

USER GUIDE

# Arima-HiC<sup>+</sup> Kit

A160509

For use with:

A510008 | Arima-HiC<sup>+</sup> Kit, 8 rxn

A303011 | Arima Library Prep Kit, 16 rxn

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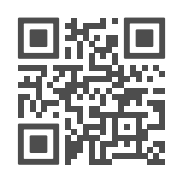
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## Revision Notes

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Specific Changes	<ul style="list-style-type: none"><li>• Modifications to Crosslinking Protocol for Low Input: Frozen Animal Tissue</li><li>• Corrected digestion master mix input</li></ul>
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Scan this QR code to download the **Arima-HiC+ Quick Reference Protocol**.



# Introduction

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## Arima-HiC Workflow Overview

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



Figure 1. Arima-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-HiC libraries are sequenced via Illumina® sequencers in “paired-end” mode. The resulting data is referred to as Arima-HiC data. The tools necessary for analyzing Arima-HiC data depend on the application. For example, for studying 3D genome conformation, we recommend processing Arima-HiC data with Juicer (Durand, 2016a). Genome organizational features such as compartments, TADs, and loops can be identified using Juicer tools and visualized using tools such as Juicebox (Durand, 2016b). To accommodate your Arima-HiC data you will need to make several minor modifications to the analysis pipeline. You will need to provide a custom Arima-HiC-specific restriction site file. In the Juicer version 1.6, this is the -y parameter in the juicer.sh command line script. Arima hosts these files for mm9, mm10, hg19, and hg38 on [ftp://ftp-arimagenomics.sdsc.edu/pub/JUICER\\_CUTSITE\\_FILES](ftp://ftp-arimagenomics.sdsc.edu/pub/JUICER_CUTSITE_FILES). We have also modified the existing Juicer

supplied python script, that generates custom cut-site files. Our modification accommodates multiple restriction enzyme cut sites and recognizes sequences which contain 'N.' This can be found at: [https://github.com/ArimaGenomics/Scripts/blob/master/generate\\_site\\_positions\\_Arima.py](https://github.com/ArimaGenomics/Scripts/blob/master/generate_site_positions_Arima.py)

For the -s argument, please type "Arima". Docker/Singularity images for Juicer can be found on Arima's GitHub: [https://github.com/ArimaGenomics/Juicer\\_pipeline\\_containers](https://github.com/ArimaGenomics/Juicer_pipeline_containers). HiC-Pro (Servant, 2015), can also be used to analyze HiC data. When using HiC-Pro, it is recommended to trim 5 bp from the 5' ends of each read using tools like fastp, Cutadapt, or Trimmomatic. In addition, to accommodate Arima HiC data, the following changes are recommended for the configuration file:

```
MIN_FRAG_SIZE = 10
```

```
MAX_FRAG_SIZE = 100000
```

```
MIN_INSERT_SIZE = 100
```

```
MAX_INSERT_SIZE = 1000
```

Pre-generated HiC-Pro cut site files for hg19, hg38, mm10 and mm9 can be found on our server at: [ftp://ftp-arimagenomics.sdsc.edu/pub/HiCPro\\_GENOME\\_FRAGMENT\\_FILES](ftp://ftp-arimagenomics.sdsc.edu/pub/HiCPro_GENOME_FRAGMENT_FILES).

Please contact tech support at [techsupport@arimagenomics.com](mailto:techsupport@arimagenomics.com) for additional information.

## How to Cite Arima-HiC+ in Publications

When citing the Arima-HiC+ protocol or kit, one may write:

*"Hi-C data was generated using the Arima-HiC+ kit, according to manufacturers protocols".*

Please reference the catalog number found on the kit packaging.

