Dovetail™ Hi-C Library Preparation Kit

Hi-C Kit Manual

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Safety Data Sheets are available at https://dovetailgenomics.com/dovetailhi-c_sds/

To learn more about Dovetail's products or services please contact info@dovetail-genomics.com

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Dovetail™ Hi-C and HiRise™ Specifications

Dovetail Hi-C Kit and HiRise Software Specifications		
Hi-C Preparation Kit	**Number of reactions**	Each kit supports 8 reactions
	Validated sample types and input amounts	Mammalian tissue 20-40 mg Mammalian blood 1 ml Mammalian cell culture 0.5 x 10^6 cells
	Supported applications	Generating Hi-C Illumina®-compatible sequencing libraries. Genome assembly with HiRise™ software.
	Required equipment and reagents	Standard genomics equipment *(see User Provided Reagents and Equipment)*
	Sequencer compatibility	All Illumina® Sequencers
HiRise™ Software	**Sequencing format**	2 x 150 paired-end reads
	Input assembly N50	> 1 Mb suggested for optimal results
	Input assembly N90	> 20 kb suggested for optimal results
	Input assembly technologies supported	Virtually all genome assembly technologies and assemblers that produce FASTA files
	Access	Service
	Hi-C sequencing coverage needed	100 M read-pairs per Gbp of haploid genome (*see Appendix 7 for more details)*

Dovetail™ Hi-C and HiRise™: From Sample to Chromosomes

The Dovetail™ Hi-C Library Preparation Kit and HiRise™ scaffolding software provide a comprehensive genome assembly pipeline for researchers.

- A Hi-C library is generated using the Dovetail Hi-C kit.
- The kit enables users to generate up to 8 Illumina-ready Hi-C sequencing library in just 2 days.

- The Hi-C library is validated using 1-2 million reads and Dovetail's QC software.
- Validated Hi-C libraries are sequenced to generate 100M read-pairs per Gbp of genome.

- The sequenced library and input assembly with a N50 of > 1 Mb and a N90 of > 20 kb are run through Dovetail's HiRise™ scaffolding pipeline. HiRise™ scaffolds up to whole chromosomes with high confidence.

Dovetail™ Hi-C Kit Workflow

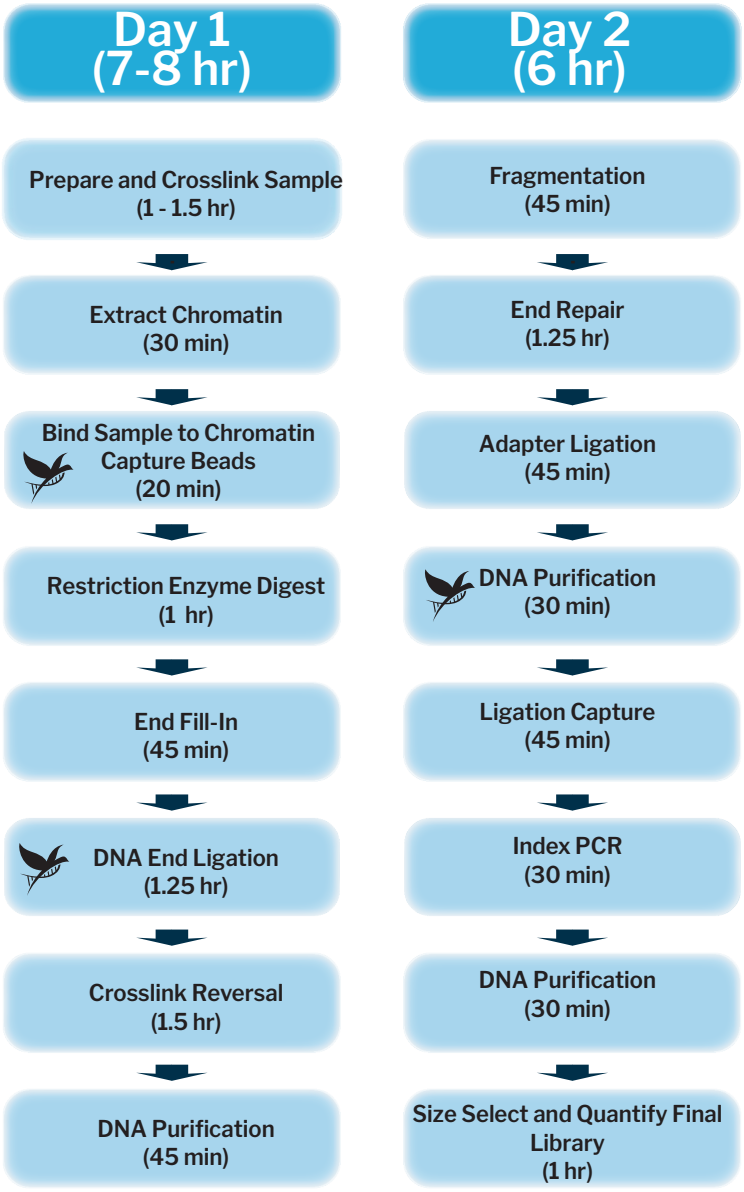
The Dovetail™ Hi-C Library Preparation Kit has been optimized through Dovetail's in-house Hi-C service.

The kit allows users to generate an Illumina®-ready Hi-C sequencing library in just 2 days.

To the right are the protocol steps for each day and the total time required.

Roughly 4 hours on Day 1, and 2.5 hours on Day 2 are hands-off incubations.

Steps that include an optional stopping point are marked with a dovetail symbol.



Kit Contents and Storage

The Hi-C Kit comes as three boxes. Store the boxes as listed below immediately upon receipt.

Box 1: Dovetail™ Hi-C Kit for Illumina® 8 Rx - Box 1 of 3 (Store at 2°C to 8°C)

Item	Item
TE Buffer pH 8.0	Chromatin Capture Beads
Wash Buffer	Crosslink Reversal Buffer
TWB Solution	Streptavidin Beads
2X NTB Solution	10X RBC Lysis Buffer
LWB Solution	40 mM Calcium Chloride
NWB Solution	20% SDS

Box 2: Dovetail™ Hi-C Kit for Illumina® 8 Rx - Box 2 of 3 (Store at -30°C to -10°C)

Item	Item
Restriction Digest Buffer	Restriction Digest Enzyme Mix
End Fill-in Buffer	End Fill-in Enzyme Mix
Intra-Aggregate Ligation Buffer	Intra-Aggregate Ligation Enzyme Mix
250 mM DTT	Adaptor
End Repair Enzyme Buffer	End Repair Enzyme Mix
Ligation Enhancer	Ligation Enzyme Mix
Universal PCR Primer	HotStart PCR Ready Mix
USER Enzyme Mix	Proteinase K
Index Primer 2	Index Primer 7
Index Primer 4	Index Primer 8
Index Primer 5	Index Primer 12
Index Primer 6	Index Primer 19

Box 3: Dovetail™ Hi-C Kit for Illumina® 8 Rx - Box 3 of 3 (Store at -30°C to -10°C)

Item
Collagenase

User Provided Reagents and Equipment

Items to be provided by users in addition to standard molecular biology equipment and reagents.

