Arima Capture-HiC+ Kit

User Guide: Arima Capture-HiC+ for Fresh Frozen Tissue, Mammalian Cell Lines, and Primary Cells

8-16 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: A510008, A302010, A302020, A302031, A302032, A302033, A302034, A302035, A303010 **Document Part Number:** A160500 v01	July 2021	Initial Release
Material Part Number: A510008, A302010, A302020, A302031, A302032, A302033, A302034, A302035, A303010 **Document Part Number:** A160500 v02	September 2021	Updated Guidance to Use the Arima-HiC+ Kit enrichment beads for Library prep with the Arima Library Prep Module. Additionally, expanded the components list at the beginning of each section.
Material Part Number: A510008, A302010, A302020, A302031, A302032, A302033, A302034, A302035, A303010 **Document Part Number:** A160500 v03	October 2021	Added T1 beads as an optional User Supplied Reagent, updated guidance for library prep to use T1 beads, updated Handling and Best Practices to avoid nuclease and PCR contamination.

Material Part Number: A510008, A101060, A303011, A302010, A302020, A302031, A302032, A302033, A302034, A302035, A303010 **Document Part Number:** A160500 v04	November 2022	Updated protocol to include support for multiplex capture. Added details towards Arima Oncology Panel. Improved formatting and instructions.

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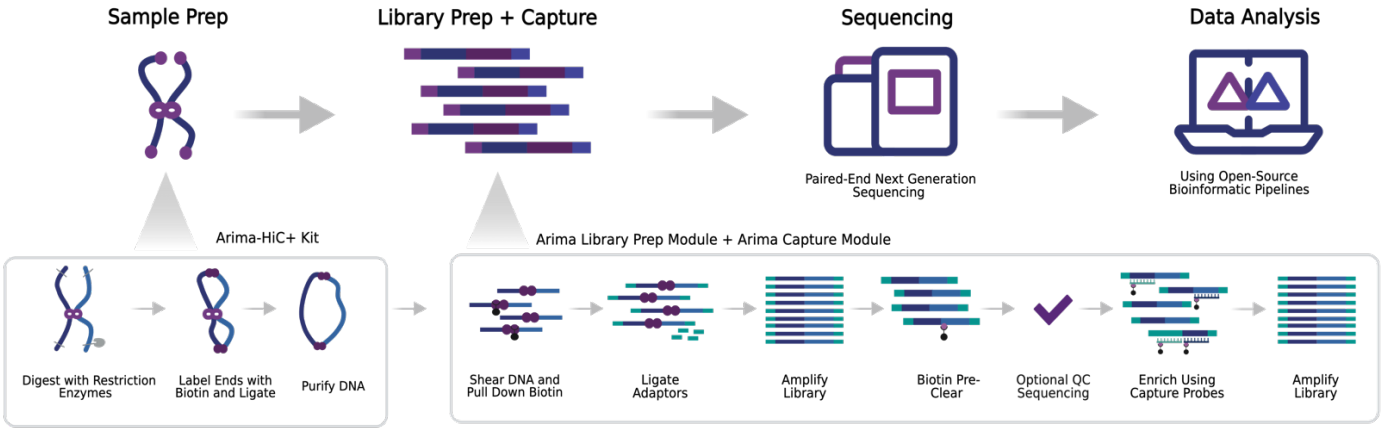


1.1 Arima Capture-HiC+ Workflow Overview

Arima Capture-HiC+ (**Figure 1)** is an experimental workflow that captures the sequence and structure (three-dimensional conformation) of target regions of genomes. **Figure 1** illustrates the Arima Capture-HiC+ workflow above. Chromatin from a sample source (tissues or cell lines) is first crosslinked to preserve the genome sequence and structure.

During "Sample Prep" with the **Arima-HiC+ kit**, 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.

During the "Library Prep and Capture" step in **Figure 1** above, the proximally-ligated DNA is fragmented, and the biotinylated fragments are enriched using T1 beads to select for molecules that capture genome structure. The enriched fragments are then subjected to a **custom** library preparation protocol utilizing the **Arima Library Prep Module** following a pre-clearing step to remove carry-over biotinylated Hi-C molecules, ensuring high on-target rate of the capture enrichment. It is recommended to perform shallow sequencing following library prep (500k-2M paired-end reads) to assess the library's quality in terms of library complexity and long-range interaction information prior to proceeding with the capture. The interactions of interest are then enriched using biotinylated RNA probes from either the **Arima Oncology Panel**, **Arima Human Promoter Panel**, the **Arima Mouse Promoter Panel**, or the **Arima Custom Panels Tier 1-5** and the **Arima Capture Reagent Module**. For **Arima Custom Panels Tier 1-5**, please contact techsupport@arimagenomics.com for help designing custom panels.

1.2 Panel Designs

Arima Oncology Panel Design

The Arima Oncology Panel is a comprehensive panel of 1,404 genes implicated in heme and solid tumors. The panel enables detection of a wide variety of structural variants (SVs), including traditional gene fusions between exons, known and novel fusion partners, and "neighborhood" SVs, which are SVs with breakpoints in intragenic regions or introns. In addition to detecting SVs, the design includes promoter sequences allowing for the simultaneous detection of the epigenetic loops contributing to the regulation of these cancer genes.

Capture probes were designed for the exons, promoters, and introns of these 1,404 cancer genes. Promoters were defined as 1,500bp upstream to 500bp downstream of the Transcription Start Site (TSS) of the gene. Each of the probes is 120bp long and is designed to have a full 120bp of complementarity to the minus strand of the reference Genome assembly, GRCH38. Target sequences were defined as a certain distance around the restriction cut sites in the **Arima-HiC+** chemistry, depending on whether the restriction fragments were in exonic and promoter regions or intronic regions.

For exons and promoters, probes were tiled at a 1x tiling density for 350bp around each Hi-C Restriction cut site contained in the genes' exon and promoter sequences. Probe sequences were filtered out for those that were designed for repetitive sequences in the genome. Probes performance is attempted to be normalized by "Boosting," in which probes are replicated in the design to increase their relative abundance in the probe pool. Probes were designed with a high density in exons compared to introns to optimize sensitivity and breakpoint resolution for traditional gene fusions involving exons.

For Introns, probes were designed exactly 5bp away from the restriction cut site using the same in silico digestion above but with some differences in how the probes were placed. Probes were not designed for fragments that were <130bp. One probe was designed to the left most cut site in fragments that were between 130bp and 259bp in size. Two probes were designed to fragments ≥260bp. Probes were removed if they had less than 40% GC and higher than 60% GC. Probes were removed if they were designed to repetitive regions in the genome. No Boosting was utilized for these probes because the GC% filters above normalize one of the major factors of probe performance resulting in all intron probe sequences in the design having the same relative abundance. Sparse coverage of the introns was included to provide better resolution for SVs that occur in the gene body but outside of exon sequences. Sparse coverage was needed in this case as opposed to the full 1x tiling for Promoters and Exons to keep the probe count down as the amount of intronic sequence in the design is much higher than the amount of exonic sequence. Putting constraints on the %GC content of the probe sequences and designing probes so that they do not overlap cut sites allowed us to filter out what we predicted to be underperforming probe sequences, creating the sparsity needed for these probes.

For a list of genes included in the **Arima Oncology Panel** and their genomic coordinates, please contact techsupport@arimagenomics.com

Arima Human Promoter Panel Design

The Arima Human Promoter Capture Probes were made to the promoters of 23,711 genes from the human GRCh38 Ensemble database, version 95, including: 18,741 protein-coding genes, 84 antisense RNAs, 170 lincRNAs, 1,878 miRNAs, 938 snoRNAs, and 1,898 snRNAs. Capture probes were designed to the restriction fragment of each of the promoters as well as to one restriction fragment upstream and one fragment

downstream of the fragment containing the promoter. The probes were manufactured using 1x tiling with repeat masking and balance boosting.

Arima Mouse Promoter Panel Design

Similar to the Arima Human Promoter Panel, the Arima Mouse Promoter Panel was designed for the promoters of 25,752 genes from the Mouse GRCm38 Ensemble database, version 94, including 21,088 protein-coding genes, 207 antisense RNA's, 544 lincRNA's, 1,015 miRNA's, 1,494 snoRNA's, and 1,383 snRNA's. Capture probes were designed to the restriction fragment of each of the promoters as well as to one restriction fragment upstream and one fragment downstream of the fragment containing the promoter. The probes were manufactured using 1x tiling with repeat masking and balance boosting.

1.3 Sequencing and Data Analysis

Arima Capture-HiC+ libraries are sequenced via Illumina® sequencers in "paired-end" mode. The resulting data is referred to as Arima Capture-HiC+ data. There are two options for analyzing Arima Capture-HiC+ data depending on the panel used to generate the Arima Capture-HiC+ library. The **Arima Oncology Pipeline** is optimized for SV and Loop calling in Arima Capture-HiC+ data generated using the **Arima Oncology Panel**. The **Arima Capture-HiC+ Pipeline** is optimized for calling loops from Arima Capture-HiC+ data generated using the **Human** or **Mouse Promoter Capture Panels** as well as **Arima Custom Panels, Tier 1-5.**

Arima Capture-HiC+ Pipeline

The Arima Capture-HiC+ Analysis Pipeline (**https://github.com/ArimaGenomics/CHiC**) and (**https://github.com/ArimaGenomics/Arima-Oncology-Pipeline**) include tools necessary for analyzing and visualizing Arima Capture-HiC+ data generated using the **Human** or **Mouse Promoter Capture Panels** as well as **Arima Custom Panels, Tier 1-5**. Briefly, the pipeline pre-processes the Arima Capture-HiC+ data using HiCUP (Wingett *et al.*, 2015) and calls loops using CHiCAGO (Cairns *et al.*, 2016). The choice of these tools was determined via a benchmarking analysis in which these tools had the lowest false positive and false negative rates compared to the other tools used in the study. This pipeline has a command line interface for easily processing the data with recommended parameters tuned for the Arima Promoter Capture probe design and the Arima 2 enzyme chemistry. The pipeline has precomputed a number of files needed for alignment and loop calling for samples captured with the **Arima Human Promoter Panel**, the **Arima Mouse Promoter Panel**. Finally, the pipeline outputs a number of files that enable an assessment of quality of the Capture-HiC libraries as well as visualizations for viewing and comparing loop calls from different samples.

1.4 How to cite Arima Capture-HiC+ in publications

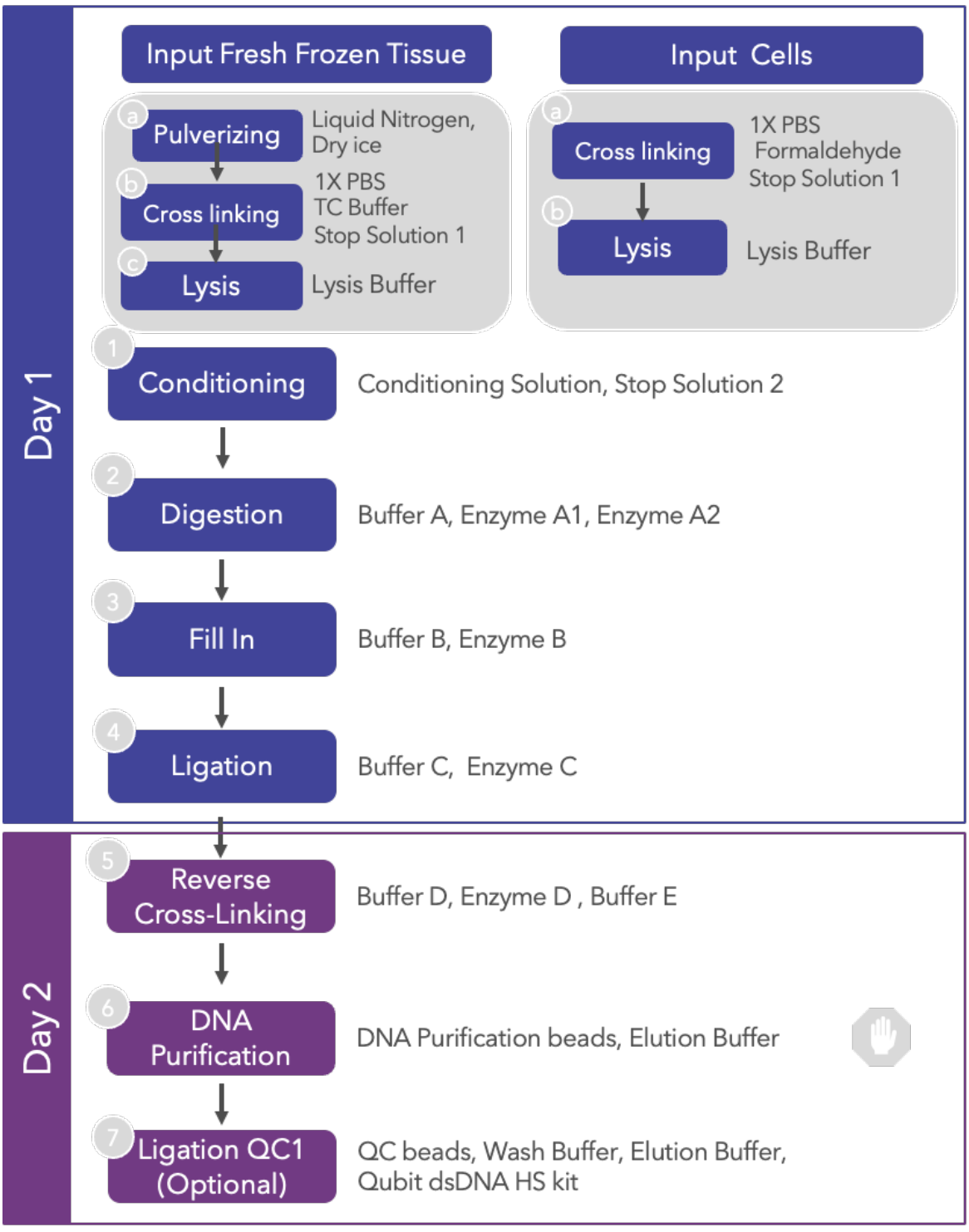
When citing the Arima Capture-HiC+ protocol or kit, one may write:
"Capture Hi-C data was generated using the **Arima-HiC+ kit**, the [Arima Capture Module used for the experiments], and the **Arima Library Prep Module** according to the **Arima Genomics** manufacturer's protocols".

