USER GUIDE

Arima CiFi Kit

v1.0
8 reactions

Material Part Number: Arima CiFi Kit
Document Part Number: V1.0
Release Date: March 2026

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

This user manual must be read in advance of using the product and strictly followed by qualified and properly trained personnel to ensure proper use of the Arima CiFi 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.

TRADEMARKS

AMPure® is a trademark of Beckman Coulter, Inc.
Qubit® is a trademark of Molecular Probes, Inc.
SSIbio® is a trademark of Scientific Specialties, Inc.
Bio-Rad® is a trademark of Bio-Rad Laboratories, Inc.
ThermoFisher Scientific® is a trademark of ThermoFisher Scientific Company, LLC.
FlashGel® is a trademark of Lonza Inc.
FEMTO Pulse™ and TapeStation are trademarks of Agilent Inc.
SMRTbell® is a trademark of Pacific Biosciences of California, Inc.

Contact

Arima Genomics
6354 Corte Del Abeto, Suite B
Carlsbad, CA 92011
USA

arimagenomics.com

Revision Notes

Document Number	v1.0
Title	User Guide – Arima CiFi Kit
Revision	Initial Release
Revision Date	March 2026
Specific Changes	
General Changes	

Table of Contents

Getting Started

1. Handling and Preparation

- Several steps during the Arima CiFi 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 CiFi 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.
- Enzyme H1 should be stored at -80°C. If stored at -20°C, the enzyme will only retain full enzymatic activity for 3 months.
- All kit reagents should be fully thawed and thoroughly mixed before use.
- Conditioning Solution and Buffer D from Box A may contain precipitates. If present, these precipitates must be dissolved before use. Heating these reagents at 37°C for 5-15 min. may be necessary to dissolve precipitates.
- During handling and preparation, reagents from Box A should be kept at RT.
- During handling and preparation, reagents from Box B should be kept on ice, except for Enzyme D, which should be kept on ice but warmed to room temperature just before use.
- Enzyme solutions from Box B are viscous and require special attention during pipetting.

2. User-supplied reagents, consumables, and equipment checklist

- 37% Formaldehyde (e.g. ThermoFisher Scientific® Cat # F79-500)
- 100% Ethanol and freshly prepared 80% and 70% Ethanol
- Phenol:Chloroform:Isoamyl Alcohol (25:24:1 v/v; Thermo Fisher Cat # 15593031)
- Glycogen (20 µg/µL; ThermoFisher Cat # 10814010)
- 7.5M Ammonium Acetate (Sigma Cat # A2706-100ML)
- AMPure XP Beads® (Beckman Coulter Cat # A63880)
- 1X PBS, pH 7.4 (e.g. ThermoFisher Cat # 50-842-949)
- Qubit® Fluorometer, dsDNA HS Assay Kit and consumables (e.g. ThermoFisher Cat # 32851, 32856)
- 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
- Thermal cycler (if performing Arima CiFi Protocol in PCR tubes or PCR plate)
- Thermomixer (if performing *Arima CiFi Protocol* in 1.7mL microcentrifuge tubes)

Crosslinking – Standard Input

Input: Cells collected from cell culture
Output: Crosslinked cells

Before you begin

The *Arima CiFi Protocol* for mammalian cell lines begins with the harvesting and crosslinking of 5-10 million mammalian cells. If fewer than 1 million cells are available, please contact technical support for our *Crosslinking – Low Input* protocol. For plants and animal tissues, refer to the crosslinking protocol starting on page 14 in the following document: https://arimagenomics.com/wp-content/files/Arima-HiC-Kit-User-Guide-A160509-v2.pdf

The *Crosslinking – Standard Input* protocol below involves several cell pelleting centrifugations. During these centrifugations, pellet your specific cell types at a speed and duration as you normally would. Alternatively, we generally recommend centrifuging for 5 min at 500 x G.

1. Harvest cells from cell culture using standard protocols and pellet cells by centrifugation.
2. Resuspend in cell culture media, obtain a cell count by hemocytometer or automated cell counting methods.
3. Transfer 5-10 million cells to be crosslinked into a new 15mL conical tube, pellet cells by centrifugation and remove supernatant.
4. Resuspend cells in 5mL of RT 1X PBS.
5. Add 286µL of 37% formaldehyde, bringing the final formaldehyde concentration to 2%.
6. Mix well by inverting 10 times and incubate at RT for 10 min.
7. Add 460µL of Stop Solution 1, mix well by inverting 10 times and incubate at RT for 5 min.
8. Place sample on ice and incubate for 15 min.
9. Pellet cells by centrifugation.
10. Discard supernatant.
11. Resuspend cells in 5mL 1X PBS.
12. 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.
13. Pellet cells in all aliquots by centrifugation.
14. Discard supernatant leaving only the crosslinked cell pellet and no residual liquid.
15. Freeze samples on dry ice or liquid nitrogen, and store at -80°C until ready to proceed to the protocol in the following section.

