

Arima-HiC Kit

User Guide for Mammalian Blood
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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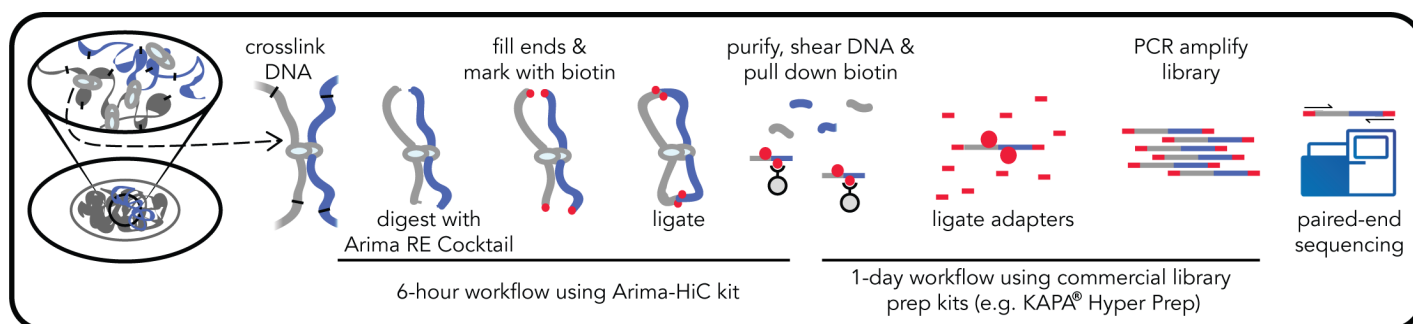
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1.1 Arima-HiC Workflow Overview

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), Hi-C Pro (Servant, 2015), or HiCUP (Wingett, 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 may 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 the Arima-HiC mapping pipeline (<https://github.com/ArimaGenomics>), and then the contigs/unitigs can be scaffolded using tools such as SALSA (Ghurye, 2018). Please contact us at techsupport@arimagenomics.com or www.arimagenomics.com for more information.

Arima-HiC Quick Reference Protocol



Kit P/N: A510008
Doc P/N: A160259
Date: Nov 2018

Arima-HiC Quick Reference Protocol



Crosslink Sample



Resuspend cells in 20 μ L **Lysis Buffer** OR nuclei in 20 μ L water¹
Incubate 15 min. at 4°C



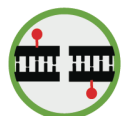
Add 24 μ L **Conditioning Solution**
Incubate 10 min. at 62°C



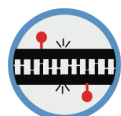
Add 20 μ L **Stop Solution 2**
Incubate 15 min. at 37°C



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



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



Add 70 μ L **Buffer C** + 12 μ L **Enzyme C**
Incubate 15 min. at 25°C



Add 10.5 μ L **Buffer D** + 25 μ L **Enzyme D**
Add 20 μ L **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



Kit P/N: A510008
Doc P/N: A160259
Date: Nov 2018

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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Getting Started

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

- ☐ Cold 1X PBS, pH 7.4 (e.g. Fisher Scientific Cat # 50-842-949)
- ☐ Histopaque®-1077 (e.g. Sigma® Cat # 10771)
- ☐ 37% Formaldehyde (e.g. Fisher Scientific Cat # F79-500)
- ☐ 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 or dry ice
- ☐ 15mL conical tubes
- ☐ 1.7mL microcentrifuge tubes, PCR tubes (SSIbio® #3247-00 recommended), or PCR plates (Bio-Rad® #HSS9641 recommended) 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 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.

The optimal sequencing depth for Arima-HiC libraries 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. We recommend obtaining this total number of read-pairs by combining data from 2 biological replicates per biological condition. In doing so, you will be able to assess the overall reproducibility of the Arima-HiC data across replicates, and then combine the replicate Arima-HiC data for high-resolution chromatin conformation analyses. 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. 1Gb genome = 200M read-pairs).

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 of thumb, each Arima-HiC library should be complex enough to sequence up to ~600M read-pairs without reaching saturation. If >600M read-pairs 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.

2.4 How to cite Arima-HiC in publications

When citing the Arima-HiC protocol or kit, please reference a publication where the Arima-HiC kit was used to generate Hi-C data and include the catalog number (A510008) where appropriate. For example, “Hi-C data was generated using the Arima-HiC kit (Link, Cell, 2018), according to the manufacturers protocols (Catalog # A510008)”.

PBMC Isolation and Crosslinking

Input: Whole blood

Output: Crosslinked peripheral blood mononuclear cells (PBMCs)

Before you begin: The Arima-HiC workflow for mammalian blood begins with the isolation of PBMCs from 2mL of freshly drawn whole blood, followed by PBMC crosslinking. The *PBMC Isolation and Crosslinking* protocol below involves several centrifugation steps. *It is critical to ensure that the centrifuge brake and accelerator features are turned OFF for Step 6.*

