

ARIMA HiC FOR HIGH COVERAGE PROTOCOL: NUCLEATED BLOOD INPUT

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Adapted from Arima protocol modified by Michael Quail of the Wellcome-Sanger Institute, and from

Arima High Coverage HiC Kit User Guide for Nucleated Blood, Document Part Number: A160177 v01,

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INTRODUCTION

Arima HiC is an experimental workflow that captures the sequence and structure (three-dimensional conformation) of genomes. Arima-HiC has been successfully performed on a wide-range of species from the plant and animal kingdoms. 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 cocktail. The 5'-overhangs are then filled in, causing the digested ends to be labelled 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.

NOTES:

- Several steps during the *Arima-HiC Protocol* require preparation of a master mix. With the excess in the kit provided by Arima there is sufficient reagent to make master mixes with 10% excess volume. Use the master mix calculation tables provided.
- Pipette carefully/accurately and check your math! Especially when formaldehyde is involved.
- Incubations times should be exact, especially when formaldehyde is involved.
- Always pipette-mix when DNA is involved, don't vortex. **Best to pipette-mix with a wide orifice pipette tip. All resuspensions should be done with wide orifice pipette tips.**
- When handling reagents, room temperature (RT) = 20-25°C.
- All kit reagents should be fully thawed and thoroughly mixed before use.
- Stop Solution 1, Conditioning Solution, and Buffer D from Box A may contain precipitates. If present, these precipitates must be dissolved before use. Pre-heat these reagents at 37°C for 5-15 minutes to dissolve precipitates *if present*.
- Reagents from **Box A** should be **kept at RT** during handling and prep.
- 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.
- Use lo-bind tubes for all protocol master mixes to minimize wastage of reagents and sample.

Equipment		Consumables
<input type="checkbox"/> Heat block	<input type="checkbox"/> Thermal cycler	<input type="checkbox"/> 50, 15, and 5 mL tubes for aliquots
<input type="checkbox"/> Thermomixer	<input type="checkbox"/> Ice block racks for 1.5 mL and 0.2 mL tubes	<input type="checkbox"/> Nitrile gloves
<input type="checkbox"/> Cold centrifuge	<input type="checkbox"/> Magnet rack for 1.5 mL tubes	<input type="checkbox"/> P200 pipette tips, regular, extra-long, and wide orifice
<input type="checkbox"/> Accurate scale	<input type="checkbox"/> Magnet rack for 0.2 mL tubes	<input type="checkbox"/> P20 / p2 pipette tips, regular
<input type="checkbox"/> Fume hood	<input type="checkbox"/> Dewar for liquid nitrogen	<input type="checkbox"/> P1000 pipette tips, regular and wide orifice
<input type="checkbox"/> Sharps container	<input type="checkbox"/> Box for dry ice	<input type="checkbox"/> 2 mL, 1.5 mL Lo-bind tubes, pointed & round bottom
<input type="checkbox"/> Pipettes	<input type="checkbox"/> Long forceps for flash freezing	<input type="checkbox"/> PCR tubes (0.2 mL)
<input type="checkbox"/> Timer	<input type="checkbox"/> Qubit HS	<input type="checkbox"/> Qubit HS reagents and consumables
<input type="checkbox"/> Tube trays and racks	<input type="checkbox"/> Mini centrifuge (benchtop)	<input type="checkbox"/> 1.7 mL microcentrifuge tubes
<input type="checkbox"/> Refrigerator (4°C)	<input type="checkbox"/> Mini vortex (benchtop)	<input type="checkbox"/> Covaris sonicator consumables
<input type="checkbox"/> Freezer (-20°C)	<input type="checkbox"/> Covaris sonicator	<input type="checkbox"/> TapeStation consumables and reagents
<input type="checkbox"/> Deep freezer (-80°C)	<input type="checkbox"/> TapeStation	

ARIMA BOX A Stored at Room Temperature (20-25°C)					
Cap	Item	Category	μL per sample tube	Day	Notes
Bottle ☉	Stop Solution 1	Solution	272.4	1	
Bottle ☉	Elution Buffer	Buffer	357.1	1,2,3	
Bottle ☉	Wash Buffer	Buffer	400	3	
White ○	Conditioning Solution	Solution	24	2	
Black ●	Stop Solution 2	Solution	20	2	
Purple ●	Buffer D	Buffer	23.65	1,2	
Purple ●	Buffer E	Buffer	40	1,2	

ARIMA BOX B Stored in the Freezer (-20°C)					
Cap	Item	Category	μL per sample tube	Day	Notes
Red ●	Lysis Buffer	Buffer	20	2	Thaw and keep on ice
Yellow ●	Buffer F	Buffer	8.25	2	Thaw and keep on ice
Yellow ●	Enzyme A2	Enzyme	1.65	2	Thaw and keep on ice
Yellow ●	Enzyme F1	Enzyme	1.65	2	Thaw and keep on ice
Yellow ●	Enzyme F3	Enzyme	1.65	2	Thaw and keep on ice
Yellow ●	Enzyme F4	Enzyme	1.65	2	Thaw and keep on ice
Blue ●	Buffer C	Buffer	77	2	Long time to thaw, take out early!
Blue ●	Enzyme C	Enzyme	13.2	2	Thaw and keep on ice
Green ●	Buffer G	Buffer	13.2	2	Long time to thaw, take out early!
Green ●	Enzyme B	Enzyme	4.4	2	Thaw and keep on ice
Purple ●	Enzyme D	Enzyme	56.3	1,2	Thaw to RT. Long time to thaw!

ARIMA BOX C Stored in the Refrigerator (4°C)					
Cap	Item	Category	μL per sample tube	Day	Notes
Clear ☉	Enrichment Beads	Beads		4	
Brown ●	QC Beads	Beads	50	3	Used in Arima QC1. Store upright!

