Arima Library Prep Module

User Guide: Library Preparation for Arima High Coverage HiC Kit

16 reactions

Material Part Numbers: A101030, A303011
Document Part Number: A160186 v02
Release Date: May 2024

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 High Coverage HiC Kit, P/N A101030, and the Arima Library Prep Module, P/N A303011, and 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 Library Prep Module. 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
Illumina™, MiSeq™, NextSeq™, HiSeq™, and NovaSeq™ are trademarks of Illumina, Inc.
AMPure™ is a trademark of Beckman Coulter, Inc.
Qubit™ is a trademark of Molecular Probes, Inc.
SSIbio™ is a trademark of Scientific Specialties, Inc.
Bio-Rad™ is a trademark of Bio-Rad Laboratories, Inc.
Fisher Scientific™ is a trademark of Fisher Scientific Company, LLC.
Bioanalyzer™ and TapeStation™ are trademarks of Agilent Technologies, Inc.
Covaris™ is a trademark of Covaris, Inc.
Diagenode™ is a trademark of Diagenode S.A.

Revision History

Document	Date	Description of Change
Material Part Number: A101030, A303011 Document Part Number: A160186 v01	June 2023	Initial Release
Material Part Number: A101030, A303011 Document Part Number: A160186 v02	May 2024	Corrected typos and fixed errors.

Table of Contents

Getting Started

Handling and Preparation

- Several steps during the *Arima Library Prep Protocol* require the preparation of a master mix. Sufficient reagent has been included in the kit to make master mixes with 10% excess volume. Use the master mix calculation tables provided.
- When handling reagents, room temperature (RT) is defined as 20 to 25°C.
- All kit reagents should be fully thawed and thoroughly mixed before use.
- To protect samples from nucleases, we recommend the use of gloves and sterilized filter pipette tips.
- If possible, performing the pre-amplification steps in a "Pre-PCR" environment and the post-amplification steps in a "Post-PCR" environment will reduce PCR contamination.
- DNA Purification Beads (e.g. AMPure XP Beads) should be warmed to RT and thoroughly mixed before each use.
- In steps with magnetic beads that require centrifugation, be careful not to pellet the beads.

Arima materials checklist

☐ Proximally Ligated DNA generated using the **Arima High Coverage HiC Kit (P/N A101030)**

☐ Arima Library Prep Module (P/N A303011)

Table 1: Reagents Included in the Arima Library Prep Module

Cap	Name	P/N	Box	Storage
	Ligation Buffer	A311035-01	A	-20°C
	T4 DNA Ligase	A311035-02	A	-20°C
	End Repair-A-Tailing Buffer	A311035-03	A	-20°C
	End Repair-A Tailing Enzyme Mix	A311035-04	A	-20°C
	Herculase II Fusion DNA Polymerase	A311035-05	A	-20°C
	5x Herculase II Buffer with dNTPs	A311035-06	A	-20°C
	Adaptor Oligo Mix	A311035-07	A	-20°C
	Index 1-16	A311036-01 – A311036-16	B	-20°C
	T1 Beads	A311042-01	C	4°C
	Binding Buffer	A311041-01	D	RT

User-supplied reagents, consumables, and equipment checklist

- ☐ Freshly prepared 80% Ethanol
- ☐ DNA Purification Beads e.g. Beckman Coulter Cat # A63880, Qubit Fluorometer, dsDNA HS Assay Kit and consumables (e.g. Thermo Fisher Scientific Cat # 32851, 32856)
- ☐ 1.7mL microcentrifuge tubes, PCR tubes (e.g. SSIbio Cat # 3247-00), or PCR plates (e.g. Bio-Rad Cat # HSS9641) and magnetic rack compatible with tube selection.
- ☐ Centrifuge
- ☐ Thermal cycler
- ☐ DNA fragmentation device (e.g Covaris or Diagenode Bioruptor Pico)
- ☐ 8-well PCR Strip Tubes with Caps
- ☐ Optional: 8- or 12-channel 200 µL Multi-Channel Pipette. Recommended when processing more than 4 samples at a time.
- ☐ Gel Electrophoresis System (e.g. Bioanalyzer, TapeStation, etc.)
- ☐ Deionized / Nuclease-free Water

How to cite Arima High Coverage HiC in publications

When citing the Arima High Coverage HiC protocol or kit, one may write: "Hi-C data was generated using the Arima High Coverage HiC kit and the Arima Library Prep Module according to the Arima Genomics manufacturer's protocols".

