April 2026

QIAseq® xHYB HRD Panel Enrichment Handbook

For hybridization-capture–based enrichment to study homologous recombination deficiency (HRD) from FFPE DNA samples

Sample to Insight

Table of Contents

Kit Contents

QIAseq xHYB HRD Panel and Reagent Kit

QIAseq xHYB HRD Panel only includes the HRD-specific probe set(s) needed for hybridization capture. The QIAseq xHYB Human Reagent Kit contains all reagents required for hybrid capture-based target enrichment and must be purchased in addition to QIAseq xHYB HRD Panel.

QIAseq xHYB HRD Panel		
Catalog no.	**334135**	**334132**
No. of hybridization reactions	**12**	**3**
Typical no. of samples	**96**	**24**
xHYB HRD Panel for DNA target enrichment for homologous recombination deficiency (HRD) assessment	48 µL	12 µL

QIAseq xHYB Human Reagent Kit Catalog no. No. of hybridization reactions Typical no. of samples	333195 12 96	333430 3 24
Box 1 of 2		
One-4-All Blocking Oligos	96 µL	24 µL
One-4-All Blocking Solution	60 µL	15 µL
Fast Hybridization Solution	240 µL	60 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
RNase-free Water	1.5 mL	1.5 mL
Box 2 of 2		
Streptavidin Binding Beads	1.2 mL	300 µL
Post Capture Binding Buffer	9.6 mL	2 × 1.2 mL
Wash Buffer A	5.4 mL	2 × 675 µL
Wash Buffer B	8.4 mL	2 × 1.05 mL

Shipping and Storage

QIAseq xHYB HRD Panel only contains hybrid capture probes, which are shipped on dry ice. Store immediately upon receipt at –30°C to –15°C in a constant-temperature freezer.

QIAseq xHYB Human Reagent Kit consists of 2 boxes. Box 1 is shipped on dry ice. Box 2 is shipped on a cool pack (4°C). Store Box 1 immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. Store Box 2 immediately upon receipt at 2–8°C.

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

Intended Use

QIAseq xHYB HRD Panel and QIAseq xHYB Human Reagent Kit are intended for molecular biology applications. 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 format 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 Human Reagent Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAseq xHYB HRD Panel provides a comprehensive assessment of homologous recombination deficiency (HRD) by interrogating genomic instability signatures using a genome-wide SNP backbone and 98 HRR-related genes, including BRCA1 and BRCA2.

The genomic instability signatures reflect DNA repair defects associated with homologous recombination deficiency (HRD), including:

- **Loss of heterozygosity (LOH)**: Regions where one parental allele is lost due to deletions, mitotic recombination, or gene conversion, representing widespread allelic imbalance typical of HRR deficient tumors
- **Large scale transitions (LST)**: Chromosomal breakpoints between adjacent regions ≥10 Mb, separated by ≤3Mb, indicating structural disorganization caused by failure in accurate double strand break repair
- **Telomeric allelic imbalance (TAI)**: Imbalance extending to the telomere without crossing the centromere, arising from replication stress and faulty resolution of stalled or collapsed replication forks

Together, these orthogonal signatures form a genomic scar profile associated with HRR loss and BRCA1/2 deficiency.

Principle of the procedure

The workflow starts with whole genome library preparation using either The QIAseq FX DNA Library Kit or the QIAseq Multimodal DNA/RNA Library Kit (Table 1). Other whole genome sequencing (WGS) library preparation kits are also compatible, as long as the libraries are prepared without modifications with biotin.

Hybrid capture is then performed using the QIAseq xHYB Human Reagent Kit (Table 2), in combination with the QIAseq xHYB HRD Panel (Table 3). During hybridization, biotinylated xHYB probes selectively enrich genome-wide SNPs, HRR-related genes and MSI loci. Streptavidin magnetic bead pull-down isolates targeted fragments, which are then amplified and purified. Final libraries undergo quality control and are sequenced to generate reads with high-depth coverage suitable for HRD scoring and variant detection.

The resulting FASTQ files are uploaded to the QIAGEN CLC Genomics Workbench for filtering, read mapping and variant calling and GIS scoring. GIS scoring is performed using a proprietary algorithm licensed from Myriad Genetics and implemented within QIAGEN CLC Genomics Workbench.

The outputs from CLC GW are uploaded to QIAGEN Clinical Insight – Interpret (QCI-I), enabling a variant-filtering cascade that facilitates prioritizing variants for evidence-based interpretation for research use only.

Note: The QIAseq FX DNA Library Kit is recommended for standard FFPE HRD workflow. For combined QIAseq xHYB CGP DNA + HRD analysis, the QIAseq Multimodal DNA Library Kit is recommended to enable UMI based detection of low VAF variants.

