GENE-SWitCH

The regulatory GENomE of SWine and CHicken: functional annotation during development

Protocol WP1 T1.6
Promoter Capture Hi-C on chicken tissues

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1 Summary

Promoter Capture Hi-C allows identifying by Next Generation Sequencing all the genomic regions in interactions with a defined set of promoters. It is indeed a key approach to identify enhancer/promoter interactions and allow to add spatial information on genome annotations.

The aim is to provide sequencing raw data of capture Hi-C libraries produced from muscle and liver of chicken and pigs samples at three developmental stages. For each species (pig and chicken), each developmental stage (early and late organogenesis, newborn) and each tissue (liver and muscle), 2 biological replicates (1 male and 1 female) were processed. It represents a total of 24 libraries.

Hi-C libraries are usually performed on single cell suspension. Single cell suspensions were performed by INRAE (https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_Capture HiC_Cell_Dissociation_20210331.pdf) for each sample by enzymatic dissociation of primary tissues. Unfixed cell pellets were then snapfrozen in liquid nitrogen and stored at -80°C

To perform Promoter Capture Hi-C, we used the ARIMA Hi-C+ kit for its strong reproducibility and efficiency, coupled with the SureSelect XT HS v2 kit from Agilent using a custom design of probes for chicken promoters.

We designed the probes necessary for the Promoter Capture Hi-C in close collaboration with Arima Genomics and Agilent technical and bioinformatic supports. We designed 62562 probes, covering a total of ~30,000 different promoters.

2 Protocol description

2.1 Instruments, kits and solutions required

2.1.1 Instruments

- Covaris fragmentation system
- Qubit fluorometer (Thermo Fischer Scientific)
- QuantStudio real-time PCR system (Applied Biosystem) (or any other device for quantification by qPCR)
- BioAnalyzer (Agilent Technologies)
- ThermoMixer (Eppendorf)
- DynaMag – PCR and DynaMag for 1.5ml tubes (Thermo Fischer Scientific)
- Thermocycler (Eppendorf)

2.1.2 Kits and reagents

- Falcon tubes (15ml)

- Eppendorf DNA low binding 1.5ml tubes
- Qubit dsDNA HS and BR assay kit
- Agilent High Sensitivity DNA Kit for BioAnalyzer
- AMPure XP purification beads (Beckman Coulter)
- Dynabeads MyOne Streptavidin T1 (Invitrogen)
- Arima-HiC+ for HiC (Arima Genomics)
- Arima Library Prep kit (Arima Genomics)
- SureSelect XT HS V2 reagent kit, target enrichment baits (custom design) (Agilent Technologies)

  Design is on the SureSelect website (https://earray.chem.agilent.com/suredesign/)

  Design ID: S3386943 (available under request to Hervé Acloque: herve.acloque@inrae.fr)

  Design contains 62562 probes covering ~30,000 promoters

2.1.3 Solutions

- PBS 1X
- Ethanol
- 2% formaldehyde solution (prepare fresh, keep at RT)
- 1M Glycine

2.2 Procedure

2.2.1 In-situ crosslinking

Preparation: cool down centrifuge for Eppendorf tubes and 15mL Falcon tubes

1. Resuspend the isolated cell pellet in 5 mL PBS 1X (~1 million cells to get at least 2.5µg of DNA)
2. Transfer each batch of cells to a 15 mL Falcon tube
3. Add 5 mL of freshly made 2% formaldehyde solution (1% final)
4. Crosslink cells tumbling for exactly 10 min at RT
5. Take 10 µL and determine the number of cell nucleus (with Kova counting blades-slide or any other counting system)
6. Add 2,5 mL 1M Glycine to quench the formaldehyde and put immediately on ice
7. Centrifuge 5 min 500g at 4°C
8. Remove the supernatant

9. Wash 2 times with 5mL PBS 1X
10. Centrifuge 5 min, 500g at 4°C
11. Remove supernatant
12. Resuspend in the appropriate volume of PSB 1X and transfer to Eppendorf Safe lock tubes to make 1 *10^6 aliquots
13. Freeze cells in liquid N_2 and store at -80°C or immediately proceed to next step

2.2.2 Lysis, restriction digest and proximity ligation

Starting material should be around 2.5µg to 5µg of DNA (~1million nuclei)

Follow Steps 7.1 to 7.21 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

We used AMPure XP Beads as DNA Purification Beads. Warm sample and AMPure XP beads to room temperature 30 min before use. Vortex Ampure beads well!

2.2.3 Arima-QC1 Quality Control

Follow Steps 8.1 to 8.16 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

High quality Arima-QC1 values are expected to be **>15%.**

2.2.4 Fragmentation and size selection

Fragmentation was performed on a Covaris to obtain an average fragment size of 400bp.

Prepare Covaris for use at least 40 min before use (add MQ to fill level 15).

We fragmented all the DNA eluted after step 7.21.

1. Add Elution Buffer to your sample to bring the volume to **100 µL**
2. Transfer to a Covaris millitube and shear using the following parameters:

Duty factor	15%
Peak incident	500
Cycles/burst	200
Duration	30 seconds

3. Transfer sample to fresh 1.5 mL Lo-bind Eppendorf tube. If necessary, add Elution Buffer to your sample to bring the volume to **100 µL**

For the size selection, follow Steps 9.2.1 to 9.2.13 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

We used AMPure XP Beads as DNA Purification Beads. Warm sample and AMPure XP beads to room temperature 30 min before use. Vortex Ampure beads well!

2.2.5 Biotin enrichment

We used **exactly 200ng** of DNA as mentioned in the protocol at step 9.3.4.

s described in the protocol

Follow Steps 9.3.1 to 9.3.17 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

Following the protocol, we used exactly 200ng of size-selected DNA

We used Streptavidin Beads (T1) from Thermo Fisher Scientific as Enrichment Beads.

We used 1.5ml low binding Eppendorf tubes instead of PCR microtubes or plates and a ThermoMixer set at 55°C.

2.2.6 End-repair, dA-tailing, and Adapter ligation (Arima Library Prep kit)

Follow Steps 9.4.1.1 to 9.4.1.9 for End-repair, 9.4.2.1 to 9.4.2.13 for Adapter ligation from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

2.2.7 Library amplification and purification

Follow Steps 9.5.1 to 9.5.8 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

We used the indexes provided by the kit (n°1 to 13).

We used 10 amplification cycles as mentioned in the protocol (when following the requirement of starting quantities written in the protocol).

For the purification follow Steps 9.6.1 to 9.6.12 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

We used AMPure XP Beads as DNA Purification Beads.

Warm sample and AMPure XP beads to room temperature 30 min before use.

Vortex Ampure beads well!

2.2.8 Pre-clearing, hybridization and capture

For the promoter capture we used the reagents from the SureSelect XT HS V2 kit and we followed Steps 10.1.1 to 10.4.23 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

Pre-clearing
We used Streptavidin Beads (T1) from Thermo Fisher Scientific for the pre-clearing.

Pre-clearing allows to remove any residual biotin from the religation step that can interfere later with the purification of the biotinylated probes.

We used 1.5ml low binding Eppendorf tubes instead of PCR microtubes or plates and a ThermoMixer set at 55°C.

Follow Steps 10.1.1 to 10.1.20 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

Hybridization
We used the capture panel from Agilent with the design ID **S3386943 (stored at -80°C).**
We used **1.5µg of DNA** as mentioned in the protocol at section 10.2.

Follow Steps 10.2.1 to 10.2.15 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

Capture of hybridized probes and DNA fragments
We used Streptavidin Beads (T1) from Thermo Fisher Scientific for the capture

We used 1.5ml low binding Eppendorf tubes instead of PCR microtubes or plates and a ThermoMixer set at 55°C.

Follow Steps 10.3.1 to 10.3.8 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

Amplification and purification

We used AMPure XP Beads as DNA Purification Beads.

Warm sample and AMPure XP beads to room temperature 30 min before use.