Input: Proximally Ligated DNA generated using the **Arima High Coverage HiC Kit (P/N A101030)**
Output: Arima High Coverage HiC library ready for sequencing
Components:

- **Arima High Coverage HiC, Box A (RT)**
  - Elution Buffer
  - Wash Buffer
- **Arima Library Prep Module Box A (-20°C)**
  - End Repair-A Tailing Enzyme Mix
  - End Repair-A Tailing Buffer
  - T4 DNA Ligase
  - Ligation Buffer
  - Adaptor Oligo Mix
  - 5X Herculase II Buffer with dNTPs
  - Herculase II Fusion DNA Polymerase
- **Arima Library Prep Module Box B (-20°C)**
  - Index Primer Pair 1 – 16
- **Arima Library Prep Module Box C (4°C)**
  - T1 Beads
- **Arima Library Prep Module Box D (RT)**
  - Binding Buffer
- **User Supplied Reagents**
  - DNA Purification Beads
  - 80% ethanol
  - Qubit assay and tubes

Overview: Library preparation begins with DNA fragmentation (Section 2.1), DNA size selection (Section 2.2), and biotin enrichment (Section 2.3). Afterward, the Arima Library Prep Module reagents are used in a custom end-repair, dA-tailing, and adapter ligation protocol (Section 2.4). This custom *Arima Library Preparation Protocol* constructs libraries while DNA is bound to T1 Beads. The final step is PCR amplification of the bead-bound Arima High Coverage HiC library using the library amplification reagents and index PCR primers from the Arima Library Prep Module, producing the final Arima High Coverage HiC library.

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 1500ng of DNA per sample, or up to 5µg (depending on the DNA fragmentation instrument manufacturer recommendations). However, for certain applications, less than 750ng of DNA could be used.

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 a 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.

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 T1 Beads provided in the Arima Library Prep. Module. 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 1 min. to air-dry the beads.
7. Remove the sample from magnet, resuspend beads in 30 µL 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™. Record this value.
10. Samples may be stored at -20°C for up to 3 days.

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 230µL 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.

Note: T1 Beads used directly below are from the Arima Library Prep Module. They should not be mistaken for and are NOT interchangeable with the Arima High Coverage HiC Enrichment Beads nor the Arima High Coverage HiC QC Beads

1. Mix T1 Beads very well before using, making sure that the solution is homogenous and there is nothing sticking to the bottom of the bottle.
2. Add 12.5µL of T1 Beads from the Arima Library Prep Box C into a well of a strip tube for each sample. Note: These beads are NOT the Enrichment Beads that come with the Arima High Coverage HiC kit.
3. Wash the T1 Beads in each tube by:
4. Add 200uL of Binding Buffer.
5. Mix by pipetting up and down 20 times, cap the tubes, and vortex at high speed for 5 - 10 seconds.
6. Place tubes against a magnet and incubate 5 minutes or until solution is clear.
7. Discard supernatant and remove the tube from magnet.
8. Repeat steps 4–7 two more times for a total of three washes.
9. Resuspend beads in 200uL of Binding Buffer.

10. Transfer exactly 200ng* 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 30µL.
**Biotin enrichment and subsequent library preparation has been optimized to deliver peak performance for DNA inputs of 200ng. Using 200ng of DNA input has been shown to build libraries with sufficient complexity for up to 600M read-pairs of sequence data. If the amount of DNA is less than 200ng, add in the entire amount.*
11. Add 200µL of washed T1 Beads in Binding Buffer, mix thoroughly by pipetting, and incubate at RT for 15 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 200µL of Wash Buffer and incubate at 55°C for 2 min. Set lid temperature to 85°C.
15. Place sample against magnet and incubate until solution is clear.
16. Discard supernatant and remove sample from magnet.
17. Wash beads by resuspending in 200µL of Wash Buffer and incubate at 55°C for 2 min. Set lid temperature to 85°C.
18. Place sample against magnet and incubate until solution is clear.
19. Discard supernatant and remove sample from magnet.
20. Wash beads by resuspending in 100µL of Elution Buffer.
21. Place sample against magnet and incubate until solution is clear.
22. Discard supernatant and remove sample from magnet.
23. Resuspend beads in 50µL of Deionized / Nuclease-free Water.