Reagents:

1. Agencourt™ AMPure™ XP Beads (Beckman Coulter, Inc., cat.no. A63880)
2. 37% Formaldehyde solution (Sigma-Aldrich, product no. F8775)
3. 1x PBS
4. 80% EtOH
5. DNase and RNase free water

Molecular Biology Equipment and Materials:

1. 1.5 ml DNA Low Binding Microcentrifuge Tubes
2. 0.2 ml PCR Microcentrifuge Tubes
3. Thermal mixer
4. Thermal cycler
5. Vortex
6. Pipets and pipet tips
7. Microcentrifuge
8. Dry ice/petri dish/razor or liquid nitrogen/mortar and pestle (for animal tissue samples)
9. Hemocytometer (for cell culture or blood samples)
10. 15 ml centrifuge tubes (for blood samples)
11. Magnetic separation stand for 1.5 ml microcentrifuge tubes
12. Magnetic separation stand for 0.2 ml microcentrifuge tubes

Genomics Equipment:

1. Illumina® sequencer and sequencing reagents
2. Qubit® Flourometer and Qubit® dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific, cat. nos. Q32851, Q32854)
3. Agilent TapeStation System or Bioanalyzer
4. Bioruptor® Pico Ultrasonicator for DNA fragmentation
5. DNA size selection system such Sage Science Pippin Prep™ for size selecting DNA in the 350-850 nt range. Alternatively, AMPure™ XP beads can be used to size select.

Details on Required Equipment

Sequencing Platform

The Hi-C Kit is designed to create Illumina®-compatible Hi-C sequencing libraries.

Microcentrifuge tubes

As written, most of the protocol is performed in 1.5 ml low bind microcentrifuge tubes. There are 3 stages on Day 2 where it is easiest to switch to a 0.2 ml PCR tube. The protocol lists the suggested microcentrifuge tube size for each of those stages on Day 2.

DNA Quantification

A Qubit® Flourometer or similar instrument is necessary to quantify DNA levels in the Hi-C protocol. A Nanodrop® is not sensitive enough to be used in the Hi-C assay.

DNA Sizing

An Agilent TapeStation, Bioanalyzer, or similar assay is required to accurately determine the size distribution of the DNA.

DNA Fragmentation

The Hi-C Protocol was validated using a Bioruptor® Pico Ultrasonicator to fragment the sample DNA. Appendix 2 includes suggestions for using a Covaris® M220.

DNA Size-Selection

A Sage Science Pippin Prep™ or similar instrument is recommended for size selecting the final library. Appendix 5 includes an alternative protocol for size selecting the library using AMPure™ XP beads.

Samples

There are 4 sample types, each with a distinct protocol for crosslinking and extracting chromatin (protocol stages 1 and 2). We recommend only performing one of the sample type-specific protocols at a time. Users can store prepared chromatin from Stage 2 at 4°C for several days, and sync up samples of different types starting at Stage 3 of the protocol.

Protocol	Validated Sample Type	Suggested Sample Types
Tissue (pg. 14)	• Mammalian tissues such as muscle, brain, heart and spleen	• Vertebrate tissues such as muscle, brain, heart, and spleen • Invertebrate muscle tissue with high cellularity and low-fat content
Cells (pg. 15)	• Mammalian cell culture	• Non-mammalian animal cell culture
Blood (pg. 16)	• Mammalian blood	
Plants (pg. 33)		• Young plant leaves • Plant cell culture

If you have a desired sample type not listed above please contact technical support at support@dovetail-genomics.com or +1 (831) 233-3780.

Sample handling guidelines

Tissue

Tissue samples with high cellularity and low-fat content perform extremely well, especially muscle, brain, heart, and spleen. Tissue samples should be taken from a live, or very recently deceased, specimen and snap-frozen in liquid nitrogen as soon as possible after harvest. Store samples at -80°C, the library and data quality will decrease for samples stored at or above -20°C. Avoid freeze-thaw cycles of tissue samples. If shipping, do so overnight on dry ice.

The kit does not support fat, bone, or similar tissue types. Do not use samples which have been preserved in RNAlater™, EtOH, or which have been freeze-dried.

Blood

Blood samples should be taken from a live or very recently deceased specimen and must have an anti-coagulant added. EDTA is the preferred anti-coagulant, Dovetail has also successfully tested samples with Heparin and Citrate (ACD-A). If using blood collection tubes, follow the manufacturer's instructions to ensure proper mixing of the anticoagulant.

Flash freeze blood samples in liquid nitrogen and keep at -80°C if the sample will be stored before preparing the library. If shipping frozen blood do so overnight on dry ice, ship fresh blood overnight on wet ice to ensure the blood remains cold throughout the shipment.

Cell Culture

Adherent cells can be disassociated using Trypsin. Wash the cells with 1x PBS before starting the Hi-C protocol.

Plants

Leaves harvested from plants at the one or two leaf seedling stage are the preferred sample type. Very young leaves from mature plants and plant tissue culture can also be used. Snap-freeze freshly harvested leaves immediately in liquid nitrogen and store samples at -80°C until use.

If shipping, we recommend sending already-frozen leaf samples overnight on plenty of dry ice.

Dovetail™ Hi-C Kit Protocol

Dovetail™ Hi-C Protocol Day 1

Getting Started

1. Day 1 should take roughly 7 hours for cells, 8 hours for tissue or blood samples.
2. Kit-supplied reagents are listed in **bold blue**, user-supplied reagents are listed in **bold black**.
3. Use standard practices for molecular biology including thawing, vortexing, and quick spinning down reagents before use.
4. There are 2 optional stopping points listed for Day 1.
5. If you are prepping several samples in tandem, prepare a master mix at the start of each step.
6. An 'agitating thermal mixer' should be set at 1250 rpm for 1.5 ml tubes, 1450 rpm for 0.2 ml/PCR tubes.
7. All centrifugation steps occur in a standard table-top microcentrifuge.
8. The times listed for resting tubes on the magnet are a recommendation. Always wait until the solution is visibly devoid of beads before continuing with the protocol.
9. To wash beads, follow these steps:
    a. Add the listed reagent to the sample tube containing beads
    b. Vortex or pipet to resuspend the beads and quick spin the sample tube to remove liquid from the tube cap
    c. Place tube on an appropriate magnet for 1-3 min
    d. Carefully pipet off and discard the supernatant
10. Table 1 lists the equipment and reagents you will need for Day 1.

