October 2020

# GeneRead™ QIAact BRCA Advanced DNA UMI Panel Handbook


For constructing targeted, molecularly bar-coded libraries from DNA for digital sequencing with next-generation sequencing (NGS)


181925

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For Research Use Only. Not for use in diagnostic procedures.

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **QIAact BRCA Advanced DNA UMI Panel** | |
|---|---|
| **Catalog no.** | **181925** |
| **Number of reactions** | **24** |
| GeneRead QIAact Library Preparation and Target Enrichment Reagents (Kit Box 1)* | |
| GeneRead QIAact BRCA Advanced UMI Kit (Kit Box 2)* | |

* Kit boxes 1 and 2 are components of the GeneRead QIAact BRCA Advanced DNA UMI Panel.

| **GeneRead QIAact Library Preparation and Target Enrichment Reagents*** | |
|---|---|
| **Number of reactions** | **24** |
| Fragmentation Buffer, 10x | 65 µl |
| Fragmentation Enzyme Mix | 130 µl |
| FERA Solution | 20 µl |
| Ligation Buffer, 5x | 260 µl |
| Ligation Solution | 200 µl |
| DNA Ligase | 130 µl |
| Nuclease-free Water | 2 x 1.9 ml |
| TEPCR Buffer, 5x | 220 µl |
| UPCR Buffer, 5x | 220 µl |
| HotStarTaq® DNA Polymerase | 2 x 50 µl |
| One bottle containing QIAact Beads (provided in separate cold-packed shipment) | 10 ml |

*Not for individual sale; to order reagents, see cat. no. 181925.

| GeneRead QIAact BRCA Advanced UMI Kit* | |
|---|---|
| **Number of reactions** | **24** |
| GeneRead QIAact BRCA1-2 Forward Primers | 30 µl |
| GeneRead QIAact BRCA1-2 Reverse Primers | 30 µl |
| GeneRead QIAact pTEN/TP53 Forward Primers | 30 µl |
| GeneRead QIAact pTEN/TP53 Reverse Primers | 30 µl |
| QIAact Adapters (contains 12 tubes with each tube corresponding to one specific 9 bp sample bar code; each tube can process up to 2 samples) | 12 µl |
| GeneReader™ TE-PCR Primer | 40 µl |
| GeneReader Universal PCR Primer A | 40 µl |
| GeneReader Universal PCR Primer B | 40 µl |
| Water for Sample Dilution | 1.9 ml |

*Not for individual sale; to order products, see cat. no. 181925.

# Shipping and Storage

The GeneRead QIAact Library Preparation and Target Enrichment Reagents (not including QIAact Beads) are shipped on dry ice and should be stored at –15 to –30°C upon arrival.

QIAact Beads are shipped on cold packs and should be stored at 2–8°C. When stored properly, all reagents are stable for up to 3 months after delivery, unless otherwise indicated on the label.

The GeneRead QIAact BRCA Advanced UMI Panel and Adaptor Kits are shipped on dry ice and should be stored at –15 to –30°C upon arrival. When stored properly, all reagents are stable for up to 3 months after delivery, unless otherwise stated on the label.

# Intended Use

The GeneRead QIAact BRCA Advanced UMI Panel and Adaptor Kit and GeneRead QIAact Library Preparation and Target Enrichment Reagents are for research use only. Not for use in diagnostic procedures.

# 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 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 the GeneRead QIAact BRCA Advanced DNA UMI Panel is tested against predetermined specifications, to ensure consistent product quality.

# Introduction

DNA sequencing is a useful tool to detect genetic variations, including somatic mutations, single nucleotide variants (SNVs), and small insertions and deletions (inDels). Targeted enrichment technology enables next-generation sequencing (NGS) platform users to sequence-specific regions of interest instead of the entire genome, effectively increasing sequencing depth and throughput with lower cost. Existing target enrichment methods, library preparation, and sequencing steps all utilize enzymes and amplification processes, which introduce substantial bias and artifacts. These biases and artifacts lead to background artifactual errors that greatly limit the detection of true low-frequency variants in heterogeneous samples such as tumors.

The GeneRead QIAact BRCA Advanced DNA UMI Panel integrates unique molecular index (UMI) technology into a gene-specific, primer-based target enrichment process, enabling sensitive variant detection of targeted genomic regions by NGS on the GeneReader system.

The QIAact BRCA Advanced DNA UMI Panel has been optimized in combination with a specially formulated enrichment chemistry to achieve highly efficient enrichment on both regular and GC-rich regions at high multiplex levels.

## Principle and procedure

The QIAact BRCA Advanced DNA UMI Panel relies on gene-specific primer enrichment and UMIs for uniform coverage and sensitive variant detection.

### Unique molecular index

The concept of unique molecular indexing is that prior to any amplification, each original DNA molecule is attached to a unique sequence index. This attachment is accomplished by the ligation of fragmented DNA with a QIAact adapter containing a UMI with 8 random bases.

The DNA molecules are then amplified by PCR for target enrichment and library amplification. Due to intrinsic noise and sequence-dependent bias, DNA molecules with UMIs may be amplified unevenly across the target regions. Even target region coverage can be achieved by counting the number of UMIs in the reads rather than counting the number of total reads for each region. Sequence reads with varying UMIs represent different original molecules, while sequence reads with the same UMI are the result of PCR duplication from one original molecule.

PCR amplification and the sequencing process generate biases and artefacts, which lead to the recording of false-positive DNA variants in the sequencing reads. These false-positive variants may mask the detection of true low-frequency variants in heterogeneous samples such as tumors. To reduce the number of false-positive variants, call variants across all reads within a UMI versus only calling variants from the original sequencing read (Figure 1).

