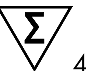
July 2016

GeneRead™ QIAact Panels, Powered by QCI™, Handbook



For targeted 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 181910

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MAT 1103411

Sample to Insight

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Kit Contents

GeneRead QIAact Panels, Powered by QCI	
Catalog no.	**181910***
Actionable Insights Tumor Panel (additional ordering information: GRTP-101X-12*): pools with 747 variant positions in 12 genes for 12 samples†	4

* Gene panel pools are labeled A1, A2, A3, and A4.

† Product no. GRTP-101X-12 must be specified when ordering cat. no. 181910.

One of the following kits with PCR reagents for use with the GeneRead QIAact Panels must be ordered separately.

Kit	Catalog no.
GeneRead DNAseq Panel PCR Kit V2 (12)	181940
GeneRead DNAseq Panel PCR Kit V2 (96)	181942

Storage

GeneRead QIAact Panels, Powered by QCI, (cat. no. 181910) are shipped on dry ice and should be stored at –30°C to –15°C in a constant-temperature freezer upon arrival. If stored under these conditions, the reagents are stable for up to 6 months after delivery.

The GeneRead DNAseq Panel PCR Kit V2 (cat. no. 181940 or 181942) is shipped on cold packs. For long-term storage, keep tubes at –30°C to –15°C. If the entire volume will not be used at once, we recommend dividing into aliquots and storing at –30°C to –15°C. Avoid repeated freezing and thawing. If stored under these conditions, the GeneRead DNAseq Panel PCR Kit V2 is stable for 6 months after receipt.

Intended Use

GeneRead QIAact Panels, Powered by QCI, and GeneRead DNAseq Panel PCR Kits V2 are 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 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 DNAseq Panel PCR Kit V2 and GeneRead QIAact Panels, Powered by QCI, are 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. Targeted 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. GeneRead QIAact Panels, Powered by QCI, use multiplex PCR-based targeted enrichment technology in combination with a sophisticated primer-design algorithm. This technique enables amplification and enrichment of any gene or targeted region in the human genome for detection of genetic variation using NGS (Figure 1, page 6). Adjacent and potentially interacting primer pairs are separated into different pools for optimal performance. GeneRead QIAact Panels, Powered by QCI, are designed to analyze a panel of genes and can be used with the QIAGEN GeneReader instrument. The targeted enrichment process is essential for the efficient utilization of medium-throughput sequencers, such as the QIAGEN GeneReader.

GeneRead QIAact Panels, Powered by QCI, have been optimized in combination with the GeneRead DNAseq Panel PCR Kit V2 to provide superior sensitivity and linear multiplex amplification. The simplicity of the PCR method makes these panels accessible for routine use in every clinical research laboratory.

The Actionable Insights Tumor Panel, one of the GeneRead QIAact Panels, Powered by QCI, was developed with an unprecedented process. The panel leverages the expertly curated QIAGEN Knowledge Base, which focuses only on clinically relevant findings, such as approved therapeutics labels, professional association practice guidelines, and active late-stage clinical trials. The design results in a unique set of genes and variants with an unparalleled level of direct relevance that provides necessary and sufficient insights.

Principle and procedure

The Actionable Insights Tumor Panel covers specific hotspots designed to detect SNVs and small (<20 bp) indels with the provided one 4-pool set. GeneRead QIAact Panels, Powered by QCI, can enrich selected genes and/or regions using as little as 40 ng genomic DNA in 3 hours for a 4-pool panel (Figure 2). DNA samples are combined with the primer mix and PCR reagent, and PCR is performed in a standard thermocycler. After they are complete, the reactions for each sample are pooled and the enriched DNA is purified (Figure 3). The purified DNA is then ready for NGS library construction and sequencing using the QIAGEN GeneReader.

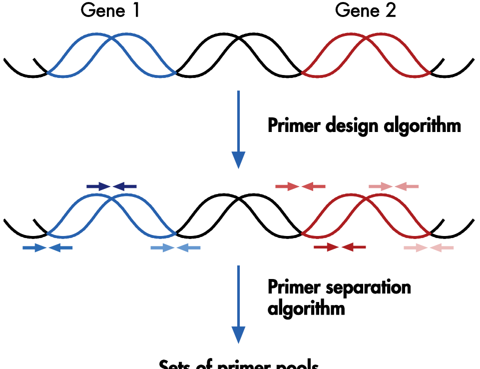

Figure 1. Multiplex PCR-based targeted enrichment scheme. Actionable Insights Tumor Panel uses multiplex PCR-based targeted enrichment technology in combination with a sophisticated primer-design algorithm to maximize design coverage and minimize nonspecific amplification. The adjacent primer sets are distributed across an appropriate number of pools to minimize nonspecific amplification products.

