Notices

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Safety notices

Reminder symbols call attention to minor details that may be easily overlooked and compromise the procedure resulting in decreased assay performance.

Caution symbols denote critical steps in the procedure where risk of protocol failure or damage to the product itself could occur if not carefully observed.

Stop symbols indicate where this procedure may be safely suspended and resumed at a later time without risk of compromised assay performance. Make note of these steps and plan your workflow accordingly.

Version History

Version	Release Date	Description of Changes
1	November 20, 2024	Initial release
2	February 6, 2025	Clarify vortexing instructions in Capture Bead & Wash Preparation and Wash (Stringent Wash) sections.
3	April 8, 2025	Added workflow diagrams; removed statement on equal representation of probes, leaving only 'individually synthesized' lingo; updated language on IDT panels and low bind plastics; consolidated / simplified wording about beads and shipping; slight adjustment of the mixing and homogeneity instructions provided in the Before Getting Started section' removed the line about compatibility with previous conditions from v1/v2; standardized format changes and alignments throughout.
4	September 10, 2026	Addition of spike-in panel recommendations; included alternate instrument for mixing; provided clarification of multiplexing capabilities; removed room temperature hold disclaimers after incubation steps; clarified library and hybrid capture reaction samples
5	September 2026	Updated Capture Program table to include BioShake iQ speed instructions.

Contents

Version History 2

Overview 4

Workflow Reference Guide 6

Before Getting Started 11

Protocol 13

APPENDIX A 21

APPENDIX B 22

APPENDIX C 23

SAFETY WARNING 24

Technical Support

Visit https://www.idtdna.com/pages/support/faqs for helpful answers to frequently asked questions or contact us directly at applicationsupport@idtdna.com.

Overview

This protocol includes the steps necessary for target enrichment of a next generation sequencing library prepared from DNA or RNA and target capture with xGen™ Hyb Capture Panel (xGen custom or xGen stocked panels).

Visit User guides & protocols [https://www.idtdna.com/pages/support/guides-and-protocols] to verify that you are using the most current version of this Protocol for xGen™ Hyb and Wash Reagents v3 Kit.

Important: **Do not** use this protocol with any older version of the xGen™ Hybridization and Wash v2 Kit (V2, xGen Lockdown Reagents, etc.)

In brief, the steps of this protocol include:

1. Pool libraries and prepare hybridization reaction
    a. Be sure to use the correct adapter blocking oligos. Visit our website for more information.
2. Perform Hybridization with libraries and xGen Capture panels
3. Prepare xGen Beads and Wash Buffers
4. Bind xGen beads to capture probes.
5. Perform washes.
6. Post-capture PCR to amplify the library for sequencing.

After preparing an Illumina NGS library, this procedure includes the addition of IDT xGen Universal Blockers to prevent off-target fragments from annealing to the intended target sequence via adapter-to-adapter hybridization. The type of xGen Universal Blockers depends upon the chosen NGS adapter. IDT offers a variety of different options that can be reviewed in NGS Blocking Oligos for Hybridization Capture | IDT (https://www.idtdna.com/pages/products/next-generation-sequencing/workflow/xgen-ngs-hybridization-capture/xgen-universal-blockers).

In addition to using adapter blockers, the protocol describes the conditions necessary for hybridization with one of the xGen Predesigned Hybridization Capture Panels or one of the xGen Custom Hybridization Capture Panels. These panels contain capture probes that were individually synthesized and pooled, which ensures xGen Custom Hyb Panels can be used as a spike-in to supplement the target space of one of the predesigned panels. Please refer to the **Before Getting Started** section.

The hybridization procedure presented here is specific to the IDT xGen™ Hyb and Wash Reagents v3 and xGen™ Hyb and Wash Beads v3. Any of the IDT xGen Hybridization Panels (custom or stocked) are compatible with this kit.

The hybridization, capture, and washes can be performed either in low bind 96-well plates or low bind tubes.

After the probes are hybridized to the target fragments, the procedure for separating the desired DNA targets from the non-targeted fragments is presented. Since each probe has a 5' biotin modification, this procedure describes the use of streptavidin-coated magnetic beads to capture the probe and targeted DNA duplexes. Post-capture washes are performed to further remove non-targeted DNA. IDT provides a selection of compatible suppliers of the necessary reagents and magnetic stands in the Materials Required and Equipment section.

