Arima-HiC Kit

User Guide for Plant Tissues

8 reactions

Material Part Number: A510008
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Revision History

Document	Date	Description of Change
Material Part Number: A510008 **Document Part Number:** A160135 v00	November 2018	Initial Release
Material Part Number: A510008 **Document Part Number:** A160135 v01	October 2019	• Added *Crosslinking* protocol and associated user-supplied reagents, consumables, equipment, and buffer recipes. • Replaced previous *Nuclei Isolation* protocol with a *Nuclei Isolation* protocol using Sigma CelLytic™ kit. Added associated user-supplied reagents, consumables, and equipment. • 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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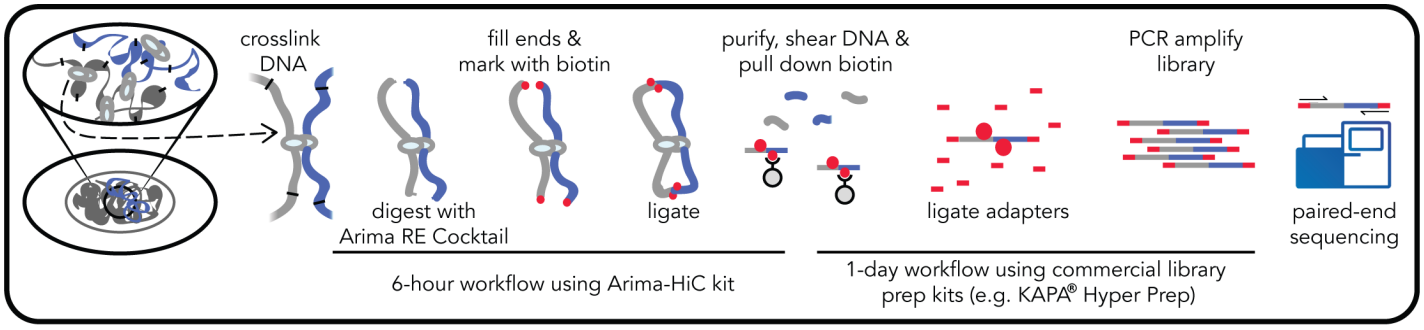

1.1 Arima-HiC Workflow Overview

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 Kit Contents and Storage Info

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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

- ☐ **PC2 Buffer** and **PQ2 Buffer** (see Section 2.3 for recipes)
- ☐ CelLytic™ Plant Nuclei Extraction Kit (Sigma Cat # CELLYTPN1)
- ☐ DTT (Sigma Cat # D9779)
- ☐ Protease Inhibitor Cocktail for Plants (Sigma Cat # P9599)
- ☐ Freshly prepared 80% Ethanol
- ☐ DNA Purification Beads (e.g. Beckman Coulter Cat # A63880)
- ☐ Qubit® 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
- ☐ 50mL conical tubes with 7 holes punched through the caps using a 20-gauge needle or similarly sized hole punch (to allow for air flow)
- ☐ Vacuum source and tubing

- Desiccator with stopcock (e.g. Thermo Scientific™ Cat # 5310-0250)
- Small cloth bag with drawstring (e.g. ULINE Cat # S-17831 or S-870), or comparable bag that is permeable to liquid and can be sealed shut.
- 1.7mL microcentrifuge tubes, PCR tubes (SSIbio® Cat # 3247-00), or PCR plates (Bio-Rad® Cat # HSS9641) and magnetic rack compatible with tube selection.
- Centrifuge
- Thermal cycler (if performing Arima-HiC in PCR tubes or PCR plate)
- Thermomixer (if performing Arima-HiC in 1.7mL microcentrifuge tubes)

2.3 Buffer Recipes

PC2 Buffer – The **PC2 Buffer** must be prepared fresh and used the same day as it is prepared. The following recipe is sufficient for at least 8 samples. Scale up or down as needed. After the **PC2 Buffer** is prepared, mix gently by inversion until homogeneous and store at room temperature (RT) until use.

Reagent	Stock Vendor	Stock Cat #	Stock Concentration	Final Concentration	Stock Amount
Water	Fisher Scientific®	50-843-406			389.1mL
Formaldehyde	Fisher Scientific®	F79-500	37%	1%	10.9mL
				Total	**400mL**

PQ2 Buffer – The **PQ2 Buffer** may be prepared in advance using sterile technique to avoid contamination. The following recipe is sufficient for at least 8 samples. Scale up or down as needed. After the **PQ2 Buffer** is prepared, mix by inversion and shaking until homogeneous and store at room temperature (RT) until use.

Reagent	Stock Vendor	Stock Cat #	Final Concentration	Stock Amount
Glycine	Sigma®	G7126-100G	125mM	3.75g
Water	Fisher Scientific®	50-843-406		Fill to 400mL
			Total	**400mL**

2.4 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.

2.5 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

Input: Fresh or frozen plant tissue diced in 2cm pieces
Output: Crosslinked plant tissue

Before you begin: The following protocol crosslinks diced plant tissue using formaldehyde under vacuum pressure to help penetrate the tissue. If possible, set the vacuum pressure to 400 millibar (~5.8 psi), although standard tissue culture vacuum pressure will suffice. Prior to crosslinking, it is important to weigh the plant tissue and record the mass. Use between 1-2 grams of diced plant tissue for crosslinking.