Table 1. Recommended kits for whole genome library preparation prior to hybrid capture

QIAseq Library Kit	Application
QIAseq FX DNA Library Kits*	• Standard library preparation from FFPE DNA • Enzymatic fragmentation of genomic and formalin-compromised DNA • Recommended for routine HRD analysis without UMIs
QIAseq Multimodal DNA Kits	• Library preparation from FFPE DNA with UMIs • Recommended when combining the QIAseq xHYB HRD Panel with the QIAseq xHYB CGP DNA Panel • UMIs support improved detection of low-variant allele frequency (VAF) variants

* Follow the dedicated QIAseq FX protocol in this handbook.

† Follow QIAseq Multimodal DNA-EnzFrag Kit Supplementary Protocol on **www.qiagen.com/QIAseqMultimodalDNA-RNALibKit**

Table 2. QIAseq xHYB Human Reagent Kit

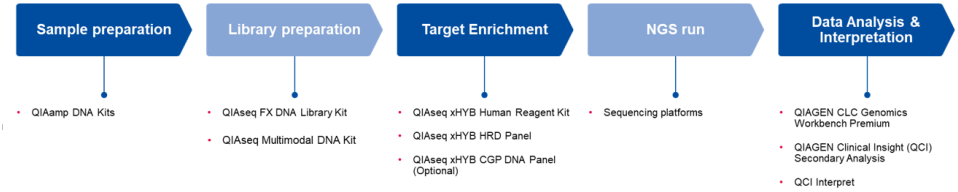
Cat. no. (24/96)	Product name	Panel size	Kit type
333430/333195	QIAseq xHYB Human Reagent Kit	–	Reagent Kit*

* The QIAseq xHYB Human Reagent Kit contains all reagent necessary for hybridization capture and postcapture library amplification for use with QIAseq xHYB HRD Panel.

Table 3. QIAseq xHYB HRD Panels

Cat. no. (24/96)	Product name	Panel size
334132/334135	QIAseq xHYB HRD Panel	1 Mb

Sample to Insight workflow for QIAseq xHYB HRD Panel



Equipment and Reagents to be Supplied by User

- QIAseq Beads (cat. nos. 333923, 333903, 333927) or Agencourt® AMPure® XP Beads (cat. no. A63880, A63881) for bead-based library purification
- 100% ethanol (ACS grade)
- Nuclease-free water
- Buffer EB (cat. no. 19086)
- PCR tubes or plates
- Pipette tips and pipettes
- DNA LoBind tubes (from Axygen or Eppendorf)
- Vortexer
- Microcentrifuge
- Magnetic racks for magnetic beads separation (e.g., Thermo Fisher Scientific DynaMag™ Magnet)
- Thermal cycler with heated lid
- Capillary electrophoresis device, e.g., QIAGEN QIAxcel®, Agilent® Bioanalyzer®, or similar to evaluate the DNA fragmentation profile (optional)
- 2 heating blocks for 1.5–2.0 mL tubes
- An evaporation device (e.g., Eppendorf Concentrator Plus, cat. no. 305000100) equipped with a rotor for 1.5 mL tube and/or a rotor for 0.2 mL PCR tubes/plates.

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 used in this protocol is in good working condition and has 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 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 Advanced System or the Agilent 2100 Bioanalyzer using High Sensitivity DNA chips.
- A typical indexed whole genome library (QIAseq FX DNA Library Kit) has an average size distribution centered around 400 bp and is free of adapter dimers at a typical yield of >500 ng (Figure 1).
- Libraries from physically sheared DNA (QIAseq Ultralow Input Kit) will reflect the size distribution of the sheared input DNA.

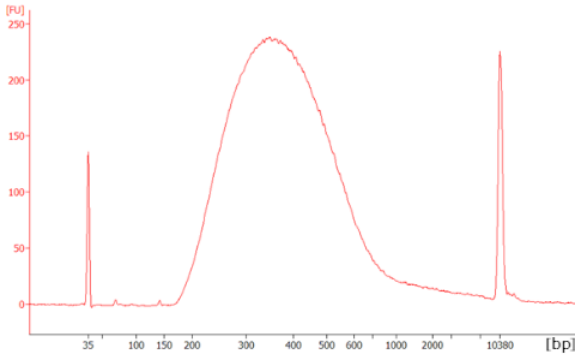


Figure 1. Capillary electrophoresis device traces ofa typical hybridization capture input library. QIAseq FX DNA Library with a typical mean size distribution of 350–450 bp that is free of adapter dimers.

Indexing recommendations

- Use QIAseq UDI Y-Adapters to generate whole genome libraries for hybridization capture.
- Use different sample indexes for all samples within a hybridization capture pool.
- Consider using different sample indexes also across hybridization capture pools to allow postcapture multiplexing depending on your sequencing instrument.