After removal of the non-specific DNA from the sample, the procedure for post-capture PCR is described using a PCR master mix with the IDT xGen Library Amplification Primer Mix. IDT offers suggestions for the number of cycles for amplification based on the number of probes in the panel as a starting point to create sufficient DNA library for sequencing the final captured library.

Product Description

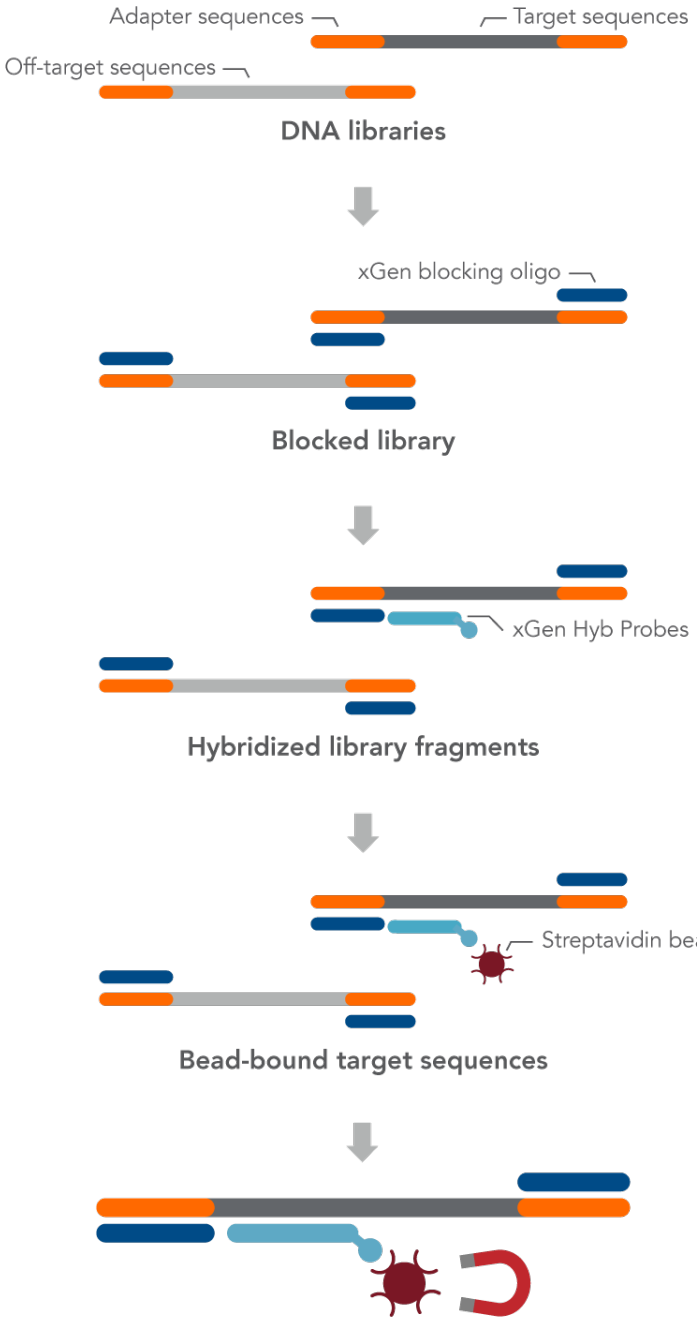
The xGen™ Hyb and Wash Reagents v3 kit is optimized for use with the xGen family of products, including library prep kits, Hybridization Capture panels, blockers, and related reagents, for targeted enrichment from libraries intended for Illumina sequencing, with adapter sequences that are less than 80nt on each end.

