



Proximo™ Hi-C Kit

Version 3.0

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Proximo Hi-C (Plant) Kit Protocol



For Crude-Sample Proximity Ligation Library Prep from Plant Samples,
for Illumina® Sequencing

This document applies to Proximo Hi-C (Plant) Prep Kits KT3030.

Please read through this protocol before beginning your experiment. For customer support, please email support@phasegenomics.com or reference our [Frequently Asked Questions](#).

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Kit Specifications

Kit Contents

| Cap/label color | Ref. Code | Top label | Component name | Volume | Number of tubes | Storage temp. (°C) | Used in Step | Before Starting |
|-----------------|-----------|---------------------------|----------------------------------|-----------|-----------------|--------------------|---------------------------------|--|
| | KS0002 | Crosslink Solution | Crosslinking Solution | 10 mL | 1 | -25 to +25 °C | 1.1 | Thaw to RT |
| | KS0003 | Quench Solution | Quenching Solution | 1 mL | 1 | -25 to +25 °C | 1.3 | Thaw to RT* |
| | KB0002 | Lysis Buffer 1 | Lysis Buffer 1 | 4 mL | 1 | -25 to +25 °C | 2.1-2.2 | Thaw to RT |
| | KB0003 | Lysis Buffer 2 | Lysis Buffer 2 | 1 mL | 1 | -25 to +25 °C | 2.7 | Thaw to RT |
| | KB0006 | Fragment Buffer | Fragmentation Buffer | 300 µL | 1 | -25 to -15 °C | 3.1 | Thaw on ice |
| | KE0004 | Fragment Enzyme | Fragmentation Enzyme | 6 µL | 1 | -25 to -15 °C | 3.2 | Thaw on ice |
| | KE0016 | Finishing Enzyme | Finishing Enzyme | 6 µL | 1 | -25 to -15 °C | 3.4 | Thaw on ice |
| | KS0004 | Stop Solution | Stop Solution | 15 µL | 1 | -25 to -15 °C | 3.5 | Thaw to RT |
| | KB0007 | Ligation Buffer | Ligation Buffer | 200 µL | 1 | -25 to -15 °C | 4.1 | Thaw on ice |
| | KE0005 | Ligation Enzyme | Ligation Enzyme | 10 µL | 1 | -25 to -15 °C | 4.2 | Thaw to RT |
| | KE0007 | RX Enzyme | RX Enzyme | 10 µL | 1 | -25 to -15 °C | 5.1 | Thaw on ice |
| | KB0015 | Elution Buffer | Elution Buffer | 300 µL | 1 | -25 to +25 °C | 6.8, 10.12 | Thaw to RT |
| | KR0011 | Recovery Beads | Recovery Beads | 600 µL | 1 | +2 to +8 °C | 2.9, 3.1, 4.1, 10.3, 10.6 | Warm to RT |
| | KB0035 | Recovery Wash Concentrate | Recovery Wash Concentrate | 400 µL | 1 | +2 to +8 °C | 6.6, 10.9 | Warm to RT. Mix. 200 µL concentrate with 1 mL 95% Ethanol ** |
| | KR0002 | Strept Beads | Streptavidin Beads | 40 µL | 1 | +2 to +8 °C | 7.1 | Warm to RT |
| | KB0012 | Bead Bind | Bead Binding Buffer | 250 µL | 1 | -25 to +25 °C | 7.8 | Warm to RT |
| | KR0001 | Bead Reagent | Bead Reagent | 25 µL | 1 | -25 to -15 °C | 7.11 | Thaw on ice |
| | KB0011 | Wash Buffer 1 | Wash Buffer 1 | 3 mL | 1 | -25 to +25 °C | 7.4, 7.7, 7.19, 8.16 | Thaw to RT |
| | KB0012 | Wash Buffer 2 | Wash Buffer 2 | 3 mL | 1 | -25 to +25 °C | 7.15, 7.18, 8.12, 8.15 | Thaw to RT |
| | KB0009 | Library Buffer | Library Buffer | 50 µL | 1 | -25 to -15 °C | 8.5 | Thaw on ice |
| | KE0009 | LRI/Library Reagent 1 | Library Reagent 1 | 10 µL | 1 | -25 to -15 °C | 8.6 | Thaw on ice |
| | KS0005 | Library Reagent 2 | Library Reagent 2 | 10 µL | 1 | -25 to +25 °C | 8.8 | Thaw to RT |
| | KE0011 | HSR Mix | PCR Hot Start Ready Mix/ HSR Mix | 55 µL | 1 | -25 to -15 °C | 9.2 | Thaw on ice |
| | KP000N*** | Index | Index Mix | 5 µL each | 2 | -25 to -15 °C | 9.3 | Thaw on ice |
| | KB0017 | 10X CRB | 10X CRB | 1.6 mL | 1 | -25 to +25 °C | 1.6, 1.11, 2.6, 2.13, 3.8, 3.11 | Dilute to 1X before use.*** |

