Arima-HiC Kit

User Guide for Animal Tissues

8 reactions

Material Part Number: A510008
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This product is intended for research use only. This product is not intended for diagnostic purposes.

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This user manual must be read in advance of using the product and strictly followed by qualified and properly trained personnel to ensure proper use of the Arima-HiC kit. Failure to do so may result in damage to the product, injury to persons, and/or damage to other property. Arima Genomics does not assume any liability resulting from improper use of its products or others referenced herein.

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

Revision History

Document	Date	Description of Change
Material Part Number: A510008 **Document Part Number:** A160132 v00	November 2018	Initial Release
Material Part Number: A510008 **Document Part Number:** A160132 v01	October 2019	• Reduced recommended tissue input in *Crosslinking – Large Animals* section. • Revised tissue input amount in *Estimating Input Amount – Large Animals* section. • Revised Enzyme D incubation at 68°C from 90 min. to overnight in *Estimating Input Amount* sections. • Added guidance for handling particularly fatty or low cellularity tissues in *Arima-HiC Protocol* section. • Revised Lysis Buffer incubation from 15 to 30 min. in *Arima-HiC Protocol* section. • Revised Enzyme A1 and A2 incubation from 30 to 60 min. in *Arima-HiC Protocol* section. • Added guidance for optional overnight Enzyme D incubation in *Arima-HiC Protocol* section.

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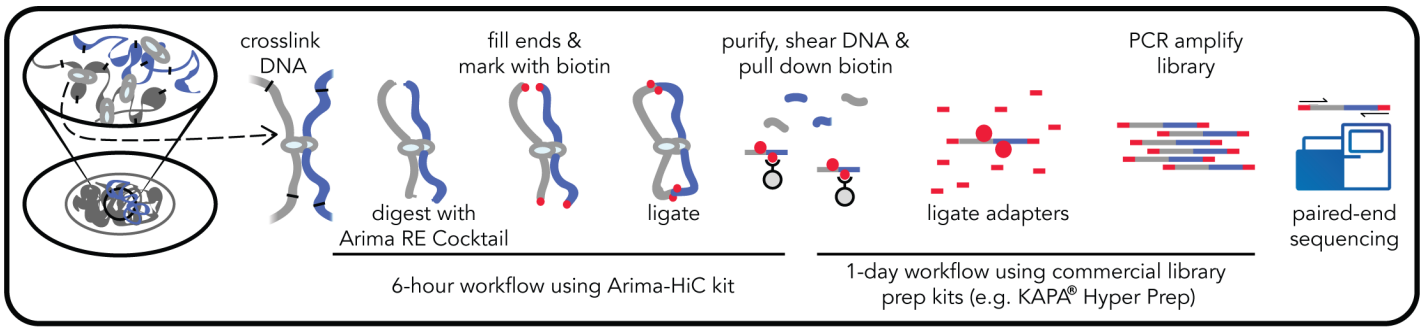

1.1 Arima-HiC Workflow Overview

Arima-HiC is an experimental workflow that captures the sequence and structure (three-dimensional conformation) of genomes. Arima-HiC has been successfully performed on a wide-range of species from the plant and animal kingdoms. As illustrated in the Arima-HiC workflow schematic above, chromatin from a sample source (tissues, cell lines, or blood) is first crosslinked to preserve the genome sequence and structure. The crosslinked chromatin is then digested using a restriction enzyme (RE) cocktail. The 5'-overhangs are then filled in, causing the digested ends to be labeled with a biotinylated nucleotide. Next, 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 proximally-ligated DNA is then fragmented, and the biotinylated fragments are enriched. The enriched fragments are then subjected to a custom library preparation protocol utilizing a range of supported commercially available library prep kits. Depending on the choice of library prep kit, a separate Arima-HiC Library Prep user guide is provided that contains a custom protocol for converting proximally-ligated DNA to Arima-HiC libraries.

