April 2018

GeneRead™ QIAact BRCA 1/2 Panel Handbook

Σ 21

For target enrichment prior to next-generation sequencing (NGS) applications that use the QIAGEN GeneReader® instrument

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

REF 181920

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

R2

Sample to Insight

QIAGEN

Contents

Kit Contents

GeneRead QIAact BRCA 1/2 Panel	**(21)**
Catalog number	**181920**
Number of reactions	**21***
BRCA Primer Mix 1†	290 µl
BRCA Primer Mix 2†	290 µl
BRCA Primer Mix 3†	290 µl
BRCA Primer Mix 4†	290 µl
Taq DNA Polymerase (HotStarTaq®, 5 units/µl)	2 x 85 µl
GR NGS Panel 5x PCR Buffer V2	465 µl
Nuclease-Free Water (clear cap)	1900 µl

* The number of reactions includes samples and any positive controls used. The positive control must be ordered separately.

† The target region is covered by 253 primer pairs distributed in these 4 tubes.

Storage

The GeneRead QIAact BRCA 1/2 Panel is shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. If stored under these conditions, the reagents are stable until the stated expiration date.

If any component of the GeneRead QIAact BRCA 1/2 Panel is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note or the reagents, contact QIAGEN Technical Services or local distributors (visit **www.qiagen.com**).

Once opened, reagents can be stored in their original packaging at –30°C to –15°C until the stated expiration date shown on the packaging. Do not exceed a maximum of 5 freeze–thaw cycles.

Intended Use

The GeneRead QIAact BRCA 1/2 Panel is intended for Research Use Only. Not for use in diagnostic procedures.

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 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 1/2 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, SNPs and small insertions and deletions. Target enrichment technology enables NGS-platform users to sequence specific regions of interest instead of the entire genome, effectively increasing sequencing depth and throughput with lower cost. The GeneRead QIAact BRCA 1/2 Panel uses multiplex-PCR-based target enrichment technology in combination with a sophisticated primer-design algorithm. This technique enables amplification and enrichment of target regions in the human genome for detection of genetic variation using NGS (Figure 1). Adjacent and potentially interacting primer pairs are separated into different pools for optimal performance. GeneRead QIAact BRCA 1/2 Panel is designed to be used with the QIAGEN GeneReader instrument. The target-enrichment process is essential for the efficient use of medium-throughput sequencers, such as the QIAGEN GeneReader.

Principle of the procedure

The GeneRead QIAact BRCA 1/2 Panel is a PCR amplicon-based assay using 4 multiplexed primer mixes designed to amplify all coding regions of the *BRCA1* and *BRCA2* genes, including the 20 intronic nucleotides adjacent to each exon. The sequences of the amplicons are determined and compared with the reference sequences for the *BRCA1/2* genes.

In the first step of the GeneRead QIAact BRCA 1/2 Panel workflow ("Protocol: Target PCR", page 11), the full coding regions of the *BRCA1/2* genes (including at least 20 nucleotides adjacent) are amplified in 4 separate multiplex PCR for each sample (Figure 1).

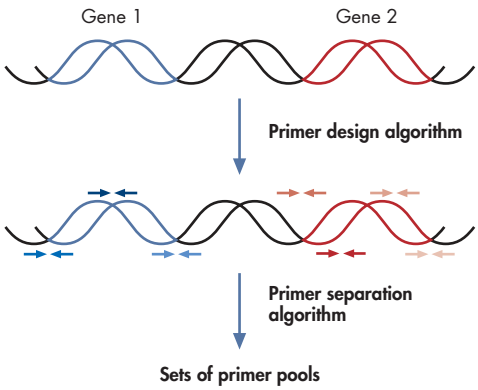


Figure 1. Multiplex PCR-based targeted enrichment scheme. The GeneRead QIAact BRCA 1/2 Panel Kit uses multiplex PCR-based targeted enrichment technology. The adjacent primer sets are distributed across four pools to minimize nonspecific amplification products.

In the second step of the workflow ("Protocol: Sample Pooling and Purification", page 13), the purified PCR products for each sample (total of 4 reactions) are pooled before proceeding to bead purification.

