NGS DNA Library Prep Kit

(ion torrent platform)

Catalog No. 30051S: 24 reactions
Catalog No. 30051L: 48 reactions

Description

The NGS DNA Library Prep Kit is developed for construction of high quality libraries for next-generation sequencing (Ion Torrent platform). The kit needs double strand DNA fragments (blunt and/or sticky) as input DNA for NGS library construction, and is compatible with DNA fragments generated from both enzymatic methods (dsDNA fragmentase etc.) and physical methods (sonication, nebulization etc.). Our unique technology increases library conversion rate and eliminates insert concatemer ligation.

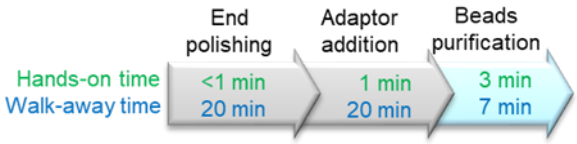

Fig. 1

Features

- Simple: three steps in one tube (Fig.1)
- Fast (Fig.2)
  - Total time: <1 hr
  - Hands-on time: ~5 min
- Guaranteed quality: higher library conversion efficiency as compared to other kits (Fig. 3)
- Input DNA amount: from 50 ng to 1 ug
- No insert concatemer ligation (Fig. 4)

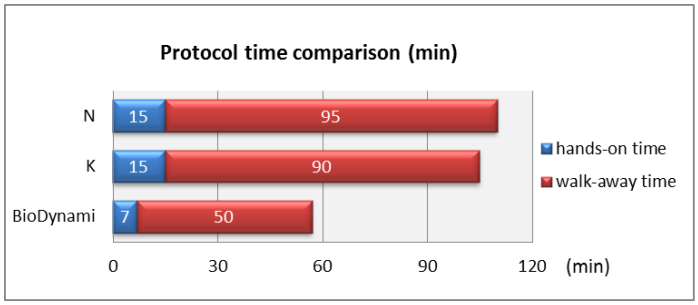

Fig. 2

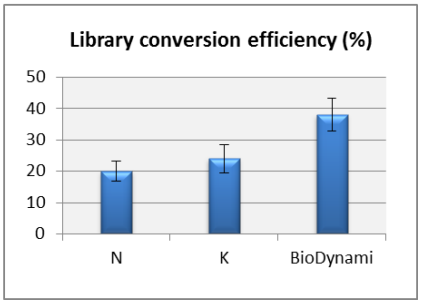

Fig. 3

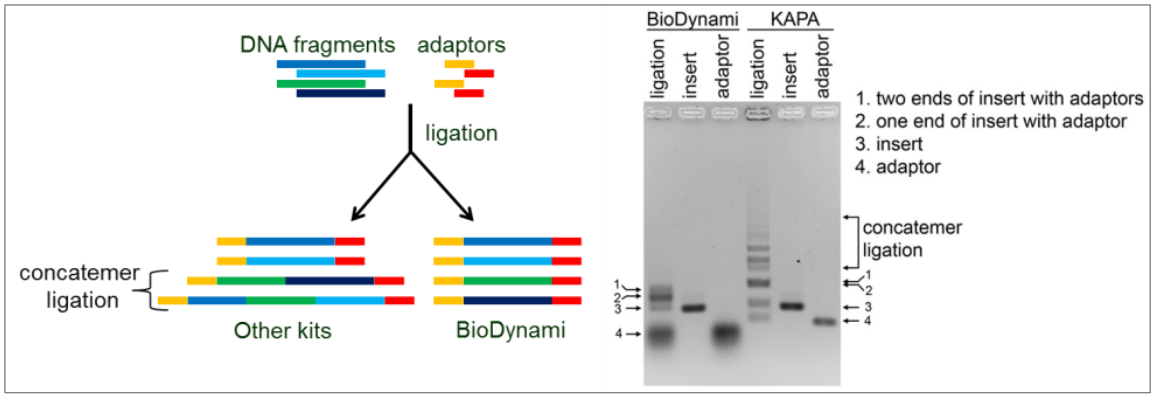

Fig. 4

Component

	Cat.# 30051S	Cat.# 30051L
• DT1 Buffer	96 ul	192 ul
• DT1 Enzyme	96 ul	192 ul
• DT2 Buffer	672 ul	1344 ul
• DT2 Enzyme	48 ul	96 ul
• Sodium Chloride (2M)*	960 ul	1920 ul

Storage Condition

- Store kit at -20°C, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- PCR thermal cycler
- 96-well PCR plate
- 80% ethanol (prepare before use)
- Sodium Chloride (2 M)*
- Magnetic Beads (BioDynami Cat.# 40051) or equivalent

*Sometimes the tube of Sodium Chloride (2 M) may crack during dry ice shipping. Customers need to prepare Sodium Chloride (2 M) in this case.

Protocol

Step 1: End polishing

1) Add the following to one well of a 96-well PCR plate:

Sheared DNA*	42 ul (50 ng~1 ug)
DT1 Buffer	4 ul
DT1 Enzyme	4 ul
Total	50 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 15 min, 70°C for 5 min. Proceed immediately to step 2.

* We recommend purifying the sheared DNA using AMPure XP beads before Step 1. Purifying DNA can improve the quality of the library. We also recommend DNA concentration to be determined by fluorometric methods (picogreen/Qubit) for accuracy.

Step 2: Adaptor addition

1) Add the following to Step 1 reaction mixture. Slow pipetting of the viscous DT2 Buffer is needed for precise aliquot. The total reaction volume is 80 ul.

DT2 Buffer	28 ul
DT2 Enzyme	2 ul
Total	80 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 20 min.
4) Add Sodium Chloride (2 M) 40 ul to the reaction mixture. Proceed immediately to step 3.

Step 3: Beads purification

1) Resuspend Magnetic Beads and transfer 40 ul to the above reaction mixture, mix by pipetting and incubate for 3 min.
2) Load the plate on a magnet, incubate for 2 min, and discard the supernatant carefully.
3) Add 180 ul of 80% ethanol, incubate for 30 sec, and discard the supernatant carefully. Remove all residual ethanol without disturbing the beads.
4) Remove the plate from the magnet, resuspend the beads in 22 ul of water or Tris-HCl (10 mM).
5) Load the plate on the magnet, incubate for 1 min, and transfer 20 ul supernatant (containing library) to a new tube without disturbing the beads.

Attention: PCR prior to sequencing

Amplification of the DNA library created from this kit may be necessary dependent on your project and sequencing platform. Prior to proceeding to PCR or other downstream steps, quantify your library to determine whether the library is enough for your application. Amplify your library according to the instruction from your platform provider.

Attention: Structure of NGS library

NGS DNA library created from this kit will generate an additional C (in red) between insert DNA fragment and adaptor A (Fig.6). Justification may be needed for alignment/assembly of sequences.

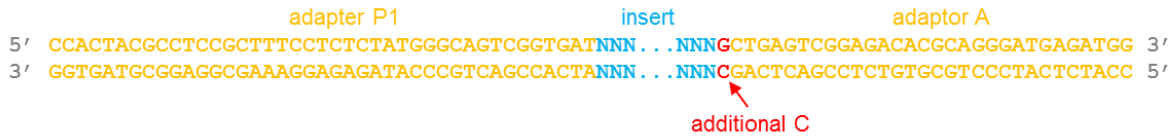

Fig. 6

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

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