PHASE GENOMICS

ProxiMeta™ Hi-C Kit

ProxiMeta Hi-C Kit Protocol

For crude-sample proximity ligation library prep from Microbe samples, for Illumina® sequencing.

This document applies to ProxiMeta Hi-C Kit KT5045.

Please review this protocol thoroughly before you start processing your samples. If you have any questions, please contact us at support@phasegenomics.com or visit our FAQs.

Version 4.5.4 June 2024

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Introduction

Proximity ligation or Hi-C is one of a number of "chromosome conformation capture" (3C) methods, originally designed to study the spatial organization of chromatin.[1,2] Hi-C employs cost-effective, high-throughput, short-read sequencing to identify the nucleotide sequences of genomic loci that are in close proximity in three-dimensional space, but may be megabases apart in the linear genome sequence. This powerful methodology has enabled significant improvements in genome assembly of humans and other species, as well as structural variant and epigenetic analysis.[3] In addition, it has unlocked many applications in metagenomics and microbiology.[4]

Phase Genomics' ProxiMeta Platform employs Hi-C to measure the physical proximity between DNA sequences in the same cell. This Proximo Hi-C kit is designed for the preparation of eight dual-indexed Hi-C libraries from whole-cell microbial samples. The entire protocol, from sample to sequencing-ready library for Illumina paired-end sequencing can be completed in 1.5 to 3 days.

This kit is suitable for all types of whole-cell microbial inputs. Any microbial sample type (from soil to feces) may be used, but extracted DNA is not a suitable input. Please refer to the **Sample Types and Preparation** section to determine if your type of microbial sample requires additional preparation or reagents.

The Proximo Genome Scaffolding computational tool combines Hi-C sequencing data with draft short- or long-read assemblies to assign contigs to scaffolds, arranges contigs in linear order, and then orients contigs in such a way as to maximize the likelihood of having generated the observed Hi-C data. Contact us at support@phasegenomics.com to find out how to use Proximo and FALCON-Phase™ to produce high-quality, chromosome-scale, haplotype-resolved "gold" or "platinum" reference genomes.

The ProxiMeta Platform (library preparation and analysis) is illustrated in Figure 1 on the next page.

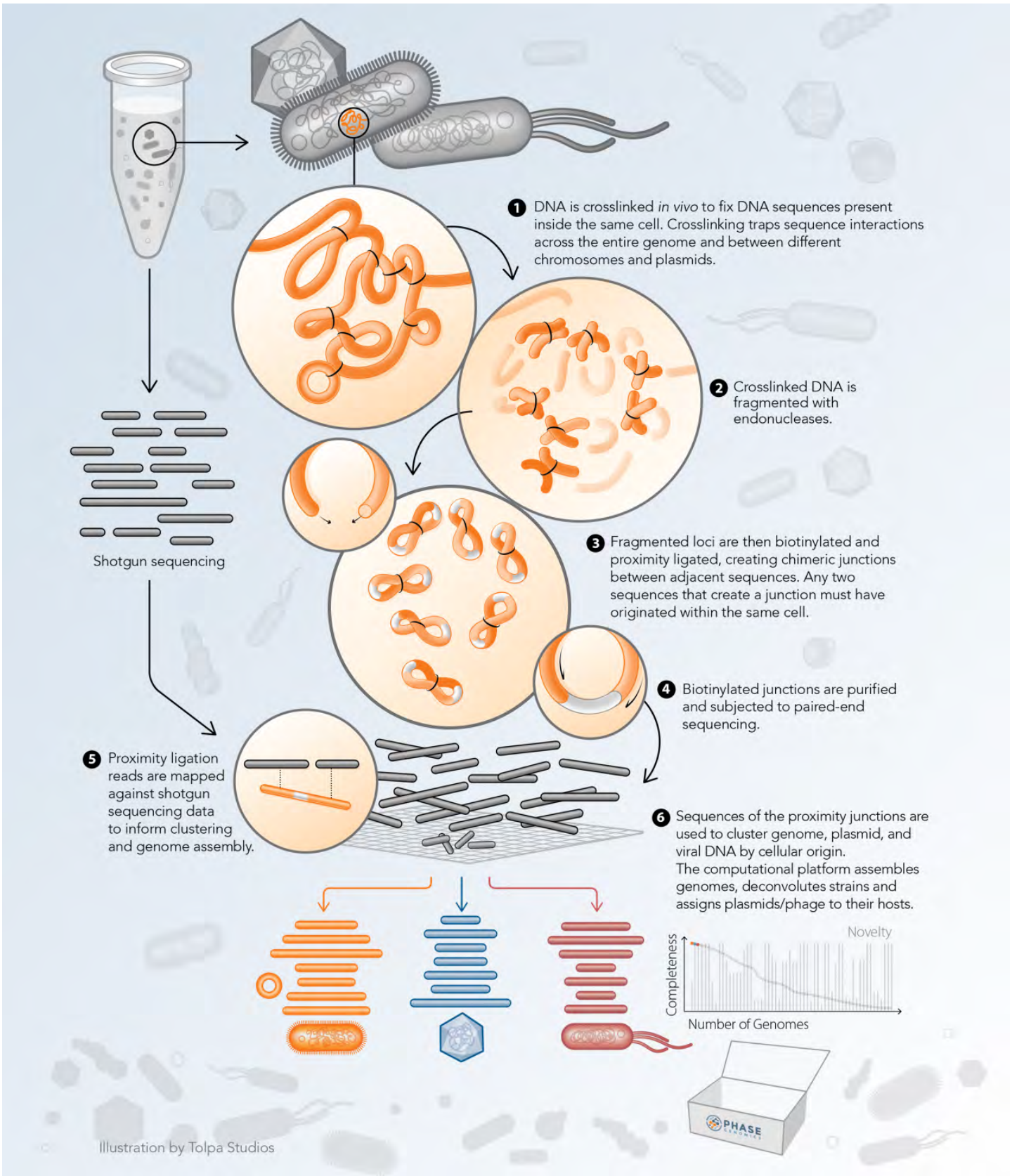


Figure 1. How the ProxiMeta Platform works

References

1. Lieberman-Aiden E, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 2009; 326 (5950): 289-293. doi: 10.1126/science.1181369.

2. Van Berkum NL, et al. Hi-C: a method to study the three-dimensional architecture of genomes. J. Vis. Exp. 2010; 39: e1869. doi: 10.3791/1869.

