USER GUIDE

Arima High Coverage HiC Kit

A160667, Rev A

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

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

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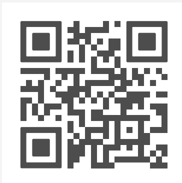

Scan this QR code to download the **Arima High Coverage HiC Quick Reference Protocol**.

Introduction

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Arima High Coverage HiC Workflow Overview

The Arima High Coverage HiC workflow (Figure 1) captures the sequence and structure (three-dimensional conformation) of genomes.

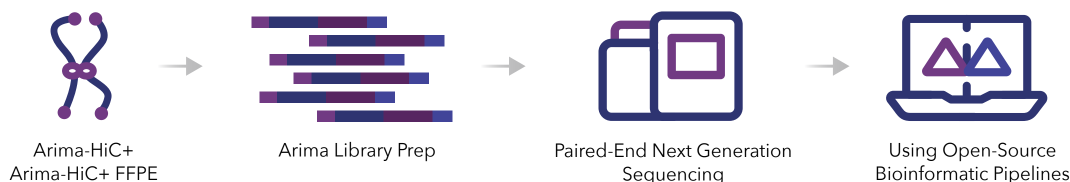

Figure 1. Arima High Coverage HiC workflow

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.

The tools necessary for analyzing Arima High Coverage HiC data depend on the application. For example, for studying 3D genome conformation, Arima High Coverage HiC data can be processed using publicly available tools such as Juicer (Durand, 2016a; https://github.com/aidenlab/juicer/wiki) or Hi-C Pro (Servant, 2015; https://github.com/nservant/HiC-Pro), and genome organizational features such as compartments, TADs, and loops can be identified and visualized using tools such as Juicebox (Durand, 2016b; https://github.com/aidenlab/Juicebox/wiki).

These tools require usage modifications and/or custom input files that are specific to Arima High Coverage HiC data, so please contact Technical Support for assistance implementing these tools. Additionally, because paired-end reads of Arima High Coverage HiC data can originate from distal sequences along the linear genome, the data captures short- and long-range DNA contiguity information that is valuable for applications such as de novo assembly, genome scaffolding, and haplotype phasing. Arima High Coverage HiC data can be mapped to contigs/unitigs using our mapping pipeline (https://github.com/ArimaGenomics/mapping_pipeline) or Juicer, and then the contigs/unitigs can be scaffolded using tools such as SALSA (Ghurye, 2019; https://github.com/marbl/SALSA) or 3D-DNA (Dudchenko, 2017; https://github.com/aidenlab/3d-dna). Lastly, because the Arima High Coverage HiC data provides uniform per base genome coverage while maintaining the highest long-range signal contiguity, it greatly benefits variant discovery, base polishing, scaffolding, and phasing. Please contact Technical Support for more information.

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, according to manufacturers protocols".

Please reference the catalog number found on the kit packaging.

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Handling and Preparation

- Several steps during the Arima High Coverage HiC 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.
- If the Arima High Coverage HiC Protocol is performed in PCR plates or PCR tubes, ensure to have a total volume capacity of at least 320µL. Also, ensure that plates and/or tubes are compatible with thermal cyclers and other required equipment. Using seals and caps for PCR plates and tubes is required.
- All kit reagents should be fully thawed and thoroughly mixed before use.
- Stop Solution 1, Conditioning Solution, and Buffer D from Box A may contain precipitates. If present, these precipitates must be dissolved before use. Heating these reagents at 37°C for 5-15 minutes may be necessary to dissolve precipitates.
- Enzyme D may contain precipitates. If present, these precipitates must be dissolved before use. Heating this reagent at 37-42°C for 5-10 minutes may be necessary to dissolve precipitates.
- During handling and preparation, reagents from Box A should be kept at RT.
- During handling and preparation, reagents from Box B should be kept on ice, except for Enzyme D, which should be kept on ice but warmed to room temperature just before use.
- Enzyme solutions from Box B are viscous and require special attention during pipetting.
- To prevent contamination of reagents and probes by nuclease, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease free aerosol-resistant tips.
- If possible, performing the pre-amplification steps in a “Pre-PCR” environment and the post-amplification steps in a “Post-PCR” environment to reduce PCR contamination.
- Maintain clean work areas. Clean pre-PCR surfaces that pose highest risk of contamination with 10% bleach solution or equivalent, wait 10 to 15 minutes and then clean the surfaces with DI-Water.
- When working with liquid nitrogen wear safety goggles and cold resistant gloves.
- DNA Purification Beads (e.g., AMPure™ XP Beads) should be warmed to RT and thoroughly mixed before each use. Aspirate and dispense Purification beads slowly due to the viscosity of the solution.
- All thermal cycler incubations must be performed with heated lid on except when samples are incubated at RT (20 to 25°C), which heated lid should be off.
- Possible stopping points, where samples may be stored at -20°C, are marked in the protocol. Do not subject the samples to multiple freeze/thaw cycles.

Required Reagents and Equipment

Arima Material Checklist | Arima High Coverage HiC Kit

Description	Arima P/N
Arima High Coverage HiC Kit	**A101030, A101031**
Box A (20 to 25°C)	
☐ Stop Solution 1 ☐ Elution Buffer ☐ Wash Buffer ☐ Conditioning Solution	☐ Stop Solution 2 ☐ Buffer D ☐ Buffer E
Box B (-20°C)	A410110
☐ Lysis Buffer ☐ Buffer F ☐ Enzyme F1 ☐ Enzyme A2 ☐ Enzyme F3 ☐ Enzyme F4	☐ Buffer G ☐ Enzyme B ☐ Buffer C ☐ Enzyme C ☐ Enzyme D
Box C (2 to 8°C)	A410110
☐ Enrichment Beads	☐ QC Beads

Arima Material Checklist | Arima Library Prep Module

Description	Arima P/N
Arima Library Prep Module	**A303011**
Box A (-20°C)	A311035
☐ Ligation Buffer ☐ T4 DNA Ligase ☐ End Repair-A Tailing Buffer ☐ End Repair-A Tailing Enzyme Mix	☐ Herculase II Fusion DNA Polymerase ☐ 5× Herculase II Buffer with dNTPs ☐ Adaptor Oligo Mix
Box B (-20°C)	A311036
☐ Index 1 ☐ Index 2 ☐ Index 3 ☐ Index 4 ☐ Index 5 ☐ Index 6 ☐ Index 7 ☐ Index 8	☐ Index 9 ☐ Index 10 ☐ Index 11 ☐ Index 12 ☐ Index 13 ☐ Index 14 ☐ Index 15 ☐ Index 16
Box C (4°C)	A311042
☐ T1Beads	
Box D (20 to 25°C)	A311041
☐ Binding Buffer	

Equipment Checklist

Description	Vendor and Part Number
☐ Thermal Cycler with 96 well, 0.2 ml block	Various Models, Suppliers
☐ Qubit Fluorometer	ThermoFisher Scientific P/N Q33238
☐ Vortex Mixer	General Laboratory Supplier
☐ Gel Electrophoresis System	e.g. Bioanalyzer™, TapeStation™, etc.
☐ DNA Shearing Sonicator	Diagnode, Covaris
☐ 96-well plate Mixer	General Laboratory Supplier
Magnetic Separators	
☐ 0.2ml Magnetic Separator	PERMAGEN P/N MSR812
☐ Plate Magnetic Stand-96	ThermoFisher P/N AM10027
☐ 1.7ml Magnetic Separator	ThermoFisher DynaMag-2 P/N 12321D
☐ P2, P20, P200, P1000 Pipettes	Rainin Pipettes or equivalent
☐ Multi-Channel Pipette	Rainin Pipettes or equivalent
☐ Microcentrifuge	Eppendorft microcentrifuge, model 5417C or equivalent
☐ Ceramic Mortar and Pestel[1]	Cole-Parmer® P/N UX-63100-63 or equivalent
☐ Metal Spatula[1]	Cole-Parmer® P/N SI-06369-16
☐ Cold Resistant Gloves[1]	Various Supplier
☐ Liquid Nitrogen and Dry Ice[1]	Various Supplier
☐ Centrifuge with refrigeration	Various Supplier
☐ Thermomixer or Plate Shaker	Various Supplier
☐ Powermasher II (optional)	DiagnoCine Cat # 891300
☐ Biomasher II Pestle and Tube Set (optional)	DiagnoCine Cat # 320103

