Arima-HiC+ FFPE Kit

User Guide for Formalin Fixed Paraffin Embedded (FFPE) Tissues

8 reactions

Material Part Number: A101060
Document Part Number: A160172 v01
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This product is intended for research use only. This product is not intended for diagnostic purposes.

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

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Revision History

Document	Date	Description of Change
Material Part Number: A101060 **Document Part Number:** A160172 v01	February 2022	Initial Release

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1. Introduction

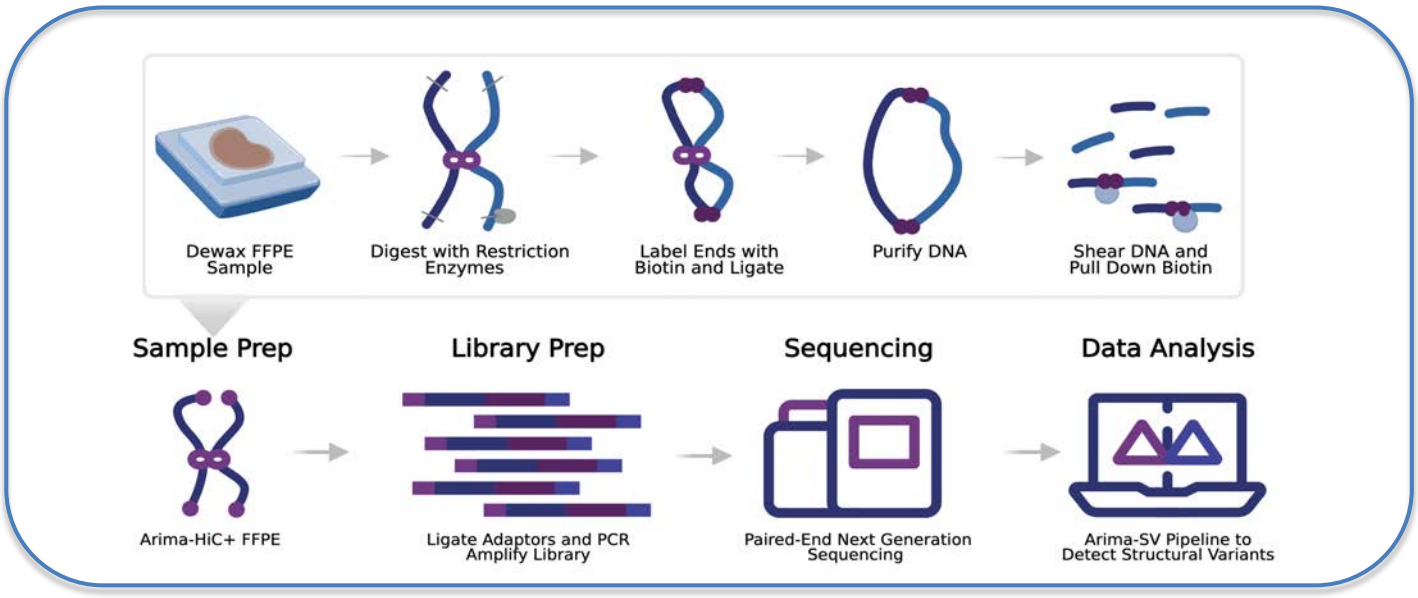

Figure 1-Arima Hi-C Workflow for FFPE Tissues

1.1 Arima Hi-C for FFPE Tissues Workflow Overview

Arima Hi-C (**Figure 1**) is an experimental workflow that capture a genome's sequence and structure (three-dimensional conformation). Arima Hi-C has been successfully performed on a wide range of species from the plant and animal kingdoms. This user guide provides instructions for how to use the Arima-HiC+ FFPE Kit (SKU: A101060) for processing Formalin Fixed Paraffin Embedded Tissues (FFPE). FFPE tissue is crosslinked with formalin prior to embedding into Paraffin way. Thin sections are made from the tissue using a microtome and the sections are dewaxed and rehydrated then the cross-linked chromatin is 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 the Swift Accel-NGS 2S Plus Library prep kit using the: **User Guide: Library Preparation using Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit (Doc. PN: A160140)**.