# Getting Started



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## Handling and Preparation

- Several steps during the Arima-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 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 min. 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 min. 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-HiC+ Kit

Description	Arima P/N
<b>Arima-HiC+ Kit</b>	<b>A101030, A101031</b>
Box A (20 to 25°C)	A410231
<input type="checkbox"/> Stop Solution 1 <input type="checkbox"/> Elution Buffer <input type="checkbox"/> Wash Buffer <input type="checkbox"/> Conditioning Solution	<input type="checkbox"/> Stop Solution 2 <input type="checkbox"/> Buffer D <input type="checkbox"/> Buffer E
Box B (-20°C)	A410232
<input type="checkbox"/> Lysis Buffer <input type="checkbox"/> Enzyme A2 <input type="checkbox"/> Buffer A <input type="checkbox"/> Buffer G <input type="checkbox"/> Enzyme A1 <input type="checkbox"/> Enzyme B	<input type="checkbox"/> Buffer B <input type="checkbox"/> Enzyme C <input type="checkbox"/> Enzyme D
Box C (4°C)	A410239
<input type="checkbox"/> Enrichment Beads	<input type="checkbox"/> QC Beads

### Arima Material Checklist | Arima Library Prep Module

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

## Equipment Checklist

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

<sup>1</sup> Only if processing Fresh Frozen Tissues

## User Supplied Reagents and Consumables

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

<sup>1</sup> If working with "low-input" cell cultures (<1M cells)

<sup>2</sup> If working with whole nucleated blood

<sup>3</sup> If working with PBMCs

<sup>4</sup> If working with plant tissues

<sup>5</sup> 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-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 50 $\mu$ L 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 50 $\mu$ L when pooled at the bottom of a 1.5mL microfuge tube.

## Optimal Read Length, Sequencing Depth, and Number of Arima-HiC Reactions Per Sample

Arima-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-HiC data alignment quality, although shorter read lengths (e.g. 2x100bp) and lower throughput instruments can certainly be used for certain applications of Arima-HiC data such as 3D genome conformation analysis and genome scaffolding.

The optimal sequencing depth for Arima-HiC libraries also depends on the application. 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-HiC data across replicates, and then use the combined replicate Arima-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.

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-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).

As a general rule, each Arima-HiC library should be complex enough to sequence up to ~600M read-pairs without reaching saturation. If >600M read-pairs of Arima-HiC data are needed, it may be more efficient to sequence a second Arima-HiC library than sequence deeper into the first Arima-HiC library.

# Crosslinking Protocols



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## Introduction

The Arima-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-HiC workflow for animal tissues starts with pulverization and crosslinking. For vertebrates and large invertebrates with dense tissues, weigh 50-200mg 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 min. 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	ThermoFisher Scientific	50-843-406			1.67mL
Sodium Chloride	ThermoFisher Scientific	PR-V4221	5M	100mM	100µL
EDTA	ThermoFisher Scientific	PR-V4231	0.5M	1mM	10µL
EGTA	ThermoFisher Scientific	BM-151	0.5M	0.5mM	5µL
HEPES pH 8.0	ThermoFisher Scientific	H1090	1M	50mM	250µL
Formaldehyde*	ThermoFisher Scientific	F79-500	37%	22%	3mL
				Total	5mL

\* 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
- 1X PBS, pH 7.4 (User Supplied)
- Dry ice and liquid nitrogen
- 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 500 $\mu$ L 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 289 $\mu$ L 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 100 $\mu$ L (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 50 $\mu$ L (5% = 10mg) rather than 100 $\mu$ L (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-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  $\leq 50\text{mg}$  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 min. 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	Thermo Fisher Scientific	50-843-406			1.67mL
Sodium Chloride	Thermo Fisher Scientific	PR-V4221	5M	100mM	100 $\mu$ L
EDTA	Thermo Fisher Scientific	PR-V4231	0.5M	1mM	10 $\mu$ L
EGTA	Thermo Fisher Scientific	BM-151	0.5M	0.5mM	5 $\mu$ L
HEPES pH 8.0	Thermo Fisher Scientific	H1090	1M	50mM	250 $\mu$ L
Formaldehyde*	Thermo Fisher Scientific	F79-500	37%	22%	3mL
Total					5mL

