User Guide

Arima High Coverage HiC Kit

Library Preparation using Illumina® TruSeq® DNA PCR-Free Library Prep Kit

Material Part Number: Arima High Coverage HiC
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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: Arima High Coverage HiC Early Access Kit **Document Part Number:** A160174 v00	October 2019	Initial Release
Material Part Number: Arima High Coverage HiC Early Access Kit **Document Part Number:** A160174 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 Early Access 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 Illumina® TruSeq® DNA PCR-Free Library Prep Kit. Other required reagents are either supplied in the Arima High Coverage HiC Early Access 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.
- To maximize efficient use of enzyme reagents, thaw enzymes for at least 10 min. on ice, then mix by inversion (not vortexing) and spin down enzyme reagents prior to pipetting.

1.3 User-supplied reagents, consumables and equipment checklist

- ☐ Illumina® TruSeq® DNA PCR-Free Library Prep Kit (Illumina® Cat # 20015962)
- ☐ KAPA® Library Quantification Kit for Illumina® Platforms (e.g. KAPA® Cat # KK4824)
- ☐ KAPA® Library Amplification Kit (KAPA® Cat # KK2620 or KK2621)
- ☐ 15µM Illumina® TruSeq® sequencing adapters (e.g. Illumina® Cat # 20020590)
- ☐ 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 (Sections 2.2), and biotin enrichment (Section 2.3). Afterwards, Illumina® TruSeq® DNA PCR-Free Library Prep kit reagents are used in a custom end-repair and adapter ligation protocol (Section 2.4). This custom protocol contains specific modifications to the standard Illumina® TruSeq® DNA PCR-Free Library Prep kit protocol that must be performed. All buffers and enzymes provided by the Illumina® TruSeq® DNA PCR-Free Library Prep kit are used, and most additional required reagents are provided in the Arima High Coverage HiC Early Access kit. This custom *Library Preparation* protocol constructs libraries while DNA is bound to the **Enrichment Beads**. After the *Library Preparation* protocol, there is a recommended Arima-QC2 Quality Control checkpoint. Lastly, while library preparation is performed using a "PCR-Free" library preparation kit, the Arima High Coverage HiC workflow still requires PCR amplification of the bead-bound Arima High Coverage HiC library using the KAPA® HiFi Hot Start Ready Mix Kit library amplification reagents, which produces 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. DO NOT over-fragment the DNA or else a significant portion of DNA will be lost during Size Selection (Sections 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).

2.2.1 DNA Size Selection – Standard Input (>500 ng)

Before you begin: Fragmented DNA must be size-selected to have a size distribution between 400 – 700bp. 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 Early Access 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 65µL 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 ~195uL *of supernatant* to a new sample tube or well of a PCR plate. Discard beads.
5. Add 26µL of **DNA Purification Beads** to the ~195µL 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.

2.2.2 DNA Size Selection – Low Input (<500 ng)

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 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 High Coverage HiC Early Access 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 200µL of **80% ethanol**, and incubate at RT for 1 min.
5. Discard supernatant. While sample is still against magnet, add 200µL 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 **up to 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 input amounts up to 2µg. While input amounts closer to 2µg will result in more complex Arima High Coverage HiC libraries, input amounts of 125ng or less have 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 and Adapter Ligation

Before you begin: This custom protocol *resembles* the standard Illumina® TruSeq® DNA PCR-Free 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 High Coverage HiC Early Access kit are used during this protocol. This protocol 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 150µL of sample volume. Select a uniquely indexed Illumina® TruSeq® sequencing adapter for each sample.

Note: Steps 1, 15, and 18 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 50µL of a master mix containing the following reagents to 50µL of bead-bound, biotin-enriched DNA:

Reagent	Volume per reaction	10% extra		# reactions		Final
Nuclease-free water	10µL	11µL	x	2	=	22µL
ERP2 or ERP3	40µL	44µL	x	2	=	88µL
Total	**50µL**					110µL

2. Mix thoroughly by pipetting until homogeneous, and incubate as follows in a thermal cycler with the heated lid set to 100°C. Once completed, immediately proceed to the next step.