Table 1

User Supplied Material or Equipment	Stage Used In
Low-bind microcentrifuge 1.5 ml tubes	Throughout
PCR Tubes (optional for day 1)	Throughout
Magnet holders for tubes	Throughout
Thermal mixer	Throughout
1x PBS	1
37% Formaldehyde Solution	1
Dry ice, petri dish, razor blade OR liquid nitrogen, mortar, pestle	1 Tissue sample
Hemocytometer	1 Cell/Blood samples
DNase and RNase free water	1 Blood sample
Beckman-Coulter AMPure™ XP Beads	8
80% EtOH	8
Qubit®	8

Stage 1. Crosslink Tissue And Prepare Chromatin

In Stage 1 the sample will be crosslinked, and chromatin prepared. Make sure the PBS/formaldehyde added to each sample is freshly mixed, and keep the timing of the crosslinking consistent across all samples. There are 3 separate protocols for crosslinking, 1 each for tissue, blood, and cell culture on the following 3 pages. Follow the protocol for your sample type, then continue the protocol at Stage 2.

Stage 1: Crosslinking Animal Tissue Samples

Note: Keep animal tissue frozen until it is in the formaldehyde-containing solution. Incubate the kit-supplied 20% SDS at 37°C for 15 min before use.

1. Weigh out 20-40 mg of frozen tissue sample.
2. Disrupt the tissue either by coarsely chopping it with a razor blade on a petri dish placed on dry ice, OR by grinding it with mortar and pestle in liquid nitrogen.
3. Transfer the disrupted tissue sample to a 1.5 ml microcentrifuge tube.
4. Add to sample tube:
   1 ml **1x PBS**
   40.5 µl **37% Formaldehyde**
5. Vortex mix tube.
6. Rotate tube for 20 min at room temperature. Tissue should not settle.
7. Pellet tissue for 1 min at 13,000 rpm (~15,000 x *g*). Repeat spin if any debris are floating. Pipet off and discard supernatant.
8. Add 300 µl **Wash Buffer** to tube, vortex to resuspend the tissue.
9. Pellet tissue for 1 min at 13,000 rpm (~15,000 x *g*). Pipet off and discard supernatant.
10. Repeat steps 8-9 twice, for a total of 3 washes.
11. Add to sample tube:
    99 µl **Wash Buffer**
    1 µl **40 mM Calcium Chloride**
    25 µl **1 mg/ml Collagenase**
12. Incubate for 1 hr at 37°C in an agitating thermal mixer.
13. Do not spin tube, transfer the liquid fraction to new tube. Tube containing the liquid fraction is now the sample. Discard remaining tissue.
14. Add 6.3 µl **20% SDS** to sample.
15. Vortex tube for >30 sec. Use a pipet to break up any large clumps.
16. Continue protocol at Stage 2: Normalize Chromatin Samples.

Stage 1: Crosslinking Cell Culture Samples

Note: Incubate the kit-supplied 20% SDS at 37°C for 15 min before use.

1. Pellet 0.5 x 10^6 cells for 5 min at 5,200 rpm (~2,500 x *g*). Pipet off and discard supernatant.
2. Add to pellet:
   1 ml **1x PBS**
   40.5 µl **37% Formaldehyde**
3. Pipet gently to resuspend pellet.
4. Rotate tube at room temperature for 20 min. Cells should not settle.
5. Pellet cells for 5 min at 5,200 rpm (~2,500 x *g*). Pipet off and discard supernatant.
6. Add 1 ml **Wash Buffer** and pipet gently to resuspend pellet.
7. Pellet cells for 5 min at 5,200 rpm (~2,500 x *g*). Pipet off and discard supernatant.
8. Add to pellet:
   50 µl **Wash Buffer**
   2.5 µl **20% SDS**
9. Vortex tube for >30 sec. Use a pipet to break up any large clumps.
10. Continue protocol at Stage 2: Normalize Chromatin Samples.

Stage 1: Crosslinking Blood Samples

Notes: The sample should be normalized to 0.5 x 10^6 white blood cells (step 6 below). This will generally require 300 µl – 1000 µl of blood. If less input is used, the yield will be decreased. Make 1x RBC Lysis Buffer by diluting the kit-supplied 10x RBC Lysis Buffer to 1x in DNase and RNase free water**.** Incubate the kit-supplied 20% SDS at 37°C for 15 min before use.

1. Place in 15 ml tube:
   1 volume **Fresh or thawed mammalian blood sample**
   10 volumes **1x RBC Lysis Buffer**
2. Pipet mix the sample and incubate at room temperature for 5 min.
3. Pellet sample for 5 min at 2,300 rpm (~500 x *g*). Pipet off and discard supernatant.
4. Use a 100 µl pipet tip to remove any remaining red blood lysate.
5. Resuspend cell pellet in 1ml **1x PBS**. Carefully remove any cell clumps that do not break apart with pipetting.
6. Normalize the sample to contain 0.5 x 10^6 white blood cells.
7. Pellet sample for 5 min at 5,200 rpm (~2,500 x *g*). Pipet off and discard supernatant.
8. Add to sample pellet:
   1 ml **1x PBS**
   40.5 µl **37% Formaldehyde**
9. Pipet gently to resuspend pellet.
10. Rotate tube at room temperature for 20 min. Cells should not settle.
11. Pellet sample for 5 min at 5,200 rpm (~2,500 x *g*). Pipet off and discard supernatant.
12. Add 1 ml **Wash Buffer**, gently pipet to resuspend pellet.
13. Pellet sample for 5 min at 5,200 rpm (~2,500 x *g*). Pipet off and discard supernatant.
14. Add to pellet:
    50 µl **Wash Buffer**
    2.5 µl **20% SDS**
15. Vortex mix for >30 sec. Pipet to break up any clumps.
16. Continue protocol at Stage 2: Normalize Chromatin Samples.

Stage 2. Normalize Chromatin Samples (Box 1)

Note: See Appendix 1 for an alternative protocol to normalize chromatin.

1. Quantify 1 µl of the crosslinked sample using a Qubit and Qubit dsDNA HS Kit.
2. Add 500 ng of sample DNA to a new tube. Bring the total volume in the sample tube up to 50 µl with **Wash Buffer**.
   If a tissue sample contains 50-500 ng total DNA, use all the sample up to 100 µl in Stage 3, Step 1. If less than 50 ng total DNA is extracted do not continue with the sample.
3. Store remaining chromatin at 4°C for up to 5 days.

