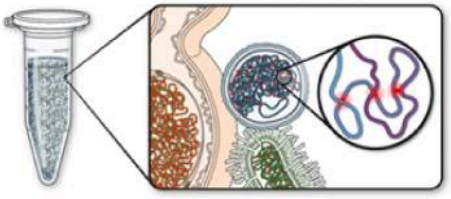
PHASE GENOMICS

ProxiMeta™ Hi-C Protocol

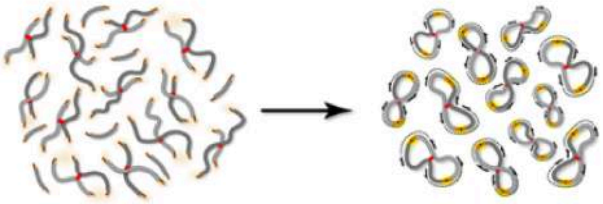
www.phasegenomics.com **Version: 1.0** Email: support@phasegenomics.com

Overview

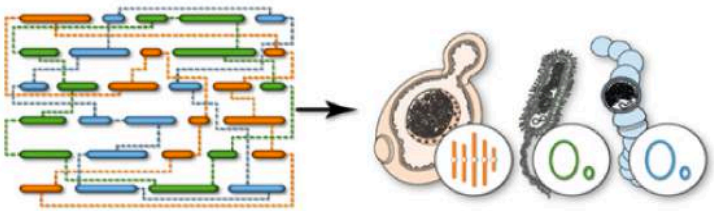
ProxiMeta™ Hi-C Metagenome Deconvolution



In vivo crosslinking traps intra-cellular DNA contacts, including inter-chromosomal and plasmid-genome interactions. Because crosslinking is performed *in vivo*, inter-cellular interactions are negligible.



Crosslinked loci are fragmented and proximity ligated, creating chimeric junctions between sequences originating from the same cell. Paired-end sequencing of these junctions yields proximity signal that is used to group sequences by cellular origin.



Intracellular proximity signal is used to deconvolute metagenomes by grouping sequences into species- and strain-specific clusters. Multi-chromosome genomes can also be assembled, and plasmids can be assigned to host organisms.

Included in this Kit:

Bead Binding Buffer	DNA Binding Buffer	Library Reagent 1	PCR Hot Start Mix	Quenching Solution
Bead Reagent	Elution Buffer	Library Reagent 2	PCR Index Mixes	RX Enzyme
Bead Wash Buffer 1	Fragmentation Buffer	Lysis Buffer 1	PCR Primer Mix	Spin Columns
Bead Wash Buffer 2	Fragmentation Enzyme	Lysis Buffer 2	Proximity Ligation Buffer	SPRI Beads
Crosslinking Solution	Library Buffer	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
- Deionized Water
- 2 mL microcentrifuge tubes and PCR tubes

Additional Notes

- Store beads at 4°C and other kit contents at -20 °C.
- Protocol takes approximately one and a half 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)

• Transfer sample according to chart below to a 2 mL centrifuge tube

Sample Type	Cell Pellet	Fecal	Soil
Sample Size	10^8 cells or 50 µL pellet	100 µL sample pellet	200 µL sample pellet
Notes	Sample needs no additional work	Resuspend in TBS, spin down pellet and discard supernatant twice before crosslinking. Final pellet volume should be about 100 µL.	Resuspend in 1 mL of TBS, allow sediment to fall to the bottom for 10-30 seconds, transfer supernatant to clean 2 mL tube and spin down. Final pellet volume should be about 200 µL.

• Add 1 mL of **Crosslinking Solution** and resuspend.

• Incubate for 20 minutes at room temperature with occasional mixing by inversion, brief vortexing, or rotation.

• Add 100 µL of **Quenching Solution** and incubate for 20 minutes with occasional mixing.

• Centrifuge at 17K g for 5 minutes.

• Remove and discard supernatant without dislodging the pellet.

2. Cell Lysis (Orange)

• Resuspend pellet in 700 µL of **Lysis Buffer 1**, the pellet may not fully resuspend, this is normal.

• Add sample to **Lysis Tube** and 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.

• Transfer lysate 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 for 5 minutes, discard supernatant.

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

• Resuspend in 500 µL of **Lysis Buffer 2**, incubate at 65°C for 15 minutes.

• Centrifuge down 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 to break the pellet into small pieces and continue to next step.

3. Fragmentation (Pink)

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

• Add 22 µL of **Fragmentation Enzyme** and incubate at 37°C for one hour.

• Centrifuge at max speed 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 florescence 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 if needed.

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 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.
- Cenrifuge 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 on 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 on 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 23 µL of deionized water.

• Add 25 µL of **Library Buffer**.

• Add 2 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 on 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 on 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 on magnetic rack for 30 seconds and transfer the eluted **ProxiMeta Hi-C 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.
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

For support, email support@phasegenomics.com