Subsequently the resulting PCR amplicons are subjected to library construction. Each purified PCR product is individually bar coded with gene-specific, GeneReader™-compatible adapters at each end in a universal PCR amplification (Figure 2). The prepared libraries are quantified using the QIAxcel® instrument and pooled in equimolar amounts. The pooled amplified libraries are subjected to clonal amplification by emulsion PCR before sequencing on the GeneReader platform. Following sequencing, demultiplexed raw data files (FASTQ files) are imported to the QCI-A software for deeper analysis to identify variant positions compared with the reference sequences for the *BRCA1/2* genes.

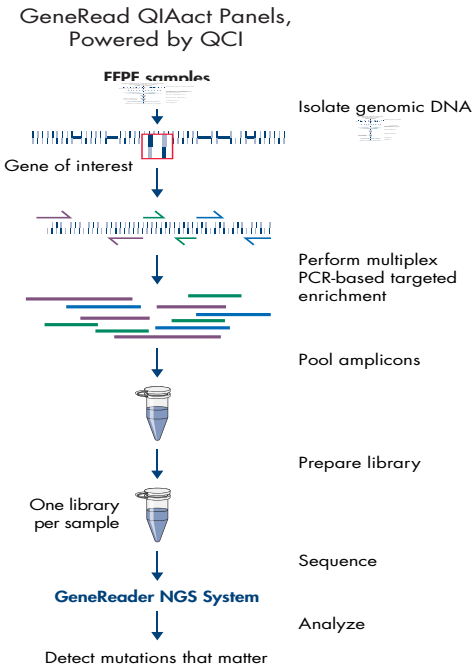


Figure 2. GeneRead QIAact 1/2 BRCA Panel procedure.

To ensure good quality results, in-process control criteria are used throughout the workflow ("Appendix C: In-Process Control Criteria", page 20). These criteria allow validation of the different steps of the workflow to identify samples that give poor sequencing results.

Recommendation for multiplexing and clonal amplification input

More than one DNA sample can be sequenced in one flow cell, based on the sample-specific bar code that is added during library preparation (see the "Protocol: End Repair, Adapter Ligation and Size Selection of DNA" section of the *GeneRead DNA Library Q Handbook* for more information). The sequencing libraries prepared from the corresponding samples must be pooled prior to clonal amplification to be sequenced together in one flow cell (see the "Protocol: Library Concentration Normalization and Pooling" section of the *GeneRead Clonal Amp Q Handbook* for more information). Based on the total number of amplicons that are produced by the GeneRead QIAact BRCA 1/2 Panel and the sequencing kit output, we recommend using a maximum multiplex of 7 samples per sequencing flow cell. The protocol in this handbook describes the preparation of 7 samples per flow cell; however, the procedure can be scaled up for multiple batches. After target enrichment and library preparation, use 400 pg pooled DNA in the clonal amplification process (see "Preparing Libraries for Emulsion Making" in the *GeneRead Clonal Amp Q Handbook* for more information).

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, please consult the appropriate safety data sheets (SDSs) available from the product supplier.

For DNA isolation

See "DNA preparation", page 10 for specific recommendations.

For target enrichment

- High-quality, nuclease-free water (e.g., Nuclease-Free Water [10 x 50 ml], cat. no. 129114). **Do not use DEPC treated water.**
- 96–100% ethanol
- Agencourt® AMPure® XP Kit (Beckman Coulter cat. no. A63880)
- Magnetic stand for 1.5 ml microcentrifuge tubes (e.g., Life Technologies® cat. no. 12321D)
- Microcentrifuge*
- 1.5 ml LoBind tubes (e.g., Eppendorf® AG)
- 0.2 ml PCR tubes, 96-well reaction plates or PCR strips and caps
- Thermal cycler* (e.g., Bio-Rad® C1000 Touch™ or Applied Biosystems 2720)
- Pipette and pipette tips
- Qubit® 3.0 Fluorometer* (Life Technologies, cat. no. Q33216) or comparable
- Qubit dsDNA BR Assay Kit (Life Technologies, cat. no. Q32853)
- Qubit assay tubes (e.g., Life Technologies, cat. no. Q32856)
- QIAxcel® Advanced instrument* (for information, visit **www.qiagen.com**)
- QIAxcel DNA High Resolution Kit (1200) (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)

* Make sure that instruments have been checked, maintained and calibrated regularly according to the manufacturer's instructions.

