Tinzyme Co., Limited

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Tn5 Transposase

Product Number: NG0105

Shipping and Storage

-20 ℃, dry ice transportation.

Component

Component	Volume
Tn5 transposase	50μL
5×FA Reaction Buffer	1.0mL
10×TPS buffer solution	1.0mL

Description

Tn5 transposase is a highly active mutant of wild-type Tn5 transposase, which can efficiently insert Tn5 transposons into target sequences. Tn5 transposase recognizes the inner end (IE), outer end (OE), and chimeric end (ME) sequences of Tn5 transposase sequences, with the highest in vitro transposition efficiency achieved by fragments containing ME sequences. The insertion site of Tn5 transposase has high randomness, making it widely used in fields such as in vitro transgenic (integration of exogenous genes into host cells) and second-generation sequencing library construction.

Application

In vitro genetic modification (integration of exogenous genes into host cells), high-throughput sequencing, CUT&Tag.

Features

1. Product source: Escherichia coli strain containing Tn5 transposase sequence.
2. Stock solution: 50 mM Tris HCl (pH 8.0 @ 25 ° C), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100, and 50% glycerol (V/V).

Protocol

1. Taking the second-generation database construction as an example

1.1. Construction of Transposome

1.1.1. Prepare the following reaction system (using ME as an example for identification sequence)

Name	Volume
Mix with ME sequence (concentration) [*]	2μL
10 x TPS buffer solution	2μL
Tn5 transposase (unit)	2μL(Adjust according to actual needs)
ddH_2O	14μL

[*]: The ME sequence in the adapter must be double stranded and phosphorylated at the end. The adapter mix should be prepared according to the sequencing platform. For example, adapter 1 (Phos represents phosphorylation):

5'-Phos-CTGTCTCTTATACACATCT

3 '- GACAGAATGTGTGATGA+connector 1

Connector 2 (Phos stands for phosphorylation):

5'-Phos-CTGTCTCTTATACACATCT

3 '- GACAGAATGTGTGATGA+connector 2

1.1.2. Mix the reactants well and react at 25°C for 30 minutes. After the reaction is completed, it is 1.2. Transposomes in the 1-step system, spare

1.2. Tagmentation reaction

1.2.1. Prepare the following reaction system

Name	Volume
Genomic DNA	50ng
10 x TPS buffer solution	10μL
Transposome	5μL
water	To 50μL

1.2.2. Fragmented reaction program

Temperature	Time
Hot cover at 105 ℃	On
55 ℃	10min
4 ℃	Hold

Note: If the fragment is larger after fragmentation, the amount of transposase can be changed in the transposon construction step, or the amount of transposon can be changed in the fragmentation reaction step.

1.3. Fragmentation termination purification

The fragment obtained in the previous step can be purified using magnetic beads. If the enzyme is inactivated and the fragment is directly enriched by PCR without purification, the transposase in the fragment may affect the PCR process.

1.4. PCR enrichment

After DNA is digested by transposase, it undergoes cleavage or translation, which requires 72°C and 3 minutes for cleavage repair before PCR amplification. The number of PCR cycles is determined based on the initial amount of DNA, usually 5-15 cycles. The PCR enriched products can be sequenced on the machine after purification and other steps.