April 2022

# QIAseq® xHYB Human Hybrid Capture Panel Handbook

For hybridization capture–based enrichment from human whole genome NGS libraries for Illumina® instruments

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **QIAseq xHYB Human Hybrid Capture Panels** | **(24)** | **(96)** |
|---|---|---|
| **Catalog no.** | | |
| **QIAseq xHYB Actionable Exome Panel** | **333372** | **333375** |
| **QIAseq xHYB Carrier Panel** | **333362** | **333365** |
| **No. of hybridization reactions** | **3** | **12** |
| **Typical no. of samples** | **24** | **96** |
| **Box 1 of 2** | | |
| xHYB panel-specific Probe Set | 12 µl | 48 µl |
| One-4-All Blocking Oligos | 24 µl | 96 µl |
| One-4-All Blocking Solution | 15 µl | 60 µl |
| Fast Hybridization Solution | 60 µl | 240 µl |
| Vapor-Lock | 500 µl | 500 µl |
| Post Hybrid-Capture PCR Mix (2x) | 660 µl | 660 µl |
| Primer Mix Illumina Library Amplification 12 rxn | 20 µl | 3 x 20 µl |
| RNase-free Water | 1.5 ml | 1.5 ml |
| **Box 2 of 2** | | |
| Streptavidin Binding Beads | 300 µl | 1.2 ml |
| Post Capture Binding Buffer | 2 x 1.2 ml | 9.6 ml |
| Wash Buffer A | 2 x 675 µl | 5.4 ml |
| Wash Buffer B | 2 x 1.05 ml | 8.4 ml |

| **QIAseq xHYB Spike-in Panel** | **(24)** | **(96)** |
|---|---|---|
| **Catalog no.** | | |
| **QIAseq xHYB Mitochondrial Panel** | **333382** | **333385** |
| **No. of reactions** | **3** | **12** |
| xHYB Spike-in Probe Set | 12 µl | 48 µl |

| **QIAseq xHYB Automation Kit** | |
|---|---|
| **Catalog no.** | **333430** |
| Streptavidin Binding Beads | 300 µl |
| Post Capture Binding Buffer | 2 x 1.2 ml |
| Wash Buffer A | 2 x 675 µl |
| Wash Buffer B | 2 x 1.05 ml |

# Shipping and Storage

QIAseq xHYB panels consist 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 to –15°C in a constant-temperature freezer. Store Box 2 immediately upon receipt at 2–8°C.

QIAseq xHYB Spike-in Panels are shipped on dry ice. Store spike-in panels immediately upon receipt at –30 to –15°C in a constant-temperature freezer. The QIAseq xHYB Automation Kit is shipped on a cool pack (4°C). Store the automation kit 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 Panels 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's ISO-certified Quality Management System, each lot of QIAseq xHYB Human DNA Panels is tested against predetermined specifications to ensure consistent product quality.

# Introduction

QIAseq xHYB Panels make use of QIAGEN's fast and powerful hybridization capture technology to enable sample-to-insight, targeted next-generation sequencing of DNA. The optimized QIAseq Human Exome Kit and QIAseq xHYB solutions facilitate highly uniform enrichment of target sequences from indexed whole-genome libraries and allow comprehensive variant detection with minimal sequencing effort. The flexible xHYB workflow allows simultaneous hybridization from up to 8 indexed libraries in as little as 30 minutes. QIAseq xHYB enrichment is fully compatible with upstream library preparation methods such as the QIAseq FX DNA Library or the QIAseq Ultralow Input Library workflow. This enables library preparation from blood, cells, tissue, and biofluids, and thus allows hybrid capture-based target enrichment from a broad variety of sample types, including high-quality gDNA, cfDNA, formalin-compromised DNA, and physically sheared DNA. Highly efficient target capture mediated by double-stranded DNA probes and the xHYB fast hybridization chemistry require as little as 20 ng of input DNA to yield highly complex libraries. Depending on the DNA input type, we recommend using the library preparation kits listed in Table 1.

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

| QIAseq Library Kit | Application |
|---|---|
| QIAseq FX DNA Library Kits* | Enzymatic fragmentation of genomic DNA<br>Enzymatic fragmentation of formalin-compromised DNA |
| QIAseq Ultralow Input Library Kits† | Physical shearing of genomic DNA<br>Physical shearing of formalin-compromised DNA<br>Library preparation from cfDNA |

* Follow the dedicated QIAseq FX protocol in this handbook.