2 Arima Capture-HiC+ Overview Workflow

2.1 Workflow: Proximity-Ligated DNA

The following diagrams (**Figure 2**) show the overall workflow for preparation of Arima proximity-ligated DNA using Cell lines, or Fresh Frozen Tissues as inputs.

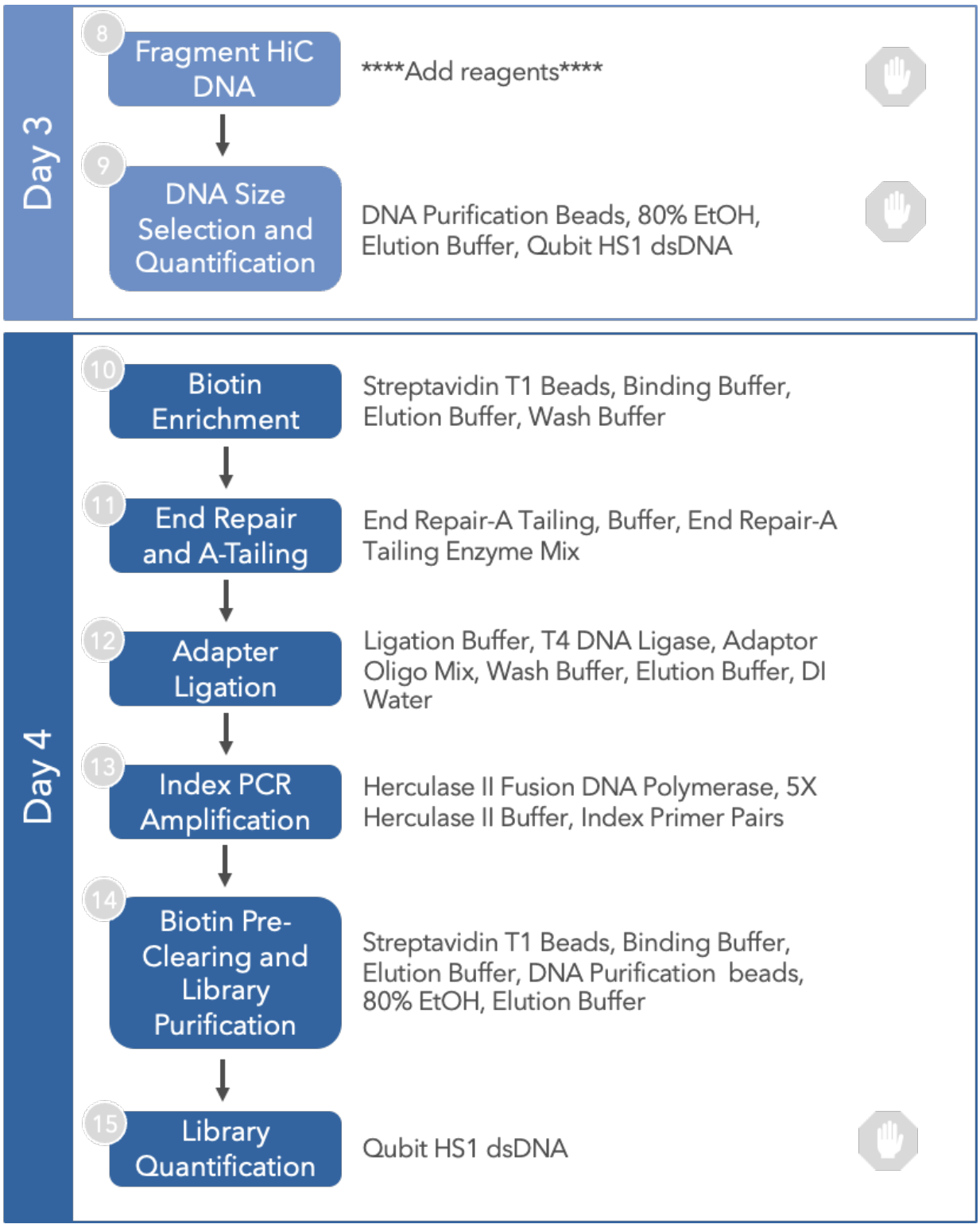
Figure 2- Arima Sample Crosslinking and DNA Hi-C Workflow



2.2 Workflow: Library Preparation from Proximity-Ligated DNA

The following diagram (**Figure 3)** shows the overall workflow for preparation of libraries using the **Arima Library Prep Module**.

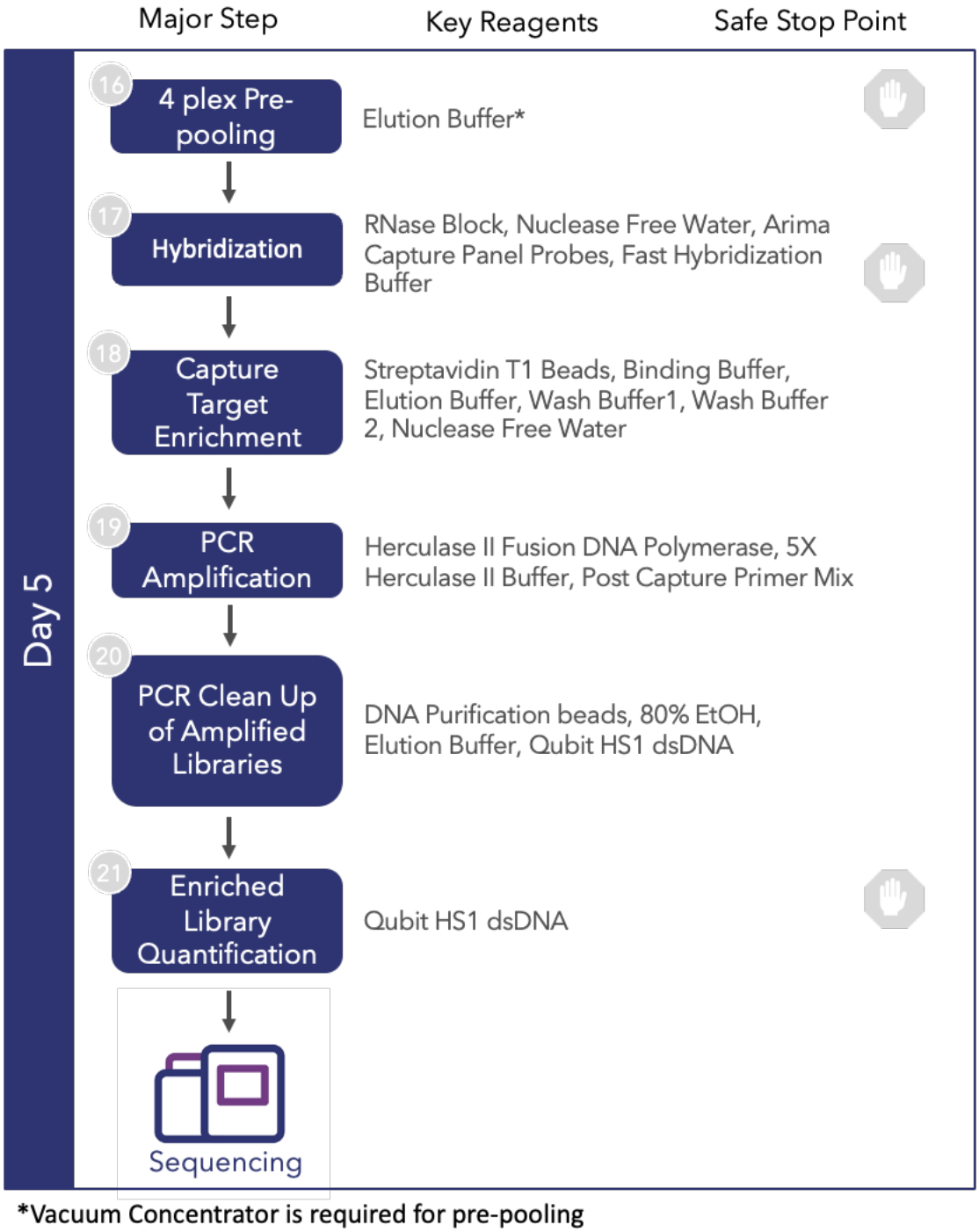
Figure 3- Arima Library Prep Workflow



2.3 Workflow: Capture Enrichment

The Arima Capture workflow using pre-capture pooling is illustrated in **Figure 4**.

Figure 4- Arima Capture Enrichment Workflow



3 Arima Capture-HiC+ Box Contents and Storage Info

Before proceeding, confirm the kit contents listed in Figures 6-10. Also, ensure that you have the necessary consumables and equipment to complete the assay (**Table 2** and **Table 3**).

Figure 5- **Arima-HiC+ Kit** (A510008) Content (For mammalian cells and Fresh Frozen Tissues)

Box A Pre- PCR P/N A410231		
Components	**Cap**	**Storage Temperature**
Stop Solution 1		20 to 25°C
Elution Buffer		
Wash Buffer		
Conditioning Solution		
Stop Solution 2		
Buffer D		
Buffer E		

Box B Pre-PCR P/N A410232		
Components	**Cap**	**Storage Temperature**
Lysis Buffer		-20°C
Buffer A		
Enzyme A1		
Enzyme A2		
Buffer B		
Enzyme B		
Buffer C		
Enzyme C		
Enzyme D		

Box C Pre-PCR P/N A410239		
Components	**Cap**	**Storage Temperature**
Enrichment Beads		2 to 8°C
QC Beads		

Figure 6- **Arima Library Prep Module** (A303010) Content

Box A Pre-PCR P/N A311035		
Components	**Cap**	**Storage Temperature**
End A Buffer		-20°C
End Repair- A Tailing Enzyme mix		
Ligation Buffer		
T4 DNA Ligase		
Adaptor Oligo Mix		
Herculase II Fusion DNA Polymerase		
5X Herculase II Buffer with dNTPs		

Box B Pre-PCR P/N A311036		
Components	**Cap**	**Storage Temperature**
Indexes Strip Tubes (1-16)		-20°C

Box C Pre-PCR P/N A311042		
Components	**Cap**	**Storage Temperature**
Streptavidin Beads (T1)		2 to 8°C

Box D Pre-PCR P/N A311041		
Components	**Cap**	**Storage Temperature**
Binding Buffer		20 to 25°C

Figure 7- **Arima Capture Module Kit** Content

Box A Post-PCR P/N A311032		
Components	**Cap**	**Storage Temperature**
Wash Buffer 1		20 to 25°C
Binding Buffer (x3)		
Wash Buffer 2		

Box B Post PCR P/N A311033		
Components	**Cap**	**Storage Temperature**
Fast Hybridization Buffer		-20°C
RNAase Block		
Blocker Mix		
Herculase II Fusion DNA Polymerase		
5X Herculase II Buffer with dNTPs		
Post Capture Primer Mix		

Box C Post-PCR P/N A311034		
Components	**Cap**	**Storage Temperature**
Streptavidin Beads (T1) (x2)		2 to 8°C

The following capture probes are in unique boxes and shall be ordered separately depending on the choice of organism and intended use. For more information, please contact Arima Technical Support.

Figure 8- List of Arima Capture Probes and Part Number

Arima Capture Probe Panels Post PCR				
Components	**Cap**	**Organism**	**P/N**	**Storage Temperature**
Arima Oncology Panel		Human	A311051	-80°C
Arima Human Promoter Panel		Human	A311025	
Arima Mouse Promoter Panel		Mouse	A311026	
Arima Custom Panel, Tier 1		Human	A311027	
Arima Custom Panel, Tier 2		Human	A311028	
Arima Custom Panel, Tier 3		Human	A311029	
Arima Custom Panel, Tier 4		Human	A311030	
Arima Custom Panel, Tier 5		Human	A311031	

4 Getting Started

4.1 Handling and Preparation

- Several steps during the *Arima Capture-HiC+ Protocol* require preparation of a master mix. Sufficient reagent has been included in the kit to make master mixes with 10 to 12% overage volume. Use the master mix calculation tables provided.
- When handling reagents, room temperature (RT) is defined as 20 to 25°C.
- If the *Arima Capture-HiC+ Protocol* is performed in PCR plates or PCR tubes, ensure at least 230µl volume capacity for each reaction listed in each section. If needed, samples may be transferred to 1.5ml low bind tubes for the high-volume protocol steps. 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.
- Arima Library Prep takes place on beads, make sure the beads are mixed well while adding the reagents and before each incubation step.
- **The Arima-HiC+** kits support multiple different applications, so some of the component may be in excess. In addition, **Enrichment bead** provided in these kits are not used in this user guide.
- **Stop Solution 1**, ○**Conditioning Solution**, and ●**Buffer D** from **Arima-HiC+ Box A** may contain precipitates. If present, heating these reagents at 37°C for 5 to 15 minutes may be necessary to dissolve precipitates.
- During handling and preparation, reagents from the **Arima-HiC+ Box A** and the **Arima Capture Module, Box A (Ambient),** should be kept at RT.
- During handling and preparation, reagents from the **Arima-HiC+ Box B, Arima Capture Module, Box B (-20°C), Arima Library Prep Module, Box A (-20°C),** and the **Arima Library Prep Module, Box B (-20°C)** should be kept on ice, except for **Enzyme D,** which should be kept on ice but warmed to room temperature just before use.
- ●**Enzyme D** may contain precipitates. If present, heating this reagent at 37°C for approximately 5 minutes may be necessary to dissolve precipitates.
- Enzyme solutions from the **Arima-HiC+ Box B**, the **Arima Capture Module, Box B (-20°C),** and the **Arima Library Prep Module, Box A (-20°C)** are viscous and require special attention during pipetting.
- All Arima Capture Probes including the **Arima Oncology Panel**, the **Arima Human Promoter Panel**, the **Arima Mouse Promoter Panel**, the **Arima Oncology Panel**, and **Arima Custom Capture Panel Tier 1-5** are RNA probes and must be stored at -80°C to prevent degradation of the RNA probes.
- To prevent contamination of reagents and probes by nuclease, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease free aerosol-resistant tips.
- If possible, performing the pre-amplification steps in a "Pre-PCR" environment and the post-amplification steps in a "Post-PCR" environment to reduce PCR contamination.