Description of protocols

The "Protocol: QIAseq FX DNA Library Kit for Use with the QIAseq xHYB HRD Panel" describes the generation of whole genome libraries using an optimized workflow for the QIAseq FX DNA Library Kit. To use the QIAseq Multimodal DNA Kit, see **www.qiagen.com/HB-3703**

The "Protocol: Target Enrichment from Indexed Whole-Genome Libraries Using QIAseq xHYB HRD Panel" describes the hybridization capture workflow for QIAseq xHYB HRD Panel.

Protocol: QIAseq FX DNA Library Kit for Use with the QIAseq xHYB HRD Panel

This protocol describes the generation of indexed whole genome libraries using the QIAseq FX DNA Library Kit.

Fragmentation, end repair, and A-addition

This protocol describes the FX reaction for single-tube fragmentation, end repair, and A-addition.

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 protocol in Appendix: Removal of Divalent Cations and EDTA from Input Nucleic Acid.
- 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 brief vortexing to avoid any localized concentration gradients. Briefly spin down the reagents before use.
- Program the thermal cyclers. For increased speed and convenience, all incubation steps can be preprogrammed and saved in advance.

Procedure

1. Program a thermal cycler according to Table 4. Be sure to use the instrument's heated lid, and if possible, set the temperature of the heated lid to 70°C.

Table 4. 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 5. We recommend using 50 ng of high quality input DNA. Mix well by gently pipetting (do not vortex).

Table 5. 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**

* Input amounts of below 20 ng may result in reduced library complexity and are therefore not recommended.

4. Add 10 µL FX enzyme mix to each reaction and mix well by pipetting up and down 6–8 times. Keep the reactions on ice throughout the 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 samples and place them on ice.
7. Immediately proceed with adapter ligation as described in the next protocol.

Adapter ligation

This protocol describes adapter ligation. Make sure to use QIAseq UDI or CDI Y-Adapters, or other TruSeq-compatible adapters that are fully compatible with QIAGEN One-4-All Blocking Oligos for hybridization capture. If adapters from another supplier are used, follow the manufacturer's instructions. QIAseq Universal Blockers are designed to work with TruSeq-compatible dual-indexed adapters with up to 12 bp index motives.

Things to do before starting

- Equilibrate Agencourt AMPure XP beads or QIAseq Beads to room temperature (15–25°C) for 20–30 min before use.
- Vortex and spin down the thawed adapter plate before use.

Procedure

8. Remove the protective adapter plate lid, pierce the foil seal for each adapter well to be used, and transfer 5 µL from one DNA adapter well to each 50 µL sample from the previous protocol. Track the barcodes from each adapter well used for each sample.
9. Replace the adapter plate lid and freeze unused adapters. The adapter plate is stable for a minimum of 10 freeze–thaw cycles.

   Important: Use only a single adapter per ligation reaction. Do not reuse adapter wells once the foil seal has been pierced.
10. Prepare the ligation master mix (per DNA sample) according to Table 6 in a separate PCR plate or tube on ice and mix well by pipetting.

Table 6. Ligation master mix setup (per sample)

Component	Volume (µL)
DNA Ligase Buffer, 5×	20
DNA ligase	10
Nuclease-free water	15
Total	**45**

11. Add 45 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.

12. Proceed immediately to adapter ligation cleanup using 0.8× (80 µL) Agencourt AMPure XP beads or QIAseq Beads.

13. Add 80 µL of resuspended Agencourt AMPure XP beads or QIAseq Beads to each ligated sample and mix well by pipetting.

14. 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.

15. 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.

16. 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.

17. Elute by resuspending in 52 µL Buffer EB or 10 mM TrisCl, pH 8.0. Pellet the beads and transfer 50 µL supernatant to a new tube.
18. Add 1.0× (50 µL) of resuspended Agencourt AMPure XP beads or 1.1× (55 µL) of QIAseq Beads and mix well by pipetting.
19. Perform steps 14–16, and then elute by resuspending in 26 µL Buffer EB or 10 mM TrisCl, pH 8.0.
20. Pellet the beads and carefully transfer 23.5 µL to a new PCR tube. If not proceeding immediately, the sample can be stored at –30°C to –15°C.

Amplification of library DNA

PCR-based library amplification is always required prior to hybridization capture. This protocol is for high-fidelity amplification of the DNA library using the amplification reagents provided in the QIAseq FX DNA Library Kit.

Things to do before starting

- Thaw QIAseq HiFi PCR Master Mix and Primer Mix on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations.
- Always start with the cycling conditions specified in this protocol. The cycling has been optimized for use with QIAseq HiFi PCR Master Mix for even and high-fidelity amplification of sequencing libraries.

Procedure

21. Program a thermal cycler with a heated lid according to Table 7.

Table 7. 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.

22. Prepare a reaction mix on ice according to Table 8. Mix the components in a PCR tube or 96-well PCR plate.

Table 8. Reaction mix for library enrichment

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

23. Transfer the PCR tube or plate to the thermal cycler and start the program.

24. 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.

25. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.

26. 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.

27. 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.