3. http://phasegenomics.com/applications/human-genomics-epigenomics/

4. http://phasegenomics.com/applications/metagenomics-microbiology/

Kit Specifications

Kit Contents

Cap/ Label Color	Reference Code	Top Label	Tube Label	Volume per tube	No. of Tubes	Storage Temperature (˚C)	Used in Step	Before Starting
	KS0016	Crosslink Solution	Crosslinking Solution	10 mL	1	-25 to +8˚C	1.1	Thaw and warm to RT
	KS0003	Quench Solution	Quenching Solution	1 mL	1	-25 to +25˚C	1.3	Thaw and warm to RT[1]
	KB0037	Lysis Buffer 1	Lysis Buffer 1	6 mL	1	-25 to +25˚C	2.1-2.2	Thaw and warm to RT
	KB0003	Lysis Buffer 2	Lysis Buffer 2	1 mL	1	-25 to +25˚C	2.7	Thaw and warm to RT
	KC0001	Lysis Tube	Lysis Tube	500 µL	8	-25 to +25˚C	2.2	
	KB0050	Fragment Buffer	Fragmentation Buffer	1.2 mL	1	-25 to -15˚C	3.3	Thaw on ice
	KE0022	Fragment Enzyme	Fragmentation Enzyme	90 µL	1	-25 to -15˚C	3.4	Thaw on ice
	KB0053	Ligation Buffer	10X Ligation Buffer	80 µL	1	-25 to -15˚C	4.2	Thaw on ice
	KE0028	Ligation Enzyme	Ligation Enzyme	40 µL	1	-25 to -15˚C	4.3	Thaw and warm to RT
	KE0017	RX Enzyme	RX Enzyme	40 µL	1	-25 to -15˚C	5.1	Thaw on ice
	KB0028	Elution Buffer	Elution Buffer	1.1 mL	1	-25 to +25˚C	6.7, 10.10	Thaw and warm to RT
	KR0011	Recovery Beads	Recovery Beads	1.2 mL	2	+2 to +8˚C	2.9, 6.2, 10.3, 10.6	Thaw and warm to RT
	KB0040	Recovery Wash Buffer	Recovery Wash Buffer	2 mL	1	+2 to +8˚C	6.4, 6.5, 10.9	Warm to RT. Add 95%-100% Ethanol according to the instructions on the bottle.[2]
	KR0005	Strept Beads	Streptavidin Beads	160 µL	1	+2 to +8˚C	7.1	Thaw and warm to RT
	KB0025	Bead Bind	Bead Binding Buffer	800 µL	1	-25 to +25˚C	7.4	Thaw and warm to RT
	KB0047	Wash Buffer 1	Wash Buffer 1	7 mL	1	-25 to +25˚C	7.2, 7.3, 7.9, 8.15	Thaw and warm to RT
	KB0048	Wash Buffer 2	Wash Buffer 2	7 mL	1	-25 to +25˚C	7.7, 7.9, 8.13, 8.15	Thaw and warmx to RT
	KB0045	FERAT Buffer	Frag, Repair & A-Tail Buffer	32 µL	1	-25 to -15˚C	8.5	Thaw on ice
	KE0031	FERAT Enzyme	Frag, Repair, & A-Tail Enzyme	48 µL	1	-25 to -15˚C	8.6	Thaw on ice
	KS0013	Universal Adapter	Universal Adapter	40 µL	1	-25 to -15˚C	8.8	Thaw on ice
	KE0034	Adapter Ligation Mix	Adapter LIgation Mix	160 µL	1	-25 to -15˚C		Thaw on ice
	KE0037	Hot Start Mix	Hot Start PCR Mix	200 µL	1	-25 to -15˚C	9.2	Thaw on ice
	KP000N[3]	Index	Index Mix	5 µL each	8	-25 to -15˚C	9.3	Thaw on ice
	KB0054	10X CRB	10X CRB	1.6 mL	2	-25 to -15˚C	1.6, 2.6, 2.11, 3.4, 3.5	Dilute to 1X in molecular biology-grade water before use.[4]

[1]May be warmed to 37˚C to dissolve any precipitate that is present after freezing and thawing
[2]Prepared Recovery Wash Buffer may be stored at +2 to +8˚C for up to 6 months
[3]Reference code varies depending on your unique index mixes
[4]1X CRB is stable when stored at room temperature for up to 1 year

Shipping, Storage, and Handling

ProxiMeta Hi-C Kits are shipped on cold packs. Upon receipt, remove the inner container with the **Recovery Beads** 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 Adapter Ligation Mix 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 https://phasegenomics.com/product-literature/

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.

- 95 - 100% ethanol
- Molecular biology-grade water

Reagents for Low abundance Soil Preparation (Appendix B-1)

- OptiPrep™ (Or similar 60% iodixanol solution)
- Formaldehyde
- Glycine

Equipment and Consumables

The following general laboratory equipment and consumables are needed for this protocol.

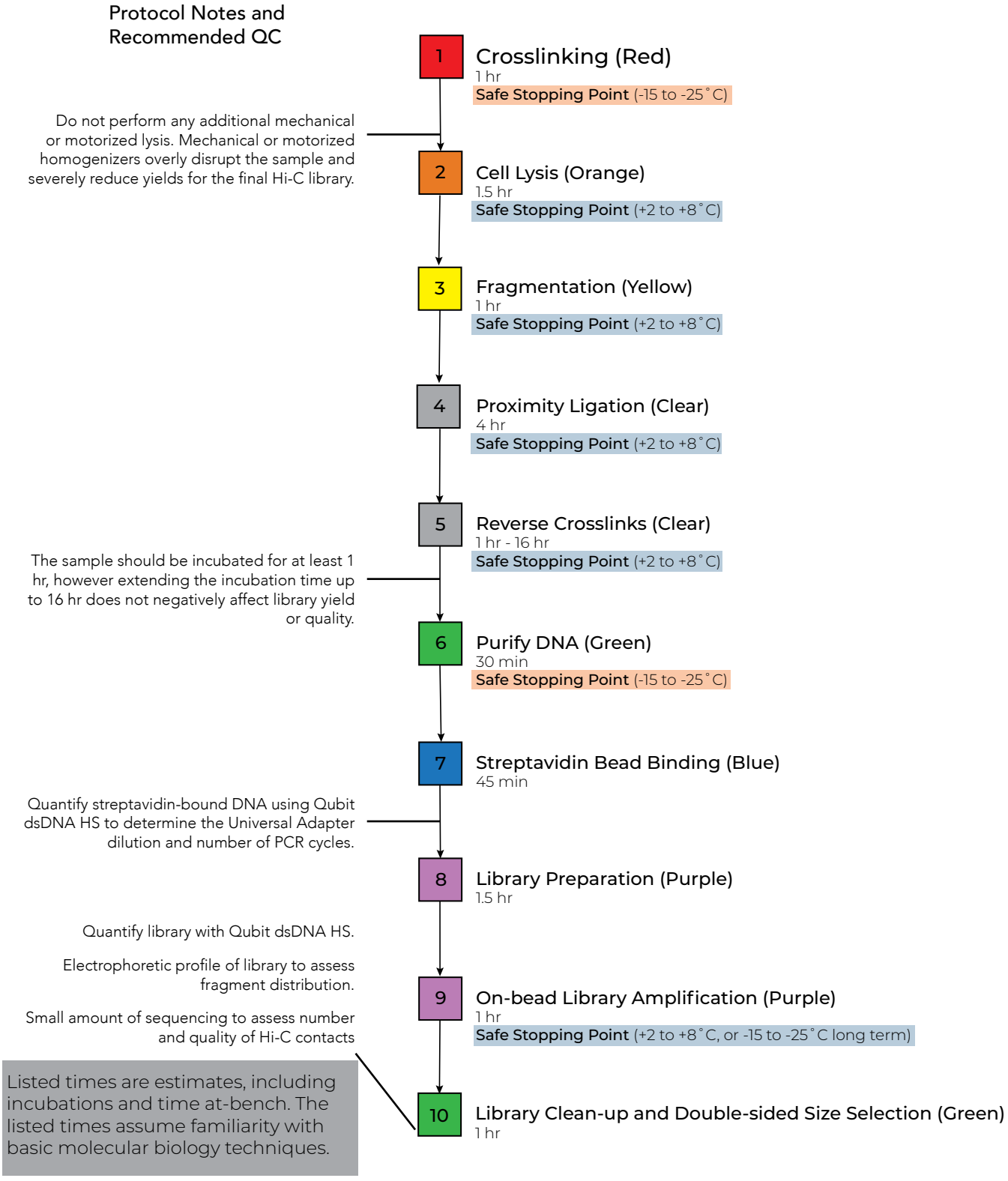
- Calibrated 2 – 10 µL pipette and filtered tips
- Calibrated 10 – 100 µL pipette and filtered tips
- Calibrated 200 – 1000 µL pipette and filtered tips
- 1.5 or 2 mL microcentrifuge tubes
- 0.2 mL PCR tubes
- Magnetic tube rack/magnet for 2 mL microcentrifuge tubes or 0.2 mL PCR tubes (depending on tube type used in step 2.7).
- Microcentrifuge capable of ≥6,000 *x g*
- Thermocycler
- Vortex mixer
- Qubit™ Fluorometer and Qubit dsDNA DNA HS Assay Kit (Thermo Fisher Scientific), or similar fluorometric assay for the quantification of double-stranded DNA