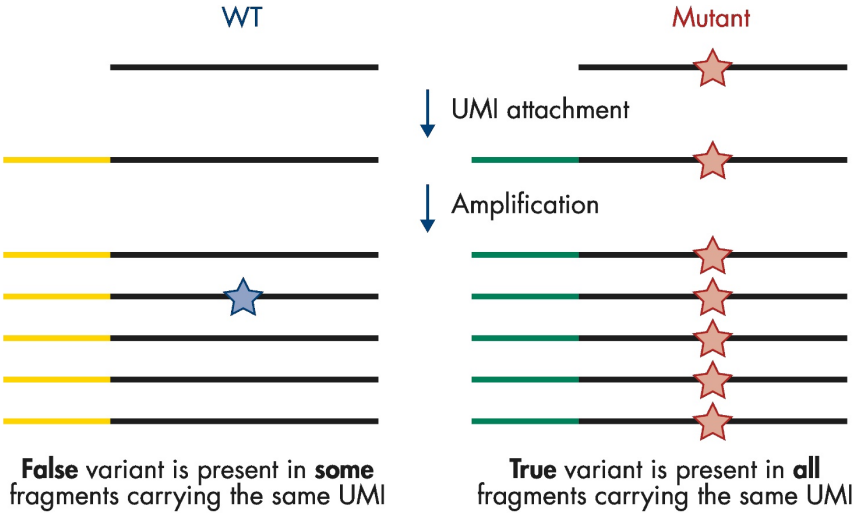


**Figure 1. Principle of variant detection with UMI.** Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while errors are mutations present in only one or a few reads within a UMI. Description of the variant calling algorithm can be found at **www.qiagen.com**.

## Procedure

The QIAact BRCA Advanced DNA UMI Panel is provided as 4 primer mix tubes, with up to 219 primers per tube. The QIAact BRCA Advanced DNA UMI Panel comprises 2 separate target enrichment panels, QIAact BRCA1-2 and QIAact pTEN/TP53, designed to enrich specific target regions in BRCA1, BRCA2 and pTEN, TP53, respectively. When used in combination, the QIAact BRCA Advanced DNA UMI Panel selectively targets all 4 genes.

Genomic DNA samples are first fragmented, end-repaired, and A-tailed using a single, controlled multi-enzyme reaction. The prepared DNA fragments are then ligated at their 5′ ends to a GeneReader-specific adapter containing a UMI and a 9 base-pair (bp) sample-specific bar code.

Ligated DNA molecules are subject to limited cycles of target enrichment PCR, with one gene-specific primer targeting a region and one universal forward primer complimentary to an adapter sequence. This reaction ensures that intended targets and UMIs are enriched sufficiently to be represented in the final library. A universal PCR with GeneReader-specific sequences is then carried out to amplify the targets and complete the library.

Once the library is sequenced, results can be analyzed using the GeneRead QIAact BRCA Advanced DNA UMI Panel workflow, which will automatically perform all steps necessary to generate a DNA sequence variant report from your raw NGS data. All detected variants can be further interpreted by QIAGEN Clinical Insight (QCI®) analysis.

## Recommendation for multiplexing and clonal amplification input

More than one DNA sample can be sequenced in one flow cell and this is made possible by the addition of a 9 bp sample-specific bar code that is added during library preparation (see "Protocol: Adapter Ligation", page 18). The sequencing libraries prepared from the corresponding samples must be pooled prior to clonal amplification to allow them to be sequenced together in one flow cell (see "Protocol: Library Concentration Normalization and Pooling" in the *GeneRead Clonal Amp Q Handbook*, **www.qiagen.com/HB-2027**, for more

information). Based on the total number of amplicons that are produced by the GeneRead QIAact BRCA Advanced DNA UMI Panel, we recommend a multiplex maximum of 8 samples for FFPE and 12 samples for whole blood.

After target enrichment and library preparation, use 625 pg pooled DNA in the clonal amplification process (see "Preparing Libraries for Emulsion Making" in the *GeneRead Clonal Amp Q Handbook* for more information).

## Important note

The cycling conditions for emulsion making using QIAact BRCA Advanced libraries are listed in the table below.

**Note**: This is a deviation from the *GeneRead Clonal Amp Q Handbook*'s Table 4 (in "Protocol: Emulsion Making"), to reduce the effect of sequence-specific artefacts.

**Table 1. Cycling conditions***

| Time | Temperature | Number of cycles |
|---|---|---|
| 6 minutes | 94°C | 1 |
| 15 seconds<br>30 seconds<br>60 seconds | 94°C<br>57°C<br>74°C | 60 |
| 2 minutes | 72°C | 1 |
| Hold | 4°C | ∞ |
| Lid | 105°C | – |

* Total volume used: 125 µl; program thermal cycler accordingly (125 µl or maximum volume allowed).

## Limitations of the assay

The BRCA Advanced panel is designed for targeted sequencing of full coding regions for the BRCA1, BRCA2, PTEN, and TP53 genes plus 20 bp into flanking introns. However, there are a few exceptions. See Appendix A (page 36) for details.

# Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

In addition to the QIAact Library Preparation and Target Enrichment Reagents and GeneRead QIAact BRCA Advanced DNA UMI Panel, the following supplies are required:

**For DNA isolation**

- See "Recommended genomic DNA preparation methods", page 13

**For library construction and targeted enrichment**

- High-quality, nuclease-free water. **Important**: Do not use DEPC-treated water.
- 80% ethanol, made fresh
- Microcentrifuge
- 1.5 ml LoBind® tubes (Eppendorf)
- 0.2 ml PCR tubes, 96-well PCR plates or PCR strips and caps
- Thermal cycler (e.g., Bio-Rad® C1000™)
- Multichannel pipettor
- Single-channel pipettor
- DNase-free pipette tips and tubes
- QIAxcel® Advanced instrument (for information, visit **www.qiagen.com**)
- QIAxcel DNA High Resolution Kit (cat. no. 929002)
- QX DNA Size Marker 50–800 bp (50 µl) (cat. no. 929561)
- QX Alignment Marker 15 bp/3 kb (1.5 ml) (cat. no. 929522)

- QX Nitrogen Cylinder (x 6) (cat. no. 929705)
- Qubit® 3.0 or higher Fluorometer (Thermo Fisher Scientific cat. no. Q33216) or equivalent
- Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific cat. no. Q32851)
- Qubit assay tubes (e.g., Thermo Fisher Scientific cat. no. Q32856)
- DynaMag™-2 Magnet (Thermo Fisher Scientific cat. no. 12321D)

**Optional**

- QIAxpert® spectrophotometer (cat. no. 9002340) or equivalent
- Agilent® 2100 Bioanalyzer®
- Agilent High Sensitivity DNA Kit (Agilent cat. no. 5067-4626)

# Important Notes

## DNA preparation

### Maximizing DNA quality is essential for obtaining good sequencing results

The most important prerequisite for sequence analysis is maximizing the DNA quality of every experimental sample. Therefore, sample handling and DNA isolation procedures are critical to the success of the experiment.

Residual traces of proteins, salts, or other contaminants may either degrade the DNA or decrease the efficiency of (if not block completely) the enzyme activities necessary for optimal targeted genome amplification.

### Recommended DNA preparation methods

The QIAGEN QIAamp® DNA Blood Midi Kit (cat. no. 51183), GeneRead DNA FFPE Kit (cat. no. 180134), or QS GeneRead DNA FFPE Treatment Kit (cat. no. 185306) in combination with the QIAsymphony® DSP DNA Mini Kit (cat. no. 937236) are highly recommended for the preparation of genomic DNA samples from different sample types.

**Important**: Do not omit the recommended RNase treatment step to remove RNA.

For best results, all DNA samples should be resuspended in the recommended buffer or DNase-free 10 mM Tris* buffer pH 8.0.

**Important**: Do not use DEPC-treated water.

* 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), available from the product supplier.

### Recommended genomic DNA preparation method from whole blood samples

To maximize DNA yields, we recommend extraction from a minimum 2.0 ml of whole blood as instructed by the *QIAamp DNA Blood Midi/Maxi Handbook*, **www.qiagen.com/HB-2533**, (cat. no. 51183).

## DNA quantification

For best results, all DNA samples should also demonstrate consistent quality according to the following criteria:

### DNA purity determined by UV spectrophotometry

The purity of DNA should be determined by measuring absorbance in a spectrophotometer such as the QIAxpert System. Prepare dilutions and measure absorbance in 10 mM Tris·Cl* buffer, pH 8.0. The spectral properties of nucleic acids are highly dependent on pH.

$A_{260}/A_{280}$ ratio should be greater than 1.8.

### DNA concentration

The concentration of DNA should be determined by fluorometric quantitation using the Qubit 3.0 or higher Fluorometer (Thermo Fisher Scientific cat. no. Q33216). DNA input of 40–160 ng is recommended for the GeneRead QIAact BRCA Advanced DNA UMI Kit. For high quality DNA from reference cell lines or extracted from whole blood, DNA input of 40 ng is recommended. For unknown or lower quality FFPE DNA, DNA input between 80 and 160 ng is recommended. For FFPE DNA, lower input amounts down to 40 ng are possible; however, this will lead to fewer sequenced UMIs and depending on the DNA quality may lead to reduced variant detection sensitivity.

# Protocol: Fragmentation, End-repair, and A-addition

## Important points before starting

- Ensure input DNA is in 10 mM Tris, for example, QIAGEN's Buffer EB, QIAGEN's Buffer ATE or low TE (0.1 x TE, 0.1 mM EDTA)*
- Pre-chill thermal cycler to 4°C

## Procedure

1. If using 40 ng of DNA as input, dilute DNA to 10 ng/µl with nuclease-free water in a LoBind tube.

2. For each sample, add 4 µl of diluted DNA (40 ng total) for fragmentation to a separate 0.2 ml PCR tube.

   **Note**: If available DNA concentration is below 10 ng/µl ensure that final DNA input for fragmentation is 40 ng and reduce the volume of nuclease-free water added to the reaction accordingly (Table 2).

   **Note**: If using an increased DNA input dilute DNA accordingly, for example, if using 100 ng, dilute DNA to 25 ng/µl with nuclease-free water in a LoBind tube.

**Table 2. Preparation of mixture for fragmentation, end-repair, and A-addition**

| Component | 1x volume (µl) | 8x volume (µl) | 12x volume (µl) |
|---|---|---|---|
| DNA | 4 | – | – |
| Fragmentation Buffer, 10x | 2.50 | 21.2 | 31.2 |
| FERA Solution | 0.75 | 6.4 | 9.4 |
| Nuclease-free Water | 12.75 | 108.4 | 159.4 |
| **Total volume** | **20.0** | **136.0** | **200.0** |

* 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), available from the product supplier.

3. Prepare a reaction mixture for fragmentation, end-repair, and A-addition according to Table 2. Mix the components well by pipetting up and down 10 times using a pipette set to 50 µl.

4. Add 16 µl master mix to each 0.2ml PCR tube containing a separate DNA sample (from step 2). It is important to keep the mixture on ice and mix the components well by pipetting up and down 10 times using a pipette set to 10 µl volume.

5. Add 5 µl Fragmentation Enzyme Mix to each reaction and ensure that the reaction solution is mixed well by pipetting up and down 10 times using a pipette set to 15 µl volume.

