Hyperactive pG-Tn5 Transposase for CUT&Tag

Catalog# S602

Version 9.1

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Introduction

Hyperactive pG-Tn5 Transposase for CUT&Tag is a CUT&Tag technology specifically designed for research on protein-genomic interaction. It combines Protein G with engineered ultra-high activity Tn5 transposase to form a novel fusion enzyme with transposase and Protein G activity. Compared with the traditional protein-genomic interaction research method ChIP-Seq, CUT&Tag has significant advantages. The technology shows short operation time, high signal-to-noise ratio, good repeatability and low cell input, and is especially suitable for research on early embryo development, stem cells, tumors, and epigenetics.

Components

Components	S602-01 (10 μg)	S602-02 (20 μg)	
Hyperactive pG-Tn5 Transposase (500ng/μl)[a]	20 μl	40 μl	
5 × Tagment Buffer L[b]	250 μl	500 μl	
Coupling Buffer	250 μl	500 μl	
Annealing Buffer	500 μl	1 ml	

a. The mass concentration of #S602 is 500 ng/μl, converted to a molar concentration of 7.5 pmol/μl;

b. 5 × Tagment Buffer L contains Mg2+, which is used to test the effect of the DNA fragmentation by prepared transposon. It is not CUT&Tag working Buffer.

Storage

The entire kit should be stored at -85°C ~ -65°C and transported on dry ice. After receiving, please store Hyperactive pG-Tn5 Transposase at -85°C ~ -65°C, and store other components at -30°C ~ -15°C.

The prepared transposon should be stored at -30°C ~ -15°C and is valid for one year.

Protocol (for generating a transposon for the Illumina platform)

1.Preparation of Adapter (Adapter Mix):

a. The name and sequence of reference primers for Illumina platform：

Primer A：5'-phos-CTGTCTCTTATACACATCT-NH_2-3'

Primer B*：5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′

Primer C*：5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′

b. Dissolve Primer A, Primer B, Primer C with Annealing Buffer to 100 μM.

c. Prepare the following reaction systems：

Reaction 1			Reaction 2		
Primer A	(100 μM)	10 μl	Primer A	(100 μM)	10 μl
Primer B*	(100 μM)	10 μl	Primer C*	(100 μM)	10 μl
In total		20 μl	In total		20 μl

* The reaction system can be changed in proportion according to the actual needs. The adapter sequence except ME also can be customized.

d. 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℃	On
75℃	15 min
60℃	10 min
50℃	10 min
40℃	10 min
25℃	30 min

e. After the reaction, mix the reaction 1 and the reaction 2 in an equal volume, named Adapter Mix, store at -30°C ~ -15°C.

Protocol

2.Transposon Formation

a. Prepare the following components to a sterile PCR tube in order:

	2 μg preparation system	10 μg preparation system
Hyperactive pG-Tn5 Transposase (500 ng/μl)	4 μl	20 μl
Adapter Mix	0.7 μl	3.5 μl
Coupling Buffer	2.8 μl	14 μl
In total*	7.5 μl	37.5 μl

b. Mix thoroughly by pipetting 20 times. (IMPORTANT!)

c. The reaction was carried out at 30°C for 1 hour. The product was named TTE Mix and can be directly applied to CUT&Tag experiments, or stored at -30°C ~ -15°C.

* The final concentration of the transposon prepared according to this reaction system is 4 pmol/μl, which can be changed in proportion according to actual needs;

Transposon Activity Assay

1. DNA Tagmentation

a. Thaw 5 × Tagment Buffer L at room temperature, mix upside down before use.

b. Prepare the following components to a sterile PCR tube in order:

ddH2O	15-x μl
5 × Tagment Buffer L	4 μl
50 - 100 ng DNA*	x μl
TTE Mix*	1 μl
In total	20 μl

* If you need to improve the degree of fragmentation, please increase the amount of TTE Mix used or reduce the amount of DNA input; On the contrary, if you need to reduce the degree of fragmentation n, please reduce the amount of enzyme used or increase the amount of DNA input.

c. Mix thoroughly by pipetting 20 times. (IMPORTANT!)

d. Place the tubes in a Thermocycler and perform the following program:

Hot lid of 105℃	On
55℃	10 min
4℃	Hold

Take out PCR tube after the sample temperature was lowered to 4°C.

e. Perform purification process of fragmented product. Column or magnetic bead purification can be selected. Elute the final fragmented product using 22 μl of sterilized distilled water. Do not use an elution buffer containing a metal-ion complexing agent such as TE!

2. Amplification of Fragmented Product

a. The name and sequence of reference primers for Illumina platform;b. Prepare the following components to a sterile PCR tube in order:

Primer 1：5′-AATGATACGGCGACCACCGA-3′

Primer 2：5′-CAAGCAGAAGACGGCATACGA-3′

N5：5′-AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC-3′ (add appropriate index 2 sequence at i5)

N7：5′-CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG-3′ (add appropriate index 1 sequence at i7)

b. Place a sterile PCR tube in an ice water bath and add the following reaction components in order:

ddH2O	15 μl
Purified product	20 μl
5 × TAB[a]	10 μl
Primer1[b] (20 μM)	1 μl
Primer2[b] (20 μM)	1 μl
N5[b] (20 μM)	1 μl
N7[b] (20 μM)	1 μl
TAE[a]	1 μl
In total	50 μl

a.This Kit does not contain TAE (TruePrep Amplify Enzyme) and 5 × TAB (TruePrep Amplify Buffer). Amplification module should be purchased separately. Vazyme #TD601 is recommended.

b.This Kit does not contain Primer 1*/Primer 2*, N5/N7 components which should be designed by yourself. Vazyme #TD202-207 is recommended.

c. Mix thoroughly by pipetting 10 times.

d. Place the PCR tube in the PCR Thermocycler and run the following program:

Hot lid of 105℃	On	
72℃	3 min	
98℃	30 sec	
98℃	15 sec	7 - 9 cycles
60℃	30 sec	
72℃	3 min	
72℃	5 min	
4℃	Hold	

3. Product Quality Control

a. Length distribution detection* can be performed using 2% agarose gel electrophoresis or Agilent Technologies 2100 Bioanalyzer.

b. Concentration determination can be performed using the Qubit or VAHTS® Library Quantification Kit (Vazyme #NQ101-NQ106).

* According to the above detection system, 50 ng - 100 ng of genomic DNA can be fragmented into a 200 bp-500 bp of library by 1 μl of transposon.