PHASE GENOMICS

Proximo™ Hi-C Kit

Proximo™ Hi-C (Human) Kit Protocol

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

This document applies to Proximo™ Hi-C Kit (Human) Kits KT1245, KT1345 and KT1445.

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 June 2026

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Introduction

Proximity ligation or Hi-C is one of several "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]

This Proximo™ Hi-C (Human) Kit is designed for the preparation of two, eight or twenty-four dual-indexed Hi-C libraries from whole-cell human samples. The entire protocol, from sample to sequencing-ready library for Illumina paired-end sequencing can be completed with approximately 6.5 hours of hands-on time.

The Proximo™ Hi-C (Human) kit is compatible with all types of whole-cell human inputs (100K - 500K cells) and has been specially developed for use with cells or blood. Extracted DNA is not a suitable input. For preparation of solid tissue samples or material that will require mechanical lysis, we recommend using one of our Proximo kits (KT2045).

For more information or recommendations regarding data analysis, please contact us at support@phasegenomics.com.

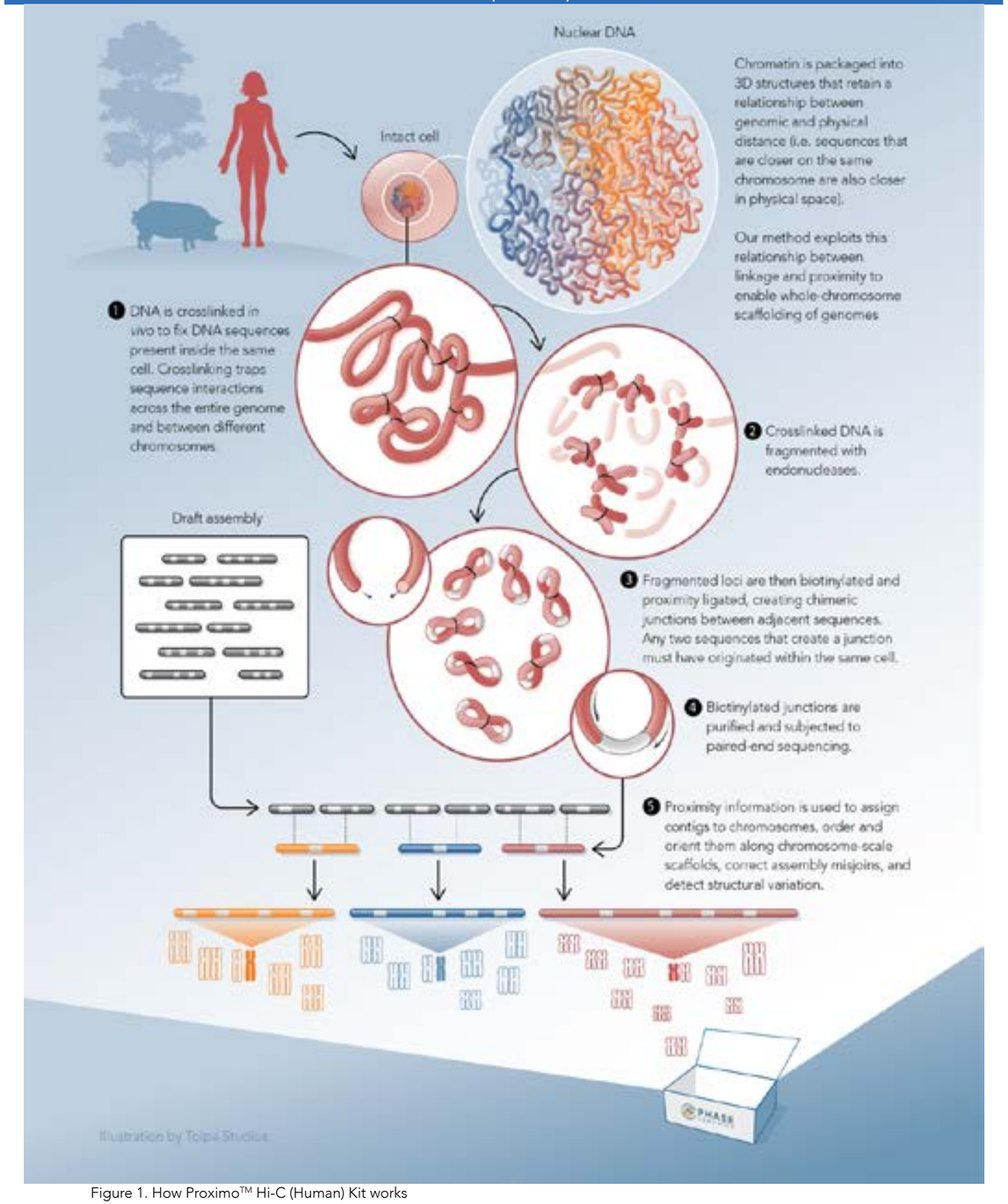

Figure 1. How Proximo™ Hi-C (Human) Kit works

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

Reagent Storage and Preparation

Cap Color	Cap Label	Tube Label	Storage Temperature (˚C)	Used in Step	Before Starting
	Crosslink Solution	Crosslinking Solution	-25 to +8˚C	1.2	Thaw and warm to RT
	Quench Solution	Quenching Solution	-25 to +25˚C	1.4	Thaw and warm to RT[1]
	Lysis Buffer 1	Lysis Buffer 1	-25 to +25˚C	2.1-2.3	Thaw and warm to RT
	Lysis Buffer 2	Lysis Buffer 2	-25 to +25˚C	2.9	Thaw and warm to RT
	Fragment Buffer	Fragment Buffer	-25 to -15˚C	3.3	Thaw on ice
	Fragment Enzyme	Fragment Enzyme	-25 to -15˚C	3.4	Thaw on ice
	Finishing Enzyme	Finishing Enzyme	-25 to -15˚C	3.6	Thaw on ice
	Stop Solution	Stop Solution	-25 to -15˚C	3.8	Thaw and warm to RT
	10X Ligation Buffer	10X Ligation Buffer	-25 to -15˚C	4.1	Thaw on ice
	Ligation Enzyme	Ligation Enzyme	-25 to -15˚C	4.2	Thaw on ice
	RX Enzyme	RX Enzyme	-25 to -15˚C	5.1	Thaw on ice
	Elution Buffer	Elution Buffer	-25 to -15˚C	6.7. 10.10	Thaw and warm to RT
	Recovery Beads	Recovery Beads	+2 to +8˚C	2.11, 6.2, 10.3, 10.6	Warm to RT
	Recovery Wash Buffer	Recovery Wash Buffer	-25 to +8˚C	6.4-6.5, 10.8-10.9	Warm to RT. Add 5 mL 95%-100% Ethanol to the provided bottle[2]
	Strept Beads	Streptavidin Beads	+2 to +8˚C	7.1	Warm to RT
	Bead Bind	Bead Binding Buffer	-25 to +25˚C	7.4	Thaw and warm to RT
	Wash Buffer 1	Wash Buffer 1	-25 to +25˚C	7.2-7.3, 7.9, 8.15,	Thaw and warm to RT
	Wash Buffer 2	Wash Buffer 2	-25 to +25˚C	7.7-7.8, 8.13-8.14	Thaw and warm to RT
	FERAT Buffer	Frag, Repair, A-Tail Buffer	-25 to -15˚C	8.9	Thaw on ice
	FERAT Enzyme	Frag, Repair, A-Tail Enzyme	-25 to -15˚C	8.6	Thaw on ice