*Can heat at 37 °C to re-dissolve any precipitate that has crashed out after freezing

**Prepared Recovery Wash Buffer can be stored at +2 to +8 °C for up to 6 months

***1X CRB is stable stored at room temperature for up to 1 year

Shipping, Storage and Handling

Proximo Hi-C Kits (Plant) are shipped on cold packs. Upon receipt, remove inner container with Recovery Beads, Recovery Buffer Concentrate, and Streptavidin beads and store this at +2 to +8 °C. Store the remainder of the kit between -25 and -15 °C. When stored under these conditions, and handled appropriately, all kit components will retain full activity until the expiration date indicated on the kit label.

Always ensure that all components are fully thawed and thoroughly mixed prior to use. Keep all enzymes and Library Reagent 1 on ice at all times during use.

Safety Information

When working with chemicals, always wear personal protective gear, such as a lab coat, disposable gloves, and safety glasses. For more information consult the appropriate safety data sheets (SDS). These are available online at www.phasegenomics.com

Other Reagents, Equipment and Consumables Required

Reagents

The following molecular-biology grade reagents are required to complete this protocol. Ensure that reagents are free of DNA, RNA and nucleases.

- 80% Ethanol
- Molecular biology-grade Water

Equipment and Consumables

No specialist equipment is required for this protocol. The following general laboratory equipment and consumables are needed.

- Calibrated 2 – 10 µL pipette and filtered tips
- Calibrated 10 – 100 µL pipette and filtered tips
- Calibrated 200 – 1000 µL pipette and filtered tips
- 1.5 or 2 mL microcentrifuge tubes
- 0.2 mL PCR tubes
- Magnetic tube rack/magnet for 2 mL microcentrifuge tubes or 0.2 mL PCR tubes (depending on tube type used in step 2.8).
- Microcentrifuge capable of $\geq 6,000 \times g$
- Thermocycler
- Heating block or water bath that can maintain a temperature of 12°C and 65°C (alternatively use a thermocycler)
- Vortexer
- liquid nitrogen or dry ice
- sterilized mortar and pestle or comparable tool
- Qubit™ Fluorometer and Qubit™ dsDNA DNA HS Assay Kit (Thermo Fisher Scientific), or similar fluorometric assay for the quantification of double-stranded DNA

Workflow Overview

Notes

While both leaf number and mass inputs are provided, sample not limiting, err on the side of more sample than less.

The sample should be incubated at least 1 hr, however extending the incubation time up to 16 hours is not detrimental to sample preparation.