- Maintain clean work areas. Clean pre-PCR surfaces that pose highest risk of contamination with 10% bleach solution or equivalent, wait 10 to 15 minutes and then clean the surfaces with DI-Water.
- When working with liquid nitrogen wear safety goggles and cold resistant gloves.
- DNA Purification Beads (e.g., AMPure® XP Beads) should be warmed to RT and thoroughly mixed before each use. Aspirate and dispense Purification beads slowly due to the viscosity of the solution.
- All thermal cycler incubations must be performed with heated lid on except when samples are incubated at RT (20 to 25°C), which heated lid should be off.
- Possible stopping points, where samples may be stored at -20°C, are marked in the protocol. Do not subject the samples to multiple freeze/thaw cycles.

4.2 Required Reagents and Equipment

Arima Materials Checklist

Table 1- Arima Material Checklist with Part Numbers

Description	Sample Size	Arima P/N
Arima-HiC+ Kit	8 reactions	A510008
Box A		A410231
Box B		A410232
Box C		A410239
Arima Library Prep Module	16 reactions	A303011
Box A		A311035
Box B		A311036
Box C		A311042
Box D		A311041
Arima Capture Modules	*See below*	*Various see below*
Box A		A311032
Box B		A311033
Box C		A311034
Arima Human Promoter Panel	8 reactions	A302010
Arima Mouse Promoter Panel	8 reactions	A302020
Arima Oncology Panel	8 reactions	A303010
Arima Custom Capture Panel, Tier 1	16 reactions	A302031
Arima Custom Capture Panel, Tier 2	16 reactions	A302032
Arima Custom Capture Panel, Tier 3	16 reactions	A302033
Arima Custom Capture Panel, Tier 4	16 reactions	A302034
Arima Custom Capture Panel, Tier 5	16 reactions	A302035

User-Supplied Reagents, Consumables, and Equipment

Table 2- Required Equipment

Description	Vendor and Part Number
Thermal Cycler with 96 well, 0.2 ml block	Various Models, Suppliers
Qubit Fluorometer	ThermoFisher Scientific P/N Q33238
Vortex Mixer	General Laboratory Supplier
Gel Electrophoresis System	e.g. Bioanalyzer®, TapeStation®, etc.
DNA Shearing Sonicator	Diagnode, Covaris
96-well plate Mixer	General Laboratory Supplier
Vacuum Concentrator	Eppendorf Vacufuge System5301
Magnetic Separator	
0.2ml Magnetic Separator	PERMAGEN P/N MSR812
Plate Magnetic Stand-96	ThermoFisher P/N AM10027
1.7ml Magnetic Separator	ThermoFisher DynaMag-2 P/N 12321D
P2, P20, P200, P1000 Pipettes	Rainin Pipettes or equivalent
Multi-Channel Pipette	Rainin Pipettes or equivalent
Microcentrifuge	Eppendorft microcentrifuge, model 5417C or equivalent
Ceramic Mortar and Pestel*	Cole-Parmer® P/N UX-63100-63 or equivalent
Metal Spatula*	Cole-Parmer® P/N SI-06369-16
Cold Resistant Gloves *	Various Supplier
Liquid Nitrogen and Dry Ice*	Various Supplier
Centrifuge with refrigeration	Various Supplier
Thermomixer or Plate Shaker	Various Supplier
Powermasher II (optional)	DiagnoCine Cat # 891300
Biomasher II Pestle and Tube Set (optional)	DiagnoCine Cat # 320103

*Only if processing Fresh Frozen Tissues

Table 3- User Supplied Reagents and Consumables

Description	Vendor and Part Number
1X PBS, pH 7.4	Fisher Scientific P/N 50-842-949
37% Formaldehyde	Fisher Scientific P/N F79-500
100% Ethanol, molecular biology grade	Sigma Aldrich P/N 459836
Xylene	IMEB Inc. P/N XY-110
Freshly Made TC Buffer	See **Table 5** for recipe
DNA Purification Beads	KAPA@ Pure beads P/N KS8002
Qubit@ dsDNA HS Assay kit	ThermoFisher P/N Q33231
Qubit@ assay tubes	ThermoFisher P/N Q32856
Liquid Nitrogen or dry ice	Various Supplier
15 ml conical tubes	Various Supplier

1.7 ml LoBind tubes	Various Supplier
Plastic ware compatible with Thermal Cycler	
8-well micro Strip tubes and Caps	SSIbio® P/N 3247-00 or equivalent
or Hard Shell PCR Plates	BioRad P/N HSS9601
Adhesive Plate Seal	BioRad P/N MSB1001
Bioruptor or Covaris Sample holder	
Covaris MicroTube for individual Sample processing.	Covaris P/N 520045
Bioruptor NGS 0.65 Microtubes	Diagnode P/N C30010011
Nuclease Free Water	Various Supplier
Sterile, Nuclease free aerosol barrier pipette tips	General laboratory supplier

4.3 Optimal Read Length, Sequencing Depth, and Number of HiC Reactions per Sample

Arima Capture-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 Capture-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 Capture-HiC+ libraries depends on the application and the genomic resolution needed for that application. For loop calling at a resolution of 3kb we recommend 100 million read-pairs for the **Arima Human Promoter Panel**, the **Arima Mouse Promoter Panel.** For loop calls with the **Arima Oncology Panel** we recommend 100 million reads, and for structural variant detection please reach out to Arima technical support**.** For the **Arima Custom Capture Panel Tier 1-5** we recommend 10 million read-pairs per 1Mb of capture region. We recommend processing at least 2 biological replicates for each experimental condition using the Arima Capture-HiC+ protocol. In doing so, you will be able to assess the overall reproducibility of the Arima Capture-HiC+ data across replicates, and then use the combined replicate Arima Capture-HiC+ dataset for high-resolution chromatin conformation analyses. The Arima Capture-HiC+ and the Arima Oncology Pipeline can be used to analyze shallow (0.5 to 2 million read-pairs) sequencing data for each Arima Capture-HiC+ library. These Pipelines will generate a number of QC metrics for assessing the quality of the Arima Capture-HiC+ libraries. For help estimating the optimal sequencing depth for different genome sizes or analysis goals, please contact Technical Support.

Lastly, it is important to note that each Arima Capture-HiC+ library should pass the shallow sequencing metrics for Long-range cis (intra-chromosomal read-pairs greater than 15kb apart in the linear genome), percent of reads overlapping the capture probes (On-target %), and library complexity (PCR duplicates) prior to deep sequencing. Typical libraries will have <20% trans reads and >40% Long-range cis reads of the total unique Hi-C contacts. On-target rates are typically greater than 60% but are often 80-90%. As a general rule, each Arima Capture-HiC+ library should be complex enough to sequence up to ~100 to 200 million read-pairs without reaching saturation. If >200 million read-pairs of Arima Capture-HiC+ data are needed, it may be more efficient to sequence a second Arima Capture-HiC+ library than sequence deeper into the first Arima Capture-HiC+ library.

4.5 Input Sample Type Recommendation

Arima Capture-HiC+ workflow is optimized to prepare Hi-C libraries from cells collected from cell culture, and fresh frozen animal tissues. Proceed to the appropriate sections listed below for upfront preparation of proximity-ligated DNA. The library prep and capture hybridization are common procedures and are the same for all proximity ligated Hi-C DNA.

Crosslinking - Input Sample Type Fresh Frozen Tissues
Input: Fresh-frozen animal tissue
Output: Pulverized crosslinked animal tissue

Table 4- Reagent Kits Required for Cross-Linking and Estimating input DNA using Fresh Frozen Tissues

Reagent Kit	Box ID	Storage Temperature
Arima-HiC+ (P/N A510008)	Box A	20 to 25°C
	Box B	-20°C

Overview: The Arima-HiC+ workflow for animal tissues begins with the pulverization and crosslinking of fresh-frozen large animal tissue. For most vertebrates that comprise dense tissues, begin by weighing 50 to 200mg of fresh frozen tissue, and record this measured mass. The measured mass will be used later in this protocol for *Estimating Input Amount*.

Also, note that this crosslinking protocol requires the handling of liquid nitrogen, dry ice, and severely cold equipment. Please use extra caution and wear cold-resistant gloves as needed.

4.4 Pulverization of Fresh Frozen Tissues

Consumables

- 15ml conical tubes
- Dry ice and liquid nitrogen
- 1X PBS, pH 7.4 (User Supplied)
- TC Buffer (User Supplied, See **Table 5** formulation)
- Stop Solution 1

Before you begin:

- The crosslinking protocol requires the handling of liquid nitrogen and dry ice. Use extra caution and **wear cold-resistant gloves and goggles while handing liquid nitrogen**.
- Before using Mortar and pestle sterilize the equipment by spraying down with 10% bleach and then rinse with water in a sink, then spray down with 80% ethanol and dry with paper towel and at the end rinse mortar/pestle with DI-water and dry with paper towel.
- Cool down pestle and mortar and spatulas before beginning pulverization step by incubating in dry ice/liquid nitrogen at least 15 minutes. Keep them cold during the entire procedure.
- Use eye protection and cold resistant gloves while handling liquid nitrogen.
- **TC Buffer** must be prepared fresh directly before performing in the *Crosslinking* protocols. The following recipe is enough for crosslinking 8 samples. This recipe should be scaled accordingly if more or less than 8 samples are processed simultaneously. If using a 16% formaldehyde stock, please contact Technical Support for a different **TC Buffer** formulation. The table below includes a suggested vendor and catalog number for each reagent. After the **TC Buffer** is prepared, store at RT until ready to use.

Table 5- Formulation of TC Buffer

Component	Supplier	Stock Cat#	Stock Conc.	Final Conc.	Stock Volume
Water	Fisher Scientific®	50-843-406			1.67ml
Sodium Chloride	Fisher Scientific®	PR-V4221	5M	100mM	100µl
EDTA	Fisher Scientific®	PR-V4231	0.5M	1mM	10µl
EGTA	Fisher Scientific®	BM-151	0.5M	0.5mM	5µl
HEPES pH 8.0	Fisher Scientific®	H1090	1M	50mM	250µl
Formaldehyde	Fisher Scientific®	F79-500	37%	22%	3ml
				Total	**5ml**

1. Place a mortar and a 15ml conical tube onto a bed of dry ice; place a pestle into the mortar. Cool a spatula at -20°C or colder for later use.
2. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Allow liquid nitrogen to evaporate completely.

3. Pour liquid nitrogen into the mortar until the entire pestle tip is submerged. Transfer frozen animal tissue into mortar containing liquid nitrogen. Allow liquid nitrogen to evaporate just enough for the animal tissue to stay submerged.
4. Pulverize animal tissue in the mortar using the pestle until the sample resembles a fine powder. Ensure the animal tissue is always submerged in liquid nitrogen. *Carefully re-fill the mortar with liquid nitrogen as necessary*. The pulverization process takes *at least* 5 min per sample and for some tissue types, may take longer. The goal is to pulverize until the tissue resembles a fine powder without visible chunks.
5. Once the sample resembles a fine powder, allow liquid nitrogen in the mortar to evaporate entirely.
6. Carefully transfer pulverized animal tissue from the mortar into the 15ml conical tube using the cooled spatula.
7. Submerge 15ml conical tube into dry ice to keep all the pulverized animal tissue frozen.

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

8. Allow liquid nitrogen in 15ml conical tube to evaporate completely, then cap the tube.

***Tips*:** If pulverizing multiple animal tissue samples in a single day, keep the pulverized sample on dry ice and repeat Steps 1-8 on the remaining samples using clean equipment and consumables until all samples have been pulverized. Then, proceed to the next step and complete the remaining protocol on all samples simultaneously.

9. Remove the sample tube from dry ice, thaw for 2 min, then
10. Add 5ml of **1X PBS** at RT. Mix gently by inversion.
11. Add 500µl of fresh **TC Buffer**, bringing the final concentration of formaldehyde to 2%. Mix thoroughly by inverting the tube 5 to 10 times.
12. Incubate at RT for 20 minutes.
13. Add 289µl of **Stop Solution 1,** mix well by inverting 10 times and incubate at RT for 5 min.
14. Pellet sample by centrifugation at 2,500 x G at RT for 15 min.
15. Discard supernatant without disturbing the pellet.

Tips: Gently decant the tube to discard the majority of the supernatant, then use a P200 pipette to remove the remaining the liquid

16. Resuspend the sample in 1ml **1X PBS**.
17. To prepare for the *Estimating Input Amount Procedure* in the following section, mix the sample by inversion and then immediately aliquot 50µl (5%) of the sample in 1.7ml microcentrifuge tube. To reduce multiple freeze and thaw cycles, it is recommended to aliquot the remaining into smaller volumes in multiple 1.7ml microcentrifuge tubes, each contains the equivalent of ~20 to 25% of the pulverized animal tissue to prevent from multiple freeze and thaws. Make sure to mix samples by inversion between aliquots to ensure all aliquots are equally homogeneous.
18. Pellet all samples at 2,500 x G at RT for 10 min.
19. Discard supernatant leaving behind only the sample pellet and no residual liquid.