	Adapter Ligation	Adapter Ligation Mix	-25 to -15˚C	8.7	Thaw on ice
	Universal Adapter	Universal Adapter	-25 to -15˚C	8.11	Thaw on ice
	Hot Start Mix	Hot Start PCR Mix	-25 to -15˚C	9.3	Thaw on ice
	Primer	PCR Primer Mix	-25 to -15˚C	9.2	Thaw on ice
	10X CRB	10X CRB	-25 to -15˚C	1.7, 2.8, 2.13, 3.7-3.8, 3.11	Dilute to 1X in molecµLar biology-grade water before use.[4]
	PGShield	PGShield	-25 to +8˚C	1.2-1.3, 2.2	

[1]May be warmed to 37˚C to dissolve any precipitate that is present after freezing and thawing, however complete dissolution of precipitate is not necessary for reagent use.
[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

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Kit Contents

		KT1245			KT1345			KT1445			
Cap Color	Tube Label	Item Ref #	Volume per tube	No. of Tubes	Item Ref #	Volume per tube	No. of Tubes	Item Ref #	Volume per tube	No. of Tubes	Storage Temperature (˚C)
	Crosslinking Solution	KS0021	500 µL	1	KS0021	500 µL	1	KS0017	960 µL	1	-25 to +8˚C
	Quenching Solution	KS0022	100 µL	1	KS0022	100 µL	1	KS0018	240 µL	1	-25 to +25˚C
	Lysis Buffer 1	KB0066	800 µL	1	KB0066	800 µL	1	KB0055	1200 µL	2	-25 to +25˚C
	Lysis Buffer 2	KB0067	800 µL	1	KB0067	800 µL	1	KB0056	1200 µL	2	-25 to +25˚C
	Fragment Buffer	KB0006	300 µL	1	KB0068	1.2 mL	1	KB0057	3.6 mL	1	-25 to -15˚C
	Fragment Enzyme	KE0038	5 µL	1	KE0047	20 µL	1	KE0040	60 µL	1	-25 to -15˚C
	Finishing Enzyme	KE0016	5 µL	1	KE0048	20 µL	1	KE0041	60 µL	1	-25 to -15˚C
	Stop Solution	KS0004	15 µL	1	KS0023	50 µL	1	KS0020	180 µL	1	-25 to -15˚C
	10X Ligation Buffer	KB0051	20 µL	1	KB0053	80 µL	1	KB0058	240 µL	1	-25 to -15˚C
	Ligation Enzyme	KE0026	10 µL	1	KE0028	40 µL	1	KE0042	120 µL	1	-25 to -15˚C
	RX Enzyme	KE0007	10 µL	1	KE0017	40 µL	1	KE0043	120 µL	1	-25 to -15˚C
	Elution Buffer	KB0015	350 µL	1	KB0028	1.1 mL	1	KB0059	1.7 mL	2	-25 to -15˚C
	Recovery Beads	KR0011	600 µL	1	KR0012	1.2 mL	2	KR0014	6.6 mL	1	+2 to +8˚C
	Recovery Wash Buffer	KB0041	500 µL	1	KB0040	2 mL	1	KB0060	2 mL	2	-25 to +8˚C
	Streptavidin Beads	KR0002	40 µL	1	KR0005	160 µL	1	KR0015	480 µL	1	+2 to +8˚C
	Bead Binding Buffer	KB0012	250 µL	1	KB0025	800 µL	1	KB0061	1.5 mL	2	-25 to +25˚C
	Wash Buffer 1	KB0047	7 mL	1	KB0047	7 mL	1	KB0062	10 mL	2	-25 to +25˚C
	Wash Buffer 2	KB0048	7 mL	1	KB0048	7 mL	1	KB0063	10 mL	2	-25 to +25˚C
	Frag, Repair, A-Tail Buffer	KS0011	10 µL	1	KB0045	32 µL	1	KB0064	96 µL	1	-25 to -15˚C
	Frag, Repair, A-Tail Enzyme	KB0043	8 µL	1	KE0031	48 µL	1	KE0044	144 µL	1	-25 to -15˚C
	Adapter Ligation Mix	KE0029	12 µL	1	KS0013	40 µL	1	KE0045	480 µL	1	-25 to -15˚C
	Universal Adapter	KE0032	40 µL	1	KE0034	160 µL	1	KS0019	120 µL	1	-25 to -15˚C
	Hot Start PCR Mix	KE0035	50 µL	1	KE0037	200 µL	1	KE0046	600 µL	1	-25 to -15˚C
	PCR Primer Mix	KP000N	5 µL each	2	KP000N[3]	5 µL each	8		10 µL each	24	-25 to -15˚C
	10X CRB	KB0054	1.6 mL	1	KB0069	1 mL	1	KB0065	4.8 mL	1	-25 to -15˚C
	PGShield	KR0013	10 mL	1	KR0013	10 mL	1	KR0013	10 mL	1	-25 to +8˚C

