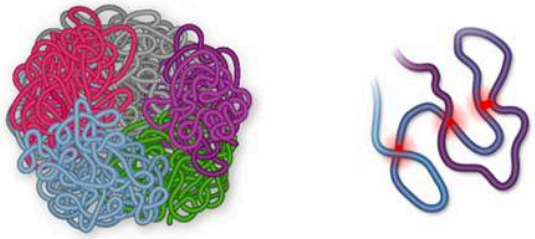
PHASE GENOMICS

Proximo™ Hi-C Plant Protocol

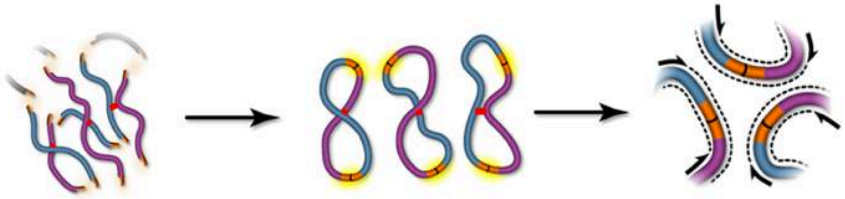
www.phasegenomics.com **Version: 1.0** Email: support@phasegenomics.com

Overview

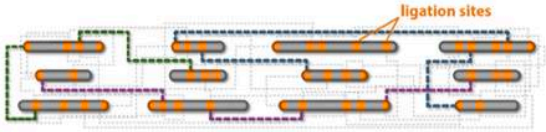
Proximo™ Hi-C Chromosome-Scale Scaffolding



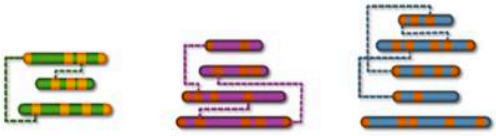
Physical proximity of nuclear DNA is inversely correlated with genomic distance. Chromatin proximity is captured through *in vivo* crosslinking, preserving contiguity information across entire chromosomes.



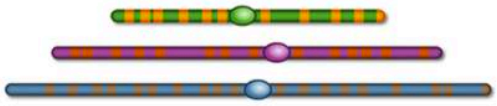
Crosslinked chromatin is fragmented and junctions are extracted. Fragmented junctions are proximity ligated and paired-end sequenced. Sequencing data encapsulates the chromatin proximity signal.



Proximity data establishes relationships among contigs at ligation sites.



Contigs are placed into chromosome groups based on proximity signal...



...then ordered and oriented by proximity signal onto chromosome-scale scaffolds.

Included in this Kit:

Bead Bind Buffer	DNA Binding Buffer	Library Reagent 1	PCR Hot Start Ready Mix	Quenching Solution
Bead Reagent	Elution Buffer	Library Reagent 2	PCR Index Mixes	RX Enzyme
Bead Wash Buffer 1	Plant Fragmentation Buffer	Plant Lysis Buffer 1	PCR Primer Mix	Spin Columns
Bead Wash Buffer 2	Plant Fragmentation Enzyme	Plant Lysis Buffer 2	Proximity Ligation Buffer	SPRI Beads
Crosslinking Solution	Library Buffer	Plant Lysis Tubes	Proximity Ligation Enzyme	Streptavidin Beads

Necessary items not included in this kit:

- Magnetic tube rack for 2 mL centrifuge tubes
- Microcentrifuge capable of 17K g or greater
- 80% Ethanol
- Thermocycler
- Heat block or waterbath that can reach 70 °C
- Vortexer (bead-beater attachment optional)
- TBS Buffer (1X Tris Buffered Saline, pH7-8)
- Deionized Water
- 2 mL microcentrifuge tubes, PCR tubes, 15 mL conical tubes
- Mortar and Pestle, liquid nitrogen

Additional Notes

- Store beads at 4°C and other kit contents at -20 °C.
- Protocol takes approximately two days to complete.
- Pellets can be stored overnight at -20°C.
- All buffers and beads should be warmed to room temperature before use and resuspended if precipitates form.
- Keep enzymes on ice.
- See Troubleshooting help on **page 7**.

