igNext™ NGS DNA Library Prep Kit for Illumina

Intact Genomics®

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Table of Contents

The Library Kit Includes

The contents of each kit are sufficient for preparation of up to 12, 48, 96 and more reactions. For best performance, all reagents should be stored at the recommended conditions.

a. igNext™ End Prep Reaction Buffer (10X)
b. igNext™ End Prep Enzyme Mix
c. igNext™ Ligation Master Mix
d. igNext™ Universal Adaptor
e. igNext™ i7 HiFi PCR Master Mix (2X)
f. igNext™ NGS Library Prep with Sample Purification Beads (AMPure® XP Beads)
g. 1X TE Buffer

Materials & Equipment Required *(not included)*

- 80% ethanol (must be freshly prepared)
- Nuclease-free Tubes (1.7 ml / 0.5 ml)
- Magnetic rack/stand
- PCR machine
- DNA shearing machine
- DNA quantification equipment (i.e. Qubit, Agilent bioanalyzer)
- Vortex, centrifuge, etc.

Additional Reagents *(not included)*

- igNext™ Unique dual index primer sets (unless selecting product option w/index included)

Product description

The igNext™ NGS DNA Library Prep Kit includes high-quality buffers and enzymes needed to make DNA fragment libraries for whole genome sequencing on the Illumina platform. This kit facilitates consistent and proven performance for various types of samples. For optimal performance, the igNext™ NGS DNA Library Prep Kit should be used in conjunction with our unique, specific index primer sets. These primer sets provide 48 unique dual indexes and up to 2,304 dual-index combinations, offering a huge range of options for library multiplexing.

At Intact Genomics, we strictly test each batch of products ensuring they meet, or exceed, our published performance specifications.

Information on large volume requirements, customized packaging and specific index combination is available by contacting: sales@intactgenomics.com.

Protocol

Starting Material: 1 – 100 ng fragmented DNA. * It is best to shear the DNA sample in 1X TE buffer. The final volume of fragmented DNA (generally 100~1,000 bp) should be 50 µl.

***Note:** This amount DNA is based on the measurement of Qubit Fluorometer or similar instrument.

Preparation before the library prep procedure:

- Sample Purification Beads need to warm to room temperature for at least 30 min prior to use.
- Set the program for End prep, Adaptor ligation, and PCR in the thermocycler.
- Thaw the Enzyme Mix, buffers, and igNext™ index primer sets on ice at least 30 min before use.
- All reagents should be thoroughly mixed by vortex before use.
- Freshly prepare a sufficient volume of 80% Ethanol.

For a Single Reaction:

1. End Prep

1.1 Add the following components to a PCR tube in order:

- 50 µl Fragmented DNA (1 – 100 ng)
- 6 µl IG igNext™ End Prep Reaction Buffer
- 4 µl igNext™ End Prep Enzyme Mix
- Total Volume 60 ul

1.2 Mix thoroughly with pipette or vortex and briefly centrifuge.
Caution: It is important to mix well and do a brief centrifuge for optimal results

1.3 Place in a thermocycler, with the headed lid set to 100 °C, and run the following program:

- 12 °C for 15 minutes
- 37 °C for 15 minutes
- 72 °C for 30 minutes
- Hold at 4 °C

2. Adaptor Ligation

2.1 Add the following components directly to the End Prep Reaction Mixture in order:

- 60 µl End Prep Reaction Mixture (Step 1.3)
- 30 µl igNext™ Ligation Master Mix
- 10 µl igNext™ Universal Adaptor
- Total Volume 100 µl

2.2 Mix thoroughly with pipette or vortex.
Caution: It is important to mix well and do a brief centrifuge for optimal results

2.3 Place in a thermocycler, without a heated lid, and run the following program:

- 30 °C for 15 minutes
- Hold at 4 °C

Safely stop point: Sample can be stored overnight at 4 °C.

3. Size selection*

- Size selection is an optional step. If the starting materials are less than 50 ng, size selection is not recommended, skip to step 3, and move to step 4 for cleanup without size selection.
- The following size selection steps are specific to mechanically sheared DNA and is suitable for libraries with 300-600 bp inserts. If the experiment requires enzymatically fragmented DNA or a different size range, the volumes of beads used in this section need to be experimentally optimized.
- Final library size is the sum of the insert size, including the size of indexes and primers, around 120 bp.
- Sample Purification Beads need to warm to room temperature for at least 30 min and re-suspended prior to use.

3.1 Add 53 µl **re-suspended Sample Purification Beads** to the 100 µl ligation reaction mixture (step 2.3) and mix thoroughly with pipette or vortex.
Caution: It is important to mix well and do a brief centrifuge for optimal results.