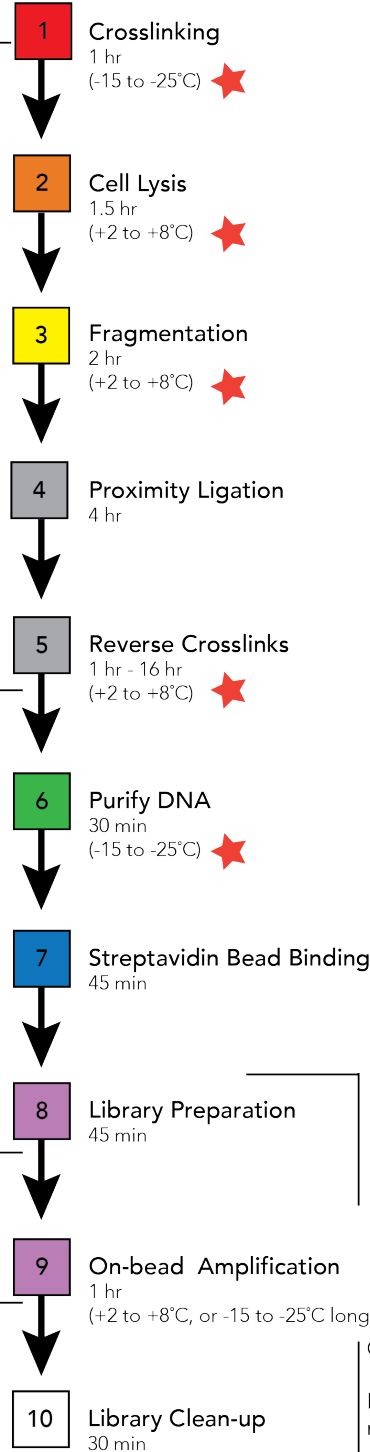
The appropriate amount of Library Reagent 1 is determined during on-bead quantification after streptavidin binding. See table at step 8.6.

Choose the number of PCR cycles based on yield determined during on-bead quantification after Step 7.

Recommended QC

Listed times are estimates including incubations and time at-bench. The listed times assume familiarity with basic molecular biology techniques.

★ indicates a safe stopping point and recommended storage temperature



Quantify streptavidin-bound DNA using Qubit dsDNA HS to ensure appropriate amount of DNA is carried into library preparation. It is essential that the beads are well resuspended prior to quantitation.

Quantify library with Qubit dsDNA HS.

Electrophoretic profile of library to assess fragment distribution

Small amount of sequencing to assess number and quality of Hi-C contacts

Detailed Protocol

1 Crosslinking (Red)

- 1.1 Finely chop 2-3 leaves or plant seedling tissue.

Use 0.5 – 1 g of plant tissue total, disposing of the stem and other “woody” plant tissue. Younger leaves generally yield superior results.

- 1.2 Transfer tissue to a 15 mL or 50 mL conical and add 10 mL **Crosslinking Solution**.

- 1.3 Incubate for 15 min at room temperature with occasional mixing by inversion or rotation.

- 1.4 Add 1 mL **Quenching Solution**.

- 1.5 Incubate for 20 min at room temperature with occasional mixing by inversion or rotation.

- 1.6 Wash the pellet with 1 mL **1X CRB** (prepared from included 10X CRB) and spin the microcentrifuge tube for 5 min at 6,000 x *g* to gently compact the cellular material. Carefully remove and discard the supernatant.

*Perform 1 min 1,000 x *g* spin as needed to assist in removal of supernatant.*

SAFE STOPPING POINT: Pellet can be stored at -15 to -25 °C

2 Cell Lysis (Orange)

Pre-heat a heating block, water bath, or thermocycler to 65° C (for use in step 2.9)
Warm Recovery Beads to room temperature (for use in step 2.10)

- 2.1 Chill in liquid nitrogen or dry ice and grind to a fine powder.
- 2.2 Divide the ground tissue between several (2-4) 2 mL microcentrifuge tubes and add 600 µL of **Lysis Buffer 1** to each tube.
- 2.3 Incubate for 20 min at room temperature with occasional gentle mixing.
- 2.4 Centrifuge at 500 x g for 1 min
- 2.5 Carefully transfer the **chromatin-containing supernatant** to a fresh microcentrifuge tube. Avoid disturbing the pellet.

Avoid transferring large amounts of debris, but a small amount of contamination from a pellet is not cause for concern.

