User Guide

Arima High Coverage 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: A410110 **Document Part Number:** A160171 v00	June 2020	Initial Release
Material Part Number: A410110 **Document Part Number:** A160171 v01	November 2021	Changed Arima-HiC 2.0 to Arima High Coverage HiC

Table of Contents

1.1 Kit Contents and Storage

- The Arima High Coverage 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 High Coverage 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)
- ☐ Custom Truncated Adaptor and Custom indexing PCR primers
- ☐ KAPA® Library Amplification Kit (KAPA® Cat # KK2611 or KK2612)
- ☐ 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 High Coverage 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) using custom truncated sequencing adapters *not* provided in the NEBNext® Ultra™ II DNA Library Prep. 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 High Coverage 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 High Coverage HiC library using KAPA® library amplification reagents and custom indexing PCR primers, producing the final sequence-ready Arima High Coverage HiC library.

2.1 DNA Fragmentation

Before you begin: The output of the *Arima High Coverage 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 130µL of **Elution Buffer**. If sample quantity is not limiting, it is recommended to fragment at least 500ng of DNA per sample, or up to 5µg (depending on the DNA fragmentation instrument manufacturer recommendations). However for certain applications, less than 500ng of DNA could be used.

Note: DNA fragmentation is especially important to the analytical performance of the Arima High Coverage HiC data. Below, DNA is fragmented to a relatively larger size (~550-600bp). If your DNA fragmentation instrument doesn't have default settings for fragmenting DNA to 550-600bp, please error on the side of caution by fragmenting to larger sizes, checking DNA size by gel electrophoresis, and then add additional shearing if necessary. Please do not over-fragment the DNA or else a significant portion of DNA will be lost during Size Selection (Section 2.2).

1. If necessary, add **Elution Buffer** to bring the sample volume to 130µL.
2. Fragment DNA to obtain an average fragment size of 550-600bp. *Please use the DNA fragmentation instrument manufacturer default settings for obtaining a target fragment size of 550-600bp. If manufacturer default settings for 550-600bp are not available, then we recommend using slightly less shearing time than that of a target size of 500bp.*

Exemplary Covaris® E220 settings are noted below for obtaining an target fragment size of 550-600bp.

Setting	Value
Temperature	7°C
Peak Incident Power	105
Duty Factor	5%
Cycles per Burst	200
Treatment time (s)	70

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 550-600bp. Please note that different gel electrophoresis systems can produce slightly different results. For more information, please see the Application Note from Covaris (link).

2.2 DNA Size Selection

Before you begin: Fragmented DNA must be size-selected to have a size distribution >400bp. 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 225µL 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 High Coverage 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 130µL.
2. Add 91µL 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. Discard supernatant. While sample is still against magnet, add 225µL of **80% ethanol**, and incubate at RT for 1 min.
5. Discard supernatant. While sample is still against magnet, add 225µL of **80% ethanol**, and incubate at RT for 1 min.

6. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
7. Remove the sample from magnet, resuspend beads in 100µL of **Elution Buffer**, and incubate at RT for 5 min.
8. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new sample tube or well of a PCR plate.
9. Quantify sample using Qubit®.
10. 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 High Coverage HiC libraries, 125ng of input has been shown to build libraries with sufficient complexity for 600M read-pairs of sequence data. Please contact Technical Support for more information about low input library preparation.

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**, and for use with custom truncated sequencing adapters rather than the adapters provided in the NEBNext Ultra II DNA Library Prep kit. The **Wash Buffer** and **Elution Buffer** provided in the Arima High Coverage 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 31µL 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
Total	**31µL**					68.2µL

4. Add 2.5µL of an unindexed **Custom Truncated Adaptor** and mix thoroughly by pipetting.

5. 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

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 20µL 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 utilizes the PCR reagents included in the KAPA® Library Amplification Kit along with **custom indexing PCR primers**. If >125ng of DNA was used as input in the *Biotin Enrichment* section, we recommend using 10 cycles of PCR to obtain enough library material for DNA sequencing. 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. Add 25µL of **2X HiFi HotStart Ready Mix** to 20µL of bead-bound Arima High Coverage HiC library.
2. Add 5µL* of **custom indexing PCR primers**.

* The addition of 5µL should bring the final concentration of each primer to 0.5-2µM

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

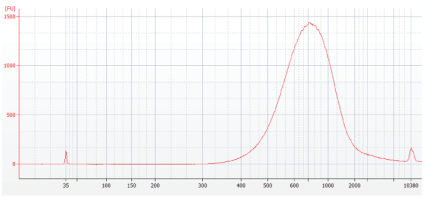
Cycles	Temperature	Time
1 X	98°C	45 sec.
User-defined (e.g. 10 X)	98°C	15 sec.
	60°C*	30 sec.
	72°C	30 sec.
1 X	72°C	60 sec.
1 X	10°C	Hold

* Optimization of annealing temperature may be required for certain adapter/primer combinations.

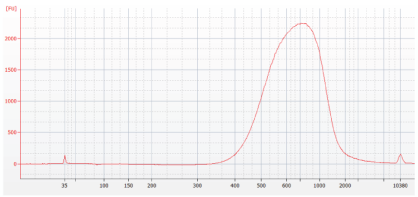
Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Enrichment Beads or QC Beads provided in the Arima High Coverage HiC kit.

4. Add 35µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.
5. Place sample against magnet, and incubate until solution is clear.
6. Discard supernatant. While sample is still against magnet, add 150µL of **80% ethanol**, and incubate at RT for 1 min.
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, incubate beads at RT for 3 – 5 min. to air-dry the beads.

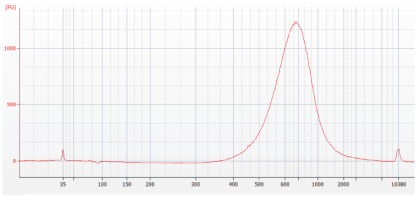
9. Remove the sample from magnet, resuspend beads in 20µL of **Elution Buffer**, and incubate at RT for 5 min.
10. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
11. Quantify sample using Qubit®. Successfully amplified libraries should have a concentration of at least 2.31ng/µL, which is equivalent to 5nM assuming the library size is 700bp including adapters.
12. Store Arima High Coverage HiC libraries at -20°C until standard library QC (Bioanalyzer®, qPCR) and sequencing. Exemplary Bioanalyzer® traces from the High Sensitivity DNA Kit are below for reference.



Sample: Zebrafish Tissue
Peak Size: 733bp



Sample: Mouse Tissue
Peak Size: 703bp



Sample: Rat Tissue
Peak Size: 674bp

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CONTACT US

Technical Support: techsupport@arimagenomics.com

Order Support: ordersupport@arimagenomics.com