FFPE Reagent Module (Sold Separately)		KT1245F			KT1345F			KT1445F			
	Tube Label	Item Ref #	Volume per tube	No. of Tubes	Item Ref #	Volume per tube	No. of Tubes	Item Ref #	Volume per tube	No. of Tubes	Storage Temperature (˚C)
	Deparaffinization Reagent	KR0016	50 µL	1	KR0016	50 µL	1	KR0017	100 µL	1	-25 to +25˚C
	FFPE Lysis Buffer	KB0071	100 µL	1	KB0071	100 µL	1	KB0072	300 µL	1	-25 to +25˚C
	FFPE RX Enzyme	KE0049	25 µL	1	KE0049	25 µL	1	KE0050	75 µL	1	-25 to -15˚C
	Recovery Beads	KR0011	600 µL	1	KR0012	1.2 mL	1	KR0014	6.6 mL	1	+2 to +8˚C
	Recovery Wash Buffer	KB0041	500 µL	1	KB0060	2 mL	1	KB0060	2 mL	2	-25 to +8˚C

Shipping, Storage, and Handling

Proximo™ Hi-C Kit (Human) Kits are shipped on cold packs. Upon receipt, remove the inner container with the **Recovery Beads and Streptavidin Beads**, and store at +2 to +8˚C. If either the **Recovery Beads and Streptavidin Beads** are stored at freezing temperatures (below 0˚C) they will no longer functional and should be replaced. 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/

Phase Genomics PGShield™

Phase Genomics PGShield™ is a new preservative system for room temperature stabilization of biological samples. Unlike many lysis-based approaches, PGShield™ is designed to keep cells and cell structures intact until processing.

General Properties

- Nontoxic to humans and the environment
- Nonflammable
- Bacteriostatic/Bacteriocidal
- Slightly viscous
- Extended storage conditions
  - -80°C indefinitely
  - 4°C up to 1 year
  - Room temperature for up to 4 months

Sample types

- Cultured cells
- Tissues & cells
- Purified white blood cells
  - Blood will coagulate quickly in PGShield™ making the sample difficult to work with. We recommend removing RBCs before using PGShield™. We have not tested if heparin stops this effect.
- FFPE (formalin-fixed paraffin-embedded) tissue (requires purchase of KT1245F, KT1345F or KT1445F in addition to the Proximo Kit)

Important Notes for Use

PGShield™ is best used as an immersive agent for cells/tissue at maximum concentration. Do not dilute PGShield™ before use, and keep it at no less than 90% concentration.

For blood samples, lysing red blood cells prior to resuspending in PGShield™ is required.

For tissue we recommend complete immersion with enough PGShield™ to fully saturate.

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
- 10X Tris-buffered Saline

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
- Microcentrifuge of ≥500 x g compatible with PCR tubes.
- Plate centrifuge of ≥500 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
- Phase Genomics FFPE Proximo™ Hi-C Kit (Human) additional reagents (Purchased separately)
  - ► Requires ultrasonicator, such as Diagenode, Covaris, or PIXUL instruments.

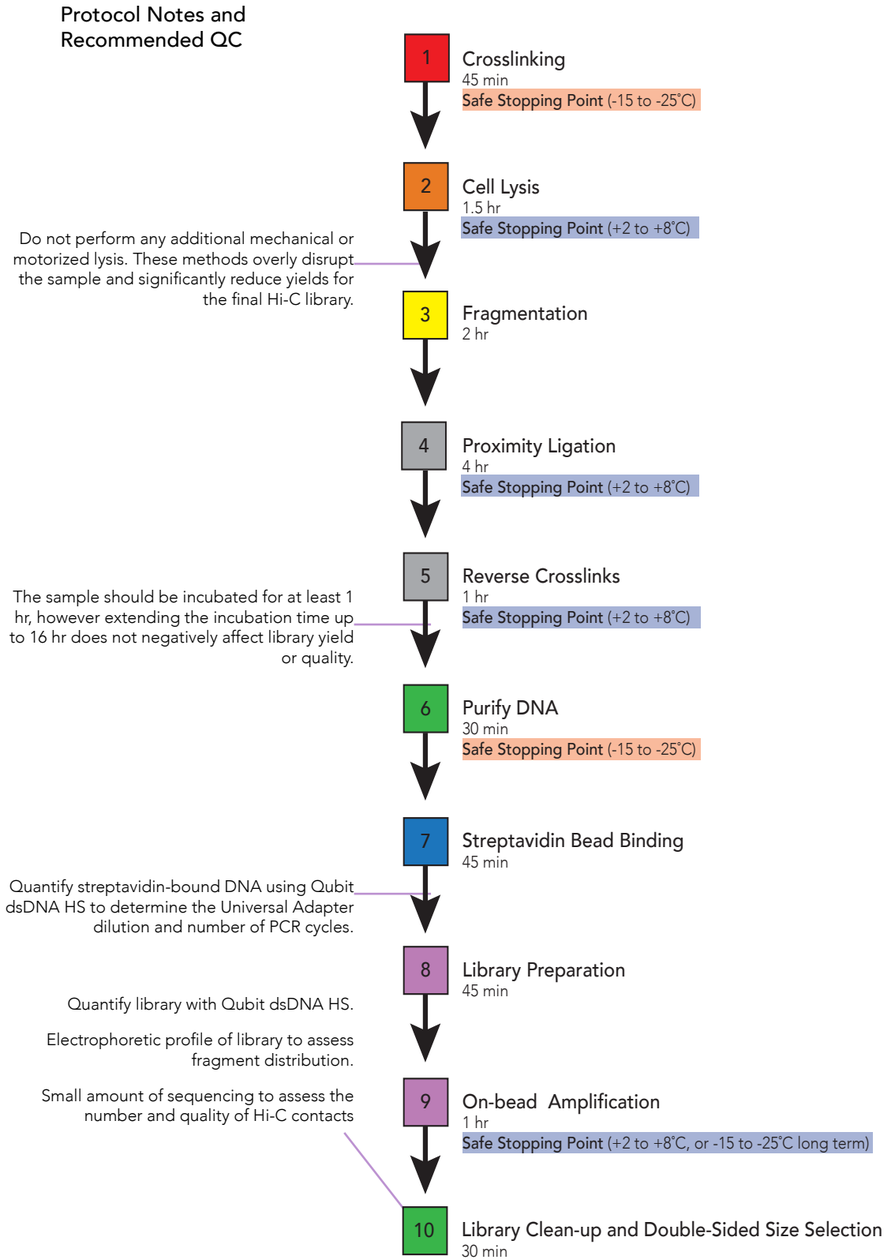
Sample Types and Preparation

This protocol is suitable for a wide range of Human types, from cultured cells, to isolated soft tissue and certain tumor material. For solid tissue or material that will require mechanical lysis, we recommend using the Proximo (Animal) Kit (KT2045). Consult the table below for required inputs and modifications to the standard protocol for your sample type.