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

Note: Steps 5 and 8 require incubations at 55°C. Set your thermal device to hold at 55°C.

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 150µL 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 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 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 15µL of **Elution Buffer.**
15. Add 15µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Nuclease-free water	2.5µL	2.75µL	x	2	=	5.5µL
ALT or ALT2	12.5µL	13.75µL	x	2	=	27.5µL
Total	**15µL**					33µL

16. Mix thoroughly by pipetting until homogeneous, and incubate as follows in a thermal cycler with the heated lid set to 100°C. Once completed, immediately proceed to the next step.

Temperature	Time
37°C	30 min.
70°C	5 min.
4°C	5 min.
4°C	Hold

17. Add 1µL of **Illumina® TruSeq® sequencing adapter (15µM)**.
18. Add 6.5µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Nuclease-free water	4µL	4.4µL	x	2	=	8.8µL
LIG2	2.5µL	2.75µL	x	2	=	5.5µL
Total	**6.5µL**					14.3µL

19. Mix thoroughly by pipetting until homogeneous, and incubate as follows in a thermal cycler with the heated lid set to 100°C. Once completed, immediately proceed to the next step.

Temperature	Time
30°C	10 min.
4°C	Hold

20. Add 5µL of STL, mix by pipetting and incubate at RT for 1 min.

Note: Steps 23 and 26 require incubations at 55°C. Set your thermal device to hold at 55°C.

21. Place sample against magnet, and incubate until solution is clear.
22. Discard supernatant, and remove sample from magnet.

23. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
24. Place sample against magnet, and incubate until solution is clear.
25. Discard supernatant, and remove sample from magnet.
26. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
27. Place sample against magnet, and incubate until solution is clear.
28. Discard supernatant, and remove sample from magnet.
29. Wash beads by resuspending in 100µL of **Elution Buffer**.
30. Place sample against magnet, and incubate until solution is clear.
31. Discard supernatant, and remove sample from magnet.
32. Resuspend beads in 22µL of **Elution Buffer**.
33. Samples may be stored at 4°C for up to 3 days.

Arima-QC2 Quality Control

Before you begin: The following protocol utilizes the KAPA® Library Quantification Kit (qPCR assay) to determine the **Arima-QC2** values and estimate the appropriate number of PCR cycles needed for library amplification. After completing the qPCR assay, use the provided **Arima High Coverage HiC QC Worksheet** to determine the Arima-QC2 values and PCR cycle numbers. If performing Capture-HiC, please contact Technical Support for additional guidance.

Note: Step 2 requires the addition of buffer and enzyme reagents, which should be prepared as a master mix and added in a single pipetting step.

1. Prepare a 1:1000 dilution of each bead-bound Arima High Coverage HiC library. To do this, first vigorously mix a bead-bound Arima High Coverage HiC library by vortexing and pipetting until homogeneous. Then, immediately add 1µL of bead-bound library to 999 µL of water.
2. Add 16µL of a master mix containing the following reagents to each well of the qPCR plate that will receive either standards, water, or samples (see Step 3 for recommended plate layout):

Reagent	Volume per reaction	10% extra		# reactions		Final
qPCR Master Mix	10µL	11µL	x	27	=	297µL
Illumina® Primer Mix	2µL	2.2µL	x	27	=	59.4µL
Water	4µL	4.4µL	x	27	=	118.8µL
Total	**16µL**					475.2µL

3. Add 4µL of each Standard, 1:1000 diluted bead-bound Arima High Coverage HiC library, or water to each well containing 16µL of master mix prepared during Step 2. Use the same water source as was used to prepare the qPCR Master Mix. A suggested plate layout is provided below:

Arima-QC2 Recommended Plate Layout											
Std_1	Std_1	Std_1	Std_2	Std_2	Std_2	Std_3	Std_3	Std_3	Std_4	Std_4	Std_4
Std_5	Std_5	Std_5	Std_6	Std_6	Std_6	H_2O	H_2O	H_2O	Lib1	Lib1	Lib1
Lib2	Lib2	Lib2	Lib3	Lib3	Lib3	Lib4	Lib4	Lib4	Lib5	Lib5	Lib5
Lib6	Lib6	Lib6	Lib7	Lib7	Lib7	Lib8	Lib8	Lib8			

Note: "Std" refers to the qPCR Standards provided in the KAPA® Library Quantification Kit. "Lib" refers to each 1:1000 diluted, bead-bound Arima High Coverage HiC library.