1.2 Sequencing and Data Analysis

Arima Hi-C libraries are sequenced via Illumina® sequencer in "paired-end" mode. The resulting data is referred to as Arima Hi-C data. Because paired-end reads of Arima Hi-C 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 structural variation analysis. The Arima-SV Pipeline is used for calling and visualizing Structural Variants (SV) from Arima Hi-C data. This pipeline preprocesses the Arima Hi-C data using HiCUP (Wingett et al. 2015) and calls SV's using hic_breakfinder (Dixon et al. 2018). Additionally, Juicer (Durand et al. 2016b) is run with HiCUP to generate .hic files for visualizing the Arima Hi-C data with Juicebox (Durand et al. 2016a). The Arima-SV Pipeline comes pre-installed and pre-tested in a Singularity image along with all required accessory files for processing human Hi-C data for SV detection. Guidance on downloading and running the Arima-SV Pipeline can be found at the tool's GitHub page https://github.com/ArimaGenomics/Arima-SV-Pipeline and in the **Bioinformatics User Guide Arima Structural Variant Pipeline (Doc. PN: A160602).**

2. Arima Hi-C Kit Contents and Storage Info

ARIMA GENOMICS

Arima Hi-C Box Contents & Storage Info

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

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

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

* Required for library prep and Arima QC1

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

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

3. Handling and Preparation

3.1 Getting Ready

- Several steps during the *Arima Hi-C Protocol* require the 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.
- The *Arima Hi-C Protocol* is performed in PCR plates or PCR tubes. Ensure that the PCR tube or plates have a total volume capacity of at least 320µL. See Section 3.2 for recommended consumables. Ensure that PCR plates and/or tubes are compatible with thermal cyclers. 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.
- If possible, performing the pre-amplification steps in a "Pre-PCR" environment and the post-amplification steps in a "post-PCR" environment will reduce PCR contamination.
- DNA Purification Beads (e.g., AMPure® XP Beads) should be warmed to RT and thoroughly mixed before each use.
- Xylene is an organic solvent; the use of Personal Protective Equipment (e.g., gloves, lab coat, and eye protection) and the use of a chemical fume hood are strongly recommended.

3.2 User-supplied reagents, consumables, and equipment checklist

- ☐ Xylene (e.g. IMEB Inc. Cat # XY-110)
- ☐ 100% Ethanol
- ☐ Freshly prepared 80% Ethanol
- ☐ DNA Purification Beads (e.g., KAPA® Pure beads Cat # KS8002)
- ☐ Qubit® Fluorometer, dsDNA HS Assay Kit and required consumables (e.g., Thermo Fisher Scientific Cat # 32851, 32856)
- ☐ 15mL conical tubes
- ☐ 1.7mL microcentrifuge tubes, PCR tubes (SSIbio® #3247-00), or PCR plates (Bio-Rad® #HSS9641) and magnetic rack compatible with tube selection.
- ☐ Centrifuge
- ☐ Thermal cycler
- ☐ Benchtop rotating mixer (e.g., Benchmark Scientific Cat # R5010, or equivalent)
- ☐ 0.2 mL PCR Strip Magnetic Separator (e.g., Permagen Cat # MSR812)

3.3 Optimal read length, sequencing depth, and number of Arima Hi-C reactions per sample

Arima Hi-C 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 Hi-C data alignment quality, although shorter read lengths (e.g., 2x50bp, 2x100bp) and lower throughput instruments can certainly be used for specific applications of Arima Hi-C data such as 3D genome conformation and structural variation analysis.

The optimal sequencing depth for Arima Hi-C libraries also depends on the application. For example, 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 paired- reads *per biological condition* for high-resolution analyses of A/B compartments, TADs, and chromatin loops. One way of getting 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 Hi-C data across replicates and then use the combined replicate Arima Hi-C dataset for high-resolution chromatin conformation analyses. Alternatively, one can obtain at least 600 million paired-reads *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 paired-reads 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 structural variation analysis, the required sequencing depth can vary depending on factors such as the heterogeneity and clonality of the sample (e.g., the mutant allele fraction), the size of the structural variant and whether it's an intra- or inter-chromosomal structural rearrangement. However, benchmarking analysis has revealed that ~100-150M reads per sample is needed for structural variant calling. The Arima-SV Pipeline will provide a recommended sequencing depth in its shallow QC output file.

