



Arima-HiC⁺ Kit

HiChIP Library Preparation using Swift Biosciences[®] Accel-NGS[®] 2S Plus DNA Library Kit

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U.S. Patent No. US 9,434,985 and 9,708,648 pertains to the use of this product.

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Revision History

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Getting Started

1.1 Kit Contents and Storage

- The Arima-HiC⁺ Kit contains the following reagents to be used during Library Preparation:

Kit Location	Reagent	Storage Temperature
Box A	Wash Buffer Elution Buffer	RT
Box C	Enrichment Beads	2 to 8°C

1.2 Handling and Preparation

- The majority of required reagents for the *Library Preparation* protocol are included in the Swift Biosciences[®] Accel-NGS[®] 2S Plus DNA Library Kit and one of the various Swift Biosciences[®] Indexing Kits. Other required reagents are either supplied in the Arima-HiC⁺ kit, or, listed below in Section 1.3.
- Safe stopping points are indicated where the option is available.
- The **Enrichment Beads** should be thoroughly mixed by pipetting or vortexing until homogeneous directly before use.
- The **Wash Buffer** and **Elution Buffer** should be kept at room temperature during handling and preparation.
- To maximize efficient use of enzyme reagents, thaw enzymes for at least 10 min. on ice, then mix by inversion (not vortexing) and spin down enzyme reagents prior to pipetting.

1.3 User-supplied reagents, consumables and equipment checklist

- Swift Biosciences[®] Accel-NGS[®] 2S Plus DNA Library Kit (Cat # 21024 or 21096)
- Swift Biosciences[®] Indexing Kit (Cat # 26148, 26248, 26396, 26596, 26696, 26796, 26896, or 269384)
- KAPA[®] Library Quantification Kit for Illumina[®] Platforms (e.g. KAPA[®] Cat # KK4824)
- KAPA[®] Library Amplification Kit (KAPA[®] Cat # KK2620 or KK2621)
- DNA Purification Beads (e.g. Beckman Coulter Cat # A63880)
- Qubit[®] Fluorometer, dsDNA HS Assay Kit and required consumables (e.g. Thermo Fisher Scientific Cat # 32851, 32856)
- Freshly prepared 80% Ethanol
- 1.7mL microcentrifuge tubes, PCR tubes or PCR plates
- Magnetic rack for 1.7mL microcentrifuge tubes, PCR tubes or PCR plates
- Thermal cycler
- Thermomixer (if doing some steps in 1.7mL microcentrifuge tubes)
- Gel Electrophoresis System (e.g. Bioanalyzer[®], TapeStation[®], FlashGel[™], etc.)

Library Preparation

Input: Immunoprecipitated Proximally-ligated DNA

Output: Bead-bound Arima-HiChIP library

Overview: Library preparation begins with Biotin Enrichment (Section 2.1). Afterwards, Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit and Swift Biosciences® Indexing Kit reagents are used in a custom end-repair and adapter ligation protocol (Section 2.2). This custom protocol contains specific modifications to the standard Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit protocol that must be performed. All buffers and enzymes provided by the Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit are used, and most additional required reagents are provided in the Arima-HiC⁺ kit. This custom *Library Preparation* protocol constructs libraries while DNA is bound to the **Enrichment Beads**. After the *Library Preparation* protocol, there is a recommended *Library Complexity Quality Control* checkpoint. Lastly, while the Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit includes PCR reagents, library amplification of the bead-bound Arima-HiChIP library must be performed using KAPA® library amplification reagents, which produces the final sequence-ready Arima-HiChIP library.

2.1 Biotin Enrichment

Before you begin: The output of the *Arima-HiChIP Protocol* is fragmented and biotin-labelled proximally-ligated DNA molecules that were bound to a protein of interest. These DNA fragments must be enriched for biotin-labelled fragments prior to the *Library Preparation* protocol. 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.

1. Add Elution Buffer to bring the sample volume to 100µL.
2. Add 100µL of **Enrichment Beads**, mix thoroughly by pipetting, incubate at RT for 15 min.
3. Place sample against magnet, and incubate until solution is clear.
4. Discard supernatant, and remove sample from magnet.
5. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant, and remove sample from magnet.
8. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
9. Place sample against magnet, and incubate until solution is clear.
10. Discard supernatant, and remove sample from magnet.
11. Wash beads by resuspending in 100µL of **Elution Buffer**.

