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QIAseq[®] xHYB Pro Capture Kit Handbook

For hybridization- capture– based enrichment from human whole genome/transcriptome libraries

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Kit Contents

QIAseq xHYB Pro Capture Kits

QIAseq xHYB Pro Capture Kits include two boxes containing all reagents required for hybridization capture, post-capture washing, and post-capture amplification.

QIAseq xHYB human or custom probe sets, as well as the reusable QIAseq xHYB Pro Capture Frame, are sold separately.

QIAseq xHYB Pro Capture Kit Catalog no. No. of hybridization reactions Typical no. of samples	334145 (12) 96	334142 (3) 24
Box 1 of 2		
xHYB Pro Hybridization Reagent	12 tubes	3 tubes
One-4-All Blocking Oligos	96 µL	24 µL
xHYB Pro Blocking Solution	60 µL	15 µL
Vapor-Lock	500 µL	500 µL
Post Hybrid-Capture PCR Mix (2x)	660 µL	660 µL
Primer Mix Illumina Library Amplification 12 rxn	3 × 20 µL	20 µL
Post Hybrid-Capture PCR Buffer	800 µL	800 µL
RNase-free Water	2 × 1.5 mL	1.5 mL
8-cap Strips (3)	1 bag	1 bag
Box 2 of 2		
xHYB Pro Streptavidin Beads	600 µL	150 µL
xHYB Pro Wash Buffer 1	3 × 800 µL	800 µL
xHYB Pro Wash Buffer 2	3 × 800 µL	800 µL
QIAseq Beads	10 mL	10 mL

QIAseq xHYB Pro Capture Frame

QIAseq xHYB Pro Capture Frame Catalog no.	334141
xHYB Pro Capture Frame	1 unit (reusable)

The QIAseq xHYB Pro Capture Frame is a reusable 96-well plate accessory that does not contain tubes and must be purchased separately before the first use. It is designed to securely hold individual xHYB Pro Hybridization Reagent tubes, enabling flexible sample throughput in a stable, plate-based format.

While use of the Capture Frame is not strictly required, it is strongly recommended to ensure sample integrity and ease of handling. Use of individual tubes without the frame can lead to mechanical deformation under the pressure of the thermal cycler heated lid, which may hinder pipette access and impact downstream steps.

In addition, the QIAseq xHYB Pro Capture Frame supports ergonomic handling during critical workflow steps, such as on-cycler bead addition at 60°C, where managing individual tubes is impractical and increases the risk of handling errors.

For reliable performance and consistent workflow execution, the QIAseq xHYB Pro Capture Frame is considered an essential accessory.

Shipping and Storage

QIAseq xHYB Pro Capture Kits consists of 2 boxes.

Box 1 is shipped on dry ice. Store Box 1 immediately upon receipt at -30°C to -15°C in a constant-temperature freezer.

Box 2 is shipped on a cool pack (4°C). Store Box 2 immediately upon receipt at $2-8^{\circ}\text{C}$. Do not freeze Box 2.

Under these conditions, the components are stable until the expiry date indicated on the label.

Intended Use

QIAseq xHYB Pro Capture Kits are intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in a convenient and compact PDF at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN ISO-certified Quality Management System, each lot of QIAseq xHYB Pro Capture Kits are tested against predetermined specifications to ensure consistent product quality.

Introduction

QIAseq xHYB Pro Capture Kits enable rapid, high-performance hybridization capture for targeted enrichment from indexed whole-genome and whole-transcriptome libraries. Building on the QIAGEN xHYB chemistry, the system delivers significant improvements in workflow speed, handling simplicity and support for multimodal inputs.

The kit uses a pre-dried, pre-dispensed hybridization reagent, which eliminates vacuum concentration steps and simplifies workflow setup. This formulation supports hybridization in as little as 5 minutes, while maintaining high specificity and uniform coverage. As a result, the workflow reduces hands-on time and variability, and shortens overall turnaround time.

The system supports flexible and multiplexed hybridization, enabling multiple indexed libraries to be combined in a single enrichment reaction for processing of high-plex sample pools. Optimized bead-binding and wash conditions further reduce manual intervention while supporting the removal of non-target fragments and target recovery.

The workflow accommodates a broad range of sample types, including genomic DNA (gDNA), formalin-fixed paraffin-embedded (FFPE) DNA, cell-free DNA (cfDNA), and RNA-derived libraries. It is also compatible with QIAseq library preparation solutions and xHYB probe panels. Supported applications include whole-exome sequencing, comprehensive genomic profiling (CGP), and multimodal DNA/RNA analysis.

The system can be used in manual and automated workflows, and incorporates ready-to-use reagents and plate-based handling, ensuring reproducibility and scalability, and completion of the sample-to-sequencer workflow within one day.

Principle

QIAseq xHYB Pro Capture Kits use hybridization capture technology for targeted enrichment of next-generation sequencing libraries. Indexed DNA- and RNA- libraries serve as input for the target enrichment. The Kits are optimized for use with QIAseq library preparation solutions, and are also compatible with appropriately prepared libraries generated using other library preparation methods.

During enrichment, library fragments hybridize to biotinylated, double-stranded DNA capture probes that are complementary to target regions. Universal Blocking Oligos are added during hybridization to prevent nonspecific binding of adapter sequences and ensure selective target capture. For RNA-derived libraries, QIAseq N Blocking Oligos are used to block RNA adapter sequences.

The xHYB Pro system incorporates a pre-dried, pre-dispensed hybridization reagent, and does not require vacuum concentration. This design enables efficient and specific enrichment of target regions from complex library pools while maintaining uniform coverage.

After hybridization, probe–target complexes are isolated using streptavidin-coated magnetic beads, and non-target fragments are removed by washing. The enriched libraries are then amplified to generate sufficient material for downstream sequencing while maintaining representation across regions with varying GC content.

The system supports multiplexed hybridization capture, enabling multiple indexed libraries to be processed in a single enrichment reaction.



QIAseq xHYB Pro Capture Kit

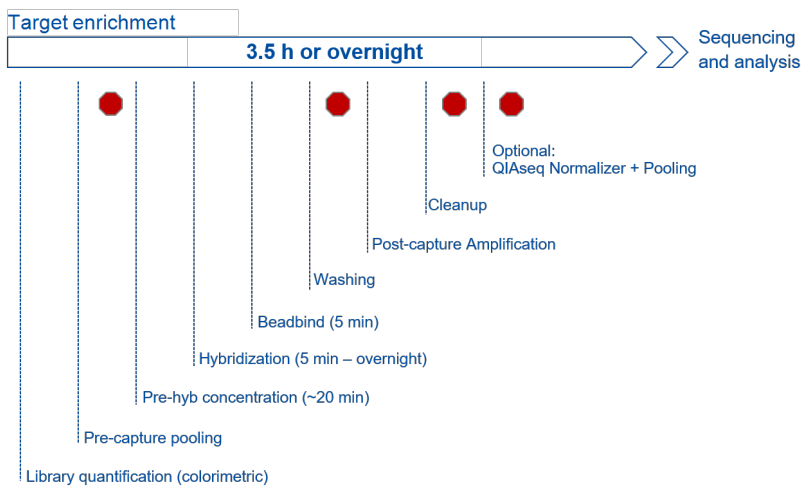


Figure 1. Workflow principle for QIAseq xHYB Pro Capture Kits. Red marks indicate safe stopping points.

Compatible Library Preparation Solutions

QIAseq xHYB Pro Capture Kits are compatible with indexed libraries generated using the following QIAseq library preparation solutions:

Table 1. Recommended library preparation kits to be used for hybridization capture

QIAseq library preparation solution	Supported input types	Notes
QIAseq FX DNA Library Kit*†	gDNA, FFPE DNA	Enzymatic fragmentation workflow
QIAseq Ultralow Input DNA Library Kit‡	cfDNA, low-input DNA	Optimized for low-input and fragmented samples
QIAseq Multimodal DNA/RNA Library Prep Kits§	DNA and RNA	Supports combined DNA and RNA analysis and incorporates unique molecular identifiers (UMIs) for improved error correction
QIAseq Multimodal DNA/RNA Modular Kits¶	DNA and RNA	Flexible modular workflow configurations with support for UMI-based workflows
QIAseq FastSelect Library Kits*	RNA	Enables selective depletion of abundant transcripts to improve target detection

*† Follow the dedicated QIAseq FX protocol in “Appendix A” on www.qiagen.com/HB-2015. Alternatively, follow the Ultrafast FX DNA Workflow for high quality DNA in “Appendix A”.

‡ Follow the protocol for Multimodal DNA Library preparation with enzymatic fragmentation on www.qiagen.com/HB-3703

‡ Follow the protocol for Ultralow Input Library Kits on www.qiagen.com/UltraLowInputLibraryKit

§ Follow the protocol for Multimodal DNA/RNA Library preparation on www.qiagen.com/HB-3579

¶ Follow the protocol for Multimodal RNA Library preparation on www.qiagen.com/HB-3705

Equipment and Reagents to be Supplied by User

- QIAseq xHYB Probe Panel (see “Kit Contents” on page 4)
- 1 re-usable xHYB Pro Capture Frame (see “Kit Contents” on page 4)
- If using RNA panels: QIAseq N Blocking Oligos (12) (cat. no. 334871)
- 100% ethanol (ACS grade)
- Nuclease-Free Water (cat. no. 129117 or 129115)
- Buffer EB (250 mL) (cat. no. 19086)
- PCR tubes or plates
- Pipette tips and pipettes
- DNA LoBind® tubes (from either Axygen® or Eppendorf®)
- Vortexer
- Microcentrifuge
- Magnetic racks for magnetic beads separation (e.g., DynaMag™ Magnet, Thermo Fisher Scientific, Inc.)
- Thermal cycler (96-well block) with heated lid (e.g., BioRad C-1000)
- Capillary electrophoresis device (e.g., QIAGEN QIAxcel®, Agilent® Bioanalyzer®, or similar to evaluate the DNA fragmentation profile [optional])
- Colorimetric DNA quantification method (e.g., Qubit® HS DNA Assay, Thermo Fisher Scientific, Inc.)
- 1 or 2 heating blocks for 1.5–2.0 mL tubes

Important Notes

General precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile and DNase- and RNase-free.
- Before starting, wipe down work area and pipettes with an RNase- and DNA-cleaning product.
- For consistent hybridization capture and amplification, ensure the thermal cycler and heating blocks used in this protocol are in good working condition and have been calibrated within the manufacturer's specifications.
- Read the entire protocol before beginning. Take note of stopping points where samples can be frozen at -30°C to -15°C and plan your workflow accordingly.