- 2.6 Centrifuge at 6,000 x g for 5 min and discard the supernatant. **The chromatin is now in the pellet.**
- 2.7 Resuspend the pellet in 500 µL **1X CRB** and centrifuge at 6,000 x g for 5 min. Gently remove and discard the supernatant.
- 2.8 Resuspend the pellet in 100 µL **Lysis Buffer 2**.

If using a thermocycler for subsequent incubation steps, the chromatin-containing solution should be transferred to a 0.2 mL PCR tube at this time.

- 2.9 Incubate at 65° C for 15 min.
- 2.10 Allow sample tube to cool to room temperature. Thoroughly resuspend **Recovery Beads** and add 100 µL to sample tube. Mix thoroughly by vortexing or by pipetting.

*Chromatin binds irreversibly to **Recovery Beads**. The crosslinked DNA-protein complexes will remain bound to the beads until completion of **Step 5: Reverse Crosslinks**.*

- 2.11 Incubate at room temperature for 10 min.

- 2.12 Place the sample tube on a magnetic tube rack or magnet.
- 2.13 Once the solution has cleared, remove the supernatant without disrupting the beads.
- 2.14 Remove the tube from the magnet. Gently resuspend in 200 μ L 1X CRB to wash.

SAFE STOPPING POINT:

Beads suspended in 1X CRB may be stored overnight at +2 to +8 °C.

- 2.15 Return the sample tube to the magnetic rack.

Some samples may take a while to adhere to the magnet at this step. Just be patient, and careful when pipetting to remove the supernatant!

- 2.16 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.

3 Fragmentation (Yellow)

If using thermocycler program:

make sure program is setup before beginning. (see step 3.3)

If using heat blocks:

Pre-heat thermocycler or heat block to 37 °C (for use in step 3.3)

Pre-cool a thermocycler or cooling block to 12 °C (for use in steps 3.3 and 3.4)

3.1 Resuspend the pellet fully in 150 µL **Fragmentation Buffer**.

3.2 Add 2.5 µL **Fragmentation Enzyme**.

3.3 Incubate the sample according to the following:

| Step | Temperature (°C) | Time |
|---------------|------------------|------|
| Fragmentation | 37 | 1 hr |
| Cooling | 4 | Hold |

3.4 Once sample has cooled to 4 °C, add 2.5 µL **Finishing Enzyme** to the reaction and mix by gently vortexing or thoroughly pipetting. Return the tube to the thermocycler/cooling block used in step 3.3 and proceed with the incubation as follows:

Do not add Finishing Enzyme until sample has fully cooled to 4 °C

| Step | Temperature (°C) | Time |
|-----------|------------------|--------|
| Finishing | 12 | 30 min |

3.5 Add 6 µL **Stop Solution** and mix by gently vortexing or thoroughly pipetting to terminate the fragmentation reaction.

*Promptly add **Stop Solution** after 30 minutes at 12 °C. Extended incubation at 12 °C is detrimental to the library prep.*

3.6 Place the sample tube on a magnetic tube rack or magnet.

3.7 Once the solution has cleared, remove the supernatant without disrupting the beads.

- 3.8 Remove the tube from the magnet. Gently resuspend the beads in 200 μ L **1X CRB** to wash.
- 3.9 Return the sample tube to the magnet.
- 3.10 Once the Solution has cleared, remove and discard the supernatant without disrupting the beads.
- 3.11 Repeat steps 3.8 – 3.10 one more time with 200 μ L **1X CRB** per wash, for a total of two washes.

SAFE STOPPING POINT: Store beads at +2 to +8 °C.