1.2 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, Arima-HiC data can be processed using publicly available tools such as Juicer (Durand, 2016a) or Hi-C Pro (Servant, 2015), and genome organizational features such as compartments, TADs, and loops can be identified and visualized using tools such as Juicebox (Durand, 2016b). These tools require usage modifications and/or custom input files that are specific to Arima-HiC data, so please contact Technical Support for assistance implementing these tools. Additionally, because paired-end reads of Arima-HiC data can originate from distal sequences along the linear genome, these data capture short- and long-range DNA contiguity information that is valuable for applications such as *de novo* assembly and genome scaffolding. Therefore, Arima-HiC data can be mapped to contigs/unitigs using our mapping pipeline (https://github .com/ArimaGenomics) or Juicer, and then the contigs/unitigs can be scaffolded using tools such as SALSA (Ghurye, 2019) or 3D-DNA (Dudchenko, 2017). Please contact Technical Support for more information.

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Arima-HiC Quick Reference Protocol

Crosslink Sample

Resuspend cells in 20µL ● **Lysis Buffer** OR nuclei in 20µL water[1]
Incubate 15 min. at 4°C

Add 24µL ○ **Conditioning Solution**
Incubate 10 min. at 62°C

Add 20µL ● **Stop Solution 2**
Incubate 15 min. at 37°C

Add 7µL ○ **Buffer A** + 1µL ○ **Enzyme A1** + 4µL ○ **Enzyme A2**
Incubate 30 min. at 37°C followed by 20 min. at 62°C

Add 12µL ● **Buffer B** + 4µL ● **Enzyme B**
Incubate 45 min. at 25°C

Add 70µL ● **Buffer C** + 12µL ● **Enzyme C**
Incubate 15 min. at 25°C

Add 10.5µL ● **Buffer D** + 25µL ● **Enzyme D**
Add 20µL ● **Buffer E**
Incubate 30 min. at 55°C followed by 90 min. at 68°C

Purify DNA
Proceed to library prep using the unique protocol outlined in the User Guide

[1] If nuclei have been isolated prior to lysis step (e.g. from plant tissue, FAC-sorted nuclei)

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Arima-HiC Box Contents & Storage Info

Box A		
Component	**Cap**	**Storage**
Stop Solution 1		20 to 25°C
Elution Buffer		
Wash Buffer*		
Conditioning Solution		
Stop Solution 2		
Buffer D		
Buffer E		

Box B		
Component	**Cap**	**Storage**
Lysis Buffer		-20°C
Buffer A		
Enzyme A1		
Enzyme A2		
Buffer B		
Enzyme B		
Buffer C		
Enzyme C		
Enzyme D		

Box C		
Component	**Cap**	**Storage**
Enrichment Beads*		2 to 8°C
QC Beads**		

* Required for library prep. Depending on choice of library prep kit, the User Guide has a unique library prep protocol to be followed

** Required for Arima-QC1. QC Beads are not interchangeable with Enrichment Beads

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2.1 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. See Section 2.2 for recommended PCR plates and PCR tubes. 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.
- 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.

2.2 User-supplied reagents, consumables, and equipment checklist

- ☐ Freshly prepared **TC Buffer** (see Section 2.3 for recipe)
- ☐ 1X PBS, pH 7.4 (e.g. Fisher Scientific® Cat # 50-842-949)
- ☐ Freshly prepared 80% Ethanol
- ☐ DNA Purification Beads (e.g. Beckman Coulter Cat # A63880)
- ☐ Qubit® Fluorometer, dsDNA HS Assay Kit and required consumables (e.g. Thermo Fisher Scientific Cat # 32851, 32856)
- ☐ Liquid nitrogen and dry ice
- ☐ 15mL conical tubes
- ☐ 1.7mL microcentrifuge tubes, PCR tubes (e.g. SSIbio® Cat # 3247-00), or PCR plates (e.g. Bio-Rad® Cat # HSS9641) and magnetic rack compatible with tube selection.
- ☐ Centrifuge
- ☐ Thermal cycler or thermomixer (if performing Arima-HiC in PCR tubes/plates, or microfuge tubes, respectively)
- ☐ Ceramic mortar and pestle (e.g. Cole-Parmer® Cat # UX-63100-63)
- ☐ Metal spatula (Cole-Parmer® Cat # SI-06369-16)
- ☐ Cold-resistant gloves

2.3 Buffer Recipes

TC Buffer – The **TC Buffer** must be prepared fresh directly before use in the *Crosslinking* protocols. 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 **TC Buffer** formulation. The table below includes a *suggested* vendor and catalog number for each reagent. After the **TC Buffer** is prepared, store at RT until use.