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

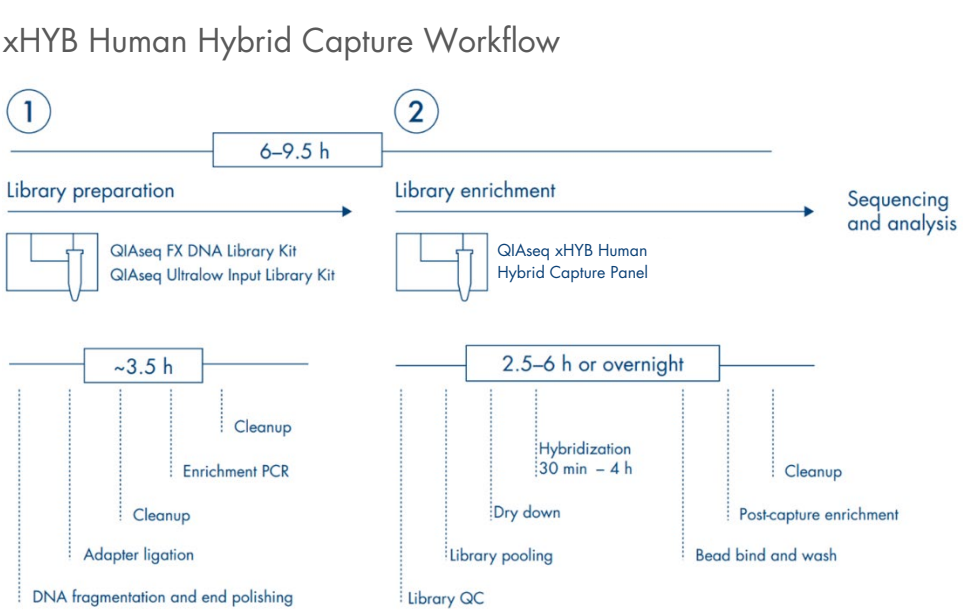



**Figure 1. Workflow principle of the QIAseq xHYB Human Hybrid Capture Panels.** Step 1: Generation of indexed libraries. Step 2: Enrichment of xHYB targets.

### Step 1: Generation of indexed libraries

Whole genome libraries are prepared in the first step prior to hybrid capture enrichment. Libraries can be prepared from a variety of DNA analytes including gDNA or formalin-compromised DNA. The QIAseq FX DNA Library Kits feature a single-tube reaction for enzymatic DNA fragmentation, end-repair, and adapter ligation. Alternatively, the QIAseq Ultralow Input Kit can be used to process physically sheared DNA or small amounts of cfDNA that are compatible for exome enrichment. Both library kits include QIAseq Unique Dual-Index (UDI) or Combinatorial Dual-Index (CDI) Adapters that are fully compatible for use with QIAseq xHYB Human Hybrid Capture Panel workflows and allow multiplexing of up to 384 samples per sequencing run. Please refer to the respective library kit handbook for more information on UDI and CDI Y-Adapters.

Step 2: Enrichment of human xHYB targets

During xHYB enrichment, indexed library fragments are bound to biotinylated double-stranded DNA capture probes with highly flexible hybridization times ranging from 30 minutes to overnight incubation. Bound fragments are immobilized on streptavidin beads and non-targeted fragments are washed away. Enriched library fragments are amplified using a proprietary postcapture amplification mix that allows even amplification of DNA regions with vastly different GC contents, which minimizes sequencing bias caused by PCR.

## QIAseq One-4-All Blocking Oligos

QIAseq xHYB Human Hybrid Capture Panels include One-4-All Blocking Oligos designed to effectively bind and block all Illumina TruSeq®-compatible NGS adapters with index sequence length of up to 12nt. All QIAseq Dual-Index Y-Adapters (Unique and Combinatorial) are fully compatible with One-4-All Blocking Oligos for hybridization capture. Using these blocking oligos, the target capture specificity is largely enhanced by preventing capture of library fragments via their adapter sequences during the hybridization reaction.