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.

Sample Type	Suggested Input	Additional Preparation
Cells	200,000 - 500,000 intact cells	Perform Step **1. Recommended Sample Pre-Processing**
Blood	200 - 300 µL	Do not perform **Step 1. Recommended Sample Pre-Processing** on raw blood. We recommend performing RBC (Red blood cell) Lysis with any commercially available RBC Lysis buffer according to the manufacturer's protocol. Remove all remaining buffer from the cell pellet before addition of PGShield.
FFPE	3-5 5 µm curls or equivalent	Process according to instructions in Appendix A-1, then proceed to Step 4. Fragmentation

Workflow Overview



Quick Protocol

This section provides a quick-step guide for experienced users. If this is your first time using the Proximo, please refer to the detailed protocol on *p. 17**. This quick protocol assumes samples are being prepared one at a time. For instructions regarding batch prepping samples, please view the Detailed Protocol.*

Step	Protocol	Incubations and notes
1. Recommended Pre-processing	▪ Count cells according to preferred cell counting method ▪ Resuspend cells in **PG Shield** (provided) to a concentration of 25,000 cells/µL ▪ Allow cells to sit in **PG Shield** for a minimum of 1 hour before proceeding. For best results, incubate cells in **PG Shield** overnight.	
2. Crosslinking (Red)	▪ Add 250,000 cells stored in **PG Shield** to a clean PCR tube. ▪ Add volume of **PG Shield** necessary to bring volume to 50 µL. ▪ Add 40 µL **Crosslinking Solution** to each sample tube.	Incubate at room temperature for 20 min.
	▪ Add 10 µL of **Quenching Solution**.	Incubate at room temperature for 15 min.
	▪ Add 100 µL **Recovery Beads** to the sample.	Incubate at room temperature for 10 min.
	▪ Wash the beads: ▸ Place the sample tubes on a magnetic rack ▸ Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads ▸ Remove the tube from the magnetic rack and, for each reaction, gently resuspend the beads in 150 µL **1X CRB**.	Proceed directly to step 3.
3. Lysis (Orange)	▪ Place the sample tubes on a magnetic rack ▪ Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.	
	▪ Resuspend cells in 100 µL of **Lysis Buffer 1.**	Incubate at room temperature for 20 min.
	▪ Place the sample tubes on a magnetic rack ▪ Once the solution in each tube has cleared, remove the supernatant without disrupting the beads	
	▪ Resuspend the pellet in 100 µL of **Lysis Buffer 2.**	Incubate at 65˚C for 15 min.
	▪ Wash the beads: ▸ Place the sample tubes on a magnetic rack ▸ Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads ▸ Remove the tube from the magnetic rack and, for each reaction, gently resuspend the beads in 150 µL **1X CRB**.	**SAFE STOPPING POINT:** Store sample at +2 to +8˚C overnight.

Step	Protocol	Incubations and notes
4. Fragmentation (Yellow)	■ Place the sample tubes on a magnetic rack ■ Once the solution in each tube has cleared, remove the supernatant without disrupting the beads ■ Remove the tube from the magnetic rack and, for each reaction, gently resuspend the beads in 147.5 µL of **Fragmentation Buffer.**	
	■ Add 2.5 µL of **Fragmentation Enzyme.**	Incubate at 37˚C for 1 hr, then cool to 4˚C
	■ Add 2.5 µL of **Finishing Enzyme.**	Incubate at 12˚C for 30 min.
	■ While on the chilled block, add 6 µL of **Stop Solution.**	
	■ Wash the beads: ► Place the sample tubes on a magnetic rack ► Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads ► Remove the tube from the magnetic rack and, for each reaction, gently resuspend the beads in 150 µL **1X CRB**. ■ Repeat the bead wash steps for a total of 2 washes with **1X CRB.**	
5. Proximity Ligation (Clear)	■ Remove **1X CRB** from beads. ■ Add 85 µL of **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.
6. 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.

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Step	Protocol	Incubations and notes
7. Purify 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 tubes on a magnetic rack. ► Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads. ► Keeping the beads on the magnet, gently rinse the beads with 150 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** ■ Air dry the beads.	Leave tubes with caps open on the magnet at room temperature for 10 - 15 min.
	■ Resuspend the beads in 100 µL of **Elution Buffer**.	Incubate at room temperature for 5 min.
	■ Place the sample tubes on a magnetic tube rack or magnet. ■ Once the solution in each tube has cleared, recover the **DNA-containing supernatant** and transfer to a fresh tube.	

Step	Protocol	Incubations and notes
8. Streptavidin Bead Binding (Blue)	**Prepare the Beads** ■ Transfer 20 µL of **Streptavidin Beads** into a new 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 in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads. ■ Wash the beads: ► Place the sample tubes on a magnetic rack ► Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads ► Remove the tube from the magnetic rack and, for each reaction, gently resuspend the beads in 150 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 tubes on a magnetic rack ► Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads ► Remove the tube from the magnetic rack and, for each reaction, gently resuspend the beads in 150 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** for a total of 4 washes. ■ 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
9. Library Preparation (Purple)	■ Transfer no more than 200 ng of DNA-containing Streptavidin Beads to a fresh microcentrifuge tube. ■ Place the sample tubes on a magnetic tube rack or magnet. ■ Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads. ■ Place the tube on a pre-cooled thermocycler.	Pre-cool thermocycler to 4˚C.
	■ To beads add: ► 40 µL of Molecular biology-grade water ► 4 µL of Frag, Repair, & A-Tail Buffer ► 6 µL of Frag, Repair, & A-Tail Enzyme	Fragment, end-repair, and A-tail using the thermocycler program listed in Step 9.
	■ 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 9.10. Incubate at 20˚C for 15 min, no heated lid.
	■ Wash the beads: ► Place the sample tubes on a magnetic rack ► Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads ► Remove the tube from the magnetic rack and, for each reaction, gently resuspend the beads in 150 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.	
10. 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 the PCR protocol given in Step 10 of the detailed protocol.