Quality control of input libraries

- Control and quantify each indexed library using a capillary electrophoresis method such as the QIAxcel System or the Agilent 2100 Bioanalyzer using High Sensitivity DNA chips.
- A typical indexed whole genome library has an average size distribution centered around 400 bp and is free of adapter dimers at a typical yield of >500 ng (Figure 2A). The size distribution of libraries generated from severely degraded FFPE material may vary.
- A typical indexed cfDNA library has 1 or 2 side peaks representing the N2 and N3 fraction at a typical yield of >200 ng (Figure 2B).
- Libraries from physically sheared DNA will reflect the size distribution of the sheared input DNA.

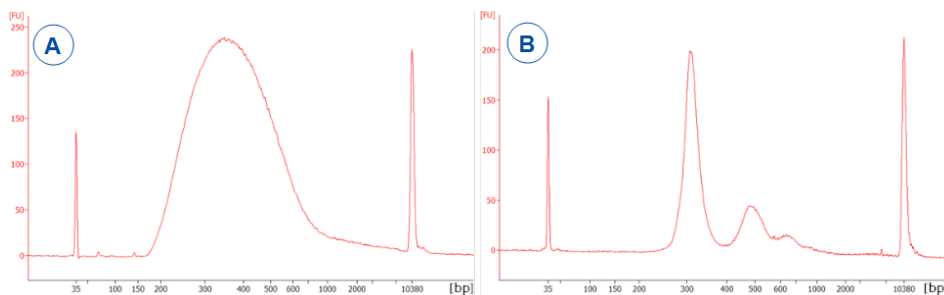


Figure 2. Capillary electrophoresis device traces of different hybridization capture input libraries. **A:** Library generated from genomic DNA by enzymatic fragmentation with a typical mean size distribution of 350–450 bp that is free of adapter dimers. **B:** A typical cfDNA library with the major library peak at 300 bp and additional peaks representing the N2 and N3 fractions. An example electrophoresis trace of the final post-capture library pool is shown at the end of the hybrid capture workflow protocol (see “Protocol: Target Enrichment from Indexed WGS/WTS Libraries Using the QIAseq xHYB Pro Capture Kit” on page 17).

Indexing recommendations

- Use QIAseq indexed adapters or indexed primers that are recommended for use with the selected library preparation kit.
- When using non-QIAseq adapters, non-QIAseq indexed primers, or a non-QIAseq library preparation method, make sure the adapter architecture is Illumina® TruSeq® compatible and refer to the manufacturer’s instructions for adapter dilution and sample indexes.
- Use different sample indexes for all samples within a hybridization capture pool.
- Consider using different sample indexes also across hybridization capture pools to allow post-capture multiplexing depending on your sequencing instrument.

Description of protocols

The “Protocol: Target Enrichment from Indexed WGS/WTS Libraries Using the QIAseq xHYB Pro Capture Kit” describes the hybridization capture-based enrichment from indexed whole genome or indexed whole transcriptome libraries using any xHYB Human panel for short read sequencing.

Generate libraries before hybridization capture. For library preparation, follow the protocols of the respective library preparation handbook or adhere to “Appendix A” for fast library preparation from high quality genomic DNA using QIAseq FX DNA Library UDI Kits.

Protocol: Target Enrichment from Indexed WGS/WTS Libraries Using the QIAseq xHYB Pro Capture Kit

Things to do before starting

- Generate indexed whole genome and/or whole transcriptome libraries using one of the library preparation methods outlined in Table 2. Alternatively, indexed whole genome libraries can be prepared with many third-party methods that are TruSeq-compatible.
- Quantify the amplified, indexed libraries using a colorimetric method (e.g., Qubit HS DNA Assay) or by electrophoresis (e.g., QIAxcel, Agilent Bioanalyzer, etc.).
- Prepare fresh 80% ethanol.
- Allow QIAseq Beads to equilibrate to room temperature for 30 minutes. Vortex vigorously to homogenize.
- Thaw the xHYB Probe Set(s), One-4-All Blocking Oligos, xHYB Pro Blocking solution, and Vapor Lock. Pulse vortex and spin down.
- Per hybridization reaction, obtain 1 tube of xHYB Pro Hybridization Reagent containing the dried hybridization chemistry. Leave the sealed tube on the benchtop until needed.
- If hybridizing for less than 1 hour, prepare the following before starting:
 - Equilibrate xHYB Pro Streptavidin Beads to room temperature (15–25°C).
 - Obtain tube(s) of xHYB Pro Wash Buffer 1 and preheat to 68°C in a heating block. One tube is sufficient for 4 hybrid capture reactions.
 - Obtain tube(s) of xHYB Pro Wash Buffer 2 and preheat to 58°C in a heating block. One tube is sufficient for 4 hybrid capture reactions.

Prepare the hybridization pool and hybridize

Pooling of amplified, indexed whole genome libraries enables processing multiple libraries in a single hybridization-capture reaction. We recommend pooling 8 indexed libraries for one hybridization capture reaction, but using fewer or more than 8 libraries per pool is possible. Make sure to only pool PCR-amplified libraries for hybridization capture that have been quantified using the same method.

During the hybridization step, QIAseq xHYB Probes bind specifically to targeted library fragments present in the pool. The ultrafast protocol (see “Compatible Library Preparation Solutions”) allows for the hybridization to take place within 5 minutes, while hybridizing for longer than 5 minutes can improve the capture performance. Incubating for longer than 30 minutes or overnight will not enhance performance significantly but may be considered for highest flexibility in experiment planning.

Procedure

1. For each capture pool, determine the per-library amount (ng) and calculate the volume (µL) of each amplified library needed for pooling. For example, for a library concentration of 25 ng/µL, 20 µL are needed to add 500 ng of indexed library to the capture. Follow the guidelines in Table 2 and Table 3.

Note: Take note of the index motifs of all libraries within a pool, as well as the total volume of all libraries of a pool.

Table 2. Guidelines for pooling indexed libraries for hybridization capture

Library pooling guidelines	Amount
Recommended number of libraries per capture pool	8
Maximum total input per capture pool	4000 ng
Minimal input per library	200 ng

Table 3. Recommended library pooling strategy for hybridization capture

Number of indexed samples per pool	Amount of each indexed library per pool (ng)
1	200–500
2	200–500
3	200–500
4	200–500
6	200–500
8	200–500
16	200–250
20	200

Note: To maintain equal representation of libraries during sequencing, use the same input amount for all libraries. Using less than 200 ng input per library may result in reduced complexity.

- Pool libraries into a 1.5 mL Eppendorf tube. To achieve equal read counts per sample, pool equal masses of different libraries.

Optional: Pooled libraries can be stored at -20°C

- Add 2 volumes of homogenized QIAseq Beads and mix well. For example, for a 200 μL library pool, add 400 μL QIAseq beads.
- Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) for 5 min or until the solution has cleared. Carefully discard the supernatant.
- Wash the beads by adding 200 μL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant.

Note: During the ethanol wash, the tube can remain on the magnetic stand. The beads do not need to be resuspended.

6. Repeat the wash once for a total of 2 ethanol washes. Remove and discard the ethanol supernatant.
7. Pulse-spin the tube and place back on the magnet. Then, using a small pipette tip, remove as much excess ethanol as possible.
8. Incubate on the magnetic stand for 10–15 min, depending on the pellet size. Ensure that the beads are dry. Overdrying does not adversely affect QIAseq Beads.
9. While the beads are drying, prepare an elution mix for every capture pool according to Table 4. When using the same xHYB Probe Panel for all pools, you can prepare a master mix and scale it up as needed.

Alternatively, the elution mix can be prepared directly before starting the bead-based concentration and kept on ice until needed.

10. After the beads have dried, remove tube from the magnet.
11. Pipet 25 μ L of elution mix directly onto the bead pellet. Vortex vigorously until the beads are completely resuspended.

Note: Ensure that all beads have detached from the tube wall and are thoroughly homogenized.

12. Pulse-spin and place the tube on the magnetic stand for 2 min or until the solution is clear.

Table 4. Preparation of the elution mix for every library pool at room temperature

Component	Volume (μL)
xHYB Probe Set*	4
Up to 2 additional spike-in probe sets (Optional)†	8
One-4-All Blocking Oligos	8
xHYB Pro Blocking Solution	5
Water	Variable
Total volume	25

*QIAseq xHYB Custom Human Panels may consist of single or multiple probe sets. Add 4 μL of every probe set supplied for your custom panel.

†The Mitochondrial Spike-in Probe Set must be diluted 1:10 in water before use. Then, use 4 μL of the diluted probe set.

13. For every hybridization reaction, remove the seal of a xHYB Pro Hybridization Reagent tube containing the dry hybridization reagent. Then insert the tube into the xHYB Capture Frame. Apply pressure to the top of the tube until it audibly clicks into place. For convenience the frame may be placed on a 96-well PCR-plate rack to prevent bending of the frame when applying pressure.

Important: The dried chemicals in the hybridization tube may not be firmly attached to the bottom of the tube. Take caution not to lose any material when removing the seal or while locking the tube into place.