Reagent	Stock Vendor	Stock Cat #	Stock Concentration	Final Concentration	Stock Amount
Water	Fisher Scientific®	50-843-406			1.67mL
Sodium	Fisher Scientific®	PR-V4221	5M	100mM	100µL
EDTA	Fisher Scientific®	PR-V4231	0.5M	1mM	10µL
EGTA	Fisher Scientific®	BM-151	0.5M	0.5mM	5µL
HEPES pH 8.0	Fisher Scientific®	H1090	1M	50mM	250µL
Formaldehyde	Fisher Scientific®	F79-500	37%	22%	3mL
				Total	**5mL**

2.4 Determining whether the sample should be classified as a large or small animal

In this user guide, there are three crosslinking protocols and two protocols for estimating the optimal animal tissue input amount for an Arima-HiC reaction. For two of the crosslinking protocols, the amount of animal tissue to pulverize and crosslink depends on whether the sample is from a large or small animal. Animal classifications for Arima-HiC are as follows:

- Most vertebrates and large invertebrates are considered large animals. These large animals comprise dense tissues, and the amount of input tissue required for pulverization and crosslinking (50-200mg) is typically obtained from a portion of one animal (e.g. tumor, liver or muscle tissue) or sometimes a few whole animals in the case of certain invertebrates.
- Small animals are those in which the tissue input amount to pulverization and crosslinking is not well approximated by mass (e.g. flies, mosquitoes, small worms). Instead, the optimal number of whole animals is determined by the volume they occupy (~50µL).

Please contact Technical Support for additional guidance.

2.5 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. 2x50bp, 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 used 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).

Lastly, it is important to note that each Arima-HiC library should pass the Arima-QC2 assay and be evaluated for library complexity prior to deep sequencing. 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.

2.6 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 the manufacturers protocols". Please reference the catalog number found on the kit packaging.

Crosslinking – Large Animals

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

Before you begin: The Arima-HiC workflow for large animal tissues begins with the pulverization and crosslinking of fresh-frozen large animal tissue. For most vertebrates and large invertebrates that comprise dense tissues, begin by weighing 50-200mg of fresh frozen tissue, and record this measured mass. The measured mass will be used later in this protocol and the following *Estimating Input Amount – Large Animals* protocol. For some applications, less than 50mg can be used, particularly when sample quantity is scarce. If less than 50mg of tissue is available, please contact Technical Support for additional guidance. Also, note that this crosslinking protocol requires the handling of liquid nitrogen, dry ice, and severely cold equipment. Please use extra caution and wear cold-resistant gloves as needed.

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 large animal tissue into mortar containing liquid nitrogen. Allow liquid nitrogen to evaporate just enough for the large animal tissue to stay submerged.
4. Pulverize large animal tissue in the mortar using the pestle until the sample resembles a fine powder. Ensure the large animal tissue is always submerged in liquid nitrogen. *Carefully re-fill the mortar with liquid nitrogen as necessary*. 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.

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 large animal 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 large animal tissue from the mortar into the 15mL conical tube.
8. 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.

9. 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 1-9 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. Remove sample tube from dry ice, 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 well by inverting 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 2,000 x G at RT for 15 min.
15. Discard supernatant.
16. Resuspend sample in 1mL **1X PBS**.
17. To prepare for the *Estimating Input Amount – Large Animals* protocol in a following section, mix the sample by inversion and then immediately aliquot sample such that 1 aliquot contains the equivalent of 10%* of the original pulverized large animal tissue, while the rest of the aliquots each contain the equivalent of ~20-25% of the pulverized large animal tissue. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.

* The 10% aliquot will be used in the *Estimating Input Amount – Large Animals* protocol. The 10% aliquot should not contain more than the equivalent of 10mg of the original pulverized large animal tissue. For example, if one is preparing 200mg of pulverized large animal tissue, then the small aliquot should be 5% (10mg) rather than 10% (20mg). The remaining 3 aliquots containing 20-25% are meant to be saved as sample material for the *Arima-HiC Protocol*.