4 Proximity Ligation (Clear)

- 4.1 Resuspend the pellet in 95 μ L **Ligation Buffer**.
- 4.2 Add 5 μ L **Ligation Enzyme** and mix.
- 4.3 Incubate the sample according to the following:

| Step | Temperature (°C) | Time |
|---------------------|------------------|--------|
| Ligation | 25 | 4 hr |
| Enzyme Inactivation | 65 | 10 min |
| | 4 | Hold |

5 Reverse Crosslinks (Clear)

Pre-heat thermocycler or heat block to 65°C (for use in step 5.2)

- 5.1 Add 5 μ L **RX Enzyme** to the ligation reaction and mix well by vortexing or pipetting.
- 5.2 Incubate at 65°C for at least 1 hr (up to 18 hours). **Your sample is now in the supernatant and no longer bound to the beads.**

SAFE STOPPING POINT: The reaction may be incubated at 65°C overnight, or stored at +2 to +8°C overnight after the 1 hr incubation at 65°C.

6 Purify DNA (Green)

Prepare Recovery Wash Buffer.

To prepare, add 1 mL 95% Ethanol to 200 µL provided Recovery Wash Concentrate and mix well.

- 6.1 Allow sample tube to cool to room temperature.
- 6.2 Resuspend **Recovery Beads** and add 100 µL **Recovery Beads** to the sample tube and mix thoroughly by vortexing or pipetting.

DNA has been released back into the supernatant during 5 Reverse Crosslinks. More beads are added here to re-bind the DNA to the beads.

- 6.3 Incubate at room temperature for 10 min.
- 6.4 Place the sample tube on a magnetic tube rack or magnet.
- 6.5 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- 6.6 Keeping beads on the magnet, gently wash beads twice with 200 µL **Recovery Wash Buffer** without disrupting the beads, leaving buffer on the beads for at least 30 sec between washes.
- 6.7 Air dry the beads at room temperature 10-15 min on the magnet with the cap open.
Over-drying is not problematic for Recovery Beads. They can be left drying for up to an hour, but should not need more than 10-15 minutes.
- 6.8 Remove the sample tube from the magnet and thoroughly resuspend the beads in 100 µL **Elution Buffer**.
- 6.9 Incubate for 10 min at room temperature to elute the DNA.
- 6.10 Place the sample tube on a magnetic tube rack or magnet.

- 6.11 Once the solution has cleared, recover the DNA-containing-supernatant and transfer to a fresh tube. Discard the beads.

SAFE STOPPING POINT:

Purified, proximity-ligated DNA may be stored at -15 to -25° C (indefinitely)

7 Streptavidin Bead Binding (Blue)

A. Prepare the Beads

- 7.1 Thoroughly resuspend the Streptavidin Beads and transfer 20 μ L into a new 2 mL microcentrifuge tube (or 0.2 mL PCR tube).
- 7.2 Place the tube on a magnetic tube rack or magnet for at least 30 sec.
- 7.3 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.

Do not yet combine the beads with the DNA recovered in Step 6.

- 7.4 Remove the tube from the magnet. Gently resuspend the beads in 200 μ L **Wash Buffer 1**.
 - 7.5 Return the sample tube to the magnet.
 - 7.6 Once the solution has cleared, remove and discard the supernatant without disrupting the beads
- 7.7 Repeat steps 7.4 – 7.6 one more time with 200 μ L **Wash Buffer 1** per wash, for a total of two washes.
 - 7.8 Remove beads from the magnet and resuspend in 100 μ L **Bead Binding Buffer**.

B. Bind the Sample to the Beads

- 7.9 Transfer 100 μ L of purified DNA (from Step 6) to the **washed Streptavidin Beads** (from Step 7.8) and mix thoroughly by gentle vortexing or pipetting.
- 7.10 Incubate 5 min at room temperature, mixing occasionally by gentle vortexing or inversion.
- 7.11 Add 10 μ L **Bead Reagent** and mix thoroughly by gentle vortexing or pipetting.
- 7.12 Incubate 5 min at room temperature, mixing occasionally by gentle vortexing or inversion.
- 7.13 Return the sample tube to the magnet.
- 7.14 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- 7.15 Remove the tube from the magnet. Gently resuspend the beads in 200 μ L **Wash Buffer 2**.
- 7.16 Return the sample tube to the magnet.
- 7.17 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- 7.18 Repeat steps 7.15 – 7.17 one more time with 200 μ L **Wash Buffer 2** per wash, for a total of two washes.
- 7.19 Repeat steps 7.15 – 7.17 one time with 200 μ L **Wash Buffer 1**.
- 7.20 Remove the tube from the magnet. Gently resuspend the beads in 200 μ L of **molecular biology-grade water**.
- 7.21 Measure the concentration of DNA (while still bound to the streptavidin beads) using a Qubit™ dsDNA HS Assay Kit or similar fluorometric assay.

