Arima High Coverage HiC Kit

User Guide for Plant Tissues

8 reactions

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

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Revision History

Document	Date	Description of Change
Material Part Number: A410110 Document Part Number: A160163 v00	June 2020	Initial Release
Material Part Number: A410110 Document Part Number: A160163 v01	November 2021	Changed Arima-HiC 2.0 to Arima High Coverage HiC
Material Part Number: A410110 Document Part Number: A160163 v02	April 2023	Introduced nuclei isolation step prior to crosslinking to improve performance and reliability

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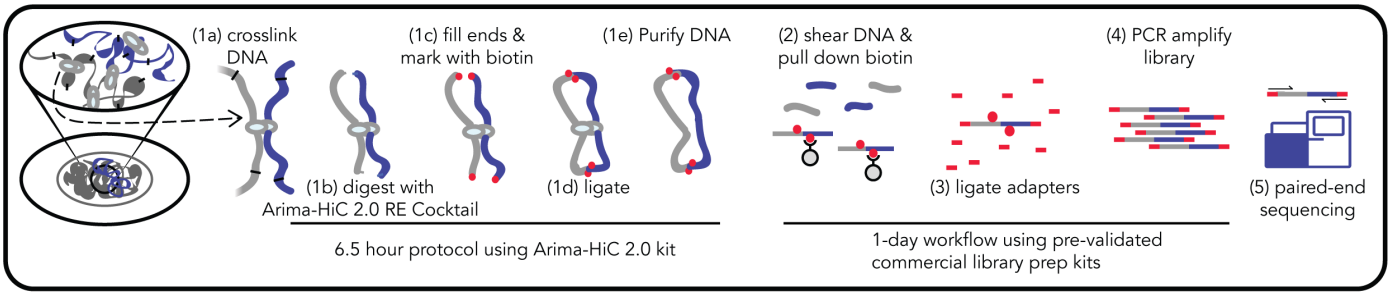
1 Introduction



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

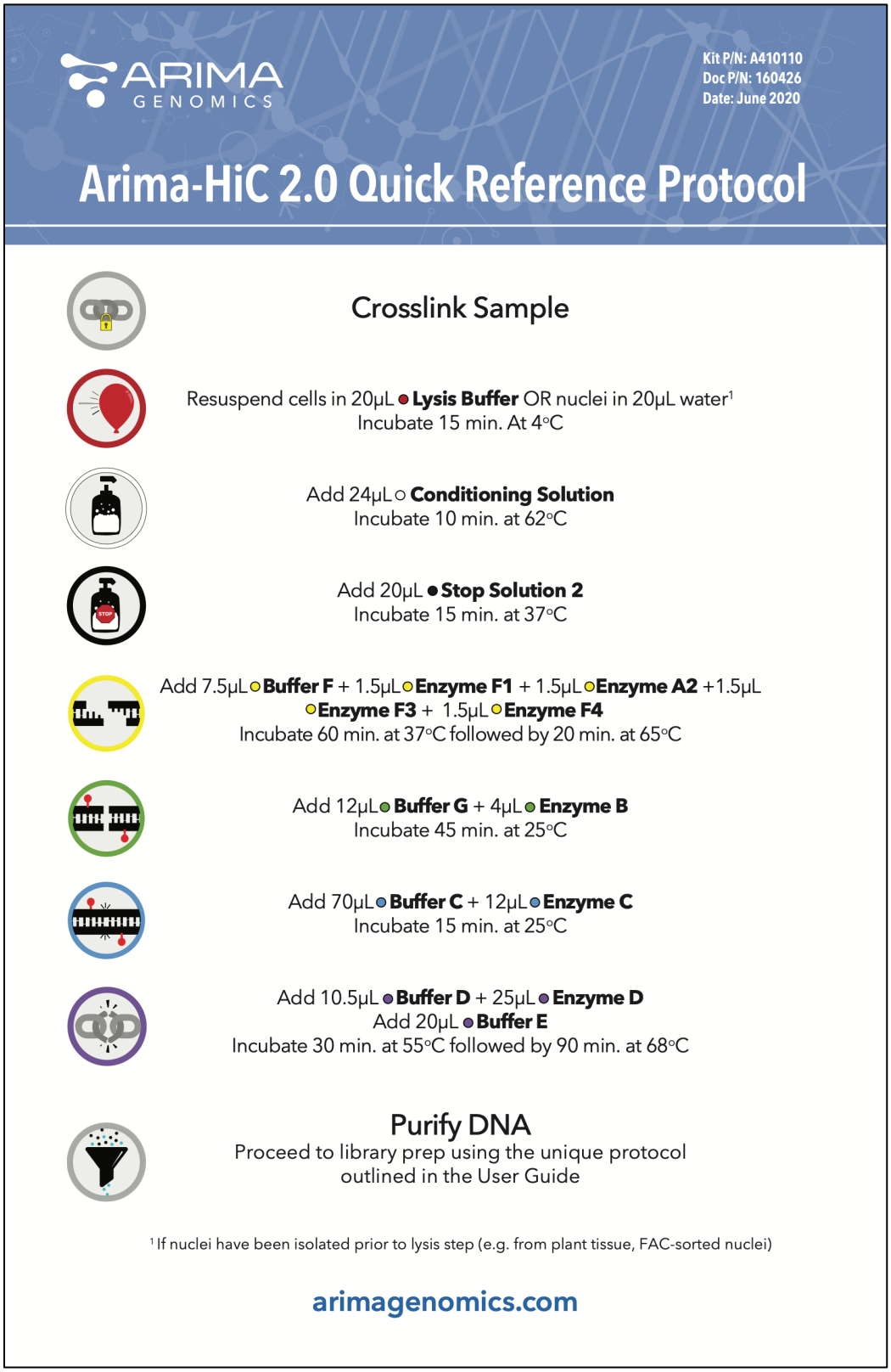
Arima High Coverage HiC libraries are sequenced via Illumina® sequencers in "paired-end" mode. The resulting data is referred to as Arima High Coverage HiC data. The tools necessary for analyzing Arima High Coverage HiC data depend on the application. For example, for studying 3D genome conformation, 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. Please refer to our Arima HiC Bioinformatics User Guide for more information. Custom cut site files for use with juicer can be found on our ftp server:

ftp://ftp-arimagenomics.sdsc.edu/pub/JUICER_CUTSITE_FILES_for_High_Coverage_HiC_4E

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/mapping_pipeline) 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.

Arima High Coverage HiC Quick Reference Protocol



Arima High Coverage HiC Kit Contents and Storage Info

ARIMA GENOMICS

Kit P/N: A410110
Doc P/N: 160426
Date: June 2020

Arima High Coverage 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 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 Getting Started

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 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

- ☐ CelLyticTM Plant Nuclei Extraction Kit (Sigma™ Cat # CELLYTPN1)
- ☐ DTT (SigmaÒ Cat # D9779)
- ☐ Protease Inhibitor Cocktail for Plants (Sigma™ Cat # P9599)
- ☐ Freshly prepared 80% Ethanol
- ☐ DNA Purification Beads (e.g. Beckman Coulter Cat # A63880)
- ☐ QubitÒ Fluorometer, dsDNA HS Assay Kit and consumables (e.g. Thermo Fisher Scientific Cat # 32851, 32856)
- ☐ Liquid nitrogen and dry ice
- ☐ Funnel
- ☐ Ceramic mortar and pestle (Cole-Parmer™ Cat # UX-63100-63)
- ☐ Metal spatula (Cole-Parmer™ Cat # SI-06369-16)
- ☐ Cold-resistant gloves
- ☐ Miracloth (e.g., Sigma Aldrich Cat# 475855) 37% formaldehyde 15ml conical tubes1.7mL 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 (if performing Arima High Coverage HiC in PCR tubes or PCR plate)
- ☐ Thermomixer (if performing Arima High Coverage HiC in 1.7mL microcentrifuge tubes)

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

Arima High Coverage HiC libraries can be sequenced using paired-end mode on most Illumina® sequencing machines (e.g. MiSeq®, NextSeq®, HiSeq®, NovaSeq®) with a variety of read lengths. While shorter read lengths (e.g. 2x50bp, 2x100bp) can be used for certain applications, we strongly recommend 2x150bp.

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.

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.

2.4 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.

3 Nuclei Isolation

Input: Fresh-frozen plant tissue

Output: Isolated nuclei Nuclei

Before you begin:

This protocol requires pulverization of fresh-frozen plant tissue, typically 2-3g, although less can be used if sample quantity is scarce. Extra caution should be taken due to the use of liquid nitrogen, dry ice, and severely cold equipment, and cold-resistant gloves should be worn.

1. Place a mortar and a 15mL conical tube onto a bed of dry ice, and place a pestle into the mortar. Cool a spatula at -20°C or colder for later use.
2. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Allow liquid nitrogen to evaporate completely.
3. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Transfer frozen plant tissue into mortar containing liquid nitrogen. Allow liquid nitrogen to evaporate just enough for the plant tissue to stay submerged.
4. Pulverize plant tissue in the mortar using the pestle until the sample resembles a fine powder. Ensure plant tissue is always submerged in liquid nitrogen. Carefully re-fill the mortar with liquid nitrogen as necessary. The pulverization process should take at least 8 min per sample and some tissue types may take longer. The goal is to pulverize until the tissue resembles a fine powder without visible chunks.

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

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

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

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

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

10. Complete a semi-pure plant nuclei extraction using the CelLyticTM Plant Nuclei Isolation/Extraction Kit according to the manufacturer's recommendations for Cell Lysis (Section A) and Semi-pure Preparation of Nuclei (Section B2). For the tissue grinding (Section A3), we recommend grinding for 20 min. We recommend adding the 10% TRITONTM X-100 to a final concentration of 0.5%, however this will vary between species (section A7). For the sucrose solution (Section B2), we recommend using a 1.5 M dilution instead of 2.3 M Sucrose.
11. Resuspend the nuclei pellet in 100µL of Nuclei Pure Storage buffer and store at -80°C until ready to proceed to crosslinking.