28. Elute by resuspending in 25 µL of Buffer EB or 10 mM TrisCl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 23 µL of the supernatant into a new tube.

29. Assess the quality and quantity of the library using a capillary electrophoresis device such as QIAGEN QIAxcel or Agilent Bioanalyzer. Check for the expected size distribution (see Figure 1).

    Note: The library should show a distribution centered around approximately 350 bp.

30. 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.

Protocol: Target Enrichment from Indexed Whole-Genome Libraries Using QIAseq xHYB HRD Panel

Pool libraries and dry down the indexed library pool

Pooling of amplified, indexed whole genome libraries enables processing multiple libraries in a single hybridization-capture reaction. We recommend pooling of up to 8 indexed libraries for one hybridization capture reaction. Alternatively, hybrid-capture may be performed for a single library. The amount of indexed libraries to be pooled depends on the number of samples per pool. Make sure to only use PCR-amplified libraries for hybridization capture.

Things to do before starting

- Generate indexed whole genome libraries using the QIAseq FX DNA Library protocol or any other compatible library preparation workflow (see guidelines in "Introduction" and Table 1).
- Quantify the amplified, indexed whole genome libraries (see guidelines in "Important Notes" and Figure 1).
- Thaw the xHYB Probe Set, All-4-One Blocking Oligos, and All-4-One Blocking Solution on ice, and then pulse vortex and pulse-spin.
- To proceed immediately to hybridization capture after library pool dry-down, equilibrate the Vapor-Lock reagent to room temperature and heat the Fast Hybridization Solution to 65°C before pool evaporation is complete. Alternatively, the dried library pool can be stored at –30°C to –15°C for up to 7 days.

Procedure

1. For each capture pool, determine the per-library amount (ng) according to the desired pool size referring to the guidelines in Table 9 or follow the recommendations in Table 10. Make sure to not exceed 3200 ng total input per capture pool.

Table 9. Guidelines for pooling indexed libraries for hybridization capture

Library pooling guidelines	
Recommended number of libraries per capture pool	8
Maximum total input per capture pool	3200 ng
Minimal input per library	200 ng

Table 10. 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	200–400 *

* Do not exceed a total DNA input of 3200 ng per pool.

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.

2. Calculate the volume (µL) of each amplified library needed for pooling, e.g., for a library concentration of 25 ng/µL, 16 µL are needed to add 400 ng of indexed library to the capture pool.

3. Transfer the calculated volumes for each library to a 0.2 mL PCR tube/strip/plate. If the total library pool volume exceeds 180 µL, use a1.5 mL LoBind tube. Use multiple tubes/wells if performing multiple hybridization reactions at once.

   Recommendation: If the volume allows, pool libraries in a 0.2 mL PCR tube/strip or plate.

4. Add the xHYB HRD Probe Set and blocking reagents to each library pool according to Table 11.

Table 11. Complement the library pool with probes and blocking reagents

Component	Volume (µL)
Pool of indexed libraries (from step 3)	Variable
QIAseq xHYB HRD Panel	4
One-4-All Blocking Oligos	8
One-4-All Blocking Solution	5

5. Completely evaporate all liquid content of the complemented library pool by using a SpeedVac system (or a similar evaporator device) equipped with an appropriate rotor for tubes and/or 96-well plates. If needed, accelerate the evaporation of larger volumes by setting the temperature to 60°C.

   Alternative: Evaporate all liquid content by placing the opened tube/plate in a heating block or thermal cycler with the lid opened. Evaporate at 60°C over night or until all liquid has evaporated.

6. When evaporation of the complemented library pool is complete, store the dried library pool on ice and proceed immediately to step 7 (Hybridization capture protocol). Alternatively, the dried library pool can be stored at –30 to –15°C for up to 7 days.

Hybridization capture

During the hybridization step, QIAseq xHYB HRD Probes bind specifically to targeted library fragments present in the pool. The flexible hybridization protocol allows adjusting the duration of hybridization from as little as 30 min to 4 h. Incubating for 1 h or longer can improve the capture performance. Incubating for longer than 4 h or overnight will not enhance performance significantly, but may be considered for highest flexibility in experiment planning.

Things to do before starting

- Thaw the Vapor-Lock reagent and equilibrate to room temperature.
- Heat the Fast Hybridization Solution to 65°C in a heating block for at least 10 min and make sure all precipitate is dissolved.
- When performing the hybridization capture reaction for 1 h or less, start preparing reagents needed for binding the hybridized targets to Streptavidin binding beads as outlined at the beginning of the next chapter.

Procedure

7. Thaw and equilibrate the Vapor-Lock reagent to room temperature. Heat the Fast Hybridization Solution to 65°C for 10 min in a heating block. Vortex and make sure all precipitate are dissolved. Keep the Fast Hybridization Solution at 65°C until used.
8. Program a thermal cycler with a heated lid according to Protocol: Target Enrichment from Indexed Whole-Genome Libraries Using QIAseq xHYB HRD Panel.