\* 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. If sample is not already in a 1.5mL Eppendorf tube, carefully transfer tissue to a 1.5mL microfuge tube and place on dry ice.
2. Keep the tube on dry ice, add a small amount of liquid nitrogen while pulverizing, and manually grind the tissue using a disposable plastic pestle, (Ex. Fisher Scientific # 12-141-368). Grind until the tissue resembles a fine powder. Let any residual liquid nitrogen evaporate.
3. Thaw sample tube for 2 min. Add 1mL of 1X PBS and mix gently by inversion.
4. Add 100 $\mu$ L of fresh TC buffer, bringing the final concentration of formaldehyde to 2%.
5. Mix well by inverting 10 times and incubate at RT for 20 min.
6. Add 57.8 $\mu$ L of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
7. Pellet sample by centrifugation a 2,500 X G at RT for 5 min.
8. Carefully discard the supernatant.

**Note:** For low sample quantities (e.g., 5mg of vertebrate tissue or a single mosquito portion), flash-freeze the pellet in liquid nitrogen or on dry ice and store at  $-80^{\circ}\text{C}$  unless proceeding to the Arima-HiC Protocol immediately. The entire homogenized sample will be used as input for the Arima-HiC Protocol. If sample quantity is sufficient, proceed to steps 9-12 below.

9. Add 1mL of 1X PBS. To prepare for the Estimating Input Amount protocol in the next section, mix the sample by inversion and then immediately aliquot sample such that 1 aliquot contains the equivalent of 10% of the original homogenized animal tissue, while the rest of the aliquots each contain the equivalent of approx. 20-25% of the homogenized animal tissue. Mix sample by inversion between aliquots to ensure all aliquots are equally homogenous.

**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-HiC Protocol.

10. Pellet samples by centrifugation at 2,500 X G at RT for 5 min.
11. Carefully discard the supernatant.
12. Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input Amount Protocol in the following section.

⊘ **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-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 2mL 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 min. before pulverization. Keep them cold throughout the procedure.

### Consumables

#### Arima Box A

- Stop Solution 1

#### User Supplied

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

## 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 min. 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 min. before pulverization. Keep them cold throughout the procedure.

### **Consumables**

#### Arima Box A

- Stop Solution 1

#### User Supplied

- 15mL conical tubes
- 1X PBS, pH 7.4 (User Supplied)
- Dry ice or liquid nitrogen
- 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 286 $\mu$ L 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 460 $\mu$ L 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  $1 \times 10^6$  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  $-80^{\circ}\text{C}$  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 min.) 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 min. before pulverization. Keep them cold throughout the procedure.

### Consumables

#### Arima Box A

- Stop Solution 1

#### User Supplied

- 1.7mL microfuge
- 1X PBS containing 3% BSA (v/v)
- Dry ice or liquid nitrogen
- 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 57 $\mu$ L of 37% formaldehyde, mix well by inverting 10 times and incubate at RT for 10 min. with occasional inversion.
4. Add 91.9 $\mu$ L 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-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 min.).
- 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 min. before pulverization. Keep them cold throughout the procedure.

### Consumables

#### Arima Box A

- Stop Solution 1

#### User Supplied

- 15mL conical tubes
- 1X PBS, pH 7.4 (User Supplied)
- Dry ice or liquid nitrogen
- 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  $1 \times 10^6$  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 $\mu$ L of whole nucleated blood is needed for one Hi-C reaction, but crosslink 25 $\mu$ L if sufficient blood is available. For example, if 50  $\mu$ L of blood was preserved in 1mL of ethanol, use 500  $\mu$ L of this ethanol-diluted blood for crosslinking, assuming it contains 25 $\mu$ L of nucleated blood. Centrifuge for 5 min. 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 min. 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	Thermo Fisher Scientific	50-842-949			54.45mL
FBS	Thermo Fisher Scientific	A3160501	100%	1%	550 $\mu$ L
				Total	55mL

## 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-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  $-80^{\circ}\text{C}$  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 2-3g 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 min. 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.