[1] Only if processing Fresh Frozen Tissues

User Supplied Reagents and Consumables

Description	Vendor and Part Number
☐ 1X PBS, pH 7.4	Fisher Scientific P/N 50-842-949
☐ 1X PBS containing 3% BSA (v/v)[1]	Fischer Scientific P/N AAJ61655AP
☐ FBS[2]	Fisher Scientific P/N A3160501
☐ 37% Formaldehyde	Fisher Scientific P/N F79-500
☐ 100% Ethanol, molecular biology grade	Sigma-Aldrich P/N 459836
☐ Histopaque®-1077[3]	Sigma-Aldrich P/N 10771-100ML
☐ CellLytic™ Plant Nuclei Isolation/Extraction Kit[4]	Sigma-Aldrich P/N CELLYTPN1
☐ Freshly Made TC Buffer[5]	See Table 1 for recipe
☐ DNA Purification Beads	KAPA@ Pure beads P/N KS8002
☐ Qubit@ dsDNA HS Assay kit	ThermoFisher P/N Q33231
☐ Qubit@ assay tubes	ThermoFisher P/N Q32856
☐ Liquid Nitrogen or dry ice	Various Suppliers
☐ 15 ml conical tubes	Various Suppliers
☐ 1.7 ml LoBind tubes	Various Suppliers
Plasticware compatible with Thermal Cycler	
☐ 8-well micro Strip tubes and Caps	SSIbio™ P/N 3247-00 or equivalent
☐ or Hard Shell PCR Plates	BioRad P/N HSS9601
☐ Adhesive Plate Seal	BioRad P/N MSB1001
☐ Bioruptor or Covaris Sample holder	
☐ Covaris MicroTube for individual Sample processing.	Covaris P/N 520045
☐ Bioruptor NGS 0.65 Microtubes	Diagnode P/N C30010011
☐ Nuclease Free Water	Various Supplier
☐ Sterile, Nuclease free aerosol barrier pipette tips	General laboratory supplier

[1] If working with “low-input” cell cultures (<1M cells)
[2] If working with whole nucleated blood
[3] If working with PBMCs
[4] If working with plant tissues
[5] If working with animal tissues (standard and low input)

Classifying Samples as Standard or Low Input for Crosslinking

In this user guide, there are two crosslinking protocols depending on whether the input sample is considered standard or low input. The Standard Input protocol has been widely used across numerous animal tissue types. It may also be applied to some Low Input sample types with success, but requires considerable care to avoid sample loss. It is also lower throughput.

The Low Input protocol may also be applied to some Standard Input sample types because >50mg of some tissues is more sample material than what is necessary for Arima High Coverage HiC. For these cases, simply dissect a ~50mg piece of tissue from the larger tissue sample and use the ~50mg sample as input to the Crosslinking – Low Input protocol. It is also higher throughput. Please contact Technical Support for additional guidance.

Classifications for each input type are as follows:

Standard Input:

- Large animal tissues (e.g. tumor, liver, muscle) with a mass greater than ~50mg (50-200mg)
- Small animal tissues (e.g. whole mosquitos, flies, worms) where the volume of the tissue(s) occupy greater than 50µL when pooled at the bottom of a 1.5mL microfuge tube.

Low Input:

- Large animal tissues (e.g. tumor, liver, muscle) with a mass less than ~50mg.
- Small animal tissues (e.g. single or partial mosquitos, flies, worms) where the volume of the tissue(s) occupy less than 50µL when pooled at the bottom of a 1.5mL microfuge tube.

Sequencing Specifications

Arima High Coverage HiC libraries must be sequenced in paired-end mode, and are compatible with most Illumina® sequencing machines (e.g. MiSeq®, NextSeq®, HiSeq®, NovaSeq®) and a variety of read lengths. We generally recommend 2x150bp read length on the HiSeq® or NovaSeq™ instruments to optimize for sequencing throughput and Arima High Coverage HiC data alignment quality, although shorter read lengths (e.g. 2x50bp, 2x100bp) and lower throughput instruments can certainly be used for certain applications of Arima High Coverage HiC data such as 3D genome conformation analysis and genome scaffolding. For applications such as base polishing, genomic variant discovery, or haplotype phasing, 2x150bp read length is strongly recommended to maximize the performance of the data.

3D Genome Architecture

For studying 3D genome conformation, the ability to detect certain genome organization features depends on the sequencing depth. For ~3Gb genomes such as mouse and human, we generally recommend obtaining at least 600 million read-pairs per biological condition for high-resolution analyses of A/B compartments, TADs, and chromatin loops. One way of obtaining at least 600 million read-pairs is by combining at least 300 million read-pairs from 2 biological replicates. In doing so, you will be able to assess the overall reproducibility of the Arima High Coverage HiC data across replicates, and then used the combined replicate Arima High Coverage HiC dataset for high-resolution chromatin conformation analyses. Alternatively, one can obtain at least 600 million read-pairs per biological replicate and then use the common set of identified genome conformational features across replicates as a "high confidence" set of structural features supported by their observation in both replicates. For lower resolution analyses of A/B compartments and TADs, we generally recommend obtaining at least 300 million read-pairs per biological condition. For help estimating the optimal sequencing depth for different genome sizes or analysis goals, please contact Technical Support.

Genome Assembly

For applications such as de novo assembly and genome scaffolding, the required sequencing depth can vary depending on the quality of contig/unitigs that are being scaffolded using Arima High Coverage HiC data. For a 3Gb genome, we recommend obtaining up to 600M read-pairs, as this is the amount of sequencing that is currently utilized from Arima-HiC libraries for genome scaffolding by the Vertebrate Genome Project (VGP) consortia. The amount of sequencing required scales linearly with the genome size (e.g. up to 200M read-pairs for a 1Gb genome).

For applications such as base polishing, genomic variant discovery, or haplotype phasing, we recommend sequencing to at least 30X depth using a 2x150bp read length.

Lastly, it is important to note that each Arima High Coverage HiC library should pass the Arima-QC2 assay and be evaluated for library complexity prior to deep sequencing. As a general rule, each Arima High Coverage HiC library should be complex enough to sequence up to ~600M read-pairs without reaching saturation. If >600M read-pairs of Arima High Coverage HiC data are needed, it may be more efficient to sequence a second Arima High Coverage HiC library than sequence deeper into the first Arima High Coverage HiC library.

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Introduction

The Arima High Coverage HiC workflow is optimized to prepare Hi-C libraries from a variety of sample types, including cells collected from cell culture, fresh frozen animal tissues, plant tissues, peripheral blood mononuclear cells (PBMCs), cryopreserved cells, and whole nucleated blood. Proceed to the appropriate sections listed below for upfront preparation of proximity-ligated DNA. The library prep procedure is the same for all proximity ligated Hi-C DNA.

Standard Input: Fresh Frozen Animal Tissue

Input: Fresh-frozen animal tissue
Output: Pulverized crosslinked animal tissue

Before you begin:

- The Arima High Coverage HiC workflow for animal tissues starts with pulverization and crosslinking. For vertebrates and large invertebrates with dense tissues, weigh 50-200 mg of fresh frozen tissue and record this mass for later use in the Estimating Input Amount protocol. For smaller sample quantities (less than 50 mg), follow the Crosslinking – Low Input protocol.
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

TC Buffer must be prepared fresh directly before performing the Crosslinking protocols for animal tissues The following recipe is enough for crosslinking 8 samples. This recipe should be scaled accordingly if more or less than 8 samples are processed simultaneously.

The table below includes a suggested vendor and catalog number for each reagent. After the TC Buffer is prepared, store at RT until ready to use.

Table 1. Formulation of TC Buffer

Component	Supplier	Stock Cat#	Stock Conc.	Final Conc.	Stock Volume
Water	Fisher Scientific™	50-843-406			1.67 ml
Sodium Chloride	Fisher Scientific™	PR-V4221	5M	100 mM	100 µl
EDTA	Fisher Scientific™	PR-V4231	0.5M	1 mM	10 µl
EGTA	Fisher Scientific™	BM-151	0.5M	0.5 mM	5 µl
HEPES pH 8.0	Fisher Scientific™	H1090	1M	50 mM	250 µl
Formaldehyde*	Fisher Scientific™	F79-500	37%	22%	3 ml
				Total	5 ml

* If using a 16% formaldehyde stock, please contact Technical Support for a different TC Buffer formulation.