User-Supplied Reagents						
Stock conc.	Item	Temp.	Storage	μL per sample tube	Day	Notes
	Water	RT	Chemical shelf	Excess	1,2,3,4	
37%	Formaldehyde	RT	Fume hood	3028.6	1	Fixation, Double Fixation
1X	PBS, pH 7.4	RT	Chemical shelf	5000	2	Blood Prep, Double Fixation
	Fetal Bovine Serum (FBS)	-20	Freezer	A few μL	1	Blood Prep
1	EtOH	RT	Flammables cabinet	Excess	2,3	Bead cleaning
	Dry ice	-80°C	Deep freeze	Excess	1	Blood Prep, Double Fixation
	Liquid nitrogen	-80°C	LN2 Tank	Excess	1	Double Fixation
	DMSO (dimethyl sulfoxide)	RT	Chemical shelf	10	1	Double Fixation
	DSG (disuccinimidyl glutarate)	4°C	Refrigerator	One tube	1	Double Fixation
	DNA Purification Beads	RT	Above bench	250	2,3	
	Qubit HS Reagents	4°C	Refrigerator	Excess	2,3	

DAY 1: BLOOD PREPARATION AND CROSSLINKING

Before you begin: If you have flash-frozen blood, you can begin directly with the protocol below. If your blood is preserved in ethanol, you should first pellet the blood by centrifugation (2000 x G for 5 minutes at a time), remove the ethanol, and then proceed with the protocol.

The protocol assumes one knows how much whole blood (in μL) was originally collected and then preserved in ethanol. Less than 25 μL of whole nucleated blood (i.e. not the volume of the ethanol diluted blood) is typically needed for a single Arima High Coverage HiC reaction, but we recommend crosslinking 25 μL of whole nucleated blood if sufficient blood is available. For example if 50 μL of whole blood was collected in 1 mL of ethanol, then 500 μL of the ethanol diluted blood would be used for crosslinking because it contains the equivalent of 25 μL of whole nucleated blood.

Prep:

- ☐ Obtain dry ice.
- ☐ Ensure you have access to liquid nitrogen & a dewar.
- ☐ Pre-cool the centrifuge to 4°C.
- ☐ Pre-label six 1.5 mL LoBind tubes with sample ID for splitting up the sample at end of day. See steps 23 and 24 for labeling instructions.

Prepare Aliquots:

- ☐ FBS (fridge after thawing at RT)
- ☐ 1X PBS
- ☐ Formaldehyde (keep in hood)
- ☐ DMSO

BLOOD PREPARATION

- Input requirement is around 1 million cells. Keep frozen on dry ice until ready.
- Prepare fresh **Resuspension Buffer**. Store in the refrigerator (4°C) until use.

Reagent	Stock conc.	Final conc.	Stock volume
1X PBS			54.45 mL
Fetal Bovine Serum (FBS)		1%	550 μL
Total μL buffer mix			55 mL

- Log your sample information in the table below:

Date started	Sample ID	Species	Blood amount	Notes

- Remove sample tube from dry ice, add **1 mL Resuspension Buffer** and mix gently by inversion to resuspend the cell pellet.
- Add **57.2 μL of 37% formaldehyde stock**; this brings the final concentration of formaldehyde to 2%.
- ⌚ Mix sample well by inverting 10 times and incubate at RT for 10 minutes. Make sure the blood is moving in the solution so it's permeated by the reagents. When incubation is done, move immediately to the next step.
- Immediately add **92 μL Stop Solution 1** ⌚, mix well by inverting 10 times and incubate on ice for 15 minutes.
- Centrifuge at 2000 x G at RT for 5 minutes to pellet the sample.
- Discard supernatant carefully. If the sample dislodges during pipetting, pellet by centrifugation again and discard remaining supernatant.
- Add **1 mL 1X PBS** and resuspend the pellet.
- Proceed to double fixation. The double fixation step helps to ensure that you have adequately fixed the cells prior to the rest of the protocol. It is not specifically optimized for any particular input material type.

DOUBLE FIXATION

12. In the fume hood, add **28.6 μ L 37% formaldehyde** stock to each tube, to reach final formaldehyde concentration of 1%.
13. ⌚ Mix well by inverting 10 times and incubate at RT for 10 minutes.
➡ Prepare the DSG 🌱 while sample incubating ►►►
14. Add **53.6 μ L Stop Solution 1** ☹, mix well by inverting 10 times and incubate at RT for 5 minutes.
15. Wash sample with 1X PBS:
 - 1) Spin 500 x G at 4°C in the cold centrifuge for 4 minutes.
 - 2) Remove the supernatant carefully; use the long p200 tips and go down to p20 if needed.
 - 3) Add **1 mL 1X PBS**, gently pipette mix to resuspend cells.
 - 4) Spin 500 x G at 4°C in the cold centrifuge for 4 minutes.
 - 5) Remove the supernatant carefully.
16. Add **1 mL 1X PBS** and **10 μ L 0.3M DSG** 🌱 to the sample tube, and pipette-mix thoroughly to resuspend.
➡ Pipette-mix the DSG solution and draw up exactly 10 μ L, make sure there are no air bubbles in the pipette tip. Dispense all the DSG into the sample tube by pipette-mixing a few times.
17. ⌚ Incubate for 40 minutes with rotation at RT. Rotate at various angles, not just up and down.
➡ This is a good time to go eat lunch and obtain the liquid nitrogen. BUT don't leave before completing the next two steps.
18. Take out Enzyme D 🟣 to thaw and put on ice until right before *determining input concentration*.
19. Pre-heat thermomixer to 37°C.
20. Add **161 μ L Stop Solution 1** ☹, mix well by inverting 10 times and incubate at RT for 5 minutes.
21. Wash sample with 1X PBS:
 - 1) Spin 500 x G at 4°C in the cold centrifuge for 4 minutes.
 - 2) Remove the supernatant carefully.
 - 3) Add **1 mL 1X PBS**, gently pipette mix to resuspend the cells.
 - 4) Spin 500 x G at 4°C in the cold centrifuge for 4 minutes.
 - 5) Remove the supernatant carefully.
22. Resuspend pellet in **1 mL 1X PBS**.
23. Pipette-mix well and take 100 μ L aliquot (10%) for input QC. Place in the fridge while doing the next few steps.
24. Separate remaining sample into five 1.5 mL lo-bind tubes: four tubes with 200 μ L each, one tube with 100 μ L of sample. Label appropriately according to contents.
25. Spin 500 x G at 4°C in the cold centrifuge for 4 minutes.
26. Remove the supernatant carefully. Try to remove as much as possible without sample loss.
27. Flash freeze sample tubes and store at -80°C.