Step	Protocol	Incubations and notes
11. Library Clean-up and Double-Sided Selection (Green)	■ Place the sample tubes on a magnetic tube rack or magnet and allow the 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 tubes on a magnetic tube rack or magnet.	**Your library is in the supernatant. Do not discard.**
	■ Transfer the supernatant (105 µL) to a new tube containing 15 µL of **Recovery Beads**.	Incubate at room temperature for 10 min.
	■ Rinse the beads: ► Place the sample tubes on a magnetic rack. ► Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads. ► Keeping the beads on the magnet, gently rinse the beads with 150 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 tubes on a magnetic tube rack or magnet. ■ Once the solution in each tube has cleared, recover the **Proximo Hi-C library-containing supernatant** and transfer to a fresh microcentrifuge tube.	See Step 12 in the detailed Protocol for recommended QC to determine if your library is sufficient.

Detailed Protocol

1. Recommended Sample Pre-processing

1.1 Count cells according to preferred cell counting method.

1.2 Remove any remaining buffer or media from cells.

The solution must contain more than 80% PG Shield. Overly diluting PG Shield not only negates any benefit of PG Shield but can actually damage the cells, significantly decreasing the likelihood of a successful library prep.

1.3 Resuspend cells in **PG Shield**.

*Recommended concentration between 10,000-50,000 cells/µL, but if cell count is unknown resuspend in 55 µL **PG Shield***

1.4 Allow cells to sit in **PG Shield** for at least 12 hours before proceeding for best results, but minimally 1 hour.

*Cells can be stored in **PG Shield** at +2 to +8˚C for up to 1 year.*

2. Crosslinking (Red)

Bring Recovery Beads to room temperature.

2.1 For each sample, transfer up to 50 µL of cells prepared in step 1.3 (between 100,000 and 500,000 cells) to a clean PCR tube.

2.2 If required, add additional **PG Shield** to each sample, bringing the final volume to 50 µL.

2.3 Add 40 µL **Crosslinking Solution** to each sample tube and mix well by vortexing gently.

2.4 Incubate at room temperature for 20 min.

2.5 Add 10 µL of **Quenching Solution** to each sample tube and mix well by vortexing gently.

2.6 Incubate at room temperature for 15 min.

2.7 Thoroughly resuspend **Recovery Beads** and add 100 µL of beads to each sample tube. Mix well by vortexing gently or pipetting thoroughly.

2.8 Incubate at room temperature for 10 min.

2.9 Wash the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.
- Remove the tubes from the magnet and gently resuspend the beads in 150 µL of **1X CRB.**

Proceed Directly to Step 3. Cell Lysis

3. Cell Lysis (Orange)

Pre-heat a thermocycler to 65˚C (for use in Step 3.8, with lid heated to 105˚C).

3.1 Place the sample tubes on a magnetic rack or on a magnet.

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

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

3.4 Resuspend each sample in 100 µL of **Lysis Buffer 1** and mix well by vortexing gently.

Pipette mixing is not recommended as bead-bound chromatin can stick to the pipette tip.

3.5 Incubate for 20 min at room temperature.

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

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

3.8 Resuspend each pellet in 100 µL of **Lysis Buffer 2.**

3.9 Incubate at 65˚C for 15 min.

3.10 Briefly allow the sample tubes to cool.

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3.11 Wash the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.
- Remove the tubes from the magnet and gently resuspend the beads in 150 µL of **1X CRB**.

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

4. Fragmentation (Yellow)

Set up the following Fragmentation program:

Step	Temperature (˚C)	Time
Lid temperature	105	
Fragmentation	37	1 hr
Cool	4	Hold
Finishing	12	30 min
Hold	4	Hold

4.1 Prepare **Fragmentation Mix:**

Reagent	Volume 1 reaction (µL)	Volume 8.2 reactions (µL)
Fragmentation Buffer	147.5	1209.5
Fragmentation Enzyme	2.5	20.5
Total	150	1230

4.2 Place the sample tubes on a magnetic rack or on a magnet.

4.3 Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.

4.4 Remove the tubes from the magnetic rack and gently resuspend the beads for each sample in 150 µL of prepared **Fragmentation Mix.**

4.5 Place the samples on the thermocycler and initiate the run of the above **Fragmentation** program (Programmed at the beginning of Step 4. Fragmentation).

4.6 Once the samples have cooled to 4˚C and keeping the samples on the thermocycler, open the lid and add 2.5 µL of **Finishing Enzyme** to each sample tube and mix by vortexing gently or pipetting thoroughly.

4.7 Progress the thermocycler program to the **Finishing** step.

4.8 While samples are still chilling in the thermocycler, add 6 µL of **Stop Solution** to each sample tube, then remove from the thermocycler and mix by vortexing gently or pipetting thoroughly to quench the reaction.

Promptly add Stop Solution after 30 minutes at 12°C. Extended incubation after the 30 minute Finishing step is complete may result in a low quality library.

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4.9 Wash the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads.
- Remove the tubes from the magnet and gently resuspend the beads in 150 µL of **1X CRB**.

4.10 Repeat the bead wash steps two more times using 150 µL of **1X CRB** per wash for a total of three washes.

5. Proximity Ligation (Clear)

Set up the following Ligation program:

Step	Temperature (˚C)	Time
Lid temperature	105	
Ligation	25	4 hr
Enzyme inactivation	65	10 min
Final hold	4	Hold

5.1 Prepare **Ligation Mix**:

Reagent	Volume 1 reaction (µL)	Volume 8.2 reactions (µL)
Water	85	697
10X Ligation Buffer	10	82
Ligation Enzyme	5	41
Total	100	820

5.2 Place the sample tubes on a magnetic rack or on a magnet

5.3 Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.

5.4 Remove the tubes from the magnetic rack and gently resuspend the beads for each sample in 100 µL of prepared **Ligation Mix**.