Consumables

Arima Box A

- [ ] Stop Solution 1

User Supplied

- [ ] 15ml conical tubes
- [ ] Dry ice and liquid nitrogen
- [ ] 1X PBS, pH 7.4 (User Supplied)
- [ ] TC Buffer (User Supplied, See Table 1)

Protocol

1. Place a mortar and a 15mL conical tube onto a bed of dry ice, and place a pestle into the mortar. Cool a spatula at -20°C or colder for later use.
2. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Allow liquid nitrogen to evaporate completely.
3. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Transfer frozen animal tissue into mortar containing liquid nitrogen. Allow liquid nitrogen to evaporate just enough for the animal tissue to stay submerged.

Note: Using cold-resistant gloves is highly recommended to handle the mortar.

4. Pulverize animal tissue in the mortar using the pestle until the sample resembles a fine powder. Ensure the animal tissue is always submerged in liquid nitrogen. Carefully re-fill the mortar with liquid nitrogen as necessary.

Note: The pulverization process should take at least 5 min per sample and some tissue types may take longer. The goal is to pulverize until the tissue resembles a fine powder without visible chunks.

5. Once the sample resembles a fine powder, allow liquid nitrogen in the mortar to evaporate entirely.
6. Carefully transfer pulverized animal tissue from the mortar into the 15mL conical tube using the cooled spatula.
7. Submerge 15mL conical tube into dry ice to keep all the pulverized animal tissue frozen.

Note: Do NOT cap the 15mL conical tube until all the liquid nitrogen has completely evaporated.

8. Allow liquid nitrogen in 15mL conical tube to evaporate completely, then cap the tube.

Note: If pulverizing multiple animal tissue samples in a single day, keep the pulverized sample on dry ice and repeat Steps 1 – 8 on the remaining samples using clean equipment and consumables until all samples have been pulverized. Then, proceed to the next step and complete the remaining protocol on all samples simultaneously.

9. Remove sample tube from dry ice, thaw at RT for 2 min.
10. Add 5mL of 1X PBS and mix gently by inversion.
11. Add 500µL of fresh TC Buffer, bringing the final concentration of formaldehyde to 2%.
12. Mix thoroughly by inverting the tube 10 times and incubate at RT for 20 min.
13. Add 289µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
14. Pellet sample by centrifugation at 2,500 x G at RT for 5 min.
15. Discard the supernatant without disturbing the pellet. Gently decant the tube to discard the majority of the supernatant, then use a P200 pipette to remove the remaining liquid.
16. Resuspend sample in 1mL 1X PBS.

    Prepare for the Estimating Input Amount protocol in the following section:

    a. Mix the sample by inversion and then immediately aliquot 100uL (10%*) of the original pulverized animal tissue into a 1.7mL microcentrifuge tube.

       *The 10% aliquot will be used in the Estimating Input Amount protocol. The 10% aliquot should not contain more than 10mg of the original pulverized animal tissue. For example, if you have 200mg of pulverized animal tissue, the aliquot should be 50uL (5% = 10mg) rather than 100uL (10% = 20mg).

    b. To minimize the risk of degradation from repeated freeze - thaw cycles, aliquot the remaining material into multiple 1.7ml microcentrifuge tubes, each containing the equivalent of ~20 to 25% of the pulverized animal tissue.

Note: Mix samples by inversion between aliquots to ensure all aliquots are equally homogeneous. The aliquots containing 20-25% are meant to be saved as sample material for the Arima High Coverage HiC Protocol.

17. Pellet all samples by centrifugation at 2,500 x G at RT for 5 min.
18. Discard supernatant leaving behind only the sample pellet and no residual liquid.

⃠ **Safe Stopping Point:** Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input Amount protocol in a following section.

Low Input: Fresh Frozen Animal Tissue

Input: Fresh-frozen animal tissue
Output: Homogenized crosslinked animal tissue

Before you begin:

- For large animal tissues weighing ≤50 mg or small quantities of small animal tissues, begin by weighing the fresh frozen tissue and record the mass for later use in the protocol and Estimating Input Amount. Use the Crosslinking – Low Input protocol for these cases.
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

TC Buffer must be prepared fresh directly before performing the Crosslinking protocols for animal tissues. The following recipe is enough for crosslinking 8 samples. This recipe should be scaled accordingly if more or less than 8 samples are processed simultaneously.

The table below includes a suggested vendor and catalog number for each reagent. After the TC Buffer is prepared, store at RT until ready to use.

Table 2. Formulation of TC Buffer

Component	Supplier	Stock Cat#	Stock Conc.	Final Conc.	Stock Volume
Water	Fisher Scientific™	50-843-406			1.67 ml
Sodium Chloride	Fisher Scientific™	PR-V4221	5M	100 mM	100 µl
EDTA	Fisher Scientific™	PR-V4231	0.5M	1 mM	10 µl
EGTA	Fisher Scientific™	BM-151	0.5M	0.5 mM	5 µl
HEPES pH 8.0	Fisher Scientific™	H1090	1M	50 mM	250 µl
Formaldehyde*	Fisher Scientific™	F79-500	37%	22%	3 ml
				Total	5 ml

* If using a 16% formaldehyde stock, please contact Technical Support for a different TC Buffer formulation.

Consumables

Arima Box A

- ☐ Stop Solution 1

User Supplied

- ☐ 1.5mL microfuge tube
- ☐ Dry ice or liquid nitrogen
- ☐ 1X PBS, pH 7.4 (User Supplied)
- ☐ TC Buffer (User Supplied, See Table 1)

Protocol

1. Transfer animal tissue to 1.5mL microfuge tube, thaw for 2-3 min.
2. Add 1mL of 1X PBS.
3. Add 100µL of fresh TC Buffer, bringing the final concentration of formaldehyde to 2%.
4. Mix thoroughly by inverting 10 times and incubate at RT for 20 min.
5. Add 57.8µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
6. Pellet sample by centrifugation at 2,500 x G at RT for 5 min.
7. Carefully discard supernatant. If the sample dislodges during pipetting, pellet by centrifugation again at a higher speed and discard remaining supernatant. Some insects (e.g. mosquitoes) will not pellet. In these cases, use pipette tip to place insect against the tube wall and then discard the supernatant.
8. Add 500µL of 1X PBS, mix well by inversion, and transfer to a sterile Biomasher® II tube.

Note: If homogenizing multiple samples in a single day, keep all crosslinked samples on ice and complete the following tissue homogenization (Step 8) on each individual sample using clean disposable pestles until all crosslinked samples have been homogenized. Then, proceed to Step 9 and complete the remaining protocol on all samples simultaneously.

9. Homogenize the tissue using the handheld Powermasher II instrument and accompanying disposable plastic pestles. Homogenize for 2-3 min. or until thoroughly homogenized with periodic lifting and pushing down into the tube.

Prepare for the Estimating Input Amount protocol in the following section:

a. Mix the sample by inversion and then immediately aliquot 100uL (10%*) of the original pulverized animal tissue into a 1.7mL microcentrifuge tube.

   *The 10% aliquot will be used in the Estimating Input Amount protocol. The 10% aliquot should not contain more than 10mg of the original pulverized animal tissue. For example, if you have 200mg of pulverized animal tissue, the aliquot should be 50uL (5% = 10mg) rather than 100uL (10% = 20mg).

b. To minimize the risk of degradation from repeated freeze - thaw cycles, aliquot the remaining material into multiple 1.7ml microcentrifuge tubes, each containing the equivalent of ~20 to 25% of the pulverized animal tissue.

Note: Mix samples by inversion between aliquots to ensure all aliquots are equally homogeneous. The aliquots containing 20-25% are meant to be saved as sample material for the Arima High Coverage HiC Protocol.

10. Pellet all samples by centrifugation at 2,500 x G at RT for 5 min.

11. Carefully discard supernatant. If no sample is visible or if the sample dislodges during pipetting, pellet by centrifugation again at a higher speed and discard remaining supernatant.

⃠ **Safe Stopping Point:** Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input Amount protocol or Arima High Coverage HiC Protocol depending on the input amount.