Recommendation: For optimal heating conditions in some thermal cyclers or automated liquid handling systems, use the well positions in the center of the plate. See Figure 3 for reference.

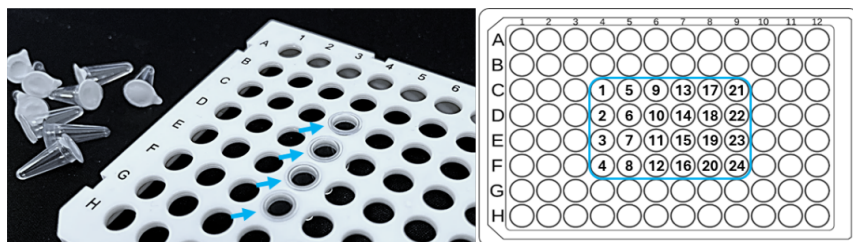


Figure 3. Place and lock hybridization tubes in the xHYB Capture Frame. For optimal heating conditions, use the inside well positions of the frame for up to 24 parallel hybrid capture reactions as shown in the image. As an example, the photo shows 4 hybridization tubes placed at the positions C4 to F4.

14. Aspirate the complete 25 μ L eluate (supernatant from step 12) and transfer it to a hybridization tube (from step 13). Transferring some beads does not affect the procedure.

Note: Pipet directly onto the dried buffer. Do not submerge the pipet tip in the dried buffer and do not pipet to mix.

15. Use a cap strip delivered with the kit to close the tubes.

Note: Do not use third-party cap strips, as they may not seal the tubes tightly.

16. Program a thermal cycler with a heated lid according to Table 5 and start the program so it preheats and holds at 95°C. Set the heated lid to 105°C

Important: Ensure that the thermal cycler heating block can accommodate fully skirted 96-well plates. The hybridization tubes must be secured in the Capture Frame and are fully lowered into the heating block. If needed, insert single hybridization tubes into the cycler without using the Capture Frame. Single hybridization tubes can be connected by using the cap strips delivered with the kit.

Table 5. Conditions for hybridization capture

Temperature (°C)	Time	Step	Purpose
95	Hold	1	Reconstitution
95	5 min	2	Denaturation
60	5 min to 16 h*	3	Hybridization
60	Hold	4	Hold temperature
60	5 min	5	Beadbind

*Hybridizing for longer than 5 min may improve performance. However, exceeding 30 min of hybridization time will typically not improve the performance further, but may be considered for flexible experiment planning.

17. Place the xHYB Capture Frame into the thermal cycler and incubate for 5 min at 95°C.
18. Remove the xHYB Capture Frame from the cycler and vortex the plate for 30 s at maximum intensity.

Caution: The plate is hot.

Note: Vortex the plate by touching the outer skirt to the vortex head, rather than pressing down flat from above. Cover the plate with a lab wipe and hold it firmly with your hand while vortexing to prevent individual tubes from popping out.

If using single hybridization tubes connected by cap strips, use a 96-well PCR rack to stabilize the tubes while vortexing.

19. Repeat steps 17–18 for another round of reconstitution.
20. Spin down the plate to collect all liquid at the bottom.
21. Add 30 µL of Vapor Lock to each hybridization pool. Recap the tubes afterwards.

Note: Vapor Lock will form a distinct phase on top of the aqueous hybridization reagents.

22. Place the plate back to the thermal cycler and advance to program step 2.
- Note:** When hybridization is complete, leave the reaction to hold temperature (step 4) until ready to continue. Leaving the reaction at 60°C does not adversely affect performance.

Post-capture bead bind and wash

In this step, the biotin-coupled xHYB Probes and their hybridized targets will be captured on Streptavidin beads. Nonspecific targets will be washed away during a series of washing steps. Start preparing the xHYB Pro Streptavidin Beads and wash buffers for at least 30 min before hybridization is complete.

Most catalog and custom xHYB panels use the standard xHYB Pro wash conditions. Panels requiring alternative wash temperatures are listed in Table 6. When using one of these panels, or when combining panels with different wash requirements, use the wash buffer preheating and incubation temperatures specified below.

Table 6. Panels requiring alternative wash temperatures

Panel	xHYB Pro Wash Buffer 1 (preheat and incubation) (°C)	xHYB Pro Wash Buffer 2 (preheat and incubation) (°C)
QIAseq Human Exome Panel	68	58
QIAseq xHYB Actionable Exome Panel	68	58
QIAseq xHYB Custom Human panels*	68	58
QIAseq xHYB CGP Panel	68	58
QIAseq xHYB HRD Panel	66	58

Table 6. Panels requiring alternative wash temperatures (continued)

Panel	xHYB Pro Wash Buffer 1 (preheat and incubation) (°C)	xHYB Pro Wash Buffer 2 (preheat and incubation) (°C)
QIAseq xHYB HRD Panel in combination with the QIAseq xHYB CGP DNA Panel and/or QIAseq xHYB Custom Human Panel(s)	66	58

*Standard xHYB Pro wash conditions are suitable for most QIAseq xHYB Custom Human Panels. Depending on panel content and design characteristics, optimization of wash temperatures may be required

Things to do before starting

- Equilibrate xHYB Pro Streptavidin Beads to room temperature and homogenize by vigorously vortexing for 1 minute.
- Obtain tube(s) of xHYB Pro Wash Buffer 1 and preheat to 68°C or 66°C (see Table 6) in a heating block. One tube is sufficient for 4 hybrid capture reactions.
- Obtain tube(s) of xHYB Pro Wash Buffer 2 and preheat to 58°C in a heating block. One tube is sufficient for 4 hybrid capture reactions.
- Thaw Nuclease-Free Water and keep it at room temperature until use.
- Thaw xHYB Pro PCR Buffer and vortex to homogenize. Keep it at room temperature until use.
- Thaw Post-Hybrid-Capture PCR MM and Illumina Library Amp Primer on ice. Pulse vortex to homogenize. Then, spin down.
- If using QIAseq Normalizer for post-capture library adjustment, thaw Normalizer Primer Mix on ice. Pulse vortex to homogenize. Then, spin down.

Procedure

23. Once the hybridization program has reached step 4 (hold at 60°C), open the lid of the thermal cycler and remove the cap strips from the hybridization tubes.

Caution: The cap may still be hot from the heated lid. If needed, allow caps to cool down for a minute.

Note: Leave the xHYB Capture Frame inside the thermal cycler.

24. Homogenize xHYB Pro Streptavidin Beads by vortexing vigorously.
25. Transfer 50 μ L Streptavidin Beads to each hybridization tube. Dispense beads at the bottom of the tube. Then, mix the beads with the hybridization solution, and Vapor Lock 10 times by pipetting up and down.
26. Recap the tubes, close the lid of the thermal cycler, and advance to program step 5.
27. Once the program has completed, remove the plate from the thermal cycler and transfer the full content (approx. 105 μ L) of each well to a 1.5 mL or 2 mL Eppendorf tube.

Note: The 1.5 mL or 2 mL Eppendorf tubes will be used later for the washing steps. Choose the type of Eppendorf tube to match the shape of the used heating block to ensure optimal heat transfer. For example, do not use a conical tube in a round bottom heating block.

28. Place on a magnetic stand until the solution is clear.
29. Remove and discard the cleared supernatant. Do not aspirate the beads. Then, remove the tube from the magnetic stand.
30. Pulse-spin the tube and place back on the magnet. Then, using a small pipette tip, remove as much residual supernatant as possible. Remove tube from the magnetic stand.
31. Vortex a tube of preheated xHYB Pro Wash Buffer 1 to homogenize. Then, add 200 μ L of xHYB Pro Wash Buffer 1 to each bead pellet, and homogenize by vortexing vigorously.

32. Incubate the bead slurry at 68°C or 66°C (see Table 6) in a heating block for 15 min.
33. Pulse-spin the tubes to collect all liquid at the bottom. Then, place on a magnetic stand until the solution is clear.
34. Remove and discard the cleared supernatant. Do not aspirate the beads. Then, remove the tube from the magnetic stand.
35. Pulse-spin the tube and place back on the magnet. Then, using a small pipette tip, remove as much residual supernatant as possible. Remove tube from the magnetic stand.
36. Vortex a tube of pre-heated xHYB Pro Wash Buffer 2 to homogenize. Then, add 200 µL of xHYB Pro Wash Buffer 2 to each bead pellet and homogenize by vortexing vigorously.
37. Incubate the bead slurry at 58°C in a heating block for 15 minutes.
38. Pulse-spin the tubes to collect all liquid at the bottom. Then, place on a magnetic stand until the solution is clear.
39. Remove and discard the cleared supernatant. Do not aspirate the beads. Then, remove the tube from the magnetic stand.
40. Pulse-spin the tube and place back on the magnet. Then, using a small pipette tip, remove as much residual supernatant as possible. Remove tube from the magnetic stand.
41. Add 50 µL of Nuclease-Free Water to each bead pellet and homogenize by vortexing vigorously.
42. Place on a magnetic stand until the solution is clear.
43. Remove and discard the cleared supernatant. Do not aspirate the beads. Then, remove the tube from the magnetic stand.

Note: Captured library fragments are still bound to the beads.

44. Add 45 µL of Post Hybrid Capture PCR Buffer to each bead pellet and homogenize by pipetting. Store the bead slurry on ice.
45. Split the homogenized bead slurry into two thin-walled 0.2 mL strip tubes (22.5 µL each).

Note: One slurry is needed for post-capture amplification. Bead slurries can be stored at -30°C to -15°C for later amplification.

