Arima-HiC+ Kit

User Guide: Library Preparation using the Arima Library Prep Module

16 reactions

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Revision History

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Section 1: Getting Started

1.1 Handling and Preparation

- Several steps during the *Arima Library Prep Protocol* require 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 and from Nucleases we recommend the use of gloves and sterilized filter 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 the pellet the beads.

1.2 Arima materials checklist

- ☐ Proximally Ligated DNA generated using the **Arima HiC+ Kit (PN A510008)**
- ☐ **Arima Library Prep Module (PN A303011)**

Table 1: Reagents Included in the Arima Library Prep Module

Name	PN	Box	Storage	Cap Color
Ligation Buffer	A311035-01	A	-20°C	Purple
T4 DNA Ligase	A311035-02	A	-20°C	Blue
End Repair-A-Tailing Buffer	A311035-03	A	-20°C	Yellow
End Repair-A Tailing Enzyme Mix	A311035-04	A	-20°C	Orange
Herculase II Fusion DNA Polymerase	A311035-05	A	-20°C	Red
5x Herculase II Buffer with dNTPs	A311035-06	A	-20°C	Clear
Adaptor Oligo Mix	A311035-07	A	-20°C	White
Index 1-16	A311036-01 – A311036-16	B	-20°C	Foil cover
T1 Beads	A311042-01	C	4°C	White
Binding Buffer	A311041-01	D	RT	White

1.3 User-supplied reagents, consumables, and equipment checklist

- ☐ Freshly prepared 80% Ethanol
- ☐ DNA Purification Beads (SPRI, e.g. Beckman Coulter Cat # A63880, Approx. 750µL per sample)
- ☐ 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
- ☐ 8-well PCR Strip Tubes with Caps
- ☐ Optional: 8- or 12-channel 200 mL 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

1.4 How to cite Arima HiC in publications

When citing the Arima HiC protocol or kit, one may write: “Hi-C data was generated using the Arima-HiC+ kit, and the Arima Library Prep Module according to the manufacturer’s protocols”.

Section 2: Library Preparation

Input: Proximally Ligated DNA generated using the **Arima HiC+ Kit (PN A510008)**
Output: Arima-HiC library ready for sequencing
Components:

- **Arima-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). Afterwards, 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-HiC library using the library amplification reagents and index PCR primers from the Arima Library Prep Module, producing the final 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 (Arima HiC+, Box A)**. 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 1,500ng 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.

2.1.1 If necessary, add **Elution Buffer** to bring the sample volume to 100µL. Do not exceed 100µL of volume for DNA fragmentation.

2.1.2 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.

2.1.3 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 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.

2.2.1 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.

2.2.2 Add 60µL of **DNA Purification Beads**, mix thoroughly by pipetting, and incubate at RT for 5 min.

2.2.3 Place sample against magnet and incubate until solution is clear.

2.2.4 Transfer ~160uL *of supernatant* to a new sample tube or well of a PCR plate. Discard beads.

2.2.5 Add 40µL of **DNA Purification Beads** to the ~160µL of supernatant, mix thoroughly by pipetting, and incubate at RT for 5 min.

2.2.6 Place sample against magnet and incubate until solution is clear.

2.2.7 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.

2.2.8 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.

2.2.9 Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.

2.2.10 Remove the sample from magnet, resuspend beads in 30µL of **Elution Buffer**, and incubate at RT for 5 min.

2.2.11 Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new sample tube or well of a PCR plate.

2.2.12 Quantify sample using Qubit®. Record this value.

2.2.13 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 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-HiC+ Enrichment Beads nor the Arima-HiC+ QC Beads

2.3.1 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.

2.3.2 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 HiC+ kit.**

2.3.3 Wash the **T1 Beads** in each tube by:

2.3.4 Add 200uL of **Binding Buffer**.

2.3.5 Mix by pipetting up and down 20 times, cap the tubes, and vortex at high speed for 5 - 10 seconds.

2.3.6 Place tubes against a magnet and incubate 5 minutes or until solution is clear.

2.3.7 Discard supernatant and remove the tube from magnet.

2.3.8 Repeat steps 2.3.2.1 – 2.3.2.4 two more times for a total of three washes.

2.3.9 Resuspend beads in 200uL of **Binding Buffer**.

2.3.10 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. (Refer to Table 11 for amplification guidance).**

2.3.11 Add 200µL of washed **T1 Beads** in **Binding Buffer**, mix thoroughly by pipetting, and incubate at RT for 15 min.

2.3.12 Place sample against magnet and incubate until solution is clear.

2.3.13 Discard supernatant and remove sample from magnet.

2.3.14 Wash beads by resuspending in 200µL of **Wash Buffer** and incubate at 55°C for 2 min. Set lid temperature to 85°C.

2.3.15 Place sample against magnet and incubate until solution is clear.

2.3.16 Discard supernatant and remove sample from magnet.

2.3.17 Wash beads by resuspending in 200µL of **Wash Buffer** and incubate at 55°C for 2 min. Set lid temperature to 85°C.

