MCLAB
Molecular Cloning Laboratories

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Product name: Tn5 Transposase

Cat #: TN5-100, TN5-200

Description:

MCLAB's Tn5 Transposase represents a highly active variant of the Tn5 transposase enzyme. This enzyme facilitates the random insertion of a Tn5 Transposon into target DNA in vitro. Efficient transposition requires the presence of a specific 19-bp transposase recognition sequence (Mosaic End or ME sequence) at each end of the Tn5 Transposon.

The transposition process catalyzed by the Tn5 Transposase involves a multi-step "cut and paste" mechanism. Initially, the enzyme binds to the 19-bp ME of the transposon, forming a complex known as the Transposome. Subsequently, the Transposome initiates random attacks on the phosphodiester backbone of the target DNA, leading to cleavage. 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. This transposition event results in the creation of a 9-bp sequence duplication immediately flanking the site of transposon insertion.

Protocols:

General Protocol for generating a transposon for Illumina platform

1. Preparation of Adapter Mix

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
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

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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

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

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

2. Preparation of Transposon

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

Component	**Concentration**	**Volume**
Adapter Mix (50 μM)	50 μM	4 μl
Tn5 Transposase	10 pmol/μl	20 μl

2. Mix thoroughly by pipetting 20 times.
3. Incubate at RT (25°C) for 1 h, the obtained transposon can be directly used for DNA tagmentation, or stored at -20°C.

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3. DNA Tagmentation

1. Thaw each component at room temperature, mix upside down before use.
2. Prepare the following components to a sterile PCR tube:

Component	**Volume/Amount**
10X Reaction Buffer	2 µl
Transposon	1 µl
DNA Sample	50-100 ng
ddH_2O	to 20 µl

3. Mix thoroughly by pipetting 20 times.
4. Incubate at 55°C for 10 min, then add 5 µL of 10X 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.