Arima High Coverage HiC Kit

User Guide for Animal Tissues

8 reactions

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Revision History

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Table of Contents

Introduction 5

Arima High Coverage HiC Quick Reference Protocol 7

Arima High Coverage HiC Kit Contents and Storage Info 8

Getting Started 9-12

Crosslinking – Standard Input 13-14

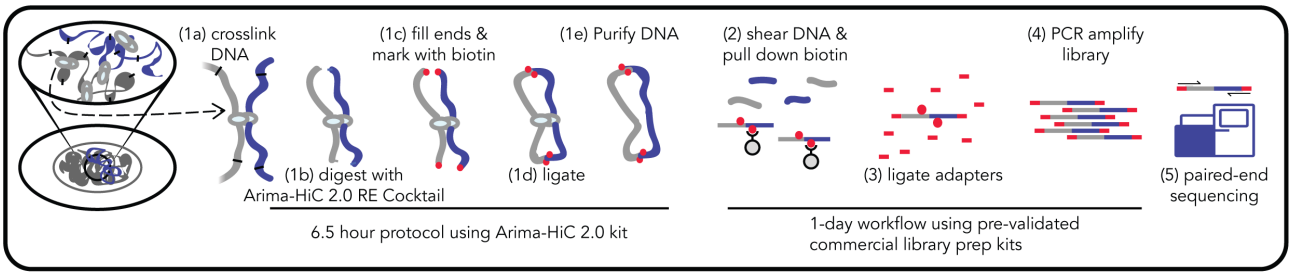
Crosslinking – Low Input 15-16

Estimating Input Amount 17-18

Arima High Coverage HiC Protocol 19-22

Arima-QC1 Quality Control 23

Warranty and Contact Info 25



1.1 Arima High Coverage HiC Workflow Overview

Arima High Coverage HiC is an experimental workflow that captures the sequence and structure (three-dimensional conformation) of genomes. As illustrated in the Arima High Coverage 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 optimized for coverage uniformity across a wide range of genomic sequence compositions. 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 High Coverage HiC Library Prep user guide is provided that contains a custom protocol for converting proximally-ligated DNA to Arima High Coverage HiC libraries.

1.2 Sequencing and Data Analysis

Arima High Coverage HiC libraries are sequenced via Illumina® sequencers in "paired-end" mode. The resulting data is referred to as Arima High Coverage HiC data. The tools necessary for analyzing Arima High Coverage HiC data depend on the application. For example, for studying 3D genome conformation, Arima High Coverage HiC data can be processed using publicly available tools such as Juicer (Durand, 2016a) 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 High Coverage HiC data, so please contact Technical Support for assistance implementing these tools. Additionally, because paired-end reads of Arima High Coverage HiC data can originate from distal sequences along the linear genome, these data capture short- and long-range DNA contiguity information that is valuable for applications such as *de novo* assembly, genome scaffolding, and haplotype phasing. Therefore, Arima High Coverage 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). Lastly, because the Arima High Coverage HiC data provides uniform per base genome coverage while maintaining the highest long-range contiguity signal, it uniquely benefits

analyses such as variant discovery, base polishing, scaffolding, and phasing. Please contact Technical Support for more information.

Arima High Coverage HiC Quick Reference Protocol

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Date: June 2020

Arima High Coverage 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 7.5µL ○ **Buffer F** + 1.5µL ○ **Enzyme F1** + 1.5µL ○ **Enzyme A2** +1.5µL ○ **Enzyme F3** + 1.5µL ○ **Enzyme F4**
Incubate 60 min. at 37°C followed by 20 min. at 65°C

Add 12µL ● **Buffer G** + 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 High Coverage HiC Kit Contents and Storage Info

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Kit P/N: A410110
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Arima High Coverage HiC
Box Content & 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 F		
Enzyme F1		
Enzyme A2		
Enzyme F3		
Enzyme F4		
Buffer G		
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 using the Arima-HiC 2.0 Library Preparation user guide.

** 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 High Coverage HiC Protocol require preparation of a master mix. Sufficient reagent has been included in the kit to make master mixes with 10% excess volume. Use the master mix calculation tables provided.
- When handling reagents, room temperature (RT) is defined as 20 to 25°C.
- If the Arima High Coverage HiC Protocol is performed in PCR plates or PCR tubes, ensure to have a total volume capacity of at least 320µL. 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.
- ● **Enzyme D** may contain precipitates. If present, these precipitates must be dissolved before use. Heating these reagents at 37-42°C for 5-10 minutes may be necessary to dissolve precipitates.
- During handling and preparation, reagents from **Box A** should be kept at RT.
- During handling and preparation, reagents from **Box B** should be kept on ice, except for **Enzyme D,** which should be kept on ice but warmed to room temperature just before use.
- Enzyme solutions from **Box B** are viscous and require special attention during pipetting.