## xHYB Human Hybrid Capture Panels

Hybridization capture is a powerful tool to capture DNA targets by sequence-specific interaction between probes and their target molecules. All xHYB Human Hybrid Capture probe sets consist of 5'-biotinylated double-stranded 120 nt DNA probes designed to enrich targeted library molecules specifically and uniformly. xHYB panels are designed based on the hg38 genome build and use GENCODE annotations for the definition of transcripts and coding sequences. Target region definition files for all human xHYB panels are available in BED format for download at **www.qiagen.com/qiaseq-xhyb-human-hybrid-capture-panels**. A summary of all available xHYB Human Hybrid Capture Panels and accessory products is shown in Table 2.

**Table 2. xHYB Human Hybrid Capture Panels and accessory kits**

| Cat. no. (24/96) | Product name | Panel size | Kit type |
|---|---|---|---|
| 333372/333375 | QIAseq xHYB Actionable Exome Panel | 12.3 Mb | Complete kit* |
| 333362/333365 | QIAseq xHYB Carrier Panel | 1.4 Mb | Complete kit* |
| 333382/333385 | QIAseq xHYB Mitochondrial Panel | 16.6 Kb | Spike-in panel† |
| 333430 | QIAseq xHYB Automation Kit | – | Accessory‡ |

* Complete kits include the xHYB probe set and all reagents needed for hybridization capture and postcapture library amplification.

† Spike-in panels only include a probe set for use in conjunction with a complete kit.

‡ The accessory automation kit for xHYB panels contains additional wash buffers and reagents for automated hybridization workflows where larger reagent volumes are required.

## xHYB Actionable Exome Panel

As of today, there are more than 6,000 monogenic disorders with a known molecular basis linked to far more than 4,000 genes (www.omim.org). In addition, an even greater number of genes are involved in complex diseases or phenotypes with strong genetic heterogeneity, or have been revealed as susceptibility factors or genetic modifiers for well-known phenotypes. The Human Gene Mutation Database (HGMD; **www.digitalinsights.qiagen.com/products-overview/clinical-insights-portfolio/human-gene-mutation-database**) currently lists far more than 13,000 disease-related genes — 9,000 of which are clearly associated or likely associated with an inherited disease. Targeting the coding sequence of all 13,000 genes would result in a relatively large target region and does not enable high target coverage at reasonable sequencing cost. In contrast, smaller clinical exome panels only focusing on (~4,500) genes with clear association to monogenic disease will save sequencing cost, but will fail to detect a large number of disease-related variants.

The QIAseq xHYB Actionable Exome Panel combines comprehensive detection of disease-causing and disease-associated variants with the compact target region design of a classical clinical exome panel. The xHYB panel targets all (100%) disease-causing and likely disease-causing variants listed at HGMD (>300,000 variants) including variants in regulatory regions and splice sites, as well as deep intronic variants. Due to its proprietary bimodal design, the

target region size is reduced to 12.3 Mb, while still targeting variants in more than 10,000 genes. More than 4,300 genes comprise a high number of known disease-causing variants and will be enriched entirely. This includes the whole coding sequence and 10 bp splice regions. Regulatory and deep intronic regions are added to include disease-relevant HGMD loci. Genes with a low number of disease-related variants are enriched partially, but selectively to include known disease-relevant variant loci in exons, splice regions, introns, and regulatory regions. Please obtain more information on panel content, bimodal design, and gene lists at **www.qiagen.com/qiaseq-xhyb-human-hybrid-capture-panels**.

## xHYB Carrier Screening Panel

Rare diseases with an incidence of 1 in 2000 births or less affect hundreds of million people worldwide. Around 80% of rare diseases have a genetic origin. While dominant diseases are passed on from generation to generation with a 50% chance, parents of individuals with a recessively inherited condition are typically unaffected carriers. Carrier screening was first conducted selectively for conditions with a high prevalence in specific ethnicities, for example, sickle cell disease in Black individuals or Tay-Sachs disease in the Ashkenazi Jewish. After Cystic Fibrosis and Spinal Muscular Atrophy were recommended for ancestry-independent screening, modern high throughput sequencing technologies enabled the inclusion of a growing number of genes causing autosomal recessive or X-linked conditions into carrier screening tests.