Sample Types and Preparation

This protocol is suitable for a wide range of microbial inputs, from soil to fecal samples.

Sample Type	Protocol Notes	Suggested Input
Microbial Cell Pellet		1 - 20 million cells
Fecal Sample		50 - 100 uL
Soil	Crosslinking, see appendix A-1 and A-2	Variable
Other (including low input)	contact support@phasegenomics.com	variable

Workflow Overview



Quick Protocol

This section provides a quick-step guide for experienced users. If this is your first time using the Proximo Hi-C Kit (Microbe), please refer to the detailed protocol on *p. 17**.*

Step	Protocol	Incubations and notes
1. Crosslinking (Red)	■ Transfer sample to a 2 mL microcentrifuge tube and add 1 mL of **Crosslinking Solution**.	Incubate at room temperature for 20 min while rotating.
	■ Add 100 µL of **Quenching Solution**.	Incubate for room temperature for 15 min while rotating.
	■ Centrifuge at 17,000 *x g* for 5 min to pellet all sample material. ■ Remove and discard the supernatant. ■ Wash the pellet with 1 mL of **1X CRB.** ■ Centrifuge at 17,000 *x g* for 5 min. ■ Carefully remove and discard the supernatant.	
2. Lysis (Orange)	■ Resuspend cells in 700 µL of **Lysis Buffer 1** and transfer to **Lysis Tube**.	Vortex for at room temperature for 20 min, using a bead-beater attachment if available.
	■ Centrifuge for 10 sec in benchtop centrifuge.	
	■ Transfer the supernatant to a clean microcentrifuge tube.	**The chromatin is in the supernatant.**
	■ Centrifuge the supernatant at 17,000 *x g* for 5 min. ■ Discard the supernatant.	**The chromatin is now in the pellet.**
	■ Resuspend the pellet in 500 µL of **1X CRB**. ■ Centrifuge at 17,000 *x g* for 5 min. ■ Carefully remove and discard the supernatant.	**SAFE STOPPING POINT**: Pellet may be stored at -25˚C to -15˚C for up to 1 month.
	■ Resuspend the pellet in 100 µL of **Lysis Buffer 2**.	Incubate at 65˚C for 15 min.
	■ Add 100 µL **Recovery Beads** to sample.	Incubate at room temperature for 10 min.
	■ Wash the beads: ▸ Place the sample tube on a magnetic rack ▸ Once the solution has cleared, remove and discard the supernatant without disrupting the beads ▸ Remove the tube from the magnetic rack and gently resuspend the beads in 200 µL **1X CRB.**	**SAFE STOPPING POINT**: Store sample at +2 to +8˚C overnight.

Step	Protocol	Incubations and notes
3. Fragmentation (Yellow)	■ Place the sample tube on a magnetic rack ■ Once the solution has cleared, remove the supernatant without disrupting the beads ■ Remove the tube from the magnetic rack and gently resuspend the beads in 139 µL of **Fragmentation Buffer**.	
	■ Add 11 µL of **Fragmentation Enzyme**.	Incubate at 37˚C for 1 hr.
	■ Wash the beads: ► Place the sample tube on a magnetic rack ► Once the solution has cleared, remove and discard the supernatant without disrupting the beads ► Remove the tube from the magnetic rack and gently resuspend the beads in 200 µL **1X CRB.** ■ Repeat the bead wash steps for a total of 2 washes with **1X CRB.**	**SAFE STOPPING POINT**: Store bead-bound sample in **1X CRB** at +2 to +8˚C overnight.
4. Proximity Ligation (Clear)	■ Remove **1X CRB** from beads. ■ Add 85 µL **molecular biology-grade water.** ■ Add 10 µL of **10X Ligation Buffer**.	
	■ Add 5 µL of **Ligation Enzyme.**	Incubate at 25˚C for 4 hr, followed by 65˚C for 10 min
		SAFE STOPPING POINT: Store sample at +2 to +8˚C overnight.
5. Reverse Crosslinks (Clear)	■ Add 5 µL of **RX Enzyme.**	Incubate at 65˚C for 1 hr
		SAFE STOPPING POINT: Store sample at +2 to +8˚C overnight.

Step	Protocol	Incubations and notes
6. Purifiy DNA (Green)	■ Add 100 µL of **Recovery Beads** to the sample tube.	Incubate at room temp for 10 min.
	■ Rinse the beads: ► Place the sample tube on a magnetic rack. ► Once the solution has cleared, remove and discard the supernatant without disrupting the beads. ► Keeping the beads on the magnet, gently rinse the beads with 200 µL of **Recovery Wash Buffer** without disrupting the beads, leaving the buffer on the beads for 30 sec - 1 min between washes. ■ Repeat the bead wash steps for a total of 2 washes with **Recovery Wash Buffer** ■ Remove **Recovery Wash Buffer** and air dry the beads.	To air dry, leave tubes with caps open on the magnet at room temperature for 5 - 15 min.
	■ Resuspend the beads in 100 µL of **Elution Buffer**.	Incubate at room temperature for 5 min.
	■ Place the sample tube on a magnetic tube rack or magnet. ■ Once the solution has cleared, recover the **DNA-containing supernatan**t and transfer to a fresh tube.	