1. Collect at least 2mL of whole blood and store at the temperature recommended by the blood collection tube manufacturer. If less than 2mL of whole blood is collected, please contact Technical Support.
2. Warm **Histopaque®-1077** to RT by letting the bottle sit at RT for 30 min.
3. Add 2mL of whole blood and 2mL of cold **1X PBS** to a new 15mL conical tube. Mix by gentle inversion until homogeneous.
4. Prepare a separate 15mL conical tube with 4mL of RT **Histopaque®-1077**.
5. Aspirate 4mL of diluted blood from step 3 with a serological pipette. Tilt tube from step 4 at 45-degree angle and place the tip of the serological pipette just above the top of the **Histopaque®-1077** solution. Slowly add the 4mL of diluted blood on top of the **Histopaque®-1077** solution. Adjust the height of the pipette during pipetting so that the tip remains just above the top of the **Histopaque®-1077** solution throughout the pipetting. Once finished, proceed immediately to the next step.
6. Centrifuge sample *with the accelerator and break OFF* at 400 x G at 4°C for 30 min. Once complete, gently remove the sample tube and place upright into a tube rack. The top yellow-colored layer in the sample tube is the plasma layer, and the very thin white layer directly below the plasma are PBMCs.
7. Carefully transfer the PBMCs (i.e. “buffy coat”) to a new 15mL conical tube and safely discard the remaining non-PBMC blood sample material.
8. Add cold **1X PBS** to the PBMCs to bring the total volume to 5mL.
9. Obtain a cell count by hemocytometer or automated cell counting methods.
10. Centrifuge sample at 500 x G at RT for 10 min.
11. Discard supernatant.
12. Resuspend cells in 1mL cold **1X PBS**.
13. Add 57µL of **37% formaldehyde**, bringing the final formaldehyde concentration to 2%.
14. Mix well by inverting 10 times, and incubate at RT for 10 min. with occasional inversion.

15. Add 91.9µL of **Stop Solution 1**, mix well by inverting 10 times and incubate at RT for 5 min. with occasional inversion.
16. Place sample on ice and incubate for 15 min.
17. Pellet cells by centrifugation at 500 x G at RT for 5 min.
18. Discard supernatant.
19. Resuspend cells in 1mL cold **1X PBS**.
20. Aliquot cells into separate microcentrifuge tubes, with 0.5 million cells per aliquot. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.
21. Pellet cells in all aliquots by centrifugation at 500 x G at RT for 5 min.
22. Discard supernatant leaving only the crosslinked cell pellets and minimal residual liquid.
23. Freeze the crosslinked cell pellets by submerging in liquid nitrogen or by placing on dry ice, and store at -80°C until ready to proceed to the *Estimating Input Amount* section.

Estimating Input Amount

Input: Crosslinked peripheral blood mononuclear cells (PBMCs)

Output: Purified genomic DNA

Before you begin: The *Estimating Input Amount* protocol is required if one does not know how many crosslinked cells will comprise 750ng-5µg of DNA, and if sufficient cells are available to perform this protocol. Arima-HiC reactions are optimally performed on crosslinked cells comprising ~750ng-5µg of DNA. The *Estimating Input Amount* protocol measures the amount of DNA obtained per 0.5×10^6 crosslinked cells, which guides the calculation of the optimal cellular 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 estimate the optimal number of crosslinked cells 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 0.5×10^6 cells prepared during the *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	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 0.5×10^6 crosslinked PBMCs.
12. Estimate how many crosslinked PBMCs to use per Arima-HiC reaction. See the example description below:

Example: In the following *Arima-HiC Protocol*, it is recommended to use crosslinked cells corresponding to at least 750ng of DNA per Arima-HiC reaction, but no more than 5µg of DNA. If 375ng of DNA was obtained from 0.5×10^6 crosslinked PBMCs as calculated in step 11, one can estimate that at least 1×10^6 crosslinked cells should be used per Arima-HiC reaction (~750ng of DNA). More crosslinked cells should be used if available, as long as the total DNA per reaction is not more than 5µg. If possible, we recommend aiming to use crosslinked cells comprising 3µg of DNA per Arima-HiC reaction. Additionally, please note that the crosslinked cell pellet for one Arima-HiC reaction should occupy no more than 20µL of volume in the sample tube. If the crosslinked cell pellet comprises 750ng-5µg of DNA but occupies greater than 20µL of volume, aliquot the cells into multiple Arima-HiC reactions such that the sum of the DNA input from all reactions is at least 750ng and each cell 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 cells comprising at least 750ng of DNA is only a *general* recommendation. If crosslinked cells comprising at least 750ng 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.

Arima-HiC Protocol

Input: Crosslinked PBMCs containing ~750ng-5µg of DNA

Output: Proximally-ligated DNA

Before you begin: The cell pellet for one Arima-HiC reaction should occupy no more than 20µL of volume and should be devoid of any residual liquid. If the cell pellet occupies greater than 20µL of volume, aliquot the cells such that the sum of the DNA input from all reactions is between 750ng-5µg and each cell 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.

Note: Choose to perform either Step 1a if the input sample type is crosslinked PBMCs, or Step 1b only if the input sample type is crosslinked *nuclei* that have been previously purified from PBMCs.

- 1a. Resuspend one reaction of crosslinked PBMCs in 20µL of ● **Lysis Buffer** in a tube or a well of a PCR plate, and incubate at 4°C for 15 min.
- 1b. Resuspend one reaction of purified crosslinked nuclei in 20µL of **Water** in a tube or a well of a PCR plate and proceed 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	30 min.*
65°C	20 min.
25°C	10 min.

* Optimal performance can be achieved with incubation durations between 30 and 60 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 in the workflow, this incubation can also be held overnight at 4°C. Do not incubate at 68°C for longer than 90 min.

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®.
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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CONTACT US

Technical Support: techsupport@arimagenomix.com

Order Support: ordersupport@arimagenomix.com