Prepare DSG 🌱: Use 1 DSG tube per sample. Spin down DSG tube; put the DSG tube inside of a capless 1.5 mL tube to allow it to fit for spinning down. You may need to use a razor blade to trim off the top few millimeters of the green DSG cap to make it fit in the mini benchtop centrifuge. Add **10 μ L DMSO** to the **DSG tube**. Slowly pipette the DMSO along the inside of the tube, let it go all the way to the bottom, recap it, flick it a few times, then spin it down. Make sure DSG is completely dissolved in the DMSO.

DETERMINING INPUT CONCENTRATION

1. Set the thermomixer to 37°C and put Buffer D and Buffer E to warm – this helps to dissolve any precipitates.
2. Bring Enzyme D 🟣, Buffer D, and Buffer E to RT before use. Buffer D & E and Elution Buffer are stored at RT.
3. Set the thermomixer to 55°C.
4. Calculate the total amount of master mix and prepare in a 1.5 mL tube:

Component	μ L for 1 sample (calculated with 15% excess)	# tubes	μ L in Master Mix
Arima Elution Buffer ☹	85.1	x	=
Buffer D 🟣	12.1	x	=
Enzyme D 🟣	28.8	x	=
Total	126	x	=

5. Add **109.5 μ L master mix** to the 100 μ L QC tube(s) set aside earlier.
6. Add **20 μ L Buffer E** 🟣 to each sample tube, and pipette mix gently.
7. ⌚ Put the tubes in the thermomixer for 30 minutes at 55°C, no shaking.
8. ⌚⌚ Change the thermomixer to 68°C and incubate overnight (minimum 90 minutes). **NOTE START TIME.**

DAY 2: CONTINUE INPUT AMOUNT DETERMINATION & HiC PROTOCOL

Prep:

- Bring DNA Purification beads to RT and mix. □ Prepare 10 mL fresh 80% EtOH.

- ☞ Note: 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.
- ☞ DO NOT LET THE BEADS DRY OUT AND CRACK. Always keep beads wet – unless they are in the drying step, in which case you should keep a close eye on them.

CONTINUE INPUT AMOUNT DETERMINATION

1. Transfer the samples from the thermomixer to the refrigerator (4°C). Keep in fridge until ready to proceed, but no more than ~15 minutes to keep the samples from getting too cold / gloppy. NOTE TIME YOU TOOK THEM OUT.
2. Add **150 µL DNA Purification Beads** to sample tube, pipette-mix thoroughly, and incubate at RT for 5 minutes.
3. Place sample on magnet, and wait until solution is clearer.
4. With sample on magnet, discard supernatant, add **450 µL 80% EtOH**, and incubate at RT for 1 minute. Make sure there are no beads in the supernatant when discarding.
5. Repeat step 4 two more times for a total of three washes (or more if needed).
6. With sample on magnet, open the tube cap and incubate beads at RT for a few minutes to air-dry the beads. Use a p20 to remove any excess EtOH from the bottom of the tube. Watch the beads closely!
 - ☞ At the beginning of the drying time, the mass of beads will look shiny because it's still wet with 80% EtOH. As the alcohol evaporates, the beads will become less shiny. You are waiting for the beads to reach a "leather matte" look, but making sure they don't go beyond this and dry out to an ashy state. The purpose of this drying step is to reduce/eliminate alcohol mixing into your elution buffer in the following step.
7. Remove sample from magnet, and use a wide orifice p200 tip to thoroughly resuspend the beads in **20 µL Arima Kit Elution Buffer** ☺, then incubate at RT for 5 minutes. While you wait, label a fresh tube for the clean elution. Given the small volume of the elution, consider using a PCR microcentrifuge tube (200 µL PCR tube) for the next step.
8. Place sample on magnet, wait until solution is clear, and transfer supernatant to a new, labeled tube.
9. Quantify 2 µL of sample using Qubit HS. If concentration is very high you may need to dilute an aliquot of the sample and re-quantify. Use the dilution formula: $C_1 \times V_1 = C_2 \times V_2$
10. Calculate DNA yield and amount to determine how much of each sample to use for the Arima-HiC reaction:

☞ If the sample is very dense, initial bead migration may take longer. Just give it the time it needs.

☞ You may also need to do more 80% EtOH washes if the sample is really dense, or do several washes to gradually replace the supernatant with EtOH without removing sample. Take your time getting the sample clean before moving on to drying and elution.

☞ If you get an aberrant result (e.g., far too high concentration for the Qubit to read), it's best to repeat the input amount determination for the sample(s) in question using the 100 µL reserved tube.

Sample ID	Qubit measured conc.	Total ng DNA in QC tube	ng DNA in 100 µL reserved tube	ng DNA in 200 µL reserved tube
	ng/µL x 20 µL =	=	=	x 2 =
	ng/µL x 20 µL =	=	=	x 2 =
	ng/µL x 20 µL =	=	=	x 2 =
	ng/µL x 20 µL =	=	=	x 2 =

For large complex genomes 750-2000 ng is recommended; standardize on 2000 ng where possible. For smaller genomes <750 ng may be sufficient. Calculate how much you need to reach 2000 ng from the quant above.

For example, 50 ng/ µL x 20 µL = 1000 ng ⇒⇒ 1000 ng x 2 = 2000 ng

In this example, exactly one of the 20% (200 µL) reserved tube would be needed; the rest can be kept for backup.



If the input amount determination protocol yielded considerably more than 50 ng/μL, you will need to split one of the reserved tubes to take the amount needed to reach 2000 ng of input material. Resuspend one of the 200 μL reserved tubes, because those were easier to prepare at the end of the last step (remove the supernatant and flash freeze) and so likely have the amount of material in them that is expected for carrying forward.

