Tn5 Transposase

Catalog No.: 5000

Expressed in: *E. coli*

Contents:
Tn5 Transposase is supplied at a concentration of 1 U/µL with 10X Tn5 reaction buffer (100 mM $MgCl_2$, 100 mM Tris-HCl, pH 7.5) and 40X Stop Solution (2% SDS). Adapters not included.

Background:
Tn5 Transposase is a hyperactive form of the wild type enzyme. Tn5 can be used to randomly insert unique oligonucleotide sequences into any target DNA *in vitro*. Efficient transposition requires the assembly of a specific 19-bp transposase recognition sequence (Mosaic End or ME sequence) with the Tn5 Transposase. The assembled transposon catalyzes a random "cut and paste" reaction that adds the ME sequence to the target DNA, creating a 9-bp sequence duplication immediately flanking the transposon insertion site.

Application Notes:
Construction of random libraries for second-generation sequencing and *in vitro* transgenic experiments. Tn5 is utilized in genome sequencing to covalently attach oligonucleotide adaptors and fragment the DNA in a single enzymatic reaction, reducing the time and input requirements over traditional Next Generation Sequencing library preparation.

*These products are intended for research use only, not for diagnostic use. The safety and efficacy of these products in diagnostic or other clinical uses has not been established.

Shipping and Storage
Tn5 Transposase is stored at -20 °C in 10 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5.

Tn5 Transposase is shipped on dry or blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided.

Quality Control:
- Tn5 Transposase activity: The activity of Tn5 is determined by fragmentation of 100 ng of λ phage DNA by 1 Unit of Tn5 in 30 minutes at 55 °C to fragments of 200 - 1000 bp in length as assessed by agarose gel electrophoresis.
- Purity: >95% as determined by SDS-PAGE analysis
- Tn5 Transposase is free of detectable RNase and DNase (exo- and endonuclease).
- <0.2 ng contaminating host DNA per Unit

Generating a Transposon for Illumina NGS

Preparation of Adapter Mix

The name and sequence of reference primers for Illumina platform：

Primer ME A**:** 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

Primer ME B**:** 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Primer ME rev: 5'-pCTGTCTCTTATACACATCT-3'

Dissolve Primer ME A, Primer ME B, Primer ME rev with Annealing Buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.5) to 1 mM.

Example for mixing and annealing adapters	**ME A/rev**	**ME B/rev**
Component	Volume	Volume
Tn5 ME rev [1 mM]	5 µL	5 µL
Tn5 ME A [1 mM]	5 µL	
Tn5 ME B [1 mM]		5 µL
1X annealing buffer	40 µL	40 µL
Total volume	50 µL	50 µL

Use thermocycler for annealing adapters with the following program:

95 °C, 5 minutes

65 °C, 5 minutes

25 °C, 30 minutes

Mix A/rev and B/rev together in equal amounts after annealing.

Final concentration is 50 µM each.

Transposon Assembly

Component	Volume
50 µM each ME A/rev + ME B/rev	2 µL
Tn5 transposase	10 µL

Incubate at 25 °C with lid set to 55 °C for 30 min.

Transposon assembly may be stored at -20 °C until ready to use.

Tagmentation Reaction Example

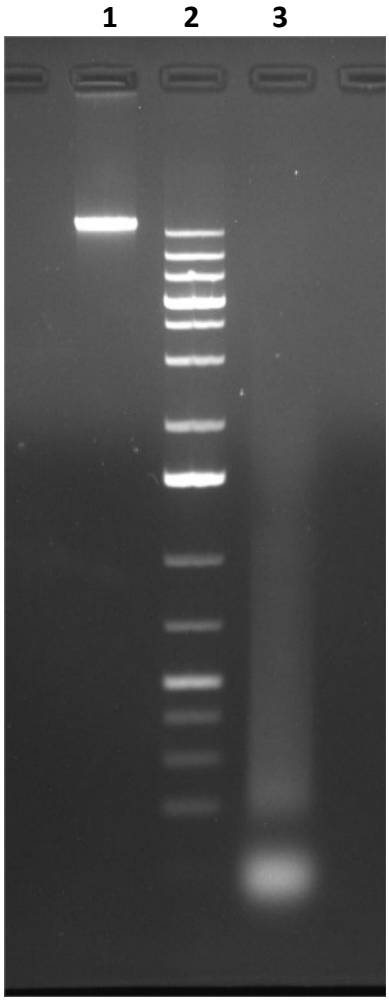
Component	Volume
10X Tn5 reaction buffer	2 µL
Lambda DNA [100 ng/µL]	1 µL
water	15 µL
Transposon assembly mix	2 µL
Total volume	20 µL

Incubate samples at 55 °C in thermocycler with lid set to 55 °C for 30 min.

Add 0.5 µL of 40X Stop Solution (2% SDS) to samples, heat at 55 °C for 10 min.

1.2 % agarose gel electrophoresis at 80 V for 1.5 hrs.

Performance of Tn5 Transposase on Lambda DNA



Lane:

1. Uncut λ DNA
2. GeneRuler 1 kb plus DNA Ladder
3. Tn5-treated λ DNA