   **Important**: Do not vortex.

   **Important**: It is critical to keep the PCR tube or plate on ice for the entire time during reaction setup.

6. Program a thermal cycler according to Table 3. Set the reaction volume to 25 µl and be sure to use the instrument's heated lid (e.g., set to 103°C).

**Table 3. Thermal cycler conditions**

| Time | Temperature | Number of cycles |
|---|---|---|
| 1 min | 4°C | 1 |
| 24 min | 32°C | 1 |
| 30 min | 72°C | 1 |
| ∞* | 4°C | Hold |

* Samples should not remain at 4°C for a prolonged period of time.

7. Start the program. When the thermal cycler block reaches 4°C, pause the program.

8. Transfer the PCR tube to the prechilled thermal cycler and resume the cycling program.

9. When the thermal cycler program is complete and the sample block has returned to 4°C, remove the samples and place them on ice.

10. Immediately proceed to “Protocol: Adapter Ligation”, page 18.

# Protocol: Adapter Ligation

## Procedure

1. Prepare the ligation reaction master mix on ice according to Table 4. Mix well by pipetting up and down 10 times using a pipette set to 50 µl volume.

**Table 4. Reaction setup for adapter ligation**

| Component | 1x volume (µl) | 8x volume (µl) | 12x volume (µl) |
|---|---|---|---|
| Ligation Buffer, 5x | 10.0 | 85.0 | 125.0 |
| DNA Ligase | 5.0 | 42.5 | 62.5 |
| Ligation Solution (PEG6000, 50%) | 7.2 | 61.2 | 90.0 |
| **Total volume** | **22.2** | **188.7** | **277.5** |

2. Each QIAact Adapter has a different 9 bp sample-specific bar code. Transfer 2.8 µl of one QIAact Adapter for each sample being prepared into a separate 0.2 ml PCR tube(s).

   **Note:** Only one single QIAact adapter should be used per ligation reaction. Open one adapter tube at a time to avoid cross-contamination. It is also recommended to change gloves between each adapter addition to avoid cross-contamination.

3. Transfer 25 µl of each fragmentation, end-repair, and A-addition product into each 0.2 ml PCR tube(s), which contains an adapter.

4. Add 22.2 µl of ligation master mix to each 0.2 ml PCR tube(s) and mix gently by pipetting up and down 7 times with a pipette set to 25 µl. Centrifuge briefly (10–15 s), and then place on ice.

   **Note**: The final ligation reaction volume may be less than 50 µl. It is important to measure the ligation reaction volume. If the volume is less than 50 µl, add the appropriate volume of nuclease-free water to bring the final volume to 50 µl.

5. Program a thermal cycler to 20°C.

6. Transfer tube(s) to the thermal cycler and incubate reaction for 15 min at 20°C.

   **Important**: Do not use the heated lid.

7. After the reaction is complete, place the reactions on ice and proceed to "Protocol: Cleanup of Adapter-Ligated DNA with QIAact Beads".

   **Note:** If reactions are to be stored after ligation, transfer them to a –20°C freezer. Samples are stable for 3 days.

# Protocol: Cleanup of Adapter-Ligated DNA with QIAact Beads

## Procedure

1. Let the QIAact Beads come to room temperature (15–25°C) for at least 30 min, and vortex thoroughly before use.

   **Recommendation**: Vortex for 1 min at maximum speed.

2. Transfer 50 µl ligation reaction from "Protocol: Adapter Ligation", page 18, to a 1.5 ml LoBind tube. Add nuclease-free water to bring the volume to 100 µl.

   **Note**: The final ligation reaction volume may be less than 50 µl due to evaporation. It is important to measure the ligation reaction volume from "Protocol: Adapter Ligation", and then add the appropriate volume of nuclease-free water to bring the final volume to 100 µl.

3. Add 100 µl (1.0 x volume) QIAact Beads to 100 µl diluted DNA solution. Mix well by pipetting up and down 10 times using a pipette set to 100 µl. Use a fresh tip for every sample.

4. Incubate for 5 min at room temperature.

5. Place the tube on the magnetic rack for 10 min to separate beads from supernatant. After the solution is clear, carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the DNA target.

   **Important**: Do not discard the beads.

6. Completely remove residual supernatant (it is recommended to use a 10 µl tip to aspirate the trace amount of residual supernatant after the first aspiration).

7. Add 200 µl freshly made 80% ethanol to the tube while it is on the magnetic rack. Rotate the tube 180 degrees on the magnetic rack 4 times, shifting the position of the pellet, to wash the beads. Once complete, wait until solution is clear (2–3 min). Completely remove ethanol.

8. Repeat the previous step once.

9. After completely removing the ethanol, close the tube lid.

10. Remove the tube from the magnetic rack and centrifuge briefly (10–15 s).

11. Replace the tube on the magnetic rack and wait until solution is clear (2–3 min). Open the lid and then use a 10 µl tip to remove any residual ethanol. Keeping the tube lid open, air-dry the beads for up to 10 min on the rack.

    **Note**: Avoid overdrying the beads. As drying depends on temperature and airflow, the drying time may vary. Adapt the drying time until the beads no longer appear shiny.

    **Note**: The beads in different tubes may dry at different rates. Once the beads in a tube are dry, close the lid to avoid overdrying.

12. Add 52 µl nuclease-free water to the beads to elute the DNA. Mix well by pipetting up and down 10 times using a pipette set to 25 µl. Use a fresh tip for every sample. Place the tube on the magnetic rack until solution is clear (5–10 min).