Optional (not included)

- Positive control DNA to test target PCR efficiency: e.g., NA12878 from Coriell Institute for Medical Research repository

- QIAseq™ DNA QuantiMIZE Array Kit (cat. no. 333404) and QIAseq DNA QuantiMIZE Assay Kit (cat. no. 333414) can be used to assess sample integrity following DNA extraction from formalin-fixed, paraffin-embedded (FFPE) samples (see "Appendix A: FFPE-Purified DNA Quality" page 17).

Important Notes

DNA preparation

GeneRead QIAact BRCA 1/2 assay is only compatible with samples from FFPE tissues.

The most important prerequisite for DNA sequence analysis is consistent, high-quality DNA from every experimental sample. Therefore, sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, alcohol or other contaminants can degrade the DNA or decrease the efficiency or block completely the enzyme activities necessary for optimal targeted genome amplification.

QIAGEN's GeneRead DNA FFPE Kit (cat. no. 180134) is highly recommended for manual and semi-automated (QIAcube) preparation of genomic DNA samples from FFPE tissue samples. Ensure that samples have been treated to remove RNA, as RNA contamination will cause inaccuracies in DNA concentration measurements. For higher throughputs, the DNA extraction can be automated on the QIAsymphony® instrument using the QIAsymphony GeneRead DNA FFPE Treatment Kit (cat. no. 185306) in combination with the QIAsymphony DSP DNA Mini Kit (cat. no. 937236).

DNA quantification

The concentration of DNA should be determined by fluorometric quantitation using the Qubit Fluorometer and the Qubit dsDNA BR Assay Kit. We recommend using a DNA concentration of 2.5 ng/µl for target enrichment from FFPE.

Protocol: Target PCR

The GeneRead QIAact BRCA 1/2 Panel allows testing of a total of 21 samples in three sequencing flow cells. We recommend performing each sequencing run with a maximum plexity of 7 samples per flow cell (an optional positive control, such as NA12878, can be included to verify efficiency of Target PCR). An example plate layout is shown in Figure 3.

Be sure to use only the protocols provided in the *GeneRead QIAact BRCA 1/2 Panel Handbook* when using the GeneRead QIAact BRCA 1/2 Panel.

		1	2	3	4	5	6	7	8	9	10	11	12
	A	–	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–	–
Primer Mix 1	C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	–	–	–	–	–
Primer Mix 2	D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	–	–	–	–	–
Primer Mix 3	E	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	–	–	–	–	–
Primer Mix 4	F	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	–	–	–	–	–
	G	–	–	–	–	–	–	–	–	–	–	–	–
	H	–	–	–	–	–	–	–	–	–	–	–	–

Figure 3. Layout scheme of the 96-well target PCR plate.

Procedure

1. Thaw reagents on ice.
2. Dilute DNA to 2.5 ng/µl with nuclease-free water in a LoBind tube. For each sample, 10 ng (4 µl, 2.5 ng/µl) is required for each of 4 PCRs (i.e., a total of 40 ng per sample).

   We recommend testing no more than 7 samples per sequencing flow cell with this assay.
3. Prepare PCR strips or a 96-well PCR plate according to the number of reactions. Label with sample names and pool numbers.
4. Prepare the 4 target PCR mixes according to Table 1. For each sample, 4 target PCR mixes are required. Mix gently by pipetting up and down.

Table 1. Preparation of target PCR mixes for each primer mix pool

Component	1 reaction (µl)	7 reactions (µl)
BRCA Primer Mix 1, 2, 3 or 4	11	77
Taq DNA Polymerase (HotStarTaq, 5 units/µl)	1.5	10.5
GR NGS Panel 5x PCR Buffer V2	4.4	30.8
Nuclease-Free Water	0.7	4.9
Total volume*	**17.6***	**123.2***

* Includes additional volume for pipetting loss: use 16 µl per sample.

