Arima-HiC Kit

Library Preparation using NEBNext® Ultra™ II DNA Library Prep Kit

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U.S. Patent No. US 9,434,985 pertains to the use of this product.

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Revision History

Document	Date	Description of Change
Material Part Number: A510008 **Document Part Number:** A160141 v00	November 2018	Initial Release
Material Part Number: A510008 **Document Part Number:** A160141 v01	October 2019	• Added procedure to detach Arima-HiC library from Enrichment Beads prior to PCR in *Library Amplification* section.

Table of Contents

1.1 Kit Contents and Storage

- The Arima-HiC Kit contains the following reagents to be used during Library Preparation:

Kit Location	Reagent	Storage Temperature
Box A	Wash Buffer Elution Buffer	RT
Box C	Enrichment Beads	2 to 8°C

1.2 Handling and Preparation

- The majority of required reagents for the *Library Preparation* protocol are included in the NEBNext® Ultra™ II DNA Library Prep kit. Other required reagents are either supplied in the Arima-HiC kit, or, listed below in Section 1.3.
- Safe stopping points are indicated where the option is available.
- The **Enrichment Beads** should be thoroughly mixed by pipetting or vortexing until homogeneous directly before use.
- The **Wash Buffer** and **Elution Buffer** should be kept at room temperature during handling and preparation.

1.3 User-supplied reagents, consumables and equipment checklist

- ☐ NEBNext® Ultra™ II DNA Library Prep kit (e.g. NEB Cat # E7645S or E7645L)
- ☐ NEBNext® Singleplex or Multiplex Oligos for Illumina® (e.g. NEB Cat # E7350, E7335, E7500, E7710, E7730, E7600, or E6609)
- ☐ DNA Purification Beads (e.g. Beckman Coulter Cat # A63880)
- ☐ Qubit® Fluorometer, dsDNA HS Assay Kit and required consumables (e.g. Thermo Fisher Scientific Cat # 32851, 32856)
- ☐ Freshly prepared 80% Ethanol
- ☐ 1.7mL microcentrifuge tubes, PCR tubes or PCR plates
- ☐ Magnetic rack for 1.7mL microcentrifuge tubes, PCR tubes or PCR plates
- ☐ Instrument for DNA Fragmentation (e.g. Covaris® or Diagenode®) and consumables.
- ☐ Thermal cycler
- ☐ Thermomixer (if doing some steps in 1.7mL microcentrifuge tubes)
- ☐ Gel Electrophoresis System (e.g. Bioanalyzer®, TapeStation®, etc.)

Input: Proximally-ligated DNA
Output: Bead-bound Arima-HiC library

Overview: Library preparation begins with DNA fragmentation (Section 2.1), DNA size selection (Section 2.2), and biotin enrichment (Section 2.3). Afterwards, NEBNext® Ultra™ II DNA Library Prep reagents are used in a custom end-repair, dA-tailing and adapter ligation protocol (Section 2.4). This custom protocol contains specific modifications to the standard NEBNext® Ultra™ II DNA Library Prep protocol that must be performed. All buffers and enzymes provided by the NEBNext® Ultra™ II DNA Library Prep kit are used, and most additional required reagents are provided in the Arima-HiC kit. This custom *Library Preparation* protocol constructs libraries while DNA is bound to the **Enrichment Beads**. The final step is PCR amplification of the Arima-HiC library using NEBNext® Ultra™ II DNA Library Prep library amplification reagents, producing the final sequence-ready Arima-HiC library.

2.1 DNA Fragmentation

Before you begin: The output of the *Arima-HiC Protocol* is large proximally-ligated DNA molecules. These large DNA molecules must be fragmented using mechanical methods to limit sequence bias, and then prepared as a sequencing library that is compatible with Illumina® sequencing instruments. Covaris® instruments are recommended for mechanical fragmentation of DNA, although Diagenode® instruments have also been tested and yield comparable results. DNA should be fragmented in 100µL of **Elution Buffer**. Some Covaris® protocols recommend DNA fragmentation in 130µL, but 100µL must be used for DNA fragmentation in the Arima-HiC library preparation protocol. It is recommended to fragment at least 750ng of DNA per sample, or up to 5µg (depending on the DNA fragmentation instrument manufacturer recommendations). For certain applications, less than 750ng of DNA could be used.

1. If necessary, add **Elution Buffer** to bring the sample volume to 100µL. Do not exceed 100µL of volume for DNA fragmentation.
2. Fragment DNA to obtain an average fragment size of 400bp. *Please use the DNA fragmentation instrument manufacturer default settings for obtaining a target fragment size of 400bp.* For example, Covaris® publishes optimal DNA fragmentation Power, Duty Factor, Cycles per Burst, and Time for obtaining a target fragment size of 400bp.
3. Samples may be stored at -20°C for up to 3 days.