Step	Protocol	Incubations and notes
7. Streptavidin Bead Binding (Blue)	**Prepare the Beads** ■ Transfer 20 µL of Streptavidin Beads into a new 2 mL microcentrifuge tube (or 0.2 mL PCR tube). ■ Place the tube on a magnetic tube rack or magnet for at least 30 sec. ■ Once the solution has cleared, remove and discard the supernatant without disrupting the beads. ■ Wash the beads: ► Place the sample tube on a magnetic rack ► Once the solution has cleared, remove and discard the supernatant without disrupting the beads ► Remove the tube from the magnetic rack and gently resuspend the beads in 200 µL **Wash Buffer 1.** ■ Repeat the bead wash steps for a total of 2 washes with **Wash Buffer 1.** ■ Resuspend beads in 100 µL of **Bead Binding Buffer**.	
	Bind the Sample to the Beads. ■ Transfer 100 µL of purified DNA from step 6 to the washed Streptavidin Beads.	Incubate at room temperature for 10 min.
	■ Wash the beads: ► Place the sample tube on a magnetic rack ► Once the solution has cleared, remove and discard the supernatant without disrupting the beads ► Remove the tube from the magnetic rack and gently resuspend the beads in 200 µL **Wash Buffer 2.** ■ Repeat the bead wash steps for a total of 2 washes with **Wash Buffer 2.** ■ Repeat the bead wash steps once with **Wash Buffer 1.** ■ Resuspend the beads in 200 µL of **molecular biology-grade water.** ■ Measure the concentration of DNA (while still bound to the streptavidin beads) using a Qubit™ dsDNA HS Assay Kit or similar fluorometric assay.	

Step	Protocol	Incubations and notes
8. Library Preparation (Purple)	■ Transfer no more than **500 ng of DNA-containing Streptavidin Beads** to a fresh microcentrifuge tube. ■ Place the sample tube on a magnetic tube rack or magnet. ■ Once the solution has cleared, remove and discard the supernatant without disrupting the beads. ■ Place tube on pre-cooled thermocycler.	Pre-cool thermocycler to 4˚C.
	■ To beads add: ■ 40 µL of **Molecular biology-grade water** ■ Cool to 4˚C, then add: ■ 4 µL of **Frag, Repair, & A-Tail Buffer** ■ 6 µL of **Frag, Repair, & A-Tail Enzyme**	Fragment, end-repair, and A-tail using thermocycler program listed in Step 8.8.
	■ To sample add: ■ 5 µL of **Universal Adapter** (diluted if necessary) ■ 20 µL **Adapter Ligation Mix**	Dilute Universal Adapter according to the table listed in Step 8.9. Incubate at 20˚C for 15 min, no heated lid.
	■ Wash the beads: ► Place the sample tube on a magnetic rack ► Once the solution has cleared, remove and discard the supernatant without disrupting the beads ► Remove the tube from the magnetic rack and gently resuspend the beads in 200 µL **Wash Buffer 2.** ■ Repeat the bead wash steps for a total of 2 washes with **Wash Buffer 2.** ■ Repeat the bead wash steps once with **Wash Buffer 1.** ■ Repeat the bead wash steps once with **molecular biology-grade water** for a total of 4 washes.	
9. On-bead Amplification (Purple)	To beads add: ■ 20 µL of **molecular biology-grade water** ■ 25 µL **Hot Start PCR Mix** ■ 5 µL of one **PCR Primer Mix**	Amplify with PCR protocol given in Step 9.4 of the detailed protocol.

Step	Protocol	Incubations and notes
10. Library Clean-up (Clean-up)	■ Place the sample tube on a magnetic tube rack or magnet and allow solution to clear. ■ Transfer 50 µL of the **library-containing supernatant** to a new tube.	
	■ Add 57.5 µL of **Recovery Beads.**	Incubate at room temperature for 10 min.
	■ Place the sample tube on a magnetic tube rack or magnet.	**Your library is in the supernatant. Do not discard.**
	■ Transfer the supernatant (107.5 µL) to a new tube containing 15 µL of **Recovery Beads.**	Incubate at room temperature for 10 min.
	■ Rinse the beads: ► Place the sample tube on a magnetic rack. ► Once the solution has cleared, remove the supernatant without disrupting the beads. ► Keeping the beads on the magnet, gently rinse the beads with 200 µL of **Recovery Wash Buffer** without disrupting the beads, leaving the buffer on the beads for 30 sec - 1 min between washes. ■ Repeat the bead rinse steps for a total of 2 washes with **Recovery Wash Buffer** ■ Air dry the beads.	Leave tubes with caps open on the magnet at room temperature for 10 - 15 min.
	■ Resuspend the beads in 30 µL of **Elution Buffer**.	Incubate at room temperature for 5 min.
	■ Place the sample tube on a magnetic tube rack or magnet. ■ Once the solution has cleared, recover the **Proximo Hi-C library-containing-supernatant** and transfer to a fresh microcentrifuge tube.	See Step 11 in detailed Protocol for recommended QC to determine if your library is sufficient.

Detailed Protocol

1.Crosslinking (Red)

*See **Sample Types and Preparation** for sample input and possible protocol modification recommendations based on sample type.*

1.1 Resuspend sample in 1 mL of **Crosslinking Solution.**

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

1.3 Add 100 µL of **Quenching Solution**.

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

1.5 Centrifuge at 17,000 *x g* for 5 min to pellet all sample material. Remove and discard the supernatant.

1.6 Wash the pellet with 1 mL of **1X CRB** (prepared as described on *p. 6*) and centrifuge at 17,000 *x g* for 1 min to gently conpact the cellular material. Carefully remove and discard the 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 2.8)

2.1 Vortex **Lysis Buffer 1** to resuspend any particulates that may have settled out.

2.2 Resuspend cells in 700 µL of **Lysis Buffer 1** and add to **Lysis Tube.**

2.3 Vortex at room temperature for 20 min using a bead-beater attachment if available.

Other types of bead-beading shakers can be used. The appropriate duration and intensity will vary between instruments. Refer to manufacturer's recommendations.

However, do not perform any additional mechanical or motorized lysis. Mechanical or motorized homogenizers overly disrupt the sample and severely reduce yields of the final Hi-C library.

2.4 Centrifuge at 500 *x g* for 10 sec to collapse bubbles and pellet debris, then transfer the supernatant to a clean microcentrifuge tube. **The chromatin is in the supernatant.**

2.5 Centrifuge the supernatant from Step 2.4 at 17,000 *x g* for 5 min and discard the supernatant. **The chromatin is now in the pellet**.

2.6 Resuspend the pellet in 500 µL of **1X CRB** and centrifuge at 17,000 *x g* for 5 min. Discard the supernatant.

SAFE STOPPING POINT: Sample pellet may be stored at -15 to -25˚C for up to 1 month.

2.7 Resuspend the pellet in 100 µL of **Lysis Buffer 2** and transfer the sample to a PCR tube.

2.8 Incubate at 65˚C for 15 min.

2.9 Briefly allow sample tube to cool. Thoroughly resuspend **Recovery Beads** and add 100 µL of beads to sample tube. mix well by vortexing gently or pipetting thoroughly.