Workflow Reference Guide

1	Combine DNA with blockers Dry down DNA ⛔ Perform hybridization incubation	Total time: 15 minutes Total time: Variable Total time: 1–16 hours
2	Wash streptavidin beads	Total time: 15 minutes
3	Bead capture incubation	Total time: 30 minutes
4	Perform post-capture washes	Incubation time: 25 minutes
5	Perform post-capture PCR ⛔	Total time: Variable
6	Post-capture PCR clean up ⛔	Total time: Variable*

*Time varies based on batch size

Target capture workflow



Add blocking oligos and Cot-1 DNA

Hybridize targets to capture probes

Incubate with magnetic streptavidin beads

Isolate targets with magnet

xGen™ Hyb and Wash Reagents v3 Kit Components

Materials Supplied				
Description	Storage (°C)	Shipping (°C)	Part Number	
			16 Reaction (10028311)	96 Reaction (10028312)
Human Cot DNA	-20	-20	1080684	1080684
xGen 1X Hybridization Buffer	-20	-20	10027941	10027946
xGen 2X Bead Wash Buffer	4	-20	10027943	10027948
xGen 1X Stringent Wash Buffer	4	-20	10027942	10027947
xGen 1X Wash Buffer I	4	-20	10027444	10027949
xGen 1X Wash Buffer II	4	-20	10027945	10027950
xGen 2X HiFi PCR Mix	-20	-20	10010205	10010204
xGen Hyb and Wash Beads v3*	4	Room Temp	10025272	1002573

*xGen Hyb and Wash Beads v3 are sold together with xGen™ Hyb and Wash Reagents v3 but will be provided in a separate box (Catalog Number 1002572 or 10025273) to enable the different shipping conditions.

Materials Required But Not Supplied in Kit

Materials required but not supplied, have been optimized for proper application of the xGen™ Hyb and Wash Reagents v3 and Beads v3 kit. Use of other materials has not been tested by IDT.

Materials Required, but not supplied		
Description	**Supplier**	**Part Number**
xGen Universal Blockers	IDT	Varies
xGen Hybridization Panel (custom or stocked)	IDT	Varies
xGen Library Amplification Primer Mix 16 reaction 96 reaction 192 reaction	IDT	 1077675 1077676 1077677
Nuclease-Free Water	General laboratory supplier	Varies
200 or 190 proof Ethanol (Dilute to 80%)	General laboratory supplier	Varies
Buffer EB (or equivalent: 10 mM Tris-HCl, pH 8.5)	QIAGEN, or general laboratory supplier	19086
AMPure® XP Beads	Beckman Coulter	A63880
twin.tec™ PCR Plates LoBind® or TempAssure® PCR Flex-Free 8-Tube Strips	Eppendorf USA Scientific	0030129504 1402-4700
Microseal® 'B' PCR Plate Sealing Film, adhesive, optical	Bio-Rad	MSB1001
High Sensitivity D1000 ScreenTape®	Agilent	5067-5584
High Sensitivity D1000 Reagents	Agilent	5067-5585
Qubit® dsDNA HS Assay Kit or equivalent kits for alternate fluorometers	Thermo Fisher Scientific	Q32851 or Q32854

Equipment		
Item	**Supplier**	**Part Number**
T100 Thermal Cycler or Veriti™ 96-Well Fast Thermal Cycler or equivalent	Bio-Rad Applied Biosystems	1861096 A48141
ThermoMixer® C BioShake iQ + 96-well adapter plate or equivalent	Eppendorf Qinstruments	5382000023 1808-0506, 1808-1041
SmartBlock™ PCR 96	Eppendorf	5306000006
DynaMag®-2 Magnet	Invitrogen	12321D
96-Well Side Pull Bar Magnet or Magnum EX	Permagen Alpaqua	MSP750 A000380
Plate centrifuge	General laboratory supplier	Varies
Microcentrifuge	General laboratory supplier	Varies
Vacuum concentrator (aka SpeedVac)	General laboratory supplier	Varies
Vortex mixer	General laboratory supplier	Varies
Qubit® Fluorometer or Qubit® Flex Fluorometer or equivalent	Thermo Fisher Scientific	Q33238
TapeStation® System or equivalent	Agilent	G2991BA