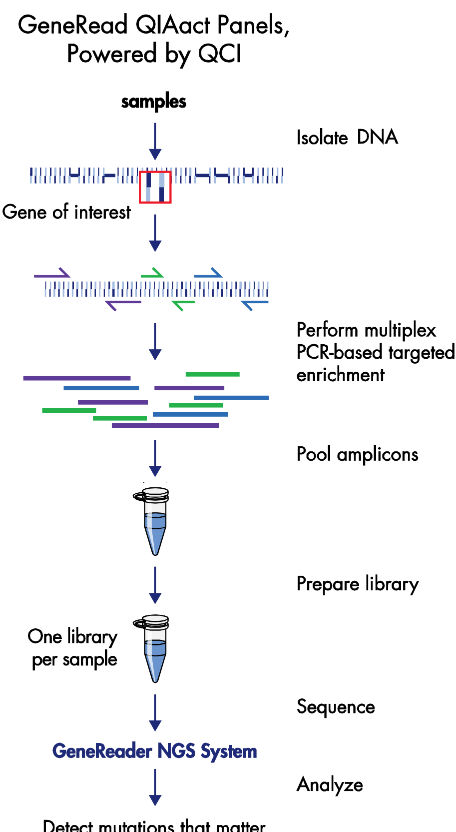

Figure 2. The GeneRead QIAact Panels, Powered by QCI, procedure.

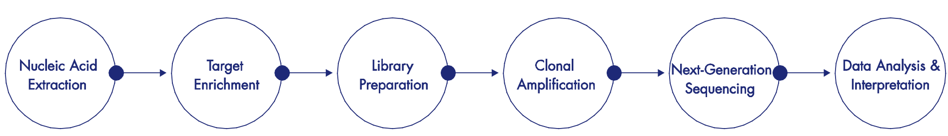

Figure 3. Overview of the complete NGS workflow with Actionable Insights Tumor Panel. The complete sample-to-interpretation procedure begins with DNA extraction, followed by targeted enrichment with Actionable Insights Tumor Panel, NGS library construction, sequencing, and data analysis.

Recommendation for multiplexing and clonal amplification input

Based on the total number of amplicons that are produced by the GRTP-101X panel, we recommend multiplexing up to 10-plex for FFPE samples and 6-plex for liquid biopsy samples. After target enrichment and library preparation, use 400 pg pooled DNA in the clonal amplification process (see the "Preparing Libraries for Emulsion Making" procedure section in the *QIAGEN 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, consult the appropriate safety data sheets (SDSs), available from the product supplier.

In addition to the Actionable Insights Tumor Panel and GeneRead DNAseq Panel PCR Kit V2, the following supplies are required:

For genomic DNA isolation

- See “Recommended genomic DNA preparation method” on page 11 for specific recommendations.

For targeted enrichment

- GeneRead DNAseq Panel PCR Kit V2 (cat. no. 181940 for 12 samples or 181942 for 96 samples)
- High-quality, nuclease-free water (e.g., Nuclease-Free Water [10 x 50 ml], cat. no. 129114). **Do not use DEPC-treated water**.
- 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*

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

- Pipet tips and pipetsQIAxcel® Advanced instrument* (for information, visit **www.qiagen.com**)
- QIAxcel DNA High Resolution Kit (1200) (100 runs/12 samples) (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)

Optional

- GeneRead DNA QuantiMIZE Array or Assay Kit (e.g., QIAGEN cat. nos. 180642 to180654) if using formalin-fixed, paraffin-embedded (FFPE) samples

Important Notes

DNA preparation and quality control

High-quality DNA is essential for obtaining good sequencing results

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 and real-time PCR performance.

Recommended genomic DNA preparation method

QIAGEN's GeneRead DNA FFPE Kit (cat. no. 180134) is highly recommended for the preparation of genomic DNA samples from fresh tissues and FFPE tissue samples. Ensure that samples have been treated to remove RNA, as RNA contamination will cause inaccuracies in DNA concentration measurements. **Do not omit** the recommended RNase treatment step to remove RNA. If genomic DNA samples are to be harvested from biological samples for which kits are not available, please contact QIAGEN Technical Services for suggestions.

DNA quantification and quality control

For best results, all DNA samples should also demonstrate consistent quality according to the criteria described in the following sections.

Concentration and purity determined by UV spectrophotometry

The concentration and purity of DNA should be determined by measuring the absorbance in a spectrophotometer. Prepare dilutions and measure absorbance in 10 mM Tris·Cl,* 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

Concentration determined by A_{260} should be >2.5 μg/ml DNA.