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

2.10 Incubate at room temperature for 10 min.

2.11 Wash the beads:

- Place the sample tube in a magnetic rack or on a magnet.
- Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- Remove the tube from the magnet and gently resuspend the beads in 200 µL of **1X CRB**.

If after several minutes your sample is not clearly adhering to the magnet, briefly centrifuge the sample to collect the bead-bound sample in the bottom of your tube and remove the supernatant, avoiding transfer of any particulate sample. Then resuspend the beads in 100 µL of 1X CRB. Repeat as needed until the beads better adhere to the magnet.

SAFE STOPPING POINT: Bead-bound sample may be stored in **1X CRB** at +2 to +8˚C overnight.

3. Fragmentation (Yellow)

Pre-heat a heating block, water bath, or thermocycler to 37˚C (for use in Step 3.3)

3.1 Place the sample tube on a magnetic rack

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

3.3 Remove the tube from the magnetic rack and gently resuspend the beads in 139 µL of **Fragmentation Buffer** to the sample.

*If your bead-bound sample was stored in **1X CRB**, remove the buffer before adding Fragmentation Buffer.*

3.4 Add 11 µL of **Fragmentation Enzyme** to the sample and mix by vortexing gently or pipetting thoroughly.

3.5 Incubate the sample at 37˚C for 1 hr.

3.6 Wash the beads:

- Place the sample tube in a magnet.
- Once the solution has cleared, remove the supernatant without disrupting the beads.
- Remove the tube from the magnet and gently resuspend the beads in 200 µL of **1X CRB**.

3.7 Repeat the bead wash steps one more time with 200 µL of **1X CRB** per wash, for a total of two washes.

SAFE STOPPING POINT: Store bead-bound sample in **1X CRB** at +2 to +8˚C overnight.

4. Proximity Ligation (Clear)

4.1 Place the sample tube on a magnetic rack.

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

4.3 Remove the tube from the magnetic rack and gently resuspend the beads in 85 µL of **Molecular Biology-grade Water** to the bead-bound sample.

4.4 Add 10 µL of **10X Ligation Buffer**.

4.5 Add 5 µL of **Ligation Enzyme** and mix by vortexing gently or pipetting thoroughly.

4.6 Incubate the sample as follows:

Step	Temperature (˚C)	Time
Ligation	25	4 hr
Enzyme inactivation	65	10 min
Final hold	4	Hold

SAFE STOPPING POINT: Store sample at +2 to +8˚C overnight.

5. Reverse Crosslinks (Clear)

Heat thermocycler to 65˚C (for use in Step 5.2).

5.1 Add 5 µL of **RX Enzyme** to the ligation reaction and mix well by vortexing or pipetting.

5.2 Incubate at 65˚C for 1 hr.

The sample is no longer bound to the beads and has been released into solution.

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 by adding 10 mL of 95-100% ethanol to the 2 mL of provided Recovery Wash Buffer bottle and mix well.

6.1 Allow sample tube to cool to room temperature.

6.2 Thoroughly resuspend the **Recovery Beads** and add 100 µL of **Recovery Beads** to the sample tube and mix thoroughly by vortexing or pipetting.

6.3 Incubate at room temperature for 10 min.

6.4 Rinse the beads:

- Place the sample tube in a magnetic rack or on a magnet.
- Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- Keeping the beads on the magnet, gently rinse the beads with 200 µL of **Recovery Wash Buffer** without disrupting the beads, leaving the buffer on the beads for 30 sec to 1 min between washes.

6.5 Repeat the bead rinse steps for a total of 2 rinses with **Recovery Wash Buffer**.

6.6 Air dry the beads at room temperature for 5 - 15 min on the magnet with the cap open.

*Over-drying is not problematic for **Recovery Beads**. Air dry the beads by leaving the tube on the magnet for 5 - 15 min with the cap open.*

6.7 Remove the sample tube from the magnet and thoroughly resuspend the beads in 100 µL of **Elution Buffer**.

6.8 Incubate at room temperature for 5 minutes to elute the DNA.

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

6.10 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 -25 to -15˚C (indefinitely)

7. Streptavidin Bead Binding (Blue)

A. Prepare the Beads

Do not yet combine the beads with the DNA recovered in Step 6. DNA-binding will occur after beads are prepared in section B.

7.1 Thoroughly resuspend the **Streptavidin Beads** and transfer 20 µL into a new microcentrifuge tube (or 0.2 mL PCR tube).

7.2 Wash the Beads:

- Place the sample tube in a magnetic rack or on a magnet.
- Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- Remove the tube from the magnet and gently resuspend the beads in 200 µL of **Wash Buffer 1**.

7.3 Repeat the bead wash steps one more time with 200 µL of **Wash Buffer 1** for a total of two washes.

7.4 Remove beads from the magnet and resuspend in 100 µL of **Bead Binding Buffer**.

B. Bind the Sample to the Beads

7.5 Transfer 100 µL of purified DNA (from Step 6) to the washed **Streptavidin Beads** (from Step 7.4) and mix by vortexing gently or pipetting thoroughly.

7.6 Incubate at room temperature for 10 min, mixing occasionally by gentle vortexing or inversion.

7.7 Wash the beads:

- Place the sample tube in a magnetic rack or on a magnet.
- Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- Remove the tube from the magnet and gently resuspend the beads in 200 µL of **Wash Buffer 2**.

7.8 Repeat the bead wash steps one more time with 200 µL of **Wash Buffer 2** for a total of two washes.

7.9 Repeat the bead wash steps one more time with 200 µL of **Wash Buffer 1**.

7.10 Repeat the bead wash steps one more time with 200 µL of **molecular biology-grade water.**

7.11 With your bead-bound sample suspended in 200 µL of water, 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.

Beads will interfere with spectrophotometric quantitation of bound DNA. Use of fluorometric assay is a requirement.

A concentration of less than 10 ng at this stage does NOT necessarily indicate failure. Proceed through the remainder of the protocol as written.

8. Library Preparation (Purple)

Pre-cool a thermocycler to 4˚C (see Step 8.8).

8.1 Transfer no more than 500 ng of streptavidin-bound DNA to a fresh microcentrifuge tube.

8.2 Place the sample tube in a magnetic rack or on a magnet.

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

8.4 Resuspend the beads in 40 µL of **molecular biology-grade water.**

8.5 Place the sample in the pre-cooled thermocycler and then cool to 4˚C for at least 1 min.

8.6 Add 4 µL of **Frag, Repair, & A-Tail Buffer**.

8.7 Add 6 µL of **Frag, Repair, & A-Tail Enzyme** and mix by vortexing gently or pipetting thoroughly.

Vortex for at least 5 sec or pipette at least 25 µL of the reaction up and down a minimum of 10 times to ensure proper mixing.