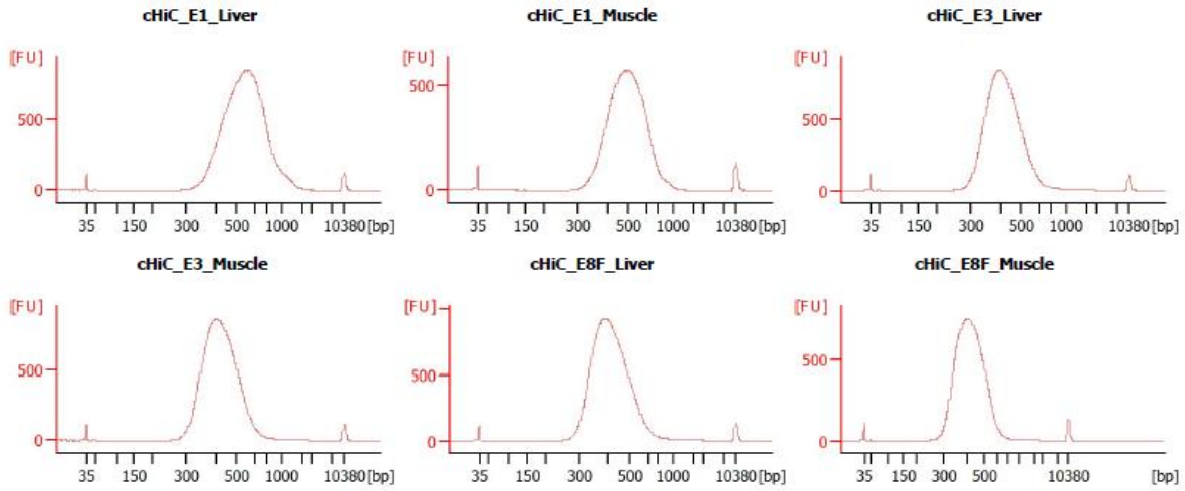
Vortex Ampure beads well!

We used 13 amplification cycles as mentioned in the protocol (when following the requirement of starting quantities written in the protocol).

Follow Steps 10.4.1 to 10.4.23 from the User Guide Mammalian cell lines ref: Doc A160500 v03 from Arima Capture-HiC kit (in Annex at the end of the document).

2.2.9 Final libraries' QC

Libraries were quantified using Qubit dsDNA HS and checked on a BioAnalyzer HS DNA chip.



BioAnalyzer profiles of 6 libraries used for the GENE-SWitCH project

Index name	Sequence i7 –i5	Tubes	Lib name	Taille lib (bp)	Qty ng/ul	Tissue	Sex	Stage
1	CAAGGTGA-ATGGTTAG	E1-Liver	SSC_INRAE_GS_WP1_CHiC_Liver_HC_1	553	11	Liver	F	HC
2	TAGACCAA-CAAGGTGA	E1-Muscle	SSC_INRAE_GS_WP1_CHiC_Muscle_HC_1	503	9	Muscle	F	HC
3	AGTCGCGA-TAGACCAA	E3-Liver	SSC_INRAE_GS_WP1_CHiC_Liver_HC_2	423	14	Liver	M	HC
4	CGGTAGAG-AGTCGCGA	E3-Muscle	SSC_INRAE_GS_WP1_CHiC_Muscle_HC_2	429	11	Muscle	M	HC
9	CTACCGAA-AAGTGTCT	E8F-Liver	SSC_INRAE_GS_WP1_CHiC_Liver_E8_1	421	13	Liver	F	E8
10	TAGAGCTC-CTACCGAA	E8F-Muscle	SSC_INRAE_GS_WP1_CHiC_Muscle_E8_1	426	13	Muscle	F	E8
13	GACTTGAC-GCATCATA	E8M-Liver	SSC_INRAE_GS_WP1_CHiC_Liver_E8_2	431	10	Liver	M	E8
12	GCATCATA-ATGTCAAG	E8M-Muscle	SSC_INRAE_GS_WP1_CHiC_Muscle_E8_2	451	19	Muscle	M	E8
5	TCAGCATC-AAGGAGCG	E13-Liver	SSC_INRAE_GS_WP1_CHiC_Liver_E15_1	441	12	Liver	F	E15
6	AGAAGCAA-TCAGCATC	E13-Muscle	SSC_INRAE_GS_WP1_CHiC_Muscle_E15_1	439	10	Muscle	F	E15
7	GCAGGTTC-AGAAGCAA	E15-Liver	SSC_INRAE_GS_WP1_CHiC_Liver_E15_2	428	10	Liver	M	E15
8	AAGTGTCT-GCAGGTTC	E15-Muscle	SSC_INRAE_GS_WP1_CHiC_Muscle_E15_2	454	11	Muscle	M	E15

Libraries produced using this protocol on chicken tissues.

3 Annex : User Guide Arima-Capture HiC (Doc A160500 v03)

Arima Capture-HiC Kit

User Guide for Mammalian Cell Lines

8-16 reactions

Material Part Numbers: A510008, A302010, A302020, A302031, A302032, A302033, A302034, A302035, A303010
Document Part Number: A160500 v03
Release Date: October, 2021

This product is intended for research use only. This product is not intended for diagnostic purposes.

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

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.

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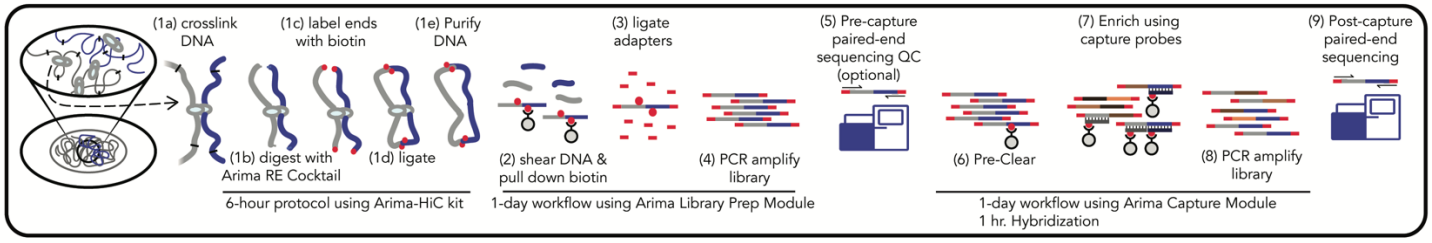
1. Introduction



1.1 Arima Capture-HiC Workflow Overview

Arima Capture-HiC is an experimental workflow that captures the sequence and structure (three-dimensional conformation) of genomes. As illustrated in the Arima Capture-HiC workflow schematic above, chromatin from a sample source (tissues, cell lines, or blood) is first crosslinked to preserve the genome sequence and structure. The crosslinked chromatin is then digested using a restriction enzyme (RE) cocktail. The 5'-overhangs are then filled in, causing the digested ends to be labeled with a biotinylated nucleotide. Next, spatially proximal digested ends of DNA are ligated, capturing the sequence and structure of the genome. The ligated DNA is then purified, producing pure proximally-ligated DNA. The proximally-ligated DNA is then fragmented, and the biotinylated fragments are enriched. The enriched fragments are then subjected to a **custom** library preparation protocol utilizing the **Arima Library Prep Module**. Following Library prep, it is recommended to perform a shallow sequencing (500k-2M paired-end reads) to assess the libraries quality in terms of library complexity and long-range interaction information prior to proceeding with the capture. Once HiC is complete, the sample is pre-cleared to remove carry over biotinylated HiC molecules. The interactions of interest are then enriched using biotinylated RNA probes from either the **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 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 RNA's, 170 lincRNA's, 1,878 miRNA's, 938 snoRNA's, and 1,898 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 Arima Mouse Promoter Panel Design

Similar to the Arima Human Promoter Panel, the Arima Mouse Promoter Panel was designed to 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.

1.4 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. The tools necessary for analyzing and visualizing Arima Capture-HiC data are included in the Arima Capture-HiC Analysis Pipeline. See the Arima Capture-HiC Bioinformatics User Guide for more details. Briefly, the pipeline pre-processes the Arima Capture-HiC data using HiCUP (Wingett *et al.*, 2019) 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 rate 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 which enable an assessment of quality of the Capture-HiC libraries as well as visualizations for viewing and comparing loop calls from different samples.