5. Dispense 16 µl target PCR mix into wells of a 96-well plate according to the plate layout described in Figure 3.
6. Add 4 µl of each DNA sample to the reaction mix of each PCR according to the plate layout shown in Figure 3, page 11. Mix gently by pipetting up and down.
7. Seal the wells before loading into a thermal cycle. Program the cycling conditions as listed in Table 2.

Table 2. Cycling conditions

Time	Temperature	Cycles
15 minutes	95°C	1
15 seconds 150 seconds	95°C 60°C	26
10 minutes	72°C	1
Hold	4°C	∞

Note: The launch of the target PCR can be delayed up to 6 hours if reactions mixes are stored at 2–8°C.

8. After the reaction is complete, proceed with "Protocol: Sample Pooling and Purification", below.

   Note: If the samples are to be stored prior to purification, store them at –30°C to –15°C freezer for up to 72 hours.

Protocol: Sample Pooling and Purification

This protocol describes the procedure for sample pooling and purification. For PCR set up, see "Protocol: Sample Pooling and Purification", page 11.

IMPORTANT: Equilibrate the AMPure XP beads at room temperature (15–25°C) before use. Vortex the AMPure XP beads thoroughly before use.

1. Centrifuge the tubes, plates or strips briefly to collect liquid at the bottom. For each sample, combine all 4 reactions into one PCR plate/strip. If the volume is less than 80 µl (4 x 20 µl), add water to a final volume of 80 µl to ensure the correct ratio of beads to sample. Mix thoroughly.
2. Dispense 72 µl AMPure XP beads into a 1.5 ml LoBind tube for purification.
3. Transfer 80 µl pooled sample reactions to the AMPure XP beads (0.9x volume). Mix well using a vortex mixer or by pipetting up and down at least 10 times. Pulse-spin the tube.
4. Incubate for 5 minutes at room temperature.

   Place the tube on magnetic rack to separate beads from supernatant. After the solution is clear (approximately 5 minutes), carefully transfer 140 µl supernatant to a new 1.5 ml LoBind tube without disturbing the beads. Do not discard the supernatant. Discard the beads, which contain unwanted large DNA fragments.

   IMPORTANT: Transferring 140 µl supernatant will leave behind approximately 12 µl. This remainder is left is to ensure that beads are not carried over with the transferred supernatant.
5. Add 128 µl AMPure XP beads to the supernatant, mix well, pulse-spin and incubate for 5 minutes at room temperature.
6. Place the tube on a magnetic rack and wait until solution is clear (about 5 minutes). Carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the target DNA.

   IMPORTANT: Do not discard the beads.
7. Add 400 µl fresh 80% ethanol to the tube while it is on the magnetic rack. Incubate at room temperature for 30 seconds and then carefully remove and discard the supernatant.
8. Repeat previous step once.
9. Briefly spin the tube and place on the magnetic rack. Completely remove residual ethanol and dry the beads for 15 minutes (or until the beads are dry) while the tube is on the rack with the lid open.

10. Transfer 28 µl nuclease-free water to the beads. Mix well by vortexing. Spin down briefly and place the tube on the magnetic rack until the solution is clear. Transfer 25 µl supernatant to a clean 1.5 ml nuclease-free LoBind tube.
11. Determine the amount of PCR-enriched DNA using the QIAxcel Advanced instrument and the QIAxcel DNA High Resolution Kit.

    Note: See "Appendix B: Target-enriched DNA Quality Control Methods", page 18.

    Note: See "Appendix C: In-process control criteria", page 20.
12. Proceed to library construction according to the *GeneRead DNA Library Q Handbook*.

    Note: If reactions are to be stored prior to library construction, transfer them to a –30° to –15°C freezer. Samples are stable for 3 days.

    Note: Before proceeding to library preparation, the concentrations of the positive control and the samples must be >20 ng/µl and >4 ng/µl, respectively.

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**).

For troubleshooting information relating to other kits, please refer to the corresponding kit handbooks.