Post-capture amplification

Captured targets bound to the Streptavidin Beads will be amplified using QIAGENs Post Hybrid-Capture PCR Mix. The amplified hybrid capture library will be purified and is ready for QC and sequencing on Illumina instruments. Optionally, Normalizer Primer Mix can be used for post-capture amplification, which will allow fast library normalization to ~ 4 nmol/L using QIAseq Library Normalizer Kits (Normalizer workflow B) (refer to *QIAseq Normalizer Kit Handbook* at www.qiagen.com/HB-3361).

Things to do before starting

- Prepare fresh 80% ethanol.
- If stored at -30°C to -15°C , thaw a 22.5 μL aliquot of the Streptavidin Binding Bead slurry on ice (from step 45).
- Allow QIAseq Beads to equilibrate to room temperature for 30 minutes. Vortex vigorously to homogenize.
- Thaw Post Hybrid-Capture PCR Mix and Primer Mix Illumina Library Amplification on ice. Once reagents are thawed, mix by pulse-vortexing to avoid any localized concentrations. Before use, briefly spin down vortexed reagents..
- If using QIAseq Normalizer for post-capture library adjustment, thaw Normalizer Primer Mix on ice. Pulse vortex to homogenize. Then, spin down.

Procedure

46. Program a thermal cycler with a heated lid according to Table 7 and Table 8.

Table 7. Post-capture amplification conditions

Time	Temperature (°C)	Number of cycles
2 min	98	1
20 s	98	See Table 8
30 s	60	See Table 8
30 s	72	See Table 8
1 min	72	1
∞	4	Hold

Note: See Table 8 for the recommended cycle numbers depending on input amount and xHYB panel used.

Table 8. Recommended number of post-capture amplification cycles

Total captured target size* (Mb)	<500 ng capture input (Cycles)	500 ng to 2.0 µg capture input (Cycles)	>2.0 µg capture input (Cycles)
Custom Panels			
<1 [†]	≤ 20	≤ 20	≤ 20
>1 – 2.5	13	12	11
>2.5 – 5	12	11	10
>5 – 10	10	9	8
>10 – 15	9	8	7
>15 – 20	8	8	7
>20	8	7	7
Cataloged Panels			
Human Exome Panel	8	7	7
Actionable Exome Panel	9	8	7
CGP Panel	12	11	10
HRD Panel	12	11	10
CGP+HRD Panels combined	11	10	9

* If combining multiple panels in a single hybrid capture, get the sum of the individual panel sizes to obtain the total captured target size. Do not take the sum of the size of spike-in panels that correct poorly enriched regions (booster panels), as these do not increase the total target region size.

[†]Custom panels of less than 1 Mb target region size or very small panels with less than 100 Kb target region size may require up to 20 cycles of post-capture amplification.

Note: Add the following number of extra cycles to the post-capture amplification if they meet the conditions:

- 1 extra cycle: If the hybridization time was 15 minutes or less.
- 1 extra cycle: If using the QIAseq Normalizer.
- 2 extra cycles: If using the QIAseq Normalizer and the hybridization time was 15 minutes or less.

47. Prepare the reaction mix on ice according to Table 9. Mix the components in a PCR tube or 96-well PCR plate.

Note: If using the QIAseq Normalizer to adjust the post-capture library concentration, prepare the reaction mix according to Table 10.

Table 9. Reaction mix for post-capture amplification

Component	Volume (µL)
Bead slurry with captured targets (from step 45)	22.5
Post Hybrid-Capture PCR Mix	25
Primer Mix Illumina Library Amplification*	2.5
Total reaction volume	50

*Primer Mix Illumina Library Amplification is included in the QIAseq xHYB Pro Capture Kit (Box1).

Table 10. Reaction mix for post-capture amplification and Normalization using QIAseq Normalizer Library Kits

Component	Volume (µL)
Bead slurry with captured targets (from step 45)	22.5
Post Hybrid-Capture PCR Mix	25
Normalizer Primer Mix*	1.5
Nuclease-Free Water	1
Total reaction volume	50

*Normalizer Primer Mix is part of the QIAseq Normalizer Primer Kit.

48. Transfer the PCR tube or plate to the thermal cycler and start the program.
49. Once PCR is complete, add 75 μL of resuspended QIAseq beads to each reaction (50 μL) and mix by vortexing.
50. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand, and carefully discard the supernatant.
51. Wash the beads by adding 200 μL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant.
52. Repeat the wash once for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
53. Incubate on the magnetic stand for 5–10 min. Ensure that the pellet is completely dry by visual inspection.
54. Elute by resuspending in 32 μL of Buffer EB, 10 mM Tris-Cl, pH 8.0, or RNase-free water. Incubate at room temperature for 2 min.
55. Pellet the beads on the magnetic stand. Carefully transfer 30 μL of the clear supernatant into a new tube.
56. Assess the quality and size distribution of the library using a capillary electrophoresis device such as the QIAxcel Advanced or the Agilent Bioanalyzer 2100. The library should show a distribution similar to the whole genome/whole transcriptome libraries used for the respective capture pool.

Note: The QIAseq Normalizer allows for fast library normalization and pooling of post-capture libraries (Appendix B). Alternatively, quantitate the captured library using a qPCR assay such as the QIAseq Library Quant Assay Kit (cat. no. 333314) or compatible method.
57. The purified library can be safely stored at -30°C to -15°C in a DNA LoBind tube until ready to use for sequencing.

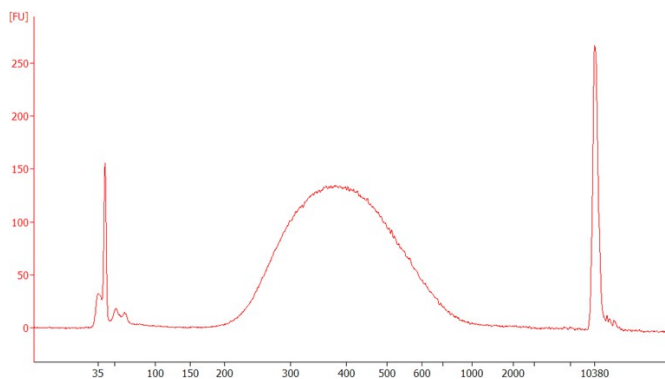


Figure 4. Electrophoresis trace data of a final hybrid capture library pool.

Sequencing Guidelines

Deep sequencing considerations

Deep sequencing of QIAseq xHYB libraries enables detection of variants with low allele frequencies, such as those found in tumor tissue or cell-free DNA (cfDNA). Increasing sequencing depth generally improves sensitivity for low-frequency variant detection, but the benefit plateaus: beyond a certain point, higher coverage mainly increases duplicate reads rather than adding new information. This effect is most pronounced with low-input or compromised samples (e.g., FFPE or cfDNA), which require more amplification and therefore yield higher duplicate rates.

To address this, QIAGEN recommends the QIAseq Multimodal DNA/RNA Library Kit (cat. no. 334842) or QIAseq Multimodal DNA Kit EnzFrag (cat. no. 334965) for deep sequencing applications. Both incorporate Unique Molecular Indexes (UMIs), which label individual DNA molecules before amplification. This allows PCR duplicates to be accurately removed during analysis, improving error correction and supporting confident detection of true low-frequency variants even in highly duplicated datasets.

Multiplexing guidelines

Depending on desired coverage and the sequencing instrument used, combining multiple hybridization capture pools may be advisable for cost efficient sequencing. To determine the total number of samples to be sequenced in a single sequencing run, consider specifications of the sequencing instrument, the safe target cluster density, the size of the targeted region, and the desired coverage per sample.

Determining your target coverage

- Set coverage by application.

Sequencing coverage requirements depend on the intended application. Germline variant detection is typically well served by an average target region coverage of approximately 100x, whereas somatic applications — such as detecting low-frequency variants in FFPE or plasma samples — require substantially greater depth (1200x or greater).

- Accounting for coverage uniformity.

QIAseq xHYB Panels achieve high coverage uniformity across the target region, with a typical Fold-80 base penalty of 1.3–1.5 or better. To ensure that 80% of covered target bases reach your desired minimum coverage, plan for an average coverage approximately 1.3- to 1.5-fold higher than the minimum. For example, to reliably achieve 100x across the target region, plan for an average coverage of approximately 140x.

Germline applications (~100x coverage)

For germline variant detection, an average target region coverage of approximately 100x is typically sufficient. Refer to Table 11 for custom panels and Table 12 for cataloged human panels. All values assume 2 × 150 bp paired-end sequencing on Element[®] Biosciences Aviti[™] or Illumina instrument.

Table 11. Multiplexing guidelines for QIAseq xHYB Custom Human Panels (2 × 150 bp paired-end) to achieve a minimal average target coverage of 100x

Note: Actual coverage may vary depending on input DNA quality, the characteristics of the panel used, and the sequencing instrument.

Instrument	Paired Reads (M)	1 Mb	2 Mb	5 Mb	10 Mb	15 Mb	20 Mb	50 Mb
Element Aviti Low Output	500	192	96	40	20	12	10	4
Element Aviti Medium Output	1000	384	192	80	40	24	20	8
Element Aviti High Output	2000	768	384	160	80	52	40	16
MiniSeq® (High Output)	45	16	8	3	1	1	–	–
MiSeq® (v2)	30	12	6	2	1	–	–	–
MiSeq i100 (25M)	50	18	8	4	2	1	1	–
MiSeq i100 Plus (50M)	100	36	16	8	4	2	2	–
MiSeq i100 Plus (100M)	200	72	32	16	8	5	4	1
NextSeq® 500/550 (Mid Output)	260	96	48	20	10	6	5	2
NextSeq 500/550 (High Output)	800	312	152	64	32	20	16	6
NextSeq 1000/2000 (P1)	200	72	32	16	8	5	4	1
NextSeq 1000/2000 (P2)	800	312	152	64	32	20	16	6
NextSeq 2000 (P3)	2400	936	456	192	96	64	48	18
NextSeq 2000 (P4)	3600	1248	624	272	136	88	64	28
NovaSeq® 6000 (SP)	1500	576	288	112	56	40	28	12
NovaSeq 6000 (S1)	3000	1176	576	232	112	76	56	24
NovaSeq 6000 (S2)	8000	>1536	1536	624	312	208	152	64

Table 11. Multiplexing guidelines for QIAseq xHYB Custom Human Panels (2 × 150 bp paired-end) to achieve a minimal average target coverage of 100x (continued)

Note: Actual coverage may vary depending on input DNA quality, the characteristics of the panel used, and the sequencing instrument.