Peripheral Blood Mononuclear cells (PBMCs)

Input: Whole blood
Output: Crosslinked PBMCs

Before you begin:

- The workflow for mammalian blood involves isolating PBMCs from 2 mL of freshly drawn whole blood, followed by crosslinking. During the PBMC Isolation and Crosslinking protocol, ensure that the centrifuge brake and accelerator features are turned off during Step 6.
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

Consumables

Arima Box A

- ☐ Stop Solution 1

User Supplied

- ☐ Histopaque®-1077
- ☐ 15ml conical tubes
- ☐ Dry ice and liquid nitrogen
- ☐ 1X PBS, pH 7.4 (User Supplied)
- ☐ 37% formaldehyde

Protocol

1. Collect at least 2mL of whole blood and store at the temperature recommended by the blood collection tube manufacturer. If less than 2mL of whole blood is collected, please contact Technical Support.
2. Warm Histopaque®-1077 to RT by letting the bottle sit at RT for 30 min.
3. Add 2mL of whole blood and 2mL of cold 1X PBS to a new 15mL conical tube. Mix by gentle inversion until homogeneous.
4. Prepare a separate 15mL conical tube with 4mL of RT Histopaque®-1077.
5. Aspirate 4mL of diluted blood from step 3 with a serological pipette. Tilt tube from step 4 at 45-degree angle and place the tip of the serological pipette just above the top of the Histopaque®-1077 solution. Slowly add the 4mL of diluted blood on top of the Histopaque®-1077 solution. Adjust the height of the pipette during pipetting so that the tip remains just above the top of the Histopaque®-1077 solution throughout the pipetting. Once finished, proceed immediately to the next step.
6. Centrifuge sample with the accelerator and break OFF at 400 x G at 4°C for 30 min. Once complete, gently remove the sample tube and place upright into a tube rack. The top yellow-colored layer in the sample tube is the plasma layer, and the very thin white layer directly below the plasma are PBMCs.
7. Carefully transfer the PBMCs (i.e. "buffy coat") to a new 15mL conical tube and safely discard the remaining non-PBMC blood sample material.
8. Add cold 1X PBS to the PBMCs to bring the total volume to 5mL.
9. Obtain a cell count by hemocytometer or automated cell counting methods.
10. Centrifuge sample at 500 x G at RT for 10 min.
11. Discard supernatant.
12. Resuspend cells in 1mL cold 1X PBS.
13. Add 57µL of 37% formaldehyde, bringing the final formaldehyde concentration to 2%.
14. Mix well by inverting 10 times, and incubate at RT for 10 min. with occasional inversion.
15. Add 91.9µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min. with occasional inversion.
16. Place sample on ice and incubate for 15 min.
17. Pellet cells by centrifugation at 500 x G at RT for 5 min.
18. Discard supernatant.
19. Resuspend cells in 1mL cold 1X PBS.

20. Aliquot cells into separate microcentrifuge tubes, with 0.5 million cells per aliquot. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.
21. Pellet cells in all aliquots by centrifugation at 500 x G at RT for 5 min.
22. Discard supernatant leaving only the crosslinked cell pellets and minimal residual liquid.

Safe Stopping Point: Freeze the crosslinked cell pellets by submerging in liquid nitrogen or by placing on dry ice, and store at -80°C until ready to proceed to the Estimating Input Amount section.

Standard Input: Cell Culture Cells

Input: Cells collected from cell culture
Output: Crosslinked cells

Before you begin:

- Begin with the harvesting and crosslinking of at least 1 million cells, with optimal performance achieved with 5-10 million cells. If fewer than 1 million cells are available, use the Crosslinking – Low Input protocol. During centrifugation, pellet cells at 500 x G for 5 minutes or at a speed and duration appropriate for your specific cell type.
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

Consumables

Arima Box A

- [ ] Stop Solution 1

User Supplied

- [ ] 15ml conical tubes
- [ ] Dry ice or liquid nitrogen
- [ ] 1X PBS, pH 7.4 (User Supplied)
- [ ] 37% formaldehyde

Protocol

1. Harvest cells from cell culture using standard protocols and pellet cells by centrifugation.
2. Resuspend in cell culture media, obtain a cell count by hemocytometer or automated cell counting methods.
3. Transfer 5-10 million cells to be crosslinked into a new 15mL conical tube, pellet cells by centrifugation and remove supernatant.
4. Resuspend cells in 5mL of RT 1X PBS.
5. Add 286µL of 37% formaldehyde, bringing the final formaldehyde concentration to 2%.
6. Mix well by inverting 10 times and incubate at RT for 10 min.
7. Add 460µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
8. Place sample on ice and incubate for 15 min.
9. Pellet cells by centrifugation.
10. Discard supernatant.
11. Resuspend cells in 5mL 1X PBS.
12. Aliquot cells into several new tubes, with 1 x 106 cells per aliquot. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.
13. Pellet cells in all aliquots by centrifugation.
14. Discard supernatant leaving only the crosslinked cell pellet and no residual liquid.

Safe Stopping Point: Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input Amount – Standard Input protocol.

Low Input: Cell Culture, Cell Sorting, Other Cells

Input: Cells collected from cell culture, cell sorting, or other sources
Output: Crosslinked cells

Before you begin:

- For fewer than 1 million cells, follow the Crosslinking – Low Input protocol. Centrifuge at a higher speed and duration (e.g., 2500 x G for at least 5 minutes) to ensure minimal sample loss. Cell pellets may be difficult to see, so carefully note where the pellet should be located to avoid disturbing it during pipetting.
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

Consumables

Arima Box A

- ☐ Stop Solution 1

User Supplied

- ☐ 1.7mL microfuge
- ☐ Dry ice or liquid nitrogen
- ☐ 1X PBS containing 3% BSA (v/v)
- ☐ 37% formaldehyde

Protocol

Note: Steps 2 – 4 and Step 8 involve the addition of reagents pertaining to crosslinking or washing. Please note that each step involves mixing by inversion. Do not mix by pipetting to ensure minimal sample loss during the crosslinking workflow.

1. Collect cells in a 1.7mL microfuge tube and pellet cells by centrifugation.
2. Add 1mL of RT 1X PBS containing 3% BSA (v/v) and mix by inverting 5 times.
3. Add 57µL of 37% formaldehyde, mix well by inverting 10 times and incubate at RT for 10 min. with occasional inversion.
4. Add 91.9µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min. with occasional inversion.
5. Place sample on ice and incubate for 15 min.
6. Pellet cells by centrifugation.
7. Discard supernatant.
8. Add 1mL of RT 1X PBS containing 3% BSA (v/v) and mix by inverting 5 times.
9. Pellet cells by centrifugation.
10. Discard supernatant leaving only the crosslinked cell pellet and no residual liquid.

⃠ **Safe Stopping Point:** Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed directly to the Arima High Coverage HiC Protocol section

Cryopreserved Cells

Input: Cryopreserved cells
Output: Crosslinked cells

Before you begin:

- The workflow can also apply to cells preserved in cryogenic "freeze" media (e.g., cell culture media with FBS and DMSO). Thaw and crosslink these cells as you would freshly harvested cells, following the same centrifugation guidelines (typically 500 x G for 5 minutes).
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

Consumables

Arima Box A

- ☐ Stop Solution 1

User Supplied

- ☐ 15ml conical tubes
- ☐ Dry ice or liquid nitrogen
- ☐ 1X PBS, pH 7.4 (User Supplied)
- ☐ 37% formaldehyde

Protocol

1. Fill a 15mL conical tube with 4mL of 1X PBS.
2. Thaw the cryopreserved cells in a 37°C water bath.

Note: In the following step, the entire contents of the cryopreserved cell sample (i.e. cells and the cryogenic media) are transferred into the conical tube containing PBS. Do not centrifuge the cells to try and remove the cryogenic freeze media. The following step also assumes the cells are preserved in 1mL of cryogenic freeze media, and transferring the cells into the PBS will bring the total volume to 5mL. If the cells are not frozen in 1mL of cryogenic freeze media, adjust the volume of PBS so that the total sample volume after Step 3 will be 5mL.

3. Gently transfer cells, including the cryogenic freeze media, into the conical tube containing 4mL of 1X PBS, bringing the total volume to 5mL.
4. Add 286µL of 37% formaldehyde, bringing the final formaldehyde concentration to 2%.
5. Mix well by inverting 10 times and incubate at RT for 10 min.
6. Add 460µL of Stop Solution 1, mix by inverting 10 times and incubate at RT for 5 min.
7. Place sample on ice and incubate for 15 min.
8. Pellet cells by centrifugation and discard supernatant.
9. Resuspend cells in 5mL 1X PBS.
10. Aliquot cells into several new tubes, with 1 x 106 cells per aliquot. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.
11. Pellet cells in all aliquots by centrifugation.
12. Discard supernatant leaving only the crosslinked cell pellet and no residual liquid.

⃠ **Safe Stopping Point:** Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input Amount – Standard Input protocol.

