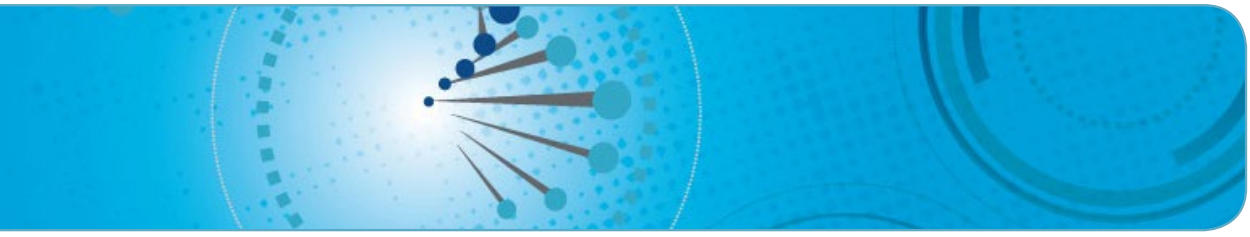
xGen® Lockdown® Probes and Panels:

Hybridization capture of Illumina Nextera® DNA libraries



For use with:

- Illumina Nextera libraries
- xGen Universal Blockers—NXT Mix
  (Catalog # 1079584, 1079585, 1079586)
- xGen Lockdown Reagents
  (Catalog # 1072280, 1072281)

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Introduction

This protocol includes the steps necessary for target enrichment of an Illumina Nextera library prepared from genomic DNA. Target enrichment is achieved through hybridization capture using xGen Lockdown Probes or Panels.

xGen Lockdown Probes or Panels

xGen Lockdown Probes are individually synthesized, 5'-biotinylated oligos for target capture applications in next generation sequencing. These probes are useful for creating custom capture panels that can be optimized, expanded, and combined with other panels. xGen Lockdown Probes can also be used to enhance the performance of existing capture panels by rescuing poorly represented regions, such as areas of high GC content. If you plan to use xGen Lockdown Probes for spike-ins into existing probe sets or panels, please contact our technical support group at applicationsupport@idtdna.com, who will provide tailored recommendations for your specific experimental design.

xGen Lockdown Panels are inventoried enrichment panels for targeted next generation sequencing and are typically based on 1X tiling of xGen Lockdown Probes. Several research panels are available for the human genome, including panels for the whole exome, disease genes, and sample identification. Visit www.idtdna.com/LockdownPanels for a complete list.

xGen Universal Blockers—NXT Mix

xGen Universal Blockers—NXT Mix will bind to Nextera adapter sequences on a designated strand to reduce non-specific binding, improve the number of reads on target, and increase the depth of enrichment. Universal Blockers—NXT Mix works for Nextera libraries with 8 bp indexes. If you have a custom index that is longer or shorter than 8 bp, please contact our technical support group at applicationsupport@idtdna.com for additional guidance.

xGen Lockdown Reagents

The components of the xGen Lockdown Reagents kit have been optimized for the hybridization and wash steps in target capture protocols using xGen Lockdown Probes and Panels.

Protocol overview

	Protocol step	Approximate time
A	Prepare capture probes	45 minutes
B	Combine and dry blocking oligos, Cot-1 DNA, and genomic DNA library	
STOP		
C	Hybridize capture probes with the library	4 hours
D–E	Prepare buffers and streptavidin beads	1.75 hours
F	Bind hybridized targets to streptavidin beads	
G	Wash streptavidin beads to remove unbound DNA	
H	Perform PCR enrichment	
STOP		
I	Purify postcapture PCR fragments	2.5 hours
STOP		
J	Validate and quantify library	
STOP		
	Ready for sequencing	**Total: 9 hours**

STOP Optional stopping points

Reagents, kits, and equipment

Oligos and reagents from IDT

	Size	Storage conditions	Ordering information
Target capture			
xGen Lockdown Probes	Varies	–20°C*	www.idtdna.com/xGen
xGen Lockdown Panels	16 or 96 rxn	–20°C*	
xGen Gene Capture Pools	16 or 96 rxn	–20°C*	
xGen Universal Blockers—NXT Mix	16, 96, or 4 x 96 rxn	–20°C*	Cat # 1079584, 1079585, or 1079586
xGen Lockdown Reagents	16 or 96 rxn	–20°C	Cat # 1072280 or 1072281
Custom DNA oligonucleotides			
Illumina P5 primer: AATGATACGGCGACCACCGA	Varies	–20°C	Custom DNA Oligos (www.idtdna.com/DNA)
Illumina P7 primer: CAAGCAGAAGACGGCATACGA	Varies	–20°C	
Reagents			
IDTE pH 8.0 (1X TE solution)	10 × 2 mL	Room temp. (15–25°C)	Cat # 11-01-02-05
Nuclease-Free Water	10 × 2 mL	Room temp. (15–25°C)	Cat # 11-04-02-01

* See resuspension and storage instructions at www.idtdna.com/protocols.