12. Place sample against magnet, and incubate until solution is clear.
13. Discard supernatant, and remove sample from magnet.
14. Resuspend beads in 40µL of **Elution Buffer**.

2.2 End Repair and Adapter Ligation

Before you begin: This custom protocol *resembles* the standard Swift Biosciences® Accel-NGS® 2S Plus DNA Library Kit protocol but has been modified for library preparation while DNA is bound to the **Enrichment Beads**. The **Wash Buffer** and **Elution Buffer** provided in the Arima-HiC⁺ kit are used during this protocol. This protocol 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 150µL of sample volume. Select a uniquely indexed sequencing adapter from the Swift Biosciences® Indexing Kit for each sample.

Note: Steps 1, 14, 27 and 41 require the addition of buffer and enzyme reagents, which should be prepared as a master mix, spun down, and added in a single pipetting step.

1. Add 20µL of a master mix containing the following reagents to 40µL of bead-bound, biotin-enriched DNA:

Reagent	Volume per reaction	5% extra		# reactions		Final
Low EDTA TE	13µL	13.65µL	x	2	=	27.3µL
Buffer W1	6µL	6.3µL	x	2	=	12.6µL
Enzyme W2	1µL	1.05µL	x	2	=	2.1µL
Total	20µL					42µL

2. Mix thoroughly by pipetting until homogeneous, and incubate at 37°C for 10 min. in a thermal cycler with the heated lid turned OFF. Once completed, immediately proceed to the next step.

Note: Steps 5 and 8 require incubations at 55°C. Set your thermal device to hold at 55°C.

3. Place sample against magnet, and incubate until solution is clear.
4. Discard supernatant, and remove sample from magnet.
5. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant, and remove sample from magnet.
8. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
9. Place sample against magnet, and incubate until solution is clear.
10. Discard supernatant, and remove sample from magnet.

11. Wash beads by resuspending in 100µL of **Elution Buffer**.
12. Place sample against magnet, and incubate until solution is clear.
13. Discard supernatant, and remove sample from magnet.
14. Resuspend beads in 50µL of a master mix containing the following reagents:

Reagent	Volume per reaction	5% extra		# reactions		Final
Low EDTA TE	30µL	31.5µL	x	2	=	63µL
Buffer G1	5µL	5.25µL	x	2	=	10.5µL
Reagent G2	13µL	13.65µL	x	2	=	27.3µL
Enzyme G3	1µL	1.05µL	x	2	=	2.1µL
Enzyme G4	1µL	1.05µL	x	2	=	2.1µL
Total	50µL					105µL

15. Mix thoroughly by pipetting until homogeneous, and incubate at 20°C for 20 min. in a thermal cycler with the heated lid turned OFF. Once completed, immediately proceed to the next step.

Note: Steps 18 and 21 require incubations at 55°C. Set your thermal device to hold at 55°C.

16. Place sample against magnet, and incubate until solution is clear.
17. Discard supernatant, and remove sample from magnet.
18. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
19. Place sample against magnet, and incubate until solution is clear.
20. Discard supernatant, and remove sample from magnet.
21. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
22. Place sample against magnet, and incubate until solution is clear.
23. Discard supernatant, and remove sample from magnet.
24. Wash beads by resuspending in 100µL of **Elution Buffer**.
25. Place sample against magnet, and incubate until solution is clear.
26. Discard supernatant, and remove sample from magnet.
27. Resuspend beads in 25µL of a master mix containing the following reagents:

Reagent	Volume per reaction	5% extra		# reactions		Final
Low EDTA TE	20µL	21µL	x	2	=	42µL
Buffer Y1	3µL	3.15µL	x	2	=	6.3µL
Enzyme Y3	2µL	2.1µL	x	2	=	4.2µL
Total	25µL					52.5µL

28. Add 5µL of a uniquely indexed **Reagent Y2** and mix thoroughly by pipetting. **Reagent Y2** is included in the Swift Biosciences® Indexing Kit. Record the index added to each sample.
29. Incubate at 25°C for 15 min. in a thermal cycler with the heated lid turned OFF. Once completed, immediately proceed to the next step.