Safe Stop Point: Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the

4.5 Estimating Input Amount in Fresh Frozen Tissues

Input: 50µl (5%) Pulverized or homogenized crosslinked animal tissue
Output: Purified genomic DNA

Overview: Arima-HiC+ reactions are optimally performed on pulverized or homogenized crosslinked animal tissue comprising ~2.5-5µg of DNA. The *Estimating Input Amount* protocol is required if one does *not* know how much pulverized or homogenized crosslinked animal tissue will comprise 2.5-5µg of DNA, and if sufficient tissue is available to perform this protocol.

The *Estimating Input Amount* protocol measures the amount of DNA obtained from **50µl (5%)** of the original pulverized or homogenized crosslinked animal tissue, which guides the calculation of the optimal tissue input for an Arima-HiC+ reaction. The Arima-HiC+ kit contains enough reagents to perform an *Estimating Input Amount* protocol on 8 samples. This protocol concludes with a descriptive example of how to estimate the optimal amount of pulverized or homogenized crosslinked animal tissue to use per the Arima-HiC+ reaction.

Consumables

- 1.7ml Microcentrifuge Tubes
- PCR Strip
- Buffer D
- Enzyme D
- Elution Buffer
- DNA Purification Beads
- Freshly made 80% Ethanol
- HS dsDNA Qubit® Assay
- Qubit® tubes

Before you begin:

- **Enzyme D** should be warmed to RT before use to help prevent precipitation in the master mix. Gently vortex the tube at low speed or warm up the tube to 37°C for 5 to 10 minutes if a lot of precipitation is observed; please note that it is acceptable to use Enzyme D if the reagent stays slightly cloudy.
- 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.
- Prepare the following (**Table 6)** reagents found in Arima-HiC+ Box A and Box B.

Table 6- List of Reagents Required for Estimating Input Amount in Fresh Frozen Tissues

Reagent	Thaw Temp.	Mix	Box
Buffer E	RT	Vortex	Box A, Arima-HiC+
Enzyme D	RT	Vortex	Box B, Arima-HiC+
Buffer D	RT	Vortex	Box B, Arima-HiC+
DNA Purification Beads	RT	Vortex	User Supplied
Freshly Made 80% Ethanol	RT	Vortex	User Supplied

1. Preprogram a thermal cycler as shown in **Table 7.** Set the lid temperature to 85°C and name "**RXlink"**

Table 7- Thermal Cycler Program for Input Quantification

Temperature	Time
55°C	30 minutes
68°C	90*minutes
25°C	5 minutes

*The reaction can be incubated up to 16 hours at 68°C

2. Thaw the aliquot containing 50µl (5%) of pulverized crosslinked animal tissue prepared during previous *Crosslinking* step and transfer to a PCR Strip tube.
3. Add 124µl of **Elution buffer** to adjust the total volume of cross-linked samples to174µl.
4. Add 35.5µl of a reverse crosslinking master mix containing the following reagents listed in **Table 8.**

Table 8- Reagent Volumes for Preparing Reverse Crosslinking Master Mix

Reagent	Volume per reaction	10% extra		# Reactions		Final
●Buffer D	10.5µl	11.55µl		8	=	92.4µl
●Enzyme D	25µl	27.5µl	x	8	=	220µl
Total	35.5µl					312.4µl

5. Add 20µl of ● **Buffer E**, mix gently by pipetting.
6. Incubate on the thermal cycler using "**RXlink** "program. Use a reaction volume setting of 100µl, if required for thermal cycler setup.
7. Transfer the entire volume of the reaction (229.5µl) to a fresh1.7ml microcentrifuge tube.
8. While samples are in the thermal cycler a) Take DNA Purification Beads out of 4°C and let them warm to RT for at least 30 minutes. b) Prepare 1ml of fresh 80% ethanol per reaction; this includes the total volume needed per reaction plus additional overage.
9. Resuspend DNA purification beads thoroughly by vortexing on high speed for 1 minute.
10. Add 150µl of **DNA Purification Beads**, mix thoroughly by pipetting up and down, incubate at RT for 5 min.
11. Place sample against a magnetic stand and incubate until solution is clear.
12. Discard supernatant. While sample is still against the magnetic stand, add 400µl of 80% ethanol, and incubate at RT for 30 seconds.
13. Discard supernatant. While sample is still against the magnetic stand, add 400µl of 80% ethanol, and incubate at RT for 30 seconds.
14. Discard supernatant and briefly spin the tube to collect the residual ethanol. While sample is against magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 1 – 3 minutes to air-dry the beads
15. Remove sample from magnetic stand and resuspend thoroughly in 20µl of **Elution Buffer**

16. Incubate at RT for 5 min.
17. Place sample against magnetic stand and incubate until solution is clear. Transfer supernatant to a new tube.
18. Quantify sample using Qubit®assay. Multiply the Qubit®quantification by 20 (the total volume=1ml 1XPBS) to calculate the total DNA yield obtained from *Input Estimate protocol.*

    (The total DNA yield corresponds to the amount of DNA obtained from 50µl (50%) of the pulverized or homogenized crosslinked animal tissue) X 20
19. Estimate how much pulverized or homogenized crosslinked animal tissue to use per Arima High Coverage HiC reaction. See the descriptive example below:

Example: In the following *Arima-HiC+ Protocol*, it is recommended to use pulverized crosslinked animal tissue comprising at least 2.5µg of DNA per Arima-HiC+ reaction but no more than 5µg of DNA. If 125ng of DNA was obtained from 5% of the pulverized crosslinked small animal tissue as calculated above, one could estimate that *at least* 20% of the pulverized crosslinked small animal tissue (~500ng of DNA) is needed for Arima-HiC+. If possible, we recommend aiming to use pulverized crosslinked small animal tissue comprising 3µg of DNA per Arima-HiC+ reaction. Additionally, please note that the pulverized crosslinked animal tissue pellet for one Arima-HiC+ reaction should occupy no more than 20µl of volume in the sample tube. If the pulverized crosslinked small animal tissue pellet comprises 2.5-5µg 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 tissue pellet occupies no more than 20µl of volume or contact Technical Support for additional guidance.

The recommendation to use pulverized crosslinked small animal tissue comprising at least 500ng of DNA is only a *general* recommendation. If crosslinked animal tissue comprising at least 500ng of DNA cannot be obtained, one still should proceed with the *Arima-HiC+ Protocol* in this user guide.

5 Crosslinking - Input Sample Type Mammalian Cells

Input: Cells collected from cell culture

Output: Crosslinked cells

Table 9- Reagent Kits Required for Crosslinking Mammalian Cells

Reagent Kit	Box ID	Storage Temperature
Arima-HiC+ (P/N A510008)	Box A	20 to 25°C
	Box B	-20°C

Overview In this section Chromatin from a sample source (Cell line or blood) is crosslinked to preserve the genomic sequence and structure**.** Then the estimating input will be performed to calculate how many crosslinked cells will comprise 2.5 to 5µg of DNA, and if sufficient cells are available to perform this protocol.

Consumables

- 1X PBS
- 37% Formaldehyde
- Stop Solution 1
- 15ml Conical Tubes
- 1.7ml Microcentrifuge Tubes

5.1 Crosslinking Standard Input

Before you begin: The Arima Capture-HiC+ workflow for mammalian cell lines begins with the harvesting and crosslinking of at least 1 million cells but performs optimally with 5-10 million mammalian cells. The crosslinking protocol below involves several cell pelleting centrifugations. During these centrifugations, pellet your specific cell types at a speed and duration as you normally would. Alternatively, we generally recommend centrifuging for 5 min at 500 x G.

1. Harvest cells from cell culture using standard protocols and pellet cells by centrifugation.
2. Resuspend in cell culture media, obtain a cell count by hemocytometer or automate cell counting methods.
3. Transfer 5-10 million cells to be crosslinked into a new 15ml conical tube, pellet cells by centrifugation and remove supernatant.
4. Resuspend cells in 5ml of RT **1X PBS**.
5. Add 286µl of **37% formaldehyde**, bringing the final formaldehyde concentration to 2%.
6. Mix well by inverting 10 times and incubate at RT for 10 min.
7. Add 460µl of **Stop Solution 1,** mix well by inverting 10 times and incubate at RT for 5 min.
8. Place sample on ice and incubate for 15 min.
9. Pellet cells by centrifugation.
10. Discard supernatant.
11. Resuspend cells in 5ml **1X PBS**.

12. **Optional:** To prevent multiple freeze and thaw cycles, aliquot cross-linked cells in multiple smaller volumes in 1.7ml microcentrifuge tubes with approximately 1 million cells per aliquot. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.
13. Prepare a 50ul (~1 x 10^6) aliquot for estimating input amount.
14. Pellet cells in all aliquots by centrifugation at 5000xg for 10 min at 4°C.
15. Discard supernatant leaving only the crosslinked cell pellet and no residual liquid.
16. Freeze samples on dry ice or liquid nitrogen for 5 minutes, and store at -80°C until ready to perform the Arima-HiC+ Workflow. Proceed to *Estimating Input Amount* protocol in the following section.

5.2 Estimating Input Amount - Mammalian Cells

Input: Crosslinked cells
Output: Purified genomic DNA
Consumables

- Buffer D
- Buffer E
- Elution Buffer
- Enzyme D
- DNA Purification Beads
- Freshly made 80% Ethanol
- HS dsDNA Qubit® Assay
- Qubit® tubes

Before you begin: The *Estimating Input Amount* protocol is required if one does *not* know how many crosslinked cells will comprise 2.5µg-5µg of DNA, and if sufficient cells are available to perform this protocol. The *Estimating Input Amount* protocol measures the amount of DNA obtained per 50µl (or ~1 x 10^6) crosslinked cells, which guides the calculation of the optimal cellular input for an Arima Capture-HiC+ reaction. The Arima Capture-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 Capture-HiC+ reaction.

Prepare the following reagents listed in **Table 10.**

Table 10- List of Reagents Required to Estimate Input DNA in Cells

Reagent	Thaw Temp.	Mix	Box
Buffer E	RT	Vortex	Box A, Arima-HiC+
Enzyme D	RT	Vortex	Box B, Arima-HiC+
Buffer D	RT	Vortex	Box A, Arima-HiC+
DNA Purification Beads	RT	Vortex	User Supplied
Freshly Made 80% Ethanol	RT	Vortex	User Supplied

1. Preprogram a thermal cycler as shown in **Table 11.** Set the lid temperature to 85°C and name "**RXlink"**

Table 11- Thermal Cycler program for Estimating Input

Temperature	Time
55°C	30 minutes
68°C	90 minutes
25°C	5 minutes

2. If previously frozen, thaw one aliquot of 50µl cell pellets prepared during the *Crosslinking – Standard Input* protocol.

Note: If sample is pelleted, resuspend in 50µl of 1X PBS

3. Add 120µl **Elution Buffer** to bring the total volume to 170µl. Gently resuspend the cells in Elution buffer.
4. Add 35.5µl of a reverse crosslinking master mix containing the following reagents listed in **Table 12.** Gently mix by pipetting up and down 10 times.

Table 12- Reverse Cross-Linking Master Mix

Reagent	Volume per reaction	10% extra		# Reactions		Final
●Buffer D	10.5µl	11.55µl		8	=	92.4µl
●Enzyme D	25µl	27.5µl	x	8	=	220µl
Total	35.5µl					312.4µl

5. Add 20µl of ● **Buffer E**, mix gently by pipetting up and down 10 times.
6. Incubate the sample in a thermal cycler using "**RXlink"** program, set the lid temperature to 85°C. Use a reaction volume setting of 100µl, if required for thermal cycler setup.
7. While samples are in the thermal cycler:
    a. Take DNA Purification Beads out of 4°C and let them warm to RT for at least 30 minutes.
    b. Prepare 1ml of fresh 80% ethanol per reaction, this includes the total volume needed per reaction plus additional overage.
8. Transfer the entire volume of the reaction (229.5 µl) to a 1.7ml microcentrifuge tube.
9. Resuspend DNA purification beads thoroughly by vortexing on high speed for 1 minute.
10. Add 150µl of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.
11. Place sample against magnetic stand and incubate until solution is clear.
12. Remove and Discard supernatant. While sample is still against magnet, add 400µl of **80% ethanol**, and incubate at RT for 30 seconds.
13. Remove and discard supernatant. While sample is still against magnet, add 400µl of **80% ethanol**, and incubate at RT for 30 seconds.
14. Discard supernatant and briefly spin the tube to collect the residual ethanol. While sample is against magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 1 – 3 minutes to air-dry the beads
15. Remove sample from magnetic stand, resuspend beads thoroughly in 20µl of **Elution Buffer**, and incubate at RT for 5 min.
16. Place sample against magnetic stand, incubate until solution is clear.
17. Transfer supernatant to a new tube.
18. Quantify sample using HS dsDNA Qubit® Assay.
19. The total DNA yield corresponds to the amount of DNA obtained from 100µl mammalian cells.
20. Estimate how many mammalian cells to use per Arima Capture-HiC+ reaction. See the example description below:

Example: In the following *Arima Capture-HiC+ Protocol*, it is recommended to use crosslinked cells corresponding to at least 2.5µg of DNA per Arima Capture-HiC+ reaction, but no more than 5µg of DNA. If

1,250ng of DNA was obtained *per* 100µl (~1 x 10^6) mammalian cells as calculated in above, one can estimate that *at leas*t (200µl) 2 x 10^6 crosslinked cells should be used per Arima Capture-HiC+ reaction (~2.5µg 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 2.5µg of DNA per Arima Capture-HiC+ reaction. Additionally, please note that the crosslinked cell pellet for one Arima Capture-HiC+ reaction should occupy no more than 20µl of volume in the sample tube. If the crosslinked cell pellet comprises 2.5µg-5µg of DNA but occupies greater than 20µl of volume, aliquot the cells into multiple Arima Capture-HiC+ reactions such that the sum of the DNA input from all reactions is at least 2.5µg and each cell pellet occupy no more than 20µl of volume. Samples split over multiple Hi-C reactions can be pooled after completion of the Arima-HiC+ protocol and prior to library preparation. Contact Technical Support for additional guidance.