Resuspend crosslinked sample in 1000 μL 1X PBS. Pipette-mix thoroughly, then transfer the required volume for 2000 ng into a fresh 1.5 mL Eppendorf tube. Centrifuge at 500 X G for 4 minutes at 4°C and remove supernatant before proceeding to the addition of lysis buffer. If you do this, you'll need to have dry ice and liquid nitrogen available, similar to when doing the splitting of samples earlier in the protocol.

Calculation for HiC Input material based on quantification from the input amount determination protocol:

Sample ID	ng in 200 μL reserved tube	Resuspend sample in 1000 μL 1X PBS	
		ng/μL in 1000 μL	μL for 2000 ng
		/1000= ng/μL	x 0.0005= μL
		/1000= ng/μL	x 0.0005= μL
		/1000= ng/μL	x 0.0005= μL
		/1000= ng/μL	x 0.0005= μL

- ➡ You can bring several samples up to this point in the protocol and then proceed with them all together into the next step, the HiC Protocol. It's best not to exceed 2500 ng input, aim for about 2000 ng when possible.

BEGIN HiC PROTOCOL

SAMPLES

Prep:

- ☐ Set two heating devices, one to 62°C and one to 37°C.
- ☐ Thaw Arima Kit Lysis Buffer ●, pipette mix and spin down.

- Thaw the number of sample tubes needed to reach input amount from the previous step (take out of -80°C). If needed, resuspend in 1X PBS and take amount needed to reach 2000 ng of input, spin down in 4°C centrifuge, and remove supernatant before proceeding (see note on previous page).
- Add 20 µL Arima Kit Lysis Buffer ● to each tube; pipette mix.
- ⌚ Incubate tubes at 4°C (fridge) for 30 minutes.
- ⌚ Add 24 µL Conditioning Solution ○ to each tube; pipette mix then incubate at 62°C for 10 minutes. If using a thermocycler, set the lid temperature to 85°C.
- ⌚ Add 20 µL Stop Solution 2 ● to each tube; pipette mix then incubate at 37°C for 15 minutes. If using a thermocycler, set the lid temperature to 85°C.
- While the samples are incubating at 37°C, make **MASTER MIX #1:**

Component	µL per tube x 1.1	# tubes	µL in Master Mix	Notes
Buffer F ●	8.25	x	=	Thaw and keep on ice
Enzyme F1 ●	1.65	x	=	
Enzyme A2 ●	1.65	x	=	
Enzyme F3 ●	1.65	x	=	
Enzyme F4 ●	1.65	x	=	
Total	14.85			

- Add 13.5 µL Master Mix #1 to each sample tube; pipette mix.
- ⌚⌚ Incubate at 37°C for 3 hours.
 - ⌚ 2.5 hours into the three hour incubation, take out Buffer G ● to thaw at RT (it takes about an hour).
- ⌚ Move samples to 65°C, incubate for 20 minutes.
- ⌚ Move samples to RT, incubate for 10 minutes. Make sure samples are at room temp before moving on to next step, otherwise the enzyme will die.
- While the samples are incubating at room temperature, spin down Buffer G ● and Enzyme B ● and make **MASTER MIX #2:**

Component	µL per tube x 1.1	# tubes	µL in Master Mix
Buffer G ●	13.2	x	=
Enzyme B ●	4.4	x	=
Total	17.6		

- Add 16 µL Master Mix #2 to each tube; pipette mix.
- ⌚ Incubate at RT for 45 minutes.
- At the beginning of the 45 minute incubation, take out Enzyme D ● and Buffer C ● to allow them to warm up to room temperature.
- While the samples are incubating at room temperature, spin down Buffer C ● and Enzyme C ● and make **MASTER MIX #3:**

Component	µL per tube x 1.1	# tubes	µL in Master Mix
Buffer C ●	77	x	=
Enzyme C ●	13.2	x	=
Total	90.2		

- Add 82 µL Master Mix #3 to each tube; pipette mix.
- ⌚ Incubate at RT for 15 minutes.

18. Inspect Buffer D ● and if it contains precipitates, warm it to 37°C for a few minutes to dissolve them.
19. While the samples are incubating at room temperature, spin down Buffer D ● and Enzyme D ● and make

MASTER MIX #4:

Component	μL per tube x 1.1	# tubes	μL in Master Mix
Buffer D ●	11.55	x	=
Enzyme D ●	27.5	x	=
Total	39.05		

Yes, Master Mix #4 has more enzyme than buffer. This is correct.

20. Add **35.5 μL Master Mix #4** to each tube; pipette mix.
21. Add **20 μL Buffer E ●** to each tube; pipette mix.
22. ⌚ Incubate at 55°C for 30 minutes.
23. ⌚⌚ Incubate at 68°C for overnight (minimum 90 minutes). **NOTE START TIME.**

DAY 3: ELUTION & ARIMA QC-1

Prep:

- ☐ Bring DNA Purification beads to RT and mix. ☐ Prepare 10 mL fresh 80% EtOH.

BEAD CLEAN AND ELUTION

⚠ IF YOU HAVE MORE THAN ONE TUBE PER SAMPLE, THIS IS A SERIAL BEAD CLEAN AND ELUTION ⚠

- Take out DNA Purification Beads and bring to RT.
 - Note: DNA Purification Beads (e.g. AMPure XP Beads or SPRIselect) should be warmed to RT and thoroughly mixed before use. ***DNA Purification Beads are a user-supplied reagent.***
- Take samples out of incubator and put at RT for 10 minutes.