Safety data sheets (SDSs) and certificates of analysis (COAs) for xGen products may be obtained by emailing applicationsupport@idtdna.com. For SDSs and COAs for other IDT products, go to www.idtdna.com.

Additional materials and equipment

Materials	Ordering information
>80% Ethanol	General laboratory supplier
Agencourt® AMPure® XP – PCR Purification beads	Beckman-Coulter, Cat # A63880
Digital electrophoresis chips, such as: Experion™ DNA 1K Analysis Kit Agilent High Sensitivity DNA Kit Agilent High Sensitivity D1000 ScreenTape®	 Bio-Rad Laboratories, Cat # 700-7107 Agilent Technologies, Cat # 5067-4626 Agilent Technologies, Cat # 5067-5584
Dynabeads® M-270 Streptavidin	Life Technologies, Cat # 65305
Invitrogen™ Human Cot-1 DNA®	Life Technologies, Cat # 15279-011
KAPA HiFi HotStart ReadyMix	Kapa Biosystems, Cat # KK2601
Library Quantification Kit – Illumina/Universal	Kapa Biosystems, Cat # KK4824
MAXYMum Recovery® Microtubes, 1.7 mL	VWR, Cat # 22234-046
MAXYMum Recovery PCR Tubes, 0.2 mL flat cap	VWR, Cat # 22234-056
QIAGEN Buffer EB (or equivalent: 10 mM Tris-Cl, pH 8.5)	QIAGEN, Cat # 19086 (or general laboratory supplier)
(Optional) Qubit® Assay Tubes	Life Technologies, Cat # Q32856
(Optional) Qubit dsDNA HS Assay Kit	Life Technologies, Cat # Q32851
Equipment	**Ordering information**
96-well or 384-well thermal cyclers	General laboratory supplier
Digital electrophoresis system, such as: Experion Electrophoresis Station Agilent 2100 Electrophoresis Bioanalyzer Agilent 2200 TapeStation	 Bio-Rad Laboratories, Cat # 700-7010 Agilent Technologies, Cat # G2939AA Agilent Technologies, Cat # G2965AA
Magnetic separation rack, such as: 6-tube magnetic separation rack 16-tube DynaMag™-2 Magnet DiaMag02 magnetic rack	 New England Biolabs, Cat # S1506S Life Technologies, Cat # 12321D Diagenode, Cat # B04000001
Microcentrifuge	General laboratory supplier
(Optional) Qubit 3.0 Fluorometer	Life Technologies, Cat # Q33216
Vacuum concentrator or oven	General laboratory supplier
Vortex mixer	General laboratory supplier
Water bath or heating block	General laboratory supplier

Protocol

A. Prepare capture probes

xGen Lockdown Probes

If you received the xGen Lockdown Probes as a hydrated solution:

1. Thaw at room temperature (15–25°C).
2. Mix thoroughly and briefly spin down.

If you received the xGen Lockdown Probes dry:
Resuspend in IDTE pH 8.0 to a final concentration of 0.75 pmol/µL.

For additional support regarding resuspension of Lockdown Probes pools, visit **www.idtdna.com/xGen**.

xGen spike-in panels

Refer to the relevant protocol for your spike-in panel (e.g., *Expansion of xGen Lockdown Panels with xGen spike-in panels* for the xGen Human ID or Human mtDNA Research Panels) at **www.idtdna.com/xGen** for instructions on adding supplementary panels to your primary panel.

B. Combine and dry blockers, Cot-1 DNA, and genomic DNA library

1. Mix the following in a low-bind 1.7 mL PCR tube (for example, MAXYMum Recovery tube):

	Illumina Nextera libraries
Pooled, barcoded library	500 ng/library
Cot-1 DNA	5 µg
xGen Universal Blockers—NXT Mix	2 µL

2. Dry the contents of the tube using a vacuum concentrator (e.g., SpeedVac® System or a similar evaporator device) set at 70°C or lower.

Optional stopping point: After drying, tubes can be stored overnight at room temperature (15–25°C).

C. Hybridize DNA capture probes with the library

1. Thaw all xGen Lockdown Reagents buffers at room temperature.

> **Note:** Inspect the tube of 2X Hybridization Buffer for crystallization of salts. If crystals are present, heat the tube at 65°C, shaking intermittently, until the buffer is completely solubilized; this may require heating for several hours.