13. Transfer 50 µl supernatant to a clean 1.5 ml tube.

14. Add 50 µl (1.0x volume) QIAact Beads to 50 µl DNA solution from the previous step. Mix well by pipetting up and down 10 times using a pipette set to 50 µl. Use a fresh tip for every sample.

15. Incubate for 5 min at room temperature.

16. Place the tube on the magnetic rack for 10 min to separate beads from supernatant. After the solution is clear, carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the DNA target.

    **Important**: Do not discard the beads.

17. Completely remove residual supernatant (it is recommended to use a 10 µl tip to aspirate the trace amount of residual supernatant after the first aspiration).

18. Add 200 µl freshly made 80% ethanol to the tube while it is on the magnetic rack. Rotate the tube 180 degrees on the magnetic rack 4 times, shifting the position of the pellet, to wash the beads. Once complete, wait until solution is clear (2–3 min). Completely remove ethanol.

19. Repeat the previous step once.

20. After completely removing the ethanol, close the tube lid.

21. Remove the tube from the magnetic rack and centrifuge briefly (10–15 s).

22. Replace the tube on the magnetic rack and wait until solution is clear (2–3 min). Open the lid and then use a 10 µl tip to remove any residual ethanol. Keeping the tube lid open, air-dry the beads for up to 10 min on the rack.

    **Note**: Avoid overdrying the beads. As drying depends on temperature and airflow, the drying time may vary. Adapt the drying time until the beads no longer appear shiny. Ethanol carryover can affect enrichment PCR efficiency in the target enrichment PCR.

    **Note**: The beads in different tubes may dry at different rates. Once the beads in a tube are dry, close the lid to avoid overdrying.

23. Add 22 µl nuclease-free water to the beads to elute the DNA. Mix well by pipetting up and down 10 times using a pipette set to 15 µl. Use a fresh tip for every sample. Place tube on the magnetic rack until solution is clear (5–10 min).

24. Prepare 2 sets of 0.2 ml PCR tubes, one for the forward target enrichment PCR and one for the reverse target enrichment PCR.

25. From each 1.5 ml LoBind tube transfer 9.4 µl supernatant to each of 2 PCR tubes, one for the forward target enrichment PCR and one for the reverse target enrichment PCR.
    Proceed to the next protocol, "Target Enrichment PCR", page 24.

    If reactions are to be stored after QIAact Beads cleanup, transfer them to a –20°C freezer. Samples are stable for 3 days.

# Protocol: Target Enrichment PCR

## Procedure

1. Prepare the following master mixes as indicated in Table 5 and Table 6 for the BRCA1-2 panel or Table 7 and Table 8 for the combined BRCA1-2 and pTEN/TP53 panels in separate 1.5 ml LoBind tubes. A total of 2 reaction mixes should be made. Mix well by pipetting up and down 10 times using a pipette set to 30 µl volume.

**Table 5. Reaction components for forward target enrichment PCR using QIAact BRCA1-2 Panel**

| Component | 1x volume (µl) | 8x volume (µl) | 12x volume (µl) |
|---|---|---|---|
| DNA library (from "Protocol: Cleanup of Adapter-Ligated DNA with QIAact Beads") | 9.4 | – | – |
| TEPCR Buffer, 5x | 4.0 | 34.0 | 50.0 |
| GeneRead QIAact BRCA1-2 Panel Forward Primers | 1.25 | 10.6 | 15.6 |
| Water for Sample Dilution | 3.75 | 31.9 | 46.9 |
| GeneReader TE-PCR Primer | 0.8 | 6.8 | 10.0 |
| HotStarTaq DNA Polymerase | 0.8 | 6.8 | 10.0 |
| **Total volume** | **20.0** | **90.1** | **132.5** |

**Table 6. Reaction components for reverse target enrichment PCR using QIAact BRCA1-2 Panel**

| Component | 1x volume (µl) | 8x volume (µl) | 12x volume (µl) |
|---|---|---|---|
| DNA library (from "Protocol: Cleanup of Adapter-Ligated DNA with QIAact Beads") | 9.4 | – | – |
| TEPCR Buffer, 5x | 4.0 | 34.0 | 50.0 |
| GeneRead QIAact BRCA1-2 Panel Reverse Primers | 1.25 | 10.6 | 15.6 |
| Water for Sample Dilution | 3.75 | 31.9 | 46.9 |
| GeneReader TE-PCR Primer | 0.8 | 6.8 | 10.0 |
| HotStarTaq DNA Polymerase | 0.8 | 6.8 | 10.0 |
| **Total volume** | **20.0** | **90.1** | **132.5** |

**Table 7. Reaction components for forward target enrichment PCR using QIAact BRCA1-2 and pTEN/TP53 panels**

| Component | 1x volume (µl) | 8x volume (µl) | 12x volume (µl) |
|---|---|---|---|
| DNA library (from "Protocol: Cleanup of Adapter-Ligated DNA with QIAact Beads") | 9.4 | – | – |
| TEPCR Buffer, 5x | 4.0 | 34.0 | 50.0 |
| GeneRead QIAact BRCA1-2 Panel Forward Primers | 1.25 | 10.6 | 15.6 |
| GeneRead QIAact pTEN/TP53 Panel Forward Primers | 1.25 | 10.6 | 15.6 |
| Water for Sample Dilution | 2.5 | 21.3 | 31.3 |
| GeneReader TE-PCR Primer | 0.8 | 6.8 | 10.0 |
| HotStarTaq DNA Polymerase | 0.8 | 6.8 | 10.0 |
| **Total volume** | **20.0** | **90.1** | **132.5** |