Lastly, it is important to note that each Arima Hi-C library may be evaluated by the Arima-QC2 assay prior to deep sequencing if the amount of input into library preparation is quantifiable. The Arima-QC2 metric is a strong predictor of the number of long-range contacts in the Arima Hi-C Library.

3.4 How to cite Arima Hi-C in publications

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

4. De-waxing and Rehydration

Input: 5mm^3 from 5-10µm FFPE tissue section
Output: De-waxed and rehydrated formalin-fixed tissue

Table 1-List of Reagents Required for De-waxing

√	Reagent	Box	Thaw Temp.	Mixing	Cap Color
	Xylene	User	N/A	N/A	N/A
	100% Ethanol	User	N/A	N/A	N/A
	Molecular Grade Water	User	N/A	N/A	N/A

Before you begin: The Arima Hi-C workflow for FFPE tissues begins with de-waxing and rehydrating the FFPE tissue. Take special care when carrying out the protocol described in this section as FFPE tissues can be difficult to visualize within the sample tube or may not pellet during centrifugation steps and will require special care when pipetting to avoid aspirating tissue material. Also, note that due to the low DNA yield expected from a single FFPE tissue section, it is advisable to perform this protocol on multiple (1-10) FFPE tissue sections for each sample. For optimal Hi-C performance, 5mm^3 of input volume (input volume= surface area in mm^2 x Thickness in mm) of FFPE tissue is recommended and will vary by tissue type. The below protocol is written for processing 1-10x 5-10µm FFPE sections. Also note that during de-waxing and rehydration, the tissue may not appear as a "pellet" after centrifugation, but rather as a "sheet" or "shards" and tends to dislodge from the tube base or wall after centrifugation and float in the sample tube. If this happens, *carefully* pipette around the tissue initially using a 1mL pipette and then use a 200µL pipette to remove the residual volume. It is acceptable to leave ~10-20µL of residual volume during Steps 4.4, 4.7, and 4.10 that involve the discarding of supernatant to avoid aspirating tissue material. For optimal de-waxing performance, it is recommended that no more than 20 sections of 5µm or 10 sections of 10µm thickness are used per reaction, and no more than 5 sections are used per 1.7mL microfuge tube. If the Xylene turns cloudy during de-waxing, this is an indication of too much wax being dissolved in the 1mL of Xylene and can be mitigated by adding more Xylene to the sample in a 1.7mL or larger tube. If multiple de-waxing reactions are performed per sample, they can be re-combined at the lysis step in the Arima Hi-C protocol (step 5.1.1). Note: if the surface area of the sections is not known then it is recommended to process 5x 5µm sections as a default input.

Figure 2- Representative image of a 5-10µm FFPE Tissue scroll in a 1.7ml Microfuge Tube. The tip of the red arrow points to the FFPE section.

4.1 Add FFPE Sections to 1.7mL microfuge tube. This can be done by either cutting FFPE "scrolls" off an FFPE block using a microtome and transferring the scrolls into a 1.7mL microfuge tube (**Figure 2**) or by scraping an FFPE sections off a slide and carefully transferring them into a 1.7mL microfuge tube.

4.2 Transfer sample to a fume hood and add 1mL **Xylene** and incubate at RT for 10 minutes on a rotator at 20 RPM, shaker or Nutator. If a rotator, shaker and Nutator are unavailable, mix by briefly vortexing 2 sec. or by inverting 3 times every ~3 minutes during the incubation. Ensure the scroll is fully submerged in the Xylene. **Note: Wax from scrolls can be stuck to the tub cap. Open the cap gently to ensure sample material does not fall out. Mixing is critical here to ensure no material remains attached to the cap, contributing to the loss of material.**

4.3 Centrifuge at max speed (~21,000 x G) at RT for 5 minutes.

Note: Removal of Xylene from the sample tube in the step below is a particularly challenging step as the tissue may appear translucent and difficult to visualize. We recommend holding the sample tube up to the light at a 45-degree angle and turning the sample tube until the tissue becomes visible enough to carefully discard the Xylene using a pipette.