- 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.

- Once the sample resembles a fine powder, allow liquid nitrogen in the mortar to evaporate just enough for sample to stay submerged.
- 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.
- Using the cooled spatula from Step 1, transfer any remaining pulverized plant tissue from the mortar into the 15mL conical tube.
- 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.

- 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.

- Complete a semi-pure plant nuclei extraction using the CelLytic 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% Triton® 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 1.5M dilution instead of 2.3M Sucrose.

- Resuspend the nuclei pellet in 100µL 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
- 37% formaldehyde
- 1X PBS, pH 7.4 (User Supplied)
- Dry ice or liquid nitrogen

## Protocol: Crosslinking

1. Resuspend nuclei pellet in 5mL of RT 1X PBS.
2. Add 286 $\mu$ L 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 460 $\mu$ L 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 min. 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 min. 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-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 500ng – 5µg 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-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 10mg of the sample) or 1 million cells, which helps determine the optimal input for an Arima-HiC reaction. The Arima-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-HiC reaction.

**Note:** The Estimating Input Amount does not account for areas of loss within the Arima-HiC+ protocol which can lead to quantifications that overestimate sample input. On the other hand, if too much sample is input into the estimation and the aliquot is too concentrated, the quantification can underestimate sample input. Because of these potential discrepancies, we recommend considering the DNA yield of the sample from previous experiments if possible. For most cell types,  $\sim 1 \times 10^6$  will land you within the recommended range of 500ng-5µg of DNA.

### 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 37°C for 5 to 10 min. 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+ 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  HS dsDNA Qubit Assay  
 PCR Strip  Qubit tubes  
 DNA Purification Beads  
 Freshly made 80% Ethanol

## 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-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 min.
  - 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 min.
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 min. to air-dry the beads
14. Remove tube from magnet, resuspend beads thoroughly in 20 $\mu$ L 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-HiC+ reaction. See the descriptive example below:

**Example:** In the following Arima-HiC Protocol, it is recommended to use at least 500ng of DNA per Arima-HiC reaction, but no more than 5 $\mu$ g 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-HiC. If possible, we recommend aiming to use pulverized or homogenized crosslinked animal tissue comprising 1-2 $\mu$ g of DNA per Arima-HiC reaction. Additionally, please note that the pulverized or homogenized crosslinked animal tissue pellet for one Arima-HiC reaction should occupy no more than 20 $\mu$ L of volume in the sample tube. If the pulverized or homogenized crosslinked animal tissue pellet comprises 500ng-5 $\mu$ g of DNA but occupies greater than 20 $\mu$ L of volume, aliquot the sample into multiple Arima-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 20 $\mu$ L 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-HiC Protocol in this user guide and then use our validated low-input library prep protocol.

## Arima-HiC Protocol

**Input:** Crosslinked Sample

**Output:** Proximally-ligated DNA

### Before you begin:

The Arima-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-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-HiC Protocol.

## Consumables

### Arima Box A

- Conditioning Solution
- Stop Solution 2
- Buffer D

### Arima Box B

- Lysis Buffer
- Buffer A
- Enzyme A1
- Enzyme A2
- 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

**Note:** Some of the reaction volumes during incubation steps in thermal cyclers, are greater than 100 $\mu$ L. For such volumes, set the reaction volumes on the thermal cycler to 100 $\mu$ L. 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-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 20 $\mu$ L 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 40 $\mu$ L 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 20 $\mu$ L of Water in a tube or a well of a PCR plate and proceed to the next step.

- Add 24 $\mu$ L 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.
- Add 20 $\mu$ L 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.
- Preprogram a thermal cycler with program in Table 8. Set the lid temperature to 85°C and name it "Digestion."