4. Run the following qPCR cycling protocol:

Cycles	Temperature	Time
1 X	95°C	5 min.
35X	95°C	30 sec.
	60°C	45 sec.
Melt	65°C - 95°C	-

5. Extract C_q values from the qPCR instrument and follow the **Arima High Coverage HiC QC Worksheet** to calculate the Arima-QC2 values and estimate the required number of PCR cycles for library amplification. High-quality Arima-QC2 values are expected to be >0.2%. If Arima-QC1 and Arima-QC2 values both obtain a 'PASS' status, proceed to the *Library Amplification* protocol and subsequent sequencing analysis. If the Arima-QC2 value did not obtain a 'PASS' status, please contact Technical Support for troubleshooting assistance.

Library Amplification

Before you begin: The following *Library Amplification* protocol utilizes the PCR reagents included in the KAPA® Library Amplification Kit. Determining how many PCR cycles to use for library amplification can be done in 1 of 2 ways – (1) determine the required PCR cycles empirically using the *Arima-QC2 Quality Control* protocol in the previous section, or (2) use the general guideline that 10 cycles of PCR should be sufficient to obtain enough library for DNA sequencing as long as >50ng of DNA was used as input in the *Biotin Enrichment* section. If the *Arima-QC2 Quality Control* protocol was not performed and the input into biotin enrichment was <50ng, please contact Technical Support for further guidance. Additionally, please contact Technical Support for recommended library amplification modifications for Capture-HiC.

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

1. Add 30µL of a master mix containing the following reagents to 20µL of bead-bound Arima High Coverage HiC library:

Reagent	Volume per reaction	10% extra		# reactions		Final
2X HiFi HotStart Ready Mix	25µL	27.5µL	x	2	=	55µL
10X Primer Mix	5µL	5.5µL	x	2	=	11µL
Total	**30µL**					66µL

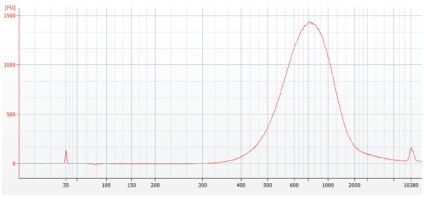
2. Mix thoroughly by pipetting until homogeneous, and run the following PCR program in a thermal cycler with the lid temperature set to 105°C:

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

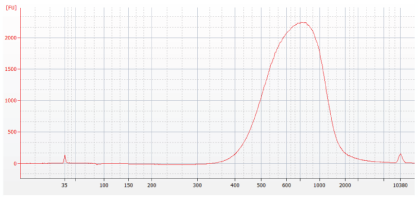
Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Enrichment Beads or QC Beads provided in the Arima High Coverage HiC Early Access kit.

3. Add 35µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.
4. Place sample against magnet, and incubate until solution is clear.

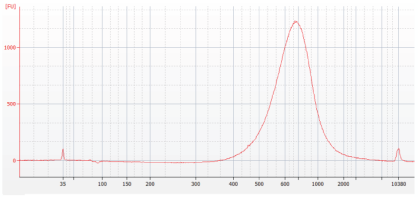
5. Discard supernatant. While sample is still against magnet, add 150µL of **80% ethanol**, and incubate at RT for 1 min.
6. Discard supernatant. While sample is still against magnet, add 150µL of **80% ethanol**, and incubate at RT for 1 min.
7. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
8. Remove the sample from magnet, resuspend beads in 20µL of **Elution Buffer**, and incubate at RT for 5 min.
9. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
10. 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.
11. 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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