
Can I use nuclei instead of genomic DNA?	Yes, nuclei can be used instead of the genomic DNA on the loading efficiency test. The nuclei have to be permeable to allow the entrance of the loaded Transposomes. For reference, 150 ng of DNA corresponds to 25,000 cells.
Where can I find Tagmentation buffer?	2x Tagmentation Buffer is available in the ATAC-Seq Buffer Set (Cat. No. 53153) or several recipes can be found in the literature.
Do the Loaded Transposomes act on single or double stranded DNA?	The loaded transposomes will only tagment double stranded DNA and DNA-RNA hybrids.