**Table 8. Reaction components for reverse target enrichment PCR using QIAact BRCA1-2 and pTEN/TP53 panels**

| Component | 1x volume (µl) | 8x volume (µl) | 12x volume (µl) |
|---|---|---|---|
| DNA library (from "Protocol: Cleanup of Adapter-Ligated DNA with QIAact Beads") | 9.4 | – | – |
| TEPCR Buffer, 5x | 4.0 | 34.0 | 50.0 |
| GeneRead QIAact BRCA1-2 Panel Reverse Primers | 1.25 | 10.6 | 15.6 |
| GeneRead QIAact pTEN/TP53 Panel Reverse Primers | 1.25 | 10.6 | 15.6 |
| Water for Sample Dilution | 2.5 | 21.3 | 31.3 |
| GeneReader TE-PCR Primer | 0.8 | 6.8 | 10.0 |
| HotStarTaq DNA Polymerase | 0.8 | 6.8 | 10.0 |
| **Total volume** | **20.00** | **90.1** | **132.5** |

2. Add 10.6 µl of master mix from either Table 5 and Table 6, or Table 7 and Table 8, to the corresponding 0.2 ml PCR tube(s) containing the purified DNA library from the previous section. Mix gently by pipetting up and down 7 times with a pipette set to 10 µl, then centrifuge briefly (10–15 s).

3. Set up the thermal cycler using the cycling conditions provided in Table 9.

   **Important**: Set the reaction volume to 20 µl, and the heated lid to 103°C.

**Table 9. Cycling conditions for target enrichment PCR**

| Time | Temperature | Number of cycles |
|---|---|---|
| 13 min<br>2 min | 95°C<br>98°C | 1 (Initial denaturation) |
| 15 s<br>10 min | 98°C<br>68°C | 8 |
| 5 min | 72°C | 1 |
| 5 min* | 4°C | 1 |
| ∞ | 4°C | Hold |

* Samples must be held at 4°C for at least 5 min.

4. When the reaction is complete, place the reactions on ice and proceed to the next protocol, "Cleanup of Target Enrichment PCR with QIAact Beads", page 27.

   **Note**: If reactions are to be stored after target enrichment PCR, transfer them to a –20°C freezer. Samples are stable for up to 3 days.

# Protocol: Cleanup of Target Enrichment PCR with QIAact Beads

## Procedure

1. Let QIAact Beads come to room temperature for at least 30 min and vortex thoroughly before use.

   **Recommendation:** Vortex for 1 min at maximum speed

2. Pulse-centrifuge the Forward and Reverse PCR reactions from "Protocol: Target Enrichment PCR", page 24, and combine them in a 1.5 ml LoBind tube. Add nuclease-free water to bring the volume to 100 µl.

   **Note**: The combined PCR reaction volume may be less than 40 µl due to evaporation. It is important to measure the combined PCR reaction volume from "Protocol: Target Enrichment PCR", and then add the appropriate volume of nuclease-free water to bring the final volume to 100 µl.

3. Add 100 µl (1.0x volume) QIAact Beads to 100 µl diluted PCR solution. Mix well by pipetting up and down 10 times using a pipette set to 100 µl. Use a fresh tip for every sample. Incubate for 5 min at room temperature.

4. Place the tube on the magnetic rack for 10 min to separate beads from supernatant. Carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the DNA target.

   **IMPORTANT**: Do not discard the beads.

5. Completely remove residual supernatant (it is recommended to use a 10 µl tip to aspirate the trace amount of residual supernatant after the first aspiration).

6. Add 200 µl fresh 80% ethanol to the tube while it is on the magnetic rack. Rotate the tube 180 degrees on the magnetic rack 4 times, shifting the position of the pellet, to wash the beads. Once complete, wait until solution is clear (2–3 min). Completely remove ethanol with a 200 µl pipette tip.

7. Repeat the previous step once.

8. After completely removing the ethanol, close the tube lid.

9. Remove the tube from the magnetic rack and centrifuge briefly (10–15 s).

10. Replace the tube on the magnetic rack and wait until solution is clear (2–3 min). Open the lid and use a 10 µl tip to remove any residual ethanol. Keeping the tube lid open, air-dry the beads for up to 10 min on the magnetic rack.

    **Important**: Avoid overdrying the beads. As drying depends on temperature and airflow, the drying time may vary. Adapt the drying time until the beads no longer appear shiny. Ethanol carryover can affect PCR efficiency in the universal PCR.

    **Note**: The beads in different tubes may dry at different rates. Once the beads in a tube are dry, close the tube lid to avoid overdrying.

11. Add 16 µl nuclease-free water to the beads to elute the DNA. Mix well by pipetting up and down 10 times using a pipette set to 8 µl. Use a fresh tip for every sample. Place on the magnetic rack until solution is clear (5–10 min). Transfer 13.4 µl supernatant to a clean 0.2 ml PCR tube. Proceed to “Protocol: Universal PCR Amplification”, page 29.

    **Note:** If reactions are to be stored, transfer them to a –20°C freezer. Samples are stable for up to 3 days.

# Protocol: Universal PCR Amplification

## Procedure

1. Prepare the following master mix as indicated in Table 10 in a 1.5 ml LoBind tube.

**Table 10. Reaction components for universal PCR amplification**

| Component | 1x volume (µl) | 8x volume (µl) | 12x volume (µl) |
|---|---|---|---|
| Enriched DNA (from "Cleanup of Target Enrichment PCR with QIAact Beads") | 13.4 | – | – |
| UPCR Buffer, 5x | 4.0 | 34.0 | 50.0 |
| GeneReader Universal PCR Primer A | 0.8 | 6.8 | 10.0 |
| GeneReader Universal PCR Primer B | 0.8 | 6.8 | 10.0 |
| HotStarTaq DNA Polymerase | 1.0 | 8.5 | 12.5 |
| **Total volume** | **20.0** | **56.1** | **82.5** |

2. Add 6.6 µl of master mix from Table 10 to the 0.2 ml PCR tube(s) containing the enriched DNA from the previous section. Mix gently by pipetting up and down 7 times with a pipette set to 10 µl, centrifuge briefly (10–15 s).