Before Getting Started

Important precautions

- Read through the entire protocol before starting your experiment.
- Shorter hybridization times generally exhibit higher variabilities in different applications, especially when combined with very small probe panels (≤ 1 Mb) or very low total hybridization input (100ng total input). Users are encouraged to explore suitable inputs and hybridization times for specific applications.
- The xGen Hyb and Wash Reagents v3 consist of different formulations for Hybridization Buffer, Stringent Wash Buffer, Wash Buffer I and Wash Buffer II as compared with previous kits. It is critical to use v3 Reagents and to follow the v3 workflow as described in this Protocol for xGen™ Hyb and Wash Reagents v3.
- The simplicity of the xGen Hyb and Wash Reagents v3 workflow is achieved by unique buffer formulations and by decoupling the individual steps of the workflow. It is critical to achieve homogeneity, to spin down, and to remove the residual liquid when the protocol specifies these details.
- Mixing streptavidin beads to homogeneity during the wash steps should follow the procedures and techniques established in the user's laboratory. Homogeneity cannot be achieved with insufficient mixing, while excessive agitation can compromise the sample with risks such as leakage, cross-contamination, or foaming. Care must be taken, especially with Wash Buffer I, to prevent foaming and leaking during mixing steps. Recommendations for mixing methods and durations are provided as guidance.
- Reagents should be disposed of according to local regulations.

Working with liquid reagents

- All reagents are shipped on dry ice except for the xGen Hyb and Wash Beads v3.
- Store the Human Cot DNA, xGen 1X Hybridization Buffer, and xGen 2X HiFi PCR Mix at −20°C.
- Wash Buffers can be stored at 4°C after initial use for up to 10 weeks.
- Allow all buffers to come to room temperature (RT) before using.
- Confirm the buffers are fully homogeneous (no visible fluid current when pipette mixed) at room temperature (RT) before use. Buffers can precipitate or form a gradient at cool room temperatures.

Input nucleic acid

- xGen™ Hyb and Wash Reagents v3 is compatible with 100 ng to 6 µg of total library input per reaction for overnight hybridizations. Depending on application needs, optimum hybridization time can be as short as 1-hour and as long as overnight. For short hybridization times, the lowest (100 ng) and highest (6 µg) total inputs can exhibit higher noise and lower performance than longer hybridization times, especially for very small (≤ 1 Mb) and very large (>30 Mb) Hybridization panels. Users are encouraged to explore suitable inputs and hybridization times for specific applications.
- If using a Spike-In panel, see Appendix B (custom panel) or Appendix C (stocked panel). For additional information and options, please contact IDT customer support.
- For optimal results, use a vacuum concentrator for concentrating DNA libraries before hybridization. Although a bead-based concentration method can be used, bead-based concentration can result in adverse GC-bias. Please refer to Appendix A for bead-based concentration instructions.

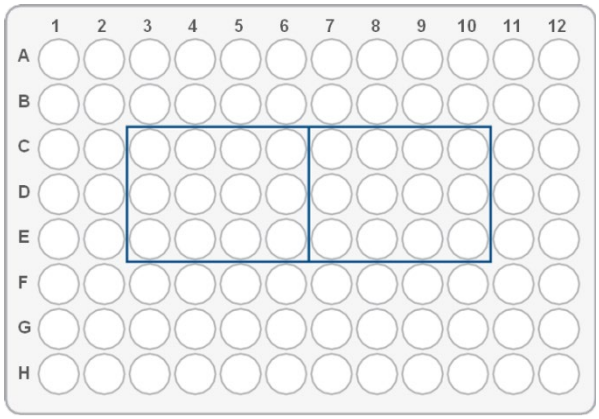
Hybridization and Wash reagents to prepare before starting

- Confirm the buffers are fully homogeneous (no visible fluid current after solution is mixed) at room temperature (RT) before use. Buffers can precipitate or form a gradient at cool room temperatures.
- xGen Bead Wash Buffer is supplied at 2X concentration. This buffer should be diluted to 1X concentration before use, as described by the protocol.
- xGen Hyb and Wash Beads v3 should be washed before use, as described by the protocol.

Protocol

Prepare library mixture

We recommend avoiding wells on the plate edges and avoiding wells on thermal cycler edges. Mark the wells that contain DNA before drying down the plate, since they will not be visually distinguishable from empty wells after dry-down. Hybrid-capture reaction samples will be concentrated via a drying method or bead based (refer to Appendix A).



1. Prepare the Blocker Master Mix in a tube. Multiply by the number of hybridization-capture reactions to be performed and add 10% excess volume for each component. Each separate hybridization-capture reaction, whether singleplex or multiplex, equals one separate capture reaction.