2. Arima Capture-HiC Quick Reference Protocol

ARIMA GENOMICS

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

Arima-HiC Quick Reference Protocol

Crosslink Sample

Resuspend cells in 20µL ● **Lysis Buffer** OR nuclei in 20µL water[1]
Incubate 15 min. at 4°C

Add 24µL ○ **Conditioning Solution**
Incubate 10 min. at 62°C

Add 20µL ● **Stop Solution 2**
Incubate 15 min. at 37°C

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

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

Add 70µL ● **Buffer C** + 12µL ● **Enzyme C**
Incubate 15 min. at 25°C

Add 10.5µL ● **Buffer D** + 25µL ● **Enzyme D**
Add 20µL ● **Buffer E**
Incubate 30 min. at 55°C followed by 90 min. at 68°C

Purify DNA
Proceed to library prep using the unique protocol outlined in the User Guide

[1] If nuclei have been isolated prior to lysis step (e.g. from plant tissue, FAC-sorted nuclei)

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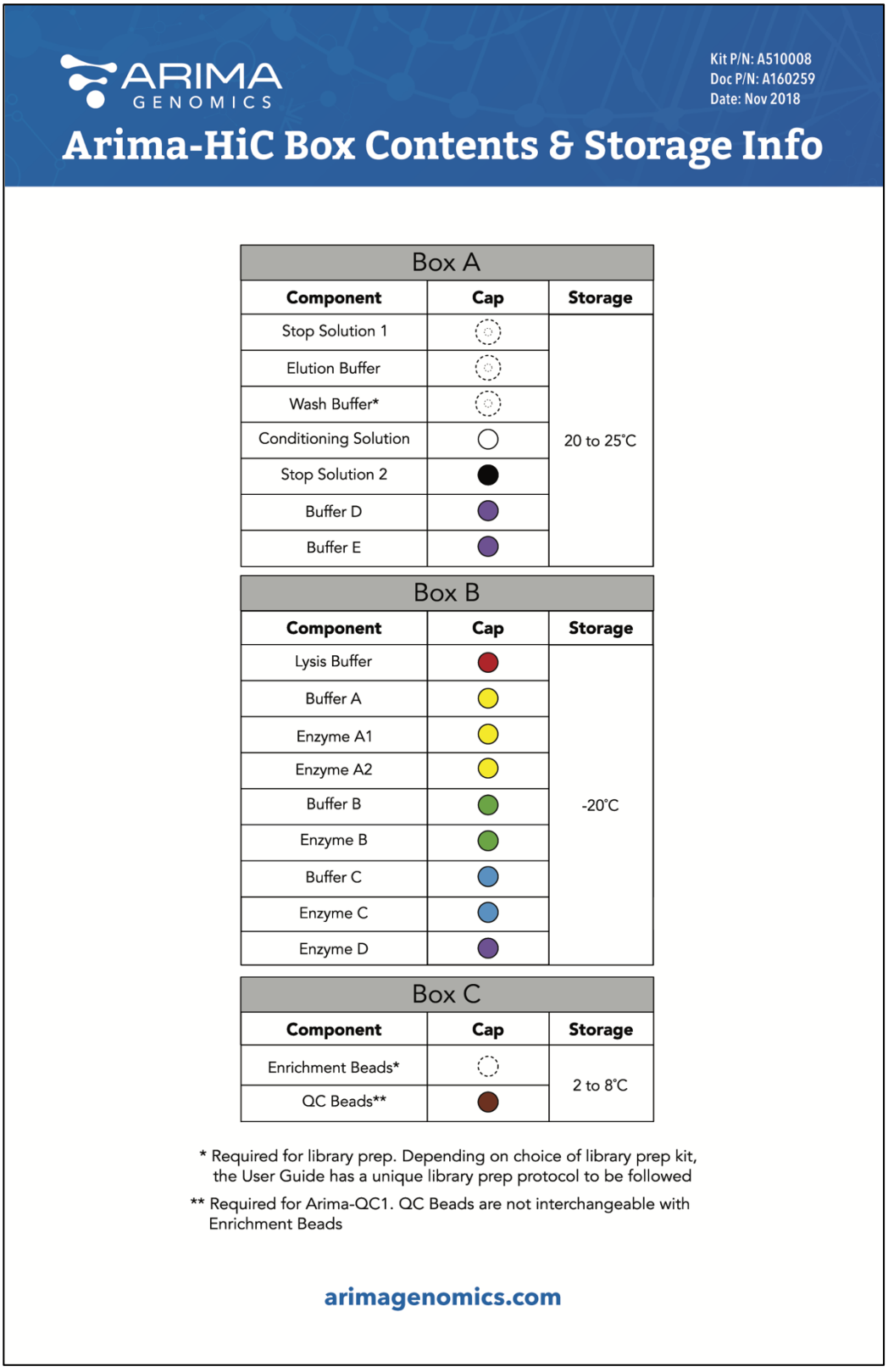
3. Arima Capture-HiC Kit Contents and Storage Info



ARIMA GENOMICS

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

Arima-HiC Box Contents & Storage Info

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

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

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

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

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

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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% excess volume. Use the master mix calculation tables provided.
- When handling reagents, room temperature (RT) is defined as 20 to 25°C.
- If the *Arima Capture-HiC Protocol* is performed in PCR plates or PCR tubes, ensure to have a total volume capacity of at least 320µL. See Section 2.2 for recommended PCR plates and PCR tubes. Also, ensure that plates and/or tubes are compatible with thermal cyclers and other required equipment. Using seals and caps for PCR plates and tubes is required.
- All kit reagents should be fully thawed and thoroughly mixed before use.
- **Stop Solution 1**, ○**Conditioning Solution**, and ●**Buffer D** from **Arima-HiC+ Box A** may contain precipitates. If present, these precipitates must be dissolved before use. Heating these reagents at 37°C for 5-15 minutes may be necessary to dissolve precipitates.
- During handling and preparation, reagents from 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, these precipitates must be dissolved before use. Heating these reagents at 37-42°C for 5-10 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.
- The Arima Promoter Capture Probes in the **Arima Human Promoter Panel**, the **Arima Mouse Promoter Panel** and any **Arima Custom Capture Panel Tier 1-5** must be stored at -80°C to prevent degradation of the RNA probes.
- To protect samples and probes from Nucleases we recommend the use of gloves and sterilized filter tips.
- If possible, performing the pre-amplification steps in a "Pre-PCR" environment and the post-amplification steps in a "Post-PCR" environment will reduce PCR contamination.
- DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before each use.

4.2 Arima materials checklist

- ☐ **Arima HiC+ Kit (A510008)**
- ☐ **Arima Library Prep kit (A303010)**
- ☐ Capture Modules containing probes and capture reagents, at least one of the following:
  - ☐ **Arima Promoter Capture module (human) (A302010)**
  - ☐ **Arima Promoter Capture module (mouse) (A302020)**
  - ☐ **Arima Custom Capture module, Tier 1 (A302031)**
  - ☐ **Arima Custom Capture module, Tier 2 (A302032)**
  - ☐ **Arima Custom Capture module, Tier 3 (A302033)**
  - ☐ **Arima Custom Capture module, Tier 4 (A302034)**
  - ☐ **Arima Custom Capture module, Tier 5 (A302035)**

4.3 User-supplied reagents, consumables, and equipment checklist

- ☐ 1X PBS, pH 7.4 (e.g. Fisher Scientific® Cat # 50-842-949)
- ☐ 37% Formaldehyde (e.g. Fisher Scientific® Cat # F79-500)
- ☐ Freshly prepared 80% Ethanol
- ☐ DNA Purification Beads (SPRI, e.g. Beckman Coulter Cat # A63880, Approx. 750µL per sample)
- ☐ Optional: Dynabeads™ MyOne™ Streptavidin T1 (Thermo Fisher Scientific Cat # 65601, 65602, 65603). **NOTE:** The T1 beads are sold along with the **Arima Promoter or Custom Capture Modules (A302010, A302020, A302031, A302032, A302033, A302034, A302035)** but **NOT** with the **Arima Library Prep Kit (A303010)**. If proceeding to library prep without having an **Arima Promoter or Custom Capture Module** on hand, then additional T1 beads are required as a user supplied reagent.
- ☐ Qubit® Fluorometer, dsDNA HS Assay Kit and consumables (e.g. Thermo Fisher Scientific Cat # 32851, 32856)
- ☐ Liquid Nitrogen or dry ice
- ☐ 15mL conical tubes
- ☐ 1.7mL microcentrifuge tubes, PCR tubes (e.g. SSIbio® Cat # 3247-00), or PCR plates (e.g. Bio-Rad® Cat # HSS9641) and magnetic rack compatible with tube selection.
- ☐ Centrifuge
- ☐ Instrument for DNA Fragmentation (e.g. Covaris® or Diagenode®) and consumables.
- ☐ Thermal cycler
- ☐ 8-well PCR Strip Tubes with Caps
- ☐ 8- or 12-channel 200 µL Multi-Channel Pipette
- ☐ Gel Electrophoresis System (e.g. Bioanalyzer®, TapeStation®, etc.)
- ☐ Deionized / Nuclease-free Water

4.4 Optimal read length, sequencing depth, and number of Arima Capture-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** and we recommend 10 million read-pairs per 1Mb of capture region for the **Arima Custom Capture Panel Tier 1-5**. 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 used the combined replicate Arima Capture-HiC dataset for high-resolution chromatin conformation analyses. The Arima Capture-HiC Bioinformatics Pipeline can be used to analyze shallow (0.5 – 2 million read-pairs) sequencing data for each Arima Capture-HiC library. This Pipeline will generate a number of QC metrics for assessing the quality of the Arima Capture-HiC libraries as well as providing a recommendation for the number of reads needed to sequence each library at a given resolution. 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 15 kb 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 HiC 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 ~100-200M read-pairs without reaching saturation. If >200M 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 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 manufacturers protocols”.