Stage 3. Bind Chromatin to Chromatin Capture Beads (Box 1)

1. Add 100 µl **Chromatin Capture Beads** to sample tube containing 50-100 µl of normalized chromatin.
2. Fully resuspend the beads and incubate for 5 min at room temperature off magnet.
3. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant. The pellet may be loose—switch to a 10 µl pipet tip to remove as much liquid as possible.
4. Wash beads twice with 200 µl **Wash Buffer**.

Optional Stopping Point: Sample can be resuspended in 200 µl of Wash Buffer and stored overnight at 4ºC. Pipet off and discard supernatant before starting Stage 4.

Stage 4. Restriction Enzyme Digest (Box 2, Purple Caps)

1. After the last wash has been removed, add to sample tube:
   50 µl **Restriction Digest Buffer**
   1 µl **Restriction Digest Enzyme Mix**
2. Fully resuspend the beads and incubate for 1 hr at 37°C in an agitating thermal mixer.

Stage 5. End Fill-In (Box 2, Green Caps)

1. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant.
2. Wash beads twice with 200 µl **Wash Buffer**.
3. Add to sample tube:
   50 µl **End Fill-in Buffer**
   1 µl **End Fill-in Enzyme Mix**
4. Fully resuspend the beads and incubate for 30 min at 25°C in an agitating thermal mixer.

Stage 6. Intra-Aggregate DNA End Ligation (Box 2, Clear Caps)

1. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant.
2. Wash beads twice with 200 µl **Wash Buffer**.
3. Add to sample tube:
   250 µl **Intra-Aggregate Ligation Buffer**
   1 µl **Intra-Aggregate Ligation Enzyme Mix**
4. Fully resuspend the beads and incubate for 1 hr at 16°C in an agitating thermal mixer.

Optional Stopping Point: Sample can be incubated at 16ºC in agitating thermal mixer for up to 16 hr.

Stage 7. Crosslink Reversal (Box 1 & 2, Brown Caps)

Note: Incubate the Crosslink Reversal Buffer for 10 min at 50°C before use.

1. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant.
2. Add to sample tube:
   50 µl **Crosslink Reversal Buffer**
   1 µl **Proteinase K**
3. Fully resuspend the beads.
4. Incubate for 15 min at 55°C, followed by 45 min at 68°C, in an agitating thermal mixer.
5. Quick spin the tube and place on the magnet for 2-5 min. Transfer 50 µl of the SUPERNATANT to a new tube. The tube containing the supernatant is now the sample. Discard beads.

Stage 8. DNA Purification on AMPure XP Beads (Box 1)

1. Add 100 µl resuspended **AMPure XP Beads** to the sample tube.
2. Fully resuspend the beads and incubate for 5 min at room temperature off magnet.
3. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant.
4. Leave tube on the magnet, and wash beads twice with 250 µl **80% EtOH**. There is no need to resuspend the beads for these washes.
5. Quick spin the tube and place on the magnet for 1 min. Use a thin pipet tip to remove traces of EtOH.
6. Air dry beads for 5 min on the magnet.
7. Add 54 µl **TE Buffer pH 8.0** to sample tube.
8. Fully resuspend the beads and incubate for 2 min at room temperature off magnet.
9. Quick spin the tube and place on the magnet for 2-5 min. Transfer 52 µl of the SUPERNATANT to a new tube. The tube containing the supernatant is now the sample. Discard beads.
10. Quantify 1 µl of the sample using a Qubit Fluorometer and Qubit dsDNA HS Kit.

For each sample you should recover more than 200 ng DNA. If less than 100 ng of sample DNA is recovered do not continue with sample.

11. Store the purified DNA sample at -20°C.

End of Day 1

Dovetail™ Hi-C Protocol Day 2

Getting Started

1. Day 2 should take roughly 7 hours. Allow an additional 2 hours if size selecting and QC sequencing.
2. Kit-supplied reagents are listed in **bold blue**, user-supplied reagents are listed in **bold black**.
3. Use standard practices for molecular biology including thawing, vortexing, and quick spinning down reagents before use.
4. There is 1 optional stopping point listed for Day 2.
5. If you are prepping several samples in tandem prepare a master mix at the start of each step.
6. An 'agitating thermal mixer' should be set at 1250 rpm for 1.5 ml tubes,1450 rpm for 0.2 ml/PCR tubes.
7. The times listed for leaving tubes on the magnets are a recommendation. In all cases wait until the solution is visibly devoid of beads before continuing with the protocol.
8. To wash beads, follow these steps:
    a. Add the listed reagent to the sample tube containing beads
    b. Vortex or pipet to resuspend the beads and quick spin the sample tube to remove liquid from the tube cap
    c. Place tube on an appropriate magnet for 1-3 min
    d. Carefully pipet off and discard the supernatant
9. Table 2 lists the equipment and reagents you will need for Day 2.

Table 2

User Supplied Material or Equipment	Stage Used In
Low-bind Eppendorf 1.5 ml Tubes	Throughout
PCR Tubes	Throughout
Magnet holders for tubes	Throughout
Thermal cycle	10,11,15
Thermal mixer	12
Diagenode Bioruptor Pico	9
Agilent TapeStation or Bioanalyzer	9, 17
Beckman-Coulter AMPure XP Beads	12, 16
80% EtOH	12, 16
Pippin Prep or AMPure XP Beads	17
Qubit	17

Stage 9. Fragmentation (Box 1)

Notes: See Appendix 2 for suggestions on fragmenting samples using a Covaris® M220 Focused-ultrasonicator. See Appendix 3 for examples of sufficient and insufficient sample fragmentation.

1. Cool the Diagenode Bioruptor Pico to 4˚C.
2. Transfer 200 ng of Day 1 purified DNA to a 0.1 ml Bioruptor tube.
3. Use **TE Buffer pH 8.0** to bring the total volume in each Bioruptor tube to 50 µl. If sample has less than 200 ng DNA, use up to 50 µl of the sample.
4. Cool DNA in Bioruptor tubes for 10 min on ice.
5. Fragment the sample as follows:
   Fragment 4 cycles of 30 sec ON/30 sec OFF
   Remove tubes from carousel. Quick spin and pipet mix
   Fragment 4 cycles of 30 sec ON/30 sec OFF
6. Check the size distribution of 2 µl of the fragmented samples on a TapeStation or Bioanalyzer. If the fragmented samples are centered substantially larger than 250-450 bp, refragment those samples for 3 more cycles of 30 sec ON/30 sec OFF. Confirm the size distribution of re-fragmented samples.
7. Save remaining DNA at 4˚C for up to 6 months.

Stage 10. End Repair (Box 2, Blue Caps)

1. Place in clean 0.2 ml tube:
   48 µl Fragmented Sample from Stage 9
   7 µl **End Repair Buffer**
   3 µl **End Repair Enzyme Mix**
   0.5 µl **250 mM DTT**
2. Vortex and quick spin sample tube.
3. Incubate for 30 min at 20˚C, followed by 30 min at 65˚C in a thermal cycler. Hold at 12˚C.