It is essential that the beads are well resuspended in the molecular biology-grade water prior to quantification by fluorometry. Vortex the beads in the fluorometric assay tube immediately prior to measuring DNA concentration to ensure an accurate measurement.

8 Library Preparation (Purple)

Pre-heat thermocycler or heat block to 55°C (for use in step 8.7)

- 8.1 Transfer no more than 50 ng of DNA-containing streptavidin beads to a fresh microcentrifuge tube.

Be sure to resuspend the beads thoroughly prior to transfer. Any remaining beads may be stored at +2 to +8°C for subsequent analysis. If the amount of DNA measured in Step 7.21 is <50 ng, or even undetectable, proceed with the full 200 µL of beads from step 7.

Additionally, if the volume of beads transferred is less than 15 µL, allow additional time for the beads to adhere to the magnet and be very careful when removing the supernatant.

- 8.2 Place the sample tube on a magnetic tube rack or magnet.
- 8.3 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- 8.4 Resuspend the beads in 20 µL of **molecular biology-grade water**.
- 8.5 Add 25 µL **Library Buffer**
- 8.6 Add the appropriate amount of **Library Reagent 1** and **molecular biology-grade water** as determined by the table below. Use no more than 50 ng sample. Mix by gentle vortexing or pipetting.

| Total Sample Measured at Step 7.21 | Library Reagent 1 Volume (µL) | Water volume (µL) |
|------------------------------------|-------------------------------|-------------------|
| ≤ 10 ng | 1 | 4 |
| 10 – 20 ng | 2 | 3 |
| 20 – 30 ng | 3 | 2 |
| 30 – 40 ng | 4 | 1 |
| 40 – 50 ng | 5 | 0 |

- 8.7 Incubate at 55°C for 10 min.
- 8.8 Add 5 µL **Library Reagent 2**. Mix by gentle vortexing or pipetting.

- 8.9 Incubate at 55° C for 10 min.
- 8.10 Place the sample tube on a magnetic tube rack or magnet.
- 8.11 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- 8.12 Remove the tube from the magnet. Gently resuspend the beads in 200 µL **Wash Buffer 2**.
- 8.13 Return the sample tube to the magnet.
- 8.14 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- 8.15 Repeat steps 8.12 – 8.14 three more times with 200 µL **Wash Buffer 2** per wash, for a total of four washes.
- 8.16 Repeat steps 8.12 – 8.14 two times with 200 µL **Wash Buffer 1** per wash.
- 8.17 Repeat steps 8.12 – 8.14 one time with 200 µL **molecular biology-grade water**.

9 On-bead Library Amplification (Purple)

- 9.1 Resuspend the beads in 19 μ L molecular biology-grade water.
- 9.2 Add 26 μ L HSR Mix (PCR Hot Start ReadyMix).
- 9.3 Add 5 μ L of one **PCR Index Mix** (use a different index for each sample. 2 unique indexes are provided with each kit. Sequences are found on page 23 of this document). Mix samples well by gentle vortexing or pipetting.
- 9.4 Amplify the library in a thermocycler programmed as follows:

| Step | Temperature (°C) | Time | Cycles |
|----------------------|------------------|--------|--------|
| | 72 | 5 min | 1 |
| Initial Denaturation | 98 | 30 sec | 1 |
| Denaturation | 98 | 10 sec | 12* |
| Annealing | 62 | 20 sec | |
| Extension | 72 | 50 sec | |
| | 4 | Hold | 1 |

**If less than 20 ng DNA was carried into step 8 (Library preparation), increase the number of PCR cycles to 15*

SAFE STOPPING POINT:

PCR reaction can be held overnight at +2 to +8 °C, or stored at -15 to -25 °C (indefinitely)

10 Library Clean-up and Double-sided Size Selection (White)

Use Recovery Wash Buffer Prepared at step 6.