Estimating Input Amount – Standard Input

Input: Crosslinked cells
Output: Purified genomic DNA

Before you begin

The *Estimating Input Amount* protocol is required if one does not know how many crosslinked cells will comprise ~500ng to 5µg of DNA, and if sufficient cells are available to perform this protocol. Arima CiFi reactions are optimally performed on crosslinked cells comprising ~500ng to 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 CiFi reaction. The Arima CiFi 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 CiFi 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.

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

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: AMPure XP Beads should be warmed to RT and thoroughly mixed before use. The AMPure XP Beads are a *user-supplied reagent*.

4. Add 150µL of AMPure XP Beads, mix thoroughly, and incubate at RT for 5 min.
5. Place sample against magnet, and incubate until solution is clear.
6. Discard supernatant. While sample is still against magnet, add 400µL of 80% ethanol, and incubate at RT for 1 min.
7. Discard supernatant. While sample is still against magnet, add 400µL of 80% ethanol, and incubate at RT for 1 min.
8. Discard supernatant. While sample is still against magnet, incubate beads at RT for 3 – 5 min. to air-dry the beads.
9. Remove sample from magnet, resuspend beads thoroughly in 20µL of Elution Buffer, and incubate at RT for 5 min.
10. Place sample against magnet, incubate until solution is clear, and transfer supernatant to a new tube.
11. Quantify sample using Qubit. The total DNA yield corresponds to the amount of DNA obtained from 1 x 10^6 mammalian cells.

12. Estimate how many mammalian cells to use per Arima-3C CiFi reaction. See the example description below:

    Example: In the following *Arima CiFi Protocol*, it is recommended to use crosslinked cells corresponding to ~500ng-5µg of DNA per Arima CiFi reaction. If 1.5µg of DNA was obtained per 1 x 10^6 *mammalian cells* as calculated in step 11, one can estimate that 2 x 10^6 crosslinked cells should be used per Arima CiFi reaction (~3µg of DNA). Additionally, please note that the crosslinked cell pellet for one Arima CiFi reaction should occupy no more than 20µL of volume in the sample tube. If the crosslinked cell pellet comprises ~500ng-5µg of DNA but occupies greater than 20µL of volume, aliquot the cells into multiple Arima CiFi reactions such that the sum of the DNA input from all reactions is ~500ng-5µg and each cell pellet occupies no more than 20µL of volume, or contact Technical Support for additional guidance.

    Recommended HiC Input Amount Explanation: The recommendation to use crosslinked cells comprising ~500ng-5µg of DNA is only a *general* recommendation. If cells are limited and less than 3µg of DNA can be obtained, one can proceed at risk with the *Arima CiFi Protocol* as described in this user guide, but with an expectation that there might not be sufficient DNA for Library Prep and sequencing.

Arima CiFi Protocol

Input: Crosslinked cells containing ~500ng-5µg of DNA
Output: Proximally ligated DNA

Before you begin

The cell pellet for one Arima CiFi 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 ~500ng-5µg and each cell pellet occupies no more than 20µL of volume, or contact Technical Support for additional guidance. Note that steps 2 – 3 require consecutive heated incubations at different temperatures. Make sure your thermal device(s) are set to 62°C and 37°C for these incubations. Also note that if the input sample type is crosslinked nuclei that have been previously purified from cells or tissues, please contact Technical Support.

1. 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.
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.
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 4, 7, and 9 require addition of several reagents in the same step. These reagents should be combined into master mixes following the master mix tables.

4. Add 28µL of a master mix containing the following reagents:

Reagent	Vol/Rxn	10% extra		# reactions		Final
Water	11µL	12.1µL	x	2	=	24.2µL
Buffer H	9µL	9.9µL	x	2	=	19.8µL
Enzyme H2	8µL	8.8µL	x	2	=	17µL
Total	28µL					61.6µL

Note: Of the two enzymes provided (H1 and H2), we recommend starting with enzyme H2 which employs the same cut site the CiFi method was optimized with. H1 can be used if users wish to explore an alternate cut site.

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	~18 hours
65°C	20 min.
16°C	10 min.