Thorough mixing at this stage is extremely important! *Improper mixing will result in a poorly fragmented library and will negatively affect your sequencable yield.*

8.8 Proceed to fragmentation, end-repair, and A-tailing according to the following program:

Step	Temperature (˚C)	Time (min)
Lid temperature	105	
Pre-cooling	4	Hold
Fragmentation, end-repair, and A-tailing	30	7
	65	30
Final hold	4	Hold

8.9 If the amount of library measured at step 7.11 was less than 10 ng, dilute the **Universal Adapter (provided tube is 15 µM)** as according to the table below.

Either molecular biology-grade water or 10 mM Tris-HCl, pH 8.0 can be used for the dilution.

Input Mass (ng)*	Adapter Concentration	Volume Water or Tris (µL)	Volume 15 µM Adapter (µM)
> 10	15 µM	do not dilute	
1 - 10	1 µM	14	1

*Measured in Step 7.11

8.10 Add 5 uL of **Universal Adapter** (see step 8.9 for dilution instructions) to the sample and mix by vortexing gently or pipetting thoroughly.

8.11 Add 20 µL of **Adapter Ligation Mix.** Mix by pipetting thoroughly.

*Do not vortex **Adapter Ligation Mix.***

8.12 Incubate the sample as follows:

Step	Temperature (˚C)	Time (min)
Lid temperature	off	
Ligation	20	15

8.13 Wash the beads:

- Place the sample tube in a magnetic rack or on a magnet.
- Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- Remove the tube from the magnet and gently resuspend the beads in 200 µL of **Wash Buffer 2**.

8.14 Repeat the bead wash steps one more time with 200 µL of **Wash Buffer 2** for a total of two washes.

8.15 Repeat the bead wash steps one more time with 200 µL of **Wash Buffer 1**.

8.16 Repeat the bead wash steps one more time with 200 µL of **molecular biology-grade water.**

9. On-bead Library Amplification (Purple)

9.1 Thoroughly resuspend the beads in 20 µL of **molecular biology-grade water**.

9.2 Add 5 µL one **PCR Primer Mix** and mix by vortexing gently or pipetting thoroughly.

Use a different primer for each sample. Sufficient primers with unique index sequences are provided with each kit. See Index Sequences for more information).

9.3 Add 25 µL of **Hot Start PCR Mix**.

9.4 Determine how many PCR cycles each of your samples requires according to the following table:

Mass used at step 8.1 (ng)	Recommended Number of PCR Cycles
<10	14
10-50	12
50-200	10
200-500	8

Amplifying your libraries beyond the above suggested number of cycles can negatively impact the final data quality. This may require you to separate your samples into separate PCR runs

9.5 Amplify the library in a thermocycler programmed as follows:

Step	Temperature (˚C)	Time (sec)	Cycles
Initial denaturation	98	45	1
Denaturation	98	15	12*
Annealing	60	30	
Extension	72	30	
Final extension	72	60	1
Hold	12	hold	

*If less than 10 ng DNA was carried into Step 8 (Library preparation), increase the number of PCR cycles to 14

SAFE STOPPING POINT: PCR reaction can be held overnight at +2 to +8˚C, or stored at -25 to -15˚C (indefinitely)

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

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.

*Streptavidin beads can be stored in **1X CRB** for troubleshooting if needed. Otherwise they can be discarded.*

10.3 Add 57.5 µL (1.15X volume) of thoroughly resuspended **Recovery Beads** to the tube containing the library (from Step 10.2).

Unwanted high molecular weight fragments will be 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 2 min, or once the solution has cleared, transfer the supernatant (107.5 µL) to a new tube containing 15 µL of **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 Rinse the beads:

- Place the sample tube in a magnetic rack or on a magnet.
- Once the solution has cleared, remove and discard the supernatant without disrupting the beads.
- Keeping the beads on the magnet, gently rinse the beads with 200 µL of **Recovery Wash Buffer** without disrupting the beads, leaving the buffer on the beads for 30 sec - 1 min between washes.

10.9 Repeat the bead rinse steps for a total of two rinses with **Recovery Wash Buffer**. 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**. Air dry the beads by leaving the tube on the magnet for 5 - 15 min with the cap open.*

10.10 Remove the sample tube from the magnet and thoroughly resuspend the beads in 30 µL of **Elution Buffer**.

10.11 Incubate at room temperature for 5 min to elute the DNA.

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

10.13 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 that library preparation has been successful. The library can be stored at -15 to -25˚C indefinitely.

11.2 Assess library fragment size using BioAnalyzer or similar instrument.

The expected average library size is between 400-500 bp.

Before performing a full sequencing run, it is highly recommended that you perform low-pass sequencing (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 analysis tools (available from https://github.com/phasegenomics/hic_qc).

12. Sequencing

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

Population Complexity	Hi-C Sequencing Recommendation
Low-to-mid-complexity communities (e.g. fecal microbiomes)	> 50 million pairs (2 x 75 bp or longer)
High-complexity communities (e.g. rumen, sludge, wastewater)	> 50 million pairs (2 x 75 bp or longer)

For mixed-community experiments, producing a shotgun library from the same input sample is recommended (not included in this kit).

Population Complexity	Shotgun Sequencing Recommendation
Low-to-mid-complexity communities (e.g. fecal microbiomes)	> 100 million pairs (2 x 75 bp or longer)
High-complexity communities (e.g. rumen, sludge, wastewater)	> 200 million pairs (2 x 75 bp or longer)

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

13. Analysis

Take advantage of our expertise! Interested in additional computational analyses? Contact us to learn more about the services listed below:

ProxiMeta Metagenome Deconvolution Platform
Assemble high-quality genomes directly from the microbiome, or associate plasmids, phages and antimicrobial resistance genes (ARGs) with their hosts

Proximo™ Genome Scaffolding
Chromosome-scale genome scaffolding for virtually any organism, no high-molecular weight DNA required.

Proximo SV
Identify large-scale structural variation and determine epigenetic changes using Hi-C data.

FALCON-Phase™
Integrate PacBio long-read assemblies with Hi-C data to generate phased, diploid genome assemblies.and services.

Appendix A-1

This appendix describes modifications to the protocol for soil samples. If you are unsure about the optimal preparation for your sample, please reach out to support@phasegenomics.com.

Basic Soil Protocol

This protocol is designed for rich soil (i.e. top soil).

A1.1 Mix 5 mL of water per gram of sediment.

For rich soils use 1 - 2 g of soil.

A1.2 Vortex for 5 minutes.

A1.3 Centrifuge at 500 *x g* for 5 min to settle out heavy particles.

A1.4 Transfer the supernatant to a fresh microcentrifuge tube(s).

A1.5 Centrifuge the supernatant at 17,000 *x g* for 5 min.

A1.6 Remove and discard the supernatant.

A1.7 Proceed with pellet to step 1. Crosslinking .

Appendix A-2

This protocol is intended for soils with low levels of live organisms. For soils with abundant microbial life, follow the soil protocol included in **Appendix A-1**.

Differential Centrifugation of Soil or Sludge Samples

Additional reagents not included in the kit are required.

A2.1 Mix 5 mL of water per gram of sediment.

A2.2 Vortex for 5 minutes.

Recommended starting amount: 2 tubes of 25 mL water + 5 g sediment.