Library Preparation of Enriched Ligation products

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.

End Repair

1. Thaw reagents and mix reagents according to Table 2.
Note: Thaw ligation buffer and vortex on high to make sure homogenous (buffer is highly viscous).

Table 2: Thawing and Mixing Instructions for End Repeat

Reagent	Thaw Temp.	Mix	Cap
End Repair-A Tailing Buffer	On Ice	Vortex	Yellow
Ligation Buffer	On Ice	Vortex	Purple
End Repair-A Tailing Enzyme Mix	Ice Just Before Use	Inversion	Orange
T4 DNA Ligase	Ice Just Before Use	Inversion	Blue
Adaptor Oligo Mix	On Ice	Vortex	Clear

2. Prepare Ligation master mix to allow equilibration to room temperature before use (see Table 3, which includes 12.5% master mix overage for 8 reactions).

Table 3: Ligation Master Mix Worksheet

Reagent	Volume per reaction	12.5% extra		# reactions		Final
Ligation Buffer	23µL	25.88µL	x	8	=	207µL
T4 DNA Ligase	2µL	2.25µL	x	8	=	18µL
Total	25µL					225µL

Note: Ligation Master Mix will be used in Adapter Ligation Step Below (After End Repair and dA Tailing)

3. Keep Ligation Master Mix at room temperature for 30 to 45 minutes before use.

Prepare End Repair-A Tailing Master Mix

1. Vortex thawed vial of End Repair-A Tailing Buffer for 15 seconds – continue vortexing until no solids are observed.
2. Prepare End Repair/dA-Tailing master mix by combining reagents as listed in
3. Table 4, mix well and spin down.

Table 4: End Repair/dA Tailing Master Mix Worksheet

Reagent	Volume per Reaction	12.5% extra		# Reactions		Final
End Repair-A Tailing Buffer	16µL	18µL	x	8	=	144µL
End Repair-A Tailing Enzyme Mix	4µL	4.5µL	x	8	=	36µL
Total	20µL					180µL

4. Add 20uL of the End Repair/dA-Tailing master mix to each sample containing 50uL of Bead bound HiC library from the previous section. Mix well.

Adapter Ligation

1. Program thermal cycler for End Repair and dA-Tailing using the parameters in Table 5. Set reaction volume for 70µL, and the heated lid to 85°C, and press start. Total run time is approx. 30 min.

Table 5: End Repair and dA-Tailing Thermal Cycler Program

Temperature	Time
20°C	15 min
72°C	15 min
4°C	Hold

2. Once thermal cycler has reached 4°C hold step, transfer samples to ice while preparing the ligation reaction.
3. Add 25µL of Ligation Master Mix, to the 70µL of bead-bound, end-repaired and dA-tailed HiC library. Mix well.
4. Add 5µL of Adaptor Oligo Mix to each sample. Mix well.
5. Briefly spin tubes with the bead-bound HiC library, Ligation master mix, and Adaptor Oligo Mix.
6. Program the thermal cycler for the ligation step with the program specified in
7. Table 6 below.
8. Set the reaction volume to 100µL and press start. Total time is approx. 30 min.

Table 6: Adapter Ligation Thermal Cycler Program

Temperature	Time
20°C	30 min
4°C	Hold

9. After the "Ligation" program completes, remove the samples from the thermocycler and quick spin the tubes to remove any liquid from the caps.
10. Magnetize beads until liquid is clear. Remove and discard supernatant.
11. Resuspend beads in 200µL Wash Buffer. Mix by pipetting. Incubate at 55C for 2 min. Set lid temperature to 85°C
12. Magnetize beads until liquid is clear. Remove and discard supernatant.
13. Resuspend beads in 100µL Elution Buffer.
14. Magnetize beads until liquid is clear. Remove and discard supernatant.
15. Resuspend the beads in 34µL of Deionized Water and proceed immediately to Library Amplification below.