1. Crosslinking (Red)

• Finely chop 2-3 leaves or plant seedling tissue (about 0.2 grams of plant tissue total), dispose of the stem and other "woody" plant tissue.

• Transfer tissue to a 15 mL conical tube and fill conical with 1 bottle (about 10 mL) of **Crosslinking Solution.**

• Incubate for 15-20 minutes at room temperature with occasional mixing by inversion or rotation.

• Add 1 ml of **Quenching Solution** and incubate for 15-20 minutes with occasional mixing.

• Remove supernatant and wash with 2 mL of TBS

• Grind the plant sample using mortar and pestle with liquid nitrogen.

• Split sample and put into 3 **Plant Lysis Tubes** (no more than 200 µL per tube).

2. Cell Lysis (Orange)

• Add 600 µL of **Plant Lysis Buffer 1** to each of the 3 **Plant Lysis Tubes**

• Vortex for 20 minutes using bead-beater attachment if available.

• Sample may foam heavily, centrifuge briefly to collapse bubbles then resuspend by pipetting up and down and mixing beads.

• Transfer lysate from each tube into a new, clean, 2 mL centrifuge tube by pressing the pipette tip to the bottom of the tube in order to avoid transferring beads.

• Centrifuge at 17K g (13 K RPM) for 5 minutes, discard supernatant.

• Resuspend in 500 µL of TBS and centrifuge at 17K g for 5 minutes, discard supernatant.

• Combine the 3 samples and resuspend Resuspend in 500 µL of **Plant Lysis Buffer 2**, incubate at 65°C for 15 minutes.

• Centrifuge at 17K g for 30 seconds, discard the supernatant.

• Resuspend in 500 µL of TBS, centrifuge for 5 minutes at 17K g, discard supernatant. (If the sample becomes rubbery use a pipette tip to break the pellet into small pieces and continue to next step; this is normal)

3. Fragmentation (Pink)

• Resuspend the pellet in 300 µL of **Plant Fragmentation Buffer**.

• Add 17 µL of **Plant Fragmentation Enzyme** and incubate at 37°C for one hour.

• Centrifuge at 17K g for 5 minutes, discard supernatant.

4. Proximity Ligation (Clear)

• Resuspend the pellet in 500 µL of deionized water.

• Quantify DNA concentration. Low concentrations are not concerning at this stage, including out of range low measurements.
(Qubit or other fluorescence based quantification system recommended).

When quantifying, be sure to resuspend sample before transferring and measuring.

• Transfer no more than 1000 ng of DNA to a 2 mL centrifuge tube, if there is less than 1000 ng, transfer the entire sample.

- Add 1500 µL of **Proximity Ligation Buffer**.
- Add sufficient water to bring the final volume to 2 mL.
- Add 10 µL of **Proximity Ligation Enzyme**.
- Incubate for 4 hours at room temperature with occasional mixing.

Reverse Crosslinks

- Centrifuge the ligated sample at 17K g for 5 minutes.
- Remove 1.5 ml of supernatant, leaving 500 µL of solution and pellet.
- Add 20 µL of **RX Enzyme** and resuspend.
- Incubate at 60°C for at least one hour (this can be left overnight at 60°C if needed, a possible pausing point)

5. Purify DNA (Black)

- Add 1 mL **DNA Binding Buffer** and pipette up and down to mix.
- Aliquot sample into two **DNA Purification Spin Columns** with disposable collection tubes (750 µL of sample per tube).
- Centrifuge for 30 seconds at 17K g then discard flow-through.
- Add 500 µL of 80% ethanol to each tube.
- Centrifuge at 17K g for 30 seconds then discard flow-through.
- Centrifuge column again at 17K g for 30 seconds to dry, then discard collection tube, replace with a clean 2 mL centrifuge tube.
- Add 75 µL of **Elution Buffer** to each column and incubate for 5 minutes at room temperature. **Keep remaining Elution Buffer as it will be used later.**
- Centrifuge at max speed for 30 seconds and combine the two eluates into a single tube (150 µL total), set aside.