Instrument	Paired Reads (M)	1 Mb	2 Mb	5 Mb	10 Mb	15 Mb	20 Mb	50 Mb
NovaSeq 6000 (S4)	18,000	>1536	>1536	1392	696	456	352	140
NovaSeq X Series (1.5B)	3200	1248	624	240	120	80	64	24
NovaSeq X Series (10B)	20,000	>1536	>1536	1536	768	504	384	152
NovaSeq X Series (25B)	52,000	>1536	>1536	>1536	>1536	1344	1008	384

* Libraries per flow cell refers to the number of single libraries with distinct sample indexes. Each hybridization capture pool can contain multiple individually barcoded libraries.

Table 12. Multiplexing guidelines for QIAseq xHYB Human Panels for the Element Biosciences Aviti or Illumina instruments using 2 × 150 bp paired-end sequencing to achieve a minimal average target coverage of 100x, which is typically sufficient for germline applications

Note: Actual coverage may vary depending on input DNA quality and the sequencing instrument.

Instrument	Paired Reads (M)	QIAseq Human Exome	QIAseq Actionable Exome
Element Aviti Low Output	500	7	16
Element Aviti Medium Output	1000	15	32
Element Aviti High Output	2000	30	64
MiniSeq (High Output)	45	–	1
MiSeq (v2)	30	–	1
MiSeq i100 (25M)	50	–	1
MiSeq i100 Plus (50M)	100	1	3
MiSeq i100 Plus (100M)	200	3	6
NextSeq 500/550 (Mid Output)	260	3	8
NextSeq 500/550 (High Output)	800	12	24
NextSeq 1000/2000 (P1)	200	3	6
NextSeq 1000/2000 (P2)	800	12	24
NextSeq 2000 (P3)	2400	36	72
NextSeq 2000 (P4)	3600	48	108
NovaSeq 6000 (SP)	1500	20	40
NovaSeq 6000 (S1)	3000	40	80
NovaSeq 6000 (S2)	8000	120	240
NovaSeq 6000 (S4)	18,000	264	552
NovaSeq X Series (1.5B)	3200	40	96
NovaSeq X Series (10B)	20,000	288	600
NovaSeq X Series (25B)	52,000	768	1536

* Libraries per flow cell refers to the number of single libraries with distinct sample indexes. Each hybridization capture pool can contain multiple individually barcoded libraries.

Somatic applications

Somatic variant detection requires substantially higher coverage to reliably identify variants with low allele frequencies. Scale your read budget to the target coverage required and reduce the number of libraries per flow cell proportionally.

Example: Increasing target coverage from 100x (germline) to 1200x (somatic) is a 12-fold increase in reads per sample, so the number of libraries per flow cell decreases by roughly the same factor. For instance, an instrument that supports 24 libraries at 100x would support only about 2 libraries at 1,200x ($24 \div 12 \approx 2$).

Use Table 13 for custom panels run at 1200x. QIAseq xHYB Panels for Comprehensive Genomic Profiling (CGP) and Homologous Recombination Deficiency (HRD) testing require higher average coverage, especially from FFPE samples; refer to Table 14 for read budgets and Table 15 for multiplexing guidelines.

Table 13. Multiplexing guidelines for QIAseq xHYB Custom Human Panels (2 x 150 bp paired-end) to achieve a minimal average target coverage of 1200x

Note: Actual coverage may vary depending on input DNA quality, the characteristics of the used panel used, and the sequencing instrument.

Instrument	Paired Reads (M)	1 Mb	2 Mb	5 Mb	10 Mb	15 Mb	20 Mb
Element Aviti Low Output	500	16	8	3	1	1	0
Element Aviti Medium Output	1000	32	16	6	3	2	1
Element Aviti High Output	2000	64	32	13	6	4	3
MiSeq i100 Plus (50M)	100	3	1	0	0	0	0
MiSeq i100 Plus (100M)	200	6	3	1	0	0	0
NextSeq 500/550 (Mid Output)	260	8	4	1	0	0	0

Table 13. Multiplexing guidelines for QIAseq xHYB Custom Human Panels (2 × 150 bp paired-end) to achieve a minimal average target coverage of 1200x (continued)

Note: Actual coverage may vary depending on input DNA quality, the characteristics of the used panel used, and the sequencing instrument.

Instrument	Paired Reads (M)	1 Mb	2 Mb	5 Mb	10 Mb	15 Mb	20 Mb
NextSeq 500/550 (High Output)	800	26	13	5	2	1	1
NextSeq 1000/2000 (P1)	200	6	3	1	0	0	0
NextSeq 1000/2000 (P2)	800	24	12	5	2	1	1
NextSeq 2000 (P3)	2400	74	37	15	7	5	3
NextSeq 2000 (P4)	3600	116	58	23	11	7	5
NovaSeq 6000 (SP)	1500	48	24	9	4	3	2
NovaSeq 6000 (S1)	3000	96	48	19	9	6	4
NovaSeq 6000 (S2)	8000	264	132	52	26	17	13
NovaSeq 6000 (S4)	18,000	576	288	119	59	39	29
NovaSeq X Series (1.5B)	3200	104	52	21	10	7	5
NovaSeq X Series (10B)	20,000	656	330	132	66	44	33
NovaSeq X Series (25B)	52,000	1536	848	336	168	112	84

* Libraries per flow cell refers to the number of single libraries with distinct sample indexes. Each hybridization capture pool can contain multiple individually barcoded libraries.

Note: The listed values are to be used as a starting point for multiplexing optimization. The values shown assume approximately 65% reads-on-target. Depending on the targeted regions of a custom panel the actual enrichment specificity may be better or worse.

Table 14. Read budget recommendations for QIAseq xHYB CGP and HRD Panels for FFPE samples for the Element Biosciences Aviti or Illumina instruments using 2 × 150 bp paired-end sequencing

Panel	Paired reads (M) (2 ×150 bp)
QIAseq xHYB CGP DNA Panel	75
QIAseq xHYB CGP RNA Panel*	50
QIAseq xHYB HRD Panel	25
QIAseq xHYB CGP and HRD Panels (combined capture)	150

*When sequencing an RNA library alone (without DNA), we recommend adding 20% PhiX.

Table 15. Multiplexing guidelines for QIAseq xHYB CGP and HRD Panels for FFPE sample for the Element Biosciences AVITI or Illumina instruments using 2 × 150 bp paired-end sequencing

Note: Actual coverage may vary depending on input DNA quality and the sequencing instrument.

Instrument	Paired Reads (M)	CGP DNA	CGP RNA	CGP DNA +RNA	HRD	CGP DNA +HRD
Element AVITI Low Output	500	6	10	4	20	4
Element AVITI Medium Output	1000	13	20	8	40	8
Element AVITI High Output	2000	26	40	16	80	16
MiniSeq (High Output)	45	–	–	–	1	–
MiSeq (v2)	30	–	–	–	1	–
MiSeq i100 (25M)	50	–	1	–	2	–
MiSeq i100 Plus (50M)	100	1	2	–	4	–
MiSeq i100 Plus (100M)	200	2	4	1	8	1
NextSeq 500/550 (Mid Output)	260	3	5	2	10	2
NextSeq 500/550 (High Output)	800	10	16	6	32	6
NextSeq 1000/2000 (P1)	200	2	4	1	8	1

Table 15. Multiplexing guidelines for QIAseq xHYB CGP and HRD Panels for FFPE sample for the Element Biosciences AVITI or Illumina instruments using 2 × 150 bp paired-end sequencing (continued)

Note: Actual coverage may vary depending on input DNA quality and the sequencing instrument.

Instrument	Paired Reads (M)	CGP DNA	CGP RNA	CGP DNA +RNA	HRD	CGP DNA +HRD
NextSeq 1000/2000 (P2)	800	10	16	6	32	6
NextSeq 2000 (P3)	2400	32	48	18	96	19
NextSeq 2000 (P4)	3600	48	72	28	144	28
NovaSeq 6000 (SP)	1500	20	30	12	60	12
NovaSeq 6000 (S1)	3000	40	60	24	120	24
NovaSeq 6000 (S2)	8000	104	160	64	320	64
NovaSeq 6000 (S4)	18,000	240	360	144	720	144
NovaSeq X Series (1.5B)	3200	42	64	25	128	25
NovaSeq X Series (10B)	20,000	266	384	160	800	160
NovaSeq X Series (25B)	52,000	672	1040	416	>1536	416

* Libraries per flow cell refers to the number of single libraries with distinct sample indexes. Each hybridization capture pool can contain multiple individually barcoded libraries.

Pooling guidelines

- Be sure all post-capture libraries to be pooled contain different indexes to allow data demultiplexing after sequencing.
- If libraries were frozen, thaw them completely, mix by pulse-vortexing, and spin down to avoid localized concentrations.
- Dilute all post-capture libraries to a fixed concentration (e.g., 5 or 10 nmol/L) based on the result of your quantification method.

- If the QIAseq Normalizer was used, all libraries have a concentration of approx. 4 nmol/L and do not have to be diluted any further before pooling. Quantifying the pool of normalized libraries is optional, but may be considered if using the QIAseq Normalizer for the first time.
- The final library pool has a global concentration equal to the single libraries (e.g., 4 nmol/L) and must be further diluted depending on the sequencing instrument and loading protocol.