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 High Coverage HiC in PCR tubes/plates, or microfuge tubes, respectively)

- Powermasher II (DiagnoCine Cat # 891300)
- Biomasher® II Pestle and Tube Set (DiagnoCine Cat # 320103)
- 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 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. Classifications for each input type are as follows:

2.4.1 Standard Input:

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

2.4.2 Low Input:

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

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

2.5 Optimal read length, sequencing depth, and number of Arima High Coverage HiC reactions per sample

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

The optimal sequencing depth for Arima High Coverage 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 High Coverage HiC data across replicates, and then used the combined replicate Arima High Coverage HiC dataset for high-resolution chromatin conformation analyses. Alternatively, one can obtain at least 600 million read-pairs *per biological replicate* and then use the common set of identified genome conformational features across replicates as a "high confidence" set of structural features supported by their observation in both replicates. For lower resolution analyses of A/B compartments and TADs, we generally recommend obtaining at least 300 million read-pairs per biological condition. For help estimating the optimal sequencing depth for different genome sizes or analysis goals, please contact Technical Support.

For applications such as *de novo* assembly and genome scaffolding, the required sequencing depth can vary depending on the quality of contig/unitigs that are being scaffolded using Arima High Coverage HiC data. For a 3Gb genome, we recommend obtaining up to 600M read-pairs, as this is the amount of sequencing that is currently utilized from Arima-HiC libraries for genome scaffolding

by the Vertebrate Genome Project (VGP) consortia. The amount of sequencing required scales linearly with the genome size (e.g. up to 200M read-pairs for a 1Gb genome).

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

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

2.6 How to cite Arima High Coverage HiC in publications

When citing the Arima High Coverage HiC protocol or kit, one may write: "Hi-C data was generated using the Arima High Coverage HiC kit, according to the manufacturers protocols". Please reference the catalog number found on the kit packaging.

Crosslinking – Standard Input

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

Before you begin: The Arima High Coverage HiC workflow for Standard Input animal tissues begins with pulverization and crosslinking. 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* protocol. For some applications, less than 50mg can be used, particularly when sample quantity is scarce and great care is taken during this protocol to avoid sample loss. If less than 50mg of tissue is available, please follow the *Crosslinking – Low Input* protocol in the next section. Also, note that this crosslinking protocol requires the handling of liquid nitrogen, dry ice, and extremely 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 animal tissue into mortar containing liquid nitrogen. Allow liquid nitrogen to evaporate just enough for the animal tissue to stay submerged.
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*. 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 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 for 2 min., then add 5mL of **1X PBS** and mix gently by inversion.
10. Add 500µL of fresh **TC Buffer**, bringing the final concentration of formaldehyde to 2%.
11. Mix well by inverting 10 times and incubate at RT for 20 min.
12. Add 289µL of **Stop Solution 1,** mix well by inverting 10 times and incubate at RT for 5 min.
13. Pellet sample by centrifugation at 2,500 x G at RT for 5 min.
14. Discard supernatant.
15. Resuspend sample in 1mL **1X PBS**.
16. To prepare for the *Estimating Input Amount* 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 animal tissue, while the rest of the aliquots each contain the equivalent of ~20-25% of the pulverized 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* protocol. The 10% aliquot should not contain more than the equivalent of 10mg of the original pulverized animal tissue. For example, if one is preparing 200mg of pulverized 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 High Coverage HiC Protocol*.

17. Pellet all samples by centrifugation at 2,500 x G at RT for 5 min.
18. Discard supernatant leaving behind only the sample pellet and no residual liquid.
19. 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.

Crosslinking – Low Input

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

Before you begin: The Arima High Coverage HiC workflow for Low Input animal tissues begins with crosslinking and tissue homogenization of dense large animal tissues with a mass less than or equal to ~50mg or small quantities of small animal tissues. See Getting Started Section 2.4 for more information. For most vertebrates and large invertebrates that comprise dense tissues, begin by weighing the fresh frozen tissue and record this measured mass. The measured mass will be used later in this protocol and the *Estimating Input Amount* protocol in the next section.

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

   Note: If homogenizing multiple samples in a single day, keep all crosslinked samples on ice and complete the following tissue homogenization (Step 8) on each individual sample using clean disposable pestles until all crosslinked samples have been homogenized. Then, proceed to Step 9 and complete the remaining protocol on all samples simultaneously.
8. Homogenize the tissue using the handheld Powermasher II instrument and accompanying disposable plastic pestles. Homogenize for 2-3 min. or until thoroughly homogenized with periodic lifting and pushing down into the tube.
9. 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 ~20-25% of the homogenized 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* protocol. If the sample quantity is very low (e.g. 5mg of vertebrate tissue or portion of a single mosquito), do not prepare these aliquots and instead use the entire homogenized tissue sample as input to the *Arima High Coverage HiC Protocol.*

10. Pellet all samples by centrifugation at 2,500 x G at RT for 5 min.
11. Carefully discard supernatant. If no sample is visible or if the sample dislodges during pipetting, pellet by centrifugation again at a higher speed and discard remaining supernatant.
12. Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the *Estimating Input Amount* protocol or *Arima High Coverage HiC Protocol* depending on the input amount.