Blocker Master Mix Components	Volume Per Reaction (µL)
Human Cot DNA	5
xGen Universal Blockers	2
Total	7

2. Vortex to mix well.
3. Add 7 µL of the Blocker Master Mix to each well of a LoBind plate or tube.
4. Add the desired amount of library (ex. 500 ng) to each well containing Blocker Master Mix.
   - This protocol is compatible with 100ng to 6µg of total library into each hybridization-capture reaction.
   - For multiplex captures, add the pooled libraries such that the total library input per hybridization-capture

reaction is 100ng to 6µg and does not exceed application-specific limit of 6µg total.

5. Concentrate the library/blocker mixture using one of the following methods:
   a. Standard Option: Dry down the mixture in a SpeedVac system (or equivalent) according to the manufacturer's instructions.
   b. Bead Option: Concentrate the library using the AMPure XP Beads DNA Concentration Protocol provided in **Appendix A**.

Optional stopping point: Be sure to seal the sample plate or tube. Store the plate at RT overnight, or −20°C for up to 72 hours.

6. Proceed to Hybridization.

Hybridization

Inspect xGen Hybridization Buffer. If it does not appear homogeneous or if precipitation is visible, gently warm solution to 45°C and mix until homogeneous. Allow Hybridization Buffer to come back to RT before using.

Note: Hybridization Buffer volume is temperature sensitive. Vortex and spin down the Hybridization Buffer at room temperature before aliquoting for Master Mix assembly. Draw up and expel Hybridization Buffer very slowly for accurate handling of viscous liquid.

1. Prepare Hybridization Master Mix in a tube. Multiply by the number of samples and add 10% overfill for each component.

   If a stocked or custom IDT spike-in panel is desired, see Appendix B or Appendix C respectively.

Hybridization Master Mix Components	Volume Per Reaction (µL)
xGen 1X Hybridization Buffer	14
xGen Hybridization Panel	4
Total	18

2. Vortex to mix well.
3. Add 18 µL of the Hybridization Master Mix to each sample well containing dried NGS library and blocker mix.
4. Seal the plate or tube, vortex briefly to mix and spin down.
5. Let the plate or tube sit at RT for 5 min to allow the libraries to resuspend.
6. Incubate the plate or tube in a thermal cycler running the Hybridization program.

Hybridization Program*			
Step	**Block Temperature (°C)**	**Lid Temperature (°C)**	**Time (min)**
1	95	105	5
2	70	105	Variable: 1-19 h
3	70	105	Hold

Capture bead and buffer preparation

Prepare xGen Hybridization and Wash Beads (capture beads) within 2 hours before use. Ensure the washed beads are ready before the hybridization program ends, as samples are transferred directly to the capture bead binding step upon completion of hybridization.

Allow the xGen Hyb and Wash Beads to equilibrate to room temperature after removal from 4°C storage. Mix thoroughly until homogeneous right before aliquoting for bead washing. For individual samples, wash beads in 0.2mL strip tubes or plate wells. Alternatively, wash beads in bulk in 1.5 mL or 2.0 mL tubes for multiple samples. Aspirate and dispense slowly for accurate pipetting of viscous liquid.

1. Dilute the following buffer to create a 1X working solution as follows: multiply by the required number of hybridization-capture reactions and add 10% overfill.

Component	Nuclease-Free Water (µL)	Buffer (µL)	Total (µL)
xGen 2X Bead Wash Buffer	159	159	318

2. Add 50 µL of capture bead-mix per hybridization-capture reaction to a LoBind tube (1.5 mL, 2.0 mL, 0.2 mL strip tube, or plate well).
3. Collect the beads on a magnet and remove the supernatant.
4. Add twice the volume (of bead-mix) of 1X Bead Wash Buffer to the tube. For example, if 500 µL of bead-mix is being washed, use 1 mL of Bead Wash Buffer.
5. Remove from the magnet and briefly vortex the beads to resuspend, then spin down.
6. Collect the beads on a magnet and remove the supernatant.
7. Repeat steps 4−6 twice more for a total of 3 washes.
8. Following the last wash, collect the beads on the magnet and remove the supernatant.
9. Remove from magnet and resuspend the beads in 18 µL of 1X Bead Wash Buffer per reaction. Mix solution *thoroughly by vortexing or pipetting until homogenous (vortex at moderate speed for at least 20 seconds is recommended). If necessary, spin down briefly at low speed to avoid bead pelleting.*

Capture

The Eppendorf ThermoMixer takes time to reach the designated temperature. Turn it on before use and let it come to 23°C or RT before starting capture.