The QIAseq xHYB Carrier Screening Panel is a hybrid-capture based solution for comprehensive carrier screening including 448 genes in total. The QIAGEN xHYB technology and its compact target region design with a size of only 1.4 Mb enable highly uniform target coverage – also on smaller benchtop sequencing instruments. The content design strongly adheres to the latest recommendations for screening autosomal recessive and X-linked conditions given by the American College of Medical Genetics and Genomics (ACMG) (1). Therefore, the xHYB Carrier Screening Panel includes all 113 genes recommended for an unbiased ethnicity-neutral screening approach. Beyond that, the target region comprises a broad range of additional genes for extended carrier screening including genes linked to conditions with low average carrier frequencies of 1 in 200 or less.

# Equipment and Reagents to be Supplied by User

- 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)
- Thermocycler 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 the work area and pipettes with an RNase- and DNA-cleaning product.
- For consistent hybridization capture and amplification, ensure the thermocycler 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 2A).
- A typical indexed cfDNA library (QIAseq Ultralow Input Library Kit) 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 (QIAseq Ultralow Input Kit) will reflect the size distribution of the sheared input DNA.

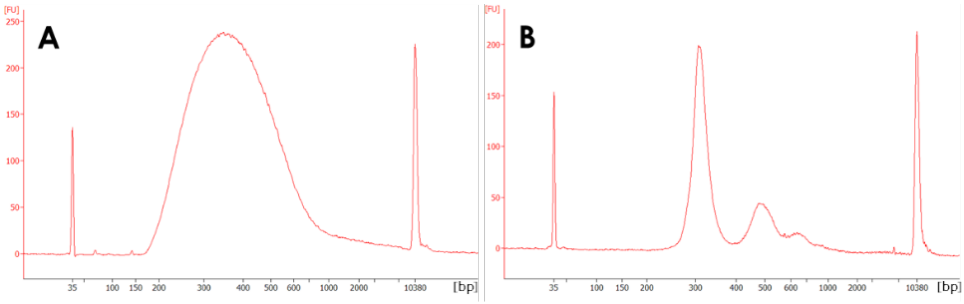



**Figure 2. Capillary electrophoresis device traces of different hybridization capture input libraries. A**: QIAseq FX DNA Library with a typical mean size distribution of 350–450 bp that is free of adapter dimers. **B**: A typical cfDNA library with the major library peak at 300 bp and additional peaks representing the N2 and N3 fractions. An example electrophoresis trace of the final postcapture library pool is shown at the end of the hybrid capture workflow protocol.

## Indexing recommendations

- Use QIAseq UDI Y-Adapters or QIAseq CDI Y-Adapters to generate whole genome libraries for hybridization capture.
- When using non-QIAseq Y-Adapters, make sure the adapters are 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 postcapture multiplexing depending on your sequencing instrument.

## Description of protocols

The QIAseq FX DNA Library Kit for use with QIAseq xHYB Human Hybrid Capture protocols (page 17) describes the generation of whole genome libraries using an optimized workflow for the QIAseq FX DNA Library Kit. To use the QIAseq Ultralow Input Library Kit, please refer to the respective handbook at **www.qiagen.com**.

The QIAseq xHYB Human Hybrid Capture Panel protocol (page 23) describes the hybridization capture workflow for any human xHYB panel and is suitable for input libraries generated using QIAseq FX DNA Library Kits, QIAseq Ultralow Input Library Kits, or alternative methods.

# Protocol: QIAseq FX DNA Library Kit for Use with xHYB Human Hybrid Capture Panels

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's Buffer EB or low TE (0.1x TE, 0.1 mM EDTA). If input DNA is in 1x TE, please remove EDTA from the DNA by following the protocol in Appendix A, page 42.
- 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.
- Program thermocyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advance.

## Procedure

1. Program a thermocycler according to Table 3. Be sure to use the instrument's heated lid, and if possible, set the temperature of the heated lid to 70°C.

**Table 3. Fragmentation, end-repair, and A-addition cycling conditions**

| Step | Incubation temperature | Incubation time |
|---|---|---|
| 1 | 4°C | 1 min |
| 2 | 32°C | 22 min* |
| 3 | 65°C | 30 min |
| 4 | 4°C | 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 thermocycler block reaches 4°C, pause the program.
3. Prepare the FX reaction mix in a PCR plate or tube on ice according to Table 4. We recommend using 50 ng of high quality input DNA. Mix well by gently pipetting (do not vortex to mix).