5. Crosslinking – Standard Input

Input: Cells collected from cell culture
Output: Crosslinked cells
Components:

- **Arima-HiC+ Kit box A**
  - **Stop Solution 1**
- **User Supplied Reagents**
  - **37% formaldehyde**
  - **1X PBS**

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.

5.1 Harvest cells from cell culture using standard protocols and pellet cells by centrifugation.

5.2 Resuspend in cell culture media, obtain a cell count by hemocytometer or automated cell counting methods.

5.3 Transfer 5-10 million cells to be crosslinked into a new 15mL conical tube, pellet cells by centrifugation and remove supernatant.

5.4 Resuspend cells in 5mL of RT **1X PBS**.

5.5 Add 286µL of **37% formaldehyde**, bringing the final formaldehyde concentration to 2%.

5.6 Mix well by inverting 10 times and incubate at RT for 10 min.

5.7 Add 460µL of **Stop Solution 1,** mix well by inverting 10 times and incubate at RT for 5 min.

5.8 Place sample on ice and incubate for 15 min.

5.9 Pellet cells by centrifugation.

5.10 Discard supernatant.

5.11 Resuspend cells in 5mL **1X PBS**.

5.12 Aliquot cells into several new tubes, with 1 x 10^6 cells per aliquot. Mix sample by inversion between aliquots to ensure all aliquots are equally homogeneous.

5.13 Pellet cells in all aliquots by centrifugation.

5.14 Discard supernatant leaving only the crosslinked cell pellet and no residual liquid.

5.15 Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the *Estimating Input Amount – Standard Input* protocol in a following section.

6. Estimating Input Amount – Standard Input

Input: Crosslinked cells
Output: Purified genomic DNA
Components:

- **Arima-HiC+ Kit box A**
  - **Buffer D**
  - **Buffer E**
  - **Elution Buffer**
- **Arima-HiC+ Kit box B**
  - **Enzyme D**
- **User Supplied Reagents**
  - **DNA Purification Beads**
  - **80% ethanol**
  - **Qubit® assay and 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. Arima Capture-HiC reactions are optimally performed on crosslinked cells comprising ~2.5µg-5µg of DNA. The *Estimating Input Amount* protocol measures the amount of DNA obtained per 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.

Note: Step 2 requires addition of several reagents in the same step. These reagents should be combined into master mixes with 10% excess volume before use.

6.1 Thaw one aliquot of 1 x 10^6 cells prepared during the *Crosslinking – Standard Input* protocol.

6.2 Add 209.5µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
Elution Buffer	174µL	191.4µL	x	2	=	382.8µL
● Buffer D	10.5µL	11.55µL	x	2	=	23.1µL
● Enzyme D	25µL	27.5µL	x	2	=	55µL
Total	**209.5µL**					460.9µL

6.3 Add 20µL of ● **Buffer E**, mix gently by pipetting, and incubate as follows. If using a thermal cycler, set the lid temperature to 85°C.

Temperature	Time
55°C	30 min.
68°C	90 min.
4°C	∞

Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Enrichment Beads or QC Beads provided in the Arima Capture-HiC kit.

6.4 Add 150µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.

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

6.6 Discard supernatant. While sample is still against magnet, add 400µL of **80% ethanol**, and incubate at RT for 1 min.

6.7 Discard supernatant. While sample is still against magnet, add 400µL of **80% ethanol**, and incubate at RT for 1 min.

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

6.9 Remove sample from magnet, resuspend beads thoroughly in 20µL of **Elution Buffer**, and incubate at RT for 5 min.

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

6.11 Quantify sample using Qubit®. The total DNA yield corresponds to the amount of DNA obtained from 1 x 10^6 mammalian cells.

6.12 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 1 x 10^6 mammalian cells* as calculated in step 6.11, one can estimate that *at least* 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 3µg 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

occupies no more than 20µL of volume. Samples split over multiple HiC reactions can be pooled after completion of the HiC protocol and prior to library preparation on page 18. Contact Technical Support for additional guidance.

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

7. Arima-HiC Protocol

Input: Crosslinked cells containing ~2.5µg-5µg of DNA
Output: Proximally-ligated DNA
Components:

- Arima-HiC+ Kit box A
  - Conditioning Solution
  - Stop Solution 2
  - Buffer D
  - Buffer E
  - Elution Buffer
- Arima-HiC+ Kit box B
  - Lysis Buffer
  - Buffer A
  - Enzyme A1
  - Enzyme A2
  - Buffer B
  - Enzyme B
  - Buffer C
  - Enzyme C
  - Enzyme D
- User Supplied Reagents
  - DNA Purification Beads
  - 80% ethanol
  - Qubit® assay and tubes

Before you begin: The cell pellet for one Arima Capture-HiC reaction should occupy no more than 20µL of volume and should be devoid of any residual liquid. If the cell pellet occupies greater than 20µL of volume, aliquot the cells such that the sum of the DNA input from all reactions is between 2.5µg-5µg and each cell pellet occupies no more than 20µL of volume or contact Technical Support for additional guidance. Note that steps 7.2 – 7.3 require consecutive heated incubations. Make sure your thermal device(s) are set to 62°C and 37°C for these incubations. The safe stopping point in this section is after completing Step 7.21.

Note: Choose to perform either Step 7.1a if the input sample type is crosslinked cells, or Step 7.1b only if the input sample type is crosslinked *nuclei* that have been previously purified from cells.

7.1a Resuspend one reaction of crosslinked cells in 20µL of ● **Lysis Buffer** in a tube or a well of a PCR plate, and incubate at 4°C for 15 min.

7.1b Resuspend one reaction of purified crosslinked nuclei in 20µL of **Water** in a tube or a well of a PCR plate and procced to the next step.

7.2 Add 24µL of ○ **Conditioning Solution**, mix gently by pipetting, and incubate at 62°C for 10 min. If using a thermal cycler, set the lid temperature to 85°C.

7.3 Add 20µL of ● **Stop Solution 2**, mix gently by pipetting, and incubate at 37°C for 15 min. If using a thermal cycler, set the lid temperature to 85°C.

Note: Steps 7.4, 7.6, 7.8 and 7.10 require addition of several reagents in the same step. These reagents should be combined into master mixes following the master mix tables.

7.4 Add 12µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
○ Buffer A	7µL	7.7µL	x	2	=	15.4µL
○ Enzyme A1	1µL	1.1µL	x	2	=	2.2µL
○ Enzyme A2	4µL	4.4µL	x	2	=	8.8µL
Total	**12µL**					26.4µL

7.5 Mix gently by pipetting and incubate as follows. If using a thermal cycler, set the lid temperature to 85°C. Note that there are sequential incubations at different temperatures:

Temperature	Time
37°C	30 min.*
65°C	20 min.
25°C	10 min.

* Optimal performance can be achieved with incubation durations between 30 and 60min.

7.6 Add 16µL of a master mix containing the following reagents:

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

7.7 Mix gently by pipetting, and incubate at room temperature (RT) for 45 min.

7.8 Add 82µL of a master mix containing the following reagents:

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

7.9 Mix gently by pipetting, and incubate at RT for 15 min.

Note: Enzyme D should be warmed to 37°C - 42°C for 5-10 min. to prevent precipitation in the below master mix.

7.10 Add 35.5µL of a master mix containing the following reagents:

Reagent	Volume per reaction	10% extra		# reactions		Final
● Buffer D	10.5µL	11.55µL	x	2	=	23.1µL
● Enzyme D	25µL	27.5µL	x	2	=	55µL
Total	**35.5µL**					78.1µL

7.11 Add 20µL of ● **Buffer E**, mix gently by pipetting, and incubate as follows. If using a thermal cycler, set the lid temperature to 85°C.