Before adding the beads to your samples, vortex or pipette mix the washed bead-mix to ensure uniformity.

1. Remove samples from thermal cycler and stop Hybridization program.
2. Spin down the plate or tubes briefly to ensure no sample or condensation is left on the seal or tube cap.
3. Ensure washed capture beads are homogenously resuspended immediately before aliquoting.
4. Add 18 µL of resuspended washed capture beads to each sample well at RT and seal plate.
5. Gently vortex the plate or tubes to resuspend the mixture.
6. Mix the plate or tubes on an Eppendorf ThermoMixer C or BioShake iQ using the Capture program.

Capture Program			
Instrument	**Speed (RPM)**	**Temperature (°C)**	**Time (min)**
Eppendorf ThermoMixer C	1200	23 (or RT)	30
BioShake iQ	1800		

Wash

Inspect the xGen 1X Stringent Wash Buffer and xGen 1X Wash Buffer I. If they do not appear homogeneous or if precipitation is visible, gently warm solution to 45°C and mix until homogeneous. Allow Stringent Wash Buffer and Wash Buffer I to cool to RT after heating. Use all Wash buffers at RT.

1. After capture, spin down and place the samples on a magnet to collect the beads.
2. Remove and discard the cleared supernatant; make sure not to remove any beads.
3. Use a P20 to remove any residual liquid.
4. Add 50 µL of 1X Stringent Wash Buffer to sample wells and seal plate.
5. Remove from magnet and briefly pulse vortex to completely resuspend the beads. Avoid getting samples on the seal. Briefly spin down the mixture away from the seal if necessary. If the beads separate or pellet, vortex again and spin down briefly.
6. Place the plate or tube in a thermal cycler and run the Stringent wash program.

Stringent Wash		
Temperature (°C)	**Lid Temperature (°C)**	**Time (min)**
54	105	20

7. Remove the plate or tubes from the thermal cycler after Stringent wash incubation.
8. Collect the beads on a magnet. Allow the beads to pellet and solution to clear. As soon as the supernatant clears, remove the cleared supernatant quickly.
9. Seal the plate or tubes, briefly spin down, and place back onto the magnet.
10. Use a P20 to remove any residual liquid.
11. Remove the samples from the magnet and add 150 µL of 1X Wash Buffer I to sample wells at RT.
12. *Resuspend solution thoroughly by vortexing or pipetting until homogenous. Wash Buffer 1 can foam and leak easily. Ensure plate or tube is securely sealed before vortex mixing (vortex at moderate speed for at least 20 seconds is recommended). Spin down briefly at low speed to avoid bead pelleting.*

Important: For hybridization capture to work consistently, it is important to mix thoroughly and spin down after mixing.

13. Collect the beads on a magnet and remove all supernatant.
14. Seal the plate or tubes, briefly spin down, and place back onto the magnet.
15. Use a P20 to remove any residual liquid.
16. Remove plate or tubes from magnet and add 150 µL of 1X Wash Buffer II at RT.
17. *Resuspend solution thoroughly by vortexing or pipetting until homogenous. Ensure plate or tube is securely sealed before vortex mixing (vortex at moderate speed for at least 20 seconds is recommended). Spin down briefly at low speed to avoid bead pelleting. There is no need to spin down unless the sample has splashed on the tube walls or seal.*
18. Place the plate or tube in a thermal cycler and run the Wash II program.

Wash II*		
Temperature (°C)	**Lid Temperature (°C)**	**Time (min)**
60	105	5

19. Remove samples from the thermal cycler and spin down briefly.
20. Collect the beads on a magnet and remove all supernatant.
21. Use a P20 to remove any residual liquid.
22. Remove from magnet and resuspend beads in 20 µL of nuclease-free water.