**Table 4. 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. It is critical to 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 thermocycler (4°C). Resume the cycling program.

6. When the thermocycler 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's 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 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:** Only 1 single adapter should be used 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 5 in a separate PCR plate or tube on ice and mix well by pipetting.

**Table 5. Ligation master mix setup (per sample)**

| Component | Volume (µl) |
|---|---|
| DNA Ligase Buffer, 5x | 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 thermocycler with a heated lid.

12. Proceed immediately to adapter ligation cleanup using 0.8x (80 µl) Agencourt AMPure XP beads.
13. Add 80 µl of resuspended Agencourt AMPure XP 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, and 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.
17. Elute by resuspending in 52 µl Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads and transfer 50 µl supernatant to a new tube.
18. Add 1.0x (50 µl) of resuspended Agencourt AMPure XP beads and mix well by pipetting.
19. Perform steps 14–16, and then elute by resuspending in 26 µl Buffer EB or 10 mM Tris·Cl, 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 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 thermocycler with a heated lid according to Table 6.

**Table 6. Library amplification cycling conditions**

| Time | Temperature | Number of cycles |
|---|---|---|
| 2 min | 98°C | 1 |
| 20 s | 98°C | 6 (100 ng input DNA) |
| 30 s | 60°C | 7 (50 ng input DNA) |
| 30 s | 72°C | 8 (20 ng input DNA) |
| 1 min | 72°C | 1 |
| ∞ | 4°C | 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 7. Mix the components in a PCR tube or 96-well PCR plate.

**Table 7. 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 thermocycler and start the program.
24. Once PCR is complete, add 50 µl of resuspended Agencourt AMPure XP Beads to each reaction (50 µl) and pipet 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.
28. 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.
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 2A).

    **Note**: The library should show a distribution centered around approximately 350 bp.
30. The purified library can be safely stored at –30 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 Human Hybrid Capture Panels

## 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 on page 17 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 2).
- 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 immediately proceed 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 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 8 or follow the recommendations in Table 9. Make sure to not exceed 3200 ng total input per capture pool.

**Table 8. 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 9. Recommended library pooling strategy for hybridization capture**

| Number of indexed samples per pool | Amount of each indexed library per pool |
|---|---|
| 1 | 200–500 ng |
| 2 | 200–500 ng |
| 3 | 200–500 ng |
| 4 | 200–500 ng |
| 6 | 200–500 ng |
| 8 | 200–400 ng* |

* 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 Probe Set and blocking reagents to each library pool according to Table 10.

**Table 10. Complement the library pool with probes and blocking reagents**

| Component | Volume (µl) |
|---|---|
| Pool of indexed libraries (from step 3) | Variable |
| xHYB Probe Set | 4 |
| Diluted xHYB Mitochondrial Spike-in Probe Set (optional)* | 4 |
| One-4-All Blocking Oligos | 8 |
| One-4-All Blocking Solution | 5 |

* Dilute the Mitochondrial Spike-in Probe Set 1:10 in water before use and add 4 µl of the diluted probe set to the library pool.

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 tube 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 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 is dissolved. Keep the Fast Hybridization Solution at 65°C until used.
8. Program a thermocycler with a heated lid according to Table 11.

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

**Table 11. Conditions for hybridization capture**

| Step | Incubation temperature | Incubation time |
|---|---|---|
| 1 | 95°C | 1 min |
| 2 | 95°C | 5 min |
| 3 | 60°C | 30 min–4 h* |
| 4 | 60°C | 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 thermocycler 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 thermocycler 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. Pipet slowly to ensure accurate volume transfer. For optimal volume transfer, set the pipet 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 2 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 thermocycler 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 thermocycler.

17. Leave the hybridization reaction in the thermocycler until ready for binding of captured library fragments to Streptavidin beads. Continue with the protocol "Bind Hybridized Targets to Streptavidin Beads" at least 30 min before hybridization is complete.

    Alternatively, the hybridization reaction may remain in the thermocycler over night to continue with binding the hybridized targets to Streptavidin 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 beads

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 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 70°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 70°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 2 more times for a total of 3 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 thermocycler, 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 thermocycler 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 70°C Wash Buffer A. Mix by pipetting.
31. Incubate for 5 min at 70°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 70°C Wash Buffer A. Mix by pipetting.