The following examples provide guidance on how to calculate pooling volumes for heterogeneous post-capture pools. These are post-capture pools that contain a different number of libraries, pools that were hybridized with panels of different size, or both.

a. All libraries / capture pools are enriched with the same panel and all pools contain the same number of libraries.

- Solution:
 - Combine equal volumes of all post-capture libraries
- Example:
 - There are 3 post-capture libraries. Each pool was enriched with the same panel and each pool contains 8 libraries.
 - Each post-capture library was diluted to the same concentration or was normalized using the QIAseq Normalizer.
 - Combining equal volumes of the 3 post-capture pools (e.g., $3 \times 10 \mu\text{L}$) will result in each of the 24 libraries yielding a similar read count.

b. Libraries / capture pools are enriched with different panels.

- Solution:
 - Calculate a weighting factor to compensate for different target region sizes: Divide the target region size by the sum of all target region sizes.
 - For every post-capture library, multiply the desired pool volume with the weighting factor. Then, pool libraries asymmetrically.
- Example:
 - There are 3 post-capture libraries. Pools were enriched with different panels and each pool contains 8 libraries.
 - Each post-capture library was diluted to the same concentration or was normalized using the QIAseq Normalizer.
 - The final pool volume will be 25 µL.

	Target region size	Weighting factor	Calculation for weight	Volume to pool (µL)	Calculation for volume (µL)
Pool-A (Human Exome)	34 Mb	0.6667	$34/(34 + 14 + 3)$	~16.7	0.6667×25
Pool-B (Actionable Exome)	14 Mb	0.2745	$14/(34 + 14 + 3)$	~6.9	0.2745×25
Pool-C (a custom panel)	3 Mb	0.0588	$3/(34 + 14 + 3)$	~1.5	0.0588×25

c. All libraries / capture pools are enriched with the same panel, but pools have a different library count

- Solution:
 - Calculate the weighting factor to compensate for different library count per pool:
Divide the library count by the total library count.
 - For every post-capture library, multiply the desired pool volume with the weighting factor. Then, pool libraries asymmetrically.
- Example:
 - There are 3 post-capture libraries that were enriched with the same panel but contain a different number of libraries.
 - Each post-capture library was diluted to the same concentration or was normalized using the QIAseq Normalizer.
 - The final pool volume will be 25 µL.

	Number of libraries	Weighing factor	Calculation for weight	Volume to pool (µL)	Calculation for volume (µL)
Pool-A (8 libraries)	8	0.5	$8/(8 + 6 + 2)$	~12.5	0.5×25
Pool-B (6 libraries)	6	0.375	$6/(8 + 6 + 2)$	~9.4	0.375×25
Pool-C (2 libraries)	2	0.125	$2/(8 + 6 + 2)$	~3.1	0.125×25

d. **Capture pools are enriched with different panels and have a different library count.**

- Solution:
 - Calculate the target region size per post-capture pool by multiplying the library count with target region size.
 - Calculate the weighting factor on the per-pool target region size to compensate for different library count per pool and different target region sizes.
 - For every post-capture library, multiply the desired pool volume with the weighting factor. Then, pool libraries asymmetrically.
- Example:
 - There are 3 post-capture libraries. Pools were enriched with different panels and each pool contains a different number of libraries.
 - Each post-capture library was diluted to the same concentration or was normalized using the QIAseq Normalizer.
 - The final pool volume will be 25 µL.

	Number of libraries	Target region size	Libraries x size	Weighting factor	Calculation for weight	Volume to pool (µL)	Calculation for volume (µL)
Pool-A (Human Exome, 2 libraries)	2	34 Mb	68 Mb	0.425	$68 / (68 + 56 + 36)$	~10.6	0.425×25
Pool-B (Actionable Exome, 4 libraries)	4	14 Mb	56 Mb	0.35	$56 / (68 + 56 + 36)$	~8.8	0.35×25
Pool-C (a custom panel, 12 libraries)	12	3 Mb	36 Mb	0.225	$36 / (68 + 56 + 36)$	~5.6	0.225×25

Sequencing setup

The following guidelines outline the most important settings for Illumina and Element Biosciences instruments. For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific documents provided by the instrument manufacturer.

If using an Element Biosciences sequencing instrument, make use of Element’s Cloudbreak Freestyle chemistry, which allows direct loading of xHYB Pro libraries without library conversion. Also, xHYB Pro libraries that have been adjusted using QIAseq Normalizer are fully compatible with the Cloudbreak Freestyle chemistry.

Instrument	Illumina®	Element Biosciences
Read type	Paired-end	Paired-end
Index reads	2	2
Index read length for CDI	2 × 8	2 × 8
Read length (with CDI)	2 × 151	2 × 159
Index read length for UDI	2 × 10	2 × 10
Read length (with UDI)	2 × 149	2 × 157

Loading concentration guide

When preparing for sequencing runs, QIAseq xHYB Pro libraries should be treated as high-complexity libraries, behaving similarly to standard shotgun sequencing libraries. Experience across multiple platforms indicates that these libraries exhibit high stability and predictable clustering behavior. For optimal results, it is recommended to align the loading concentration closely with the manufacturer's routine specifications. QIAseq xHYB Pro libraries perform optimally using the standard operating procedures for high-complexity shotgun libraries provided by the respective manufacturers. Examples for typical loading concentrations are provided below.

Sequencing Platform	Reagent Kit / Flow Cell	Routine Loading Concentration (manufacturer)
Element Aviti	Cloudbreak / Freestyle	~10 pM
NextSeq 550	High Output (HO)	1.8 pM
NextSeq 550	Mid Output (MO)	1.5 pM
NextSeq 2000	P1, P2, P3	650 pM (On-board denaturation)
NovaSeq 6000	SP, S1, S2, S4	300–400 pM (Standard Workflow)
NovaSeq X	1.5B, 10B, 25B	~250 pM (Final concentration in 280 µL)
MiSeq i100	Standard	80–100 pM
MiSeq (Legacy)	v2 Reagents	8–10 pM
MiSeq (Legacy)	v3 Reagents	12–15 pM

Note: Actual loading concentrations may have to be optimized based on the quantification method used and individual preferences for cluster density.

Generation of sample sheets for Illumina instruments

Index sequences for QIAseq Unique Dual- Index Y- Adapters are available at www.qiagen.com/PROM-1790 for download.

The orientation in which the i5 index is sequenced varies between the different sequencing instruments. Depending on the instrument and the software used for run planning, index sequences have to be used in the forward or the reverse complement orientation. The i7 index is always sequenced in forward orientation.

Table 16. Orientation of i5 sequence reads on different sequencing instruments

Instruments reading i5 in forward orientation	Instruments reading i5 in reverse complement orientation
Illumina MiSeq	Illumina iSeq®100
Illumina HiSeq 2000/2500	Illumina MiniSeq
Element Biosciences Aviti	Illumina NextSeq 500/550
	Illumina NextSeq 1000/2000
	Illumina HiSeq 3000/4000
	Illumina NovaSeq 6000 with v1.5 reagents
	Illumina NovaSeq X / X Plus

How to demultiplex sequence data using QIAseq Y-Adapters:

Element Biosciences:

- Enter both the i7 and the i5 index in forward orientation to the run manifest.

Illumina:

- Use the i7 index in forward orientation for all instruments irrespective of the software.
- If using Illumina DRAGEN/BCL, convert with a manually created v2.0 sample sheet:
 - Use i5 forward orientation for NextSeq 1000/2000 and NovaSeq X / X Plus.
 - Use i5 reverse complement orientation for all other instruments.
- Regardless of the instrument, use the i5 index in forward orientation if:
 - BaseSpace Run Planning is used for run setup.
 - BaseSpace Prep Tab is used for run setup.
 - Illumina Experiment Manager is used for run setup.
 - Local Run Manager is used for run setup.
- For instruments reading i5 as reverse complement, use the reverse i5 orientation, if:
 - Manually creating a sample sheet for use with bcl2fastq.
 - Creating a sample sheet for manual run setup.
 - Creating a sample sheet or manifest for any software not mentioned above.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: (www.qiagen.com/FAQ/FAQList.aspx). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Issue	Comments and suggestions
Low post-capture library yields	
a. Suboptimal hybrid capture input quality or amount	Ensure using high-quality indexed whole genome libraries as input that have been quantified using a fluorometric method or capillary electrophoresis. Do not use PCR-free libraries as input. Choose a number of cycles for post-capture amplification that is appropriate for your panel size and hybrid capture input (See Table 7 on page 29 and Table 8 in “Post-capture amplification”).
b. Hybridization time too short	Capture pool input amounts smaller than 1000 ng in combination with some small custom panels may result in decreased library yields when using very short hybridization times (e.g., 5 min). Increase hybrid capture input where possible and increase the number of post-capture cycles by 2–3 relative to the recommended cycle number (See Table 7 on page 29 and Table 8 in “Post-capture amplification”).
c. Inappropriate amount of xHYB Pro Streptavidin Beads	Insufficient Streptavidin beads capture too few library fragments, whereas too many Streptavidin beads may negatively affect PCR amplification. Ensure that you use 50 µL of well-homogenized xHYB Pro Streptavidin Beads per reaction. Make sure to only amplify half of the final bead slurry per PCR.
d. Inappropriate hybridization or washing temperature	Hybridization capture performs optimally at a very narrow temperature range. Ensure that your thermal cycler and heating blocks are calibrated to accurately reach the intended temperature for the hybridization reaction and washing steps.
e. Incompatible adapters used	Using incompatible non-TruSeq-like adapters for the generation of whole genome libraries will result in failure to amplify captured library fragments. Only use QIAseq CDI or UDI Y-Adapters or other TruSeq-compatible adapters for the generation of indexed whole genome libraries.

