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ABPNGS Tn5 Transposase

Catalog Number: TN501-1, TN501-2

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
ABPNGS Tn5 Transposase (Cat. No. TN501-1)			The product is stable for at least 6 months when stored as directed.
ABPNGS Tn5 Transposase, 10 pmol/μL	20 μL	-20 °C, avoid repeated free-thaw	
5× Reaction Buffer	200 μL		
5× Stop Solution	200 μL		
ABPNGS Tn5 Transposase (Cat. No. TN501-2)			
ABPNGS Tn5 Transposase, 10 pmol/μL	100 μL	-20 °C, avoid repeated free-thaw	
5× Reaction Buffer	1 mL		
5× Stop Solution	1 mL		

Product Description

ABPNGS Tn5 Transposase is a hyperactive form of Tn5 transposase. This enzyme can be used to randomly insert Tn5 Transposon into any target DNA, *in vitro*. In addition to Tn5 Transposase, efficient transposition requires that each Tn5 Transposon have a specific 19-bp transposase recognition sequence (Mosaic End or ME sequence) at each of its ends.

ABPNGS Tn5 Transposase catalyzes a multi-step “cut and paste” transposition reaction. Initially, the enzyme binds the 19-bp ME of the transposon to form a Transposome. The transposome then randomly attacks and cleaves the phosphodiester backbone of the target DNA. Finally, the Tn5 Transposase catalyzes the covalent linkage of the 3′-OH ends of the transposon to the exposed 5′-phosphorylated ends of the target DNA. Transposition creates a 9-bp sequence duplication immediately flanking the transposon insertion site.

Applications

- *in vitro* transgenic experiment
- Construction of random library for second-generation sequencing

General Protocol for generating a transposon for Illumina platform

1. Preparation of Adapter Mix

1.1. The name and sequence of reference primers for Illumina platform：
Primer A**:** 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3’
Primer B**:** 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3’
Primer ME: 5'-pCTGTCTCTTATACACATCT-3

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

1.3. Prepare the following reaction systems：

Reaction 1		Reaction 2	
Primer A (100 μM):	10 μL	Primer B (100 μM):	10 μL
Primer ME (100 μM):	10 μL	Primer ME (100 μM):	10 μL
In Total:	20 μL	In Total:	20 μL

1.4. Mix the reaction 1 and reaction 2 thoroughly by vortexing, and briefly centrifuge to collect the solution to the bottom of the tube. Place the tubes in Thermocycler and perform the following program:

Hot lid of 105°C	On
75°C	15 min
60°C	10 min
50°C	10 min
40°C	10 min
25°C	30 min

1.5. After the reaction, mix the reaction 1 and the reaction 2 in an equal volume, named Adapter Mix, store at -20°C.

2. Preparation of Transposon

2.1. Prepare the following components to a sterile PCR tube:

Adapter Mix (50 μM)	4 μL
ABPNGS Tn5 Transposase (10 pmol/μL)	20 μL

2.2. Mix thoroughly by pipetting 20 times.

2.3. Incubate at RT (25°C) for 1 h, the obtained transposon can be directly used for DNA tagmentation, or stored at -20°C.

3. DNA Tagmentation

3.1 Thaw each components at room temperature, mix upside down before use.

3.2 Prepare the following components to a sterile PCR tube:

5× Reaction Buffer	4 μL
Transposon	1 μL
DNA Sample	50-100 ng
ddH_2O	to 20 μL

3.3 Mix thoroughly by pipetting 20 times.

3.4 Incubate at 55°C for 10 min, then add 5 μL of 5× Stop Solution, mix and incubate at 55°C for additional 5 min.

The tagment products can be used for fragment distribution analysis, or purified for next-generation library construction. If the fragment is too long, increase the amount of transposon to reduce the size of the fragment. Otherwise, reduce the amount of transposon to increase the size of the fragment.