Varizymes
OPTIONS AMPLIFIED

Tn5 Transposase

Next Generation Sequencing

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 wildtype 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 & 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

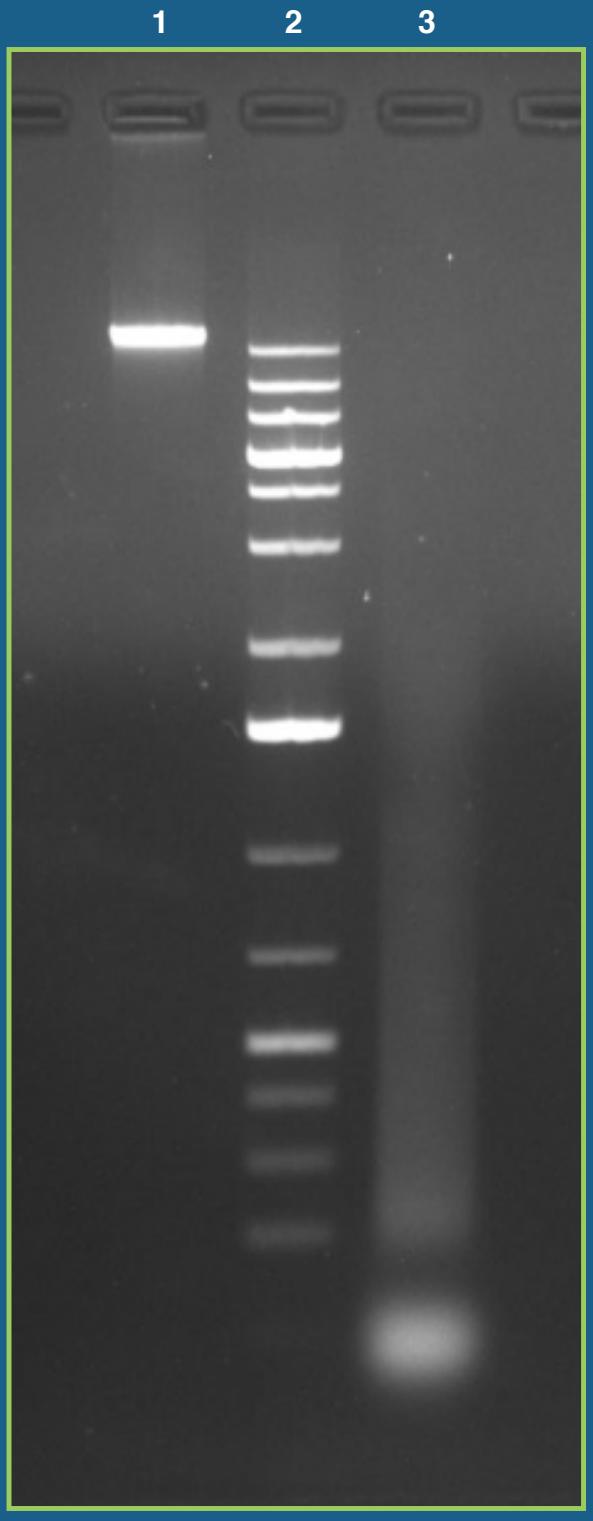
Incubate at 25 °C with lid set to 55 °C for 30 min.
Transposon assembly may be stored at -20 °C until ready to use.

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