Temperature	Time
55°C	30 min.
68°C	90 min.
25°C*	10 min.*

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

Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Arima-HiC+ Enrichment Beads, Arima-HiC+ QC Beads, the T1 beads from the Arima Capture Modules, nor the optional Dynabeads™ MyOne™ Streptavidin T1 which are also a *user-supplied reagent*.

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

7.13 Place sample against magnet and incubate until solution is clear.

7.14 Discard supernatant. While sample is still against magnet, add 300µL of 80% ethanol, and incubate at RT for 1 min.

7.15 Discard supernatant. While sample is still against magnet, add 300µL of 80% ethanol, and incubate at RT for 1 min.

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

7.17 Remove sample from magnet, resuspend beads thoroughly in 100µL of **Elution Buffer**, and incubate at RT for 5 min.

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

7.19 Quantify sample using Qubit®. Record this value in the **Arima Capture-HiC QC Worksheet** (A160501) in the "Arima Assay QC" tab in the "Qubit Concentration" column under "Ligation QC Samples" section.

Note: If the proximally-ligated DNA yield is less than 275ng, we recommend skipping the Arima-QC1 assay mentioned in Step 7.20 and described in the following *Arima-QC1 Quality Control* section and proceeding to the Arima Library Preparation Protocol Below. If less than 275ng of DNA is used for library preparation then it is recommended to repeat the Arima-HiC protocol using additional material and to pool the HiC reactions together.

Note: Separate HiC reactions for the same biological sample can be pooled at this point.

7.20 Transfer 75ng of sample into a new tube labelled "Arima-QC1**" and** add **Elution Buffer** to Arima-QC1 to bring the volume to 50µL. The "Arima-QC1" sample should now contain 75ng of proximally-ligated DNA in 50µL of **Elution Buffer**. Store at -20°C until use in the following *Arima-QC1 Quality Control* protocol.

7.21 Store all remaining samples at -20°C until ready to proceed to library preparation following an accompanying *Arima Capture-HiC Library Preparation* user guide**.**

8. Arima-QC1 Quality Control

Input: Proximally-ligated DNA
Output: Measurement of Digestion and Biotinylation Efficiency
Components:

- **Arima-HiC+ Kit box A**
  - **Wash Buffer**
  - **Elution Buffer**
- **Arima-HiC+ Kit box C**
  - **QC Beads**
- **User Supplied**
  - **Qubit® assay and tubes**

Before you begin: The following protocol quantifies the fraction of proximally-ligated DNA that has been labeled with biotin, and is a quality control metric after completing the *Arima Capture-HiC Protocol* but before proceeding to library preparation. The *Arima-QC1 Quality Control* protocol involves using **QC Beads** to enrich an aliquot of proximally-ligated DNA, which is then quantified using a Qubit® fluorometer. Unlike standard Qubit® readings which involve quantifying a transparent unobstructed DNA sample, the Arima-QC1 value is obtained by quantifying DNA that is still bound to the **QC Beads**. This protocol can be performed in either plates or tubes. Set your thermal device (thermal cycler or thermomixer) to hold at 55°C. After completing the *Arima-QC1 Quality Control* protocol, use the provided **Arima Capture-HiC QC Worksheet** to determine the Arima-QC1 values.

8.1 If necessary, thaw the "Arima-QC1" samples prepared during Step 7.20 of the *Arima Capture-HiC Protocol* in the previous section.

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

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

8.4 Discard supernatant and remove sample from magnet.

8.5 Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.

8.6 Place sample against magnet and incubate until solution is clear.

8.7 Discard supernatant and remove sample from magnet.

8.8 Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.

8.9 Place sample against magnet and incubate until solution is clear.

8.10 Discard supernatant and remove sample from magnet.

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

8.12 Place sample against magnet and incubate until solution is clear.

8.13 Discard supernatant and remove sample from magnet.

8.14 Resuspend beads in 7µL of **Elution Buffer.** Proceed to next step with resuspended beads.

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

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

8.16 Determine the **Arima-QC1** value by following the **Arima Capture-HiC QC Worksheet**. High quality Arima-QC1 values are expected to be >15%. If the Arima-QC1 value did not obtain a 'PASS' status, please contact Technical Support for troubleshooting assistance.

9. Arima Library Preparation Protocol

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

- **Arima-HiC+, Box A** (RT)
  - **Elution Buffer**
  - **Wash Buffer**
- **Arima Library Prep Module Box A** (-20°C)
  - **End Repair-A Tailing Enzyme Mix**
  - **End Repair-A Tailing Buffer**
  - **T4 DNA Ligase**
  - **Ligation Buffer**
  - **Adaptor Oligo Mix**
  - **5X Herculase II Buffer with dNTPs**
  - **Herculase II Fusion DNA Polymerase**
- **Arima Library Prep Module Box B** (-20°C)
  - **Index Primer Pair 1 – 16**

- **Arima Capture Module Box A (RT)**
  - **Binding Buffer**

- **Arima Capture Module Box C (4°C)**
  - **Streptavidin Beads (T1)**
- **User Supplied Reagents**
  - **DNA Purification Beads**
  - **80% ethanol**
  - **Qubit® assay and tubes**

Overview: Library preparation begins with DNA fragmentation (Section 1), DNA size selection (Section 2), and biotin enrichment (Section 3). Afterwards, the Arima Library Prep Module reagents are used in a custom end-repair, dA-tailing and adapter ligation protocol (Section 4). This custom *Arima Library Preparation Protocol* constructs libraries while DNA is bound to Streptavidin Beads (T1). The final step is PCR amplification of the bead-bound Arima-HiC library using the library amplification reagents and index PCR primers from the Arima Library Prep Module, producing the final sequencing and capture-ready Arima-HiC library.

9.1 DNA Fragmentation

Before you begin: 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. 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 130µL, but 100µL must be used for DNA fragmentation in the Arima-HiC library preparation protocol. It is recommended to fragment 1,500ng of DNA per sample, or up to 5µg (depending on the DNA fragmentation instrument manufacturer recommendations). For certain applications, less than 750ng of DNA could be used.

9.1.1 If necessary, add **Elution Buffer** to bring the sample volume to 100µL. Do not exceed 100µL of volume for DNA fragmentation.

9.1.2 Fragment DNA to obtain an average fragment size of 400bp. *Please use the DNA fragmentation instrument manufacturer default settings for obtaining a target fragment size of 400bp.* For example, Covaris® publishes optimal DNA fragmentation Power, Duty Factor, Cycles per Burst, and Time for obtaining a target fragment size of 400bp.

9.1.3 Samples may be stored at -20°C for up to 3 days.

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

9.2 DNA Size Selection

Before you begin: Fragmented DNA must be size-selected to have a size distribution between 200 – 600bp. This workflow can be performed in microfuge 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.

Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Enrichment Beads or QC Beads provided in the Arima-HiC kit. For the ethanol washes performed below, use sufficient 80% ethanol to fully submerge the magnetized beads.

9.2.1 Transfer fragmented DNA sample from fragmentation tube to either a microfuge tube, PCR tube, or PCR plate. If necessary, add **Elution Buffer** to bring sample volume to 100µL.

9.2.2 Add 60µL of **DNA Purification Beads**, mix thoroughly by pipetting, and incubate at RT for 5 min.

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

9.2.4 Transfer ~160uL *of supernatant* to a new sample tube or well of a PCR plate. Discard beads.

9.2.5 Add 40µL of **DNA Purification Beads** to the ~160µL of supernatant, mix thoroughly by pipetting, and incubate at RT for 5 min.

9.2.6 Place sample against magnet and incubate until solution is clear.

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

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

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

9.2.10 Remove the sample from magnet, resuspend beads in 30µL of **Elution Buffer**, and incubate at RT for 5 min.

9.2.11 Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new sample tube or well of a PCR plate.

9.2.12 Quantify sample using Qubit®. 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.

9.2.13 Samples may be stored at -20°C for up to 3 days.

9.3 Biotin Enrichment

Before you begin: This workflow can be performed in microfuge tubes as well as most PCR tubes and PCR plates. Ensure that your tubes or plates can hold up to 230µL of sample volume. Also ensure that you have a magnetic rack that fits your choice of sample tube/plate. Set your thermal device (thermal cycler or thermomixer) to hold at 55°C.