18. Pellet all samples by centrifugation at 2,000 x G at RT for 10 min.
19. Discard supernatant leaving behind only the sample pellet and no residual liquid.
20. Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the *Estimating Input Amount – Large Animals* protocol in a following section.

Crosslinking – Small Animals

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

Before you begin: The Arima-HiC workflow for small animal tissues begins with the pulverization and crosslinking of fresh-frozen small animal tissue (e.g. flies, mosquitoes, small worms). Begin by estimating how many whole animals are required for pulverization and crosslinking. The optimal number of whole animals may need to be determined on a case-by-case basis, but a reasonable starting point is to pulverize and crosslink enough whole animals that would fill a microfuge tube to the 50µL mark. As an additional reference point, approximately 5 whole mosquitos should be more than sufficient material. Please contact Technical Support for additional guidance. Also, note that this crosslinking protocol requires the handling of liquid nitrogen, dry ice, and severely cold equipment. Please use extra caution and wear cold-resistant gloves as needed.

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 small animal tissue into mortar containing liquid nitrogen. Allow liquid nitrogen to evaporate just enough for the small animal tissue to stay submerged.
4. Pulverize small animal tissue in the mortar using the pestle until the sample resembles a fine powder. Ensure the small animal tissue is always submerged in liquid nitrogen. *Carefully re-fill the mortar with liquid nitrogen as necessary*. 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.

Note: Using cold-resistant gloves is 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 small animal 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 small animal tissue from the mortar into the 15mL conical tube.
8. Submerge 15mL conical tube in 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.

9. 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 1-9 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. Remove sample tube from dry ice, 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 well by inverting 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 2,000 x G at RT for 15 min.
15. Discard supernatant.
16. Resuspend sample in 1mL **1X PBS**.
17. To prepare for the *Estimating Input Amount – Small Animals* protocol in a following section, mix the sample by inversion and then immediately aliquot sample such that 1 aliquot contains the equivalent of 10%* of the original pulverized small animal tissue, while the rest of the aliquots each contain the equivalent of ~20-25% of the pulverized small animal tissue. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.

* The 10% aliquot will be used in the *Estimating Input Amount – Small Animals* protocol. The remaining aliquots containing 20-25% are meant to be saved as sample material for the *Arima-HiC Protocol*.

18. Pellet all samples by centrifugation at 2,000 x G at RT for 10 min.
19. Discard supernatant leaving behind only the sample pellet and no residual liquid.
20. Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the *Estimating Input Amount – Small Animals* protocol in a following section.

Estimating Input Amount – Large Animals

Input: 10% aliquot of pulverized crosslinked large animal tissue
Output: Purified genomic DNA

Before you begin: Arima-HiC reactions are optimally performed on pulverized crosslinked large animal tissue comprising ~500ng-5µg of DNA. The *Estimating Input Amount – Large Animals* protocol is required if one does *not* know how much pulverized crosslinked large animal tissue will comprise 500ng-5µg of DNA, and if sufficient tissue is available to perform this protocol. If sufficient tissue is not available, please contact Technical Support for additional guidance. The *Estimating Input Amount – Large Animals* protocol measures the amount of DNA obtained from 10% of the original pulverized crosslinked large animal tissue, or no more than the equivalent of 10mg of pulverized crosslinked large animal tissue, which guides the calculation of the optimal tissue input for an Arima-HiC reaction. The Arima-HiC kit contains enough reagents to perform an *Estimating Input Amount* protocol on 8 samples. This protocol concludes with a descriptive example of how to estimate the optimal amount of pulverized crosslinked large animal tissue to use per Arima-HiC reaction.

Note: Step 2 requires addition of several reagents in the same step. These reagents should be combined into master mixes with 10% excess volume before use. Also note that Enzyme D should be warmed to RT before use to help prevent precipitation in the master mix.

1. Thaw the aliquot containing 10% of pulverized crosslinked large animal tissue prepared during the previous *Crosslinking – Large Animals* protocol.
2. Add 209.5µL of a master mix containing the following reagents:

Reagent	Volume per reaction	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

3. Add 20µL of ● **Buffer E**, mix gently by pipetting, and incubate as follows. If using a thermal cycler, set the lid temperature to 85°C.