Note: Steps 32 and 35 require incubations at 55°C. Set your thermal device to hold at 55°C.

30. Place sample against magnet, and incubate until solution is clear.
31. Discard supernatant, and remove sample from magnet.
32. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
33. Place sample against magnet, and incubate until solution is clear.
34. Discard supernatant, and remove sample from magnet.
35. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
36. Place sample against magnet, and incubate until solution is clear.
37. Discard supernatant, and remove sample from magnet.
38. Wash beads by resuspending in 100µL of **Elution Buffer**.
39. Place sample against magnet, and incubate until solution is clear.
40. Discard supernatant, and remove sample from magnet.
41. Resuspend beads in 50µL of a master mix containing the following reagents:

Reagent	Volume per reaction	5% extra		# reactions		Final
Low EDTA TE	30µL	31.5µL	x	2	=	63µL
Buffer B1	5µL	5.25µL	x	2	=	10.5µL
Reagent B2*	2µL	2.1µL	x	2	=	4.2µL
Reagent B3	9µL	9.45µL	x	2	=	18.9µL
Enzyme B4	1µL	1.05µL	x	2	=	2.1µL
Enzyme B5	2µL	2.1µL	x	2	=	4.2µL
Enzyme B6	1µL	1.05µL	x	2	=	2.1µL
Total	50µL					105µL

* **Reagent B2**, a non-indexed universal P5 adapter, is included in the Swift Biosciences® Indexing Kit.

42. Mix thoroughly by pipetting until homogeneous, and incubate at 40°C for 10 min. in a thermal cycler with the heated lid turned OFF. Once completed, immediately proceed to the next step.

Note: Steps 45 and 48 require incubations at 55°C. Set your thermal device to hold at 55°C.

43. Place sample against magnet, and incubate until solution is clear.
44. Discard supernatant, and remove sample from magnet.
45. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
46. Place sample against magnet, and incubate until solution is clear.
47. Discard supernatant, and remove sample from magnet.
48. Wash beads by resuspending in 150µL of **Wash Buffer**, and incubate at 55°C for 2 min.
49. Place sample against magnet, and incubate until solution is clear.
50. Discard supernatant, and remove sample from magnet.
51. Wash beads by resuspending in 100µL of **Elution Buffer**.
52. Place sample against magnet, and incubate until solution is clear.
53. Discard supernatant, and remove sample from magnet.
54. Resuspend beads in 22µL of **Elution Buffer**.
55. Samples may be stored at 4°C for up to 3 days.

Library Complexity Quality Control

Before you begin: The following protocol utilizes the KAPA® Library Quantification Kit (qPCR assay) to determine the appropriate number of PCR cycles needed for library amplification. After completing the qPCR assay, use the provided **Arima-HiChIP QC Worksheet** to determine the PCR cycle numbers.

Note: Step 2 requires the addition of buffer and enzyme reagents, which should be prepared as a master mix and added in a single pipetting step.

1. Prepare a 1:1000 dilution of each bead-bound Arima-HiChIP library. To do this, first vigorously mix a bead-bound Arima-HiChIP library by vortexing and pipetting until homogeneous. Then, immediately add 1µL of bead-bound library to 999 µL of water.
2. Add 16µL of a master mix containing the following reagents to each well of the qPCR plate that will receive either standards, water, or samples (see Step 3 for recommended plate layout):

Reagent	Volume per reaction	10% extra		# reactions		Final
qPCR Master Mix	10µL	11µL	x	27	=	297µL
Illumina® Primer Mix	2µL	2.2µL	x	27	=	59.4µL
Water	4µL	4.4µL	x	27	=	118.8µL
Total	16µL					475.2µL

3. Add 4µL of each Standard, 1:1000 diluted bead-bound Arima-HiChIP library, or water to each well containing 16µL of master mix prepared during Step 2. Use the same water source as was used to prepare the qPCR Master Mix. A suggested plate layout is provided below:

Recommended Plate Layout											
Std_1	Std_1	Std_1	Std_2	Std_2	Std_2	Std_3	Std_3	Std_3	Std_4	Std_4	Std_4
Std_5	Std_5	Std_5	Std_6	Std_6	Std_6	H ₂ O	H ₂ O	H ₂ O	Lib1	Lib1	Lib1
Lib2	Lib2	Lib2	Lib3	Lib3	Lib3	Lib4	Lib4	Lib4	Lib5	Lib5	Lib5
Lib6	Lib6	Lib6	Lib7	Lib7	Lib7	Lib8	Lib8	Lib8			

Note: "Std" refers to the qPCR Standards provided in the KAPA® Library Quantification Kit. "Lib" refers to each 1:1000 diluted, bead-bound Arima-HiChIP library.