PER SAMPLE TUBE, SERIALY:

- Use a wide orifice p200 pipette tip to add **100 µL DNA Purification Beads** to sample tube, pipette-mix thoroughly (at least 20 times), and incubate at RT for 5 minutes.
 - Place sample on magnet, and wait until solution is clearer. This can take up to 10 minutes (or more if sample is very dense).
 - With sample on magnet, discard supernatant, add **400 µL 80% EtOH**, and incubate at RT for 1 minute. Make sure no beads in supernatant when discarding.
 - With sample on magnet, discard supernatant, add **400 µL 80% EtOH**, and incubate at RT for 1 minute. Make sure no beads in supernatant when discarding.
 - Discard supernatant and incubate beads at RT for 3–5 minutes on the magnet to air-dry the beads. Check them and don't allow them to over dry and crack! Carefully remove any residual alcohol using a p20 pipette.
 - Remove sample from magnet, use a wide orifice p200 tip to thoroughly resuspend beads in **100 µL Arima Kit Elution Buffer** ☉, and incubate at RT for 5 minutes.
 - Pre-label fresh tubes for transferring eluted DNA.
 - Place sample on magnet, wait until solution is clear, and transfer supernatant to a new tube. ***Keep this tube and use it as the elution buffer for the next tube in the series.***
- Quantify 1 µL of the sample using Qubit HS. Note result in the chart below (Pre-QC1 Qubit).

Sample	Pre-QC1 Qubit (ng/µL) ➤DNA in Buffer◀	Post-QC1 Qubit (ng/µL) ➤Bead-bound DNA◀	Post x 7	Pre x 5	Efficiency (%)
			/	= x 100 =	
			/	= x 100 =	
			/	= x 100 =	
			/	= x 100 =	

- Transfer **5 µL sample to a new 0.2 mL PCR tube** and label it with the sample ID and "Arima-QC1". Keep the rest of the sample in the fridge.
- Add **45 µL Arima Kit Elution Buffer** ☉ to the "Arima QC-1" tube to reach a total volume of 50 µL.

Fill out Pre-QC1 column with results from step 11 above, and the Post-QC1 column with results from the next page. Use these values to calculate efficiency. 10% or higher efficiency is good. If you started with very high input amount, efficiency might be a little lower but this will still be okay.

ARIMA-QC1 (QUALITY CONTROL)

Prep:

- ☐ Set thermocycler for hold 55°C for Arima QC1 protocol.
- ☐ Thoroughly mix the **Arima QC Beads** ●.
- ☐ Ensure you have access to Covaris and TapeStation instruments, and that you have consumables for both.

The Arima QC1 protocol quantifies the fraction of proximally-ligated DNA that has been labeled with biotin, and is a quality control metric after completing the *Arima HiC Protocol* but before proceeding to library prep. The *Arima-QC1 Quality Control* protocol involves using **Arima QC Beads** ● to enrich an aliquot of proximally-ligated DNA, which is then quantified using a Qubit HS. 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.

1. Add **50 µL Arima QC Beads** ● to each sample, mix thoroughly by pipetting, and incubate at RT for 15 minutes.
2. Place sample on magnet, and wait until solution is clear.
3. Discard supernatant, and add **200 µL Wash Buffer** ○.
4. Remove sample from magnet.
5. Resuspend beads in **200 µL Wash Buffer** ○ using wide orifice tips (multichannel if multiple samples), and incubate at 55°C for 2 minutes.
6. Place sample on magnet, and wait until solution is clear.
7. Discard supernatant, and add **200 µL Wash Buffer** ○.
8. Remove sample from magnet.
9. Resuspend beads in **200 µL Wash Buffer** ○ using wide orifice tips (multichannel if multiple samples), and incubate at 55°C for 2 minutes.
10. Place sample on magnet, and wait until solution is clear.
11. Discard supernatant, and add **100 µL Arima Kit Elution Buffer** ○.
12. Remove sample from magnet.
13. Resuspend beads in **100 µL Arima Elution Buffer** ○ using wide orifice tips (multichannel if multiple samples).
14. Place sample on magnet, and wait until solution is clear.
15. Discard supernatant, working as quickly and carefully as possible to avoid over-drying the beads.
16. Remove sample from magnet.
17. Resuspend in **7 µL Arima Kit Elution Buffer** ○. Proceed to next step with resuspended beads.
 ➡ Note: The following step involves the **Qubit quantification of bead-bound DNA**.
18. Quantify the total amount of *bead-bound DNA* using Qubit. Use 2 µL thoroughly mixed bead-bound DNA for the Qubit assay. Note results in chart on previous page.
19. Calculate efficiency using chart on previous page.

Proceed immediately to Library Prep (next page) and complete the DNA Fragmentation, TapeStation, Bead-based DNA size selection, and Qubit before placing the samples in -20°C overnight.

ARIMA LIBRARY PREP

Library preparation for Arima High Coverage HiC Kit

Material parts number: A101030, A303011 | Document part number: A160186 v02

Release Date: May 2024

OVERVIEW: Library preparation begins with DNA fragmentation, DNA size selection, and biotin enrichment. The Arima Library Prep Module reagents then are used in a custom end-repair, dA-tailing, and adapter ligation protocol. This Arima Library Preparation Protocol constructs libraries while DNA is bound to T1 Beads. The final step is PCR amplification of the bead-bound Arima High Coverage HiC library using the library amplification reagents and index PCR primers from the Arima Library Prep Module, producing the final Arima High Coverage HiC library.

HANDLING AND PREPARATION

- The *Arima Library Prep Protocol* require the preparation of several master mixes. Sufficient reagent is 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.
- All kit reagents should be fully thawed and thoroughly mixed before use.
- To protect samples from nucleases, we recommend the use of gloves and sterilized filter pipette 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.
- In steps with magnetic beads that require centrifugation, be careful not to pellet the beads.
- Use lo-bind tubes for all protocol master mixes to minimize the amount of wasted reagents and sample.
- Pipette carefully and check your math!
- All resuspensions should be done with wide orifice pipette tips.

