



# **Arima-HiC Kit**

## **Library Preparation using KAPA<sup>®</sup> Hyper Prep Kit**

**Material Part Number:** A510008

**Document Part Number:** A160139 v00

**Release Date:** November 2018

This product is intended for research use only. This product is not intended for diagnostic purposes.

This document and its contents are proprietary to Arima Genomics, Inc (“Arima Genomics”). Use of this document is intended solely for Arima Genomics customers for use with the Arima-HiC Kit, PN A510008, and for no other purpose. This document and its contents shall not be used, distributed or reproduced in whole or in part and/or otherwise communicated or disclosed without the prior written consent of Arima Genomics.

This user manual must be read in advance of using the product and strictly followed by qualified and properly trained personnel to ensure proper use of the Arima-HiC kit. Failure to do so may result in damage to the product, injury to persons, and/or damage to other property. Arima Genomics does not assume any liability resulting from improper use of its products or others referenced herein.

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

#### TRADEMARKS

AMPure® is a trademark of Beckman Coulter, Inc.

KAPA® is a trademark of Roche Molecular Systems, Inc.

Covaris® is a trademark of Covaris, Inc.

Diagenode® is a trademark of Diagenode S.A.

Illumina® and TruSeq® are trademarks of Illumina, Inc.

Bioanalyzer® and TapeStation® are trademarks of Agilent Technologies, Inc.

Qubit® is a trademark of Molecular Probes, Inc.

© 2018, Arima Genomics, Inc. All rights reserved.

# Revision History

Document	Date	Description of Change
<b>Material Part Number:</b> A510008 <b>Document Part Number:</b> A160139 v00	November 2018	Initial Release

# Table of Contents

Getting Started .....	5
Library Preparation .....	6-10
Arima-QC2 Quality Control.....	11-12
Library Amplification .....	13-14
Warranty and Contact Info .....	15

# Getting Started

## 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 KAPA® Hyper 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

- Kapa® Hyper Prep Kit with Library Amplification Module (e.g. Cat # KK8500, KK8502, or KK8504)
- KAPA® Library Quantification Kit for Illumina® Platforms (e.g. KAPA® Cat # KK4824)
- 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.)

# Library Preparation

**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, KAPA® Hyper 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 KAPA® Hyper Prep protocol that must be performed. All buffers and enzymes provided by the KAPA® Hyper 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**. After the *Library Preparation* protocol, there is a recommended Arima-QC2 Quality Control checkpoint. The final step is PCR amplification of the bead-bound Arima-HiC library using KAPA® 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 KAPA® Hyper 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 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 and 4 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 Repair and A-Tailing Buffer	7µL	7.7µL	x	2	=	15.4µL
End Repair and A-Tailing Enzyme	3µL	3.3µL	x	2	=	6.6µL
<b>Total</b>	<b>10µL</b>					<b>22µL</b>

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 1µL of Illumina® TruSeq® sequencing adapter (15µM).
4. Add 49µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
PCR-grade water	9µL	9.9µL	x	2	=	19.8µL
Ligase Buffer	30µL	33µL	x	2	=	66µL
DNA Ligase	10µL	11µL	x	2	=	22µL
<b>Total</b>	<b>49µL</b>					<b>107.8µL</b>

5. 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

**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 22µL of **Elution Buffer**.
18. 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-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-HiC library. To do this, first vigorously mix a bead-bound Arima-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
<b>Total</b>	<b>16µL</b>					<b>475.2µL</b>

3. Add 4µL of each Standard, 1:1000 diluted bead-bound Arima-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 <sub>2</sub> O	H <sub>2</sub> O	H <sub>2</sub> O	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-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-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 Library Amplification Module of KAPA® Hyper Prep 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 >125ng 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 <125ng, 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-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
<b>Total</b>	<b>30µL</b>					<b>66µL</b>

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-HiC kit.

3. Add 45µL 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 50µL 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. Repeat Steps 3-7. Once Step 7 has been completed, proceed to Step 11 below.
11. Remove the sample from magnet, resuspend beads in 25µL of **Elution Buffer**, and incubate at RT for 5 min.
12. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
13. Quantify sample using Qubit®.
14. Store Arima-HiC libraries at -20°C until standard library QC (Bioanalyzer®, qPCR) and sequencing.

# Warranty and Contact Info

## WARRANTY DISCLAIMERS

THE EXPRESS WARRANTIES AND THE REMEDIES SET FORTH ABOVE ARE IN LIEU OF, AND ARIMA GENOMICS AND ITS LICENSORS, SUPPLIERS AND REPRESENTATIVES HEREBY DISCLAIM, ALL OTHER REMEDIES AND WARRANTIES, EXPRESS, STATUTORY, IMPLIED, OR OTHERWISE, INCLUDING, BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY, SATISFACTORY QUALITY, NONINFRINGEMENT OR FITNESS FOR A PARTICULAR PURPOSE, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT OR SERVICE (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), IN EACH CASE HOWEVER ARISING, INCLUDING WITHOUT LIMITATION FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE, OR OTHERWISE. TO THE MAXIMUM EXTENT PERMITTED BY APPLICABLE LAW, ARIMA AND ITS LICENSORS, SUPPLIERS AND REPRESENTATIVES SHALL NOT BE LIABLE FOR LOSS OF USE, PROFITS, REVENUE, GOODWILL, BUSINESS OR OTHER FINANCIAL LOSS OR BUSINESS INTERRUPTION, OR COSTS OF SUBSTITUTE GOODS OR SERVICES, OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, EXEMPLARY OR INDIRECT DAMAGES FOR BREACH OF WARRANTY.

## WARRANTY

All warranties are personal to the Purchaser and may not be transferred or assigned to a third-party, including an affiliate of the Purchaser. The warranty described below excludes any stand-alone third-party goods that may be acquired or used with the Product. Arima Genomics only warrants that the kit reagents will be made and tested in accordance with Arima Genomics manufacturing and quality control processes. Arima Genomics makes no warranty that the reagents provided in this kit will work as intended by the Purchaser or for the Purchaser's intended uses. ARIMA GENOMICS MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data and descriptions of Arima Genomics products appearing in Arima Genomics product literature and website may not be altered except by express written agreement signed by an officer of Arima Genomics. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

The foregoing warranties do not apply to the extent a non-conformance is due to (i) abuse, misuse, neglect, negligence, accident, improper storage, or use contrary to the Documentation or Specifications, (ii) use that is an Excluded Use, (iii) improper handling, (iv) unauthorized alterations, (v) natural disasters, or (vi) use with a third-party's good that is not specified in the product documentation. In the event of a breach of the foregoing warranty, customer shall promptly contact Arima Genomics customer support to report the non-conformance and shall cooperate with Arima Genomics in confirming or diagnosing the non-conformance. Additionally, Arima Genomics may request return shipment of the non-conforming product at Arima Genomics cost. Arima Genomics sole obligation shall be to replace the applicable product or part thereof, provided the customer notifies Arima Genomics within 90 days of any such breach. If after exercising reasonable efforts, Arima Genomics is unable to replace the product, then Arima Genomics shall refund to the Purchaser all monies paid for such applicable product.

## CONTACT US

Technical Support: [techsupport@arimagenomix.com](mailto:techsupport@arimagenomix.com)

Order Support: [ordersupport@arimagenomix.com](mailto:ordersupport@arimagenomix.com)

Arima-HiC Kit

User Guide for Library Prep using KAPA® Hyper Prep Kit

Doc A160139 v00