

# QUICK REFERENCE GUIDE

## Arima High Coverage HiC Kit

For use with:

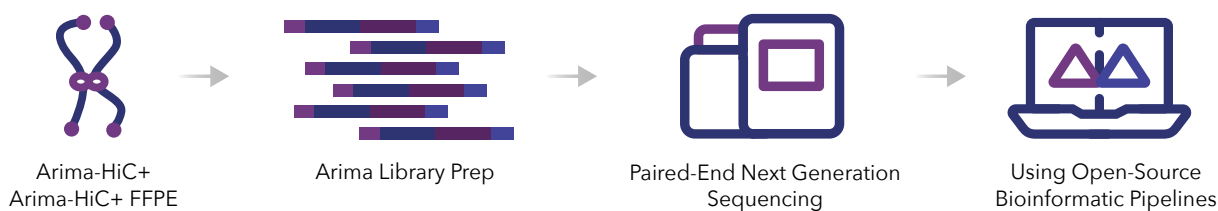
A101030 | Arima High Coverage HiC Kit, 8 rxn

A101031 | Arima High Coverage HiC Kit, 48 rxn

A303011 | Arima Library Prep Kit, 16 rxn

# Arima High Coverage HiC Workflow Overview

The Arima High Coverage HiC workflow captures the sequence and structure (three-dimensional conformation) of genomes.



Arima High Coverage HiC workflow

## Sample Prep

First, chromatin from various sample types, including tissues, cell lines, plant tissues, peripheral blood mononuclear cells (PBMCs), cryopreserved cells, and whole nucleated blood, is crosslinked to preserve its conformation. Next, the crosslinked chromatin is digested using a proprietary restriction enzyme (RE) cocktail optimized for coverage uniformity across a wide range of genomic sequence compositions. The 5'-overhangs are then filled in, incorporating biotinylated nucleotides. Finally, the spatially proximal digested ends are ligated, capturing the sequence and structure of the genome. The ligated DNA is then fragmented and purified, producing pure, proximally-ligated DNA.

## Library Prep

During the "Library Prep" step in Figure 1 above, the proximally-ligated DNA is fragmented, and the biotinylated fragments are enriched using Streptavidin beads to select for molecules that capture genome structure. The enriched fragments are then subjected to a library preparation protocol utilizing the Arima Library Preparation Kit.

## Sequencing and Data Analysis

Arima High Coverage HiC libraries are sequenced on short-read next generation sequencing platforms using 2x150 "paired-end" reads. The resulting data is referred to as Arima High Coverage HiC data.

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

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The Arima High Coverage HiC Kit user manual (document number A160667) 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 High Coverage 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

Qubit® is a trademark of Molecular Probes, Inc.



## Arima High Coverage HiC Protocol

### Resuspend

- Resuspend one reaction of crosslinked cells in 20 $\mu$ L\* or 40 $\mu$ L\*\* of Lysis Buffer or crosslinked nuclei in 20 $\mu$ L of Water

\* Crosslinked animal sample that is homogeneous

\*\* Crosslinked pulverized sample tissue that is clumpy, sticky

### Condition

- Add 24 $\mu$ L of Conditioning Solution
- Incubate 10 min. at 62°C
- Set lid temperature to 85°C

### Stop Reaction

- Add 20 $\mu$ L of Stop Solution 2
- Incubate 15 min. at 37°C

### RE Digestion

- Add 13.5 $\mu$ L of Digestion master mix
- Incubate in thermocycler (Table 8)

### Biotin Fill In

- Add 16 $\mu$ L of Fill-in master mix
- Incubate 45 min. at RT

### Ligate

- Add 82 $\mu$ L of Ligation master mix
- Incubate 15 min. at RT

### Reverse Crosslink

- Add 35.5 $\mu$ L of Reverse Crosslinking master mix
- Incubate in thermocycler (Table 12)

### Bead Purify

- Transfer to 1.7mL tube
- Add 100 $\mu$ L of DNA Purification Beads
- Incubate 5 min. at RT
- Incubate solution with magnet until clear
- Discard supernatant
- Wash with 300 $\mu$ L of 80% ethanol, incubate 1 min. at RT x 2
- Discard supernatant
- While against magnet, incubate 3 - 5 min. at RT
- Resuspend beads in 100 $\mu$ L of Elution Buffer
- Incubate 5 min. at RT
- Incubate solution with magnet until clear
- Transfer supernatant to a new tube
- Quantify sample using Qubit®

*For determining the Arima - QC1 value refer to the section of the Arima High Coverage HiC Kit user manual "Arima - QC1 Quality Control"*

Scan QR code for the  
**Arima High Coverage  
HiC Kit User Manual.**





## Arima Library Preparation

### Fragment

- Bring sample to 100 $\mu$ L with Elution Buffer
- Fragment to 550-600bp
- Qualify fragment size using Bioanalyzer or TapeStation