Recommended Hi-C Input Amount Explanation: The recommendation to use crosslinked cells comprising at least 2.5µg of DNA is required to ensure high-complexity libraries.

6 Arima-HiC+ Protocol for Fresh Frozen Tissues and Mammalian Cells

Input: Pulverized crosslinked animal tissue comprising ~2.5 to 5µg of DNA
Or Crosslinked cells containing ~2.5 to 5µg of DNA
Output: Proximally-ligated DNA

Table 13- Reagent Kits Required for Arima HiC for Fresh Frozen Tissues and Mammalian Cells

Reagent Kit	Box ID	Storage Temperature
Arima-HiC+ (P/N A510008)	Box A	20 to 25°C
	Box B	-20°C

Overview: The Arima-HiC+ reactions are optimally performed on: (a) pulverized crosslinked animal tissues comprising ~2.5 to 5µg of DNA or (b) crosslinked cells containing ~2.5 to 5µg of DNA. In the first step the cell pellets are lysed. The crosslinked chromatin is then digested using restriction enzymes. The 5' overhangs are then filled in, causing the digested ends to be labeled with biotinylated nucleotide. 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.

Consumables

- Lysis Buffer
- Conditioning Solution
- Stop Solution 2
- Buffer A
- Enzyme A1
- Enzyme A2
- Buffer B
- Enzyme B
- Buffer C
- Enzyme C
- Buffer D
- Enzyme D
- Elution Buffer
- Buffer E
- DNA Purification Beads (User Supplied)
- Freshly made 80% Ethanol (User Supplied)
- PCR Strip Tubes (User Supplied)
- Nuclease Free Water (User Supplied)

Before you begin: The tissue pellet for one Arima-HiC+ reaction should occupy no more than 20µl of volume and should be devoid of any residual liquid. If the tissue pellet occupies greater than 20µl of volume, aliquot the tissue such that the sum of the DNA input from all reactions is between 500ng to 5µg and each tissue pellet occupies no more than 20µl of volume. This may mean that for certain tissue types

(e.g., ones with low cellularity or fatty tissues), two Arima-HiC+ reactions may need to be performed on a given sample and recombined at **Step 28** of the *Arima-HiC+ Protocol*.

Note: Some of the reaction volumes during incubation steps in thermal cyclers, are greater than 100µl. For such volumes, set the reaction volumes on the thermal cycler to 100µl. The volumes have been tested and no adverse effect on the enzymatic performance of the reactions has been observed.

Prepare the following (**Table 14**) reagents found in Arima-HiC+ Box A and Box B.

Table 14- List of Reagents Required for Arima-HiC+ Workflow using Crosslinked DNA from Fresh Frozen or Mammalian Cells

Reagent	Thaw Temp.	Mix	Day needed
● Lysis Buffer	RT	Vortex	Day 1
○ Conditioning Solution	RT	Vortex	Day 1
● Stop Solution 2	RT	Vortex	Day 1
● Buffer A	On Ice	Vortex	Day 1
● Enzyme A1	On Ice	Pipetting	Day 1
● Enzyme A2	On Ice	Pipetting	Day 1
● Buffer B	On Ice	Vortex	Day 1
● Enzyme B	On Ice	Pipetting	Day 1
● Buffer C	On Ice	Vortex	Day 1
● Enzyme C	On Ice	Pipetting	Day 1
● Buffer E	RT	Vortex	Day 2
● Enzyme D	RT	Vortex	Day 2
● Buffer D	RT	Vortex	Day 2
DNA Purification Beads	RT	Vortex	Day 2
Freshly Made 80% Ethanol	RT	Vortex	Day 2

Note: Choose to perform either **Step 1a** if the input sample type is pulverized crosslinked animal tissue that is homogeneous and easy to pipette or if the input is crosslinked cells, or **Step 1b** if the sample type is pulverized crosslinked animal tissue that is clumpy, sticky, and difficult to pipette, or **Step 1c** only if the input sample type is crosslinked *nuclei* that have been previously purified from tissue.

1a. Resuspend one reaction of **pulverized crosslinked animal tissue** or **crosslinked cells** in 20µl of ● **Lysis Buffer** in a PCR tube or a well of a PCR plate, and incubate at 4°C for 30 min.

Or 1b. Resuspend one reaction of **pulverized crosslinked animal tissue** in 40µl of ● **Lysis Buffer** in a tube or a well of a PCR plate, and incubate at 4°C for 30 min. Following the incubation split into two reactions and proceed to the next step.

Or 1c. 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 minutes. Set the Thermal Cycler lid to 85°C.
3. Add 20µl of ● **Stop Solution 2**, mix gently by pipetting, and incubate at 37°C for 15 minutes. If using a thermal cycler, set the lid temperature to 85°C.
4. Preprogram a thermal cycler with program in **Table 15.** Set the lid temperature to 85°C and name it "**Digestion**."

Table 15- Thermal Cycler Program for Digestion

Temperature	Time
37°C	30 minutes for **Cells** 60 minutes for **Tissues**
65°C	20 minutes
25°C	10 minutes

5. Prepare the appropriate volume of the digestion master mix by combining the reagents in **Table 16.**

Table 16- Reagent Volumes for Preparing Digestion Master Mix

Reagent	Volume per reaction	10% extra		# Reactions		Final
○Buffer A	7µl	7.7µl	x	8	=	61.6µl
○Enzyme A1	1µl	1.1µl	x	8	=	8.8µl
○Enzyme A2	4µl	4.4µl	x	8		35.2 µl
Total	12µl					105.6µl

6. Add 12µl of the digestion master mix to each sample.
7. Mix gently by pipetting and incubate in the thermal cycler using "**Digestion**" Program,
8. Prepare the appropriate volume of Fill-in master mix by combining the reagents in **Table *17*.**

Table 17- Reagent Volumes for Preparing Fill-in Master Mix

Reagent	Volume per reaction	10% extra		# Reactions		Final
● Buffer B	12µl	13.2µl	x	8	=	105.6µl
● Enzyme B	4µl	4.4µl	x	8	=	35.2µl
Total	16µl					140.8µl

9. Add 16µl of fill-in master mix to each sample.
10. Mix gently by pipetting, and incubate at RT for 45 min.

11. Prepare the appropriate volume of ligation master mix by combining the reagents listed in **Table 18.** Mix thoroughly by pipetting up and down and spin down tube to collect liquid**.**

Table 18- Reagent Volumes for Preparing Ligation Master Mix

Reagent	Volume per reaction	10% extra		# Reactions		Final
● Buffer C	70µl	77µl	x	8	=	616µl
● Enzyme C	12µl	13.2µl	x	8	=	105.6µl
Total	82µl					721.6µl

12. Add 82µl of the ligation master mix to each sample.
13. Mix gently by pipetting, and incubate at RT for 15 min.
14. Preprogram a thermal cycler with program in **Table 19**. Set the lid temperature to 85°C and name "**RevXlink_Tissue**" or "**RevXLink_Cells**"

Note: 68°C incubation length is different for each sample type.

Table 19- Thermal Cycler Program for Reverse crosslinking

Temperature	Time
55°C	30 min.
68°C	**Cells ***(90 minutes) **Tissue** (Overnight, 16 hrs)

*The reaction can be stored at 4°C for overnight incubation

Note: Take out ●**Enzyme D** from -20°C in advance and leave at RT. Enzyme D should be warmed to RT to help to prevent precipitation in the master mix.

15. Prepare the appropriate volume of Reverse Crosslinking master mix by combining the reagents in **Table 20** and mix thoroughly**.** Make sure ● **Enzyme D** is warmed to the RT before preparing the master mix. Keep the master mix at RT until use.

Table 20-Reagent Volumes for Preparing Reverse Crosslinking Master Mix

Reagent	Volume per reaction	10% extra		# Reactions		Final
● Buffer D	10.5µl	11.55µl	x	8	=	92.4µl
● Enzyme D	25µl	27.5µl	x	8	=	220µl
Total	35.5µl					312.4µl

16. Add 35.5µl of Reverse Cross-linking master mix to each sample. Mix Gently by pipetting up and down.
17. Add 20µl of ● **Buffer E**, mix gently by pipetting up and down.

18. Incubate in the thermal cycler using "Revlink_Tissue" or "RevLink_Cells" depending on the input sample type.
19. While Samples are incubating warmed DNA Purification beads 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.
20. Prepare 1ml of freshly made 80% ethanol per reaction, this includes the total volume needed per each sample plus additional overage.
21. After incubation, transfer samples to a 1.7ml centrifuge tube or a 0.8ml MIDI plate. Make sure samples are at RT before adding DNA purification beads.
22. Vortex DNA purification beads vigorously on a vortex for 1 minute.
23. Add 100µl of **DNA Purification Beads**, mix thoroughly by pipetting up and down 10 to 15 times and incubate at RT for 5 min.
24. Place sample against a magnetic stand and incubate until solution is clear.
25. Remove and discard supernatant. While sample is still against the magnetic stand, add 400µl of 80% ethanol, and incubate at RT for 30 seconds.
26. Remove and discard supernatant.
27. While sample is still against magnet, add 400µl of 80% ethanol, and incubate at RT for 30 seconds.
28. Discard supernatant and briefly spin the tube to collect the residual ethanol. While sample is against magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 1 to 3 minutes to air-dry the beads.

Note: If 2 Arima-HiC+ reactions had to be performed due to the large volume, elute each Arima-HiC+ reaction in **50µl** of **Elution Buffer** in **Step 29**, and then combine the two samples prior to sample quantification in **Step 31**.

29. Remove sample from the magnetic stand, resuspend beads thoroughly in 100µl of **Elution Buffer**, and incubate at RT for 5 min.
30. Place sample against the magnetic stand and incubate until solution is clear, transfer supernatant to a new fresh tube.
31. Quantify sample using Qubit® HS ds DNA assay.

Note: If the proximally-ligated DNA yield is less than 300ng, we recommend skipping the Arima-QC1 assay described the following *Arima-QC1 Quality Control* section, and continue to the Arima Library Prep in **Section Arima Library Preparation Protocol**.

32. Aliquot 50ng 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 contains 50ng of proximally-ligated DNA in 50µl of **Elution Buffer**.

Safe Stop Point If you are not proceeding to Arima-QC 1 or Arima Library Prep, proximally-ligated DNA can be stored at -20°C up to 14 days.

7 Arima-QC1 Quality Control

Input: Proximally-ligated DNA
Output: QC1 measurement

Table 21- Reagent Kits Required for Arima-HiC+

Reagent Kit	Box Name	Storage Temperature
Arima-HiC+	Box C	4°C
	Box B	RT

Overview In this step the fraction of proximally-ligated DNA that has been labeled with biotin will be quantified. This 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. Arima QC1 procedure is the same for all sample types regardless of the input type (Fresh Frozen tissues , or mammalian cells).

Consumables

- QC Beads
- Elution Buffer
- Wash Buffer
- dsDNA HS Qubit® Assay
- Qubit Tubes
- PCR Strip tubes or PCR plates

Before you begin 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. P/N: A160431)** to determine the Arima-QC1 values.

1. If necessary, thaw the "Arima-QC1" samples prepared in *Arima-HiC+ Protocol* in the previous section.
2. Transfer 50ng of each sample to a fresh PCR tube. Add **Elution Buffer** to adjust the sample the volume to 50µl.
3. Mix QC beads thoroughly by vortexing vigorously for 1 minute.
4. Add 50µl of ● **QC Beads** to each 50ng sample, mix thoroughly by pipetting, and incubate at RT for 15 minutes.
5. Place sample against an appropriate magnetic stand and incubate until solution is clear.
6. Discard supernatant and remove sample from magnet.
7. Wash beads by resuspending in 200µl of **Wash Buffer** and incubate at 55°C for 2 minutes.
8. Place sample against magnetic stand and incubate until solution is clear.
9. Discard supernatant and remove sample from magnet.
10. Wash beads by resuspending in 200µl of **Wash Buffer** and incubate at 55°C for 2 minutes.
11. Place the sample against an appropriate magnetic stand and incubate until solution is clear.
12. Discard supernatant and remove sample from magnetic stand.

13. Wash beads by resuspending in 100µl of **Elution Buffer**.
14. Place the sample against an appropriate magnetic stand and incubate until solution is clear.
15. Discard supernatant and remove sample from magnet.
16. Resuspend beads in 7µl of **Elution Buffer.** Proceed to the quantification step.

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

17. Quantify the total amount of *bead-bound DNA* using Qubit®. Use 2µl of thoroughly mixed bead-bound DNA for the Qubit® assay.
18. Determine the **Arima-QC1** value by following the **Worksheet Arima-HiC+ Quality Control for Structural Variants (Doc. P/N: 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 HiC DNA. If the Arima-QC1 value did not obtain a 'PASS' status, please contact Technical Support for troubleshooting assistance.
19. If Arima-QC1 values are >15%, Proceed to library preparation procedure using **Arima Library Preparation Protocol** described in the following section.
20. After completion of quantification step, the remaining bead-bound DNA can be discarded.

8 Arima Library Preparation Protocol

Input: Proximally-ligated DNA
Output: Arima-HiC+ library ready for pre-capture sequencing and capture enrichment

Table 22- Reagent Kits Required for Arima Library Preparation

Reagent Kit	Box ID	Storage Temperature
Arima-HiC+	Box A	RT
Arima Library Prep Module	Box A	-20°C
	Box B	-20°C
	Box C	4°C
	Box D	RT

Overview: The output of the Arima-HiC+ Protocol is large proximally-ligated DNA molecules. These large DNA molecules must be fragmented using mechanical methods to limit sequence bias and then prepared as a sequencing library that is compatible with Illumina® sequencing instruments.