4.4 Working in a fume hood, carefully discard supernatant. Use a 1mL pipette set to 800µL to carefully remove 800µL of supernatant and discard. Then use a 200µL pipette set at 180µL to carefully remove 180µL of supernatant and discard. The 20µL of supernatant remaining will help to ensure sample retention.

4.5 Resuspend the deparaffinized tissue in 1mL of **100% Ethanol** and incubate at RT for 10 minutes on a rotator at 20 RPM, shaker or Nutator. If a rotator, shaker and Nutator are unavailable, mix by briefly vortexing 2 sec. or by inverting 3 times every ~3 minutes during the incubation.

4.6 Centrifuge at max speed (~21,000 x G) at RT for 5 minutes.

4.7 Carefully discard supernatant. Use a 1mL pipette set to 800µL to carefully remove 800µL of supernatant and discard. Then use a 200µL pipette set at 200µL to carefully remove and discard 200µL of supernatant. The 20µL of supernatant remaining will help to ensure sample retention.

4.8 Resuspend the deparaffinized tissue in 1mL of **Water** and incubate at RT for 10 minutes on a rotator at 20 RPM or shaker or Nutator. If a rotator, shaker and Nutator are unavailable, mix by briefly vortexing 2 sec. or by inverting 3 times every ~3 minutes during the incubation. **Note: the tissue should appear opaque and may be in many small shards of tissue or as flat sheets at this stage (Figure 3).**

Figure 3- Representative image of 5-10µm FFPE scroll following rehydration with water. The tip of the red arrow points to the FFPE section that appears as a "sheet" of tissue.

4.9 Centrifuge at max speed (~21,000 x G) at RT for 5 minutes.

4.10 Carefully discard supernatant. Use a 1mL pipette set to 800µL to carefully remove 800µL of supernatant and discard. Then use a 200µL pipette set at 200µL to carefully remove 200µL of supernatant and discard. The 20µL of supernatant remaining will help to ensure sample retention. **Note: the tissue is very easy to disturb at this point so decant with caution.**

4.11 Proceed immediately to the *Arima Hi-C Protocol* section.

5. Arima Hi-C Protocol

Input: De-waxed, rehydrated, and homogenized formalin-fixed tissue
Output: Proximally-ligated DNA

Table 2- List of Reagents Required for Preparation of Proximally- Ligated DNA

√	Reagent	Box	Thaw Temp.	Mixing	Cap Color	Day Used
	Elution Buffer	Box A, RT	N/A	Inversion	Bottle	1
	Conditioning Solution	Box A, RT	N/A	Inversion	○	1
	Stop Solution 2	Box A, RT	N/A	Inversion	●	1
	Lysis Buffer	Box B, -20°C	Ice	Inversion	●	1
	Buffer A	Box B, -20°C	Ice	Inversion	●	1
	Enzyme A1	Box B, -20°C	Ice	Inversion	●	1
	Enzyme A2	Box B, -20°C	Ice	Inversion	●	1
	Buffer B	Box B, -20°C	Ice	Inversion	●	1
	Enzyme B	Box B, -20°C	Ice	Inversion	●	1
	Buffer C	Box B, -20°C	Ice	Inversion	●	1
	Enzyme C	Box B, -20°C	Ice	Inversion	●	1
	Buffer D	Box A, RT	N/A	Inversion	●	3
	Enzyme D	Box B, -20°C	RT, then ice	Inversion	●	3
	Buffer E	Box A, RT	N/A	Inversion	●	2
	DNA Purification Beads	User Supplied	RT	Inversion	N/A	4
	80% Ethanol	User Supplied	RT	Inversion	N/A	4

Before you begin: Starting with Step 5.4, the Arima Hi-C Protocol must be carried out in PCR tubes or PCR plates and all heated incubations must be carried out in a thermal cycler. Additionally, note that steps 5.5 – 5.6 require consecutive heated incubations. Make sure your thermal cycler(s) are set to 74°C and 37°C for these incubations, respectively.