Issue	Comments and suggestions
f. Wash Buffer carryover to post-capture PCR	Incomplete removal of xHYB Pro Wash Buffer 2 and/or omitting the final wash with Nuclease-Free Water may negatively affect post-capture amplification. Remove residues of xHYB Pro Wash Buffer 2 and perform a water wash to remove inhibiting substances before PCR.

Decreased target region specificity

a. Incorrect use of Blocking Oligos or Blocking Solution	Only use xHYB One-4-All Blocking Oligos and xHYB Pro Blocking Solution as outlined in the protocol. Make sure to use N-Blockers only for hybrid capture of RNA / whole transcriptome libraries, but not for DNA libraries.
b. Wrong library adapter type used	The xHYB One-4-All Blocking Oligos delivered with the kit are designed for Illumina TruSeq compatible adapters, but not for other adapter types. If using a library preparation method from another supplier, make sure to use compatible adapters, or obtain blocking reagents that are compatible with the adapters in use.
c. Suboptimal hybridization and washing temperature	Check the thermal cycler programs and make sure that that thermal cyclers and heating blocks are properly calibrated. For some heating blocks, it may be necessary to adjust the washing temperatures by 1°C or 2°C to match the optimal washing conditions. Use reaction tubes that match the shape of the heating block well. For example, do not use conical 1.5 mL tubes in a block for 2.0 mL rounded tubes, as this will cause suboptimal heat transfer.
d. Suboptimal hybridization and washing conditions	Follow the protocol for hybridization and washing steps. Take care not to confuse the usage order of Wash Buffers 1 and 2. Ascertain that Fast Hybridization Solution is heated to 65°C before use, and neither the Fast Hybridization Solution nor the Wash Buffers show precipitates when aliquoting.
e. Custom panel design with homologous off-targets	Depending on the targeted regions, some panels may tend to enrich more off-targets than others. This can be caused by pseudogenes or regions with highly similar sequence to the targeted region.

Unequal read representation of libraries within a hybrid capture pool

a. Unequal hybrid capture input	Use equal amounts of indexed whole genome libraries to be pooled for a hybrid capture reaction. Quantify indexed whole genome libraries before hybrid capture by using a capillary electrophoresis method such as the QIAxcel Advanced System or the Agilent 2100 Bioanalyzer using High Sensitivity DNA chips.
b. Suboptimal library enrichment conditions	Do not use PCR-free libraries as input to hybrid capture. Always follow the library enrichment protocol outlined in the respective library preparation protocol. Make sure that the input libraries are not overamplified. Quantify indexed whole genome libraries using the QIAGEN Library Quant Assay and assess library quality via capillary electrophoresis.

Appendix A: Fast library preparation from genomic DNA using QIAseq FX DNA Library UDI Kits

This protocol describes the generation of indexed whole genome libraries using the QIAseq FX DNA Library UDI Kits. For library preparation from degraded material, please follow the standard protocols in the *QIAseq FX DNA Library Handbook* (www.qiagen.com/HB-2015).

Important points before starting

- Before setting up the reaction, it is critical to accurately determine the amount of input DNA.
- Ensure input DNA is in water, 10 mM Tris, QIAGEN Buffer EB or low TE (0.1× TE, 0.1 mM EDTA). If input DNA is in 1× TE, please remove EDTA from the DNA by following the instructions in the *QIAseq FX DNA Library Handbook*.
- For the preparation of indexed whole genome libraries to be used for hybridization capture, we recommend using 50 ng of input DNA.

Things to do before starting

- Prepare fresh 80% ethanol.
- Thaw reagents on ice. Once reagents are thawed, mix buffers thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.
- Equilibrate Agencourt AMPure XP (Beckman Coulter®, Inc.) beads or QIAseq Beads to room temperature.

- Program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advance.

Procedure

1. Program a thermal cycler according to Table 17 below. Make sure that you use the instrument’s heated lid, and if possible, set the temperature of the heated lid to 70°C.

Table 17. Fragmentation, end-repair, and A-addition cycling conditions

Step	Incubation temperature (°C)	Incubation time
1	4	1 min
2	32	22 min*
3	65	30 min
4	4	Hold

* For 50 ng of input gDNA, a fragmentation time of 22 min typically results in an average library fragment size centered around 400 bp. To adjust the fragmentation to DNA, input amount, and quality, please refer to the *QIAseq FX DNA Library Handbook*, (www.qiagen.com/HB-2015).

2. Start the program. When the thermal cycler block reaches 4°C, pause the program.
3. Prepare the FX reaction mix in a PCR plate or tube on ice according to Table 18. Mix well by gently pipetting (do not vortex to mix).

Table 18. FX reaction mix setup (per sample)

Component	Volume (µL)
FX buffer, 10x	5
Purified DNA (50 ng)*	Variable
Nuclease-Free Water	Variable
Total without FX Enzyme Mix	40

* It is not recommended to have input amounts of below 20 ng as this may result in reduced library complexity.

4. Add 10 μL FX enzyme mix to each reaction and mix well by pipetting up and down 6–8 times.

Important: Keep the reactions on ice for the entire time during reaction setup.

5. Briefly spin down the PCR plate/tubes and immediately transfer to the prechilled thermal cycler (4°C). Resume the cycling program.
6. When the thermal cycler program is complete and the sample block has returned to 4°C , remove the samples and place them on ice. Immediately proceed with adapter ligation.
7. Vortex and spin down the thawed adapter plate. Then, pierce the foil seal for each adapter well to be used and transfer 2.5 μL from one DNA adapter well to each 50 μL sample of fragmented DNA of step 6. Track the barcodes from each adapter well used for each sample.

Important: Only 1 single adapter should be used per ligation reaction. Do not reuse adapter wells once the foil seal has been pierced.

8. Prepare the ligation Master Mix (per DNA sample) according to Table 19 in a separate PCR plate or tube on ice and mix well by pipetting.

Table 19. Ligation master mix setup (per sample)

Component	Volume (μL)
DNA Ligase Buffer, 5x	20
DNA ligase	10
Nuclease-Free Water	17.5
Total	47.5

9. Add 47.5 μL of the ligation master mix to each sample for a total of 100 μL and mix well by pipetting. Incubate the ligation reaction at 20°C for 15 min.

Important: Do not use a thermal cycler with a heated lid.

10. Once the ligation has completed, add 50 µL of resuspended Agencourt AMPure XP beads or QIAseq Beads to each ligated sample and mix well by pipetting.
11. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) for 2 min, then carefully discard the supernatant.
12. Wash the beads by adding 200 µL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
13. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Overdrying of beads may result in lower DNA recovery. Remove from the magnetic stand.
 - a. Overdrying of Ampure XP beads may result in lower DNA recovery.
 - b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Over drying QIAseq Beads will not affect the DNA elution.
14. Elute by resuspending in 26 µL Buffer EB or 10 mM Tris-Cl, pH 8.0. Pellet the beads and transfer 23.5 µL supernatant to a new PCR tube/plate.

Note: If not proceeding immediately, the sample can be stored at –30°C to –15°C.

15. Program a thermal cycler with a heated lid according to Table 20 below.

Table 20. Library amplification cycling conditions

Time	Temperature (°C)	Number of cycles
2 min	98	1
20 s	98	6 (100 ng input DNA)
30 s	60	7 (50 ng input DNA)
30 s	72	8 (20 ng input DNA)
1 min	72	1
∞	4	Hold

Note: 6–8 amplification cycles are recommended based on the input DNA amount and quality.

16. Prepare a reaction mix on ice according to Table 21 below. Mix the components in a PCR tube or 96-well PCR plate.

Table 21. Reaction mix for library amplification

Component	Volume (μL)
HiFi PCR Master Mix, 2×	25
Primer Mix Illumina Libr. Amp (10 μM each)	1.5
Library DNA (eluate of step 14)	23.5
Total reaction volume	50

17. Transfer the PCR tube or plate to the thermal cycler and start the program.
18. Once PCR is complete, add 50 μL of resuspended Agencourt AMPure XP Beads or 60 μL of resuspended QIAseq Beads to each reaction (50 μL), and pipette up and down thoroughly to mix.
19. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
20. Wash the beads by adding 200 μL of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
21. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Overdrying of beads may result in lower DNA recovery. Remove from the magnetic stand.
- a. Overdrying of AmpureXP beads may result in lower DNA recovery.
 - b. If using QIAseq Beads, ensure that the pellet is completely dry by visual inspection. Overdrying QIAseq Beads will not affect the DNA elution.
22. Elute by resuspending in 25 μL of Buffer EB or 10 mM Tris-Cl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 23 μL of the supernatant into a new tube.

23. Determine the library concentration using a colorimetric method such as Qubit HS DNA assay. Alternatively, assess the quality and quantity of the library using a capillary electrophoresis device such as the QIAxcel or Agilent Bioanalyzer.
24. The purified library can be safely stored at -30°C to -15°C in a DNA LoBind tube until ready to use for sequencing or other applications.

Appendix B: Post-capture normalization using QIAseq Library Normalizer Kits

To normalize QIAseq xHYB Pro post-capture libraries, QIAseq Library Normalizer Kits must be purchased separately. Alternatively, the components included in QIAseq Universal Normalizer Kits can also be used. For detailed information refer to the *QIAseq Normalizer Kit Handbook* (www.qiagen.com/HB-3361).

QIAseq Library Normalizer Kit		(96)	(24)
Catalog no.		180605	180603
Number of reactions		96	24
QIAseq Normalizer Reagent Kit		Cap color	
Normalizer Reagent	black	1 × 580 µL	1 × 135 µL
Normalizer Wash Buffer	white	4 × 14 mL	1 × 14 mL
Normalizer Elution Buffer	white	2 × 1.9 mL	1 × 1.9 mL
RNase-Free Water	clear	1 × 1.9 mL	1 × 1.9 mL
QIAseq Normalizer Primer Kit		Cap color	
Normalizer Primer Mix	orange	1 × 165 µL	1 × 40 µL

Note: The QIAseq Normalizer Primer Mix is required during post-capture amplification of the xHYB Pro workflow.