3.2 Incubate at least 5 min at room temperature.

3.3 Place the tube onto magnetic stand and let sit until the solution is clear (approximately 5 mins).

3.4 **Transfer 153 µl of the supernatant into a new tube (Caution: Do not discard the supernatant!)**

3.5 Add **24** µl **re-suspended Sample Purification Beads** and mix thoroughly with pipette or vortex.
Caution: It is important to mix well and do a brief centrifuge for optimal results.

a. Incubate 10 min at room temperature.
b. Place onto magnetic stand and let sit for 5 minutes (or when the solution is clear).
c. Remove and discard supernatant (**Caution: Avoid touching the pipette tip to the accumulated beads!**)
d. Wash beads 2 times with 80% ethanol. (Incubate in ethanol for 30 seconds before removing each time).
e. Briefly centrifuge and place back onto magnetic stand, use a 10 µl pipette to remove all traces of ethanol from the bottom and let samples air dry for 2 minutes. (**Caution: Do not over dry the beads. Proceed to the next step when the beads are still dark brown and glossy looking!)**

3.6 Add 22 µl 1X TE buffer.

a. Incubate 10 minutes at room temperature.
b. Place onto magnetic stand and let sit 5 minutes.
c. Transfer 20 µl to a new PCR tube.

Safely stop point: Sample can be stored overnight at -20 °C.

4. Cleanup Adaptor-ligated DNA without Size Selection

4.1 Add 75 µl re-suspended Sample Purification Beads to the 100 µl ligation reaction mixture (step 2.3) and mix thoroughly with pipette or vortex.
Caution: It is important to mix well and do a brief centrifuge for optimal results

4.2 Incubate 10 minutes at room temperature.

4.3 Place onto magnetic stand and let sit until the solution is clear (approximately 5 minutes).

a. Remove and discard supernatant (**Caution: Avoid touching the pipette tip to the accumulated beads!**)

4.4 Wash beads 2 times with 80% ethanol.

4.5 Short centrifuge and place back onto magnetic stand, use a 10 µl pipette to remove all traces of ethanol from the bottom and let samples air dry for 2 minutes. (**Caution: Do not over dry the beads. Proceed to the next step when the beads are still dark brown and glossy looking!)**

4.6 Add 22 µl 1X TE buffer.

d. Incubate 10 minutes at room temperature.
e. Place onto magnetic stand and let sit 5 minutes.
f. Transfer 20 µl to a new PCR tube.

Safely stop point: Sample can be stored overnight at -20 °C

5. PCR Application

- **For more information regarding index primers, please refer manual in igNext™ unique dual index kit or igNext™ combinatorial dual index kit.**

5.1 Add the following components to cleanup Adaptor ligated DNA (step 3.6 or 4.7) in order:

- 20 cleanup Adaptor ligated DNA
- 25 µl IG i7 HiFi PCR Master Mix
- 5 µl index primer mix
- **Total: 50 µl reaction mix**

5.2 Mix thoroughly with pipette or vortex.
Caution: It is important to mix well and do a quick centrifuge after!

5.3 Place samples on thermocycler using the following PCR program:

Cycle step	Temp	Time	Cycles
Initial Denaturation	**98 °C**	**2 min**	**1**
Denaturation	**98 °C**	**15 sec**	**12**
Anealing	**60 °C**	**30 sec**	
Extension	**70 °C**	**45 sec**	
Final Extension	**70 °C**	**5 min**	**1**
Hold	**4 °C**	**∞**	

Safely stop point: Sample can be stored overnight at -20 °C

6. Final Cleanup

6.1 Add **60** µl **Sample Purification Beads to PCR product from step 6.3** and mix thoroughly with pipette or vortex.
Caution: It is important to mix well and do a quick centrifuge after!

6.2 Incubate 10 minutes at room temperature.

6.3 Place onto magnetic stand and let sit for 5 minutes.

6.4 Wash beads 2 times with 80% ethanol.

a. Incubate in ethanol for 30 seconds before removing each time.

6.5 Short centrifuge and place back onto magnetic stand, use a 10 µl pipette to remove all traces of ethanol from the bottom and let samples air dry for 2 minutes. (**Caution: Do not over dry the beads. Proceed to the next step when the beads are still dark brown and appear glossy!)**

6.6 Add 22 µl TLE.

a. Incubate 10 minutes at room temperature.
b. Place onto magnetic stand and let sit 5 minutes.
c. Transfer 20 µl to a new PCR tube and store at -20 °C

For multiple reactions:

- Prepare strip tubes or 96-well plates and Cover Foil.
- Multiple channel pipettes are needed.
- Make a calculation for the volume of End Prep reaction mix, ligation reaction mix, and PCR reaction mix based on how many reactions need to process.

All Steps are the same as that for single reaction.

Kit Components

Product
IG End Prep Reaction Buffer (10X)
IG End Prep Enzyme Mix
IG Ligation Master Mix
IG i7 HiFi PCR Master Mix (2X)
IG Universal Adaptor
TE Buffer
Sample Purification Beads