5.1 Resuspend one sample from step 4.10 of de-waxed and rehydrated formalin-fixed tissue in 200µL of **Lysis Buffer** in a 1.7mL microfuge tube and incubate at 4°C or on ice for 20 minutes. Use pipette to mix the tissue and fully submerge the tissue in the buffer. During the incubation, mix by a pipetting, vortexing, or quick inversion once every 5 minutes. When mixing be careful to not lose material in pipette tips or on the upper tube wall or cap and make sure the tissue remains submerged in the lysis buffer at all times. **Note: Cool Centrifuge in preparation for the next step.**

5.1.1 Optional: If multiple de-waxing reactions were used per sample then combine them at this step into a single 1.7mL microfuge tube by resuspending ALL sections, from ALL tubes for a given sample, in Lysis Buffer. For example, if 2 tubes of rehydrated tissue are combined then the total volume of Lysis Buffer will be 200µL. **Note: The tissue may be globular and sticky, so use caution to not clog the pipette tip or lose tissue material during the transfer. A cut tip or a large-bore pipette tip may help reduce sample loss during transfer. In some cases, you may need to use the pipette tip like a "scoop" to transfer the tissue.**

5.2 Centrifuge at max speed (~21,000 x G) at 4°C for 5 minutes. **Note: Make sure the centrifuge is balanced as some tubes may have more volume than others.**

5.3 Carefully discard supernatant. Use a 200µL pipette set at 200µL to carefully remove 200µL of supernatant and discard. The 20µL of supernatant remaining will help to ensure sample retention. If 2 tubes of rehydrated material were combined in step 5.1.1 then first use a 200µL pipette to remove 200µL of supernatant, then use a 20µL pipette set at 20µL to carefully remove 20µL of supernatant and discard.

5.4 Transfer the sample into PCR tube or well of a PCR plate (see Section 3.2 for required plasticware). The tissue may be globular and sticky, so use caution to not clog the pipette tip or lose tissue material during the transfer and mixing in all steps below. A cut tip or a large-bore pipette tip may help reduce sample loss during transfer and mixing. In some cases, you may need to use the pipette tip like a "scoop" to transfer the tissue. **Note: if the tissue volume, itself, is more than 20µL then split into 2 reactions.**

5.5 Add 24µL of ○ **Conditioning Solution**, mix gently by pipetting and incubate at 74°C for 40 minutes. Set the thermal cycler lid temperature to 85°C.

5.6 Add 20µL of ● **Stop Solution 2**, mix gently by pipetting and incubate at 37°C for 15 minutes. Set the thermal cycler lid temperature to 85°C.

Note: Steps 5.7, 5.9, 5.11 and 5.14 require addition of several reagents in the same step. These reagents should be combined into master mixes following the master mix tables.

5.7 Add 12µL of a master mix containing the reagents listed in **Table 3.**

Table 3- Reagent volumes Required for the Digestion step

Reagent	Volume per	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

5.8 Mix gently by pipetting, then incubate at the temperatures listed in **Table 4.** Set the thermal cycler lid temperature to 85°C.

Table 4- Incubation Temperatures for Digestion Step

Temperature	Time
37°C	60 minutes
62°C	20 minutes
25°C	10 minutes

5.9 Add 16µL of a master mix containing the reagent volumes listed in **Table 5.**

Table 5- Reagent Volumes Required for the Fill-in Step

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

5.10 Mix gently by pipetting, then incubate at room temperature (RT) for 45 minutes.

5.11 Add 82µL of a master mix containing the reagents listed in **Table 6.**

Table 6- Reagent Volumes Required for the Ligation Step

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

5.12 Mix gently by pipetting and incubate at 20°C in a thermocycler for ~16hr (overnight). Set the thermal cycler lid to OFF.

5.13 Add 16.6µL of ● **Buffer E**, mix gently by pipetting up and down, and incubate at 65°C for ~16hr (overnight). Set the thermal cycler lid temperature to 85°C.

Note: Enzyme D should be warmed to RT prior to master mixing to prevent precipitation.

5.14 Add 35.5µL of a master mix containing the reagents listed in **Table 7.**

Table 7- Reagent Volumes Required for Reverse Cross-Linking step

Reagent	Volume per reaction	10%		# 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

5.15 Mix gently by pipetting and incubate at 55°C for ~16hr (overnight). Set the thermal cycler lid temperature to 85°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 Hi-C kit.

5.16 Add 100µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 minutes.

5.17 Place sample against magnet, and incubate until solution is clear.

5.18 Discard supernatant. While sample is still against magnet, add 200µL of 80% ethanol, and incubate at RT for 1 minutes.