5.5 Place the samples on the thermocycler and initiate the above **Ligation** program run (Programmed at the beginning of Step 5. Ligation).

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

6. Reverse Crosslinks (Clear)

Set up the following Reverse Crosslinks program:

Step	Temperature (˚C)	Time
Lid temperature	105	
Reverse crosslinks	65	1 hr
Final hold	4	Hold

6.1 Add 4 µL of **RX Enzyme** to each sample tube and mix well by vortexing or pipetting.

6.2 Place the samples on the thermocycler and initiate the run of the above **Reverse Crosslinks** program (Programmed at the beginning of **Step 6. Reverse Crosslinks**).

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

SAFE STOPPING POINT: The reaction may be stored at +2 to +8˚C overnight, or the supernatant can be removed and stored at -25 to -15˚C for up to 1 month.

7. Purify DNA (Green)

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

7.1 Allow sample tubes to equilibrate to room temperature.

7.2 Thoroughly resuspend the **Recovery Beads**, then add 100 µL of **Recovery Beads** to each sample tube and mix thoroughly by vortexing or pipetting. For FFPE samples increase the volume of **Recovery Beads** to150 µL.

You do not need to remove the supernatant from the old beads before adding new Recovery Beads.

7.3 Incubate at room temperature for 10 min.

7.4 Rinse the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads.
- Keeping the beads on the magnet, gently rinse the beads with 150 µL of **Recovery Wash Buffer** without disrupting the beads, leaving the buffer on the beads for 30 sec to 1 min between washes.

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

7.6 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.

7.7 Remove the sample tubes from the magnet, add 100 µL of **Elution Buffer** to each sample and thoroughly resuspend the beads.

7.8 Incubate at room temperature for 10 minutes to elute the DNA.

*If beads were completely dry before addition of **Elution Buffer** extend elution time by 5 min.*

7.9 Place the sample tubes on a magnetic tube rack or magnet.

7.10 Once the solution in each tube has cleared, transfer the **DNA-containing-supernatant** to fresh tubes. Discard the beads.

SAFE STOPPING POINT: Purified, proximity-ligated DNA may be stored at -25 to -15˚C (indefinitely)

8. Streptavidin Bead Binding (Blue)

A. Prepare the Beads

Do not yet combine the beads with the DNA recovered in Step 7. DNA binding will occur in section B after the beads are prepared in section A.

Number of samples	Step 8.1: Volume Streptavidin beads (µL)	Step 8.3 & 8.4: Volume Wash Buffer 1 (µL)	Step 8.5: Volume Bead Binding Buffer (µL)
1	20	200	100
2	40	400	202
4	80	500	405
8	160	500	805

8.1 Determine the amount of **Streptavidin Bead** needed based on how many samples you prepare together. 20 µL **Streptavidin beads** are required per sample. (see table above)

8.2 Thoroughly resuspend the **Streptavidin Beads** and transfer the determined volume into a new tube.

8.3 Wash the Beads:

- Place the bead-containing tube on a magnetic rack or on a magnet.
- Once the solution in the tube has cleared, remove and discard the supernatant without disrupting the beads.
- Remove the tube from the magnet and gently resuspend the beads in 150 µL of **Wash Buffer 1** per 20 µL **Streptavidin Beads,** up to 500 µL maximum volume.

8.4 Repeat the bead wash steps one more time with 200 µL of **Wash Buffer 1** per 20 µL **Streptavidin Beads** up to 500 µL maximum volume for a total of two washes.

8.5 Remove beads from the magnet and resuspend in a final volume of 100 µL of **Bead Binding Buffer** per sample.

B. Bind the Sample to the Beads

8.6 For each sample transfer 100 µL of washed **Streptavidin Beads** to a sample tube containing **DNA-containing supernatant** from step 7.10 and mix well by gently vortexing.

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

8.8 Wash the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads.
- Remove the tubes from the magnet and gently resuspend the beads in 150 µL of **Wash Buffer 2**.

8.9 Repeat the bead wash steps one more time with 150 µL of **Wash Buffer 2** for a total of two washes.

8.10 Repeat the bead wash steps one more time with 150 µL of **Wash Buffer 1**.

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

8.12 With your bead-bound samples suspended in 200 µL of water, measure the concentration of DNA for each (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. To ensure an accurate measurement, vortex the beads in the fluorometric assay tube immediately prior to measuring DNA concentration.

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

Use between 2-5 µL for quantification. If at this stage your sample's concentration is less than 10 ng, including "too low - out of range" measurements, this does NOT necessarily indicate failure, but if your input was within the recommended range we suggest reaching out to support@phasegenomics.com before proceeding.

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9. Library Preparation (Purple)

Set up the following Fragmentation, End-repair, and A-tailing 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
*3 min for FFPE samples		

And set up the following Adapter Ligation Program:

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

9.1 Initiate the run of the above **Fragmentation, End-repair, and A-tailing** program (Programmed at the beginning of Step 9. Library Preparation) to pre-cool the thermocycler.

9.2 Transfer no more than 200 ng of **streptavidin-bound DNA** to a fresh tube for each sample.

Unused beads can be stored in 1X CRB at 4˚C for 1-2 days.

9.3 Place the sample tubes on a magnetic rack or on a magnet.

9.4 Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads.

9.5 Resuspend the beads of each sample in 40 µL of **molecular biology-grade water.**

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

9.7 Prepare **FERAT Master Mix:**

Reagent	Volume 1 reaction (µL)	Volume 8.2 reactions (µL)
Frag, Repair, A-Tail Enzyme	6	49.2
Frag, Repair, A-Tail Buffer	4	32.8
Total	10	82

9.8 Add 10 µL of **FERAT Mix** to each sample and mix by vortexing gently or pipetting thoroughly. Immediately place the samples back onto the thermocycler.

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.

9.9 Advance the **Fragmentation, End-repair, and A-tailing** program from the Pre-cooling step to Fragmentation, end-repair, and A-tailing.

9.10 Every library for which the total mass of DNA measured at step 8.11 was less than 10 ng, prepare diluted **Universal Adapter** (provided tube is 15 µM) 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	5
0 - 10	1 µM	14	1

*Measured in Step 8.11

9.11 Add 5 uL of appropriately diluted **Universal Adapter** (see step 9. for dilution instructions) to each sample and mix by vortexing gently or pipetting thoroughly.