2. Add the following to the tube from **step B.2**, and incubate at room temperature for 5–10 min:

	Volume (µL)
2X Hybridization Buffer	8.5
Hybridization Buffer Enhancer	2.7
Nuclease-Free Water	1.8

3. Pipette up and down to mix, and transfer to a low-bind 0.2 mL PCR tube (for example, MAXYMum Recovery tube).

4. Incubate in a thermal cycler at 95°C for 10 min.

5. Remove samples from thermal cycler and immediately add 4 µL of the xGen Lockdown Probe pool.

> **Note:** Final volume will be 17 µL. If using xGen spike-in panels, the final volume will be larger. Refer to the protocol, *Expansion of xGen Lockdown Panels with xGen spike-in panels*, at **www.idtdna.com/xGen** for use of spike-in panels.

6. Vortex and briefly spin down.

7. Incubate samples in a thermal cycler at 65°C (with the heated lid at 75°C) for 4 hr.

> **Note:** The 65°C hybridization temperature improves the percentage of on-target capture.

D. Prepare wash buffers

1. For a single capture reaction, dilute the following xGen buffers to create 1X working solutions as follows:

> **Note:** The 1X working solutions are stable at room temperature (15–25°C) for up to 4 weeks.

	Concentrated buffer (µL)	Nuclease-free water (µL)
2X Bead Wash Buffer	250	250
10X Wash Buffer I*	30	270
10X Wash Buffer II	20	180
10X Wash Buffer III	20	180
10X Stringent Wash Buffer	40	360

* If necessary, heat 10X Wash Buffer I in a 65°C water bath or heating block to resuspend particulates.

2. Prepare aliquots of Wash Buffer I and Stringent Wash Buffer from **step D.1**, and store at the temperature specified in the following table:

Buffer	Volumes of 1X working solution for each capture	Temperature for 1X working solution
Wash Buffer I	100 µL 200 µL	65°C* room temp (15–25°C)
Stringent Wash Buffer	400 µL	65°C*

* Important: Preheat buffers in a 65°C water bath before use in **step G**; may require up to 2 hr.

3. Keep the remaining 1X buffers at room temperature.

E. Prepare the streptavidin beads

Important: Beads should be prepared immediately before use. Do not allow beads to dry out.

1. Equilibrate Dynabeads M-270 Streptavidin beads at room temperature for approximately 30 min before use.

   Important: We do not recommend using alternative streptavidin magnetic beads, because many of these have delivered significantly reduced capture yields.

2. Mix the beads thoroughly by vortexing for 15 sec.

3. Aliquot 100 µL of beads per capture into a single 1.7 mL low-bind tube.

   For example: for 1 capture, prepare 100 µL of beads and for 2 captures, prepare 200 µL of beads. For more than 6 captures, you will need more than one tube.

4. Place the tube in a magnetic separation rack (magnetic rack), allowing beads to fully separate from the supernatant.
5. Remove and discard the clear supernatant, ensuring that the beads remain in the tube.
6. Perform the following wash:
    a. Add 200 µL of 1X Bead Wash Buffer per capture, and vortex for 10 sec.
    b. Place the tube in the magnetic rack, allowing beads to fully separate from the supernatant.
    c. Carefully remove and discard the clear supernatant.
7. Perform a second wash by repeating **step E.6**.
8. Add 100 µL of 1X Bead Wash Buffer per capture (refer to **step E.3**) and vortex.
9. Transfer 100 µL of the resuspended beads into a new 0.2 mL low-bind tube for each capture reaction.
10. Place the tube in a magnetic rack, allowing beads to fully separate from the supernatant.
11. Carefully remove and discard the clear supernatant.

Note: Small amounts of residual Bead Wash Buffer will not interfere with downstream binding of the DNA to the beads.

Important: Proceed immediately to the next section, **Bind hybridized target to the streptavidin beads.**

F. Bind hybridized target to the streptavidin beads

1. Transfer the hybridization samples (from **step C.7**) to the tube containing prepared beads (from **step E.11**).
2. Mix thoroughly by pipetting up and down 10 times.
3. Bind the DNA to the beads by placing the tube into a thermal cycler set to 65°C (with the heated lid at 75°C) for 45 min.
4. After 12 min, vortex the tube for 3 sec to ensure that the beads remain in suspension, and then return the tubes to the thermal cycler. Repeat after every 12 minutes.

G. Wash streptavidin beads to remove unbound DNA

Note: Use the 1X wash buffers from **step D**.