Stage 11. Adapter Ligation & USER Digest (Box 2, Red Caps)

Note: Add the Adapters For Illumina directly into the sample tube, do not make a master mix.

1. Add to 0.2 ml tube containing 58.5 µl of end-repaired sample:
   2.5 µl **Adapters for Illumina**
   30 µl **Ligation Enzyme Mix**
   1 µl **Ligation Enhancer**
2. Vortex and quick spin sample tube.
3. Incubate for 15 min at 20˚C. Hold at 12˚C.
4. Add 3 µl of **USER Enzyme Mix** to sample and pipet mix.
5. Incubate for 15 min at 37˚C. Hold at 12˚C.

Stage 12. DNA Purification (Box 1)

1. Add 80 µl resuspended **AMPure XP Beads** to the sample tube.
2. Fully resuspend the beads and incubate for 5 min at room temperature off magnet.
3. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant.
4. Leave tube on the magnet, and wash beads twice with 100 µl **80% EtOH**. There is no need to resuspend the beads for these washes.
5. Quick spin the tube and place on the magnet for 1 min. Use a thin pipet tip to remove traces of EtOH.
6. Air dry the beads for 5 min on the magnet.
7. Add 102 µl **TE Buffer pH 8.0** to sample tube.
8. Fully resuspend the beads and incubate for 2 min at room temperature off magnet.
9. Quick spin the tube and place on the magnet for 2-5 min. Transfer 100 µl of the SUPERNATANT to a new 1.5 ml tube. The tube containing the supernatant is now the sample. Discard the AMPure XP Beads.

Optional Stopping Point: Sample can be stored at -20ºC overnight.

Stage 13. Ligation Capture (Box 1)

Notes: Stage 13 does not involve any sample DNA until step 5.

1. For each sample, add 25 µl resuspended **Streptavidin Beads** to a new and empty 1.5 ml tube.
2. Place tube containing Streptavidin Beads on the magnet for 2-5 min. Pipet off and discard supernatant.
3. Wash the Streptavidin Beads twice with 200 µl **TWB**.
4. Add 100 µl **2X NTB** to the Streptavidin Beads.
5. Transfer 100 µl of the SAMPLE SUPERNATANT from Stage 12 to the tube containing the washed Streptavidin Beads and 100 µl of 2X NTB. This is now your sample tube.
6. Fully resuspend the Streptavidin Beads in the sample tube and incubate for 30 min in a 25˚C agitating thermal mixer.

Stage 14. Wash Sample on Streptavidin Beads (Box 1)

1. Quick spin tube and place on magnet for 2-5 min. Pipet off and discard supernatant.
2. Wash beads once with 200 µl **LWB**
3. Wash beads twice with 200 µl **NWB**.
4. Wash beads twice with 200 µl **Wash Buffer**.

Stage 15. Index PCR (Box 2, Black & White Caps)

Note: See Appendix 4 to choose Index Primers.

1. After the last wash has been removed, add to the sample tube:
   15 µl **DNase and RNase free H_2O**
   25 µl **HotStart PCR Ready Mix**
   5 µl **Universal PCR Primer**
   5 µl **Index Primer** (Unique to each sample)
2. Transfer sample, including Streptavidin Beads, to a 0.2 ml PCR tube. Fully resuspend the beads.
3. Amplify sample in a thermal cycler as follows:
   3 min at 98˚C
   11 cycles of:
   20 sec at 98˚C
   30 sec at 65˚C
   30 sec at 72˚C
   1 min at 72˚C
   Hold at 12˚C

Stage 16. DNA Purification on AMPure XP Beads (Box 1)

1. Quick spin the tube and place on the magnet for 2-5 min.
2. Transfer 50 µl of the SUPERNATANT to a new 1.5 ml tube. The tube containing the supernatant is now the sample. Discard beads.
3. Add 100 µl resuspended **AMPure XP Beads** to the sample tube.
4. Fully resuspend the beads and incubate for 5 min at room temperature off magnet.
5. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant.
6. Leave tube on the magnet, and wash beads twice with 200 µl **80% EtOH**. There is no need to resuspend the beads for these washes.
7. Quick spin the tube and place on the magnet for 1 min. Use a thin pipet tip to remove traces of EtOH.
8. Air dry beads for 5 min on the magnet.
9. Add 45 µl **TE Buffer pH 8.0** to sample tube.
10. Fully resuspend the beads and incubate for 2 min at room temperature off magnet.
11. Quick spin the tube and place on the magnet for 2-5 min. Transfer 43 µl of the SUPERNATANT to a new 1.5 ml tube. The tube containing the supernatant is now the Hi-C sequencing library.

Stage 17. Size Select and Quantify Final Library

1. Quantify 1 µl of the sequencing library using a Qubit and Qubit dsDNA HS Kit. Total DNA in library should be in the 1 -3 µg range.
2. Size select your sequencing library to 350-850 nt using a Sage Science Pippin Prep or similar instrument following manufacturer's instructions OR using AMPure XP Beads following the protocol in Appendix 5.
3. Qubit 1 µl of the size-selected library.
4. Use a Tapestation or Bioanalyzer to determine the average size, DNA concentration, and molarity of your size-selected library.
5. Average the Qubit and TapeStation results to determine library molarity as follows:
   $(Qubit_{ng/\mu l} \times TapeSation_{nM}) / TapeStation_{ng/\mu l} = Average\ Final_{nM}\ Library$
6. QC library on an Illumina® MiSeq or MiniSeq Instrument following manufacturer's instructions. Aim to acquire 1-2 million read pairs per library for the QC step.
7. Store the library and any remaining DNA at -20˚C.

End of Day 2

Appendix 1: Normalizing Chromatin

The chromatin normalization process in Stage 2 is imprecise due to the nature of the chromatin sample that is quantified. In our experience the protocol in Stage 2 robustly produces high-quality libraries with minimal hands-on time from the user. If more accurate chromatin normalization is desired the following alternative process can be used, it will require about 2 hours of time.