4 Crosslinking – Plant Tissue

Input: Isolated Nuclei

Output: Crosslinked Nuclei

1. Resuspend nuclei pellet in 5mL of RT 1X PBS.
2. Add 286µL of 37% formaldehyde, bringing the final formaldehyde concentration to 2%.
3. Mix well by inverting 10 times and incubate at RT for 20 min.
4. Add 460µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
5. Place sample on ice and incubate for 15 min.
6. Pellet sample by centrifugation at 2000 x g for 10 mins at RT.
7. Discard supernatant.
8. Resuspend sample in 5mL 1X PBS.
9. Divide samples into 5 1.7mL tubes with 1mL of resuspended sample each.
10. Pellet sample in all aliquots by centrifugation at 2000 x g for 10 mins at RT..
11. Discard supernatant leaving only the crosslinked nuclei pellet and no residual liquid.
12. Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the Estimating Input section.

5 Estimating Input Amount

Input: Crosslinked nuclei (e.g. derived from crosslinked seedlings or grass tissue)

Output: Purified genomic DNA

Before you begin:

The *Estimating Input Amount* protocol is required if one does *not* know how much crosslinked nuclei will comprise 500ng-5µg of DNA and if sufficient crosslinked nuclei are available to perform this protocol. Arima High Coverage HiC reactions are optimally performed on crosslinked nuclei comprising ~500ng-5µg of DNA. The *Estimating Input Amount* protocol measures the amount of DNA obtained from nuclei derived from 1/5 of the crosslinked plant tissue, which guides the calculation of the optimal input for an Arima High Coverage HiC reaction. The Arima High Coverage HiC kit contains enough reagents to perform this protocol on 8 samples. This protocol concludes with a descriptive example of how to calculate the estimate amount of crosslinked nuclei 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.

1. Thaw one aliquot of crosslinked nuclei prepared by the above *Nuclei Isolation* workflow.
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	90 min.
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. Transfer each sample to 1.7mL tubes. 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 30 secs. to air-dry the beads. Do not over-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 1/5 of the total sample. *Multiply this number times 4 to determine the total remaining DNA in the remaining 4 aliquots.*
12. Estimate how many aliquots to use per Arima High Coverage HiC reaction. See the example description below:

Example:

In the following *Arima High Coverage HiC Protocol*, it is recommended to use crosslinked nuclei corresponding to at least 500ng of DNA per Arima High Coverage HiC reaction, but no more than 5µg of DNA. If 200ng of DNA was obtained *per aliquot* of crosslinked nuclei as calculated in step 11, one can estimate that crosslinked nuclei derived from *at least* 2.5 aliquots of sample (~500ng of DNA) is needed for Arima High Coverage HiC. More crosslinked nuclei should be used if available, as long as the total DNA per reaction is not more than 5µg. Additionally, please note that the crosslinked nuclei pellet for one Arima High Coverage HiC reaction should occupy no more than 20µL of volume in the sample tube. If the crosslinked nuclei 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 nuclei pellet occupies no more than 20µL of volume, or contact Technical Support for additional guidance.

Recommended HiC Input Amount Explanation:

The recommendation to use crosslinked nuclei comprising at least 500ng of DNA is only a *general* recommendation. If crosslinked nuclei comprising at least 500ng of DNA cannot be obtained, which is frequently the case for plant tissue samples, one should still proceed with the *Arima High Coverage HiC Protocol* as described in this user guide and then use our library prep protocol.

6 Arima High Coverage HiC Protocol

Input: Crosslinked nuclei containing ~500ng-5µg of DNA

Output: Proximally-ligated DNA

Before you begin:

The nuclei 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 nuclei pellet occupies greater than 20µL of volume, aliquot the nuclei such that the sum of the DNA input from all reactions is between 500ng-5µg and each nuclei pellet occupies no more than 20µL of volume, or 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.

1. Resuspend one reaction of crosslinked nuclei in 20µL of **Water** in a tube or a well of a PCR plate.
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.*

** To provide flexibility, this incubation can also be held overnight at 4°C. Do not incubate at 68°C for longer than 90 min. unless doing so using a thermal cycler with a heated lid.*

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 30 seconds to air-dry the beads. Do not over-dry beads.

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®.
20. **Note: As mentioned in the Estimating Input Amount section, it is common for Arima High Coverage HiC reactions on plant nuclei to yield less than 500ng of proximally-ligated DNA. However, if the yield is less than 500ng, we recommend skipping the Arima-QC1 assay mentioned in Step 20 and described in the following Arima-QC1 Quality Control section.** 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 samples at -20°C until ready to proceed to library preparation following an accompanying *Arima High Coverage HiC Library Preparation* user guide**.**

7 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.

8 Warranty and Contact Info

WARRANTY

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