6. Bead Attachment (Blue)

• Resuspend **Streptavidin Beads** and aliquot 20 µL of **Streptavidin Beads** into a new 2 mL tube, place in magnetic rack for 30 seconds and remove supernatant.

• Wash beads with 200 µL of **Bead Wash Buffer 1** twice.

• Remove from magnetic rack and resuspend in 150 µL of **Bead Binding Buffer**.

For bead washes: add buffer, vortex, place on magnetic rack for one minute, then remove buffer

• Transfer purified DNA from previous steps to the washed **Streptavidin Beads** and incubate for 45 minutes at room temperature.

• Add 10 µL of **Bead Reagent** and incubate for 15 minutes at room temperature.

• Place sample in magnetic rack for 1 minute and remove supernatant without disturbing the beads.

• Wash beads twice with 200 µL of **Bead Wash Buffer 2**.

• Wash beads once with 200 µL of **Bead Wash Buffer 1**.

• Wash beads once with 200 µL of deionized water.

7. Library Preparation (Purple)

• Resuspend beads in 21 µL of deionized water.

• Add 25 µL of **Library Buffer**.

• Add 4 µL of **Library Reagent 1**.

• Incubate at 55°C for 10 minutes.

• Add 5 µL of **Library Reagent 2**.

• Incubate at 55°C for 10 minutes.

• Place sample in magnetic rack for 1 minute and remove supernatant.

• Wash four times with 200 µL of **Bead Wash Buffer 2**.

• Wash twice with 200 µL of **Bead Wash Buffer 1**.

• Wash once with with 200 µL of deionized water.

PCR

• Resuspend beads in 20 µL of water.

• Add 5 µL of **PCR Primer Mix**.

• Add 15 µL of **PCR Hot Start Ready Mix**.

• Add 10 µL of **PCR Index Mix** (use a different index for each sample).

• Run in thermocycler.

PCR Program

• 72 °C for 5 minutes
• 98 °C for 30 seconds

15 Cycles:

• 98 °C for 10 seconds
• 62 °C for 20 seconds
• 72 °C for 50 seconds

• Hold at 4°C

8. DNA Purification (Green)

• Place sample on magnetic rack and transfer supernatant to a clean 2 mL centrifuge tube.

• Add 30 µL of resuspended **SPRI Beads** to centrifuge tube containing PCR product.

• Incubate at room temperature for 15 minutes.

• Place sample in magnetic rack for 1 minute, discard supernatant.

• Wash beads twice with 200 µL of 80% Ethanol.

• Air dry the beads for 8-10 minutes on magnetic rack, with cap open.

• Add 30 µL of **Elution Buffer (Black Cap)**, resuspend, and incubate for 5 minutes at room temperature.

• Place in magnetic rack for 30 seconds and transfer the eluted **Proximo Hi-C Plant Library** to a clean centrifuge tube.

Use Qubit or similar method to quantitate library concentration, yields over 0.5ng/µL are a strong indicator of a successful library, store at -20 °C.

Troubleshooting

Problem	Solution
My pellet won't resuspend	This is a normal feature of many crosslinked samples. The pellet only needs to be broken up enough to pipette. Try pipetting up and down and use the pipet tip to break up the pellet.
My pellet is very brown in color	This could indicate that your species has a high concentration of polyphenols and may cause problems later on, please contact support@phasegenomics.com for additional advice.
I measured little or no DNA in my sample before ligation	This is not necessarily a problem, crosslinked chromatin can mask DNA that is present, giving anomalously low readings during the procedure. Proceed with the protocol as normal.
I didn't get a useable yield	This is most likely due to one of the following: - over-crosslinking: too much or too long in formaldehyde will unrecoverably damage a sample, closely follow the kit protocol to ensure proper crosslinking. - not enough sample: too little material will reduce yield below usable levels. - too much sample: the lysis tube works best with the listed quantity of sample, too much will diminish lysis efficacy and reduce final yield.
My problem isn't listed	Email support@phasegenomics.com