Alternative Stage 2. Normalize Chromatin All Samples (Box 1)

1. Remove 10% of your sample from Stage 1 and place into a new 1.5 ml tube. This is now your sample quantification tube. Use 13.1 µl from a tissue sample, 5.3 µl from a blood or cell culture sample
2. To each sample quantification tube add the following:
   50 µl **Crosslink Reversal Buffer**
   1 µl **Proteinase K**
3. Fully resuspend the beads and quick spin the tube.
4. Incubate for 15 min at 55°C in agitating thermal mixer.
5. Incubate for 45 min at 68°C in agitating thermal mixer.
6. Perform an AMPure XP bead cleanup using the following protocol (similar to Stage 8.)
   a. Add 100 µl resuspended **AMPure XP Beads** to the sample quantification tube.
   b. Fully resuspend the beads and incubate for 5 min at room temperature off magnet.
   c. Quick spin the tube and place on the magnet for 2-5 min. Pipet off and discard supernatant.
   d. Leave tube on the magnet, and wash beads twice with 250 µl **80% EtOH.** There is no need to resuspend the beads for these washes.
   e. Quick spin the tube and place on the magnet for 1 min. Use a thin pipet tip to remove traces of EtOH.
   f. Air dry beads for 5 min on the magnet.
   g. Add 21 µl **TE Buffer pH 8.0** to sample tube.
   h. Fully resuspend the beads and incubate for 5 min at room temperature off magnet.
   i. Quick spin the tube and place on the magnet for 2-5 min. Transfer 20 µl of the SUPERNATANT to a new tube. The tube containing the supernatant is now the sample to quantify. Discard beads.
7. Quantify 1-5 µl of the purified crosslink sample using a Qubit Fluorometer and Qubit dsDNA HS Kit.
8. Back calculate the amount of DNA in your sample tube from Stage 1 using your results from the sample quantification tube:
   - Sample Quantification Tube $Qubit_{ng/\mu l}$ x 20 µl x 10 = Total ng in sample tube from the end of Stage 1.
   - Divide the total ng of DNA in the sample tube by the volume of material in your sample tube to get your DNA concentration in ng/µl.
9. Proceed to Stage 2, step 2 of the main protocol.

Appendix 2: Covaris Fragmentation

The fragmentation protocol listed in Stage 9 has been optimized and validated using a Bioruptor® Pico Ultrasonicator. Covaris® instruments vary in recommended settings based on the instrument model and the volume being sonicated. To fragment DNA using a Covaris® M220 we have successfully used the following protocol. Users who plan to fragment with a Covaris should consult the documentation for their specific instrument.

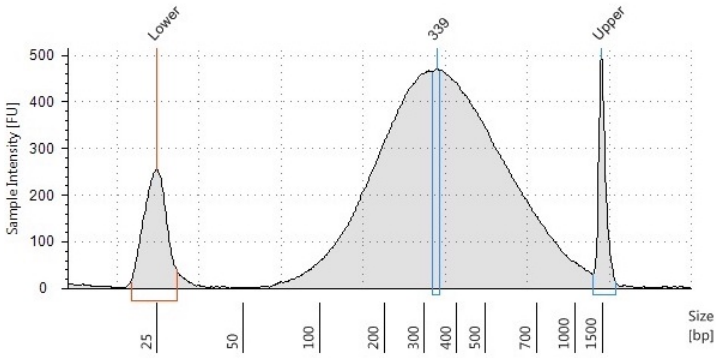
Alternative Stage 9. Covaris M220 Fragmentation

1. Transfer 200 ng of Day 1 purified DNA to a microTUBE AFA Fiber Snap-Cap (part no. 520045).
2. Use **TE Buffer pH 8.0** to bring the total volume in each tube to 55 µl. If sample has less than 200 ng DNA, use all of the sample and add buffer up to 55 µl.
3. Fragment using a Covaris M220 Focused-ultrasonicator at the following settings:
    a. Peak Incident Power (W): 50
    b. Duty Factor: 20%
    c. Cycles per Burst: 200
    d. Treatment time (s): 100
    e. Temperature (˚C): 20
    f. Sample volume (µl): 50
4. Check the size distribution of 2 µl of the fragmented samples on a TapeStation or Bioanalyzer. If the fragmented samples are centered substantially larger than 250-450 bp, refragment those samples.
5. Use 48 µl of the fragmented sample when continuing on to Stage 10.
6. Save remaining DNA at 4˚C for up to 6 months.

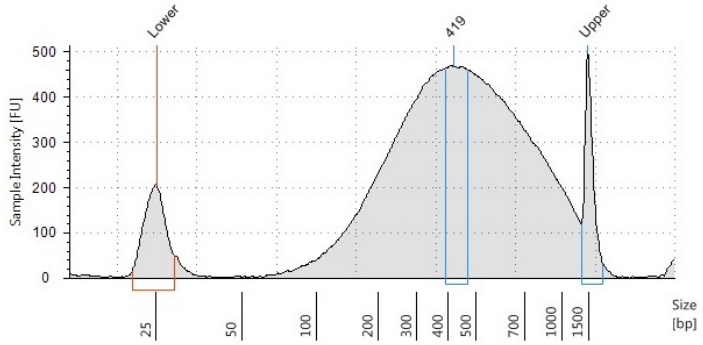
Appendix 3: Fragmentation Distributions

Sample fragmentation occurs in Stage 9. Samples should be fragmented to an average size of 250-450 bp. Below are examples of DNA size distributions after fragmentation. Panels A and B show samples that were sufficiently fragmented. Panel C shows a sample that was under-fragmented, this sample was refragmented before continuing on to End Repair (Stage 10).

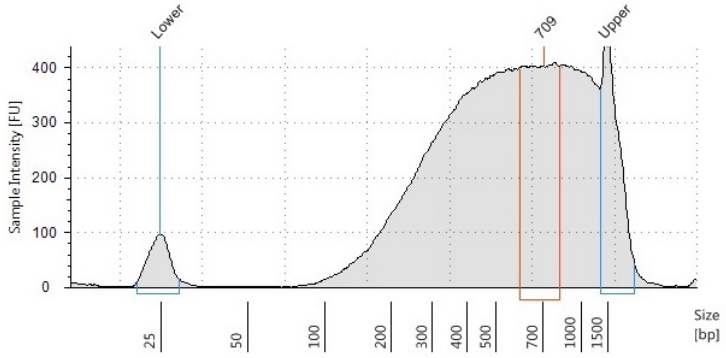
A: Sample sufficiently fragmented:



B: Sample sufficiently fragmented:



C: Sample under-fragmented, sample was refragmented:



Appendix 4: Index Multiplexing

The Hi-C kit includes 8 indexed adapter sequences, listed in this manual as "Index Primer [N]". To run multiple samples together on an Illumina® instrument, you must choose index primers that will maintain color balance. Choose index primers as listed below in Table 4. To learn more about multiplexing, see Illumina® documentation.