Whole Nucleated Blood

Input: Whole nucleated blood in ethanol
Output: Crosslinked nucleated blood cells

Before you begin:

- For nucleated blood preserved in ethanol, begin by washing the cells before crosslinking. Typically, less than 25 µL of whole nucleated blood is needed for one Hi-C reaction, but crosslink 25 µL if sufficient blood is available. For example, if 50 µL of blood was preserved in 1 mL of ethanol, use 500 µL of this ethanol-diluted blood for crosslinking, assuming it contains 25 µL of nucleated blood. Centrifuge for 5 minutes at 2000 x G.
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

Resuspension Buffer

The Resuspension Buffer must be prepared fresh directly before use in the Crosslinking protocol. The following recipe is enough for crosslinking 8 samples. This recipe should be scaled accordingly if more or less than 8 samples are processed simultaneously. If using a 16% formaldehyde stock, please contact Technical Support for a different Resuspension Buffer formulation. The table below includes a suggested vendor and catalog number for each reagent. After the Resuspension Buffer is prepared, store at 4°C until use.

Table 3. Resuspension Buffer Recipe

Reagent	Stock Vendor	Stock Cat #	Stock Concentration	Final Concentration	Stock Amount
PBS	Fisher Scientific	50-842-949			54.45 mL
FBS	Fisher Scientific	A3160501	100%	1%	550 µL
				Total	55 mL

Consumables

Arima Box A

- ☐ Stop Solution 1

User Supplied

- ☐ 1.5mL centrifuge tubes
- ☐ 15ml conical tubes
- ☐ Dry ice or liquid nitrogen
- ☐ Resuspension Buffer (See Table 2)
- ☐ 1X PBS, pH 7.4 (User Supplied)
- ☐ 37% formaldehyde

Protocol

1. Prepare Resuspension Buffer and 1X PBS. Chill buffers on ice until cold.
2. If only using a portion of whole blood preserved in ethanol for crosslinking, resuspend the whole blood in ethanol mixture thoroughly by pipetting and transfer a portion to a new 1.5mL tube for crosslinking and proceed to the next step. With the remaining blood and ethanol mixture, freeze on dry ice or liquid nitrogen and store at -80°C.
3. Pellet cells by centrifugation and remove supernatant.
4. Resuspend pellet in 1mL Resuspension Buffer.
5. Pellet cells by centrifugation and remove supernatant.
6. Resuspend pellet in 1mL Resuspension Buffer, and transfer to a 15mL conical tube.
7. Add 4mL Resuspension Buffer, bringing the total volume to 5mL.
8. Add 286µL of 37% formaldehyde, bringing the final formaldehyde concentration to 2%.
9. Mix well by inverting 10 times and incubate at RT for 10 min.
10. Add 460µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
11. Place sample on ice and incubate for 15 min.
12. Pellet cells by centrifugation and remove supernatant.
13. Resuspend cells in 1mL 1X PBS.

Prepare for the Estimating Input Amount protocol in the following section:

a. Mix the sample by inversion and then immediately aliquot sample such that 1 aliquot contains 10% of the samples, while the rest of aliquots each contain the equivalent of ~20-25% of the sample. Mix sample by inversion between aliquots to ensure all the aliquotes are equally homogenous.

Note: The 10% aliquot will be used in the Estimating Input Amount protocol. The remaining 3 aliquotes containing 20-25% are meant to be saved as sample material for the Arima High Coverage HiC Protocol.

14. Pellet cells in all aliquots by centrifugation and remove supernatant leaving only the crosslinked cell pellet and no residual liquid.

Safe Stopping Point: Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input Amount section.

Plant Tissue Nuclei Isolation and Crosslinking

Nuclei Isolation

Input: Fresh-frozen plant tissue
Output: Isolated nuclei

Before you begin:

- This workflow requires pulverization of 2-3 g of fresh-frozen plant tissue, though smaller quantities can be used if necessary. Take extra precautions when handling liquid nitrogen, dry ice, and cold equipment, and always wear cold-resistant gloves.
- Liquid Nitrogen and Dry Ice Handling: Use extra caution when handling liquid nitrogen and dry ice. Always wear cold-resistant gloves and goggles.
- Sterilization of Equipment: Before use, sterilize mortar and pestle by spraying with 10% bleach, rinsing with water, spraying with 80% ethanol, drying with a paper towel, and then rinsing with DI-water.
- Cooling Equipment: Pre-cool pestle, mortar, and spatulas in dry ice or liquid nitrogen for at least 15 minutes before pulverization. Keep them cold throughout the procedure.

Consumables

User Supplied

- ☐ 15ml conical tubes
- ☐ Dry ice and liquid nitrogen
- ☐ CelLytic™ Plant Nuclei Isolation/ Extraction Kit

Protocol: Nuclei Isolation

1. Place a mortar and a 15mL conical tube onto a bed of dry ice, and place a pestle into the mortar. Cool a spatula at -20°C or colder for later use.
2. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Allow liquid nitrogen to evaporate completely.
3. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Transfer frozen plant tissue into mortar containing liquid nitrogen. Allow liquid nitrogen to evaporate just enough for the plant tissue to stay submerged.

4. Pulverize plant tissue in the mortar using the pestle until the sample resembles a fine powder. Ensure plant tissue is always submerged in liquid nitrogen. Carefully re-fill the mortar with liquid nitrogen as necessary. The pulverization process should take at least 8 min per sample and some tissue types may take longer. The goal is to pulverize until the tissue resembles a fine powder without visible chunks.

Note: Using cold-resistant gloves is highly recommended to handle the mortar.

5. Once the sample resembles a fine powder, allow liquid nitrogen in the mortar to evaporate just enough for sample to stay submerged.
6. Carefully pour pulverized plant tissue and remaining liquid nitrogen from the mortar into the 15mL conical tube. Ensure the tube does not overflow with liquid nitrogen.
7. Using the cooled spatula from Step 1, transfer any remaining pulverized plant tissue from the mortar into the 15mL conical tube.
8. Submerge 15mL conical tube into dry ice to keep all the pulverized plant tissue frozen.

Note: Do NOT cap the 15mL conical tube until all the liquid nitrogen has completely evaporated.

9. Allow liquid nitrogen in 15mL conical tube to evaporate completely, then cap the tube.

Note: If pulverizing multiple plant samples in a single day, keep the pulverized sample on dry ice and repeat Steps 1-9 on the remaining samples using clean equipment and consumables until all samples have been pulverized. Then, proceed to the next step and complete the remaining protocol on all samples simultaneously.

10. Complete a semi-pure plant nuclei extraction using the CelLyticTM Plant Nuclei Isolation/Extraction Kit according to the manufacturer's recommendations for Cell Lysis (Section A) and Semi-pure Preparation of Nuclei (Section B2). For the tissue grinding (Section A3), we recommend grinding for 20 min. We recommend adding the 10% TRITONTM X-100 to a final concentration of 0.5%, however this will vary between species (section A7). For the sucrose solution (Section B2), we recommend using a 1.5 M dilution instead of 2.3 M Sucrose.
11. Resuspend the nuclei pellet in 100 µL of Nuclei Pure Storage buffer

Safe Stopping Point: Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to Crosslinking – Plant Tissue.

Crosslinking

Input: Isolated nuclei
Output: Crosslinked nuclei

Consumables

Arima Box A

- ☐ Stop Solution 1

User Supplied

- ☐ 1.7mL microcentrifuge tubes
- ☐ 1X PBS, pH 7.4 (User Supplied)
- ☐ 37% formaldehyde
- ☐ Dry ice or liquid nitrogen

Protocol: Crosslinking

1. Resuspend nuclei pellet in 5mL of RT 1X PBS.
2. Add 286µL of 37% formaldehyde, bringing the final formaldehyde concentration to 2%.
3. Mix well by inverting 10 times and incubate at RT for 20 min.
4. Add 460µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
5. Place sample on ice and incubate for 15 min.
6. Pellet sample by centrifugation at 2000 x g for 10 mins at RT.
7. Discard supernatant.
8. Resuspend sample in 5mL 1X PBS.
9. Divide samples into 5 1.7mL tubes with 1mL of resuspended sample each.
10. Pellet sample in all aliquots by centrifugation at 2000 x g for 10 mins at RT..
11. Discard supernatant leaving only the crosslinked nuclei pellet and no residual liquid.

⃠ **Safe Stopping Point:** Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input Amount section.

Arima High Coverage HiC

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Estimating Input Amount

Input: Crosslinked sample or nuclei
Output: Purified genomic DNA

The Estimating Input Amount protocol is required if the amount of sample needed to provide 500 ng – 5 µg of DNA is unknown, and if sufficient sample material is available to perform this protocol. For customers working with cells, it is typically advised to perform this protocol using 1 million cells. If working with low input samples, it is recommended to skip the Estimating Input Amount step and proceed directly to the Arima High Coverage HiC protocol.