		Comments and suggestions
Low yield of target PCR DNA		
a)	Check the genomic DNA concentration	We recommend using a fluorometer for genomic DNA quantitation from FFPE starting material. The genomic DNA concentration must be >2.5 ng/µl to ensure sufficient sample quantity for downstream experiments. The kit is optimized for 10 ng genomic DNA per target PCR (40 ng total for one sample).
b)	Check concentration of samples after target PCR pooling and purification	If sample concentration is <4 ng/µl (or <20 ng/µl, when the optional NA12878 positive control has been used) an error may have occurred during the target PCR or during target PCR pooling and purification step. Repeat the target PCR step for all the samples. Sample concentration below the quality control criterion of 4 ng/µl for an FFPE sample may indicate degraded DNA. Repeat DNA extraction from the failed sample.
c)	Presence of peaks of approximately 157 bp after target PCR	Peaks from fragments >157 bp represent nonspecific amplification artifacts resulting from insufficient depletion of large fragments after multiplex PCR. The presence of large fragments in the target-enriched DNA will result in the presence of large fragments after library preparation, which may affect the sequencing result. Make sure to avoid bead carryover in the first Agencourt AMPure XP bead separation. Check that the magnetic stand used for separation provides quantitative separation of Agencourt AMPure XP beads

Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
REF	Catalog number
	Manufacturer
	Use by
 <N>	Contains reagents sufficient for N reactions

Appendix A: FFPE-Purified DNA Quality

Genomic DNA present in FFPE sample archives is usually damaged and fragmented to an uncertain extent. Commonly used DNA quantification methods, including spectrometers or fluorometers, do not differentiate between amplifiable and non-amplifiable DNA. Therefore, they cannot reliably measure the amplifiable amounts of DNA that are suitable for the multiplex PCR-based target enrichment step in the NGS workflow. The QIAseq DNA QuantiMIZE and GeneRead DNA QuantiMIZE Systems are qPCR-based approaches that determine the quality of DNA that is amenable to PCR-based target enrichment prior to NGS. The system provides a sensitive and accurate approach to qualify DNA isolated from biological samples and is primarily for FFPE samples. Please refer to the corresponding kit handbook for determining FFPE sample-derived DNA quality with the QIAseq DNA QuantiMIZE or GeneRead DNA QuantiMIZE Systems. For recommendation on how to proceed based on the quality indicating QC score, see Table 3.

Table 3. Explanation of QC scores

QC score	QC call	Recommendations
≤ 0.04	High	Quality of DNA is sufficient: proceed
> 0.04	Low	1. The DNA is highly fragmented or damaged; proceed with caution if working with FFPE samples. 2. The genomic DNA may only be suitable for detection of high-frequency variants. Most low-frequency C>T or G>A variants are not reliable. Repeat genomic DNA extraction with GeneRead DNA FFPE Kit (including UNG treatment step) to reduce artificial "U" present in the FFPE sample.

Appendix B: Target-enriched DNA Quality Control Methods

After the multiplex PCR run, sample pooling and purification, the PCR product can be analyzed using the QIAxcel Advanced instrument. An example sample analysis image is provided in Figure 4. The amplicons should be in the correct size range (usually approximately 157 bp).

Recommended method for library control

To assess the quality of PCR-enriched DNA, we recommend the following analytical device:

- QIAGEN's QIAxcel Advanced instrument and ScreenGel Software 1.5 (or higher) in combination with the QIAxcel DNA High Resolution Kit (cat. no. 929002).

Refer to the instrument user manual and kit handbook for setting up the analyses of DNA samples; also, see the QIAxcel Advanced System guide *NGS Sample Quality Control using the QIAxcel Advanced System*". For sample preparation and data analysis, we recommend the setups described in the following sections.