- 10.1 Place the sample tube on a magnetic tube rack or magnet.
- 10.2 Once the solution has cleared, transfer the library-containing supernatant to a new tube. Discard the **streptavidin beads**.
- 10.3 Add 55 μL (1.1X volume) of thoroughly resuspended **Recovery Beads** to the tube containing the library (from step 10.2).

Unwanted high molecular weight fragments are binding to the beads.

- 10.4 Incubate at room temperature for 10 min.
- 10.5 Place the sample tube on a magnetic tube rack or magnet. **Your library is in the supernatant. Do not discard.**
- 10.6 After 5 min, or once the solution has cleared, transfer the supernatant (105 μL) to a new tube containing 17.5 μL **Recovery Beads**.

The library is now binding to the beads, leaving unwanted small fragments in the supernatant.

- 10.7 Incubate at room temperature for 10 min.
- 10.8 Place the sample tube on a magnetic tube rack or magnet.
- 10.9 Keeping beads on the magnet, gently wash beads twice with 200 μL **Recovery Wash Buffer** without disrupting the beads, leaving buffer on the beads for at least 30 sec between washes.
- 10.10 Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- 10.11 Air dry the beads at room temperature 10-15 min on the magnet with the cap open.

*Over-drying is not problematic for **Recovery Beads**. They can be left drying for up to an hour, but should not need more than 10-20 minutes.*

- 10.12 Remove the sample tube from the magnet and thoroughly resuspend the beads in 30 μ L **Elution Buffer**.
- 10.13 Incubate for 5 min at room temperature to elute the DNA.
- 10.14 Place the sample tube on a magnetic tube rack or magnet.
- 10.15 Once the solution has cleared, recover the **Proximo Hi-C Library-containing-supernatant** and transfer to a fresh microcentrifuge tube. Discard the beads.

11 Library QC (recommended)

- 11.1 Measure the concentration of DNA using a Qubit™ dsDNA HS Assay Kit or similar fluorometric assay

Yields over 0.5 ng/μL are a strong indication of a successful library. The library can be stored at -15 to -25 °C

- 11.2 Assess library distribution using BioAnalyzer or similar instrument.

Acceptable sequencing-ready libraries have average distributions ranging between ~ 200 – 600 bp. If resulting library is outside that range, size selection is recommended.

Before performing a full sequencing run, it is recommended to sequence a small number of reads (approximately 1 million read pairs) to assess the quality of your Hi-C library. These data can be analyzed using our open source [Hi-C tools](https://github.com/phasegenomics/hic_qc) (available at https://github.com/phasegenomics/hic_qc).

12 Sequencing

Proximo Hi-C libraries are compatible with any Illumina® sequencer

| Genome Size | Sequencing Recommendation |
|-------------------|--|
| < 0.4 Gbp | > 100 million pairs, paired-end 75 bp or greater |
| 0.4 Gbp – 1.5 Gbp | > 150 million pairs, paired-end 75 bp or greater |
| > 1.5 Gbp | > 200 Million pairs, paired-end 75 bp or greater |

Note: these are meant as guidelines for amounts of data required to scaffold genomes. Actual requirements will vary between genomes and are dependent on assembly quality.

Need help with analysis?