Post-capture PCR

1. In a tube, prepare the Amplification Reaction Mix as follows: multiply by the number of capture reactions on the plate and add 10% overfill.

Amplification Master Mix Components	Volume Per Reaction (µL)
xGen 2X HiFi PCR Mix	25
xGen Library Amplification Primer Mix	1.25
Nuclease-Free Water	3.75
Total	30

2. Add 30 µL of Amplification Master Mix to each sample for a final reaction volume of 50 µL.

   Note: Volume is not additive, so the final reaction volume may not be consistently 50 µL after adding the Amplification Reaction Mix.

3. Securely seal the sample plate or tubes, then gently vortex to thoroughly mix the reaction.
4. Briefly centrifuge the plate or tubes.
5. Place the plate or tubes in a thermal cycler, and run the following program with the lid temperature set to 105°C:

PCR Program			
PCR Step	**Number of Cycles**	**Temperature (°C)**	**Time**
Polymerase Activation	1	98	45 sec
Amplification: Denaturation Annealing Extension	Variable—refer to the panel table below	 98 60 72	 15 sec 30 sec 30 sec
Final Extension	1	72	1 min
Hold	1	4	Hold

The number of PCR cycles should be optimized per panel, input into capture, and hybridization time.

We recommend starting optimization with the following:

Panel size	Input Mass (per capture reaction well)			
	100-500 ng total input	2 ug total input	4 ug total input	6 ug total input
>100,000 probes	10 cycles	8 cycles	7 cycles	6 cycles
10,000–100,000 probes	12 cycles	10 cycles	9 cycles	8 cycles
500–10,000 probes	13 cycles	11 cycles	10 cycles	10 cycles
<500 probes	14 cycles	12 cycles	11 cycles	11 cycles

For short hybridization (1 hour) with low input (100 ng) on small panels (15,000 probes or less) or multiplex short hybridization, it is recommended to increase PCR cycles.

Optional stopping point: Store amplified captures at -20°C up to one week or per your established internal laboratory procedures.

Post-capture PCR clean up

Ensure AMPure XP beads have been equilibrated to room temperature before proceeding.

1. Prepare 400 µL of fresh 80% ethanol per sample, multiplied by the number of samples with a 10% overfill.
2. Add 75 µL (1.5X volume) of AMPure XP beads to each amplified capture.
3. After adding the beads, pipette mix thoroughly and incubate for 5–10 min.
4. Place the plate or tubes on a magnet until the supernatant is clear (2–5 min).
5. Remove the supernatant without disturbing the beads.
6. While keeping the plate or tubes on the magnet, add 200 µL of 80% ethanol, then incubate for 1 min.
7. Remove the ethanol, then repeat another ethanol wash.
8. After the second ethanol wash, use a P20 to remove any residual ethanol. It is very important to remove all residual ethanol for sample recovery.
9. Allow the beads to air dry for 3 minutes.

   Important: Do not over-dry the beads.

10. Remove the sample plate or tubes from the magnet and elute in 22 µL of Buffer EB, or equivalent (10 mM Tris-

Cl, pH 8.5). Mix thoroughly. Alternatively, TE can be used.

11. Incubate for 5 min at RT.
12. Place the plate on a magnet until supernatant is clear (1–2 min).
13. Transfer 20 µL of eluate to a fresh plate, making sure that no beads are carried over.

Optional stopping point: Store purified PCR fragments per your established internal laboratory procedures.

Quantify the library

1. Measure the concentration of the captured library using a fluorescence-based method for DNA quantitation (such as Qubit™ dsDNA HS Assay kit) or qPCR.
2. Measure the average fragment length of the captured library on a digital electrophoresis system (e.g., the Agilent 4200 TapeStation® system using a DNA tape, or other equivalent system).

Important: High probe inputs, such as exome panels, may contain high levels of probes that inflate sample quantification. Presence of probes may appear as a peak at around 120bp on Tapestation. This is observed more frequently with short hybridization and low total library inputs.