Note: Streptavidin Beads (T1) used directly below are from the Arima Capture Module. They should not be mistaken for and are NOT interchangeable with the Arima-HiC+ Enrichment Beads nor the Arima-HiC+ QC Beads. If proceeding to library prep without having an **Arima Promoter or Custom Capture Module** on hand, then Dynabeads™ MyOne™ Streptavidin T1 (Thermo Fisher Scientific Cat # 65601, 65602, 65603) can be used as a direct replacement for the **Streptavidin Beads (T1)** from the capture modules.

9.3.1 Add 12.5µL of **Streptavidin Beads (T1)** from the **Arima Capture Module Box C** into a well of a strip tube for each sample. **Note: These beads are NOT the Enrichment Beads that come with the Arima HiC+ kit.**

9.3.2 Wash the **Streptavidin Beads (T1)** beads in each tube by:

9.3.2.1 Add 200uL of **Binding Buffer.**

9.3.2.2 Mix by pipetting up and down 20 times, cap the tubes, and vortex at high speed for 5 - 10 seconds.

9.3.2.3 Place tubes against a magnet and incubate 5 minutes or until solution is clear.

9.3.2.4 Discard supernatant and remove the tube from magnet.

9.3.2.5 Repeat steps 9.3.2.1 – 9.3.2.4 two more times for a total of three washes.

9.3.3 Resuspend beads in 200uL of **Binding Buffer.**

9.3.4 Transfer exactly **200ng*** of size-selected DNA into a new microfuge tube, PCR tube, or well of a PCR plate. If necessary, add **Elution Buffer** to bring sample volume to 30µL.

* Biotin enrichment and subsequent library preparation has been optimized to deliver peak performance for DNA inputs of 200ng. Using 200ng of DNA input has been shown to build libraries with sufficient complexity for up to 200M read-pairs of sequence data.

9.3.5 Add 200µL of washed **Streptavidin Beads (T1)** in **Binding Buffer**, mix thoroughly by pipetting, and incubate at RT for 15 min.

9.3.6 Place sample against magnet and incubate until solution is clear.

9.3.7 Discard supernatant and remove sample from magnet.

9.3.8 Wash beads by resuspending in 200µL of **Wash Buffer (Arima HiC+, Box A)**, and incubate at 55°C for 2 min.

9.3.9 Place sample against magnet and incubate until solution is clear.

9.3.10 Discard supernatant and remove sample from magnet.

9.3.11 Wash beads by resuspending in 200µL of **Wash Buffer (Arima HiC+, Box A)**, and incubate at 55°C for 2 min.

9.3.12 Place sample against magnet and incubate until solution is clear.

9.3.13 Discard supernatant and remove sample from magnet.

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

9.3.15 Place sample against magnet and incubate until solution is clear.

9.3.16 Discard supernatant and remove sample from magnet.

9.3.17 Resuspend beads in 50µL of **Deionized Water**.

9.4 Library Preparation of Enriched HiC Ligation products

Before you begin: This workflow can be performed in microfuge 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. Set your thermal device (thermal cycler or thermomixer) to hold at 55°C.

9.4.1 End Repair

Reagent	Thaw Temp.	Mix	Cap
End Repair-A Tailing Buffer	On Ice	Vortex	Yellow
Ligation Buffer	On Ice	Vortex	Purple
End Repair-A Tailing Enzyme Mix	Ice Just Before Use	Inversion	Orange
T4 DNA Ligase	Ice Just Before Use	Inversion	Blue
Adaptor Oligo Mix	On Ice	Vortex	Clear

9.4.1.1 Thaw reagents and mix reagents according to the table above.

Note: Thaw ligation buffer 15 sec and vortex on high to make sure homogenous (buffer is highly viscous).

9.4.1.2 Prepare Ligation master mix to allow equilibration to room temperature before use (see table below, table includes 12.5% master mix overage for 8 reactions).

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

Note Ligation Master Mix will be used in Step 9.4.2.4 below.

9.4.1.3 Keep Ligation Master Mix at room temperature for 30 - 45 minutes before use.

9.4.1.4 Pre-Program thermal cycler for End Repair and dA-Tailing with the program below. Set reaction volume for 70µL, and the heated lid to 85°C.

Temperature	Time
20°C	15 min
72°C	15 min
4°C	Hold

9.4.1.5 Start the Program, then Pause allowing the heated lid to reach temperature while setting up reactions. Total run time is approx. 30 mins.

9.4.1.6 Vortex thawed vial of **End Repair-A Tailing Buffer** for 15 seconds - continue vortexing until no solids are observed.

9.4.1.7 Prepare **End Repair/dA-Tailing** master mix by combining reagents below, mix well and spin down.

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

9.4.1.8 Add 20uL of the **End Repair/dA-Tailing** master mix to each sample containing 50uL of Bead bound HiC library from Step 9.3.17 in the previous section. Mix well.

9.4.1.9 Briefly spin samples, immediately place on thermal cycler. Press play on the paused "End Repair" program to advance the thermal cycling program.

9.4.2 Adapter Ligation

9.4.2.1 Once thermal cycler has reached 4°C hold step, transfer samples to ice while preparing the ligation reaction.

9.4.2.2 Pre-Program the thermal cycler for the ligation step with the program below. Set the reaction volume to 100µL and the heated lid to 85°C.

Temperature	Time
20°C	30 min
4°C	Hold

9.4.2.3 Start the Program, then Pause allowing the heated lid to reach temperature while setting up reactions. Total time is approx. 30 mins.

9.4.2.4 Add 25µL of **Ligation** Master Mix, from step 9.4.1.3 above to the 70µL of bead bound, end repaired and dA-tailed HiC library. Mix well.

9.4.2.5 Add 5µL of **Adaptor Oligo Mix** to each sample. Mix well.

9.4.2.6 Briefly spin tubes with the bead-bound HiC library, Ligation master mix, and **Adaptor Oligo Mix**. Press play button to advance the "ligation" program.

9.4.2.7 After the "Ligation" program completes, remove the samples from the thermocycler and quick spin the tubes to remove any liquid from the caps.

9.4.2.8 Magnetize beads until liquid is clear. Remove and discard supernatant.

9.4.2.9 Resuspend beads in 200µL **Wash Buffer**. Mix by pipetting. Incubate at 55C for 2 min.

9.4.2.10 Magnetize beads until liquid is clear. Remove and discard supernatant.

9.4.2.11 Resuspend beads in 100µL **Elution Buffer.**

9.4.2.12 Magnetize beads until liquid is clear. Remove and discard supernatant.

9.4.2.13 Resuspend the beads in 34µL of **Deionized Water** and proceed immediately to Library Amplification below.

9.5 Amplification of Adaptor-Ligated HiC Library and Sample Indexing

9.5.1 Thaw and mix the reagents according to the table below and keep on ice. Thaw only the index primers needed for experiment to minimize freeze-thaw cycles.

Reagent	Thaw	Mix	Cap
Herculase II Fusion DNA Polymerase	Ice	Pipette	Red
5X Herculase II Buffer with dNTPs	RT	Vortex	Clear
Index Primer Pair 1 - 16	RT	Vortex	Clear

9.5.2 Determine the unique index pair assignment for each sample from the table below. **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.**

Primer Pair #	P7 Index	P5 Index
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

9.5.3 Pre-Program the thermal cycler (with heated lid on) with the program below. Start the program and immediately pause. Set reaction volume for 50µL, and the heated lid to 105°C. The PCR program below is for a total of 10 cycles so it will go to step 2 in the program 9 times.

Cycles	Temperature	Time
1 X	98°C	2 min.
10 X	98°C	30 sec.
	60°C	30 sec.
	72°C	1 min.
1 X	72°C	5 min.
1 X	4°C	Hold

9.5.4 Start the Program, then Pause allowing the heated lid to reach temperature while setting up reactions. Total time is approx. 27 mins.

9.5.5 Prepare appropriate volume of pre-captured PCR reaction mix in table below. Mix well.

Reagent	Volume per reaction	12.5% extra		# reactions		Final
5x Herculase II Buffer with dNTPs (clear cap)	10µL	11.25µL	x	8	=	90µL
Herculase II Fusion DNA Polymerase (red cap)	1µL	1.125µL	x	8	=	9µL
Total	**11µL**					99µL

9.5.6 Add 11µL of the PCR reaction mixture prepared from the table above to 34 µL of Adaptor Ligated Bead Bound HiC Library from Step 9.4.2.13.