Take a look at what we offer at

<http://phasegenomics.com/technology/bioinformatics/>

Or ask us about our analysis platform at info@phasegenomics.com

Index Primers

Included with your kit are Phase Genomics dual-unique indexes. Two dual-unique indexes are included with each Proximo Hi-C Kit but others are available at www.phasegenomics.com.

| Index | i7 Equivalent Index | i5 Equivalent Index (For iSeq, NovaSeq, MiSeq, and HiSeq 2000/2500 sample sheet) | i5 Equivalent Index For MiniSeq, NextSeq, and HiSeq 3000/4000 sample sheet) |
|-------|---------------------|---|---|
| A1 | TCAAGATC | TGACGTAG | CTACGTCA |
| B1 | GAGCGCCA | AACTCTCC | GGAGAGTT |
| D1 | ACGACAGA | AGTCTGGT | ACCAGACT |
| E1 | TAATGATG | GTATCGAA | TTCGATAC |
| F1 | ACATTACC | AGTACAGG | CCTGTACT |
| G1 | CAGTCGAC | ACTAGCCT | AGGCTAGT |
| H1 | TGTCGTTT | TCCTAGCA | TGCTAGGA |
| A2 | CAAAGTGT | CGAGTTGC | GCAACTCG |
| B2 | GCGCGGTG | ACCCGACC | GGTCGGGT |
| C2 | AGTGTGTG | GAAATTTT | AAAATTTT |
| A3 | TCAATCCG | ACTGCGAA | TTCGCAGT |
| B3 | CGCTACAT | TAGTCTCG | CGAGACTA |
| C3 | GATCCACT | TGAGCTGT | ACAGCTCA |
| D3 | ATCCACGA | AGTATGCC | GGCATACT |
| E3 | ACGATCAG | TGGTGAAG | CTTCACCA |
| F3 | GTCCTAAG | TACTGCTC | GAGCAGTA |
| G3 | CAACTCCA | ACTCCTAC | GTAGGAGT |
| H3 | AAGCATCG | TACTCCAG | CTGGAGTA |
| I3 | GAAGACTG | TCACCTAG | CTAGGTGA |
| J3 | GAACGGTT | GATCTTGC | GCAAGATC |
| K3 | CTCTATCG | AAGCCTGA | TCAGGCTT |
| L3 | ATGCCTAG | AGTACACG | CGTGTACT |
| A4 | CCACATTG | CGACACTT | AAGTGTCTG |
| B4 | ATGTGGAC | CTCACCAA | TTGGTGAG |
| C4 | TGAGACGA | AACCAGAG | CTCTGGTT |
| D4 | GGTTGGTA | GCGTATCA | TGATACGC |
| E4 | CATCAACC | AATGACGC | GCGTCATT |
| F4 | GCAATTCC | CCACAACA | TGTTGTGG |
| G4 | ACCTCTTC | GTATTCCG | CGGAATAC |
| H4 | TTCACGGA | AGGTAGGA | TCCTACCT |
| I4 | CTGGTCAT | ACGAGAAC | GTTCTCGT |
| J4 | CCTATTGG | TGACAACC | GGTTGTCA |
| K4 | AAGACCGT | CTTAGGAC | GTCCTAAG |
| L4 | GGTGTACA | CCGCTTAA | TTAAGCGG |

Revision History

| Document Version | Date | Revision Description |
|------------------|---------|--|
| 2.0.1 | 2019-10 | <ul style="list-style-type: none"> Corrected bold formatting issues throughout protocol Added water volume table for Library Preparation Added version to header Added notes about motorized lysis Added step to discard supernatant (step 10.9) |
| 3.0 | 2020-02 | <ul style="list-style-type: none"> Switched from SPRI beads to Recovery Beads; component and volume change Ethanol wash now with diluted Recovery Wash Buffer Fragmentation Enzyme and Buffer reagents were adjusted; both volume and component change Lysis Buffer 1 reagent was adjusted Tabulated multi-step incubations Expanded index list Decreased number of CRB washes after Bead clean-up from 3 to 2 Increased first lysis step from 10 min to 20 min Increased recommended spin speed and time during crosslinking from 1k 1 min to 6k 5 min Updated water description from “deionized” to “molecular biology-grade” Corrected storage instructions after lysis to storage of beads at 2-8 °C from storage of pellet at -20 °C. General formatting update – font changed to match website, etc. |

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