The Estimating Input Amount protocol measures the amount of DNA obtained from 10% of the original sample (no more than the equivalent of 10 mg of the sample) or 1 million cells, which helps determine the optimal input for an Arima High Coverage HiC reaction. The Arima High Coverage HiC kit contains enough reagents to perform the Estimating Input Amount protocol on 8 samples.

This protocol concludes with a descriptive example of how to estimate the optimal amount of crosslinked sample material to use per Arima High Coverage HiC reaction.

Before you begin:

- Enzyme D should be warmed to RT before use to help prevent precipitation in the master mix. Gently vortex the tube at low speed or warm up the tube to 37oC for 5 to 10 minutes if a lot of precipitation is observed; please note that it is acceptable to use Enzyme D if the reagent stays slightly cloudy.
- 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.
- Prepare the following reagents found in Arima-HiC High Coverage Box A and Box B.

Table 4. List of Reagents Required for Estimating Input Amount

Reagent	Thaw Temp.	Mix	Box
Buffer E	RT	Vortex	Box A, Arima-HiC+
Enzyme D	RT	Vortex	Box B, Arima-HiC+
Buffer D	RT	Vortex	Box B, Arima-HiC+
DNA Purification Beads	RT	Vortex	User Supplied
Freshly Made 80% Ethanol	RT	Vortex	User Supplied

- Preprogram a thermal cycler as shown in Table 4. Set the lid temperature to 85°C and name "RXlink"

Table 5. Thermal Cycler Program for Input Quantification

Temperature	Time
55°C	30 min.
68°C*	Overnight
4°C	∞

* The reaction can be incubated up to 16 hours at 68°C

Consumables

Arima Box A

- ☐ Buffer D
- ☐ Elution Buffer

Arima Box B

- ☐ Enzyme D

User Supplied

- ☐ 1.7ml Microcentrifuge Tubes
- ☐ PCR Strip
- ☐ DNA Purification Beads
- ☐ Freshly made 80% Ethanol
- ☐ HS dsDNA Qubit® Assay
- ☐ Qubit® tubes

Protocol

1. Thaw the aliquot containing 10% of crosslinked sample prepared during the Crosslinking protocol.
2. Transfer the sample to a strip tube or PCR plate.
3. Add 209.5µL of a master mix containing the following reagents listed in Table 6.

Table 6. Reagent Volumes for Preparing Reverse Crosslinking Master Mix

Reagent	Vol/Rxn	10% extra		# reactions		Final
Elution Buffer	174µL	191.4µL	x	2	=	382.8µL
Buffer D	10.5µL	11.55µL	x	2	=	23.1µL
Enzyme D	25µL	27.5µL	x	2	=	55µL
Total	209.5µL					460.9µL

4. Add 20µL of Buffer E, mix gently by pipetting.
5. Incubate on the thermal cycler using "RXlink "program. Use a reaction volume setting of 100µl, if required for thermal cycler setup and set the lid temperature to 85°C.

Note: 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 kit.

6. While samples are in the thermal cycler:
   a. Take DNA Purification Beads out of 4°C and let them warm to RT for at least 30 minutes.
   b. Prepare 1ml of fresh 80% ethanol per reaction; this includes the total volume needed per reaction plus additional overage.
7. Resuspend DNA purification beads thoroughly by vortexing on high speed for 1 minute.
8. Transfer each sample to 1.7mL tubes.
9. Add 150µL of DNA Purification Beads, mix thoroughly, and incubate at RT for 5 min.
10. Place sample against magnet and incubate until solution is clear.
11. Discard supernatant. While sample is still against magnet, add 400µL of 80% ethanol, and incubate at RT for 1 min.
12. Discard supernatant. While sample is still against magnet, add 400µL of 80% ethanol, and incubate at RT for 1 min.
13. Discard supernatant and briefly spin the tube to collect the residual ethanol. While the tube is against the magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 3 - 5 minutes to air-dry the beads
14. Remove tube from magnet, resuspend beads thoroughly in 20µL of Elution Buffer.
15. Incubate at RT for 5 min.
16. Place sample against magnet stand and incubate until solution is clear. Transfer supernatant to a new tube.
17. Quantify sample using HS Qubit® assay. Multiply the Qubit™ quantification by 20 to calculate the total DNA yield obtained from Input Estimate protocol.
18. Estimate how much sample to use per Arima High Coverage HiC reaction. See the descriptive example below:

Example: In the following Arima High Coverage HiC Protocol, it is recommended to use at least 500ng of DNA per Arima High Coverage HiC reaction, but no more than 5µg of DNA. If 250ng of DNA was obtained from 10% of the pulverized or homogenized crosslinked animal tissue as calculated above in step 17, one can estimate that at least 20% of the pulverized or homogenized crosslinked animal tissue (~500ng of DNA) is needed for Arima High Coverage HiC. If possible, we recommend aiming to use pulverized or homogenized crosslinked animal tissue comprising 1-2µg of DNA per Arima High Coverage HiC reaction. Additionally, please note that the pulverized or homogenized crosslinked animal tissue pellet for one Arima High Coverage HiC reaction should occupy no more than 20µL of volume in the sample tube. If the pulverized or homogenized crosslinked animal tissue pellet comprises 500ng-5µg of DNA but occupies greater than 20µL of volume, aliquot the sample into multiple Arima High Coverage HiC reactions such that the sum of the DNA input from all reactions is at least 500ng and each tissue pellet occupies no more than 20µL of volume, or contact Technical Support for additional guidance.

Recommended Hi-C Input Amount Explanation: The recommendation to use pulverized or homogenized crosslinked animal tissue comprising at least 500ng of DNA is only a general recommendation. If crosslinked animal tissue comprising at least 500ng of DNA cannot be obtained, one should proceed with the Arima High Coverage HiC Protocol in this user guide and then use our validated low-input library prep protocol.

Arima High Coverage HiC Protocol

Input: Crosslinked Sample
Output: Proximally-ligated DNA

Before you begin:

The Arima High Coverage HiC reactions are optimally performed on crosslinked sample comprising ~500ng - 5µg of DNA. In the first step the cell pellets are lysed. The crosslinked chromatin is then digested using restriction enzymes. The 5′ overhangs are then filled in, causing the digested ends to be labeled with biotinylated nucleotide. Spatially proximal digested ends of DNA are ligated, capturing the sequence and structure of the genome. The ligated DNA is then purified, producing pure proximally-ligated DNA.

The tissue pellet for one Arima High Coverage HiC reaction should occupy no more than 20µL of volume and should be devoid of any residual liquid. If the tissue pellet occupies greater than 20µL of volume, aliquot the tissue such that the sum of the DNA input from all reactions is between 500ng-5µg and each tissue pellet occupies no more than 20µL of volume. This may mean that for certain tissue types (e.g. ones with low cellularity or fatty tissues), 2 Arima-HiC reactions may need to be performed on a given sample and recombined at Step 29 of the Arima High Coverage HiC Protocol.

Consumables

Arima Box A

- ☐ Conditioning Solution
- ☐ Stop Solution 2
- ☐ Buffer D

Arima Box B

- ☐ Lysis Buffer
- ☐ Buffer F
- ☐ Enzyme F1
- ☐ Enzyme A2
- ☐ Enzyme F3
- ☐ Enzyme F4
- ☐ Buffer G
- ☐ Enzyme B
- ☐ Buffer C
- ☐ Enzyme C
- ☐ Enzyme D
- ☐ User Supplied
- ☐ DNA Purification Beads
- ☐ Freshly made 80% Ethanol
- ☐ PCR Strip Tubes
- ☐ Nuclease Free Water

Protocol

Note: Some of the reaction volumes during incubation steps in thermal cyclers, are greater than 100µl. For such volumes, set the reaction volumes on the thermal cycler to 100µl. The volumes have been tested and no adverse effect on the enzymatic performance of the reactions has been observed. Also, steps 2 – 3 require consecutive heated incubations. Make sure your thermal device(s) are set to 62°C and 37°C for these incubations. The safe stopping point in this section is after completing Step 21.