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

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-HiC kit.

4. Add 150µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.
5. Place sample against magnet, and incubate until solution is clear.
6. Discard supernatant. While sample is still against magnet, add 400µL of 80% ethanol, and incubate at RT for 1 min.
7. Discard supernatant. While sample is still against magnet, add 400µL of 80% ethanol, and incubate at RT for 1 min.
8. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
9. Remove sample from magnet, resuspend beads thoroughly in 20µL of **Elution Buffer**, and incubate at RT for 5 min.
10. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
11. Quantify sample using Qubit®. The total DNA yield corresponds to the amount of DNA obtained from 10% of the crosslinked pulverized large animal tissue.
12. Estimate how much pulverized crosslinked large animal tissue to use per Arima-HiC reaction. See the descriptive example below:

    Example: In the following *Arima-HiC Protocol*, it is recommended to use pulverized crosslinked large animal tissue comprising at least 500ng of DNA per Arima-HiC reaction, but no more than 5µg of DNA. If 250ng of DNA was obtained from 10% of the pulverized crosslinked large animal tissue as calculated above in step 11, one can estimate that *at least* 20% of the pulverized crosslinked large animal tissue (~500ng of DNA) is needed for Arima-HiC. If possible, we recommend aiming to use pulverized crosslinked large animal tissue comprising 3µg of DNA per Arima-HiC reaction. Additionally, please note that the pulverized crosslinked large animal tissue pellet for one Arima-HiC reaction should occupy no more than 20µL of volume in the sample tube. If the pulverized crosslinked large animal tissue pellet comprises 500ng-5µg of DNA but occupies greater than 20µL 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 20µL of volume, or contact Technical Support for additional guidance.

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

Estimating Input Amount – Small Animals

Input: 10% aliquot of pulverized crosslinked small animal tissue
Output: Purified genomic DNA

Before you begin: Arima-HiC reactions are optimally performed on pulverized crosslinked small animal tissue comprising ~500ng-5µg of DNA. The *Estimating Input Amount – Small Animals* protocol is required if one does *not* know how much pulverized crosslinked small animal tissue will comprise 500ng-5µg of DNA, and if sufficient tissue is available to perform this protocol. If sufficient tissue is not available, please contact Technical Support for additional guidance. The *Estimating Input Amount – Small Animals* protocol measures the amount of DNA obtained from 10% of the original pulverized crosslinked small animal tissue, which guides the calculation of the optimal tissue input for an Arima-HiC reaction. The Arima-HiC kit contains enough reagents to perform an *Estimating Input Amount* protocol on 8 samples. This protocol concludes with a descriptive example of how to estimate the optimal amount of pulverized crosslinked small animal tissue to use per Arima-HiC reaction.

Note: Step 2 requires addition of several reagents in the same step. These reagents should be combined into master mixes with 10% excess volume before use. Also note that Enzyme D should be warmed to RT before use to help prevent precipitation in the master mix.

1. Thaw the aliquot containing 10% of pulverized crosslinked small animal tissue prepared during the previous *Crosslinking – Small Animals* protocol.
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Reagent	Volume per reaction	10% extra		# reactions		Final
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Total	**209.5µL**					460.9µL

3. Add 20µL of ● **Buffer E**, mix gently by pipetting, and incubate as follows. If using a thermal cycler, set the lid temperature to 85°C.

Temperature	Time
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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-HiC kit.

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11. Quantify sample using Qubit®. The total DNA yield corresponds to the amount of DNA obtained from 10% of the crosslinked pulverized small animal tissue.
12. Estimate how much pulverized crosslinked small animal tissue to use per Arima-HiC reaction. See the descriptive example below:

    Example: In the following *Arima-HiC Protocol*, it is recommended to use pulverized crosslinked small animal tissue comprising at least 500ng of DNA per Arima-HiC reaction, but no more than 5µg of DNA. If 250ng of DNA was obtained from 10% of the pulverized crosslinked small animal tissue as calculated above in step 11, one can estimate that *at least* 20% of the pulverized crosslinked small animal tissue (~500ng of DNA) is needed for Arima-HiC. If possible, we recommend aiming to use pulverized crosslinked small animal tissue comprising 3µg of DNA per Arima-HiC reaction. Additionally, please note that the pulverized crosslinked small animal tissue pellet for one Arima-HiC reaction should occupy no more than 20µL of volume in the sample tube. If the pulverized crosslinked small animal tissue pellet comprises 500ng-5µg of DNA but occupies greater than 20µL 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 20µL of volume, or contact Technical Support for additional guidance.