9.12 Add 20 µL of **Adapter Ligation Mix** to each sample tube**.** Mix by pipetting thoroughly.

Do not vortex Adapter Ligation Mix.

9.13 Place the samples on the thermocycler and initiate the run of the above **Adapter Ligation** program (Programmed at the beginning of Step 9. Library Preparation)

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9.14 Wash the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads.
- Remove the tubes from the magnet and gently resuspend the beads in 150 µL of **Wash Buffer 2**.

9.15 Repeat the bead wash steps one more time with 150 µL of **Wash Buffer 2** for a total of two washes.

9.16 Repeat the bead wash steps one more time with 150 µL of **Wash Buffer 1**.

9.17 Repeat the bead wash steps one more time with 150 µL of **molecular biology-grade water.**

10. On-bead Library Amplification (Purple)

Set up the following PCR Program program:

Step	Temperature (˚C)	Time (sec)	Cycles
Lid temperature	105		
Initial denaturation	98	45	1
Denaturation	98	15	6*
Annealing	60	30	
Extension	72	30	
Final extension	72	60	1
Hold	12	hold	

*Use the table in step 10. 6 below to determine the number of PCR cycles to use for each sample.

10.1 Place the sample tubes on a magnetic rack or on a magnet.

10.2 Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads.

10.3 For each sample thoroughly resuspend the beads in 20 µL of **molecular biology-grade water**.

10.4 Add 5 µL of one unique **PCR Primer Mix** to each sample 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).

10.5 Add 25 µL of **Hot Start PCR Mix** to each sample tube.

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

Mass used at step 9.1 (ng)	Recommended Number of PCR Cycles (fresh samples or cells)	Recommended Number of PCR cycles (FFPE)
<50	10	10-12
50-75	8-9	10-11
75-100	6-7	8-9
100-200	5-6	7-8

Amplifying your libraries beyond 10 cycles can negatively impact the final data quality. This may require you to separate your samples into separate PCR runs.

10.7 Place the samples on the thermocycler and initiate the run of the above **PCR** program (Programmed at the beginning of Step 10. On-bead Library Amplification).

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

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

If necessary, prepare the second Recovery Wash Buffer by adding 10 mL of 95-100% ethanol to the 2 mL of the provided Recovery Wash Buffer and mix well.

11.1 Place the sample tubes on a magnetic tube rack or magnet.

11.2 Once the solution in each tube has cleared, transfer 50 µL of the **library-containing supernatant** for each sample to a new tube.

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

11.4 Add 57.5 µL to each sample (1.15X volume) of thoroughly resuspended **Recovery Beads** to the tubes containing the libraries (from Step 11.2).

Unwanted high molecular weight fragments will be binding to the beads.

11.5 Incubate at room temperature for 10 min.

11.6 Place the sample tubes on a magnetic tube rack or magnet. **Your libraries are in the supernatant. Do not discard.**

11.7 Transfer 15 µL of **Recovery Beads** to a new PCR tube for each sample.

11.8 After 2 min, or once the solution in each tube has cleared, transfer the supernatant of each sample (105 µL) to the PCR tubes containing 15 µL of **Recovery Beads**.

The libraries are now binding to the beads, leaving unwanted small fragments in the supernatant.

11.9 Incubate at room temperature for 10 min.

11.10 Rinse the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove and discard the supernatant from each sample without disrupting the beads.
- Keeping the beads on the magnet, gently rinse the beads with 150 µL of **Recovery Wash Buffer** without disrupting the beads, leaving the buffer on the beads for 30 sec - 1 min between washes.

11.11 Repeat the bead rinse steps for a total of two rinses with **Recovery Wash Buffer**. Air dry the beads at room temperature for 10 - 15 min on the magnet with the cap open.

Over-drying is not problematic for Recovery Beads.

11.12 Remove the sample tubes from the magnet and thoroughly resuspend each of the sample beads in 30 µL of **Elution Buffer**.

11.13 Incubate at room temperature for 10 minutes to elute the DNA.

*If beads were completely dry before addition of **Elution Buffer** extend elution time by 5 min.*

11.14 Place the sample tubes on a magnetic tube rack or magnet.

11.15 Once the solution in each tube has cleared, recover the **Proximo Library-containing supernatant** for each library and transfer to fresh tubes. Discard the beads.

12. Library QC (recommended)

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

0.5 ng/µL is the minimum viable library concentration. The library can be stored at -15 to -25˚C indefinitely.

12.2 Assess library fragment size using BioAnalyzer or similar instrument.

12.3 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 Proximo library. For questions or assistance with data analysis, please reach out to us directly (contact support@phasegenomics.com for more info).

13. Sequencing

Proximo libraries are compatible with any Illumina® sequencer

Sequencing Recommendation (3D Genomes)
> 400 million pairs (2 x 75 bp or longer)

Note: this is meant as a guideline for the amount of data required.

14. Analysis

Take advantage of our expertise! Interested in additional computational analyses? Contact us to learn more about your analysis options.

Appendix A-1

Instructions for preparing FFPE Proximo libraries. This preparation uses ultrasonication for deparaffinization. We have included recommended starting parameters for several instruments but recommend optimizing for your particular type of sample input.

Before beginning, bring Recovery Beads to room temperature.

A.1 Prepare 1:10 dilution of Deparaffinization Reagent in Molecular Biology-grade Water.

Deparaffinization reagent is very viscous. Pipette slowly!

Reagent	Volume (µL)
Water	90
Deparaffinization Reagent	10

A.3 Prepare Deparaffinization Buffer as follows:

Reagent	Volume 1 mL (1.25 rxn)	Volume 10 mL (8-12 rxn)	Volume 30 mL (20-35 rxn)
Water	900	9 mL	27 mL
10X TBS (Tris-Buffered Saline)	100	1 mL	3 mL
Deparaffinization Reagent	2	20 µL	60 µL

Diluted Deparaffinization Reagent and Deparaffinization Buffer must be prepared and used same-day. It is not stable stored, even at cold temperatures.

A.5 Transfer three to five 15 µm curls or equivalent amount of FFPE tissue to a 1.5 mL microcentrifuge tube.

A.6 Add 800 µL **Deparaffinization Buffer** prepared at step A.3.