   Important: The heated lid must be set to 85°C to prevent evaporation.

Table 12. Conditions for hybridization capture

Step	Incubation temperature (°C)	Incubation time
1	95	1 min
2	95	5 min
3	60	30 min–4 h*
4	60	Hold†

* Hybridization times of less than 30 min may result in reduced target region coverage. Hybridization time may be extended to overnight incubation without negative effects on specificity or uniformity.

† Program the thermal cycler to maintain 60°C after hybridization is complete. Do not allow the hybridization reaction to cool down as this negatively affects off-target rate.

9. Start the program. When the thermal cycler block reaches 95°C, pause the program in step 1.
10. Vortex the 65°C Fast Hybridization Solution and immediately transfer 20 µL to the tube/well containing the dried complemented library pool from step 6.

    Important: Fast Hybridization Solution is viscous. Pipette slowly to ensure accurate volume transfer. For optimal volume transfer, set the pipette to 20 µL; then, slowly aspirate and dispense back into the reagent tube twice, and with the same pipette tip, slowly aspirate and transfer the entire volume to the reaction tube.
11. If the dried library pool was in a **0.2 mL PCR tube/plate**, proceed to **step 15**.

    If the dried library pool was in a **1.5 mL LoBind tube**, continue with **step 12**.
12. Vortex the tube for 5 s, and then incubate for 2 min at 65°C.
13. Repeat step 12 for two more times, and then pulse-spin the tube.

    Note: A white precipitate may form. This does not affect the hybridization procedure.

14. Transfer the complete solution to a 0.2 mL PCR tube/plate and pulse-spin. Ensure that there are no bubbles present.

15. Add 30 µL of Vapor-Lock reagent. Pulse-spin the tube to ensure all liquid is at the bottom of the tube.

    Note: Vapor-Lock will form a phase on top of the hybridization reaction after pulse-spin. This does not affect the hybridization procedure.

16. Transfer the tube/plate to the thermal cycler and continue the program at 95°C.

    Important: Make sure the heated lid is set to 85°C and the tube is tightly sealed with fresh lids/foil. Make sure to use the correct lid spacers for your thermal cycler.

17. Leave the hybridization reaction in the thermal cycler until ready for binding of captured library fragments to Streptavidin binding beads. Continue with the protocol "Bind hybridized targets to Streptavidin binding beads" at least 30 min before hybridization is complete.

    Alternatively, the hybridization reaction may remain in the thermal cycler over night to continue with binding the hybridized targets to Streptavidin binding beads the next day.

    Important: Do not allow the hybridization reaction to cool to less than 60°C after the program is complete.

Bind hybridized targets to Streptavidin binding beads

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

Things to do before starting

- Preheat a heating block for 1.5 mL tubes to 68°C.
- Preheat a heating block for 1.5 mL tubes to 48°C.
- Heat the whole reagent bottles of the Wash Buffer A and Wash Buffer B to 48°C and mix thoroughly. Leave both reagent bottles at 48°C until needed.
- Heat the whole reagent bottle of the Post Capture Binding Buffer to 48°C and mix thoroughly. Then leave the reagent bottle at room temperature until needed.
- Equilibrate Streptavidin Binding Beads to room temperature for at least 30 min.

Procedure

18. Per reaction, aliquot 450 µL Wash Buffer A and preheat to 68°C.
19. Per reaction, aliquot 700 µL Wash Buffer B and preheat to 48°C.

    Important: For steps 18 and 19, do not aliquot chilled buffers. Heat the entire reagent bottles of Wash Buffer A and Wash Buffer B to 48°C and mix thoroughly. Then, prepare aliquots.

    Note: Leave the wash buffer aliquots in the heating blocks until needed.
20. Homogenize the pre-equilibrated Streptavidin binding beads by vortexing.

21. Transfer 100 µL homogenized Streptavidin binding beads to a new 1.5 mL LoBind tube. Prepare 1 tube for each hybridization reaction.

22. Add 200 µL Post Capture Binding Buffer and mix by pipetting.

    Important: Do not use chilled Post Capture Binding Buffer. Make sure to heat the buffer to 48°C before use and mix well. Then allow the buffer to cool to room temperature.

23. Incubate the beads on a magnetic stand for 1 min or until the solution is clear, and then remove and discard the clear supernatant without disturbing the pellet. Remove from the magnetic stand.

24. Repeat steps 22 and 23 for two more times for a total of three washing steps.

25. After removing the supernatant from the third wash, add a final 200 µL Post Capture Binding Buffer and homogenize the beads by vortexing. Keep the homogenized bead slurry at room temperature until needed.

26. After the hybridization reaction is complete, open the lid of the thermal cycler, open the tube, and swiftly transfer the complete volume including Vapor-Lock into the tube containing the bead slurry from step 25. Mix well by pipetting.