4. Run the following qPCR cycling protocol:

Cycles	Temperature	Time
1 X	95°C	5 min.
35X	95°C	30 sec.
	60°C	45 sec.
Melt	65°C - 95°C	-

5. Extract C_q values from the qPCR instrument and follow the **Arima-HiChIP QC Worksheet** to calculate the required number of PCR cycles for library amplification.

Library Amplification

Before you begin: The following *Library Amplification* protocol utilizes the PCR reagents included in the KAPA® Library Amplification Kit. Determining how many PCR cycles to use for library amplification can be done in 1 of 2 ways – (1) determine the required PCR cycles empirically using the *Library Complexity Quality Control* protocol in the previous section, or (2) use the general guideline that 12 cycles of PCR should be sufficient to obtain enough library for DNA sequencing as long as >10ng of DNA was used as input in the *Biotin Enrichment* section. If the *Library Complexity Quality Control* protocol was not performed and the input into biotin enrichment was <10ng, please contact Technical Support for further guidance.

Note: Step 1 requires the addition of buffer and enzyme reagents, which should be prepared as a master mix and added in a single pipetting step.

1. Add 30µL of a master mix containing the following reagents to 20µL of bead-bound Arima-HiChIP library:

Reagent	Volume per reaction	10% extra		# reactions	=	Final
2X HiFi HotStart Ready Mix	25µL	27.5µL	x	2	=	55µL
10X Primer Mix	5µL	5.5µL	x	2	=	11µL
Total	30µL					66µL

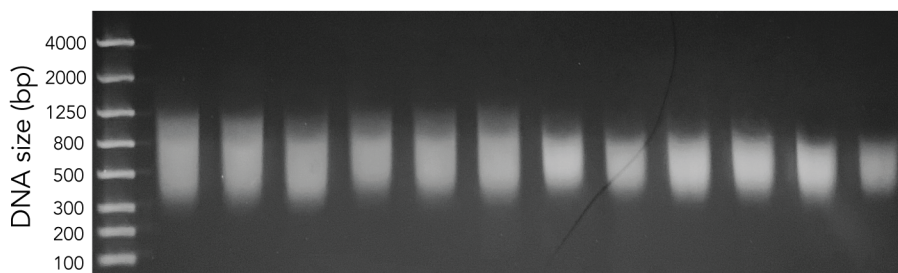
2. Mix thoroughly by pipetting until homogeneous, and run the following PCR program in a thermal cycler with the lid temperature set to 105°C:

Cycles	Temperature	Time
1 X	98°C	45 sec.
User-defined (e.g. 10 X)	98°C	15 sec.
	60°C	30 sec.
	72°C	30 sec.
1 X	72°C	60 sec.
1 X	10°C	Hold

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.

3. Add 35µL of **DNA Purification Beads**, mix thoroughly, and incubate at RT for 5 min.
4. Place sample against magnet, and incubate until solution is clear.
5. Discard supernatant. While sample is still against magnet, add 150µL of **80% ethanol**, and incubate at RT for 1 min.

6. Discard supernatant. While sample is still against magnet, add 150µL of **80% ethanol**, and incubate at RT for 1 min.
7. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
8. Remove the sample from magnet, resuspend beads in 20µL of **Elution Buffer**, and incubate at RT for 5 min.
9. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
10. Quantify sample using Qubit®.
11. Store Arima-HiChIP libraries at -20°C until standard library QC (gel electrophoresis, qPCR) and sequencing. Exemplary gel electrophoresis results from 12 Arima-HiChIP reactions run on the FlashGel™ system are below.



Warranty and Contact Info

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