9.5.7 Add 5uL of the appropriate, unique, **Primer Pair** to each sample. Make sure to take note of which index was used with each sample.

9.5.8 Place the PCR reaction in the preheated thermocycler at 98°C and press play to advance the program out of the 98°C hold.

9.6 Purify Amplified Library with DNA Purification Beads

Note: DNA Purification Beads (e.g. AMPure® XP Beads) should be warmed to RT and thoroughly mixed before use. The DNA Purification Beads are a *user-supplied reagent* and should not be mistaken for the Enrichment Beads or QC Beads provided in the Arima Capture-HiC kit.

9.6.1 Add 50 µL of **DNA Purification Beads** to each 50 µL Indexed sample. Mix well.
9.6.2 Incubate for 5 minutes at room temperature.
9.6.3 Place sample against magnet and incubate until solution is clear.
9.6.4 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
9.6.5 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
9.6.6 Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
9.6.7 Remove the sample from magnet, resuspend beads in 15µL of **Deionized Water**, and incubate at RT for 5 min.
9.6.8 Place sample against magnet and incubate until solution is clear.
9.6.9 Remove purified and complete HiC library and transfer to a fresh PCR strip tube.
9.6.10 Dilute 1 µL of sample in 9 µL of **Deionized Water** and Quantify sample using Qubit®. 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.
9.6.11 Run the Diluted sample from the previous step on a gel or other platform to determine the size distribution of the HiC library.
9.6.12 Samples may be stored at -20°C for up to 6 months.

10. Arima Capture-HiC Protocol

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

- **Arima-HiC+, Box A** (RT)
  - **Elution Buffer**
- **Arima Capture Module Box A** (RT)
  - **Binding Buffer**
  - **Wash Buffer 1**
  - **Wash Buffer 2**
- **Arima Capture Module Box B** (-20°C)
  - **Blocker Mix**
  - **RNase Block**
  - **Fast Hybridization Buffer**
  - **Herculase II Fusion DNA Polymerase**
  - **5x Herculase II Buffer with dNTPs**
  - **Post-Capture Primer Mix**
- **Arima Capture Module Box C** (4°C)
  - **Streptavidin Beads (T1)**
- **Arima Human or Mouse Promoter Panel or Arima Custom Panel Tier 1-5**
- **User Supplied Reagents**
  - **DNA Purification Beads**
  - **80% ethanol**
  - **Qubit® assay and tubes**

Overview: Capture enrichment of the Arima-HiC library begins with pre-clearing the Arima-HiC Library to remove any biotinylated HiC molecules that carry-over after amplification of the library. The pre-clearing step has been shown to be critical for high and reproducible on-target rate. Next the biotinylated probes are hybridized to the Arima-HiC Library. Streptavidin beads are used to capture the Arima-HiC library hybridized to the biotinylated probes. The beads are washed at 70°C to remove non-specifically bound molecules. **Note: 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.** The capture enrichment concludes with a second round of PCR to yield enough enriched library for deep sequencing.

10.1 Pre-clearing

Before you begin: This workflow can be performed in 1.5mL microfuge tubes. Strip tubes are not advised during this step since the volume of the of the reaction during the DNA purification is 460 µL.

10.1.1 Add 50µL of **Streptavidin Beads (T1)** to a clean 1.5 mL microfuge tube.

10.1.2 Wash the beads:

10.1.2.1 Add 200 µL of **Binding Buffer**

10.1.2.2 Mix by pipetting up and down 20 times AND cap the wells and vortex at high speed for 5 - 10 seconds

10.1.2.3 Put the tube into a magnetic separator device

10.1.2.4 Wait 5 minutes or until the solution is clear, then remove and discard the supernatant

10.1.2.5 Remove the tubes from the magnet

10.1.2.6 Repeat steps 10.1.2.1 – 10.1.2.5 two more times for a total of three washes

10.1.3 Resuspend beads in 200 µL of **Binding Buffer**

10.1.4 Re-suspend at ~1.5µg of the Arima-HiC Library from step 9.6.12 from the Arima Library Preparation Protocol above in a total volume of 30 µL of **Elution Buffer**. *Note 1.5µg of input is recommended as the input into preclearing because it will ensure 1µg of pre-cleared, pre-capture library which is essential for good library complexity. Less DNA can be put into the preclearing, down to 750ng, however, it is expected that the resulting, enriched libraries, may be under complex.*

10.1.5 Combine 30 µL of diluted Arima-HiC sample with 200 µL washed **Streptavidin Beads (T1)**.

10.1.6 Incubate it on a thermomixer 1400RPM for 30min at RT.

10.1.7 Place sample against magnet and incubate until solution is clear.

10.1.8 Remove supernatant and transfer to a new 1.5 mL tube.

10.1.9 Add 230 µL of **DNA Purification Beads** to each 230 µL pre-clearing sample. Mix well.

10.1.10 Incubate for 5 minutes at room temperature

10.1.11 Place sample against magnet and incubate until solution is clear.

10.1.12 Discard supernatant. While sample is still against magnet, add 700µL of **80% ethanol**, and incubate at RT for 1 min.

10.1.13 Discard supernatant. While sample is still against magnet, add 700µL of **80% ethanol**, and incubate at RT for 1 min.

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

10.1.15 Remove the sample from magnet, resuspend beads in **14µL** of **Deionized Water**, and incubate at RT for 5 min.

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

10.1.17 Remove purified and complete HiC library to a fresh PCR strip tube.
10.1.18 Dilute 1 µL of sample in 9 µL of **Deionized Water** and Quantify sample using Qubit®.
10.1.19 Run the Diluted sample on a gel or other platform for determine the size distribution of Nucleic Acids.
10.1.20 Pre-cleared Samples may be stored at -20°C for up to 6 Months.

10.2 Capture Enrichment - Hybridization

Before you begin: This workflow can be performed in microfuge 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. The recommended input into hybridization is 1µg of pre-cleared Arima-HiC library. Inputs as low as 500ng can be used but will result in lower library complexity.

10.2.1 Thaw Hybridization Reagents

Kit Component	Storage Location	Thawing	Mixing
Blocker Mix (blue cap)	Arima Capture Module, Box B, -20°C	Thaw on ice	Gentle Flick
RNase Block (purple cap)	Arima Capture Module, Box B, -20°	Thaw on ice	Gentle Flick
Fast Hybridization Buffer (bottle)	Arima Capture Module, Box B, -20°C	Thaw and keep at Room Temperature	Gentle Flick
Capture Panel	-80°C	Thaw on ice	Gentle Flick

10.2.2 Preprogram thermal cycler with the program pictured below and name "CHIC-HYB".

Segment	Segment Name	Cycles	Temperature	Time
1a	Denaturing	1 X	95°C	Hold
1b		1 X	95°C	5 min.
2		1 X	65°C	10 min.
3a		1 X	65°C	Hold
3b	Hybridization	1 X	65°C	1 min.
4		60 X	65°C	1 min.
			37°C	3 sec.
5		1 X	65°C	Hold

10.2.3 Start the program, allowing the block and heated lid to warm up.

10.2.4 Dilute 1µg of the Pre-Cleared Arima-HiC Library from step 10.1.20 above in a total volume of 12 µL of **Elution Buffer**.

10.2.5 Add 5µL **Blocker Mix** to the 1µg Arima-HiC library and vortex at high speed for 5 seconds to mix.

10.2.6 Quick spin to capture residual liquid

10.2.7 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.

10.2.8 Prepare 25% solution of **RNase Block** according to table below

Reagent	Volume per reaction	12.5% extra		# 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

10.2.9 Prepare Capture Library Hybridization Mix following steps 10.2.9.1, 10.2.9.2, **OR** 10.2.9.3 depending on panel size:

10.2.9.1 Prepare **Arima Human or Mouse Promoter Panel** in a clean 1.5mL microfuge tube at RT, according to the table below.

Reagent	Volume per reaction	12.5% extra		# reactions		Final
25% RNase Block solution (from step 10.2.8)	2µL	2.25µL	x	8	=	18µL
Arima Human or Mouse Promoter Panel	5µL	5.625µL	x	8	=	45µL
Fast Hybridization Buffer	6µL	6.75µL	x	8	=	54µL
Total	**13µL**					117µL

10.2.9.2 Prepare **Arima Custom Panel** (>3Mb total region size) in a clean 1.5mL microfuge tube at RT, according to the table below.