6. Mix gently by inversion, and then immediately transfer 10µL of sample into a new tube labelled "Digestion QC". Store the Digestion QC sample at -20°C until later use in the following Quality Control section, and proceed to the next step with the remaining sample.
7. Add 92µL of a master mix containing the following reagents:

Reagent	Vol/Rxn	10% extra		# reactions		Final
Water	10µL	11µL	x	2	=	22µL
Buffer C	70µL	77µL	x	2	=	154µL
Enzyme C	12µL	13.2µL	x	2	=	26.4µL
Total	92µL					202.4µL

8. Mix gently by pipetting, and incubate at 16°C for 6 hours.

Note: Enzyme D should be warmed to RT to prevent precipitation in the below master mix.

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

Reagent	Vol/Rxn	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

10. 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	~18 hours*
25°C	10 min.

* Use a thermal cycler with a heated lid to prevent condensation.

11. Proceed to the *DNA Purification – Phenol Chloroform* protocol in the next section.

DNA Purification – Phenol Chloroform

Input: Proximally ligated DNA
Output: Purified proximally ligated DNA

Note: The following protocol carries out a standard phenol chloroform extraction, followed by ethanol precipitation of DNA.

1. If the *Arima CiFi Protocol* was performed in PCR tubes or PCR plates, transfer the sample into a fresh 1.7mL microfuge tube.
2. Add 229µL of Phenol:Chloroform:Isoamyl Alcohol and then vortex for 30 sec. or invert 30-40 times.
3. Centrifuge at RT for 5 min. at 16,000 x G.
4. Carefully remove and transfer the upper aqueous phase to a fresh 1.7mL microfuge tube. Be careful not to carry over any phenol during pipetting.
5. Determine the sample volume using a pipette.
6. Add 1µL of Glycogen (20µg/µL) and mix by inversion.
7. Add 0.5X volumes of sample of 7.5M Ammonium Acetate and mix by inversion. The resulting mixture is called "Sample plus Ammonium Acetate". For example, if the sample volume was 200µL, then one would add 100µL of 7.5M Ammonium Acetate, and the resulting "Sample plus Ammonium Acetate" would be 300µL.
8. Add 2.5X volumes of Sample plus Ammonium Acetate of 100% Ethanol and mix by inversion. For example, if the Sample plus Ammonium Acetate was 300µL, then one would add 750µL of 100% Ethanol, and the resulting volume would be 1050µL.
9. Precipitate the DNA by freezing at -20°C for overnight incubation.
10. Centrifuge at 4°C for 30 min. at 16,000 x G. A DNA pellet should form at the base of the microfuge tube.
11. Carefully remove the liquid without disturbing the pellet.
12. Add 150µL of 70% Ethanol.
13. Centrifuge at 4°C for 2 min. at 16,000 x G.
14. Carefully remove the liquid without disturbing the pellet.
15. Add 150µL of 70% Ethanol.
16. Centrifuge at 4°C for 2 min. at 16,000 x G.
17. Carefully remove all the liquid without disturbing the pellet.
18. Open the microfuge tube cap and air-dry the pellet for 5-10 min.

19. Add 300µL of Elution Buffer and resuspend the DNA pellet by pipetting up and down 30-40 times.
20. Briefly centrifuge the sample and place on ice.
21. Quantify sample using Qubit.
22. Store the proximally ligated DNA samples at -20°C until ready to proceed to the Quality Control section below.
23. Complete HiFi SMRTbell CiFi Libraries following the guidance in McGinty et al 2025, CiFi: Accurate long-read chromatin conformation capture with low-input requirements, Nature Communications: https://www.nature.com/articles/s41467-025-66918-y
24. Note that before initiating SMRTbell library preparation, confirm that all user-supplied reagents listed in the most recent McGinty protocol have been purchased and are available, as specified in the PacBio procedure checklist: https://www.pacb.com/wp-content/uploads/Procedure-checklist-Amplifying-genomic-DNA-for-SMRTbell-library-preparation-and-HiFi-sequencing.pdf

Quality Control

Overview

In this section, quality control analyses are performed to assess the efficiency of Arima CiFi. First, chromatin from the Digestion QC collected during the *Arima CiFi Protocol* is reverse crosslinked, and the digested DNA is purified. Then, the size of the digested and purified proximally-ligated DNA is analyzed to determine the efficiency of the Arima CiFi digestion and ligation reactions, respectively.

Note: Step 3 requires addition of several reagents in the same step. These reagents should be combined into master mixes following the master mix tables.

1. Thaw the Digestion QC aliquot.
2. Add 90µL Elution Buffer to each Digestion QC aliquot, to bring the total volume to 100µL.

Note: Enzyme D should be warmed to RT to prevent precipitation in the below master mix.