Table 8. Thermal Cycler Program for Digestion

Temperature	Time
37°C	60 min.*
65°C	20 min.
10°C	$\infty$

**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.

- 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 A	7 $\mu$ L	7.7 $\mu$ L	x	2	=	15.4 $\mu$ L
● Enzyme A1	1 $\mu$ L	1.1 $\mu$ L	x	2	=	2.2 $\mu$ L
● Enzyme A2	4 $\mu$ L	4.4 $\mu$ L	x	2	=	8.8 $\mu$ L
Total	12 $\mu$ L					26.4 $\mu$ L

- Add 12 $\mu$ L of digestion master mix from Table 9.
- 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 B	12 $\mu$ L	13.2 $\mu$ L	x	2	=	26.4 $\mu$ L
● Enzyme B	4 $\mu$ L	4.4 $\mu$ L	x	2	=	8.8 $\mu$ L
Total	16 $\mu$ L					35.2 $\mu$ L

9. Add 16 $\mu$ L of fill-in master mix to each sample from Table 10.
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	70 $\mu$ L	77 $\mu$ L	x	2	=	154 $\mu$ L
● Enzyme C	12 $\mu$ L	13.2 $\mu$ L	x	2	=	26.4 $\mu$ L
Total	82 $\mu$ L					180.4 $\mu$ L

12. Add 82 $\mu$ L of ligation master mix to each sample from Table 11.
13. Mix gently by pipetting
14. Incubate at RT for 15 min.
15. Preprogram a thermal cycler with program in 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	$\infty$

- \* 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 min.
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 300 $\mu$ L 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-HiC reactions had to be performed due to fatty difficult tissue, elute each Arima-HiC reaction in 50 $\mu$ L 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 100 $\mu$ L 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-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 50 $\mu$ L. The "Arima-QC1" sample should now contain 75ng of proximally-ligated DNA in 50 $\mu$ L 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-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-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-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
- PCR Strip tubes or PCR plates
- Qubit Tubes

## Protocol

1. If necessary, thaw the "Arima-QC1" samples prepared in the Arima-HiC Protocol in the previous section.
2. Add 50 $\mu$ L 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 200 $\mu$ L 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 200 $\mu$ L 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 100 $\mu$ L 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 7 $\mu$ L 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 2 $\mu$ L of thoroughly mixed bead-bound DNA for the Qubit assay.
16. Determine the Arima-QC1 value by following the Arima-HiC QC Worksheet. High quality Arima-QC1 values are expected to have a value of >15% of the original 75ng input onto the beads, 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.

Poor resuspension of the bead-bound DNA can result in artificially low/failing QC1 scores.

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-HiC Library using the library amplification reagents and index PCR primers from the Arima Library Prep Module, producing the final Arima-HiC Library.

## DNA Fragmentation

**Input:** Proximally-ligated DNA

**Output:** Fragmented Proximally-ligated DNA

### **Before you begin:**

The output of the Arima-HiC Protocol is large proximally-ligated DNA molecules. These large DNA molecules must be fragmented using mechanical methods to limit sequence bias, and then prepared as a sequencing library that is compatible with Illumina sequencing instruments. Covaris instruments are recommended for mechanical fragmentation of DNA, although Diagenode® instruments have also been tested and yield comparable results. DNA should be fragmented in 100µL of Elution Buffer. 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-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 ~400bp. Please use the DNA fragmentation instrument manufacturer default settings for obtaining a target fragment size of ~400bp. If manufacturer default settings for ~400bp are not available, then we recommend using slightly less shearing time than that of a target size of 400bp. Exemplary Covaris E220 settings are noted below for obtaining a target fragment size of ~400bp.

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 between 350-550 and centered approximately around 400-450bp. 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:

In this step fragmented DNA are size-selected to remove small fragments approximately less than 200bp. 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 Hi-C+, 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(1X) of DNA Purification Beads to each sample, mix thoroughly by pipetting up and down, 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 $\mu$ L 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.

**⊘ 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 230 $\mu$ L 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-HiC Enrichment Beads nor the Arima-HiC QC Beads.

### **Consumables:**

#### Arima-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 12.5 $\mu$ L 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 HiC+ kit.