Library preparation begins with DNA fragmentation (Step1), DNA size selection (Step 2), and biotin enrichment (Step 3). Afterward the Arima Library Prep Module reagents are used in a custom end-repair, dA-tailing, and adapter ligation protocol (Step 4). This custom *Arima Library Preparation Protocol* constructs libraries while DNA is bound to Streptavidin Beads (T1). After Indexing and PCR amplification, streptavidin beads are used to remove any biotinylated HiC Molecules, and then libraries will be purified using DNA purification beads (e.g. AMPure® beads).

Prepare the following reagents (**Table 23**) found in the Arima Library Prep module and Arima Capture module.

Table 23- List of Reagents Required for Fragmentation and DNA Size Selection

Reagent	Thaw Temp.	Mix	Box
Streptavidin Beads (T1)	RT	Vortex	Box C, Arima Library Prep
Binding Buffer	RT	Not Needed	Box D Arima-HiC+
Wash Buffer	RT	Not needed	Box A Arima-HiC+
Elution Buffer	RT	Not Needed	Box A Arima-HiC+
DNA Purification Beads	RT	Vortex	User Supplied
Freshly Made 80% Ethanol	RT	Vortex	User Supplied

8.1 DNA Fragmentation

Consumables

- Elution Buffer
- Covaris tube or Diagnode tubes (depending on the choice of Sonicator available)

Before you begin: Covaris® instruments are recommended for mechanical fragmentation of DNA, although Diagenode® instruments have also been tested and yield comparable results. DNA should be fragmented in 100µl of **Elution Buffer.** Some Covaris® protocols recommend DNA fragmentation in a larger volume, but 100µl must be used for DNA fragmentation in the Arima-HiC+ library preparation protocol

- Make sure the chiller temperature is set between 2°C to 5°C.
- Check the water tank to make sure is filled with fresh water to the appropriate fill line level according to the manufacturer's recommendation
- It is recommended to fragment 500 to 2000ng of DNA per sample to improve complexity of the sample. If less than 500ng is available, continue with the library preparation. The number of PCR cycles should be adjusted based on input amount and the quality of the fragmented DNA to achieve enough indexed libraries for the capture hybridization step. See **Table 32** for the appropriate number of PCR Cycles.
- Let DNA Purification Beads (e.g., AMPure® XP Beads) come to RT at least 30 minutes before use.

1. Before fragmentation, if necessary, add **Elution Buffer** to bring the sample volume to 100µl. Do not exceed 100µl of volume for DNA fragmentation. Mix the sample and spin the tube to collect the liquid and to remove any bubbles from the bottom of the tube.
2. Shear DNA to obtain a fragment size distribution between 200 to 800bp with average around 400bp. *Note: Use the DNA fragmentation instrument manufacturer's default settings for obtaining a target fragment size of 400bp.*

Recommended QC before proceeding: Run an aliquot of fragmented DNA on a gel electrophoresis system (e.g. Bioanalyzer®, TapeStation®, or agarose gel) to confirm an appropriate fragment size distribution centered around 400bp.

8.2 DNA Size Selection

Consumables

- DNA Purification Beads (warmed up to Room Temperature)
- Freshly prepared 80% Ethanol
- Elution Buffer
- Qubit Tubes
- HS dsDNA Qubit Assay
- PCR tubes or PCR plates

Before you begin: In this step fragmented DNA are size-selected to remove small fragments less than 200bp. This workflow can be performed in microtubes as well as most PCR tubes and PCR plates. Ensure that your tubes or plates can hold >200µl of sample volume. Also, ensure that you have a magnetic stand that fits your choice of sample tube/plate.

- Let DNA Purification Beads (e.g., AMPure® XP Beads) come to RT at least 30 minutes before use.
- 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.

1. Spin down the fragmentation tubes briefly to collect sample volume.
2. Transfer fragmented DNA sample from fragmentation tube to either a PCR tube or PCR plate. If necessary, adjust total sample volume to 100µl by adding **Elution Buffer**.
3. Mix DNA Purification beads thoroughly by vortexing for 1 min to make sure it is fully resuspended.
4. Add 100µl of **DNA Purification Beads** to each sample well, mix thoroughly by pipetting up and down 20 times.
5. Incubate at RT for 5 min.
6. Place samples against magnetic stand and leave for 2 or 3 minutes or until the solution is clear.
7. Discard supernatant. While sample is still against magnet, add 200µl of 80% ethanol, and incubate at RT for 30 sec.
8. Repeat Step 7 once.
9. Discard supernatant and briefly spin the tube to collect the residual ethanol. While sample is against magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 1 to 3 minutes to air-dry the beads.
10. Remove the sample from magnetic stand, resuspend beads in 30µl of **Elution Buffer**, and incubate at RT for 5 min.
11. Place sample against magnetic stand and leave 2 to 3 minutes or until solution is clear
12. Transfer supernatant to a fresh tube or well of a PCR plate.
13. Quantify sample using Qubit® HS dsDNA Assay. Record this value in the **Arima Capture-HiC+ QC Worksheet** (A160501) in the "Arima Assay QC" tab in the "Qubit Concentration" column under "Size Selected QC Samples" section.

Safe Stop Point If you do not continue to **Biotin Enrichment**, Samples may be stored at -20°C for up to 3 days.

8.3 Biotin Enrichment

Overview in this step, a buffer exchange is performed to replace the storage buffer for Streptavidin beads (T1) with the **Binding Buffer**. Keep sheared and size-selected HiC DNA on ice while preparing T1 beads.

Consumables

- Streptavidin beads (T1)
- Binding Buffer
- Elution Buffer
- Wash Buffer
- Nuclease Free Water
- Tubes or plates that can hold 230µl or more

Before you begin: This workflow can be performed in microcentrifuge tubes as well as most PCR tubes and PCR plates. Ensure that your tubes or plates can hold a minimum 230µl of sample volume. Also ensure that you have a magnetic stand that fits your choice of sample tube/plate.

Note: T1 Beads used directly below are from the Arima Library Prep Module Box C. They should not be mistaken for and are NOT interchangeable with the Arima-HiC+ Enrichment Beads nor the Arima-HiC+ QC Beads

1. Perform pre-wash **Streptavidin Beads (T1)** before starting the library prep. The beads can be washed up to 1 hour in advance.
    a. Mix **Streptavidin Beads (T1)** thoroughly by vortexing for 1 minute, make sure beads are well resuspended.
    b. For each sample add 12.5µl of **T1 beads** to each well of a strip tube. Wash the beads in each tube by:
        i. Adding 200µl of **Binding Buffer.**
        ii. Mix by pipetting up and down 10 times, cap the tubes or seal the plate and vortex for additional 5 to 10 seconds.
        iii. If needed, quick spin to collect liquid at the bottom
        iv. Place tubes against a magnetic stand and incubate 1 minute or until solution is clear.
        v. Discard supernatant and remove the tube from the magnetic stand.
        vi. Wash the beads two more times by repeating steps i-v for a total of three times.
        vii. Resuspend beads in 200µl Binding Buffer.
2. Use **Arima Capture-HiC+ Quality Control Worksheet** (A160501) to calculate and transfer 200ng of size-selected DNA into a new microcentrifuge tube, PCR tube, or well of a PCR plate. Use Elution buffer to adjust the total sample volume to 30µl. If 200ng is not available, refer to **Table 32**.
   Note: Biotin enrichment and subsequent library preparation has been optimized to deliver peak performance for DNA inputs of 200ng.
3. Vortex washed **Streptavidin Beads (T1)** in **Binding Buffer** to mix thoroughly.
4. Add 200µl of washed **Streptavidin Beads (T1)** in **Binding Buffer** to each 30ul fragmented sample, mix thoroughly by pipetting.
5. Incubate on a thermomixer or a plate shaker at 1400RPM for 15min at RT.
6. Place sample against magnetic stand and leave 2 to 3 minutes or until solution is clear.
7. Discard supernatant and remove sample from magnet.

8. Wash beads by resuspending in 200µl of **Wash Buffer** located in Arima Library Prep module Box A. and incubate at 55°C for 2 min.
9. Place sample against magnetic stand and incubate until solution is clear.
10. Discard supernatant and remove sample from magnetic stand.
11. Wash beads by resuspending in 200µl of **Wash Buffer** and incubate at 55°C for 2 min.
12. Place sample against magnetic stand and incubate until solution is clear.
13. Discard supernatant and remove sample from magnetic stand.
14. Wash beads by resuspending in 100µl of **Elution Buffer**.
15. Place sample against magnet and incubate until solution is clear.
16. Discard supernatant and remove sample from magnetic stand.
17. Resuspend beads in 50µl of **Nuclease Free Water**.

8.4 Library Preparation of Enriched HiC Ligation products

Consumables

- End Repair-A Tailing Buffer
- End Repair A Tailing Enzyme Mix
- Ligation Buffer
- T4 DNA Ligase
- Adaptor Oligo Mix
- Wash Buffer
- Elution Buffer
- Nuclease Free Water
- Herculase II DNA Fusion Polymerase
- Herculase II Buffer with dNTPs
- Index Primer Pairs (1-16)
- DNA Purification Beads
- Streptavidin Beads (T1)
- 1.7 ml Microcentrifuge Tubes or PCR plates

Before you begin: This workflow can be performed in microcentrifuge tubes as well as most PCR tubes and PCR plates. Ensure that your tubes or plates can hold minimum 200µl of sample volume. Also, ensure that you have a magnetic stand that fits your choice of sample tube/plate. Set your thermal cycler at 55°C.

Prepare the following consumables (**Table 24**) found in Arima Library Prep Module Box A

Table 24- List of Arima Reagents Required for the HiC Library Prep

Reagent	Thaw Temp.	Mix	Box
○End Repair-A Tailing Buffer	On Ice	Vortex	Arima Library Prep Module - Box A
●Ligation Buffer	RT	Vortex	
●End Repair-A Tailing Enzyme Mix	Ice Just Before Use	Inversion	
● T4 DNA Ligase	Ice Just Before Use	Inversion	
○Adaptor Oligo Mix	RT	Vortex	
●Herculase II Fusion DNA Polymerase	On Ice	Pipette	
○5X Herculase II Buffer with dNTPs	RT	Vortex	
Index Primer Pair 1 – 16 (Foil Cap)	RT	Inversion	

Note: After thawing, vortex ligation buffer on high speed to make sure it is homogenous (buffer is highly viscous).

End Repair- dA-Tailing of the HiC Proximally-ligated DNA

1. Pre-Program thermal cycler with the program listed in **Table 25**. Set the lid temperature to 85°C and name**" A-Tail."** Set reaction volume at 70µl.

Table 25- Thermal Cycler program for End Repair and A-Tailing

Temperature	Time
20°C	15 minutes
72°C	15 minutes
10°C	Hold

2. Vortex thawed vial of **End Repair-A Tailing Buffer** for 15 seconds – visually inspect the tube and continue vortexing until all solids are dissolved.
3. Prepare **End Repair/dA-Tailing** master mix in a 1.7 ml tube by combining reagents as shown in **Table 26**, mix thoroughly by pipetting and spin down.

Table 26- Preparation of End Repair and A-Tailing Master Mix

Reagent	Volume per reaction	12.5% extra		# Reactions		Final
End Repair-A Tailing Buffer	16µl	18µl	x	8	=	144µL
End Repair-A Tailing Enzyme Mix	4µl	4.5µl	x	8	=	36µL
Total	20µl					180µL

4. Slowly add 20µl of the **End Repair/dA-Tailing** master mix to each sample containing 50 µl of Bead bound Hi-C library, ensuring that the full volume is dispensed. Mix well by pipetting up and down 15-20 times.
5. Place the tubes in the thermal cycler and start the "**A-Tailing** " program. Proceed immediately to the next step.
6. **Immediately** start preparation of the ligation master mix to allow the buffer to equilibration to room temperature for approximately 30 to 45 minutes for optimum performance (

7. **Table** 27) Make sure to include 12.5% overage as this master mix is very viscous.

Note: The Ligation Buffer used in this step is viscous; mix thoroughly by vortexing at high speed for 15 seconds before removing an aliquot for use. When combining with the T4 Ligase buffer, mix well by pipetting up and down 15 to 20 times.

a) Keep Ligation Master Mix at room temperature before use.

Table 27- Preparation of Ligation Master Mix

Reagent	Volume per reaction	12.5% extra		# Reactions		Final
Ligation Buffer	23µl	25.88µl	x	8	=	207µl
T4 DNA Ligase	2µl	2.25µl	x	8	=	18µl
Total	25µl					225µl

Ligation of Paired End Adaptors

1. Preprogram the thermal cycler for the ligation step with the program below (**Table 28).** Set the reaction volume to 100µl and do not use heated lid. Name the program "**Ligation**".

Table 28- Thermal Cycler Program for Adaptor Ligation

Temperature	Time
20°C	30 minutes
10°C	Hold

2. Once thermal cycler program for "A-Tailing" has reached 10°C hold step, transfer the samples on ice while setting up the next step.
3. Add 25µl of Ligation Master Mix to the 70µl of bead bound end repaired and dA-tailed HiC library. Mix well by pipetting up and down 10 times.
4. Add 5µl of Adaptor Oligo Mix to each sample. Mix well by pipetting up and down 10-15 times.

Note: Make sure to add ligation master mix and Adaptor Oligo Mix to the samples separately and mixing after each addition.