Estimating Input Amount

Input: Pulverized or homogenized crosslinked animal tissue
Output: Purified genomic DNA

Before you begin: Arima High Coverage HiC reactions are optimally performed on pulverized or homogenized crosslinked animal tissue comprising ~500ng-5µg of DNA. The *Estimating Input Amount* protocol is required if one does *not* know how much pulverized or homogenized crosslinked animal tissue will comprise 500ng-5µg of DNA, and if sufficient tissue is available to perform this protocol. The *Estimating Input Amount* protocol measures the amount of DNA obtained from 10% of the original pulverized or homogenized crosslinked animal tissue, or no more than the equivalent of 10mg of pulverized crosslinked animal tissue, which guides the calculation of the optimal tissue input for an Arima High Coverage HiC reaction. The Arima High Coverage 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 or homogenized crosslinked animal tissue to use per Arima High Coverage 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 animal tissue prepared during a previous *Crosslinking* 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 High Coverage 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 pulverized or homogenized crosslinked animal tissue.
12. Estimate how much pulverized or homogenized crosslinked animal tissue to use per Arima High Coverage HiC reaction. See the descriptive example below:

    Example: In the following *Arima High Coverage HiC Protocol*, it is recommended to use pulverized or homogenized crosslinked animal tissue comprising at least 500ng of DNA per Arima High Coverage HiC reaction, but no more than 5µg of DNA. If 250ng of DNA was obtained from 10% of the pulverized or homogenized crosslinked animal tissue as calculated above in step 11, one can estimate that *at least* 20% of the pulverized or homogenized crosslinked animal tissue (~500ng of DNA) is needed for Arima High Coverage HiC. If possible, we recommend aiming to use pulverized or homogenized crosslinked animal tissue comprising 1-2µg of DNA per Arima High Coverage HiC reaction. Additionally, please note that the pulverized or homogenized crosslinked animal tissue pellet for one Arima High Coverage HiC reaction should occupy no more than 20µL of volume in the sample tube. If the pulverized or homogenized crosslinked animal tissue pellet comprises 500ng-5µg of DNA but occupies greater than 20µL of volume, aliquot the sample into multiple Arima High Coverage HiC reactions such that the sum of the DNA input from all reactions is at least 500ng and each tissue pellet occupies no more than 20µL of volume, or contact Technical Support for additional guidance.

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

Arima High Coverage HiC Protocol

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

Before you begin: The tissue pellet for one Arima High Coverage HiC reaction should occupy no more than 20µL of volume and should be devoid of any residual liquid. If the tissue pellet occupies greater than 20µL of volume, aliquot the tissue such that the sum of the DNA input from all reactions is between 500ng-5µg and each tissue pellet occupies no more than 20µL of volume. This may mean that for certain tissue types (e.g. ones with low cellularity or fatty tissues), 2 Arima-HiC reactions may need to be performed on a given sample and recombined at Step 17 of the *Arima High Coverage 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 13.5µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Buffer F	7.5µL	8.25µL	x	2	=	16.5µL
Enzyme F1	1.5µL	1.65µL	x	2	=	3.3µL
Enzyme A2	1.5µL	1.65µL	x	2	=	3.3µL
Enzyme F3	1.5µL	1.65µL	x	2	=	3.3µL
Enzyme F4	1.5µL	1.65µL	x	2	=	3.3µL
Total	**13.5µL**					29.7µL

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.

* To provide flexibility in the workflow, this incubation can be held overnight at 37°C using a thermal cycler or thermomixer with a heated lid to prevent evaporation.

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

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

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 High Coverage 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 High Coverage HiC reactions had to be performed due to fatty difficult tissue, elute each Arima High Coverage HiC reaction in *50µL* of Elution Buffer in Step 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 High Coverage 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 High Coverage 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 High Coverage HiC Protocol* but before proceeding to library preparation. The *Arima-QC1 Quality Control* protocol involves using **QC Beads** to enrich an aliquot of proximally-ligated DNA, which is then quantified using a Qubit® fluorometer. Unlike standard Qubit® readings which involve quantifying a transparent unobstructed DNA sample, the Arima-QC1 value is obtained by quantifying DNA that is still bound to the **QC Beads**. This protocol can be performed in either plates or tubes. Set your thermal device (thermal cycler or thermomixer) to hold at 55°C. After completing the *Arima-QC1 Quality Control* protocol, use the provided **Arima High Coverage HiC QC Worksheet** to determine the Arima-QC1 values.

1. If necessary, thaw the "Arima-QC1" samples prepared during Step 20 of the *Arima High Coverage HiC Protocol* in the previous section.
2. Add 50µL of ● **QC Beads**, mix thoroughly by pipetting, and incubate at RT for 15 min.
3. Place sample against magnet, and incubate until solution is clear.
4. Discard supernatant, and remove sample from magnet.
5. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant, and remove sample from magnet.
8. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
9. Place sample against magnet, and incubate until solution is clear.
10. Discard supernatant, and remove sample from magnet.
11. Wash beads by resuspending in 100µL of **Elution Buffer**.
12. Place sample against magnet, and incubate until solution is clear.
13. Discard supernatant, and remove sample from magnet.
14. Resuspend beads in 7µL of **Elution Buffer.** Proceed to next step with resuspended beads.

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

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

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