Table 3: Index Primers in the Hi-C kit

Index Primer	Sequence
Index Primer 2	CGATGT
Index Primer 4	TGACCA
Index Primer 5	ACAGTG
Index Primer 6	GCCAAT
Index Primer 7	CAGATC
Index Primer 8	ACTTGA
Index Primer 12	CTTGTA
Index Primer 19	GTGAAA

Table 4: Index Primers to use for multiplexing

Libraries to Plex	Index Primer Combination
2	6 and 12
2	5 and 19
3	2, 7, and 19
3	Either of the 2-plex options and any other Index Primer
4	5, 6, 12, and 19
4	Either of the 3-plex options and any other Index Primer

Appendix 5: Library Size Selection Using AMPure XP beads

Dovetail recommends size selecting the final sequencing library to between 350-850 base pairs using a Sage Selection Pippin Prep or similar instrument. If that is not available, then AMPure XP Beads can be used to size select the library. Here we list our protocol for size selecting on AMPureXP Beads.

Select away larger DNA fragments:

1. Use a Qubit to quantify the amount of DNA in the library.
2. Place 500 ng of the library in a new tube.
3. Bring the volume of the tube up to 100 µl using **TE Buffer pH 8.0**.
4. Add 60 µl resuspended **AMPure XP Beads** to tube containing the library.
5. Fully resuspend the beads.
6. Incubate for 10 min at room temperature.
7. Quick spin the tube and place on the magnet for 2-5 min.
8. Transfer 160 µl of the SUPERNATANT to a new tube. The tube containing the supernatant is now the library. Discard beads.

Select for DNA of the desired size:

9. Add 32 µl of resuspended **AMPure XP Beads** to the library.
10. Fully resuspend the beads.
11. Incubate for 10 min at room temperature.
12. Quick spin the tube, place on the magnet for 2-5 min. Pipet off and discard supernatant.
13. Leave tube on the magnet and wash beads twice with 200 µl of **80% EtOH**. There is no need to resuspend the beads for these washes.
14. Quick spin the tube and place on the magnet for 1 min. Use a thin pipet tip to remove traces of EtOH.
15. Air dry beads for 5 min on the magnet.
16. Add 20 µl **TE Buffer pH 8.0** to tube.
17. Fully resuspend the beads and incubate for 2 min at room temperature off magnet.
18. Quick spin the tube and place on the magnet for 2-5 min. Transfer 16 µl of the SUPERNATANT to a new 1.5 ml tube. The tube containing the supernatant is your size selected library. Discard the beads.
19. Continue the main protocol at Stage 17 step 3.

Appendix 6: Plants

This appendix provides guidance to Dovetail customers who wish to use the Hi-C kit for plant samples. If you have any questions, please contact our scientific support team at support@dovetail-genomics.com.

Plant Sample Guidelines

Each Hi-C library requires 250 mg of young leaves (no stems). Leaves harvested from plants at the one or two leaf seedling stage are the preferred sample type. Very young leaves from mature plants and plant tissue culture can also be used for this protocol.

Snap-freeze freshly harvested leaves immediately in liquid nitrogen and store samples at -80°C until use.

If shipping, we recommend sending already-frozen leaf samples overnight on plenty of dry ice.

Plant-Specific Equipment

User-supplied equipment and reagents:

1. 1 ml wide bore pipet tips
2. 5 ml or larger low binding tubes
3. Mortar and pestle and liquid nitrogen
4. Becton Dickinson BD™ Sterile 1 ml Tuberculin Syringes (Cat No. B309659)
5. 50 ml Wash Buffer (see below)

Dovetail-supplied equipment:

Note: Dovetail will ship these filters to you if requested, contact support@dovetail-genomics.com or your sales representative if you wish to receive filters.

1. 8 Becton Dickinson BD™ 50 µM Syringe Filcons (Cat No. 340603)
    - 50 µM filters have green tops
2. 16 Becton Dickinson BD™ 200 µM Syringe Filcons (Cat No. 340615)
    - 200 µM filters have white tops

50 ml Wash Buffer

Reagent	Volume	Final Concentration
Molecular Biology Grade Water	48.25 ml	
1 M Tris pH 8.0	0.5 ml	10 mM
5 M NaCl	1 ml	100 mM
10% Tween 20	0.25 ml	0.05%

Dovetail™ Hi-C Protocol for Plants

Stage 1. Crosslink Tissue And Prepare Chromatin

In Stage 1 the sample will be crosslinked and chromatin prepared. Make sure the PBS/formaldehyde added to each sample is freshly mixed and keep the timing of the crosslinking consistent across samples. If not already frozen, snap-freeze the leaves in liquid nitrogen before beginning Stage 1. Use chilled 1X PBS for Stage 1.

Stage 1: Crosslinking Plant Tissue Samples

Notes: See page 35 for visual guide on grinding the plant tissue. See page 36 if you wish to use a BD™ Medimachine. Incubate the kit-supplied 20% SDS at 37°C for 15 min before use.

1. Place 250 mg of plant tissue in a small mortar sitting in liquid nitrogen.
2. Grind leaves for 10-20 minutes until they have the consistency of flour, keeping frozen.
3. Place ground leaves in a 5 ml tube. Add to tube:
   2 ml **1X PBS**
   81 µl **37% Formaldehyde**
4. Vortex mix and rotate at room temperature for 15 min.
5. Pellet sample for 5 min at 5000 x *g* at room temperature. Carefully aspirate and discard the supernatant, not disturbing the pelleted sample.
6. Resuspend sample pellet in 2 ml **Wash Buffer** by vortexing.
7. Using an attachable 1 ml syringe, gently push the 2 ml resuspended sample through a **200 µM** filter into a clean 5 ml tube.
8. If the filter clogs, replace with a new **200 µM** filter to finish filtering the sample.
9. Gently pass an additional 1 ml **Wash Buffer** though the used **200 µM** filter into the 5 ml tube containing the 2 ml filtered sample.
10. Using the same syringe, filter the now 3 ml sample through a **50 µM** filter into a clean 5 ml tube.
11. Pellet sample for 5 min at 2500 x *g* at room temperature. Carefully aspirate and discard supernatant.
12. Resuspend pellet in 1 ml of **Wash Buffer** by vortexing. Transfer to 1.5 ml tube.
13. Repeat step 11.
14. Gently resuspend the pellet in 100 µl **Wash Buffer**.
15. Add 5 µl of **20% SDS**. Vortex 30 seconds to resuspend. Pipet to break up clumps if needed.
16. Incubate at 37°C for 15 minutes, with shaking.

Stage 2. Normalize Chromatin Plant Samples (Box 1)

1. Quantify 1 µl of the crosslinked sample using a Qubit and Qubit dsDNA HS Kit.
2. Add 800 ng of sample DNA to a new tube. Bring the total volume in the sample tube up to 100 µl with **Wash Buffer**.
   If the sample contains less than 800 ng total DNA, use all the sample up to 100 µl in Stage 3, Step 1.
3. Store remaining chromatin at 4°C for up to 5 days.