3. Set up the thermal cycler using the cycling conditions provided in Table 11.

   **Important**: Set the reaction volume to 20 µl and ensure the heated lid on the thermal cycler is turned on for the PCR (e.g., set to 103°C).

**Table 11. Cycling conditions for universal PCR amplification**

| Time | Temperature | Number of cycles |
|---|---|---|
| 13 min | 95°C | 1 (Initial denaturation) |
| 2 min | 98°C | |
| 15 s | 98°C | 21 |
| 2 min | 60°C | |
| 5 min | 72°C | 1 |
| 5 min* | 4°C | 1 |
| ∞ | 4°C | Hold |

* Samples must be held at 4°C for at least 5 min.

4. When the reaction is complete, place the reactions on ice and proceed to "Protocol: Cleanup of Universal PCR with QIAact Beads", page 31.

   **Note:** If reactions are to be stored after universal PCR amplification, transfer them to a –20°C freezer. Samples are stable for up to 3 days.

# Protocol: Cleanup of Universal PCR with QIAact Beads

## Procedure

1. Let the QIAact Beads come to room temperature for at least 30 min and vortex thoroughly before use.

   **Recommendation**: Vortex for 1 min at maximum speed.

2. Transfer 20 µl PCR reaction from "Protocol: Universal PCR Amplification", page 29, to a 1.5 ml LoBind tube. Add nuclease-free water to bring the volume to 100 µl.

   **Note**: The PCR reaction volume may be less than 20 µl due to evaporation. It is important to measure the PCR reaction volume from "Protocol: Universal PCR Amplification" and then add the appropriate volume of nuclease-free water to bring the final volume to 100 µl.

3. Add 100 µl (1.0x volume) QIAact Beads to 100 µl diluted PCR solution. Mix well by pipetting up and down 10 times using a pipette set to 100 µl. Use a fresh tip for every sample. Incubate for 5 min at room temperature.

4. Place the tube on the magnetic rack for 10 min to separate beads from supernatant. Carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the DNA target.

   **Important**: Do not discard the beads.

5. Carefully remove residual supernatant (it is recommended to use a 10 µl tip to aspirate the trace amount of residual supernatant after the first aspiration).

6. Add 200 µl fresh 80% ethanol to the tube while it is on the magnetic rack. Rotate the tube 180 degrees on the magnetic rack 4 times, shifting the position of the pellet, to wash the beads. Once complete, wait until solution is clear (2–3 min). Completely remove ethanol with a 200 µl pipette tip.

7. Repeat the previous step once.

8. After completely removing the ethanol, close the tube lid.

9. Remove the tube from the magnetic rack and centrifuge briefly (10–15 s).

10. Replace the tube on the magnetic rack and wait until solution is clear (2–3 min). Open the lid and then use a 10 µl tip to remove any residual ethanol. Keeping the tube lid open, air-dry the beads for up to 10 min on the magnetic rack.

    **Note**: Avoid overdrying the beads. As drying depends on temperature and airflow, the drying time may vary. Adapt the drying time until the beads no longer appear shiny. Ethanol carryover may affect downstream processing and sample assessment.

    **Note**: The beads in different tubes may dry at different rates. Once the beads in a tube are dry, close the tube lid to avoid overdrying.

11. Add 30 µl nuclease-free water to the beads to elute the DNA. Mix well by pipetting up and down 10 times using a pipette set to 20 µl. Use a fresh tip for every sample.

12. Place the tube on the magnetic rack until solution is clear (5–10 min). Transfer 28 µl supernatant to a clean LoBind 1.5 ml tube or PCR tube.

    **Note**: Reactions can be stored after universal PCR amplification cleanup at –20°C for up to 6 months.

13. Assess the yield (ng) of PCR-enriched DNA library using a Qubit Fluorometer and Qubit dsDNA HS Assay Kit. Assess the product size (bp) using the QIAxcel Advanced instrument and the QIAxcel DNA High Resolution Kit (1200) (see Appendix B, page 37). Typically, 3–20 ng/µl of PCR product will be obtained after purification.

    **Note.** Dilute the sample 1:2 in QX DNA Dilution Buffer.

    **Note**: It is not recommended to proceed to sequencing when the yield of the Universal PCR is less than 3 ng/µl or less than 4 ng/µl for whole blood and FFPE samples respectively as this may impact performance. If the Universal PCR is less than the recommended concentration, it is recommended to repeat the library preparation with an increased DNA input.

# 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 |
|---|---|
| **Library preparation and target enrichment** | |
| a) No or low PCR product yield (<3 ng/µl) after Universal PCR | Concentration of DNA to be used for library preparation and target enrichment should be determined by fluorometric quantitation. |
| b) No or low PCR product yield (<3 ng/µl) after Universal PCR | Ensure that all reaction components are thoroughly mixed as described in this handbook before use.<br>Ensure that all reaction components are correctly added at each stage and thoroughly mixed as described in this handbook. |
| c) No or low PCR product yield (<3 ng/µl) after Universal PCR | Increase DNA input used for library preparation. If 40 ng was used initially increase input to 100 ng. If 100 ng was used initially increase input to 160 ng. |

# Symbols

| Symbol | Symbol definition |
|---|---|
|   | Contains reagents sufficient for <N> tests |
|   | Catalog number |
|   | Material number (i.e., component labeling) |
|  | Manufacturer |

# Appendix A: Limitations of the Assay

The BRCA Advanced panel is designed for targeted sequencing of full coding regions for the BRCA1, BRCA2, PTEN, and TP53 genes plus 20 bp into flanking introns. However, there are a few exceptions described here.