    Important: Do not allow the hybridization reaction to cool to less than 60°C before transferring to the Streptavidin binding beads. Rapid transfer directly from the thermal cycler is essential for minimizing the off-target rate.

27. Incubate the mixture at room temperature for 30 min on a shaker, rotator, or similar device to prevent the beads from settling.

    Alternatively, incubate the mixture at room temperature for 30 min, while agitating in 5 min intervals to prevent beads from settling.

    Note: Aggressive mixing is not required. Do not vortex.

28. Pulse-spin the tube to ensure all liquid is at the bottom of the tube.

29. Incubate the beads on a magnetic stand for 1 min or until the solution is clear, and then remove and discard the clear supernatant including Vapor-Lock without disturbing the pellet.

    Note: Small amounts of Vapor-Lock may remain after removal of supernatant and throughout each wash step. This does not affect the final capture product.

30. Remove the tube from the magnetic stand, and add 200 µL of preheated 68°C Wash Buffer A. Mix by pipetting.

31. Incubate for 5 min at 68°C.

32. Place the tube on a magnetic stand for 1 min or until the solution is clear, and then remove and discard the clear supernatant without disturbing the pellet.

    Remove the tube from the magnetic stand, and add another 200 µL of preheated 68°C Wash Buffer A. Mix by pipetting.

33. Incubate for 5 min at 68°C.

34. Pulse-spin the tube and transfer the entire volume, including the beads to a new 1.5 mL LoBind tube.

35. Place the tube on a magnetic stand for 1 min or until the solution is clear, and then remove and discard the clear supernatant without disturbing the pellet.

36. Remove the tube from the magnetic stand, and add 200 µL of preheated 48°C Wash Buffer B. Mix by pipetting.

37. Pulse-spin the tube, and then incubate for 5 min at 48°C.

38. Place the tube on a magnetic stand for 1 min or until the solution is clear, and then remove and discard the clear supernatant without disturbing the pellet.

39. Repeat steps 36 to 38 for two more times for a total of three washes.

40. After the final wash, use a 10 µL pipette to remove all residual supernatant. Proceed immediately to the next step.

    Important: Do not allow the beads to dry.

41. Remove the tube from the magnetic stand, and add 45 µL RNase-free water. Homogenize by pipetting, and store the bead slurry on ice.

42. Proceed directly to "Postcapture amplification".

    Alternatively, aliquot the bead slurry into two 22.5 µL aliquots and store at –30°C to –15°C for later use.

    Important: Do not discard the beads. The capture product is still bound to the Streptavidin binding beads.

Postcapture amplification

Captured targets bound to Streptavidin binding 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.

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 42).
- Equilibrate Agencourt AMPure XP or QIAseq Beads to room temperature for 20–30 min before use.
- 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. Briefly spin down vortexed reagents before use.

Procedure

43. Program a thermocycler with a heated lid according to Table 13 and Table 14.

Table 13. Postcapture amplification conditions

Time	Temperature (°C)	Number of cycles
2 min	98	1
20 s	98	10–12 cycles*
30 s	60	
30 s	72	
1 min	72	1
∞	4	Hold

* See Table 14 for the optimal cycle numbers depending on library input.

Table 14. Recommended number of postcapture amplification cycles

xHYB Panel	<500 ng capture input (cycles)	500 ng – 2.0 µg capture input (cycles)	>2.0 µg capture input (cycles)
HRD Panel	12	11	10

44. Prepare the reaction mix on ice according to Table 15. Mix the components in a PCR tube or 96-well PCR plate.

Table 15. Reaction mix for postcapture amplification

Component	Volume (µL)
Bead slurry with captured targets (from step 41)	22.5
Post Hybrid-Capture PCR Mix	25
Primer Mix Illumina Library Amplification	2.5
Total reaction volume	**50**

45. Transfer the PCR tube or plate to the thermal cycler and start the program.

46. Once PCR is complete, add 75 µL of resuspended Agencourt AMPure XP Beads or QIAseq Beads to each reaction (50 µL) and pipette up and down thoroughly to mix.

47. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand, and carefully discard the supernatant.

48. 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.

49. 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 AMPure XP 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.

50. Elute by resuspending in 32 µL of Buffer EB, 10 mM TrisCl, pH 8.0, or RNase-free water. Incubate at room temperature for 2 min.

51. Pellet the beads on the magnetic stand. Carefully transfer 30 µL of the clear supernatant into a new tube.

52. Assess the quality and size distribution of the library using a capillary electrophoresis device such as QIAGEN QIAxcel Advanced or Agilent Bioanalyzer 2100. Check for the expected size distribution (see Figure 2). For best sequencing results, quantitate the captured library using a qPCR assay such as the QIAseq Library Quant Assay Kit or compatible method.

    Note: The library should show a distribution similar to the whole genome libraries used for the respective capture pool.