Recommended QC before proceeding: Run an aliquot of fragmented DNA on a gel electrophoresis system (e.g. Bioanalyzer®, TapeStation®) to confirm an appropriate fragment size distribution centered around 400bp.

2.2 DNA Size Selection

Before you begin: Fragmented DNA must be size-selected to have a size distribution between 200 – 600bp. This workflow can be performed in microfuge tubes as well as most PCR tubes and PCR plates. Ensure that your tubes or plates can hold up to 200µL of sample volume. Also ensure that you have a magnetic rack that fits your choice of sample tube/plate.

Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Enrichment Beads or QC Beads provided in the Arima-HiC kit. For the ethanol washes performed below, use sufficient 80% ethanol to fully submerge the magnetized beads.

1. Transfer fragmented DNA sample from fragmentation tube to either a microfuge tube, PCR tube, or PCR plate. If necessary, add **Elution Buffer** to bring sample volume to 100µL.
2. Add 60µL of **DNA Purification Beads**, mix thoroughly by pipetting, and incubate at RT for 5 min.
3. Place sample against magnet, and incubate until solution is clear.
4. Transfer ~160uL *of supernatant* to a new sample tube or well of a PCR plate. Discard beads.
5. Add 40µL of **DNA Purification Beads** to the ~160µL of supernatant, mix thoroughly by pipetting, and incubate at RT for 5 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
8. Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
9. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
10. Remove the sample from magnet, resuspend beads in 100µL of **Elution Buffer**, and incubate at RT for 5 min.
11. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new sample tube or well of a PCR plate.
12. Quantify sample using Qubit®.
13. Samples may be stored at -20°C for up to 3 days.

2.3 Biotin Enrichment

Before you begin: This workflow can be performed in microfuge tubes as well as most PCR tubes and PCR plates. Ensure that your tubes or plates can hold up to 200µL of sample volume. Also ensure that you have a magnetic rack that fits your choice of sample tube/plate. Set your thermal device (thermal cycler or thermomixer) to hold at 55°C.

1. Transfer **125ng – 2µg*** of size-selected DNA into a new microfuge tube, PCR tube, or well of a PCR plate. If necessary, add **Elution Buffer** to bring sample volume to 100µL.

* Biotin enrichment and subsequent library preparation has been optimized to deliver peak performance for DNA inputs ranging from 125ng-2µg. While input amounts closer to 2µg will result in more complex Arima-HiC libraries, 125ng of input has been shown to build libraries with sufficient complexity for 600M read-pairs of sequence data.

2. Add 100µL of **Enrichment Beads**, mix thoroughly by pipetting, and incubate at RT for 15 min.
3. Place sample against magnet, and incubate until solution is clear.
4. Discard supernatant, and remove sample from magnet.
5. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant, and remove sample from magnet.
8. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
9. Place sample against magnet, and incubate until solution is clear.
10. Discard supernatant, and remove sample from magnet.
11. Wash beads by resuspending in 100µL of **Elution Buffer**.
12. Place sample against magnet, and incubate until solution is clear.
13. Discard supernatant, and remove sample from magnet.
14. Resuspend beads in 50µL of **Elution Buffer.**

2.4 End Repair, dA-tailing, and Adapter Ligation

Before you begin: This custom protocol *resembles* the standard NEBNext® Ultra™ II DNA Library Prep protocol but has been modified for library preparation while DNA is bound to the **Enrichment Beads**. The **Wash Buffer** and **Elution Buffer** provided in the Arima-HiC kit are used during this protocol. This protocol must be performed in PCR tubes or PCR plates. Ensure that your tubes or plates can hold up to 150µL of sample volume.

Note: Steps 1 and 3 require the addition of buffer and enzyme reagents, which should be prepared as a master mix and added in a single pipetting step.

1. Add 10µL of a master mix containing the following reagents to 50µL of bead-bound, biotin-enriched DNA:

Reagent	Volume per reaction	10% extra		# reactions		Final
End Prep Reaction Buffer	7µL	7.7µL	x	2	=	15.4µL
End Prep Enzyme Mix	3µL	3.3µL	x	2	=	6.6µL
Total	**10µL**					22µL

2. Mix thoroughly by pipetting until homogeneous, and incubate as follows in a thermal cycler with the lid temperature set to >75°C. Once completed, immediately proceed to the next step.