5. **Immediately** place the samples on the thermal cycler and start the "Ligation" program.
6. After the "Ligation" program completes, remove the samples from the thermocycler. If needed, quickly spin the tubes to remove any liquid from the caps.
7. Place samples on an appropriate magnetic stand to magnetize beads until liquid is clear. Remove and discard supernatant.
8. Resuspend beads in 200µl **Wash Buffer**. Mix by pipetting. Incubate at 55°C for 2 minutes.
9. Place samples on a magnetic stand and magnetize beads until liquid is clear. Remove and discard supernatant.
10. Resuspend beads in 100µl **Elution Buffer** at RT.
11. Place samples on a magnetic stand to magnetize beads until liquid is clear. Remove and discard supernatant.
12. Resuspend the beads in 34µl of **Nuclease-Free Water** and proceed immediately to Library Amplification below.

Index PCR Amplification of Pre-Capture libraries

13. Thaw the reagents listed in **Table 29** found in Arima Library Prep module Box A and Box B. Thaw only the index primers needed for experiment to minimize freeze-thaw cycles.

Table 29- Reagents for Pre-Capture PCR amplification

Reagent	Thaw Temp.	Mix
●Herculase II Fusion DNA Polymerase	On Ice	Pipette
○5X Herculase II Buffer with dNTPs	RT	Vortex
Index Primer Pair 1 – 16 (Foil Cap)	RT	Inversion

14. Determine the unique index pair assignment for each sample from the table below (**Table 30**).

Note: If pooling only a few samples for sequencing, then it is best practice to choose indexes that are balanced for each base at each position as much as possible. E.g. for pooling 4 samples, pool primer pairs 1 to 4 or 4 to 8, or etc. It is best practice for low-plex sequencing runs, use at least 4 libraries to provide sufficient index diversity.

Table 30- Nucleotide Sequences of Index Primer Pairs 1-16

Primer Pair #	P7 Index Forward	P5 Index Forward
1	CAAGGTGA	ATGGTTAG
2	TAGACCAA	CAAGGTGA
3	AGTCGCGA	TAGACCAA
4	CGGTAGAG	AGTCGCGA
5	TCAGCATC	AAGGAGCG
6	AGAAGCAA	TCAGCATC
7	GCAGGTTC	AGAAGCAA
8	AAGTGTCT	GCAGGTTC
9	CTACCGAA	AAGTGTCT
10	TAGAGCTC	CTACCGAA
11	ATGTCAAG	TAGAGCTC
12	GCATCATA	ATGTCAAG
13	GACTTGAC	GCATCATA
14	CTACAATG	GACTTGAC
15	TCTCAGCA	CTACAATG
16	AGACACAC	TCTCAGCA

15. Pre-Program the thermal cycler (with heated lid on) with the program listed in **Table 31.** Set reaction volume to 50µl and the heated lid to 105°C.

Note: Different libraries can produce different results based on varying DNA quality or input amount. In most cases, 12 cycles will produce an adequate yield for subsequent capture for Fresh Frozen and Cell lines samples.

Table 31- Pre-Capture PCR Thermal Cycler Program

Cycles	Temperature	Time
1	98°C	2 min.
12-16*	98°C	30 sec.
	60°C	30 sec.
	72°C	1 min.
1	72°C	5 min.
1	4°C	Hold

*Number of PCR cycles shall be adjusted based on the amount of input size selected DNA. Refer to **Table 32** to determine the appropriate PCR cycle

Table 32- Recommended PCR Cycle Numbers for Different Sample Types at Different Input Amounts

Input Sheared/Size selected into Library prep	Pre-Capture PCR Cycle Number
50-200 ng	12 cycles
<50ng	Capture is not recommended

16. Prepare appropriate volume of pre-captured PCR reaction mix as described in (**Table 33**Table 34). Mix well by pipetting up and down.

Table 33- Preparation of Arima Pre-Capture PCR Reaction Mix

Reagent	Volume per reaction	12.5% extra		# Reactions		Final
5X Herculase II Buffer with dNTPs	10µl	11.25µl	x	8	=	90µl
Herculase II Fusion DNA Polymerase	1µl	1.125µl	x	8	=	9µl
Total	11µl					99µl

17. Add 11µl of the PCR reaction mixture prepared from the table above to 34µl of Adaptor Ligated Bead Bound HiC library.
18. Add 5µl of the appropriate, unique, **Primer Pair** to each sample. Make sure to take note of which index was used with each sample.
19. Cap the tubes or cover the plate with an adhesive seal and place the PCR reaction in the thermal cycler and start the program.
    Note: Review "Biotin Pre-Clearing and Clean Up" if you plan to continue the protocol after step 28.
20. After the PCR program is completed, remove the tubes or PCR plate from thermal cycler. If there is residual on the PCR cap or on the adhesive cover, quickly spin down the samples.

21. Place the samples on an appropriate magnetic stand for 1 minute or until the solution is clear.
22. Transfer the supernatant to a clean PCR tube or a PCR plate. Proceed to Pre-Clearing and Clean up section.

Safe Stop Point If you do not continue to **Pre-Clearing and Clean up**, Samples may be stored at -20°C for up to 2 days.

Pre-Clearing and Clean Up

In this step, Streptavidin beads are used to remove any biotinylated HiC molecules that carry over after PCR amplification of the library Arima-HiC+ library. The pre-clearing step has been shown to be critical for high and reproducible on-target rate during Capture Enrichment. The Biotin pre-cleared libraries are then cleaned up with DNA purification beads (e.g., AMPure® XP beads).

Prior to using Streptavidin beads (T1 beads) for pre-clearing step, T1 beads must be washed and go through a buffer exchange to replace the storage buffer with the binding buffer. This step can be done up to one hour in advance.

Consumable

- Streptavidin Beads (T1)
- Binding Buffer
- Elution Buffer
- DNA Purification Beads
- Freshly made 80% Ethanol
- Nuclease-free water
- 1.7ml tubes or 0.8 ml plate
- Microcentrifuge tubes or PCR plates

Prepare the consumables in **Table 34** found in Arima Library Prep Module Box C and Box D and Arima Capture Module Box C.

Table 34- Arima Reagents Required for Biotin Pre-Clear and Clean Up

Reagents	Thaw Temp.	Mix	Box
○ Streptavidin Beads (T1)	RT	Vortex	Box C, Arima Capture Module
○ Binding Buffer	RT	Not Needed	Box D, Arima Library Prep
○ Elution Buffer	RT	Not needed	Box A, Arima-HiC+ kit

Before you begin: This workflow can be performed in 1.7ml microcentrifuge tubes or 0.8ml plates. Strip tubes are not advised during this step since the volume of the reaction during the DNA purification is 500µl.
Let the DNA purification beads come to room temperature for at least 30 minutes.

Note: T1 Beads used directly below are from the Arima Capture Module Box C.

23. Perform pre-wash **Streptavidin Beads (T1) during library PCR.** The beads can be washed up to 1 hour in advance.
    a. Mix **Streptavidin Beads (T1)** thoroughly by vortexing for 1 minute, make sure beads are well resuspended.
    b. For each sample, add 50µl of **Streptavidin Beads (T1)** to a clean 1.7ml microcentrifuge tube or 0.8ml plate. Wash the beads in each tube by:
        i. Adding 200µl of **Binding Buffer**
        ii. Mix by pipetting up and down 10 times, cap the tubes and vortex for additional 5 to 10 seconds
        iii. If needed, quick spin to pull liquid to bottom of the tube.
        iv. Place the tube or plate on a magnetic stand and incubate for 1 minutes or until the solution is clear.
        v. Discard the supernatant and remove the tubes from the magnetic stand.
        vi. Wash the beads two more times for a total of three times.
        vii. Resuspend beads in 200µl of **Binding Buffer**
24. Perform a quick spin of the PCR-amplified sample tubes to collect the liquid at the bottom of tubes.
25. Place the PCR-amplified sample tubes on a magnetic stand for 1 minute or until the solution is clear.
26. Transfer 50µl of supernatant from PCR amplified reaction to each well or 1.7ml tube of the washed Streptavidin (T1) beads.
27. Incubate the plate or 1.7ml tubes on a thermomixer or a plate shaker at 1400RPM for 30min at RT.
28. Place samples against magnet and incubate until solution is clear.
29. Transfer supernatant to a fresh 1.7ml tube or 0.8ml plate.
30. Vortex DNA Purification beads (e.g., AMPure® beads) for 1 minute until thoroughly mixed and are homogeneous.
31. Add 250 µl of DNA Purification Beads to each 250 µl Biotin pre-cleared samples. Mix thoroughly by pipetting up and down.
32. Incubate for 5 minutes at room temperature
33. Place sample against magnet and incubate until solution is clear (approximately 5-10minutes).
34. Discard supernatant. While sample is still against magnet, add 500µl of 80% ethanol, and incubate at RT for 30 sec.
35. Discard supernatant. While sample is still against magnet, add 500µl of 80% ethanol, and incubate at RT for 30 sec.
36. Discard supernatant and briefly spin the tube to collect the residual ethanol. While sample is against magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 1 to 3 minutes to air-dry the beads.
37. Remove the sample from magnetic stand, resuspend beads in 14µl of nuclease Water, and incubate at RT for 5 min. Use either pipette or vortex to make sure beads are well resuspended.
38. Place sample against magnetic stand and incubate until solution is clear.
39. Transfer supernatant to a fresh PCR strip tube.
40. Prepare another PCR tube and label 1:10 dilution.
    a) Dilute 1µl of amplified Hi-C libraries in 9µl of **Nuclease- Free Water**
    b) Quantify 1:10 diluted samples using Qubit® HS dsDNA assay. Record this value in the **Arima Capture-HiC+ QC Worksheet** (A160501) in the "Arima Assay QC" tab in the "Qubit Concentration" column under "Library Prep." section

c) Run 1:10 Diluted sample on a gel or other platform to determine the average size distribution of libraries. The estimated average size will be used to determine the molarity of the libraries for setting up a sequencing run.
d) Optional- it is highly recommended to perform a shallow sequencing of the pre-cleared libraries using 1:10 diluted libraries to confirm library quality

Safe Stop Point If you do not proceed to Arima **Capture-HiC+** workflow**,** libraries may be stored at -20°C for up to 6 months.

9 Hybridization and Capture-HiC+ Protocol

Input: Arima-HiC+ library
Output: Arima Capture Enriched Library

Table 35- Reagent Kit Required for Hybridization and Capture Hi-C

Reagent Kit	Box ID or P/N	Storage Temperature
Arima Capture Module	Box A	RT
	Box B	-20°C
	Box C	4°C
Arima Oncology Panel*	A303010	-80°C
Arima Mouse Promoter Panel*	A302020	
Arima Human Promoter Panel*	A302010	
Arima Custom Capture Panel, Tier 1*	A302031	
Arima Custom Capture Panel, Tier 2*	A302032	
Arima Custom Capture Panel, Tier 3*	A302033	
Arima Custom Capture Panel, Tier 4*	A302034	
Arima Custom Capture Panel, Tier 5*	A302035	

*Only one Arima capture panel is required for capture hybridization. All capture panels are individual boxes and are ordered separately.

Overview: In this section, the indexed Hi-C libraries are pooled in 4plex. The biotinylated probes are then hybridized to the pooled indexed Hi-C libraries. Streptavidin beads are subsequently used to capture the Hi-C library hybridized to the biotinylated probes. The beads are washed at 70°C to remove non-specifically bound molecules. The capture enrichment concludes with a second round of PCR to yield enough enriched library for deep sequencing.

9.1 Pool Indexed DNA Samples for Hybridization

In this step, the indexed HiC library samples are pooled and then the pooled volume is adjusted to 12µl before hybridization to the probe.

For optimum performance, each hybridization reaction requires a total of 3µg if pooled or 1µg indexed libraries for individual hybridization, the pooled samples made up of equal amount of 4 individual libraries. See **Table 36** for library pool composition recommendation.

Table 36- Pre-Capture Pooling Recommendation

Total amount of Indexed HiC library pool used for hybridization	Number of indexed HiC libraries per pool	Amount of each HiC library in pool
1 to 3µg	4	equal pico molar/indexed library
1µg	1	1000ng

***Note*:** After index library PCR step if the amount of available indexed library is <1000ng, it is highly recommended to perform the capture step in a 4-plex pool to improve the performance. The total amount of pooled indexed libraries should not be less than 1000ng. In addition, it is advisable to use equal molarities to pool indexed libraries with different fragment sizes to minimize variability of sequencing coverage between libraries within one pool. In general, the capture step performs better with higher total input DNA. Use **Arima Capture-HiC+ Quality Control Worksheet** (A160501) as a guidance for calculation of equal molar. Contact Arima Tech Support for more information.

1. Calculate the volume for each indexed HiC library needed to prepare a 4-plex pool. If the total volume for a 4 plex is greater than 12µl, combine the appropriate volume of each indexed HiC library sample in a 1.7ml tube; otherwise, combine the volume in one well of a strip tube or a PCR plate.

2. If the total volume is >12µl, use a vacuum concentrator with the heat off, to reduce the volume in each well to <12µl.

Note: Avoid completely drying the sample. Over-drying the indexed library pool negatively impact target enrichment.

3. Add sufficient nuclease-free water to each concentrated library pool to bring the final volume in each well to 12µl.

4. Cap the tubes or seal the plate and vortex to mix, spin in a centrifuge or mini-plate spinner to collect liquid, then keep the samples on ice until use for hybridization

Safe Stop Point If you do not proceed to Arima **Hybridization** Step, libraries may be stored at -20°C until ready for hybridization.

9.2 Hybridization

Overview: In this step, a pool of oligo probes specific to the region of interests hybridize to DNA libraries.

Consumables

- Blocker Mix
- RNAase Block
- Fast Hybridization Buffer
- Capture Panel
- PCR Strip tubes or PCR plate

Before you begin: This workflow can be performed in microcentrifuge tubes as well as most PCR tubes and PCR plates. Ensure that your tubes or plates can hold up to 200µl of sample volume. Also ensure that you have a magnetic rack that fits your choice of sample tube/plate.

1. Prepare the following consumables found in Arima Capture Module Box B (**Table 37**). Thaw Hybridization Reagents and mix according to the table below.