1. Prepare the following
2. Table 7 reagents found in Arima-HiC+ Box A and Box B.

Table 7. List of Reagents Required for Arima High Coverage HIC

Reagent	Thaw Temp.	Mix	Day needed
Lysis Buffer	RT	Vortex	Day 1
Conditioning Solution	RT	Vortex	Day 1
Stop Solution 2	RT	Vortex	Day 1
Buffer A	On Ice	Vortex	Day 1
Enzyme A1	On Ice	Pipetting	Day 1
Enzyme A2	On Ice	Pipetting	Day 1
Buffer B	On Ice	Vortex	Day 1
Enzyme B	On Ice	Pipetting	Day 1
Buffer C	On Ice	Vortex	Day 1
Enzyme C	On Ice	Pipetting	Day 1
Buffer E	RT	Vortex	Day 2
Enzyme D	RT	Vortex	Day 2
Buffer D	RT	Vortex	Day 2
DNA Purification Beads	RT	Vortex	Day 2
Freshly Made 80% Ethanol	RT	Vortex	Day 2

1. Choose to perform either Step 1a if the input sample type is pulverized crosslinked animal sample that is homogeneous and easy to pipette, Step 1b if the sample type is pulverized crosslinked sample tissue that is clumpy, sticky, and difficult to pipette, or Step 1c only if the input sample type is crosslinked nuclei that have been previously purified from tissue.
   a. Resuspend one reaction of pulverized crosslinked cells in 20µL of Lysis Buffer in a tube or a well of a PCR plate, and incubate at 4°C for 30 min.

b. Resuspend one reaction of pulverized crosslinked cells in 40µL of Lysis Buffer in a tube or a well of a PCR plate, and incubate at 4°C for 30 min. Following the incubation split into 2 reactions and proceed to the next step.

c. Resuspend one reaction of purified crosslinked nuclei in 20µL of Water in a tube or a well of a PCR plate and procced to the next step.

2. Add 24µL of Conditioning Solution, mix gently by pipetting, and incubate at 62°C for 10 min. If using a thermal cycler, set the lid temperature to 85°C.

3. Add 20µL of Stop Solution 2, mix gently by pipetting, and incubate at 37°C for 15 min. If using a thermal cycler, set the lid temperature to 85°C.

4. Preprogram a thermal cycler with program in Table 8. Set the lid temperature to 85oC and name it "Digestion."

Table 8. Thermal Cycler Program for Digestion

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

Note: Steps 4, 6, 8 and 10 require addition of several reagents in the same step. These reagents should be combined into master mixes following the master mix tables.

5. Prepare the appropriate volume of the digestion master mix by combining the reagents in Table 9.

Table 9. Reagent Volumes for Preparing Digestion Master Mix

Reagent	Vol/Rxn	10% extra		# reactions		Final
Buffer F	7.5µL	8.25µL	x	2	=	16.5µL
Enzyme F1	1.5µL	1.65µL	x	2	=	3.3µL
Enzyme A2	1.5µL	1.65µL	x	2	=	3.3µL
Enzyme F3	1.5µL	1.65µL	x	2	=	3.3µL
Enzyme F4	1.5µL	1.65µL	x	2	=	3.3µL
Total	13.5µL					29.7µL

6. Add 13.5µL of master mix containing the reagents in Table 9.

7. Mix gently by pipetting and incubate in the thermal cycler using "Digestion" program. If using a thermal cycler, set the lid temperature to 85°C. Note that there are sequential incubations at different temperatures.

Safe Stopping Point: To provide flexibility in the workflow, this incubation can be held overnight at 10°C using a thermal cycler or thermomixer with a heated lid to prevent evaporation.

8. Prepare the appropriate volume of Fill-in master mix by combining the reagents in Table 10.

Table 10. Reagent Volumes for Preparing Fill-in Master Mix

Reagent	Vol/Rxn	10% extra		# reactions		Final
Buffer G	12μL	13.2μL	x	2	=	26.4μL
Enzyme B	4μL	4.4μL	x	2	=	8.8μL
Total	16μL					35.2μL

9. Add 16μl of fill-in master mix to each sample.
10. Mix gently by pipetting, and incubate at room temperature (RT) for 45 min.
11. Prepare the appropriate volume of ligation master mix by combining the reagents listed in Table 11. Mix thoroughly by pipetting up and down and spin down tube to collect liquid.

Table 11. Reagent Volumes for Preparing Ligation Master Mix

Reagent	Vol/Rxn	10% extra		# reactions		Final
Buffer C	70μL	77μL	x	2	=	154μL
Enzyme C	12μL	13.2μL	x	2	=	26.4μL
Total	82μL					180.4μL

12. Add 82μL of a master mix containing the following reagents:
13. Mix gently by pipetting, and incubate at RT for 15 min.
14. Preprogram a thermal cycler with program in
15. Table 12 Set the lid temperature to 85°C and name "RevXlink_Tissue" or "RevXLink_Cells"

Table 12. Thermal Cycler Program for Reverse crosslinking

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

* Overnight incubation at 68°C must be performed if tissue particles are still readily visible. If so, this overnight incubation must be performed using a thermal cycler with a heated lid.

Safe Stopping Point: To provide flexibility in the workflow, this incubation can also be held overnight at 4°C.

Note: Take out Enzyme D from -20°C in advance and leave at RT. Enzyme D should be warmed to RT to help to prevent precipitation in the master mix.

16. Prepare the appropriate volume of Reverse Crosslinking master mix by combining the reagents in Table 13 and mix thoroughly. Make sure Enzyme D is warmed to the RT before preparing the master mix. Keep the master mix at RT until use.

Table 13. Reagent Volumes for Preparing Reverse Crosslinking Master Mix

Reagent	Vol/Rxn	10% extra		# reactions		Final
Buffer D	10.5μL	11.55μL	x	2	=	23.1μL
Enzyme D	25μL	27.5μL	x	2	=	55μL
Total	35.5μL					78.1μL

17. Add 35.5μl of Reverse Cross-linking master mix to each sample. Mix Gently by pipetting up and down.
18. Add 20μl of Buffer E, mix gently by pipetting up and down.
19. Incubate in the thermal cycler using "Revlink_Tissue" or "RevLink_Cells" depending on the input sample type.
20. While Samples are incubating warmed DNA Purification beads to RT and thoroughly mixed before use.

Note: 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.

21. Prepare 1ml of freshly made 80% ethanol per reaction, this includes the total volume needed per each sample plus additional overage.

22. After incubation, transfer samples to a 1.7ml centrifuge tube, or a 0.8ml MIDI plate. Make sure samples are at RT before adding DNA purification beads.
23. Vortex DNA purification beads vigorously on a vortex for 1 minute.
24. Add 100µL of DNA Purification Beads, mix thoroughly by pipetting up and down 10 to 15 times and incubate at RT for 5 min.
25. Place sample against magnet and incubate until solution is clear.
26. Discard supernatant. While sample is still against magnet, add 300µL of 80% ethanol, and incubate at RT for 1 min.
27. Discard supernatant. While sample is still against magnet, add 300µL of 80% ethanol, and incubate at RT for 1 min.
28. Discard supernatant and briefly spin the tube to collect the residual ethanol. While sample is against magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 3 – 5 min. to air-dry the beads.

Note: If 2 Arima High Coverage HiC reactions had to be performed due to fatty difficult tissue, elute each Arima High Coverage HiC reaction in 50µL of Elution Buffer in step 29 below, and then combine the two samples prior to sample quantification in Step 19.

29. Remove sample from magnet, resuspend beads thoroughly in 50µL of Elution Buffer, and incubate at RT for 5 min.
30. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
31. Quantify sample using Qubit®.

Note: If the proximally-ligated DNA yield is less than 275ng, we recommend skipping the Arima-QC1 assay mentioned in Step 20 and described the following Arima-QC1 Quality Control section, and strongly recommend performing the Arima-QC2 assay described in our Arima High Coverage HiC Library Preparation user guide for low input samples.

32. Transfer 75ng of sample into a new tube labelled "Arima-QC1", and if needed add Elution Buffer to Arima-QC1 to bring the volume to 50µL. The "Arima-QC1" sample should now contain 75ng of proximally-ligated DNA in 50µL of Elution Buffer. Store at -20°C until use in the following Arima-QC1 Quality Control protocol.

⊘ **Safe Stopping Point:** If you are not proceeding to Arima-QC 1 or Arima Library Prep, proximally-ligated DNA can be stored at -20°C up to 14 days.

