User Guide

Arima-HiC Kit

Library Preparation using Active Motif® Next Gen DNA Library Kit

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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 **Document Part Number:** A160136 v00	November 2018	Initial Release

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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 Active Motif® Next Gen DNA Library Kit and Active Motif® Next Gen Indexing Kit. 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

- ☐ Active Motif® Next Gen DNA Library Kit (Active Motif® Cat # 53216)
- ☐ Active Motif® Next Gen Indexing Kit (Active Motif® Cat # 53264)
- ☐ 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
- ☐ Instrument for DNA Fragmentation (e.g. Covaris® or Diagenode®) and consumables.
- ☐ Thermal cycler
- ☐ Thermomixer (if doing some steps in 1.7mL microcentrifuge tubes)
- ☐ Gel Electrophoresis System (e.g. Bioanalyzer®, TapeStation®, etc.)

Input: Proximally-ligated DNA
Output: Bead-bound Arima-HiC library

Overview: Library preparation begins with DNA fragmentation (Section 2.1), DNA size selection (Section 2.2), and biotin enrichment (Section 2.3). Afterwards, Active Motif® Next Gen DNA Library Kit and Active Motif® Next Gen Indexing Kit reagents are used in a custom end-repair, dA-tailing and adapter ligation protocol (Section 2.4). This custom protocol contains specific modifications to the standard Active Motif® Next Gen DNA Library preparation protocol that must be performed. All buffers and enzymes provided by the Active Motif® Next Gen 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 Arima-QC2 Quality Control checkpoint. Lastly, while the Active Motif® Next Gen DNA Library Kit includes PCR reagents, library amplification of the bead-bound Arima-HiC library must be performed using KAPA® library amplification reagents, which produces the final sequence-ready Arima-HiC library.

2.1 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 at least 750ng 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.

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

2.2 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.

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.
2. Add 60µL of **DNA Purification Beads**, mix thoroughly by pipetting, and incubate at RT for 5 min.
3. Place sample against magnet, and incubate until solution is clear.
4. Transfer ~160uL *of supernatant* to a new sample tube or well of a PCR plate. Discard beads.
5. Add 40µL of **DNA Purification Beads** to the ~160µL of supernatant, mix thoroughly by pipetting, and incubate at RT for 5 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
8. Discard supernatant. While sample is still against magnet, add 200µL of **80% ethanol**, and incubate at RT for 1 min.
9. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
10. Remove the sample from magnet, resuspend beads in 100µL of **Elution Buffer**, and incubate at RT for 5 min.
11. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new sample tube or well of a PCR plate.
12. Quantify sample using Qubit®.
13. Samples may be stored at -20°C for up to 3 days.

2.3 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 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. Transfer **up to 2µg*** 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 100µL.

* Biotin enrichment and subsequent library preparation has been optimized to deliver peak performance for DNA input amounts up to 2µg. While input amounts closer to 2µg will result in more complex Arima-HiC libraries, input amounts of 125ng or less have been shown to build libraries with sufficient complexity for 600M read-pairs of sequence data. Please contact Technical Support for more information about low input library preparation.

2. Add 100µL of **Enrichment Beads**, mix thoroughly by pipetting, and incubate at RT for 15 min.
3. Place sample against magnet, and incubate until solution is clear.
4. Discard supernatant, and remove sample from magnet.
5. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
6. Place sample against magnet, and incubate until solution is clear.
7. Discard supernatant, and remove sample from magnet.
8. Wash beads by resuspending in 200µL of **Wash Buffer**, and incubate at 55°C for 2 min.
9. Place sample against magnet, and incubate until solution is clear.
10. Discard supernatant, and remove sample from magnet.
11. Wash beads by resuspending in 100µL of **Elution Buffer**.
12. Place sample against magnet, and incubate until solution is clear.
13. Discard supernatant, and remove sample from magnet.
14. Resuspend beads in 40µL of **Elution Buffer**.

2.4 End Repair and Adapter Ligation

Before you begin: This custom protocol *resembles* the standard Active Motif® Next Gen DNA Library preparation 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 Active Motif® Next Gen 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 **Active Motif® Next Gen 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-MID*	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-MID** is included in the Active Motif® Next Gen 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.

Arima-QC2 Quality Control

Before you begin: The following protocol utilizes the KAPA® Library Quantification Kit (qPCR assay) to determine the **Arima-QC2** values and estimate the appropriate number of PCR cycles needed for library amplification. After completing the qPCR assay, use the provided **Arima-HiC QC Worksheet** to determine the Arima-QC2 values and PCR cycle numbers. If performing Capture-HiC, please contact Technical Support for additional guidance.

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-HiC library. To do this, first vigorously mix a bead-bound Arima-HiC 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-HiC 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:

Arima-QC2 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_2O	H_2O	H_2O	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-HiC 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-HiC QC Worksheet** to calculate the Arima-QC2 values and estimate the required number of PCR cycles for library amplification. High-quality Arima-QC2 values are expected to be >0.2%. If Arima-QC1 and Arima-QC2 values both obtain a 'PASS' status, proceed to the *Library Amplification* protocol and subsequent sequencing analysis. If the Arima-QC2 value did not obtain a 'PASS' status, please contact Technical Support for troubleshooting assistance.

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 *Arima-QC2 Quality Control* protocol in the previous section, or (2) use the general guideline that 10 cycles of PCR should be sufficient to obtain enough library for DNA sequencing as long as >50ng of DNA was used as input in the *Biotin Enrichment* section. If the *Arima-QC2 Quality Control* protocol was not performed and the input into biotin enrichment was <50ng, please contact Technical Support for further guidance. Additionally, please contact Technical Support for recommended library amplification modifications for Capture-HiC.

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-HiC 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 45µL 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 50µL 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. Repeat Steps 3-7. Once Step 7 has been completed, proceed to Step 11 below.
11. Remove the sample from magnet, resuspend beads in 25µL of **Elution Buffer**, and incubate at RT for 5 min.
12. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
13. Quantify sample using Qubit®.
14. Store Arima-HiC libraries at -20°C until standard library QC (Bioanalyzer®, qPCR) and sequencing.

Warranty and Contact Info

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