Amplification of Adaptor-Ligated HiC Library and Sample Indexing

1. Thaw and mix the reagents according to Table 7 below and keep on ice.

Table 7 : Thawing and Mixing Instructions

Reagent	Thaw	Mix	Cap
Herculase II Fusion DNA Polymerase	Ice	Pipette	Red
5X Herculase II Buffer with dNTPs	RT	Vortex	Clear
Index Primer Pair 1 - 16	RT	Vortex	Foil

2. Thaw only the index primers needed for experiment to minimize freeze-thaw cycles.
3. Determine the unique index pair assignment for each sample using Table 8 as a reference.

Table 8: Index Pairs included with the Arima Library Prep Module

Primer Pair #	P7 Index Forward	P5 Index Forward
1	CAAGGTGA	ATGGTTAG
2	TAGACCAA	CAAGGTGA
3	AGTCGCGA	TAGACCAA
4	CGGTAGAG	AGTCGCGA
5	TCAGCATC	AAGGAGCG
6	AGAAGCAA	TCAGCATC
7	GCAGGTTC	AGAAGCAA
8	AAGTGTCT	GCAGGTTC
9	CTACCGAA	AAGTGTCT
10	TAGAGCTC	CTACCGAA
11	ATGTCAAG	TAGAGCTC
12	GCATCATA	ATGTCAAG
13	GACTTGAC	GCATCATA
14	CTACAATG	GACTTGAC
15	TCTCAGCA	CTACAATG
16	AGACACAC	TCTCAGCA

4. Prepare appropriate volume of PCR reaction mix in Table 9 below. Mix well.

Table 9: PCR Reaction Mix

Reagent	Volume per Reaction	12.5% extra		# Reactions		Final
5x Herculase II Buffer with dNTPs (clear cap)	10µL	11.25µL	x	8	=	90µL
Herculase II Fusion DNA Polymerase (red cap)	1µL	1.125µL	x	8	=	9µL
Total	11µL					99µL

5. Add 11µL of the PCR reaction mixture prepared from the table above to 34µL of Adaptor Ligated Bead Bound HiC Library
6. Add 5µL of the appropriate, unique, Index Primer Pair to each sample. Make sure to take note of which index was used with each sample.
7. Program thermal cycle according to the settings in Table 10
8. Place the PCR reaction in the thermocycler and press start.

Table 10: Library Amplification Thermal Cycle Program

Cycles	Temperature	Time
1 X	98°C	2 min.
12 X*	98°C	30 sec.
	60°C	30 sec.
	72°C	1 min.
1 X	72°C	5 min.
1 X	4°C	Hold

**If you are working with challenging or lower-input samples (e.g. <200 ng), please contact technical support.*

Purify Amplified Library with Purification Beads

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 Library Prep kit.

1. Add 35µL of DNA Purification Beads to each 50µL Indexed sample. Mix well.
2. Incubate for 5 mins at room temperature.
3. Place sample against magnet and incubate until solution is clear.
4. Discard supernatant. While sample is still against magnet, add 150µL of 80% ethanol, and incubate at RT for 1 min.
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, incubate beads at RT for 3 to 5 min. to air-dry the beads.
7. Remove the sample from magnet, resuspend beads in 15µL of Deionized / Nuclease-free Water, and incubate at RT for 5 min.
8. Place sample against magnet and incubate until solution is clear.
9. Remove purified and complete HiC library and transfer to a fresh PCR strip tube.
10. Quantify sample using Qubit™ using 1µL.
11. Run the sample from the previous step on a gel or other platform to determine the size distribution of the HiC library.
12. Samples may be stored at -20°C for up to 6 months.

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.

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 INTERUPTION, OR COSTS OF SUBSTITUTE GOODS OR SERVICES, OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, EXEMPLARY OR INDIRECT DAMAGES FOR BREACH OF WARRANTY.

CONTACT US

Website: www.arimagenomics.com
Technical Support: techsupport@arimagenomics.com
Order Support: ordersupport@arimagenomics.com

Appendix A: Sample Sheets for Illumina Sequencing

A.1 Sequencing Sample Sheets

A "Sample Sheet" is a comma-separated (CSV) document that is used to specify sequencing parameters and samples for Illumina sequencing systems. Arima has pre-filled sample sheets available on the company website under Support > Documentation.