2.3.18 Place sample against magnet and incubate until solution is clear.

2.3.19 Discard supernatant and remove sample from magnet.

2.3.20 Wash beads by resuspending in 100µL of **Elution Buffer.**

2.3.21 Place sample against magnet and incubate until solution is clear.

2.3.22 Discard supernatant and remove sample from magnet.

2.3.23 Resuspend beads in 50µL of Deionized / Nuclease-free Water.

2.4 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.

2.4.1 **End Repair**

2.4.1.1 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

2.4.1.2 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 Step 2.4.3.2 below (After End Repair and dA Tailing)

2.4.1.3 Keep Ligation Master Mix at room temperature for 30 - 45 minutes before use.

2.4.2 **Prepare End Repair-A Tailing MasterMix using the table below**

2.4.2.1 Vortex thawed vial of **End Repair-A Tailing Buffer** for 15 seconds - continue vortexing until no solids are observed.

2.4.2.2 Prepare End Repair/dA-Tailing master mix by combining reagents as listed in Table 4 below, 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

2.4.2.3 Add 20uL of the End Repair/dA-Tailing master mix to each sample containing 50uL of Bead bound HiC library from 2.3.23 in the previous section. Mix well.

2.4.2.4 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

2.4.3 **Adapter Ligation**

2.4.3.1 Once thermal cycler has reached 4°C hold step, transfer samples to ice while preparing the ligation reaction.

2.4.3.2 Add 25µL of Ligation Master Mix, from step 2.4.1.3 above to the 70µL of bead bound, end repaired and dA-tailed HiC library. Mix well.

2.4.3.3 Add 5µL of **Adaptor Oligo Mix** to each sample. Mix well.

2.4.3.4 Briefly spin tubes with the bead-bound HiC library, Ligation master mix, and **Adaptor Oligo Mix**.

2.4.3.5 Program the thermal cycler for the ligation step with the program specified in **Table 6** below. Set the reaction volume to 100µL and the heated lid to 85°C, 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

2.4.3.6 After the "Ligation" program completes, remove the samples from the thermocycler and quick spin the tubes to remove any liquid from the caps.

2.4.3.7 Magnetize beads until liquid is clear. Remove and discard supernatant.

2.4.3.8 Resuspend beads in 200µL **Wash Buffer**. Mix by pipetting. Incubate at 55C for 2 min. Set lid temperature to 85°C

2.4.3.9 Magnetize beads until liquid is clear. Remove and discard supernatant.

2.4.3.10 Resuspend beads in 100µL **Elution Buffer.**

2.4.3.11 Magnetize beads until liquid is clear. Remove and discard supernatant.

2.4.3.12 Resuspend the beads in 34µL of **Deionized Water** and proceed immediately to Library Amplification below.

2.4.4 **Amplification of Adaptor-Ligated HiC Library and Sample Indexing**

2.4.4.1 Thaw and mix the reagents according to the 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

2.4.4.2 Thaw only the index primers needed for experiment to minimize freeze-thaw cycles.

2.4.4.3 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	P5 Index
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

2.4.4.4 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

2.4.4.5 Add 11µL of the PCR reaction mixture prepared from the table above to 34 µL of Adaptor Ligated Bead Bound HiC Library from step 2.4.3.12

2.4.4.6 Add 5uL of the appropriate, unique, **Index Primer Pair** to each sample. Make sure to take note of which index was used with each sample.

2.4.4.7 Program thermal cycle according to the settings in **Table 10**, and set the number of cycles according to **Table 11**

2.4.4.8 Place the PCR reaction in the thermocycler and press play

Table 10: Library Amplification Thermal Cycle Program

Cycles	Temperature	Time
1 X	98°C	2 min.
Determine cycles necessary from table 11	98°C	30 sec.
	60°C	30 sec.
	72°C	1 min.
1 X	72°C	5 min.
1 X	4°C	Hold

Note: The cycle number entered into the thermocycler will be X-1 (e.g., 5 cycles would be input as return to step 2, 4X) Set Lid Temp to 105°C and the reaction volume to 50uL

Table 11: DNA Input to Cycle Number

Amount input into Library Prep	Number of cycles
200ng – 2,000ng	5
50ng	7
5ng	10

2.5 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.

2.5.1 Add 50 µL of **DNA Purification Beads** to each 50 µL Indexed sample. Mix well.

2.5.2 Incubate for 5 minutes at room temperature.

2.5.3 Place sample against magnet and incubate until solution is clear.

2.5.4 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.

2.5.5 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.

2.5.6 Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.

2.5.7 Remove the sample from magnet, resuspend beads in 15µL of Deionized / Nuclease-free Water, and incubate at RT for 5 min.

2.5.8 Place sample against magnet and incubate until solution is clear.

2.5.9 Remove purified and complete HiC library and transfer to a fresh PCR strip tube.

2.5.10 Quantify sample using Qubit® using 1uL.

2.5.11 Run the sample from the previous step on a gel or other platform to determine the size distribution of the HiC library.

2.5.12 Samples may be stored at -20°C for up to 6 months.

Section 3: Warranty and Contact Info

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