Temperature	Time
20°C	30 min.
65°C	30 min.
4°C	Hold

3. Add 33.5µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Ligation Master Mix	30µL	33µL	x	2	=	66µL
Ligation Enhancer	1µL	1.1µL	x	2	=	2.2µL
Adaptor for Illumina®	2.5µL	2.75 µL	x	2	=	5.5µL
Total	**33.5µL**					73.7µL

4. Mix thoroughly by pipetting until homogeneous, and incubate as follows in a thermal cycler with the heated lid turned off. Once completed, immediately proceed to the next step.

Temperature	Time
20°C	15 min.
4°C	Hold

5. Add 3µL of USER™ Enzyme, mix thoroughly by pipetting, and incubate at 37°C for 15 min. in a thermal cycler with the lid temperature set to >47°C. Once completed, immediately proceed to the next step.

Note: Steps 8 and 11 require incubations at 55°C. Set your thermal device to hold at 55°C.

6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant, and remove sample from magnet.
8. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
9. Place sample against magnet, and incubate until solution is clear.
10. Discard supernatant, and remove sample from magnet.
11. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
12. Place sample against magnet, and incubate until solution is clear.
13. Discard supernatant, and remove sample from magnet.
14. Wash beads by resuspending in 100µL of **Elution Buffer**.
15. Place sample against magnet, and incubate until solution is clear.
16. Discard supernatant, and remove sample from magnet.
17. Resuspend beads in 15µL of **Elution Buffer**.
18. Samples may be stored at 4°C for up to 3 days.

Library Amplification

Before you begin: The following *Library Amplification* protocol first detaches the Arima-HiC library from the **Enrichment Beads,** and then carries out PCR without the Enrichment Beads present in the reaction. The protocol utilizes the PCR reagents included in the NEBNext® Ultra™ II DNA Library Prep Kit, as well as the various NEBNext® Oligos for Illumina® kits. We recommend using 10 cycles of PCR, as this should be sufficient to obtain enough library material for DNA sequencing as long as >125ng of DNA was used as input in the *Biotin Enrichment* section. If the input into biotin enrichment was <125ng, please contact Technical Support for further guidance. Additionally, please contact Technical Support for recommended library amplification modifications for Capture-HiC.

1. Detach the Arima-HiC library from the **Enrichment Beads** by incubating 15µL of bead-bound Arima-HiC library at 98°C for 10 min. in a thermal cycler with the lid temperature set to 105°C. Once completed, immediately proceed to the next step.
2. Place sample against magnet, incubate until solution is clear, and transfer 15µL of supernatant (i.e. the detached Arima-HiC library) to a new PCR tube.

Note: Choose to perform either Step 3a or Step 3b, depending on which NEBNext® Oligos are used for Library Amplification.

3a. For NEBNext® Singleplex or Muliplex Oligos for Illumina® where the forward and reverse primers are NOT premixed (Cat # E7350, E7335, E7500, E7710, E7730, or E7600), add 30µL of the following master mix to 15µL of Arima-HiC library. Then, add 5µL of a unique **Index Primer/i7 Primer** to each Arima-HiC library.

Reagent	Volume per reaction	10% extra		# reactions		Final
NEBNext® Ultra™ II Q5 Master	25µL	27.5µL	x	2	=	55µL
Universal PCR Primer/i5 Primer	5µL	5.5µL	x	2	=	11µL
Total	**30µL**					66µL

3b. For NEBNext® Multiplex Oligos for Illumina® where the forward and reverse primers are premixed (Cat # E6609), add 25µL of **NEBNext® Ultra™ II Q5 Master Mix** to 15µL of Arima-HiC library. Then add 10µL of a unique **Index/Universal Primer** to each Arima-HiC library.

4. Mix thoroughly by pipetting until homogeneous, and run the following PCR program in a thermal cycler with the lid temperature set to 105°C:

Cycles	Temperature	Time
1 X	98°C	30 sec.
10 X	98°C	10 sec.
	65°C	75 sec.
1 X	65°C	5 min.
1 X	10°C	Hold

Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Enrichment Beads or QC Beads provided in the Arima-HiC kit.

5. Add 45µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant. While sample is still against magnet, add 150µL of **80% ethanol**, and incubate at RT for 1 min.
8. Discard supernatant. While sample is still against magnet, add 150µL of **80% ethanol**, and incubate at RT for 1 min.
9. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
10. Remove the sample from magnet, resuspend beads in 50µL of **Elution Buffer**, and incubate at RT for 5 min.
11. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
12. Repeat Steps 5-9. Once Step 9 has been completed, proceed to Step 13 below.
13. Remove the sample from magnet, resuspend beads in 25µL of **Elution Buffer**, and incubate at RT for 5 min.
14. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
15. Quantify sample using Qubit®.
16. Store Arima-HiC libraries at -20°C until standard library QC (Bioanalyzer®, qPCR) and sequencing.

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