Sequencing

1. Perform sequencing according to the instructions for your Illumina® instrument.

APPENDIX A

AMPure XP Beads DNA concentration protocol (optional)

1. Add the desired amount of library (ex. 500 ng) to each well
   a. This protocol is compatible with 100ng to 6µg of total library into each hybridization-capture reaction.
   b. For multiplex captures, add the pooled libraries such that the total library input per hybridization-capture reaction is 100ng to 6µg and does not exceed application-specific limit of 6µg total.
2. Add 7.5 µL of Human Cot DNA.
3. Add 1.8X volume of AMPure XP beads.
4. Securely seal the plate or tube.
5. Vortex thoroughly to mix. Adjust the settings to prevent any splashing onto the seal or cap.
6. Incubate for 10 min at room temperature.
7. Incubate the plate or tubes on the magnet for at least 2 min or until supernatant is clear.
8. Remove and discard the supernatant. Keeping the plate or tubes on the magnet, add 80% ethanol to cover the surface of the beads. Incubate for 30 sec without disturbing the beads.
9. Remove and discard the supernatant, then repeat another ethanol wash for a total of two washes
10. Allow the beads to air dry for approximately 3 min.

Important: Do not over-dry.

11. Add these components to the tube to make the Hybridization Reaction Mix:

Hybridization Master Mix components	Vol per reaction (µL)
xGen 1X Hybridization Buffer	14
xGen Universal Blockers	2
xGen Hybridization Panel	4.5
Total	20.5

12. Remove the plate or tubes from magnet and vortex to mix. Ensure that the beads are fully resuspended.
13. Incubate for 5 min at room temperature.
14. After incubation, place the plate or tubes on a magnet for 5–10 min or until the supernatant is clear.
15. Transfer 18 µL of the supernatant to a new sample plate, or tube, where the hybridization will occur. Make sure to avoid bead carryover during the transfer process.

Note: The Hybridization Reaction Mix elutes the DNA from the AMPure XP beads.

16. Seal the plate or tube, vortex briefly to mix and spin down.
17. Proceed to **Hybridization Step 6**.

APPENDIX B

Custom xGen Hyb Panel Spike-In

Combine panels at equimolar amounts to achieve uniform coverage. When combining panels (during Hybridization reaction), prepare the Hybridization Master Mix based on the recommendations in **Table 1**. Multiply by the number of samples, then add a 10% overfill (See **Table 2**).

Table 1. Spike-in volumes when combining two panels

		Spike-In Panel
		≤20K probes
Main Panel	≤20K probes	3 µL Main Panel + 3 µL Spike-In Panel
	>20K probes	4 µL Main Panel + 1 µL Spike-In Panel

* Spike-In Panels containing greater than 20,000 probes will require optimization to ensure optimal performance.

Table 2. Hybridization master mix volume per component

Hybridization Master Mix	Volume per reaction (µL)
xGen 1X Hybridization Buffer	14
Main panel	See Table 1
Spike-in panel	See Table 1
Total volume*	**19-20 µL**

* Total Hybridization Master Mix volume will vary based on the size of the Main panel.

For more information regarding custom spike-in panels, contact us at www.idtdna.com/contactus.

APPENDIX C

xGen Hyb Panel Spike-In with xGen Exome Hyb Panel v2

Combine panels during Hybridization reaction according to recommendation in **Table 3** to achieve uniform coverage. Multiply by the number of samples, then add a 10% overfill.

Table 3. Hybridization master mix volume per component

Hybridization Master Mix	Volume per reaction (µL)
xGen 1X Hybridization Buffer	14
xGen Exome Hyb Panel v2	4
Stocked Spike-in panel	2
Total volume	**20 µL**

For more information regarding stocked spike-in panels, contact us at www.idtdna.com/contactus.

SAFETY WARNING

xGen™ 1X Hybridization Buffer

Signal Word
Danger
Hazard Statements
H301 – Toxic if swallowed.
H311 – Toxic in contact with skin.
H315 – Causes skin irritation.
H370 – Causes damage to organs.
H411 – Toxic to aquatic life with long lasting effects.
Precautionary Statements – EU (§28, 1272/2008)
P260 – Do not breathe dust/fume/gas/mist/vapors/spray.
P273 – Avoid release to the environment.
P280 – Wear protective gloves and protective clothing.
P301 + P310 – IF SWALLOWED: Immediately call a POISON CENTER or doctor.
P308 + P311 – IF exposed or concerned: Call a POISON CENTER or doctor.
P391 – Collect spillage