USER PROVIDED MATERIALS AND EQUIPMENT

- Freshly prepared 80% Ethanol
- DNA Purification Beads
- Qubit HS Fluorometer, reagents, and consumables
- 1.7mL microcentrifuge tubes
- PCR tubes
- Magnetic racks for both tube types
- Centrifuge
- Thermal cycler
- Covaris sonicator and consumables
- 8-well PCR Strip Tubes with Caps
- TapeStation, consumables, reagents
- Deionized / Nuclease-free Water

ARIMA KIT MATERIALS

Cap		Name	Category	Box	Storage	µL per sample tube	Part number	Notes
Purple	●	Ligation Buffer	Buffer	A	-20°C	25.88	A311035-01	Highly viscous
Blue	●	T4 DNA Ligase	Enzyme	A	-20°C	2.25	A311035-02	
Yellow	●	End Repair-A-Tailing <i>Buffer</i>	Buffer	A	-20°C	18	A311035-03	
Orange	●	End Repair-A Tailing <i>Enzyme Mix</i>	Enzyme	A	-20°C	4.5	A311035-04	
Red	●	Herculase II Fusion DNA Polymerase	Enzyme	A	-20°C	1.125	A311035-05	
Clear	⊙	5x Herculase II Buffer with dNTPs	Buffer+	A	-20°C	11.25	A311035-06	
White	○	Adaptor Oligo Mix	Mix	A	-20°C	5	A311035-07	
Foil	●	Index 1-16	Index	B	-20°C	5	A311036-01—16	Keep upright!
White	○	T1 Beads	Beads	C	4°C	12.5	A311042-01	Keep upright!
Bottle	⊙	Binding Buffer	Buffer	D	RT	800	A311041-01	

DAY 3 CONTINUED: DNA FRAGMENTATION & BEAD-BASED SIZE SELECTION

DNA FRAGMENTATION

The output of the *Arima High Coverage 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. Use a Covaris Sonication instrument for mechanical fragmentation of DNA.

➡ DNA should be fragmented in 130 μ L Elution Buffer. If sample quantity is not limiting, it is recommended to fragment at least 1500 ng of DNA per sample, or up to 5 μ g (depending on the DNA fragmentation instrument manufacturer recommendations). However, for certain applications, less than 750 ng of DNA could be used.

1. Turn on and prepare Covaris at least 30 minutes prior to running. For the **S220**, turn on the chiller, and once the water has reached 2.5°C, add distilled water to the receptacle for the sonicator head up to the 10-15 fill line.
2. Open the Covaris software, and load the HiC fragmentation protocol (parameters in chart below). Allow the pump to degas and the water to reach the right temperature before proceeding. If the water gets too cold, you can turn off the chiller for a bit until it gets back into a good temperature range.
3. Measure and note μ L sample remaining in the sample tube(s). Add **Arima Kit Elution Buffer** to reach a total sample volume of 130 μ L in each tube. Note amount added:

Sample	Volume remaining (μ L)	μ L Elution Buffer added to reach 130 μ L

4. While waiting for the Covaris to degas and equilibrate temperature, transfer samples into 130 μ L Covaris tubes. Remove the cap of each tube, pipette in 130 μ L, and close the cap carefully. Label the cap on the side with sample ID.
5. Fragment DNA to obtain an average fragment size of 550-600bp. Use the DNA fragmentation instrument manufacturer default settings for obtaining a target fragment size of 550-600bp. If default settings for 550-600bp are not available, then it's recommended to using slightly less shearing time than that of a target size of 500bp. Example Covaris E220, S220, and M220 settings are noted below for obtaining a target fragment size of 550-600bp; if using a different system, note settings in the blank column.

Setting	Covaris E220	Covaris S220	Covaris M220	
TEMPERATURE (°C)	7	7	20	
PEAK INCIDENT POWER	105	105	50	
DUTY FACTOR (%)	5	5	5	
CYCLES PER BURST	200	200	200	
TREATMENT TIME (SECONDS)	70	70	50	

6. Shear the DNA according to the proper settings for your sonication device.
7. Take out TapeStation reagents to allow them to reach room temperature (30 minutes).
8. Run a TapeStation using a **D5000** (or D5000 HS) tape to see the fragment distribution immediately following sonication – aiming for a peak around 600 bp. Print out the report and put it in your lab notebook for reference.
 - ➡ If the peak is upshifted, that's fine as long as there is sufficient concentration in the desired fragment size range. Re-sonicate if the peak is upshifted too far and there's not enough DNA in the desired fragment range.
9. Proceed directly to bead-based DNA size selection.

BEAD-BASED DNA SIZE SELECTION

Here, fragmented DNA is size-selected to have a size distribution >400bp. Perform this workflow in a PCR microcentrifuge tube (200 μ L PCR tube) and ensure you have a magnet that it can fit with.

➡ DNA Purification 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 T1 Beads provided in the Arima Library Prep Module.

➡ For the ethanol washes performed below, use sufficient 80% EtOH to fully cover the magnetized beads (you can use the 80% EtOH prepared at the beginning of the day).

1. Transfer fragmented DNA sample from fragmentation tube to PCR tube.
2. Measure how much sample you have left (should be around 127 μ L) and **add Arima Kit Elution Buffer** to bring the total volume in the tube to 130 μ L.
3. Add **91 μ L DNA Purification Beads**, mix thoroughly by pipetting, and incubate at RT for 5 minutes.
4. Place sample on magnet and wait until solution is clear.
5. On magnet, discard supernatant, add **225 μ L 80% EtOH**, and incubate at RT for 1 minute.
6. Still on magnet, discard supernatant, add **225 μ L 80% EtOH**, and incubate at RT for 1 minute.
7. Discard supernatant.
8. With sample on magnet, incubate beads at RT for 1 minute to air-dry the beads.
9. Remove the sample from magnet.
10. Resuspend beads in **30 μ L Arima Kit Elution Buffer**, and incubate at RT for 5 minutes.
11. Lay out a new tube or set of tubes, and label.
12. Place sample on magnet, wait until solution is clear, and transfer supernatant to new sample tube.
13. Quantify 1 μ L sample using Qubit, and calculate how much sample is needed for ~400-600 ng of DNA, or how many ng of DNA your total sample contains. Use this information in Day 4 to determine the number of PCR cycles to use.