33. Incubate for 5 min at 70°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 2 more times for a total of 3 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 to –15°C for later use.

    **Important:** Do not discard the beads. The capture product is still bound to the Streptavidin 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 to –15°C, thaw a 22.5 µl aliquot of the Streptavidin Binding Bead slurry on ice (from step 42).
- Equilibrate Agencourt AMPure XP 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 Tables 12 and 13.

**Table 12. Postcapture amplification conditions**

| Time | Temperature | Number of cycles |
|---|---|---|
| 2 min | 98°C | 1 |
| 20 s | 98°C | |
| 30 s | 60°C | 7–13 cycles* |
| 30 s | 72°C | |
| 1 min | 72°C | 1 |
| ∞ | 4°C | Hold |

* See Table 13 for the optimal cycle numbers depending on input amount and xHYB panel used.

**Table 13. Number of postcapture amplification cycles**

| xHYB Panel | <500 ng capture input | 500 ng – 2.0 µg capture input | >2.0 µg capture input |
|---|---|---|---|
| Actionable Exome Panel | 9 cycles | 8 cycles | 7 cycles |
| Carrier Screening Panel | 13 cycles | 12 cycles | 11 cycles |

44. Prepare the reaction mix on ice according to Table 14. Mix the components in a PCR tube or 96-well PCR plate.

**Table 14. 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 thermocycler and start the program.
46. Once PCR is complete, add 75 µl of resuspended Agencourt AMPure XP Beads to each reaction (50 µl) and pipet 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.
50. 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.
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 3). 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 to –15°C in a DNA LoBind tube until ready to use for sequencing.

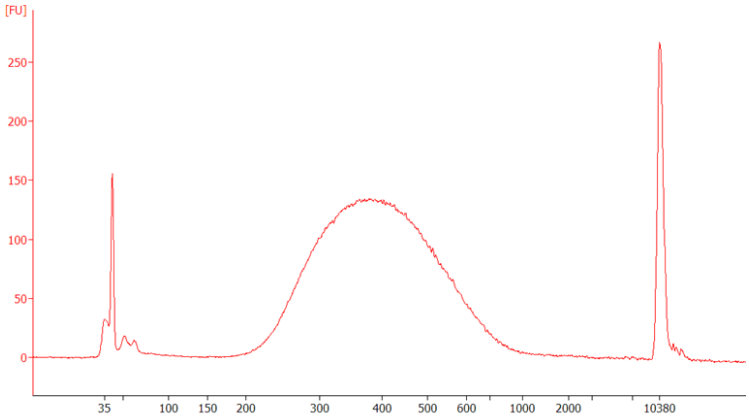



**Figure 3. Electrophoresis trace data of a final hybrid capture library pool.**

# 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 of the used xHYB panel and the desired coverage per sample. Please refer to Table 15 for initial multiplexing guidelines per sequencing run to achieve a minimal average target region coverage of 100x. 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.

xHYB panels achieve 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 15. Multiplexing guidelines for Illumina instruments using 2 x 150 bp paired-end sequencing to achieve a minimal average target coverage of 100x. Actual coverage may vary depending on input DNA quality and the sequencing instrument used**

| Sequencing Instrument | Paired reads | xHYB Actionable Exome Panel | xHYB Carrier Panel |
|---|---|---|---|
| | | Max. libraries* per flow cell | |
| MiniSeq (High Output) | 45 M | 1 | 12 |
| MiSeq (v2) | 30 M | 1 | 8 |
| NextSeq 500/550 (Mid Output) | 260 M | 8 | 72 |
| NextSeq 500/550 (High Output) | 800 M | 24 | 240 |
| NextSeq 1000/2000 (P1) | 200 M | 6 | 56 |
| NextSeq 1000/2000 (P2) | 800 M | 24 | 240 |
| NextSeq 2000 (P3) | 2400 M | 72 | (384) |
| NovaSeq 6000 (SP) | 1500 M | 48 | (384) |
| NovaSeq 6000 (S1) | 3000 M | 96 | (384) |
| NovaSeq 6000 (S2) | 8000 M | 240 | (384) |
| NovaSeq 6000 (S4) | 18000 M | (384) | (384) |