5.19 Discard supernatant. While sample is still against magnet, add 200µL of 80% ethanol, and incubate at RT for 1 minutes.

5.20 Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 minutes to air-dry the beads.

5.21 Remove sample from magnet, resuspend beads thoroughly in 30µL of **Elution Buffer**, and incubate at RT for 5 minutes.

5.22 Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.

5.23 Quantify sample using Qubit®.

5.24 If there is ≥500ng of quantified DNA, transfer 50ng into a new tube labelled "Arima-QC1**",** adjust the volume to 50µl by adding **Elution Buffer**. The "Arima-QC1" sample should now contain 50ng of proximally-ligated DNA in 50µL of **Elution Buffer**. Proceed to Arima-QC1 Quality control (Steps 6.1 through 6.16) or store Arima-QC1 tubes at -20°C until use. If there is <500ng then proceed directly to library preparation using the accompanying *Arima Hi-C Library Preparation* user guide.

5.25 If there is ≥500ng of quantified DNA, obtain a DNA size profile by gel electrophoresis using 10ng of DNA. If there is <500ng then proceed directly to library preparation using the **User Guide: Library Preparation using Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit (Doc. PN: A160140)**. FFPE DNA is more degraded than non-FFPE DNA, so typically, the size range of proximally ligated DNA from FFPE samples in between 200bp – 1kb.

5.26 Store all remaining samples at -20°C until ready to proceed to library preparation using the **User Guide: Library Preparation using Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit (Doc. PN: A160140)**.

6. Arima-QC1 Quality Control

Input: Proximally-ligated DNA
Output: QC1 measurement

Table 8- List of Reagents Required for Arima- QC1

√	Reagent	Box	Thaw Temp.	Mixing	Cap Color
	QC Beads	Box C, 4°C	N/A	Vortexing	●
	Elution Buffer	Box A, RT	N/A	Inversion	Bottle
	Wash Buffer	Box A, RT	N/A	Inversion	Bottle
	Qubit® assay	User Supplied	N/A	N/A	N/A

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 Hi-C 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: **Worksheet Arima-HiC Quality Control For Structural Variants (Doc. PN: A160431)** to determine the Arima-QC1 values.

6.1 If necessary, thaw the "Arima-QC1" samples prepared during Step 5.24 of the *Arima Hi-C Protocol* in the previous section.

6.2 Add 50µL of ● **QC Beads**, mix thoroughly by pipetting, and incubate at RT for 15 minutes.

6.3 Place sample against magnet, and incubate until solution is clear.

6.4 Discard supernatant, and remove sample from magnet.

6.5 Wash beads by resuspending in 200µL of **Wash Buffer** and incubate at 55°C for 2 minutes.

6.6 Place sample against magnet, and incubate until solution is clear.

6.7 Discard supernatant, and remove sample from magnet.

6.8 Wash beads by resuspending in 200µL of **Wash Buffer** and incubate at 55°C for 2 minutes.

6.9 Place sample against magnet, and incubate until solution is clear.

6.10 Discard supernatant, and remove sample from magnet.

6.11 Wash beads by resuspending in 100µL of **Elution Buffer**.

6.12 Place sample against magnet, and incubate until solution is clear.

6.13 Discard supernatant, and remove sample from magnet.

6.14 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.

6.15 Quantify the total amount of *bead-bound DNA* using Qubit®. Use 2µL of thoroughly mixed bead-bound DNA for the Qubit® assay.

6.16 Determine the **Arima-QC1** value by following the **Worksheet Arima-HiC Quality Control For Structural Variants (Doc. PN: A160431)**. High quality Arima-QC1 values are expected to have a value of >15% of the original 50ng input onto the beds, indicating sufficient biotinylation of the Hi-C DNA. If the Arima-QC1 value did not obtain a 'PASS' status, please contact Technical Support for troubleshooting assistance.

6.17 If Arima-QC1 values are >15%, Proceed to library preparation procedure using the **User Guide: Library Preparation using Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit (Doc. PN: A160140)**.

Note: The fragment sizes for proximally-ligated DNA are generally smaller for FFPE samples and require less shearing than non-FFEP samples.

Warranty and Contact Info

WARRANTY

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