53. The purified library can be safely stored at –30°C to –15°C in a DNA LoBind tube until ready to use for sequencing.

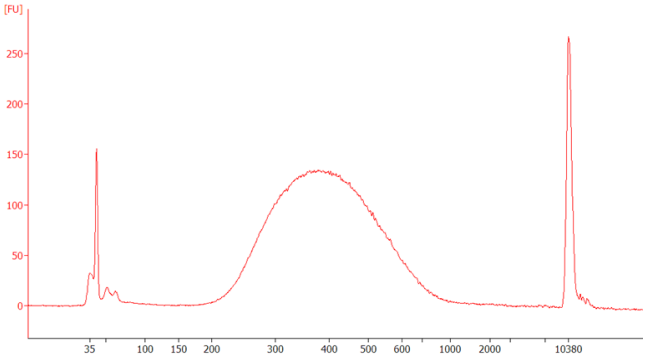


Figure 2. Electrophoresis trace data of a final hybrid capture library pool.

Sequencing Guidelines

Pooling and multiplexing guidelines

Depending on the sequencing instrument used, combining multiple hybridization capture pools may be advisable for cost efficient sequencing. Before combining equal volumes of hybrid capture pools, quantify each pool and dilute to a defined concentration (e.g., 5 nM). 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, target region size captured using QIAseq xHYB HRD Panel Protocol and the desired coverage per sample. Please refer to Table 16 for initial multiplexing guidelines per sequencing run to achieve a minimal average target region coverage of 200×. Multiplexing and loading may need to be adjusted based on your local conditions like the quantification method used and your preferences for target cluster density.

QIAseq xHYB HRD Panel achieves a very high coverage uniformity throughout the target region with a typical Fold-80 base penalty in the range of 1.3–1.5 or better. This means that the average target region coverage needs to be increased by approximately 1.4 to raise 80% of non-zero-coverage target bases to the mean coverage level. When planning the sequencing pool, consider approximately 1.3- to 1.5-fold of the desired minimal coverage to reliably obtain that coverage throughout the target region.

Table 16. Multiplexing guidelines for QIAseq xHYB HRD Panel for Illumina instruments and Element AVITI sequencer using 2 × 150 bp paired-end sequencing to achieve a minimal average target coverage of 200× from FFPE DNA samples

	1 Mb	
Sequencing instrument	**Paired end reads**	**Max. libraries* per flow cell**
NextSeq® 500/550 (Mid Output)	260 M	13
NextSeq 500/550 (High Output)	800 M	40
NextSeq 1000/2000 (P1)	200 M	10
NextSeq 1000/2000 (P2)	800 M	40
NextSeq 2000 (P3)	2400 M	120
NextSeq 2000 (P4)	3600 M	180
NovaSeq® 6000 (SP)	1500 M	75
NovaSeq 6000 (S1)	3000 M	150
NovaSeq 6000 (S2)	8000 M	400
NovaSeq 6000 (S4)	18000 M	900
NovaSeq X Series (1.5B)	3200 M	160
NovaSeq X Series (10B)	20000 M	1000
NovaSeq X Series (25B)	52000 M	1536†
Element AVITI (High Output)	2000 M	100

Note: Read requirements may be higher than indicated depending on input DNA quality.

* 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.

† Plexity is limited by the number of available Unique dual indexed adapters.

Sequencing

- Always ensure that libraries have been quantified using QIAseq Library Quant Assay or a compatible method to enable equal library representation within the sequencing pool and exact pool concentrations for optimal flow cell loading and best sequencing performance.
- For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific Illumina documents.
- Depending on the adapter type used for library preparation, please refer to the respective QIAseq Library handbook for detailed information on QIAseq Combinatorial and Unique Dual-Index Adapters.

Generation of sample sheets for Illumina instruments

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

The orientation in which the Illumina 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 17. Orientation of i5 sequence reads for all Illumina instruments

Instruments reading i5 in forward orientation	Instruments reading i5 in reverse complement orientation
MiSeq	iSeq®100
HiSeq 2000/2500	MiniSeq
NovaSeq 6000 with v1.0 reagents	NextSeq 500/550
	NextSeq 1000/2000
	HiSeq 3000/4000
	NovaSeq 6000 with v1.5 reagents
	NovaSeq X / X Plus

How to demultiplex sequence data using QIAseq Y-Adapters

- Use the i7 index in forward orientation for all instruments irrespective of the software.
- If using 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.

Sequencing setup on Illumina instruments

The following guidelines outline the most important settings for Illumina instruments. More detailed instructions on how to configure a run and how to create a sample sheet can be found in the "Product Resources" section for QIAseq Y-Adapters at **www.qiagen.com**.

- Read Type: **Paired End**
- Index Reads: **2**
- Enable Adapter Trimming
- Cycles for QIAseq CDI Y-Adapters
  - Read 1 and Read 2: **151**
  - Index 1 and Index 2: **8**
- Cycles for QIAseq UDI Y-Adapters
  - Read 1 and Read 2: **149**
  - Index 1 and Index 2: **10**

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**).