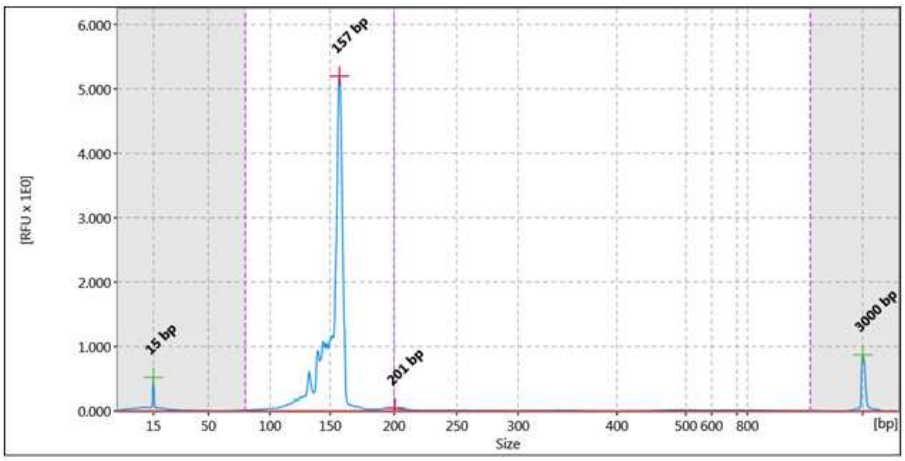


Figure 4. Trace data from the QIAxcel Advanced capillary electrophoresis instrument, showing the pooled and purified multiplex PCR product of the GeneRead QIAact BRCA1/2 Panel. A peak observed at approximately 157 bp represents the amplicons.

QIAxcel Advanced instrument setup

- For final PCR-enriched DNA analysis, use 2 µl sample and adjust volume to 10 µl with Buffer EBA.

  Note: Buffer EBA is provided in the GeneRead DNA Library Q Kit (48) (cat. no. 185444).

  Note: Adjusting the 2 µl aliquot to 10 µl with Buffer EBA results in a final dilution of 1/5. Make sure to recalculate the dilution factor for final PCR-enriched DNA quantification results. Depending on DNA quality, the final output may vary in yield. It can be necessary to re-adjust the dilution factor and repeat the analysis on the QIAxcel.

  Note: The QIAxcel Advanced instrument requires a volume of 10 µl for analysis.

  Note: The QX DNA Size Marker must be diluted with Buffer EBA for analysis.
- Use the QIAxcel ScreenGel Software version 1.5 or higher.
- QIAxcel DNA High Resolution Kit (cat. no.929002) should be used for final PCR-enriched DNA analyses.
- QX DNA Size Marker 50–800 bp (50 µl) v2.0 (cat. no. 929561) and QX Alignment Marker 15 bp/3 kb (1.5 ml) (cat. no. 929522) should be used for final PCR-enriched DNA analyses.
- For further information about PCR-enriched DNA analysis, refer to the QIAxcel Advanced System guide *NGS Sample Quality Control using the QIAxcel Advanced System*, available from **www.qiagen.com**.