Note: All reagents from the Normalizer Reagent Kit are required after the final post-capture libraries have been generated.

The following protocol describes the normalization of purified post-capture libraries that have been amplified in presence of the Normalizer Primer Mix. Using non-modified libraries will lead to normalization failure. QIAseq Normalization will yield double-stranded libraries at a

concentration of approx. 4 nmol/L. Normalized libraries can be pooled for sequencing at equal volumes without further quantification.

Things to do before starting

- Be sure xHYB Pro post-capture libraries have been amplified using the Normalizer Primer Mix in step 47 of the main protocol.
- For normalization use libraries that consistently reach concentrations of at least 15–20 nmol/L or more.
- Thoroughly mix the Normalizer Reagent by vortexing for 60 seconds. Make sure the pellet of beads is fully dissolved and the solution is well homogenized.
- Homogenize the bottle(s) of Normalizer Wash Buffer by vortexing and preheat the whole reagent bottle(s) to 55°C in a water bath. Alternatively, use a heating block for 1.5/2.0 mL tubes or Falcon tubes. Fill the homogenized wash buffer into compatible receptacles to preheat. Prepare 450 µL wash buffer (400 µL are required) per normalization reaction.
- Preheat a heating block for 1.5 mL tubes to 55°C.
- It is not required to equilibrate the Normalizer Reagent and Normalizer Elution Buffer to room temperature before use.

Procedure

1. Homogenize the bottle(s) of Normalizer Wash Buffer by vortexing and preheat the whole reagent bottle(s) to 55°C in a water bath. Alternatively, use a heating block for 1.5 or 2.0 mL tubes or Falcon tubes. Fill the homogenized wash buffer into compatible receptacles to preheat. Prepare 450 µL wash buffer (400 µL are required) per normalization reaction. Leave the wash buffer at 55°C until use.
2. Thoroughly mix the Normalizer Reagent by vortexing for 60 s. Make sure that the pellet of beads are fully dissolved and the solution is well homogenized.

3. Directly before use, pulse-spin the Normalizer Reagent at low force to collect all liquid at the bottom. Then use a 200 μ L pipette to homogenize the reagent. Make sure to disperse a sediment of beads that may have formed during pulse-spin.
4. For every library to be normalized, pipet 5 μ L of homogenized Normalizer Reagent to a 1.5 mL tube. Leave the tubes at room temperature.
5. Add 15 μ L of the purified post-capture library (from step 55 of the main protocol) to be normalized to the 1.5 mL tube containing Normalizer Reagent. Mix well by pipetting or vortexing.
6. Incubate for 10 min at room temperature.
7. Add 200 μ L pre-warmed Normalizer Wash Buffer (55°C) to each tube.
8. Pellet the beads on a magnetic stand for 2 min and wait until the solution is clear.
Important: Make sure that all beads have pelletized. If you are uncertain, leave the tubes on the magnet for 5 min.
9. Carefully discard the supernatant without disturbing the pellet.
10. With the tube on the magnet add 200 μ L pre-warmed Normalizer Wash Buffer (55°C) to each pellet.
11. Switch the tube position on the magnet to wash the beads. Then wait until all beads have pelletized. Alternatively, turn the tube by 180° to force the beads to opposite side of the tube.
12. Carefully discard the supernatant without disturbing the pellet. Remove as much remaining liquid as possible, then proceed to the next step immediately.
Note: Drying the beads is not required.
13. Add 26 μ L Normalizer Elution Buffer to each pellet and mix well by vortexing. Make sure the pellet is completely dissolved.

14. Tap the tube on the benchtop to collect the liquid at the bottom. If there is liquid remaining in the lid, pulse spin the tube at low force. Do not spin to form a compact bead pellet.
15. Incubate for 5 minutes at 55°C in a heating block.
16. Pulse-spin to collect all liquid at the bottom, then pellet the beads on a magnetic stand for 2 min and wait until the solution is clear.
17. Carefully transfer 25 µL of the supernatant to a new tube.

Important: Do not discard the supernatant. The supernatant contains the ready-to-sequence library.
18. Pool and sequence the libraries. Alternatively, the normalized libraries can be stored at -20°C for up to 3 months.

Ordering Information

QIAseq xHYB Pro Capture Kits are compatible with the QIAseq xHYB Panels and with indexed libraries prepared using the library preparation kits listed below.

QIAseq xHYB Panels contain hybrid capture probe sets only. Library preparation and hybrid capture reagents must be purchased separately.

QIAseq xHYB Pro Capture Kits

Product name	Contents	Cat. no.
QIAseq xHYB Pro Capture Kit (24)	Kit containing reagents for hybrid capture–based target enrichment for short-read human sequencing; includes 3 reactions for up to 24 samples	334142
QIAseq xHYB Pro Capture Kit (96)	Kit containing reagents for hybrid capture–based target enrichment for short-read human sequencing; includes 12 reactions for up to 96 samples	334145
QIAseq xHYB Pro Capture Frame	Reusable plate frame for xHYB Pro Hybridization Reagent tubes.	334141

QIAseq xHYB Panels

Product name	Contents	Cat. no.
QIAseq xHYB CGP DNA Panel (24)	xHYB probe panel for DNA target enrichment for comprehensive genomic profiling, fixed panel for 24 samples	333122
QIAseq xHYB CGP DNA Panel (96)	xHYB probe panel for DNA target enrichment for comprehensive genomic profiling, fixed panel for 96 samples	333125
QIAseq xHYB CGP RNA Panel (24)	xHYB probe panel for RNA target enrichment for comprehensive genomic profiling, fixed panel for 24 samples	334122
QIAseq xHYB CGP RNA Panel (96)	xHYB probe panel for RNA target enrichment for comprehensive genomic profiling, fixed panel for 96 samples	334125

Product name	Contents	Cat. no.
QIAseq xHYB CGP DNA/RNA Panels (24)	xHYB probe panels for DNA and RNA target enrichment for comprehensive genomic profiling, fixed panel for 24 samples	335122
QIAseq xHYB CGP DNA + HRD Panel (96)	xHYB probe panels for DNA target enrichment for comprehensive genomic and HRD profiling, fixed panel for 96 samples	335132
QIAseq xHYB CGP DNA + HRD Panel (24)	xHYB probe panels for DNA target enrichment for comprehensive genomic and HRD profiling, fixed panel for 24 samples	335135
QIAseq Human Exome (24)	xHYB probe panel for DNA target enrichment for Exome sequencing, fixed panel for 24 samples	333934
QIAseq Human Exome (96)	xHYB probe panel for DNA target enrichment for Exome sequencing, fixed panel for 24 samples	333938
QIAseq xHYB Actionable Exome Panel (24)	xHYB probe panel for DNA target enrichment for a focused, actionable Exome sequencing, fixed panel for 24 samples	334392
QIAseq xHYB Actionable Exome Panel (96)	xHYB probe panel for DNA target enrichment for a focused, actionable Exome sequencing, fixed panel for 96 samples	334395

Library Preparation and Index Kits

Product name	Contents	Cat. no.
QIAseq FX DNA Library UDI (A/B/C/D) Kit (96)	Buffers and reagents for DNA fragmentation, end repair, A-addition, ligation, and amplification; includes a plate containing 96 unique dual index (UDI) adapters	180479 / 180480 / 180481 / 180482
QIAseq FX DNA Library UDI Kit (24)	Buffers and reagents for DNA fragmentation, end repair, A-addition, ligation, and amplification; includes a plate containing 24 UDI adapters	180477
QIAseq Ultralow Input Library UDI (A/B/C/D) Kit (96)	Buffers and reagents for ultralow-input DNA library preparation including end polishing, ligation, and high-fidelity amplification; includes 96 UDI adapters	180497 / 180498 / 180499 / 180500
QIAseq Multimodal DNA/RNA Library Kit (24)	Reagents (excluding indices) for preparation of DNA and RNA libraries from a single sample, for 24 samples	334842

Product name	Contents	Cat. no.
QIAseq Multimodal DNA/RNA Library Kit (96)	Reagents (excluding indices) for preparation of DNA and RNA libraries from a single sample, for 96 samples	334845
QIAseq Multimodal DNA/RNA UDI (24)	DNA and RNA index adapters for multimodal library preparation workflows	334852
QIAseq Multimodal DNA/RNA UDI (96)	DNA and RNA index adapters for multimodal library preparation workflows	334855
QIAseq Multimodal DNA Kit – Mechanical Fragmentation (24)	Buffers and enzymes for DNA library preparation from pre-fragmented DNA	335022
QIAseq Multimodal DNA Kit – Mechanical Fragmentation (96)	Buffers and enzymes for DNA library preparation from pre-fragmented DNA	335025
QIAseq Multimodal DNA Kit – Enzymatic Fragmentation (24)	Buffers and enzymes for DNA library preparation with enzymatic fragmentation	334962
QIAseq Multimodal DNA Kit – Enzymatic Fragmentation (96)	Buffers and enzymes for DNA library preparation with enzymatic fragmentation	334965
QIAseq Multimodal RNA Kit (24)	Buffers and enzymes for RNA library preparation for whole-transcriptome sequencing	335032
QIAseq Multimodal RNA Kit (96)	Buffers and enzymes for RNA library preparation for whole-transcriptome sequencing	335035

Quality Control Products

Product name	Contents	Cat. no.
QIAseq Library Normalizer Kit	Reagents for normalization of NGS libraries prior to pooling	1129599
QIAseq Library Quant Assay Kit	qPCR-based assay for accurate quantification of sequencing libraries including primers, standards, and reagents	333314

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
09/2026	Initial release.

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