Optional: If DNA purified from FFPE samples will be used for GeneRead QIAact Panels, Powered by QCI, the QIAGEN GeneRead® DNA QuantiMIZE Array or Assay Kit is recommended for determining optimal DNA amount and PCR cycling conditions for each FFPE-purified DNA sample (see "Appendix B: FFPE-Purified DNA Quality and Quantity" page 25).

* 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.

Recommendations for using liquid biopsy samples

It is important to note that the method of nucleic acid preparation used and the resulting sample quality have a major impact on the quality of the sequencing data. To support nucleic acid preparation, we suggest the following to ensure optimal results from liquid biopsy samples:

- Optimal extraction of cfDNA from liquid biopsy samples can be safeguarded by drawing blood into sample collection tubes that provide efficient stabilization of the liquid biopsy sample.
- Analysis of extracted DNA using capillary electrophoresis allows detection of contamination with high-molecular-weight material, which may affect the performance of the sequencing system (e.g., with the QIAGEN QIAxcel Advanced, cat. no. 9001941).
- Extraction of DNA from 4–5 ml plasma and elution in the lowest volume stated in the *QIAamp Circulating Nucleic Acid Kit Handbook* will help maximize DNA yields from liquid biopsy samples. Low DNA yields can present challenges, such as providing insufficient input material for detecting low frequency variants. It is recommended to use a DNA concentration of 2.5 ng/µl; however, concentrations as low as 1 ng/µl can be used for NGS processing. Use of less than 1ng/µl is not recommended as this presents a risk to the outcome of the sequencing results.

Protocol: PCR Setup

This protocol describes the procedure for PCR setup. For sample pooling and purification, see "Protocol: Sample Pooling and Purification", page 17.

Procedure

1. Remove PCR reagents (QIAact Panel primer mix and DNAseq Panel PCR kit) the from freezer, and thaw at room temperature (15°C to 25°C). Do not remove the polymerase from the freezer until directly before use.
2. Determine the number of amplification reactions needed. Prepare PCR strips or a PCR plate according to the number of reactions. Label with sample names and pool numbers.

   Note: The Actionable Insights Tumor Panel consists of 4 primer mixes (A1–A4), each of which requires a separate amplification reaction (i.e., 4 reactions per sample).
3. Prepare PCR mixes according to Table 1. Mix gently by pipetting up and down or vortexing briefly.

   Note: For each sample, 4 PCR mixes corresponding to each primer mix pool (A1–A4) must be prepared for use with the Actionable Insights Tumor Panel (GRTP-101X).

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

Component	Per sample (µl)	Per *n* samples (µl)
GeneRead DNAseq Panel PCR Buffer (5x)	4.4	4.4 x *n*
Primer mix pool (2x) (A1, A2, A3, or A4)	11	11 x *n*
GeneRead HotStarTaq® DNA Polymerase (6 U/µl)	1.5	1.5 x *n*
DNase-free water	0.7	0.7 x *n*
Total volume*	**17.6***	**17.6 x *n******

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

4. Aliquot 16 µl of each PCR mix into the PCR strips or PCR plate (see Table 2 for an example layout).

Table 2. Layout example for sample positions in strips and columns

		Column											
Strip		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	Sample:	1	1	1	1	2	2	2	2	3	3	3	3
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
2	Sample:	4	4	4	4	5	5	5	5	6	6	6	6
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
3	Sample:	7	7	7	7	8	8	8	8	9	9	9	9
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
4	Sample:	10	10	10	10	11	11	11	11	12	12	12	12
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
5	Sample:	13	13	13	13	14	14	14	14	15	15	15	15
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
6	Sample:	16	16	16	16	17	17	17	17	18	18	18	18
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
7	Sample:	19	19	19	19	20	20	20	20	21	21	21	21
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
8	Sample:	22	22	22	22	23	23	23	23	24	24	24	24
	Primer:	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4

5. Dilute sample DNA to a concentration of 2.5 ng/µl with DNase-free water in a LoBind tube. For each amplification reaction, 4 µl (2.5 ng/µl) is needed.

   Note: For the Actionable Insights Tumor Panel, 4 amplification reactions with 4 µl per sample require a total volume of 16 µl; we recommend preparing, for example, 20 µl of diluted sample to ensure sufficient sample volume for all reactions.

6. Aliquot 4 µl of each DNA sample (2.5 ng/µl) into the corresponding PCR tube. Mix gently by pipetting up and down. If required, pulse-spin the tubes or plate to collect drops from the walls.