### Size Select

- Transfer fragmented DNA sample to microfuge tube, PCR tube, or PCR plate
- Bring sample to 100 $\mu$ L with Elution Buffer
- Add 100 $\mu$ L DNA Purification Beads
- Incubate for 5 min. at RT
- Incubate solution with magnet until clear
- Discard supernatant
- Wash with 200 $\mu$ L of 80% ethanol, incubate 1 min. at RT x 2
- Discard supernatant
- While against magnet, incubate 3 - 5 min. at RT
- Remove sample from magnet and resuspend beads in 30 $\mu$ L of Elution Buffer
- Incubate 5 min. at RT
- Incubate solution with magnet until clear
- Transfer supernatant to a new tube
- Quantify sample using Qubit

### Biotin Enrich

- Prepare T1 Beads (See "Biotin Enrichment", page 57 of protocol)
- Transfer 200ng of size selected DNA to new microfuge tube, PCR tube, or well of a PCR plate
- Bring sample volume to 30 $\mu$ L with Elution Buffer
- Add 200 $\mu$ L of washed T1 Beads in Binding Buffer and incubate on shaker for 15 min. at 1400 rpm.
- Incubate solution with magnet until clear
- Discard supernatant and remove sample from magnet
- Resuspend beads in 200 $\mu$ L of Wash Buffer

### Repeat 2X

- Incubate in thermocycler for 2 min. at 55 $^{\circ}$ C.
  - Incubate solution with magnet until clear
  - Discard supernatant and remove sample from magnet
- 
- Remove from magnet and resuspend beads in 100 $\mu$ L of Elution Buffer
  - Incubate solution with magnet until clear
  - Discard supernatant and remove sample from magnet
  - Resuspend beads in 50 $\mu$ L of Deionized / Nuclease-free Water



## Arima Library Preparation

### End Repair

- Prepare Ligation Master Mix (Table 16)
- Prepare End Repair/dA-Tailing Master Mix (Table 17)
- Add 20 $\mu$ L of the End Repair/dA-Tailing Master Mix

### Adapter Ligation

- Incubate sample in thermocycler (Table 18)
- Transfer sample to ice
- Add 25 $\mu$ L Ligation Master Mix
- Add 5 $\mu$ L of Adaptor Oligo Mix
- Incubate samples in thermocycler (Table 19)
- Incubate solution with magnet until clear
- Discard supernatant and remove sample from magnet
- Resuspend beads in 200 $\mu$ L Wash Buffer
- Incubate in thermocycler for 2 min. at 55°C
- Incubate solution with magnet until clear
- Discard supernatant and remove sample from magnet
- Resuspend beads in 100 $\mu$ L Elution Buffer
- Incubate solution with magnet until clear
- Discard supernatant and remove sample from magnet
- Resuspend the beads in 34 $\mu$ L of Deionized Water

### Amplify Library

- Prepare PCR reaction mix (Table 22)
- Add 11 $\mu$ L of the PCR reaction mix to 34 $\mu$ L of sample
- Add 5 $\mu$ L of each unique Index Primer Pair
- Incubate in thermocycler (Table 23)

### Purify Library

- Add 50 $\mu$ L of DNA Purification Beads
- Incubate 5 min. at RT
- Incubate solution with magnet until clear
- Discard supernatant
- With sample against magnet add 200 $\mu$ L of 80% ethanol
- Incubate 1 min. at RT
- Discard supernatant
- Repeat above step for a total of two washes
- While against magnet, incubate 3 - 5 min. at RT
- Resuspend beads in 15 $\mu$ L of Deionized / Nuclease-free Water
- Incubate 5 min. at RT
- Incubate solution with magnet until clear
- Remove supernatant and transfer to fresh strip tube
- Prepare 1:10 dilution; 1 $\mu$ L of sample with 9 $\mu$ L water
- Store samples up to 6 months at 20°C

# Reference Tables

Table 8. Thermal Cycler Program for Digestion

Temperature	Time
37°C	60 min.*
65°C	20 min.
10°C	∞

Table 12. Thermal Cycler Program for Reverse crosslinking

Temperature	Time
55°C	30 min.
68°C	90 min.*
25°C	∞

## Biotin Enrichment (pg 57)

**Input:** Size Selected Proximally-Ligated DNA

**Output:** Purified Proximally-Ligated DNA

### 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

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.

Table 16. Ligation Master Mix Worksheet

Reagent	Vol/Rxn	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

Table 17. End Repair/dA Tailing Master Mix Worksheet

Reagent	Vol/Rxn	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

Table 18. End Repair and dA-Tailing Thermal Cycler Program

Temperature	Time
20°C	15 min.
72°C	15 min.
4°C	∞

Table 19. Adapter Ligation Thermal Cycler Program

Temperature	Time
20°C	30 min
4°C	∞

Table 22. PCR Reaction Mix

Reagent	Vol/Rxn	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					9µL

Table 23. Library Amplification Thermal Cycle Program

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

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