3. Wash the T1 Beads in each tube by:
4. Add 200 $\mu$ L of Binding Buffer.
5. Mix by pipetting up and down 20 times, cap the tubes, and vortex at high speed for 5 - 10 sec.
6. Place tubes against a magnet and incubate 5 min. 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 200 $\mu$ L 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 30 $\mu$ L.  
  
\*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 200 $\mu$ L of washed T1 Beads in Binding Buffer to each 30 $\mu$ L 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 200 $\mu$ L 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 200 $\mu$ L 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 -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-HiC+, Box A (RT)

- Elution Buffer
- Wash Buffer

#### Arima Library Prep Module Box A (-20°C)

- End Repair-A Tailing Enzyme Mix
- Adaptor Oligo Mix
- End Repair-A Tailing Buffer
- 5X Herculase II Buffer with dNTPs
- T4 DNA Ligase
- Herculase II Fusion DNA Polymerase
- Ligation Buffer

#### 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
- Qubit assay and tubes
- Freshly prepared 80% ethanol

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	23µL	25.88µL	x 8	= 207µL
T4 DNA Ligase	2µL	2.25µL	x 8	= 18µL
Total	25µL			225µL

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

- Vortex thawed vial of End Repair-A Tailing Buffer for 15 sec. - continue vortexing until no solids are observed.
- 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 $\mu$ L	18 $\mu$ L	x	8	=	144 $\mu$ L
End Repair-A Tailing Enzyme Mix	4 $\mu$ L	4.5 $\mu$ L	x	8	=	36 $\mu$ L
Total	20 $\mu$ L					180 $\mu$ L

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

## Adapter Ligation

- Program thermal cycler for End Repair and dA-Tailing using the parameters in Table 18. Set reaction volume for 70 $\mu$ L, 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	$\infty$

- Once thermal cycler has reached 4°C hold step, transfer samples to ice while preparing the ligation reaction.
- Add 25 $\mu$ L of room temperature Ligation Master Mix, to the 70 $\mu$ L of bead-bound, end-repaired and dA-tailed HiC library. Mix well.
- Add 5 $\mu$ L of Adaptor Oligo Mix to each sample. Mix well.
- Briefly spin tubes with the bead-bound HiC library, Ligation master mix, and Adaptor Oligo Mix.
- Program the thermal cycler for the ligation step with the program specified in Table 19 below. Set the reaction volume to 100 $\mu$ L and press start. Total time is approx. 30 min.

Table 19. Adapter Ligation Thermal Cycler Program

Temperature	Time
20°C	30 min.
4°C	∞

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

## Amplification of Adaptor-Ligated HiC Library and Sample Indexing

- 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

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

- 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 $\mu$ L	11.25 $\mu$ L	x 8	= 90 $\mu$ L
Herculase II Fusion DNA Polymerase (red cap)	1 $\mu$ L	1.125 $\mu$ L	x 8	= 9 $\mu$ L
Total	11 $\mu$ L			99 $\mu$ L

- Add 11 $\mu$ L of the PCR reaction mixture prepared from the table above to 34 $\mu$ L of Adaptor Ligated Bead Bound HiC Library.
- Add 5 $\mu$ L of the appropriate, unique, Index Primer Pair to each sample. Make sure to take note of which index was used with each sample.
- 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.
10X*	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 <200ng of material please reference the table below for suggested cycle numbers.

Table 24. PCR Number Cycle Recommendations

DNA ng	Cycles
50 - 99ng	12
100 - 200ng	10

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

- 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.

- Add 50µL of DNA Purification Beads to each 50µL Indexed sample. Mix well.
- Incubate for 5 min. at room temperature.
- Place sample against magnet and incubate until solution is clear.
- Discard supernatant. While sample is still against magnet, add 200µL of 80% ethanol, and incubate at RT for 1 min.
- 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 15 $\mu$ L 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 (1 $\mu$ L of sample with 9 $\mu$ L of water).
11. Quantify sample using Qubit using 1 $\mu$ L.
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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