A2.3 Spin tubes at 1,000 *x g* for 10 min to settle out heavy particles.

A2.4 Transfer the supernatant to a new tube.

A2.5 Add **Formaldehyde** to a final concentration of 1% (v/v)

A2.6 Incubate at room temperature for 20 min with occasional mixing by inversion or rotation.

A2.7 Add **glycine** to a final concentration of 1% (w/v) to quench the reaction.

A2.8 Incubate at room temperature for 20 min with occasional mixing by inversion or rotation.

A2.9 Carefully layer 1 mL of supernatant on top of 1 mL of **OptiPrep™** (or similar 60% iodixanol solution, not included).

*Alternatively, you can layer larger volumes over the **OptiPrep™** cushion if you have a centrifuge that can accommodate larger volumes.*

It is also possible to sequentially load sups 1 mL at a time over the OptiPrep™ cushion.

A2.10 Centrifuge the layered supernatant and OptiPrep™ at 10,000 *x g* for 20 min to pellet. Transfer the supernatant, including cloudy interface, to a new tube and centrifuge at 17,000 *x g* for 5 min.

A2.11 Remove and discard the supernatant avoiding the pelleted cells.

A2.12 Proceed to 2. Cell Lysis with the pelleted cells.

Index Sequences

Your kit contains two sets of indexed primers which are used to generate unique dual-indexed Illumina®-compatible libraries with different sequence combinations. If you plan to pool your Hi-C libraries with other libraries for sequencing, please follow standard guidelines for multiplexed sequencing on your specific Illumina® instrument.

Please contact us at support@phasegenomics.com if additional indices or assistance with multiplexed sequencing are needed.

Plate Loc (if applicable)	Historical Index ID	Updated Index ID	i7 Equivalent Index	i5 Equivalent Index	i5 Equivalent Index (Reverse complement)
	A1	A1	TCAAGATC	TGACGTAG	CTACGTCA
	B1	B1	GAGCGCCA	AACTCTCC	GGAGAGTT
	D1	D1	ACGACAGA	AGTCTGGT	ACCAGACT
	E1	E1	TAATGATG	GTATCGAA	TTCGATAC
	F1	F1	ACATTACC	AGTACAGG	CCTGTACT
	G1	G1	CAGTCGAC	ACTAGCCT	AGGCTAGT
	H1	H1	TGTCGTTT	TCCTAGCA	TGCTAGGA
	A2	A2	CAAAGTGT	CGAGTTGC	GCAACTCG
	B2	B2	GCGCGGTG	ACCCGACC	GGTCGGGT
	C2	C2	AGTGTGTG	GAAATTTT	AAAATTTC
A1	A3	PGI1	TCAATCCG	ACTGCGAA	TTCGCAGT
A2	B3	PGI2	CGCTACAT	TAGTCTCG	CGAGACTA
A3	C3	PGI3	GATCCACT	TGAGCTGT	ACAGCTCA
A4	D3	PGI4	ATCCACGA	AGTATGCC	GGCATACT
A5	E3	PGI5	ACGATCAG	TGGTGAAG	CTTCACCA
A6	F3	PGI6	GTCCTAAG	TACTGCTC	GAGCAGTA
A7	G3	PGI7	CAACTCCA	ACTCCTAC	GTAGGAGT
A8	H3	PGI8	AAGCATCG	TACTCCAG	CTGGAGTA
A9	I3	PGI9	GAAGACTG	TCACCTAG	CTAGGTGA
A10	J3	PGI10	GAACGGTT	GATCTTGC	GCAAGATC
A11	K3	PGI11	CTCTATCG	AAGCCTGA	TCAGGCTT
A12	L3	PGI12	ATGCCTAG	AGTACACG	CGTGTACT
B1	A4	PGI13	CCACATTG	CGACACTT	AAGTGTCG
B2	B4	PGI14	ATGTGGAC	CTCACCAA	TTGGTGAG
B3	C4	PGI15	TGAGACGA	AACCAGAG	CTCTGGTT
B4	D4	PGI16	GGTTGGTA	GCGTATCA	TGATACGC
B5	E4	PGI17	CATCAACC	AATGACGC	GCGTCATT

Plate Loc (if applicable)	Historical Index ID	Updated Index ID	i7 Equivalent Index	i5 Equivalent Index	i5 Equivalent Index (Reverse complement)
B6	F4	PGI18	GCAATTCC	CCACAACA	TGTTGTGG
B7	G4	PGI19	ACCTCTTC	GTATTCCG	CGGAATAC
B8	H4	PGI20	TTCACGGA	AGGTAGGA	TCCTACCT
B9	I4	PGI21	CTGGTCAT	ACGAGAAC	GTTCTCGT
B10	J4	PGI22	CCTATTGG	TGACAACC	GGTTGTCA
B11	K4	PGI23	AAGACCGT	CTTAGGAC	GTCCTAAG
B12	L4	PGI24	GGTGTACA	CCGCTTAA	TTAAGCGG
C1		PGI25	GTGATCCA	GCTCTGTA	TACAGAGC
C2		PGI26	GGAACATG	GAACGCTT	AAGCGTTC
C3		PGI27	AGAAGCCT	AGGTCACT	AGTGACCT
C4		PGI28	ACGCTTCT	CCTATGGT	ACCATAGG
C5		PGI29	GCTACTCT	TGTTCGAG	CTCGAACA
C6		PGI30	CTTCGCAA	GTTACGCA	TGCGTAAC
C7		PGI31	ATCATGCG	GGACTGTT	AACAGTCC
C8		PGI32	TCCGATCA	GGTCTTAG	CTAAGACC
C9		PGI33	GGTACTTC	AGCAGATG	CATCTGCT
C10		PGI34	GTCTCATC	CAACACCT	AGGTGTTG
C11		PGI35	GCTGAATC	AAGAAGGC	GCCTTCTT
C12		PGI36	GCAATGAG	GTAGAGCA	TGCTCTAC
D1		PGI37	GGTTAGCT	TGTGGTAC	GTACCACA
D2		PGI38	TCTGTCGT	ACCAATGC	GCATTGGT
D3		PGI39	CTGCCATA	TACCACAG	CTGTGGTA
D4		PGI40	CAAGAAGC	GTCGGTAA	TTACCGAC
D5		PGI41	ATCGGAGA	ATGGTTGC	GCAACCAT
D6		PGI42	AATTCCGG	CACGTTGT	ACAACGTG
D7		PGI43	GGTGATGA	CTTAGTGG	CCACTAAG
D8		PGI44	CTATCCAC	ACGCCTAA	TTAGGCGT
D9		PGI45	TACTAGCG	GTGTGACA	TGTCACAC
D10		PGI46	AGAGTCCA	ACTGTGTC	GACACAGT
D11		PGI47	GGACTACT	CATACCAC	GTGGTATG
D12		PGI48	TATCGCGA	AAGCGCAT	ATGCGCTT
E1		PGI49	CTCGGTAA	GTGTTCCT	AGGAACAC
E2		PGI50	GCATCCTA	TGCTTCCA	TGGAAGCA
E3		PGI51	CCTAACAG	GTAACGAC	GTCGTTAC
E4		PGI52	CTAGCTCA	GAAGGTTC	GAACCTTC
E5		PGI53	CGGTTGTT	CGGTCATA	TATGACCG