Sample ID	Post Bead-Based Size Selection DNA Concentration (ng/ μ L)	μ L remaining, or μ L needed for ~600 ng DNA input for Biotin Enrichment

14. Store samples at -20°C overnight, or for up to 3 days.

DAY 4: LIBRARY PREP CONTINUED

Prep:

- | | |
|---|---|
| <input type="checkbox"/> Pre-heat thermal cycler to 55°C (lid temperature 85°C), set to hold. | <input type="checkbox"/> Make an aliquot of Deionized / Nuclease-free Water. |
| <input type="checkbox"/> Mix T1 beads well, by rotating and pipette mixing. They settle easily. | <input type="checkbox"/> Make fresh 80% EtOH. |
| | <input type="checkbox"/> Input and save thermal cycler programs listed in the protocol. |

BIOTIN ENRICHMENT OF FRAGMENTED DNA

Biotin enrichment and subsequent library preparation has been optimized to deliver peak performance for DNA inputs of 200-600 ng. Using 200-600 ng of DNA input has been shown to build libraries with sufficient complexity for up to 600M read-pairs of sequence data. Referring to your calculation at the very end of Day 3, check if the amount of DNA is less than 600 ng; if so, add in the entire amount.

Perform this workflow in a PCR microtube, ensure that your tubes or plates can hold up to 230 µL sample volume. Also ensure that you have a magnetic rack that fits your choice of sample tube/plate.

☞ Note: **T1 Beads are from the Arima Library Prep Module.** They are NOT interchangeable with the Arima High Coverage HiC Enrichment Beads nor the Arima High Coverage HiC QC Beads.

☞ Note: The T1 beads can be difficult to resuspend and can take a while. When resuspending, pipette-mix with a wide orifice tip until the beads “bloom” and the solution becomes a golden yellow. If you see granules and the solution doesn’t stay golden when you stop pipetting, you should keep going.

1. If your sample contains ≤600 ng of DNA, use the entire sample and carry on with the same tube it is in; if more than 600 ng are available, transfer the volume needed to a new tube. Keep sample tubes containing 600 ng of DNA in the fridge, freeze any excess fragmented DNA.

Wash the T1 beads:

2. Mix T1 Beads very well, especially right before using, making sure that the solution is homogenous and there is nothing sticking to the bottom or sides of the tube.
3. Add **12.5 µL T1 Beads** from the Arima Library Prep Box C into an empty PCR tube, one for each sample.
 - ☞ Note: These beads are **NOT the Enrichment Beads** that come with the Arima High Coverage HiC kit.
 - ☞ Note: T1 beads are not well-behaved, pipette carefully and attentively to reduce loss.
4. Wash the T1 Beads in each tube, repeat three times (check off each time to ensure wash performed three times):
 - 1) ☐ ☐ ☐ Add **200 µL Binding Buffer**.
 - 2) ☐ ☐ ☐ Resuspend: mix by pipetting up and down 20 times using a wide orifice p200 tip.
 - 3) ☐ ☐ ☐ Cap the tubes, and vortex at high speed for 10 seconds.
 - 4) ☐ ☐ ☐ Place tubes on magnet rack and wait until solution is clear.
 - 5) ☐ ☐ ☐ Discard supernatant.
 - 6) ☐ ☐ ☐ Remove tube from magnet
5. Resuspend beads in **200 µL Binding Buffer**.

Mix washed T1 beads with sample:

6. Add **200 µL washed T1 Beads in Binding Buffer**, and mix thoroughly and CAREFULLY by pipetting with a wide orifice p200 tip. This is a large volume for the tube so be mindful.
7. Incubate at RT for 15 minutes.
 - 1) ☐ ☐ Place sample on magnet and wait until solution is clear.
 - 2) ☐ ☐ Discard supernatant, and add **200 µL Wash Buffer**.
 - 3) ☐ ☐ Remove sample from magnet.
 - 4) ☐ ☐ Resuspend beads in **Wash Buffer**.
 - 5) ☐ ☐ Incubate at 55°C for 2 minutes, with lid temperature set to 85°C.
 - 6) ☐ ☐ Place sample on magnet and wait until solution is clear.
8. Discard supernatant, and add **100 µL Arima Kit Elution Buffer**.

9. Remove sample from magnet.
10. Wash beads by resuspending in **Arima Kit Elution Buffer**.
11. Place sample on magnet and wait until solution is clear.
12. Discard supernatant. Yes, discard it.
13. Remove sample from magnet.
14. Resuspend beads in **50 µL Deionized / Nuclease-free Water**.
15. Keep samples at 4°C (fridge) until ready for the next step.

LIBRARY PREPARATION OF ENRICHED LIGATION PRODUCTS

Perform this workflow in a PCR microtube, ensure that your tubes or plates can hold up to 230 µL sample volume. Also ensure that you have a magnetic rack that fits your choice of sample tube/plate.

16. Thaw and mix reagents from Arima Kit Box A as follows:

Reagent	Cap	Thaw Temperature	Mix Method	Notes
Ligation Buffer ●	Purple	On Ice	Vortex	Thaw and vortex on high to homogenize (highly viscous).
T4 DNA Ligase ●	Blue	RT, Ice just before use	Pipette-Mix	
End Repair-A Tailing Buffer ●	Yellow	On Ice	Vortex	
End Repair-A Tailing Enzyme Mix ●	Orange	RT, Ice just before use	Pipette-Mix	
Adaptor Oligo Mix ⊖	Clear	On Ice	Vortex	

17. Prepare **both** the Ligation Master Mix and the End Repair/dA Tailing Master Mix before moving forward. Ligation Master Mix needs to sit at **RT for 30-45 minutes**, so when you finish preparing it, set it aside on the bench.
18. Prepare **LIGATION MASTER MIX**, mix well, and spin down:

Component	µL per tube x 1.125	# tubes	µL in Master Mix	Notes
Ligation Buffer ●	25.88	x	=	Use wide orifice tip, buffer is viscous.
T4 DNA Ligase ●	2.25	x	=	
Total	28.13			

19. Prepare **END REPAIR/DA TAILING MASTER MIX**, mix well, and spin down:

Component	µL per tube x 1.125	# tubes	µL in Master Mix	Notes
End Repair-A Tailing Buffer ●	18	x	=	Vortex until no solids remain (~15 sec.)
End Repair-A Tailing Enzyme Mix ●	4.5	x	=	
Total	22.5			