1. Thaw tissue if frozen, and place tissue into a small cloth bag. Seal bag using drawstring.
2. Setup the crosslinking reaction by placing the sealed cloth bag containing plant tissue into the bottom of a 50mL conical tube. Prepare a desiccator by connecting the desiccator stopcock to a vacuum source using appropriately sized tubing.
3. Add **PC2 Buffer** to the 50ml conical tube containing plant tissue until the **PC2 Buffer** solution reaches the 50mL mark. The tissue MUST be fully submerged.
4. Cap the 50mL conical tubes using caps that have several puncture holes to allow airflow, place the capped sample tube upright into the desiccator, and close the desiccator lid.
5. Apply vacuum pressure by turning on the vacuum and incubate for 10 min. Subtle bubbling should be visible in the **PC2 Buffer** and persist throughout the incubation.
6. Release vacuum pressure by turning off the vacuum and incubate for 10 min.
7. Apply vacuum pressure again by turning on the vacuum and incubating for 10 min. Subtle bubbling should be visible in the **PC2 Buffer** and persist throughout the incubation.
8. Remove the desiccator lid and sample tube cap. Pour out the **PC2 Buffer** while leaving the cloth bag containing plant sample in the 50mL conical tube.
9. Add **PQ2 Buffer** to the 50ml conical tube containing plant tissue until the **PQ2 Buffer** solution reaches the 50mL mark. The tissue MUST be fully submerged.
10. Cap the 50mL conical tubes using caps that have several puncture holes to allow airflow, place the capped sample tube upright into the desiccator, and close the desiccator lid.
11. Apply vacuum pressure by turning on the vacuum and incubate for 10 min.
12. Remove the desiccator lid and sample tube cap. Pour out the **PQ2 Buffer** while leaving the cloth bag containing plant sample in the 50mL conical tube.
13. Add **Water** to the 50ml conical tube containing plant tissue until the **Water** reaches the 50mL mark and then pour out the water. Repeat this process 3 times to wash the tissue.
14. Empty plant tissue from cloth bag onto aluminum foil, pat dry, and seal by folding.
15. Snap-freeze sample by submerging the aluminum foil containing plant tissue in liquid nitrogen, and then store at -80°C until ready to proceed to the *Nuclei Isolation* protocol.

Nuclei Isolation

Input: Crosslinked plant tissue (e.g. crosslinked seedlings or grass)
Output: Crosslinked Nuclei

Before you begin: Prior to nuclei isolation, it is important to weigh the frozen crosslinked plant tissue and record the mass. Use between 1-2 grams of crosslinked frozen plant tissue for nuclei isolation. The input mass of plant tissue will be used later in the *Nuclei Isolation* protocol, as well as the following *Estimating Input Amount* protocol. Also, note that the *Nuclei Isolation* protocol requires the handling of liquid nitrogen, dry ice, and other severely cold equipment. Please use extra caution and wear cold-resistant gloves as needed.

1. Complete a semi-pure plant nuclei extraction using the CelLytic™ Plant Nuclei Isolation/ Extraction Kit according to the manufacturer's recommendations for Cell Lysis (Section A) and Semi-pure Preparation of Nuclei (Section B2). For the tissue grinding (Section A3), we recommend grinding for 20 min.
2. Upon completion the nuclei isolation, aliquot sample using **NIBA** provided in the CelLytic™ Plant Nuclei Isolation/Extraction Kit such that each aliquot contains the equivalent of ~125mg of the original plant tissue. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.
3. Pellet samples by centrifugation at 12,000 x G at RT for 5 min.
4. Discard supernatant leaving behind only a nuclei pellet and no residual liquid.
5. Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the *Estimating Input Amount* protocol in the following section.

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-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 125mg of crosslinked plant tissue, which guides the calculation of the optimal input for an Arima-HiC reaction. The Arima-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-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-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 125mg of plant tissue. *Divide this number by 125 to determine the DNA per mg of tissue*.
12. Estimate how many milligrams of plant tissue to use per Arima-HiC reaction. See the example description below:

    Example: In the following *Arima-HiC Protocol*, it is recommended to use crosslinked nuclei corresponding to at least 500ng of DNA per Arima-HiC reaction, but no more than 5µg of DNA. If 2ng of DNA was obtained *per milligram* of crosslinked nuclei as calculated in step 11, one can estimate that crosslinked nuclei derived from *at least* 250mg of plant tissue (~500ng of DNA) is needed for Arima-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-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-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-HiC Protocol* as described in this user guide and then use our validated low-input library prep protocol.

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

Before you begin: The nuclei pellet for one Arima-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 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.*

* 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-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.
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: As mentioned in the *Estimating Input Amount* section, it is common for Arima-HiC reactions on plant nuclei to yield less than 500ng of proximally-ligated DNA. However, if the yield is less than 275ng, we recommend skipping the Arima-QC1 assay mentioned in Step 20 and described in 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 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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