	Comments and suggestions
Low exome library yields	
a. Suboptimal hybrid capture input quality or amount	Make sure to use high-quality indexed whole genome libraries as input that have been quantified using a fluorometric method or capillary electrophoresis. Do not use PCR free whole genome libraries. Choose a number of cycles for postcapture amplification that is appropriate for your panel size and hybrid capture input (Table 14).
b. Hybridization time too short	Hybrid capture input amounts smaller than 1000 ng may result in decreased library yields when using hybridization times of 30 min or less. Increase hybrid capture input, hybridize for 1 h or longer and use eight cycles of postcapture amplification.
c. Inappropriate amount of Streptavidin binding beads	Insufficient Exome library yield may be a result of deficient postcapture amplification. Streptavidin Binding beads may inhibit postcapture amplification. Ensure to use no more than 100 µL of Streptavidin binding beads per hybridization capture reaction. 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 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.

	Comments and suggestions
Decreased target region specificity	
a. Incorrect use of Blocking Oligos or Blocking Solution	Only use QIAseq One-4-All Blocking Oligos and One-4-All Blocking Solution as outlined in the protocol.
b. Wrong library adapter type used	One-4-All Blockers delivered with the kit are designed for Illumina TruSeq compatible adapters, but not for other adapter types. We recommend to only use QIAseq Unique or Combinatorial Dual-index Adapters included in QIAseq Library Kits.
c. Suboptimal hybridization and washing temperature	Check thermal cycler programs and make sure 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.
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 A and B. 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.
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 whole genome libraries. Always follow the library enrichment protocol outlined in the respective library preparation protocol using QIAGENs HiFi PCR Master Mix for library enrichment. Make sure input libraries are not overamplified. Quantify indexed whole genome libraries using the QIAGEN Library Quant Assay and assess library quality via capillary electrophoresis.

References

1. Gregg, A. R., et al. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*. 23, 1793–1806.

Appendix: Removal of Divalent Cations and EDTA from Input Nucleic Acid

Refer to manufacturer's protocol for details on methods of purification.

1. If DNA is in a volume of less than 50 µL, adjust the volume to 50 µL with nuclease-free water.
2. Add 90 µL of resuspended Agencourt AMPure XP beads to the reaction for a ratio of 1.8x and mix well by pipetting. If DNA is in a volume greater than 50 µL, scale the volume of Agencourt AMPure XP beads appropriately such that the ratio of beads to DNA is 1.8x.
3. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand for 2–4 min and carefully discard the supernatant without disturbing the beads.
4. Wash the beads with 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.
5. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Remove from the magnetic stand.
6. Elute by resuspending in 45 µL of QIAGEN's Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand for 2 min. Carefully transfer 42.5 µL of supernatant into a new tube.
7. Determine the concentration of the purified DNA using Qubit™, PicoGreen®, or another fluorometric method.

Ordering Information

Product	Contents	Cat. no.
QIAseq xHYB HRD Panel (24)	xHYB probe panel for DNA target enrichment for homologous recombination deficiency (HRD) test , fixed panel for 24 samples	334132
QIAseq xHYB HRD Panel (96)	xHYB probe panel for DNA target enrichment for homologous recombination deficiency (HRD) test , fixed panel for 96 samples	334135
QIAseq xHYB Human Reagent Kit (24)	The QIAseq xHYB Human Reagent Kit contains all reagents required for hybrid capture-based target enrichment; contains 3 hybrid capture reactions, which are typically sufficient for 24 samples.	333430
QIAseq xHYB Human Reagent Kit (96)	The QIAseq xHYB Human Reagent Kit contains all reagents required for hybrid capture-based target enrichment; contains 12 hybrid capture reactions, which are typically sufficient for 96 samples.	333195
QIAseq Multimodal DNA Kit-EnzFrag (24)	QIAseq Multimodal DNA Kit-EnzFrag (24) contains reagents (except indices) necessary to process 24 samples for WG sequencing (24 WGS libraries) using enzymatic fragmentation.	335022
QIAseq Multimodal DNA Kit-EnzFrag (96)	QIAseq Multimodal DNA Kit-EnzFrag (96) contains reagents (except indices) necessary to process 96 samples for WG sequencing (96 WGS libraries) using enzymatic fragmentation.	335025
QIAseq Multimodal DNA UDI (24)	QIAseq Multimodal DNA UDI (24) contains reagents and DNA indices necessary to process 24 samples for WG sequencing - total of 24 reactions (24 sets of DNA UDIs).	334972
QIAseq Multimodal DNA UDI Set A (96)	QIAseq Multimodal DNA UDI Set A (96) contains reagents and DNA indices necessary to process 96 samples for WG sequencing - total of 96 reactions (96 sets of DNA UDIs).	334975

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.

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Document Revision History

Date	Changes
April 2026	Initial release.

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