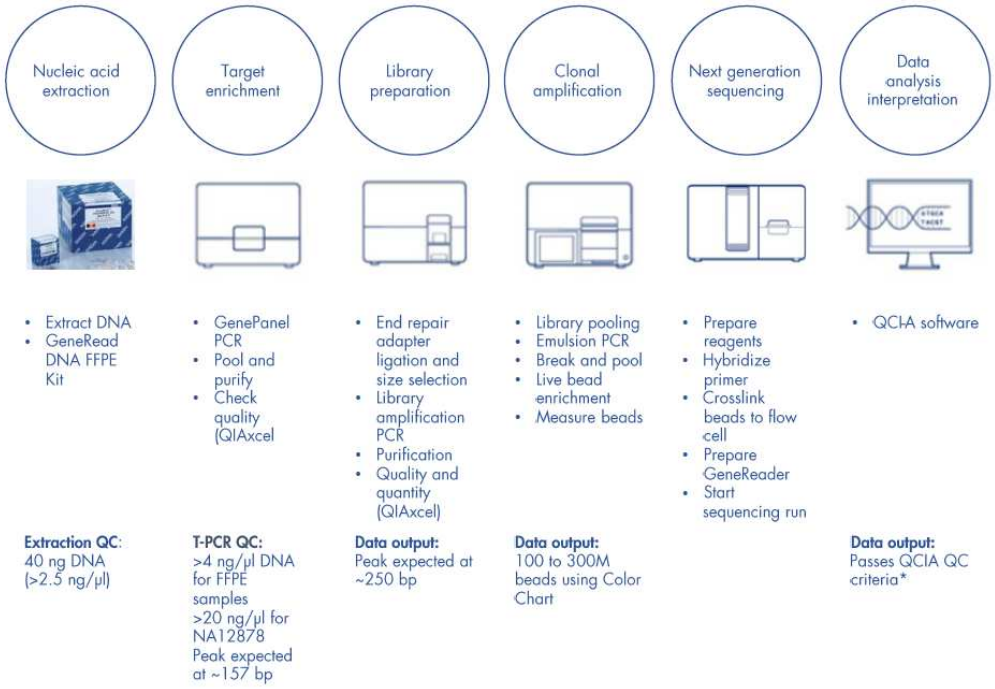
Alternative method for library control

- Alternatively, use the Agilent® Bioanalyzer® instrument in combination with the Agilent High-Sensitivity DNA Kit (Agilent Technologies® cat. no. 5067-4626).
- The Agilent Bioanalyzer instrument uses 1 µl samples. For final PCR-enriched DNA analysis, pre-dilute the sample by adjusting a 1 µl sample to 10 µl with RNase-free water.

  Note: Adjusting a 1 µl aliquot to 10 µl with RNase-free water results in a final dilution of 1/10.

Appendix C: In-Process Control Criteria

Several in-process control steps are performed in the sequencing workflow to validate sample extraction, target PCR, library preparation, clonal amplification and data analysis. The final criterion used to ensure good-quality variant calling at a given position is the minimum coverage obtained.



* "1 Summary" tab of QCIA report.

Figure 5. GeneRead QIAact BRCA 1/2 Panel workflow showing in-process quality control steps.

Ordering Information

Product	Contents	Cat. no.
GeneRead QIAact BRCA 1/2 Panel (18)	For 20 reactions: For the identification of variants in *BRCA1* and *BRCA2* with Illumina MiSeqDx platform; BRCA Primer Mix 1, BRCA Primer Mix 2, BRCA Primer Mix 3, BRCA Primer Mix 4, HotStarTaq DNA polymerase, GR NGS Panel 5x PCR Buffer V2, nuclease-free water for NTC	181920
Related products		
GeneRead DNA FFPE Kit (50)	QIAamp® MinElute® columns, Proteinase K, UNG, Collection Tubes (2 ml), Buffers, Deparaffinization Solution, RNase A	180134
QIAsymphony GeneRead DNA FFPE Treatment Kit	For uracil-N-glycosylase treatment of FFPE tissue specimens using the QIAsymphony SP Tissue_LC_200_V7_DSP protocol	185306
QIAsymphony DSP DNA Mini Kit	For 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories.	937236
QIAseq DNA QuantiMIZE Array Kit	2 arrays in Formats A, C, D, or F; or 2 discs in Format R; or 1 array in Format E or G; QuantiMIZE Control gDNA (50 µl; GeneRead qPCR SYBR Green Mastermix (2 x 1.35 ml)	333404
QIAseq DNA QuantiMIZE Assay Kit	Assay 100 and Assay 200 for 400 x 25 µl reactions (400 µl) each; QuantiMIZE Control gDNA (50 µl); RNase- and DNase-free water (1 ml x 4); GeneRead qPCR SYBR Green Mastermix (1.35 ml x 4)	333414
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX DNA Size Marker 50–800 bp (50ul) v2.0	DNA size marker with fragments of 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, and 800 bp; concentration 100 ng/µl	929561
QX Alignment Marker 15 bp/3 kb (1.5 ml)	Alignment marker with 15 bp and 3 kb fragments	929522

Product	Contents	Cat. no.
Nuclease-Free Water (10 x 50 ml)	10 x 50 ml nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); provided in 10 x 50 plastic tubes	129114
QX Nitrogen Cylinder (6)	6 QIAxcel Nitrogen Cylinders	929705

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.

Revision History

Document revision history	
R5 04/2018	Added revision history

Limited License Agreement for the GeneRead QIAact BRCA 1/2 Panel

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at **www.qiagen.com**. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see **www.qiagen.com**.

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 6,127,155, 5,677,152 (claims 1 to 23 only), 5,773,258 (claims 1 and 6 only), and claims outside the US corresponding to expired US Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.