3. Add 17.5µL of a master mix containing the following reagents:

Reagent	Vol/Rxn	10% extra		# reactions		Final
Buffer D	5µL	5.5µL	x	2	=	11µL
Enzyme D	12.5µL	13.8µL	x	2	=	27.6µL
Total	17.5µL					38.6µL

4. Add 10µL 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	90min.*
25°C	10 min.**

* Do not incubate at 68°C for longer than 90 min. unless doing so using a thermal cycler or thermomixer with a heated lid.

** To provide flexibility, this incubation can also be held overnight at 4°C, in which case, the sample may turn slightly opaque.

Note: AMPure XP Beads should be warmed to RT and thoroughly mixed before use. The AMPure XP Beads are a user-supplied reagent.

5. Add 125µL of AMPure XP Beads, mix thoroughly, 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 300µL of 80% ethanol, and incubate at RT for 1 min.
8. Discard supernatant. While sample is still against magnet, add 300µL 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 sample from magnet, resuspend beads thoroughly in 20µL 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 tube.
12. Quantify sample using Qubit. Use 2µL of DNA for the Qubit assay.
13. Analyze the DNA size of the digested DNA, and the purified proximally-ligated DNA output from the *Arima CiFi Protocol* in a previous section. Use gel electrophoresissystems such as a Bioanalyzer, TapeStation, FEMTO Pulse, or FlashGel.

Troubleshooting and Optimization Tips

Tip #1: The Conditioning Solution reaction in Step 2 of the *Arima CiFi Protocol* recommends a 10 min. incubation time for crosslinked cell inputs. Tuning the incubation time slightly up or down can influence the digestion efficiency and the molecular length of the proximally ligated DNA. For e.g. to increase the digestion efficiency and increase the number of long-range contacts, increase the Conditioning Solution reaction duration from 10 min. to 30 min.

Warranty and Contact Info

WARRANTY DISCLAIMERS

THE EXPRESS WARRANTIES AND THE REMEDIES SET FORTH ABOVE ARE IN LIEU OF, AND ARIMA GENOMICS AND ITS LICENSORS, SUPPLIERS AND REPRESENTATIVES HEREBY DISCLAIM, ALL OTHER REMEDIES AND WARRANTIES, EXPRESS, STATUTORY, IMPLIED, OR OTHERWISE, INCLUDING, BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY, SATISFACTORY QUALITY, NONINFRINGEMENT OR FITNESS FOR A PARTICULAR PURPOSE, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT OR SERVICE (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), IN EACH CASE HOWEVER ARISING, INCLUDING WITHOUT LIMITATION FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE, OR OTHERWISE. TO THE MAXIMUM EXTENT PERMITTED BY APPLICABLE LAW, ARIMA AND ITS LICENSORS, SUPPLIERS AND REPRESENTATIVES SHALL NOT BE LIABLE FOR LOSS OF USE, PROFITS, REVENUE, GOODWILL, BUSINESS OR OTHER FINANCIAL LOSS OR BUSINESS INTERUPTION, OR COSTS OF SUBSTITUTE GOODS OR SERVICES, OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, EXEMPLARY OR INDIRECT DAMAGES FOR BREACH OF WARRANTY.

WARRANTY

All warranties are personal to the Purchaser and may not be transferred or assigned to a third-party, including an affiliate of the Purchaser. The warranty described below excludes any stand-alone third-party goods that may be acquired or used with the Product. Arima Genomics only warrants that the kit reagents will be made and tested in accordance with Arima Genomics manufacturing and quality control processes. Arima Genomics makes no warranty that the reagents provided in this kit will work as intended by the Purchaser or for the Purchaser's intended uses. ARIMA GENOMICS MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data and descriptions of Arima Genomics products appearing in Arima Genomics product literature and website may not be altered except by express written agreement signed by an officer of Arima Genomics. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

The foregoing warranties do not apply to the extent a non-conformance is due to (i) abuse, misuse, neglect, negligence, accident, improper storage, or use contrary to the Documentation or Specifications, (ii) use that is an Excluded Use, (iii) improper handling, (iv) unauthorized alterations, (v) natural disasters, or (vi) use with a third-party's good that is not specified in the product documentation. In the event of a breach of the foregoing warranty, customer shall promptly contact Arima Genomics customer support to report the nonconformance and shall cooperate with Arima Genomics in confirming or diagnosing the non-conformance. Additionally, Arima Genomics may request return shipment of the non-conforming product at Arima Genomics cost. Arima Genomics sole obligation shall be to replace the applicable product or part thereof, provided the customer notifies Arima Genomics within 90 days of any such breach. If after exercising reasonable efforts, Arima Genomics is unable to replace the product, then Arima Genomics shall refund to the Purchaser all monies paid for such applicable product.

Contact Us

Technical Support:
techsupport@arimagenomics.com

Order Support:
ordersupport@arimagenomics.com