Arima-QC1 Quality Control

Input: Proximally-ligated DNA
Output: QC1 measurement

Before you begin:

The following protocol quantifies the fraction of proximally-ligated DNA that has been labeled with biotin, and is a quality control metric after completing the Arima High Coverage HiC Protocol but before proceeding to library preparation. The Arima-QC1 Quality Control protocol involves using QC Beads to enrich an aliquot of proximally-ligated DNA, which is then quantified using a Qubit® fluorometer. Unlike standard Qubit® readings which involve quantifying a transparent unobstructed DNA sample, the Arima-QC1 value is obtained by quantifying DNA that is still bound to the QC Beads. This protocol can be performed in either plates or tubes and the procedure is the same for all sample types. After completing the Arima-QC1 Quality Control protocol, use the provided Arima High Coverage HiC QC Worksheet to determine the Arima-QC1 values.

Set your thermal device (thermal cycler or thermomixer) to hold at 55°C. After completing the Arima-QC1 Quality Control protocol, use the provided: Arima High Coverage HiC QC Worksheet to determine the Arima-QC1 values.

Consumables

Arima Box B

- ☐ Elution Buffer
- ☐ Wash Buffer

Arima Box C

- ☐ QC Beads

User Supplied

- ☐ dsDNA HS Qubit® Assay
- ☐ Qubit Tubes
- ☐ PCR Strip tubes or PCR plates

Protocol

1. If necessary, thaw the "Arima-QC1" samples prepared in the Arima High Coverage HiC Protocol in the previous section.
2. Add 50μL of QC 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 7μL of Elution Buffer. Proceed to next step with resuspended beads.

Note: The following step involves the quantification of the bead-bound DNA using the Qubit® dsDNA HS Assay Kit.

15. Quantify the total amount of bead-bound DNA using Qubit®. Use 2μL of thoroughly mixed bead-bound DNA for the Qubit® assay.
16. Determine the Arima-QC1 value by following the Arima High Coverage HiC QC Worksheet. High quality Arima-QC1 values are expected to have a value of >15% of the original 50ng input onto the beds, indicating sufficient biotinylation of the HiC DNA. If the Arima-QC1 value did not obtain a 'PASS' status, please contact Technical Support for troubleshooting assistance.

If Arima-QC1 values are >15%, proceed to library prep.

After completion of quantification step, the remaining bead-bound DNA can be discarded.

Arima Library Preparation

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Introduction

Library preparation begins with DNA fragmentation, DNA size selection, and biotin enrichment. Afterward, the Arima Library Prep Module reagents are used in a custom end-repair, dA-tailing, and adapter ligation protocol. This protocol yields 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

Input: Proximally-ligated DNA
Output: Fragmented Proximally-ligated DNA

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 100µL 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.

Consumables:

Arima High Coverage HiC, Box A (RT)

- ☐ Elution Buffer

User Supplied Reagents

- ☐ Covaris MicroTube for individual Sample processing

OR

Bioruptor NGS 0.65 Microtubes

- ☐ Gel Electrophoresis System

Protocol

1. Before fragmentation, if necessary, add Elution Buffer to bring the sample volume to 100µl. Do not exceed 100µl of volume for DNA fragmentation. Mix the sample and spin the tube to collect the liquid and to remove any bubbles from the bottom of the tube.
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.

Table 14. Fragmentation Settings for Covaris E220

Setting	Value
Temperature	7°C
Peak Incident Power	105
Duty Factor	5%
Cycles per Burst	200
Treatment time (s)	70

3. 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.

Safe Stopping Point: Samples may be stored at -20°C for up to 3 days.

DNA Size Selection

Input: Fragmented Proximally-ligated DNA
Output: Size Selected Proximally-Ligated DNA

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.

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.

Consumables:

Arima High Coverage HiC, Box A (RT)

- [ ] Elution Buffer

User Supplied Reagents

- [ ] DNA Purification Beads

Protocol

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 100µL 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 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.

Safe Stopping Point: Samples may be stored at -20°C for up to 3 days.

Biotin Enrichment

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

Consumables:

Arima High Coverage HiC, Box A (RT)

- [ ] Elution Buffer
- [ ] Wash Buffer

Arima Library Prep Module Box C (4°C)

- [ ] T1 Beads

Arima Library Prep Module Box D (RT)

- [ ] Binding Buffer

User Supplied Reagents

- [ ] Nuclease-free water

Protocol

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. Using a new strip tube, add 12.5µL of T1 Beads from the Arima Library Prep Box C into each tube, one tube per 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. Wash the beads two more times by repeating steps 4–7 for a total of three washes.
9. Resuspend beads in 200uL of Binding Buffer.
10. Transfer 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. Vortex washed Streptavidin Beads (T1) in Binding Buffer to mix thoroughly.
12. Add 200µL of washed T1 Beads in Binding Buffer to each 30ul fragmented sample, mix thoroughly by pipetting, and incubate at RT for 15 min on a shaker at 1400 RPM.
13. Place sample against magnet and incubate until solution is clear.
14. Discard supernatant and remove sample from magnet.
15. Wash beads by resuspending in 200µL of Wash Buffer and incubate at 55°C for 2 min. Set lid temperature to 85°C.
16. Place sample against magnet and incubate until solution is clear.
17. Discard supernatant and remove sample from magnet.
18. Wash beads by resuspending in 200µL of Wash Buffer and incubate at 55°C for 2 min. Set lid temperature to 85°C.
19. Place sample against magnet and incubate until solution is clear.
20. Discard supernatant and remove sample from magnet.
21. Wash beads by resuspending in 100µL of Elution Buffer.
22. Place sample against magnet and incubate until solution is clear.
23. Discard supernatant and remove sample from magnet.
24. Resuspend beads in 50µL of Deionized / Nuclease-free Water.

Library Preparation of Enriched Ligation products

Input: Purified Proximally-Ligated DNA
Output: Arima High Coverage HiC Sequencing Library

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.

Consumables:

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
- [ ] Freshly prepared 80% ethanol
- [ ] Qubit assay and tubes

Protocol

End Repair

1. Thaw reagents and mix reagents according to Table 15.

Note: Thaw ligation buffer and vortex on high to make sure homogenous (buffer is highly viscous).

Table 15. Thawing and Mixing Instructions for End Repair

Reagent	Thaw Temp.	Mix	Cap
End Repair-A Tailing Buffer	On Ice	Vortex	Yellow
Ligation Buffer	Room Temp	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 16, which includes 12.5% master mix overage for 8 reactions).

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

Table 16. Ligation Master Mix Worksheet

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

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

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

4. Prepare End Repair/dA-Tailing master mix by combining reagents as listed in Table 17, mix well and spin down.

Table 17. End Repair/dA Tailing Master Mix Worksheet

Reagent	Vol/Rxn	12.5% extra		# reactions		Final
End Repair-A Tailing Buffer	16 µL	18 µL	x	8	=	144 µL
End Repair-A Tailing Enzyme Mix	4 µL	4.5 µL	x	8	=	36 µL
Total	20 µL					180 µL

5. 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 18. Set reaction volume for 70µL, and the heated lid to 85°C, and press start. Total run time is approx. 30 min.

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

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

2. Once thermal cycler has reached 4°C hold step, transfer samples to ice while preparing the ligation reaction.
3. Add 25µL of room temperature 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 Table 19 below. Set the reaction volume to 100µL and press start. Total time is approx. 30 min.

Table 19. Adapter Ligation Thermal Cycler Program

Temperature	Time
20°C	30 min
4°C	∞

7. After the "Ligation" program completes, remove the samples from the thermocycler and quick spin the tubes to remove any liquid from the caps.
8. Magnetize beads until liquid is clear. Remove and discard supernatant.
9. Resuspend beads in 200µL Wash Buffer. Mix by pipetting. Incubate at 55C for 2 min. Set lid temperature to 85°C
10. Magnetize beads until liquid is clear. Remove and discard supernatant.
11. Resuspend beads in 100µL Elution Buffer.
12. Magnetize beads until liquid is clear. Remove and discard supernatant.
13. 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 20 below and keep on ice.

Table 20. 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 21 as a reference.

Table 21. 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 22 below. Mix well.

Table 22. PCR Reaction Mix

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

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 23.

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

*If you are working with samples with <200 ng of material please reference the table below for suggested cycle numbers.

Table 24. [Needs table name]

DNA ng	Cycles
50 – 99 ng	15
100 – 200 ng	12

Note: If you are working with challenging or lower-input samples (e.g. <50 ng), please contact technical support.

8. Place the PCR reaction in the thermocycler and press start.

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 50µL 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 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 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. Prepare a 1:10 dilution (1µL of sample with 9µL of water).
11. Quantify sample using Qubit™ using 1µL.
12. Run the sample from the previous step on a gel or other platform to determine the size distribution of the HiC library.

⃠ **Safe Stopping Point:** Samples may be stored at -20°C for up to 6 months.

Warranty and Contact Info

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