Table 37- Reagents required for the Hybridization Step

Reagent	Thaw Temp	Mixing	Box
Blocker Mix	Ice	Gentle Flick	Box B, Arima Capture Module
RNAase Block	Ice	Gentle Flick	Box B, Arima Capture Module
Fast Hybridization Buffer	RT	Gentle Flick	Box B, Arima Capture Module
Capture Panel	Ice	Gentle Flick	Arima Capture Probe Panel

2. Preprogram a thermal cycler with the following program listed in **Table 38** on the thermal cycler and name "CHIC-HYB".

Table 38- Thermal Cycler Hybridization Program "CHIC-HYB"*

Segment	Segment Name	Cycles	Temperature	Time
1a	Denaturing	1X	95°C	Hold
1b		1X	95°C	5 minutes
2		1X	65°C	10 minutes
3a		1X	65°C	Hold
3b	Hybridization	1X	65°C	1 minute
4		60X	65°C	1 minute
			37°C	3 seconds
5		1X	65°C	Hold

*Use a reaction volume setting of 30 µl (final volume of hybridization reaction in segment 4) and set the lid to heat for at 105°C.

Preprogram a thermal cycler with the following program listed in **Table 38** on the thermal cycler and name "CHIC-HYB".

3. Start the program, allowing the block and heated lid to warm up.
4. To each well containing exactly 12µl of pooled Hi-C Libraries (3ug) or individual Hi-C library (1µg) add 5µl ● **Blocker Mix**. Cap the tubes or seal the wells and vortex at high speed for 5 seconds. If needed, spin briefly to collect the liquid and release any bubbles.
5. Transfer sealed tubes to thermal cycler holding on program "CHIC-HYB" and press "skip step" to move out of the 95°C hold. Total time is approx. 15 mins.
6. During segments 1 and 2 of the thermal cycler program, prepare the additional hybridization reagents as described in **steps 7** and **8** below.
7. Prepare 25% solution of ● **RNase Block** according to table below (**Table 39**) and library hybridization mix. Mix well and keep on ice.
   Note: Avoid pipetting volumes less than 1µl due to potential pipetting errors and lower accuracy.

Table 39- Preparation of 25% RNase Block Solution

Reagent	Volume per reaction	12.5% extra		# Hyb Reactions		Final
● RNase Block	0.5µl	0.563µl	x	8	=	4.5µl
Nuclease-free Water	1.5µl	1.689µl	x	8	=	13.5µl
Total	2µl					18µl

8. Prepare the appropriate volume of Capture Library Hybridization Mix in a clean 1.7ml by combining reagents listed in **Table 40**

a) Prepare Arima Human Panel, Arima Mouse Panel, or Arima Oncology Panel, or any Arima Custom panel >3Mb (total region size) according to **Table 40**.

Table 40- Reagent Volumes Required for Preparation of Hybridization Master Mix for Mouse, Human and Oncology panels

Reagents	Volume per reaction	12.5% extra		# Reactions		Final
25% RNase Block Solution **(from Step 7)**	2µl	2.25µl	x	8	=	18µl
Arima Oncology Panel **Or** Arima Human Promoter Panel **Or** Arima Mouse Promoter Panel	5µl	5.625µl	x	8	=	45µl
Fast Hybridization Buffer	6 µl	6.75µl				54µl
Total	13µl					117µl

b) Prepare Arima Custom Panel (<3Mb total region size) according to **Table 41.**

Table 41- Reagent Volumes Required for Preparation of Hybridization Master Mix for Arima Custom Panel <3Mb

Reagents	Volume per	12.5% extra		# Reactions		Final
25% RNase Block Solution (from **Step 7**)	2µl	2.25µl	x	8	=	18µl
Arima Custom Panel <3Mb total region size	2µl	2.25µl	x	8	=	18µl
Fast Hybridization Buffer	6µl	6.75µl	x	8		54µl
Nuclease Free Water	3µl	3.375µl	x	8		27 µl
Total	13µl					117µl

9. Vortex the Capture Library Hybridization Mix at high speed for 5 seconds and then quickly spin down to collect the liquid, keep the reagent master mix at RT until use.
10. Wait for the thermal cycler to reach segment 3a of the "CHIC-HYB" program from above (65°C Hold).
11. With the cycler holding at 65°C and while keeping the Arima-HiC+ Library+ Blocker samples in the thermal cycler, add 13 µl of Room Temperature Capture Hybridization Mix from **Table 40** or **Table 41** to each well containing denatured and blocked HiC library.
12. Mix well by pipetting 10 times.

13. Seal wells adequately to prevent evaporation.
    a. Press "skip step" on the thermal cycler to move the program "CHIC-HYB" out of the 65°C hold and into the hybridization segments. The approximate incubation time is about 1 hour and 30 minutes.
    b. While hybridization is taking place, proceed to **Capture Enrichment** Step 1 below, to prepare Streptavidin beads for capture Enrichment.

9.3 Capture Enrichment

Overview In this section, Streptavidin beads (T1) are used to capture the HiC library hybridized to the biotinylated probes. The beads are washed at 70°C to remove non-specifically bound molecules. The enriched libraries are then resuspended in nuclease-free water to be ready for amplification.

Consumable

- Streptavidin Beads (T1)
- Binding Buffer
- Elution Buffer
- DNA Purification Beads
- Nuclease-free water
- PCR tubes, 1.7ml Microcentrifuge tubes or 0.8 ml plate
- Freshly made 80% Ethanol
- Nuclease Free Water

Before you begin:

- The 70°C washes are critical to high and reproducible on-target performance. Take care that all washes are performed, and the sample temperature remains at 70°C. Ensure that Wash buffer 2 is pre-warmed to 70°C before use. We recommend pre-aliquoting 6x 200µl **Wash Buffer 2** per reaction and keep the tubes at 70°C in a separate Thermal Cycler to maintain temperature at 70°C during washes.
- It is important to maintain beads suspensions and mixing thoroughly at 70°C during the washing procedure to ensure specificity of capture.

1. Prepare the following the following consumables found in Arima Capture Module Box A and Box C (**Table 42**).

Table 42- List of Reagents Required for Biotin Pre-Clear and Clean up

Reagents	Thaw Temp.	Mix	Box
○ Streptavidin Beads (T1)	RT	Vortex	Box C, Arima Capture Module
○ Binding Buffer	RT	Not Needed	Box D, Arima Library Prep
○ Elution Buffer	RT	Not needed	Box A, Arima-HiC+

2. ***Note:*** T1 Beads used directly below are from the Arima Capture Module Box C.
3. Perform pre-wash **Streptavidin Beads (T1)** 1 during "CHIC-HYB" hybridization step. The beads can be washed up to 1 hour in advance.
    a. Mix **Streptavidin Beads (T1)** thoroughly by vortexing for 1 minute make sure beads are well resuspended.
    b. For each sample, transfer 50µl of **T1 beads** to a well of a fresh PCR strip tube. Wash the beads in each tube by:
        i. Adding 200µl of **Binding Buffer.**
        ii. Mix by pipetting up and down 10 times, cap the tubes and vortex for additional 5 -10 seconds.
        iii. Quick spin to pull the liquid to bottom if needed.
        iv. Place tubes against a magnetic stand and incubate 1 minute or until solution is clear.
        v. Discard supernatant and remove the tubes from the magnetic stand.
        vi. Wash the beads two more times for a total of three times.
        vii. Resuspend beads in 200µl **Binding Buffer.**
4. Wait for the hybridization step to complete and the thermal cycler to reach the 65°C hold step.
5. Quickly transfer the entire 30µl volume of the Capture Hybridization Reaction, from **Hybridization Step**, into wells containing 200µl of washed streptavidin beads. Mix by pipetting up and down.
6. Incubate the samples on a thermomixer, shaking at 1400RPM for 30mins at RT. Proceed immediately to the next step.
7. While samples are on the thermomixer pre-warm the 6x 200µl aliquots per sample of **Wash buffer 2**, for each hybridization sample, in a fresh strip tube at 70°C in a thermal cycler with the heated lid on.
8. After 30 mins binding, spin samples briefly.
9. Place samples on a magnetic stand until the solution is clear.
10. While on the magnetic stand, use a pipette to remove and discard supernatant.
11. Remove the samples from the magnetic stand.
    Resuspend the beads in 200µl **Wash Buffer 1** at RT**.** Mix by pipette 10 to15 times to fully resuspended the beads.
12. Place samples on a magnetic stand until the solution is clear.
13. While on the magnetic stand, use a pipette to remove and discard supernatant.
14. Remove the samples from the magnetic separator.

Critical Step Below:

15. Wash the beads with pre warmed **Wash buffer 2**, using steps below. ***Note:*** It is extremely important to maintain 70°C during washing procedure and thoroughly mixing the beads while being washed in **Wash buffer 2**.

    a. Thoroughly resuspend beads in 200µl of 70°C prewarmed **wash buffer 2**, pipette 10 times until fully resuspended.
    b. Incubate samples for 5 minutes at 70°C in a thermal cycler with heated lid on.
    c. Spin Briefly
    d. Quickly place strip tube on a magnetic separator at RT.
    e. Wait 30 seconds or until the solution is clear, then remove and discard supernatant.
    f. Repeat steps a-f five more times for a total of 6 washes.

16. Verify that all wash buffer has been removed by quickly spinning the tubes after removing the last wash; place the tubes back on the magnetic stand to remove the residual wash buffer 2 with P20 pipette.
17. Remove the samples from the magnetic stand and add 25µl of **nuclease-free water** to each sample well.
18. Resuspend beads by pipetting up and down 10 times and store on ice until ready for the amplification step

9.4 Amplify Capture Enriched Library

Overview in this step, universal primers (Post Capture Primer Mix) are used to amplify enriched libraries bound to the streptavidin beads.

Consumables

- Herculase II Fusion DNA Polymerase
- 5X Herculase II Buffer with dNTPs
- Post-Capture Primer Mix
- PCR Strip tubes or PCR plate
- DNA Purification Beads
- 80% Ethanol
- Nuclease Free Water
- HS dsDNA Qubit® Assay
- Qubit Tubes

Before you begin Thaw the reagents listed in **Table 43.**

Table 43- List of Reagents Required for PCR Amplification of Captured Libraries

Kit Component	Thaw Temp	Mixing Method	Box
Herculase II Fusion DNA Polymerase	Ice	Pipette up and down	Box B, Arima-Capture Module
5x Herculase II Buffer with dNTPs	Ice	Vortexing	Box B, Arima-Capture Module
Post-Capture Primer Mix	Ice	Vortexing	Box B, Arima-Capture Module
DNA Purification Beads	RT	Vortex	User Supplied
Freshly Made 80% Ethanol	RT	Vortex	User Supplied

1. Preprogram a thermal cycler with heated lid ON with program listed in **Table** 44. name the program "cHiC_amp."

Table 44- Thermal Cycler Program for amplification of Captured Enriched Libraries

Cycle Number	Temperature	Time
1X	98°C	2 minutes
13X	98°C	30 seconds
	60°C	30 seconds
	72°C	1 minute
1X	72°C	5 minutes
1X	10°C	Hold

2. Prepare appropriate amount of volume of PCR reaction mix as described in **Table 45**.

Table 45- Reagent Volumes Required for Preparation of Capture-HiC+ PCR Master Mix

Reagent	Volume per reaction	12.5% extra		# Reactions		Final
Nuclease-free water	13µl	14.625µl	x	8	=	117µL
○5x Herculase II Buffer with dNTPs	10µl	11.25µl	x	8	=	90µL
●Herculase II Fusion DNA Polymerase	1µl	1.125µl	x	8	=	9µL
○Post-Capture Primer Mix	1µl	1.125µl	x	8	=	9µL
Total	25µl					225µL

3. Add 25ul of PCR reaction mix prepared in Table above to each sample well containing 25ul of bead-bound target-enriched DNA
4. Mix the PCR reactions via pipette until homogenous. DO NOT SPIN at this step to avoid pelleting the beads.
5. Place samples on a thermal cycler and start program "cHiC_amp." Total time is approx. 50 mins.
6. Once PCR is complete, spin the PCR reactions briefly.
7. Place samples on a magnetic stand until the solution is clear.
8. While samples are on the magnetic stand, transfer 50ul **supernatant** to a fresh PCR tubes or PCR plate and discard the **Streptavidin Beads (T1)**.
9. Add 50 µl of **DNA Purification Beads** to each 50 µl Arima Capture Enriched Library.
10. Mix well by pipetting up and down 10 to 15 times.
11. Incubate at room temperature for 5 minutes.
12. Place sample against a magnetic stand for 1 minute or until solution is clear.
13. While samples are still on the magnetic stand, remove and discard supernatant.
14. Keep samples on magnetic stand and add 200µl of **80% ethanol**, incubate at RT for 30 seconds.
15. Remove and discard supernatant.
16. While sample is still against magnetic stand, add 200µl of **80% ethanol**, and incubate at RT for 30 seconds.

17. Discard supernatant and briefly spin the tube to collect the residual ethanol. While sample is against magnetic stand, remove any residual ethanol with a P20 fine pipette tip. Incubate beads at RT for 1 – 3 minutes to air-dry the beads
18. Remove sample from the magnetic stand and resuspend beads in 25µl of **Nuclease Free Water**. Incubate at RT for 5 min.
19. Place sample against magnetic stand and incubate until solution is clear.
20. Transfer 25µl of each purified library to a fresh PCR strip tube.
21. Quantify 2µl of sample using HS dsDNA Qubit® Assay.
22. Run libraries on a gel or other platform for determining the size distribution of Nucleic Acids and ensure there are no primer dimers in the reaction.
23. Sequence the Arima Capture Enriched Library using the Illumina platform of choice.

Safe Stop Point If you do not proceed to sequencing**,** libraries may be stored at -20°C for up to 6 months.

10 Warranty and Contact Info

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