Plate Loc (if applicable)	Historical Index ID	Updated Index ID	i7 Equivalent Index	i5 Equivalent Index	i5 Equivalent Index (Reverse complement)
E6		PGI54	CCGGAATA	TGTGCGTT	AACGCACA
E7		PGI55	TGGCTACA	ACGGAACA	TGTTCCGT
E8		PGI56	GGTATAGG	CGTTGAGT	ACTCAACG
E9		PGI57	ACACGAGA	CACCTGTT	AACAGGTG
E10		PGI58	GACTTGTG	TTGACAGG	CCTGTCAA
E11		PGI59	TTCGGCTA	AACGGTCA	TGACCGTT
E12		PGI60	TGCAAGAC	TCCTTAGC	GCTAAGGA
F1		PGI61	ACAACAGC	CATTCGGT	ACCGAATG
F2		PGI62	AGTCGAAG	ATGCCTGT	ACAGGCAT
F3		PGI63	TAAGTGGC	CATGGCTA	TAGCCATG
F4		PGI64	GACATCTC	AGCCAAGT	ACTTGGCT
F5		PGI65	TTGAGCTC	GCCAGTAT	ATACTGGC
F6		PGI66	GCGTTAGA	GCATACAG	CTGTATGC
F7		PGI67	ACAGAGGT	CGTTGCAA	TTGCAACG
F8		PGI68	AGGCTGAA	ATAAGGCG	CGCCTTAT
F9		PGI69	TCCAGCAA	TCAACTGG	CCAGTTGA
F10		PGI70	TCGAGAGT	TGCAGGTA	TACCTGCA
F11		PGI71	GTACACCT	TCGCTGTT	AACAGCGA
F12		PGI72	GTTCTTCG	ACCACGAT	ATCGTGGT
G1		PGI73	TCGCTATC	CAATGCGA	TCGCATTG
G2		PGI74	CTCGTTCT	GATCAAGG	CCTTGATC
G3		PGI75	GAGAGTAC	TTGGACTG	CAGTCCAA
G4		PGI76	GGACAGAT	CGGATCAA	TTGATCCG
G5		PGI77	ACCGCTAT	CGGAGTAT	ATACTCCG
G6		PGI78	AGAACCAG	TCTAGGAG	CTCCTAGA
G7		PGI79	GATACCTG	CATACGGA	TCCGTATG
G8		PGI80	CCAACACT	GCATAGTC	GACTATGC
G9		PGI81	ATTCCGCT	TCCTGACT	AGTCAGGA
G10		PGI82	CGACCTAA	ACAGCAAG	CTTGCTGT
G11		PGI83	ACCGGTTA	GAAGATCC	GGATCTTC
G12		PGI84	CAGTGCTT	GAACGAAG	CTTCGTTC
H1		PGI85	AATGGTCG	GTTATGGC	GCCATAAC
H2		PGI86	ACCATGTC	CCTATACC	GGTATAGG
H3		PGI87	TGATCACG	CAACGAGT	ACTCGTTG
H4		PGI88	TAGTCAGC	GTCCTGTT	AACAGGAC
H5		PGI89	CAATAGCC	GTTGCTGT	ACAGCAAC

Plate Loc (if applicable)	Historical Index ID	Updated Index ID	i7 Equivalent Index	i5 Equivalent Index	i5 Equivalent Index (Reverse complement)
H6		PGI90	CTTCGGTT	GGCAAGTT	AACTTGCC
H7		PGI91	CCATGAAC	GGAGTCTT	AAGACTCC
H8		PGI92	ATGAGTGC	GGCGAATA	TATTCGCC
H9		PGI93	CGGTAATC	CTAACCTG	CAGGTTAG
H10		PGI94	ACAGTGAC	CTGAACGT	ACGTTCAG
H11		PGI95	CAATCAGG	TCCTGGTA	TACCAGGA
H12		PGI96	GTAACCGA	CTTCCTTC	GAAGGAAG

Restriction Enzymes

Restriction Enzyme	Cut Sequence
Sau3AI	GATC
MluCI	AATT

Revision History

Version	Date	Revision Description
3.0	2020-01	• released
	2020-02	• tabulated incubation steps • expanded index table
4.0		• Library Preparation and On-bead Library Amplification steps protocol re-formulated • Increased Ligation Buffer concentration for better stability in long-term storage • Updated introduction • Updated links and redirects • Removed Bead Reagent addition in Streptavidin Bead Binding • Decreased sample input requirements • Added quick protocol for experienced users • Modified Workflow Overview • Adjusted index sequence IDs to match 96 well layout • moved liquid nitrogen grinding from before the lysis step to before crosslinking • added clarifying comments in crosslinking and lysis steps
4.5	2022-12	• Added restriction enzyme information • clarified buffer removal before fragmentation • decreased maximum recommended PCR cycle number from 16 to 14 • clarified dilution of CRB with molecular biology-grade water • updated formulation for crosslinking solution, 10X CRB, and Fragmentation Enzyme • Updated volumes for Wash Buffer 1 and Wash Buffer 2 • Corrected typo in step 8.9 • Updated color scheme and kit sticker image • Corrected font error in Kit Specifications • Updated formatting in quick protocol • Corrected typo in Equipment and Consumables (end of sentence was incorrectly deleted in previous iteration of the protocol) • Updated notes about DNA addition in step 8 Step 8.9 - dilution information was moved to an in-line note. • 9.2 - updated wording around number of provided primers
	2023-01	• corrected language in brief protocol in crosslinking and fragmentation steps for better clarify • Corrected capitalization error in step 2.11 • Corrected language and spelling error in subheader for streptavidin bead preparation • corrected “repar” to “repair” in table in step 8.8 • Corrected capitalization error in step 10.9 • Corrected grammatical error in Falcon-PHASE description • corrected typo in Notices section

Version	Date	Revision Description
4.5.1	2023-07	• Corrected numbering error at the beginning of step 4 • Corrected spelling of "botttom" to "bottom" on p19 • Added clarification about low on-bead measurements at step 7 • Updated final Recovery Bead clean up bead-to-volume ratios
4.5.2	2023-12	• Corrected discrepancies between quick and long protocol • Added more indexes and updated index IDs to new naming system • Converted font style • updated PCR cycle guidelines • updated adapter concentration dilution recommendations • updated index list to include more historical IDs
4.5.3	2024-04	• Increased fragmentation time from 5 to 7 min • Listed expected average fragment size in QC section
4.5.4	2024-06	• Corrected in-line Recovery Wash Buffer instructions to match instructions on bottle • Updated RX Enzyme time to 1 hour (removed extension up to 16 hours) • Added index plate locations

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Manufactured by:

Phase Genomics, Inc.
1617 8th Ave N
Seattle, WA 98109 USA
+1-833-742-7436
support@phasegenomics.com
www.phasegenomics.com