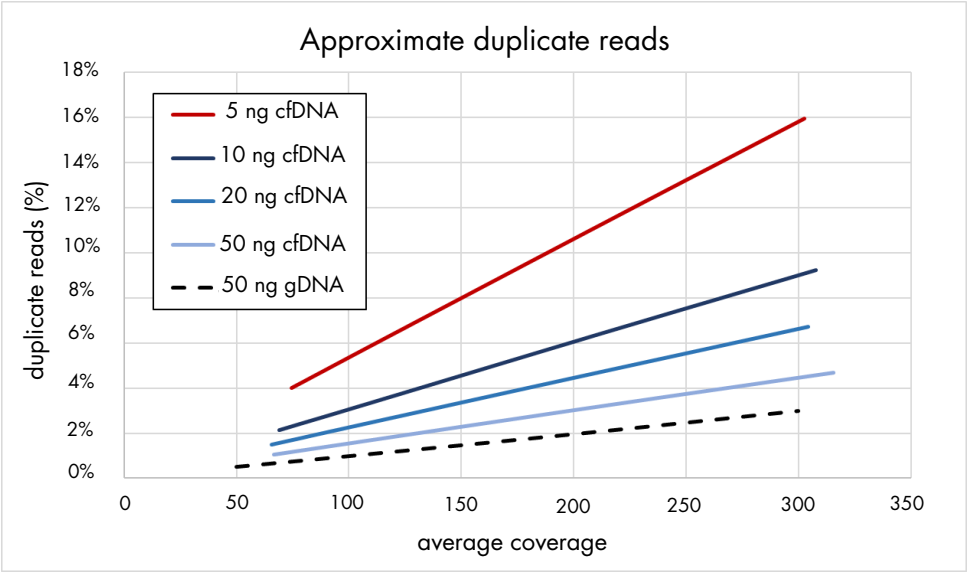
* Libraries per flow cell refers to the number of single libraries with distinct sample indexes. Each hybridization capture pool can contain multiple individually barcoded libraries.

## Deep sequencing considerations

Deep sequencing xHYB libraries enables detection of variants with low allelic frequencies, for example from human tissue or tumor samples, as well as from cell-free DNA. Higher coverage will generally enable a higher sensitivity to detect low frequency variants. However, extreme deep sequencing with more than 500x of coverage generates little additional information due to increasing duplicate rates. Besides sequencing coverage there are other factors that may impact the amount of duplicate reads. The amount and quality of the starting material will determine the library complexity, which translates to the complexity of the enriched xHYB library. This will significantly impact the amount of duplicates, as small input amounts require more amplification resulting in more duplicate reads. Generally large input amounts of high quality genomic DNA produce least duplicates. Formalin-compromised DNA may result in increased duplicate rates, and cell-free DNA typically yields highest duplicate rates. The

sequencing instrument itself can be a source of duplicates as well. For example, a NovaSeq 6000 instrument generates more duplicate reads than a NextSeq® 550 instrument. Figure 4 illustrates the amount of duplicate reads to be expected at different coverage levels and input DNA amounts.

**Figure 4. Approximate duplicate rates at a given coverage, input type, and input amount.** The input amount refers to input for whole genome library preparation using QIAseq FX DNA Library Kits for gDNA and QIAseq Ultralow Input Library Kits for cfDNA. 400 ng per indexed library has been used as input for multiplexed hybridization capture. The shown data was generated on a NextSeq 550 instrument (High Output, v2.5). Duplicate rates may vary based on input quality and the sequencing instrument used.



## 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.
- Editable and ready to use sample sheets, including all sample indexes of QIAseq CDI and UDI Y-Adapters are available for download at **www.qiagen.com**.
- A description of run setup for Illumina instruments and definition files for QIAseq CDI-Y and UDI-Y adapters to be used for Illumina Local Run Manager are available at **www.qiagen.com**.
- Sequencing on the NextSeq, HiSeq X®, HiSeq® 3000/4000 systems follow a different dual-indexing workflow than other Illumina systems. If manually creating sample sheets on these instruments, enter the reverse complement of the i5 index adapter sequence. If using BaseSpace®, or Local Run Manager to plan the run, the software will automatically reverse complement index sequences when necessary.