1. Perform 65°C washes.
    1) Add 100 µL preheated 1X Wash Buffer I to the tube from **step F.4**.
    2) **Important**: Vortex briefly, and spin to collect contents at the bottom of the tube.
    3) Transfer the mixture to a new low-bind 1.7 mL tube.
    4) **Important**: Vortex briefly.
    5) Place the tube in the magnetic rack, allowing beads to fully separate from the supernatant.
    6) Pipet and discard the supernatant, which contains unbound DNA.
    7) Perform the following wash:
        a. Add 200 µL of preheated 1X Stringent Wash Buffer, and slowly pipet up and down 10 times.

        Important: Do not create bubbles during pipetting.

        b. Incubate in a water bath at 65°C for 5 min.

        c. Place the tube in the magnetic rack, allowing beads to fully separate from the supernatant.

        d. Pipet and discard the supernatant, which contains unbound DNA.
    8) Repeat **step 7**.
2. Perform room temperature washes.
    1) Add 200 µL of room temperature 1X Wash Buffer I and vortex for 2 min.
    2) Place the tube in the magnetic rack, allowing beads to fully separate from the supernatant.
    3) Pipet and discard the supernatant.
    4) Add 200 µL of room temperature 1X Wash Buffer II and vortex for 1 min.
    5) Place the tube in the magnetic rack, allowing beads to fully separate from the supernatant.
    6) Pipet and discard the supernatant.
    7) Add 200 µL of room temperature 1X Wash Buffer III and vortex for 30 sec.

8) Place the tube in the magnetic rack, allowing beads to fully separate from the supernatant.

9) Pipet and discard the supernatant.

3. Resuspend beads.

1) Remove the tube containing the beads with captured DNA from the magnetic rack.

2) Add 20 µL of Nuclease-Free Water to the beads.

3) Pipet up and down 10 times and ensure any beads stuck to the side of the tube have been resuspended.

Important: Do not discard the beads. Use the entire 20 µL of resuspended beads with captured DNA in **step H**.

H. Perform final, postcapture PCR enrichment

1. Prepare the PCR mix in 0.2 mL low-bind PCR tubes as follows:

	Volume (µL)
2X KAPA HiFi HotStart ReadyMix	25
10 µM Illumina P5 primer	2.5
10 µM Illumina P7 primer	2.5
Beads with captured DNA (from **step G.3**)	20
Total volume	**50**

2. Briefly vortex and spin the PCR mix, but ensure that the beads remain in solution.

3. Place the PCR tube in the thermal cycler, and run the following program with the heated lid set at 105°C:

	Number of cycles	Temperature (°C)	Time
Polymerase activation	1	98	45 sec
Amplification	12		
Denaturation		98	15 sec
Annealing		60	30 sec
Extension		72	30 sec
Final extension	1	72	1 min
Hold	1	4	Hold

Cycling conditions recommended by Kapa Biosystems.

Optional stopping point: PCR-enriched captures may be stored at 4°C overnight.

I. Purify postcapture PCR fragments

1. Add 75 µL (1.5X volume) of Agencourt AMPure XP beads to each PCR-enriched capture.
2. Follow the binding and washing steps in the Agencourt AMPure protocol, **except use 80% ethanol for the washes**.
3. Elute in 22 µL of Qiagen Buffer EB or equivalent (10 mM Tris-Cl, pH 8.5).
4. Transfer 20 µL of eluted product to a fresh 1.7 mL low-bind tube, ensuring no beads are carried over.

Optional stopping point: Purified PCR fragments may be stored at –20°C for up to 1 week.

J. Validate and quantify library

1. (Optional) Measure the concentration of the captured library using a Qubit Fluorometer and the Qubit dsDNA HS Assay Kit.

> **Note:** This can be done to ensure that the concentration of the captured library is within the detection limits of the chip or tape used in **step J.2** (below) for your digital electrophoresis system.

2. Measure the average fragment length of the captured library on a digital electrophoresis system (e.g., the Bio-Rad Experion System, using a DNA 1K chip; the Agilent 2100 Bioanalyzer, using a high sensitivity DNA chip; or Agilent 2200 TapeStation, using a Agilent High Sensitivity D1000 ScreenTape).
3. Quantify libraries using the appropriate KAPA Library Quantification Kit (KAPA Biosystems) as directed by the manufacturer.

Optional stopping point: Library may be stored at –20°C overnight.

K. Perform sequencing

Perform sequencing according to the instructions for your Illumina instrument.

- Use the calculated concentration of undiluted library stock (from **step J.3**) to prepare the library for sequencing.

Revision history

Version	Date released	Description of changes
1	December 2017	Original protocol

next generation sequencing

protocol

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Technical support:

applicationsupport@idtdna.com

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