Reagent	Volume per reaction	12.5% extra		# reactions		Final
25% RNase Block solution (from step 10.2.8)	2µL	2.25µL	x	8	=	18µL
Arima Custom Panel (>3Mb total region size)	5µL	5.625µL	x	8	=	45µL
Fast Hybridization Buffer	6µL	6.75µL	x	8	=	54µL
Total	**13µL**					117µL

10.2.9.3 Prepare **Arima Custom Panel** (<3Mb total region size) in a clean 1.5mL microfuge tube at RT, according to the table below.

Reagent	Volume per reaction	12.5% extra		# reactions		Final
25% RNase Block solution (from step 10.2.8)	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
Deionized Water	3µL	3.375 µL	x	8	=	27 µL
Total	**13µL**					117µL

10.2.10 Vortex the Capture Library Hybridization Mix at high speed for 5 seconds and keep reagents at RT until use.

10.2.11 Wait for the thermal cycler to reach segment 3a of the "CHIC-HYB" program from above (65°C Hold).

10.2.12 With the cycler holding at 65°C and while keeping the Arima-HiC Library + Blocker samples in the thermal cycler, Add 13 µL of RT Capture Hybridization Mix from step 10.2.10 above to each well with denatured and blocked Arima-HiC Library. Each well now contains 30 µL of Arima-HiC library which will hybridize to the Arima Capture Probe Panel.

10.2.13 Mix by pipetting 10 times.

10.2.14 Seal wells adequately to prevent evaporation.

10.2.15 Press "skip step" on the thermal cycler to move the program "CHIC-HYB" out of the 65°C hold and into the hybridization segments. Total run time is approx. 1 hr. and 4 mins.

10.3 Capture Enrichment - Capture

10.3.1 Prepare the **Streptavidin Beads (T1)** 1 hour after starting the hybridization. Reagents need for this section are in the table below:

Kit Component	Storage Location	Thawing	Mixing
Binding Buffer	Arima Capture Module, Box A, RT	N/A	Inversion
Wash Buffer 1	Arima Capture Module, Box A, RT	N/A	Inversion
Wash Buffer 2	Arima Capture Module, Box A, RT	N/A	Inversion
Streptavidin Beads (T1)	Arima Capture Module, Box C, 4°C	N/A	Vortexing

10.3.2 Votex the **Streptavidin Beads (T1)** for 30-60 sec. to resuspend the beads so that the solution is homogeneous.

10.3.3 Add 50 µL resuspended **Streptavidin Beads (T1)** for each hybridization sample to wells of a fresh PCR strip tube.

10.3.4 Wash the **Streptavidin Beads (T1)** by:

10.3.4.1 Add 200 µL of **Binding Buffer**

10.3.4.2 Mix by pipetting up and down 20 times AND cap the wells and vortex at high speed for 5 - 10 seconds

10.3.4.3 Put the strip tube into a magnetic separator device

10.3.4.4 Wait 5 minutes or until the solution is clear, then remove and discard the supernatant

10.3.4.5 Repeat steps 10.3.4.1 – 10.3.4.4 two more times for a total of three washes

10.3.5 Resuspend beads in 200 µL of **Binding Buffer**

10.3.6 Wait for the hybridization step to complete and the thermal cycler to reach the 65°C hold step.

10.3.7 Immediately transfer the entire 30 µL volume of the Capture Hybridization Reaction, from step 2.15 above, into wells containing 200 µL of washed streptavidin beads.

10.3.8 Incubate the samples on a thermomixer, shaking at 1400RPM for 30min at RT. Proceed immediately to the next step.

Critical Step Below:

10.3.9 Pre-warm the 6x 200 µL aliquots 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.

10.3.10 Spin samples briefly.

10.3.11 Place samples on a magnetic separator to collect the beads.

10.3.12 Wait until the solution is clear then discard supernatant.

10.3.13 Resuspend in 200 µL **Wash Buffer 1**

10.3.14 Mix by pipette 10-15 times to fully resuspended the beads.

10.3.15 Place samples on a magnetic separator to collect the beads.

10.3.16 Wait until the solution is clear then discard supernatant.

10.3.17 Remove the samples from the magnetic separator and transfer to a rack at RT.

Critical Step Below:

10.3.18 Wash the beads with **Wash buffer 2**, using steps below. ***Note: It is extremely important to maintain 70°C during washing procedure and to vortex the samples.***

10.3.18.1 Resuspend the beads in 200 µL of 70°C prewarmed **wash buffer 2**, pipette until fully resuspended.

10.3.18.2 Seal the wells and then **vortex** at high speed for 8 seconds. Spin briefly DON'T pellet. MAKE SURE BEADS ARE IN SUSPENSION BEFORE PROCEEEDING.

10.3.18.3 Incubate samples for 5 minutes at 70°C in a thermal cycler with heated lid on.

10.3.18.4 Place strip tube on magnetic separator at RT.

10.3.18.5 Wait 1 min or until the solution is clear, then remove and discard supernatant.

10.3.18.6 Repeat steps 10.3.18.1 – 10.3.18.5 five more times for a total of 6 washes.

10.3.19 Verify that all wash buffer has been removed.

10.3.20 Add 25uL of **nuclease-free water** to each sample well.

10.3.21 Resuspend beads by pipetting up and down 8 times

10.3.22 Store samples on ice until they are amplified.

10.4 Capture Enrichment – Amplification

10.4.1 Thaw reagents from table below

Kit Component	Storage Location	Thawing	Mixing
Herculase II Fusion DNA Polymerase (red cap)	Arima Capture Module, Box B, -20°C	Ice	Pipette up and down 15-20 times
5x Herculase II Buffer with dNTPs (clear cap)	Arima Capture Module, Box B, -20°	Ice	Vortexing
Post-Capture Primer Mix (clear cap)	Arima Capture Module, Box B, -20°C	Ice	Vortexing

10.4.2 Preprogram thermal cycler with heated lid ON with program pictured below.

Cycles	Temperature	Time
1 X	98°C	2 min.
13 X	98°C	30 sec.
	60°C	30 sec.
	72°C	1 min.
1 X	72°C	5 min.
1 X	4°C	Hold

10.4.3 Start the PCR program, then immediately press pause button

10.4.4 Prepare appropriate amount of volume of PCR reaction mix as described in the table below

Reagent	Volume per reaction	12.5% extra		# reactions		Final
Nuclease-free water	13µL	14.625µL	x	8	=	117µL
5x Herculase II Buffer with dNTPs (clear cap)	10µL	11.25µL	x	8	=	90µL
Herculase II Fusion DNA Polymerase (red cap)	1µL	1.125µL	x	8	=	9µL
Post-Capture Primer Mix (clear cap)	1µL	1.125µL	x	8	=	9µL
Total	**25µL**					225µL

10.4.5 Add 25uL of PCR reaction mix prepared in Table above to each sample well containing 25uL of bead-bound target-enriched DNA
10.4.6 Mix the PCR reactions via pipette until homogenous. DO NOT SPIN at this step to avoid pelleting the beads.
10.4.7 Place on thermal cycler and press play. Total time is approx. 33 mins.
10.4.8 Spin the PCR reactions briefly (once PCR is complete).
10.4.9 Place on magnetic separator and transfer 50ul supernatant to fresh tubes for purification and discard the **Streptavidin Beads (T1)**.
10.4.10 Add 50 µL of **DNA Purification Beads** to each 50 µL Arima Capture Enriched Library.
10.4.11 Mix well by pipetting 10-15 times.
10.4.12 Incubate for 5 minutes at room temperature
10.4.13 Place sample against magnet, and incubate until solution is clear.
10.4.14 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
10.4.15 Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
10.4.16 Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
10.4.17 Remove the sample from magnet, resuspend beads in 25µL of **Deionized Water**, and incubate at RT for 5 min.
10.4.18 Place sample against magnet, and incubate until solution is clear.
10.4.19 Remove purified and complete HiC library to a fresh PCR strip tube.
10.4.20 Quantify 2µl of sample using **Qubit®**.
10.4.21 Run the sample on a gel or other platform for determining the size distribution of Nucleic Acids and ensure there are no primer dimers in the reaction.
10.4.22 Sequence the Arima Capture Enriched Library using the Illumina platform of choice.
10.4.23 Store the Arima Capture Enriched Library at -20°C for up to 6 Months.

11. Warranty and Contact Info

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