## 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: w**ww.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. For input amounts smaller than 1000 ng, use 8 cycles of postcapture amplification. |
| 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 8 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. |
| **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. |

| | | Comments and suggestions |
|---|---|---|
| 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.<br>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 Actionable Exome Panel (24) | For 3 hybridization reactions: Buffers, blocking reagents, Actionable Exome Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 24 whole genome libraries | 333372 |
| QIAseq xHYB Actionable Exome Panel (96) | For 12 hybridization reactions: Buffers, blocking reagents, Actionable Exome Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 96 whole genome libraries | 333375 |
| QIAseq xHYB Carrier Panel (24) | For 3 hybridization reactions: Buffers, blocking reagents, Carrier Screening Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 24 whole genome libraries | 333362 |
| QIAseq xHYB Carrier Panel(96) | For 12 hybridization reactions: Buffers, blocking reagents, Carrier Screening Probe Set, Streptavidin beads, wash buffers, and postcapture enrichment reagents for hybrid capture of up to 96 whole genome libraries | 333365 |
| QIAseq xHYB Mitochondrial Panel (24) | Mitochondrial Probe Set for spike-in for 3 hybridization reactions | 333382 |
| QIAseq xHYB Mitochondrial Panel (96) | Mitochondrial Probe Set for spike-in for 12 hybridization reactions | 333385 |

| Product | Contents | Cat. no. |
|---|---|---|
| QIAseq xHYB Automation Kit | Excess volume of Streptavidin beads, wash buffers, and Post Capture Binding Buffer for automation purposes | 333430 |
| **QIAseq library kits for the generation of indexed whole genome libraries for use in hybridization capture** | | |
| QIAseq FX DNA Library Kits (96) | QIAseq FX DNA Library UDI-A Kit (96)<br>QIAseq FX DNA Library UDI-B Kit (96)<br>QIAseq FX DNA Library UDI-C Kit (96)<br>QIAseq FX DNA Library UDI-D Kit (96)<br>QIAseq FX DNA Library CDI Kit (96)<br>For 96 reactions: Buffers and reagents for DNA fragmentation, end repair, A-addition, ligation and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate) | 180479<br>180480<br>180481<br>180482<br>180484 |
| QIAseq FX DNA Library Kits (24) | QIAseq FX DNA Library UDI Kit (24)<br>QIAseq FX DNA Library CDI Kit (24)<br>For 24 reactions: Buffers and reagents for DNA fragmentation, end repair, A-addition, ligation and library amplification; for use with Illumina instruments; includes a plate containing 24 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate) | 180477<br>180483 |

| Product | Contents | Cat. no. |
|---|---|---|
| QIAseq Ultralow Input Library Kits (96) | QIAseq Ultralow Input Lib UDI-A Kit (96)<br>QIAseq Ultralow Input Lib UDI-B Kit (96)<br>QIAseq Ultralow Input Lib UDI-C Kit (96)<br>QIAseq Ultralow Input Lib UDI-D Kit (96)<br>QIAseq Ultralow Input Lib CDI Kit (96)<br>For 96 reactions: Buffers and reagents for Ultralow Input End Polishing, Ultralow Input Ligation, and HiFi library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate) | 180497<br>180498<br>180499<br>180500<br>180501 |
| QIAseq Ultralow Input Library Kit (12) | QIAseq Ultralow Input Library Kit (12)<br>For 12 reactions: Buffers and reagents for Ultralow Input End Polishing, Ultralow Input Ligation and HiFi library amplification. For use with Illumina instruments. QIAseq UDI/CDI Y-Adapter Kits (24) need to be purchased separately | 180492 |
| QIAseq UDI Y-Adapter Kit (24) | A plate containing 24 adapters with different barcodes (UDI). Pierceable foil seal allowing usage of defined parts of plat | 180310 |
| QIAseq CDI Y-Adapter Kit (24) | A plate containing 24 adapters with different barcodes (CDI). Pierceable foil seal allowing usage of defined parts of plate | 180301 |

| Product | Contents | Cat. no. |
|---|---|---|
| **QIAseq Library Quantification Kits for use with Illumina instruments** | | |
| QIAseq Library Quant Assay Kit | Laboratory-verified forward and reverse primers for 500 x 25 µl reactions (500 µl); DNA Standard (100 µl); Dilution Buffer (30 ml); (1.35 ml x 5) GeneRead® qPCR SYBR® Green Mastermix | 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 |
|---|---|
| 04/2022 | Initial release |

## Notes

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