NGS DNA Fragmentation & Library Prep Kit

(Ion Torrent platform)

Catalog No. 30052S: 24 reactions
Catalog No. 30052L: 48 reactions

Description

The **NGS DNA Fragmentation & Library Prep Kit** (Ion torrent platform) was developed for construction of high quality libraries for next generation sequencing. The kit uses intact genomic DNA as input DNA without an additional DNA fragmentation step. Our technology provides a fast and simple workflow (Fig. 1). DNA libraries can be generated around 1 hour with only 10 min hands-on time.

Fig. 1 Kit work flow

The incorporation of DNA fragmentation in the kit makes it possible to directly use intact genomic DNA as input DNA without the need of mechanical DNA shearing or enzymatic DNA fragmentation. The protocol is optimized to generate libraries from 200 bp to 500 bp in size (Fig. 2). The library size is inversely correlated with the incubation time of step 1 at 20°C.

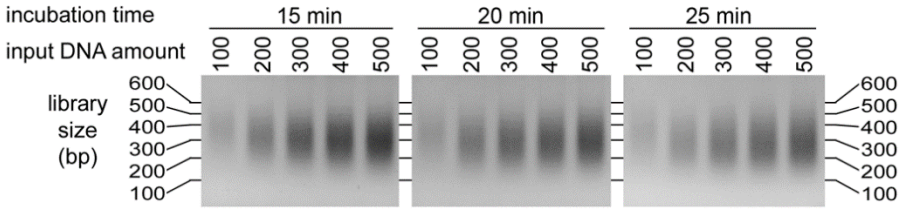

Fig. 2 library size range from 200-500 bp

Features

- Fast: 1-hour library construction from intact genomic DNA to NGS library
  - Total time: 1 hr
  - Hands-on time: 5 min
- Intact genomic DNA as input, DNA fragmentation is not needed.
- Works with both EDTA-free DNA samples and DNA samples in TE buffer
- Simple work flow
  - Less steps
  - Only one beads purification step
- Guaranteed quality: higher library conversion efficiency

Component

	Cat.# 30052S	Cat.# 30052L
• AT1 Enzyme	120 ul	240 ul
• AT2 Buffer	456 ul	912 ul
• AT2 Enzyme	24 ul	48 ul
• EF Buffer	48 ul	96 ul
• Sodium Chloride (1.25 M)*	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 Solution (1.25 M)*
- Magentic Beads (BioDynami Cat.# 40051) or equivalent

*Sometimes the tube of Sodium Chloride (1.25 M) may crack during dry ice shipping. Customers need to prepare Sodium Chloride (1.25 M) in this case.

Protocol

Attention: The quality of genomic DNA may affect DNA fragmentation.

Step 1: Fragmentation/End polishing

1) The following reaction **MUST** be assembled on ice. Add the following to a 96-well PCR plate:

For EDTA-free DNA samples:	
Genomic DNA	13 ul (100~500 ng)
EF Buffer	2 ul
AT1 Enzyme	5 ul
Total	20 ul

For DNA samples in TE buffer:	
Genomic DNA	15 ul (100~500 ng)
AT1 Enzyme	5 ul
Total	20 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 20 min*, 75°C for 10 min. Proceed immediately to step 2.
   * Incubation time affects library size. Longer time will result in shorter library size (use Fig. 2 as a reference).

Step 2: Adaptor addition

1) Add the following to the above reaction mixture. Slow pipetting of the viscous AT2 Buffer is needed for precise aliquot. The total volume is 40 ul.

AT2 Buffer	19 ul
AT2 Enzyme	1 ul
Total	40 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 20 min.
4) Add Sodium Chloride (1.25 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 the type of your library adaptor 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.3). Justification may be needed for alignment/assembly of sequences.

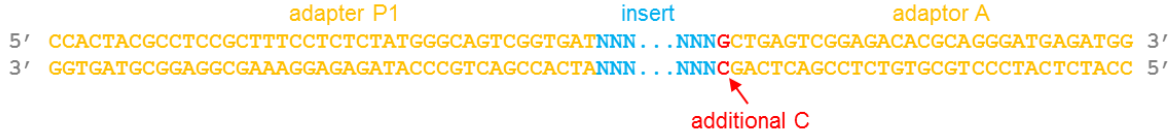

Fig. 3

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to BioDynami.com for Material Safety Data Sheet of the product.

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