A.7 Invert the tube 1-3 times to make sure most of the sample is covered by the buffer.

A.8 Incubate the samples at 80°C for 3 min.

A.9 Centrifuge at 10,000 *x g* for 2 min to gently pellet the tissue while the paraffin wax solidifies as a layer on top of the solution.

A.10 Scrape the paraffin layer using a pipette tip, being careful only to remove paraffin and leave all tissue and buffer in the tube. If tissue is still trapped in wax, it is preferred to leave some paraffin behind.

A.11 Repeat steps A.8-A.10 once.

The amount of wax to remove depends completely on the sample. It is recommended that steps A.8-A.11 be repeated until minimal wax remains. You do not need to remove all the paraffin before proceeding, but less paraffin in the sample does improve sample handling for the rest of the protocol.

A.12 Remove supernatant until 100-150 µL liquid volume remains

This does not need to be especially precise, however do your best to stay above 100 µL volume in the tube. Be careful not to remove any tissue with the supernatant.

A.13 Add 12 µL **FFPE Lysis Buffer**.

A.14 Add 3 µL **FFPE RX Enzyme.**

A.15 Incubate at 37˚C for 20 min.

A.16 If needed, transfer solution with all tissue to appropriate tube or plate for your ultrasonicator or choice.

A.17 Follow the appropriate instructions below for your preferred ultrasonicator. Please note that these instructions are starting points and it is recommended that you optimize settings based on your particular input type.

Settings	Diagenode Standard	Covaris (S220, LE220, R230)	PIXUL
Cycles/Power	5 cycles Power Level = High	Duty Factor 20% Peak incident 75W 200 cycles/burst	50 N pulse at 1 kHz 20 Hz burst rate
Time	5 minutes (30 sec on, 30 sec off)	10 minutes	10 minutes
Temperature	4˚C	17˚C	15˚C

A.19 Incubate at 80˚C for 1.5 hours.

A.20 Incubate at 55˚C for 15 min.

7.21 Thoroughly resuspend the **Recovery Beads**, then add 150 µL of **Recovery Beads** to each sample tube and mix thoroughly by vortexing or pipetting.

A.22 Incubate at room temperature for 10 min.

A.23 Wash the beads:

- Place the sample tubes on a magnetic rack or on a magnet.
- Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.
- Remove the tubes from the magnet and gently resuspend the beads in 150 µL of **1X CRB.**

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

A.24 Place the sample tubes on a magnetic rack or on a magnet.

A.25 Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.

A.26 Vortex **Lysis Buffer 1** to resuspend any particulates that may have settled out.

A.27 Resuspend each sample in 100 µL of **Lysis Buffer 1** and mix well by vortexing gently.

Pipette mixing is not recommended as bead-bound chromatin can stick to the pipette tip.

A.28 Incubate for 20 min at room temperature with occasional mixing by inversion or rotation.

A.29 Place the sample tubes on a magnetic rack or on a magnet.

A.30 Once the solution in each tube has cleared, remove the supernatant without disrupting the beads.

Proceed Directly to Step 4. Fragmentation

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Index Sequences

Your kit contains twenty-four 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 primers or assistance with multiplexed sequencing are needed.

Plate Position	Primer Mix	i7 Equivalent Index	i5 Equivalent Index	i5 Equivalent Index (Reverse Complement)
A1	PGI1	TCAATCCG	ACTGCGAA	TTCGCAGT
A2	PGI2	CGCTACAT	TAGTCTCG	CGAGACTA
A3	PGI3	GATCCACT	TGAGCTGT	ACAGCTCA
A4	PGI4	ATCCACGA	AGTATGCC	GGCATACT
A5	PGI5	ACGATCAG	TGGTGAAG	CTTCACCA
A6	PGI6	GTCCTAAG	TACTGCTC	GAGCAGTA
A7	PGI7	CAACTCCA	ACTCCTAC	GTAGGAGT
A8	PGI8	AAGCATCG	TACTCCAG	CTGGAGTA
A9	PGI9	GAAGACTG	TCACCTAG	CTAGGTGA
A10	PGI10	GAACGGTT	GATCTTGC	GCAAGATC
A11	PGI11	CTCTATCG	AAGCCTGA	TCAGGCTT
A12	PGI12	ATGCCTAG	AGTACACG	CGTGTACT
B1	PGI13	CCACATTG	CGACACTT	AAGTGTCG
B2	PGI14	ATGTGGAC	CTCACCAA	TTGGTGAG
B3	PGI15	TGAGACGA	AACCAGAG	CTCTGGTT
B4	PGI16	GGTTGGTA	GCGTATCA	TGATACGC
B5	PGI17	CATCAACC	AATGACGC	GCGTCATT
B6	PGI18	GCAATTCC	CCACAACA	TGTTGTGG
B7	PGI19	ACCTCTTC	GTATTCCG	CGGAATAC
B8	PGI20	TTCACGGA	AGGTAGGA	TCCTACCT
B9	PGI21	CTGGTCAT	ACGAGAAC	GTTCTCGT
B10	PGI22	CCTATTGG	TGACAACC	GGTTGTCA
B11	PGI23	AAGACCGT	CTTAGGAC	GTCCTAAG
B12	PGI24	GGTGTACA	CCGCTTAA	TTAAGCGG

Plate Position	Primer Mix	i7 Equivalent Index	i5 Equivalent Index	i5 Equivalent Index (Reverse Complement)
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
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

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Plate Position	Primer Mix	i7 Equivalent Index	i5 Equivalent Index	i5 Equivalent Index (Reverse Complement)
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
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
DpnII	GATC
DdeI	CTNAG
MseI	TTAA
HinfI	GANTC

Revision History

Version	Date	Revision Description
1.1	2024-02	• Updated master mix volumes • corrected item codes
1.2	2024-11	• Added FFPE processing appendix A-1 and in-line FFPE-specific modifications throughout • Corrected spelling of “Phase” in Table of Contents • Corrected spelling of “electrophoretic” in workflow outline • Corrected number of washes in step 9 of the Quick Protocol • Corrected header formatting in steps 1 and 2
	2025-10	• Added FFPE module reagents list and info
4.5	2026-02	• Combined kit format options into 1 protocol (KT1245, KT1345, KT1445)

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