20. Add **20 µL End Repair/dA Tailing Master Mix** to each sample containing 50 µL bead-bound HiC library, and pipette mix well with a wide orifice tip.
21. ⊕ Put samples in the thermal cycler and run the **END REPAIR/DA TAILING PROGRAM** (~30 minutes):

Temperature (°C)	Time (minutes)
20	15
72	15
4	Hold

Set reaction volume to 70 µL and lid temperature to 85 °C

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

23. Add **25 µL Ligation Master Mix** to the 70 µL bead-bound, end-repaired and dA-tailed HiC library. Pipette mix using a wide orifice tip.
24. Add **5 µL Adaptor Oligo Mix** to each sample. Pipette mix using a wide orifice tip.
25. Briefly spin down tubes.
26. ⌚ Put samples in the thermal cycler and run the **ADAPTOR LIGATION PROGRAM** (~30 minutes):

Temperature (°C)	Time (minutes)
20	30
4	Hold

Set reaction volume to 100 µL
and lid temperature to 85 °C

27. After the ligation program completes, remove the samples from the thermocycler and briefly spin down the tubes to remove any liquid from the caps.
28. Place tubes on magnet and wait until liquid is clear.
29. Discard supernatant and add **200 µL Wash Buffer**.
30. Off magnet, resuspend beads in **Wash Buffer** using a wide orifice tip. This may take a while, keep at it.
31. Incubate at 55°C for 2 minutes, with lid temperature set to 85°C. (Use the hold55 program on the thermal cycler)
32. Place tubes on magnet and wait until liquid is clear.
33. Discard supernatant.
34. Off magnet, resuspend beads in **100 µL Arima Kit Elution Buffer** using a wide orifice pipette tip.
35. Place tubes on magnet and wait until liquid is clear.
36. Discard supernatant. Yes, discard it.
37. Off magnet, resuspend the beads in **34 µL Deionized Water** using a wide orifice tip.
38. Place sample tubes in the refrigerator and proceed immediately to Library Amplification.

AMPLIFICATION OF ADAPTOR-LIGATED HiC LIBRARY AND SAMPLE INDEXING

39. Thaw and mix reagents from Arima Kit Boxes A and B as follows, and keep on ice:

Reagent	Cap	Thaw Temperature	Mix Method	Notes
Herculase II Fusion DNA <u>Polymerase</u> ●	Red	On Ice	Pipette-Mix	
5X Herculase II <u>Buffer</u> with dNTPs ☉	Clear	Room Temperature	Vortex	
Index Primer Pair #1-#16 ● — choose from index table below.	Foil	Room Temperature	Vortex	These are stored in a very non-user friendly way.

Index Table: Index Pairs included with Arima Library Prep Module. Fill in "Sample ID" as indexes are assigned.

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

40. Prepare **PCR REACTION MIX** as follows, and pipette mix thoroughly with a wide orifice tip:

Component	μL per tube x 1.125	# tubes	μL in Master Mix	Notes
Herculase II Fusion DNA <u>Polymerase</u> ●	1.125	x	=	
5X Herculase II <u>Buffer</u> with dNTPs ⊖	11.25	x	=	
Total	12.375			

41. Add **11 μL the PCR Reaction Mix** prepared from the table above to 34 μL Adaptor Ligated Bead Bound HiC Library.
42. Add **5 μL the appropriate, unique, Index Primer** to each sample. Make sure to note which index was used with each sample in the Index Table.
43. The number of PCR cycles will vary depending on input amount. Refer to the total ng calculated at the very end of Day 3 and use the table below to decide how many PCR cycles to use:

Total amount calculated at end of DAY 3	# of PCR cycles
Less than 50 ng	14
50-200 ng	13
200-300 ng	12
300-400 ng	10
400-600 ng	9

44. ⌚ Put samples in the thermal cycler and run the **LIBRARY AMPLIFICATION PROGRAM** (~40-50 minutes):

# of Cycles	Temperature (°C)	Time
1x	98	2 minutes
Choose number of cycles by referring to the table above.	98	30 seconds
	60	30 seconds
	72	1 minute
1x	72	5 minutes
1x	4	Hold

Note input amount and number of PCR cycles for each sample.

45. Proceed directly to bead cleaning final library.

CLEAN FINAL AMPLIFIED LIBRARY WITH DNA PURIFICATION BEADS

➡ Note: DNA Purification 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 Library Prep kit.

46. Add **35 μ L DNA Purification Beads** to each 50 μ L sample and pipette-mix well with a wide orifice tip.
47. Incubate for 5 minutes at RT.
48. Place sample on magnet and wait until solution is clear.
49. Discard supernatant.
50. With sample on magnet, add **150 μ L 80% EtOH**, and incubate at RT for 1 minute.
51. Discard supernatant.
52. With sample on magnet, add **150 μ L 80% EtOH**, and incubate at RT for 1 minute.
53. Discard supernatant.
54. With sample on magnet, incubate beads at RT to air-dry the beads. Inspect them to make sure they don't dry out and remove any remaining ethanol using a p20.
55. Remove the sample from the magnet.
56. Resuspend beads in **15 μ L Deionized / Nuclease-free Water**, and incubate at RT for 5 minutes.
57. Place sample on magnet and wait until solution is clear.
58. Lay out fresh tubes for the final libraries.
59. TRANSFER PURIFIED AND COMPLETE HiC LIBRARY TO A FRESH, LABELLED PCR TUBE.
60. Quantify 1 μ L sample on the Qubit HS.

Sample ID	Qubit - Final library concentration (ng/ μ L)	Total μ L sample remaining (should be ~14)	Total ng DNA in Final library
	x	=	
	x	=	
	x	=	
	x	=	

61. Samples may be stored at -20°C for up to 6 months.
62. QC sequencing is recommended to assess how well the protocol went. For this, a small run generating roughly a million read pairs per sample should give an indication of library complexity.