- Due to a 100% nucleotide homology with its Pseudogene PTEN-P1, a 62 bp region part of exon 9 in PTEN (ch10: 89725123 – 89725184) has been omitted from the analysis pipeline due to the inability of the system to determine the gene of origin of the detected variants.
- Eight stretches of homopolymer repeats >7 bp in various introns across the target region have been removed from the analysis due to the system's limitations to deal with these regions. Hence the region of interest (ROI) has been reduced to a minimum +2 bp to ensure the capture of splice site mutations for the following 8 introns:

    - Chr10: 89711855 – 89712036 PTEN:exon.6
    - Chr10: 89720631 – 89720895 PTEN:exon.8
    - Chr13: 32893194 – 32893482 BRCA2:exon.3
    - Chr13: 32900359 – 32900439 BRCA2:exon.6
    - Chr13: 32906389 – 32907544 BRCA2:exon.10
    - Chr13: 32968806 – 32969090 BRCA2:exon.25
    - Chr13: 32972279 – 32972927 BRCA2:exon.27
    - Chr17: 41267723 – 41267816 BRCA1:exon.3

- Should you choose to enrich just BRCA1 and BRCA2 (for example: Block A), the analysis report will flag coverage for PTEN and TP53 (Block B), because no separate analysis plug-in for the BRCA genes alone is currently offered.

# Appendix B: Analyze the Library Using QIAxcel Advanced

After the library is constructed and purified, analyze using the QIAxcel Advanced, in combination with the QIAxcel DNA High Resolution Kit (cat. no. 929002), to check the fragment size. Typically, the peak height will be within the ranges 280–450 bp, 280–350 bp, and 330–400 bp for gDNA reference standard, FFPE, and whole blood samples, respectively. The majority of the library fragments are between 200–800 bp in size (Figure 2).

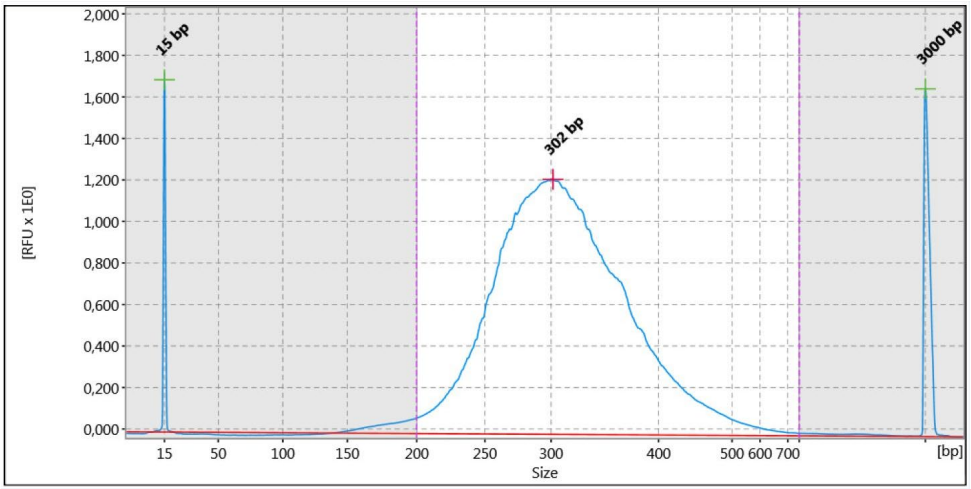


**Figure 2. Sample QIAxcel Advanced image of a GeneRead QIAact BRCA Advanced DNA UMI Panel library.** The majority of the library fragments are between 200 and 800 bp in size. Example image was generated from a gDNA reference standard. Standardly for DNA derived from FFPE tissue and whole blood samples >60% of the peak will be between 200 and 450 bp in size.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| GeneRead QIAact BRCA Advanced DNA UMI Panel (24) | GeneRead QIAact Library Preparation and Target Enrichment Reagents (24) GeneRead QIAact BRCA Advanced UMI Kit (24) | 181925 |
| **Related products** | | |
| GeneRead DNA FFPE Kit (50) | QIAamp MinElute® columns, Proteinase K, UNG, collection tubes (2 ml), buffers, Deparaffinization Solution, RNaseA | 180134 |
| QIAamp Blood DNA Midi kit (20) | QIAamp Mini Spin Columns, collection tubes (2ml), reagents and buffers | 51183 |
| QIAsymphony GeneRead DNA FFPE Treatment Kit (192) | For uracil-N-glycosylase treatment of FFPE tissue specimens using the QIAsymphony | 185306 |
| QIAsymphony DSP DNA Mini Kit (192) | For 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories | 937236 |
| QIAsymphony GeneRead DNA FFPE Treatment Kit (192) | For uracil-N-glycosylase treatment of FFPE tissue specimens using the QIAsymphony | 185306 |
| QIAsymphony DSP DNA Mini Kit (192) | For 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories. | 937236 |
| QIAsymphony GeneRead DNA FFPE Treatment Kit (192) | For uracil-N-glycosylase treatment of FFPE tissue specimens using the QIAsymphony | 185306 |

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 |
|---|---|
| 02/2020 | Added cycling conditions for emulsion making using QIAact BRCA Advanced libraries. Corrected cat. no. of "GeneRead QIAact BRCA Advanced DNA UMI Panel (24)" in "Ordering Information", from 1819251 to 181925. |
| 10/2020 | Removed mentioning of copy number variant (CNV) as it is no longer supported. Corrected errors in the titles of Tables 6 and 8. |

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