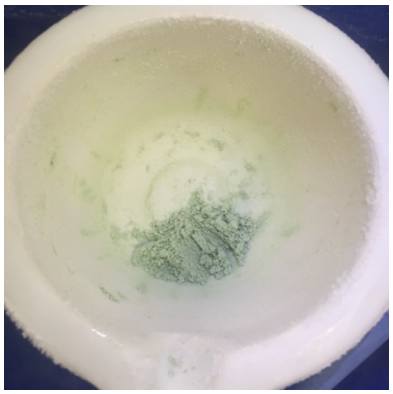
Continue Hi-C protocol at Stage 3 on page 17

Plant grinding and filtering

This protocol calls for grinding plant leaves until they are the consistency of flour. Below are examples of under and sufficiently ground samples, and the filter set up.

A: Under-ground sample

B: Sample ground to flour-like consistency



C: Filter set up

Alternative Plant Stage 1 with BD™ Medimachine

Alternative Stage 1: Crosslinking Plant Tissue Samples

Note: Incubate the kit-supplied 20% SDS at 37°C for 15 min before use.

1. Place 250 mg of plant tissue in a small mortar sitting in liquid nitrogen.
2. Grind leaves to a coarse powder keeping frozen. Transfer to a 5 ml tube.
3. Add to tube:
   2 ml **1X PBS**
   81 µl **37% Formaldehyde**
4. Vortex mix and rotate at room temperature for 15 min.
5. Pellet sample for 5 min at 5000 x *g* at room temperature. Carefully aspirate and discard the supernatant, not disturbing the pelleted sample.
6. Resuspend sample pellet in 2 ml **Wash Buffer** by vortexing.
7. Pellet sample for 5 min at 5000 x g at 4°C. Repeat spin if debris are still floating. Carefully aspirate and discard supernatant.
8. Resuspend pellet in 1 ml **Wash Buffer**. Keep tube on ice.
9. Prewet Medicon by pipetting 1 ml **Wash Buffer** onto both sides of the blade. Rotate the blade to ensure wetting the entire filter. Remove the buffer with a 1 ml syringe.
10. Using a wide bore 1ml pipet tip, transfer 1 ml of sample onto the Medicon and load into Medimachine. Retain the sample tube for the second grind.

First Grind:

11. Grind plant tissue for 60 sec. Pipet mix to redistribute sample in the Medicon. Grind tissue again for 60 sec.
12. Aspirate sample into 1 ml syringe and gently filter through a 50 µM filter into 5 ml tube. Store the tubes on ice.

Second Grind:

13. Rinse the input sample tube with 1 ml **Wash Buffer** to recover any cells/debris, and pipet onto the Medicon.
14. Grind for two cycles and filter sample through the same 50 µM filter, repeating steps 11-12. There will now be 2 mls of ground and filtered sample in the 5ml sample tube.

Third Grind:

15. Add 1 ml **Wash Buffer** onto the Medicon.
16. Grind for two cycles and filter sample through the same 50 µM filter, repeating steps 11-12. There will now be 3 mls of ground and filtered sample in the 5 ml sample tube.
17. Pellet the nuclei in the sample tube at 2500 x g for 5 min at room temperature. Carefully aspirate and discard supernatant.
18. Gently resuspend the nuclei pellet in 1 ml **Wash Buffer** using low retention pipet tips.
19. Transfer resuspended sample to a 1.5 ml low bind tube.
20. Pellet at 2500 x g for 5 min at room temperature. Carefully aspirate and discard supernatant.
21. Gently resuspend the pellet in 100 µl **Wash Buffer**.
22. Add 5 µl of **20% SDS**. Vortex 30 seconds to resuspend. Pipet to break up clumps if needed.
23. Incubate at 37°C for 15 minutes, with shaking.

Stage 2. Normalize Chromatin Plant Samples (Box 1)

4. Quantify 1 µl of the crosslinked sample using a Qubit and Qubit dsDNA HS Kit.
5. Add 800 ng of sample DNA to a new tube. Bring the total volume in the sample tube up to 100 µl with **Wash Buffer**.
   If the sample contains less than 800 ng total DNA, use all the sample up to 100 µl in Stage 3, Step 1.
6. Store remaining chromatin at 4°C for up to 5 days.

Continue Hi-C protocol at Stage 3 on page 17

Appendix 7: QC and HiRise Sequencing Guidelines

Sequencing Guidelines for QC analysis

Dovetail's QC analysis was developed as part of our in-house HiRise™ genome assembly service. It allows users to validate the quality of a Hi-C library before deep sequencing and running HiRise™. Dovetail provides all kit and HiRise™ customers with access to our QC analysis.

The QC pipeline requires an input assembly, and 1-2 million 2x75 or greater paired-end reads, sequenced from the Hi-C library. Generally this light sequencing is accomplished using an Illumina® iSeq, MiSeq, or MiniSeq platform, but can be performed on most Illumina® instruments.

Please refer to the Dovetail Sequencing and Analysis Manual for more information on QC and HiRise™.

Guidelines on Input Assembly and Sequencing for HiRise™

Dovetail's HiRise™ software scaffolds an input genome using the long-range information available from Hi-C libraries. For best results HiRise™ users should provide a draft input assembly with a N50 greater than 1 Mb and a N90 greater than 20 kb, in addition to the Hi-C library data.

See the table below for guidelines on how to determine the number of Hi-C libraries to generate, and the minimum number of read pairs to sequence for your genome if running HiRise™*. The number of read pairs is from a combined pool of the Hi-C libraries. For example, for a 2 Gb complex genome we recommend generating 2 Hi-C libraries, combining them, and then sequencing at least 250 million read pairs from the combined pool. These are minimum sequencing guidelines, sequencing additional read pairs will increase the contiguity of the HiRise™ assembly.

	Simple Genomes		**Complex Genomes**	
Genome size (Gb)	No. Hi-C libraries	No. Read pairs to sequence (Millions)	No. Hi-C libraries	No. Read pairs to sequence (Millions)
1	1	100	1	100
2	1	200	2	250
3	1	300	2	450
4	2	400	3	500
5	2	500	3	850

"**Simple Genomes**" are diploid or haploid, have repetitive content of less than 30%, and heterozygosity of less than 0.005%. Humans, many mammals, and some fish and birds are examples of simple genomes.

"**Complex Genomes**" contain any of the following: polyploidy, repeat content above 30%, heterozygosity above 0.005%. If any of the above metrics are unknown, use the complex genome sequencing guidelines. Many plants, salmonid fish, and amphibians are examples of complex genomes.

*Customers not using HiRise™ will need to determine how much sequencing is required for their specific applications and software.

Please refer to the Dovetail Sequencing and Analysis Manual for more information on QC and HiRise™.