TIANSeq Fast Ligation Module

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Cat.no. 4992354/4992355

Kit Contents

Contents	4992354 (24 rxn)	4992355 (96 rxn)
TIANSeq DNA Ligase	240 µl	960 µl
5×Ligation Buffer	500 µl	2×1 ml
Nuclease-Free ddH_2O	1 ml	2×1 ml
Handbook	1	1

Storage Conditions

TIANSeq Fast Ligation Module should be stored at -30~-15°C for one year. Avoid repeated freezing and thawing.

Product Description

The TIANSeq Fast Ligation module is a premixed enzyme module optimized for illumina high-throughput sequencing platform. This module can be used to quickly ligase adapters to the DNA fragments with dA-tailing at the 3'-end. Compared to the conventional method, this module adopts the one-step reaction process, in which the DNA fragments obtained from the TIANSeq Fragment/Repair/Tailing Module (Cat# 4992350/4992351) or TIANSeq End Repair/dA-Tailing Module (Cat# 4992352/4992353) reaction could be used directly to the adapter ligation without the need for purification.

This module eliminates the need for a multi-step magnetic beads purification step, which makes the operation much easier and higher library construction efficiency can be achieved.

Application: Quick ligation of DNA adapters to the DNA library fragments (for example, the DNA fragments obtained from the TIANSeq Fragmen/Repair/Tailing Module (Cat# 4992350/4992351) or TIANSeq End Repair/dA-Tailing Module (Cat# 4992352/4992353).

Sample input amount: 0.25 ng-1 μg DNA.

Other Recommended Reagents

1. TIANSeq Fragment/Repair/Tailing Module(Cat# 4992350/4992351)
2. TIANSeq End Repair/dA-Tailing Module(Cat# 4992352/4992353)
3. TIANSeq Single-Indexed Adapter (illumina)(Cat# 4992641/4992642/4992378)
4. TIANSeq Size Selection DNA Beads(Cat# 4992358/4992359/4992979)

Product Highlights

1. The rapid ligation of adapters and DNA fragments can be performed without the need for purification.
2. High ligation efficiency for the DNA-adapter ligation, even for low DNA input amounts.

Precautions **Please carefully read these precautions before using this kit.**

1. Attention should be paid in the operating process to avoid cross-contamination between nucleic acid samples and products.
2. Please use RNase- or DNase-free pipette tips or EP tubes for the experiment.

3. Before starting, wipe down work area with RNase and DNase cleaning reagents such as RNase Away (Molecular BioProducts, Inc). Make sure there is no contamination of RNase and DNase.
4. Before proceeding related operation, make sure the thermal cycler is calibrated and in a stable state.
5. Please read the protocol carefully before the experiment. If test suspension is needed or the downstream test is not needed to be carried out immediately, the test products can be frozen and stored at -20°C and the subsequent test can be planned accordingly.

Protocol:

1. After the end repair/dA-tailing reaction, add Y µl adapter solution to the 50 µl reaction mix, mix gently by pipetting and put on ice.

   Notes: this kit does not contain the DNA adapter for sequencing. Please refer to the usage conditions provided by the adapter supplier. TIANSeq Single-Indexed Adapter (Illumina) (Cat# 4992641/ 4992642/ 4992378) is recommended. To achieve higher ligation efficiency, we recommend the molar ratio of the adapter to the DNA fragments in the reaction mix to be between 10:1 to 200:1. For details, please refer to the TIANSeq Single-Indexed Adapter (Illumina) manual.

2. Prepare the reaction master mix according to the table below. Mix gently by pipetting and then keep it on ice.

Components	Volume (µl)
5×Ligation Buffer	20
TIANSeq DNA Ligase	10
Nuclease-Free ddH_2O	(20-Y)
Total volume	(50-Y)

   Note: for multiple reactions, calculate the total volume of reagents required and increase the volume by 10% to compensate for the pipetting loss, thus to ensure that the solution is sufficient for desired numbers of samples.

3. Add the prepared (50-Y) µl ligation master mix to the reaction solution prepared in Step 1 to generate a 100 µl reaction mix, then gently mix by pipetting up and down for 10 times. Incubate the ligation reaction at 20°C for 15 min.

   Notes: if this step is performed using a thermal cycler, turn on the hot lid and set the temperature ≤ 40°C.

4. It is recommended to use 1× (100 µl) TIANSeq Size Selection DNA Beads (Cat#

4992358/4992359/4992979) for the purification of ligation products. The steps are as follows:

(1) Equilibrate magnetic beads at room temperature for 20 min.

(2) Vortex the magnetic beads to fully suspension. Add 100 µl of the thoroughly vortexed the magnetic beads to the solution in Step 3, and mix well by pipetting up and down for 10 times.

(3) Incubate the mix for 5 min at room temperature. Place the reaction tube on the magnetic stand for 5 min. After the magnetic beads are completely attached, carefully remove the supernatant.

(4) Place the tube on the magnetic stand and add 200-500 µl freshly prepared 80% ethanol (the ethanol should be just enough to immerse all the beads) to the reaction tube, then gently pipette up and down for 3-5 times to wash the magnetic beads (do not disturb the beads). Pellet the magnetic beads with a magnetic stand for 30 sec and discard the supernatant.

(5) Repeat the washing in Step (4) once.

(6) Place the reaction tube containing magnetic beads on the magnetic stand, open the lid and keep it at room temperature for 5-10 min until it is dried.

Note: do not over-dry magnetic beads, as this will cause a decrease in the yield.

(7) Remove the reaction tube from the magnetic stand, and thoroughly resuspend the magnetic beads by adding 22.5 µl 10 mM Tris-HCl (pH 8.0) to the centrifuge tube and gently mix by pipetting up and down for 10 times. Place the tube at room temperature for 5 min, then put the reaction tube on the magnetic stand for 5 min. When the magnetic beads are completely attached, transfer about 20 µl of the supernatant to a new centrifuge tube for subsequent PCR amplification.

(8)If size selection is needed, use 102.5 µl Nuclease-free ddH_2O for beads elution. Transfer 100 µl supernatant to a new centrifuge tube for subsequent size selection.

Note:For size selection, please refer to the fragment size selection protocol in TIANSeq DirectFast DNA Library Kit(illumina)(Cat# 4992259/4992260). Alternatively, if library amplification is not intended for the ligation product, add 12.5 µl of 10mM Tris-HCl (pH 8.0) to Step (6) for the elution of DNA, and 10 µl of the purified DNA can be transferred for subsequent application. If not proceeding immediately, please keep samples stored at -20°C.