    Recommended HiC Input Amount Explanation: The recommendation to use pulverized crosslinked small animal tissue comprising at least 500ng of DNA is only a *general* recommendation. If crosslinked small 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.

Input: Pulverized crosslinked animal tissue comprising ~500ng-5µg of DNA
Output: Proximally-ligated DNA

Before you begin: 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 17 of the *Arima-HiC Protocol*. Please contact Technical Support for additional guidance. Note that 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.

Note: Choose to perform either Step 1a if the input sample type is pulverized crosslinked animal tissue that is homogeneous and easy to pipette, Step 1b if the sample type is pulverized crosslinked animal 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.

1a. Resuspend one reaction of pulverized crosslinked animal tissue in 20µL of ● **Lysis Buffer** in a tube or a well of a PCR plate, and incubate at 4°C for 30 min.

1b. Resuspend one reaction of pulverized crosslinked animal tissue 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.

1c. 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.

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.

4. Add 12µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Buffer A	7µL	7.7µL	x	2	=	15.4µL
Enzyme A1	1µL	1.1µL	x	2	=	2.2µL
Enzyme A2	4µL	4.4µL	x	2	=	8.8µL
Total	**12µL**					26.4µL

5. Mix gently by pipetting, and incubate as follows. If using a thermal cycler, set the lid temperature to 85°C. Note that there are sequential incubations at different temperatures:

Temperature	Time
37°C	60 min.
65°C	20 min.
25°C	10 min.

6. Add 16µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Buffer B	12µL	13.2µL	x	2	=	26.4µL
Enzyme B	4µL	4.4µL	x	2	=	8.8µL
Total	**16µL**					35.2µL

7. Mix gently by pipetting, and incubate at room temperature (RT) for 45 min.
8. Add 82µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Buffer C	70µL	77µL	x	2	=	154µL
Enzyme C	12µL	13.2µL	x	2	=	26.4µL
Total	**82µL**					180.4µL

9. Mix gently by pipetting, and incubate at RT for 15 min.

Note: Enzyme D should be warmed to RT to prevent precipitation in the below master mix.

10. Add 35.5µL of a master mix containing the following reagents:

Reagent	Volume per reaction	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

11. Add 20µL of ● **Buffer E**, mix gently by pipetting, and incubate as follows. If using a thermal cycler, set the lid temperature to 85°C.

Temperature	Time
55°C	30 min.
68°C	90 min.*
25°C**	10 min.**

* 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.

** To provide flexibility in the workflow, this incubation can also be held overnight at 4°C.

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-HiC kit.

12. Add 100µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.
13. Place sample against magnet, and incubate until solution is clear.
14. Discard supernatant. While sample is still against magnet, add 300µL of 80% ethanol, and incubate at RT for 1 min.
15. Discard supernatant. While sample is still against magnet, add 300µL of 80% ethanol, and incubate at RT for 1 min.
16. Discard supernatant. While sample is still against magnet, 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 *50µL* of Elution Buffer in Step 17 below, and then combine the two samples prior to sample quantification in Step 19.

17. Remove sample from magnet, resuspend beads thoroughly in 100µL of **Elution Buffer**, and incubate at RT for 5 min.
18. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
19. 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.

20. Transfer 75ng of sample into a new tube labelled "Arima-QC1", and 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.
21. Store all remaining samples at -20°C until ready to proceed to library preparation following an accompanying *Arima-HiC Library Preparation* user guide.

Arima-QC1 Quality Control

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. 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.

1. If necessary, thaw the "Arima-QC1" samples prepared during Step 20 of the *Arima-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-HiC QC Worksheet**. High quality Arima-QC1 values are expected to be >15%. If the Arima-QC1 value did not obtain a 'PASS' status, please contact Technical Support for troubleshooting assistance.

Warranty and Contact Info

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