7. **Note**: Samples with concentrations less than 2.5 ng/µl can be used for targeted enrichment PCR but might lead to low amplification concentrations. If samples less than 2.5 ng/µl are used, use 4 µl undiluted sample for each PCR tube.
8. Seal the wells with PCR tube caps. Place strips or plate in thermocycler, and set up reaction parameters according to Table 3.

   Note: If the GeneRead DNA QuantiMIZE Array or Assay Kit is used, adjust the cycles according to the recommendation from the QuantiMIZE data analysis. If GeneRead DNA QuantiMIZE Kits are not used to adjust the number of PCR cycles, proceed with 26 cycles for all samples. The use of the GeneRead DNA QuantiMIZE Kits is recommended for quantification and quality control of the DNA.

Table 3. Cycling conditions

Time	Temperature	Number of cycles
15 min	95°C	1
15 s	95°C	26
4 min	60°C	26
10 min	72°C	1
Hold	4°C	∞

9. After the reaction is complete, place samples on ice, and proceed with sample pooling and purification using Agencourt AMPure XP beads.

   Note: If the samples are to be stored prior to purification, transfer them to a –20°C freezer for up to 72 h.

Protocol: Sample Pooling and Purification

This protocol describes the procedure for sample pooling and purification. For PCR setup, see "Protocol: PCR Setup", page 14.

Procedure

1. Combine all 4 reactions from the same sample into one well of a PCR plate or strip. Mix thoroughly. The volume of each sample should be approximately 80 µl.
2. Transfer 80 µl from each sample to a 1.5 ml LoBind tube for purification.

   Note: If the volume is not 80 µl, adjust the volume by adding water to the sample.
3. Add 72 µl (0.9x volume) Agencourt AMPure XP beads to 80 µl PCR product. Mix well on a vortex mixer or by pipetting up and down at least 10 times.

   Note: Amplicons larger than intended size will bind to the beads and be removed. Using a volume of <0.9x AMPure beads will result in inefficient cleanup of unwanted larger amplicons, whereas >0.9x can lead to loss of intended amplicons.
4. Incubate for 5 min at room temperature (15°C to 25°C).
5. Pulse-spin the tube. Place the tube on the magnetic rack to separate the beads from the supernatant.
6. After the solution is clear (approximately 5 min), carefully transfer 140 µl supernatant to a new tube, without disturbing the beads. Discard the beads, which contain unwanted large DNA fragments.

   Note: Do not discard the supernatant.

   IMPORTANT: Removing 140 µl supernatant will leave behind approximately 12 µl supernatant, ensuring that no beads are carried over into the supernatant. Bead carryover can result in a significant amount of large fragments present in the library, which will affect sequencing specificity.

7. Add 128 µl Agencourt AMPure XP beads (this is 1.6x the original volume of PCR product, which was 80 µl) to the supernatant, mix well and incubate for 5 min at room temperature. Agencourt AMPure XP beads should be stored at 2–8°C after use.

   Note: Intended amplicons will bind to the beads allowing recovery. Using <1.6x volume AMPure bead ratio can potentially result in loss of desired amplicons, whereas >1.6x can lead to carryover of primer–dimers.
8. Pulse-spin the tube. Place the tube on the magnetic rack, and wait until the solution is clear (approximately 5 min).
9. Carefully remove and discard the supernatant. Be careful not to disturb the beads, which contain the DNA targets.

   Note: Do not discard the beads.
10. Add 400 µl fresh 80% ethanol to the tube while it is on the magnetic rack. Incubate at room temperature for 30 s, and then carefully remove and discard the supernatant.
11. Repeat step 10 once.
12. Briefly spin the tube, and place on the magnetic rack. Completely remove residual ethanol, and dry beads for 15 min while the tube is on the rack with the lid open.
13. Elute DNA target beads with 28 µl nuclease-free water. Mix well by vortexing. Spin down briefly, and place the tube on the rack until the solution is clear. Transfer 25 µl supernatant to a clean LoBind tube.
14. Determine the amount PCR-enriched DNA using the QIAxcel Advanced instrument and the QIAxcel DNA High Resolution Kit (1200). Normally, 25–350 ng PCR product is obtained after purification.

    Note: See “Appendix A: PCR-enriched DNA Quality Control Methods”, page 22.
15. Proceed to library construction according to the *QIAGEN GeneRead DNA Library Q Handbook*.

    Note: If reactions are to be stored prior to library construction, transfer them to a –20°C freezer for up to 72 h.

Troubleshooting Guide

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

	Comments and suggestions
Low yields of PCR-enriched DNA	
a) Suboptimal reaction conditions due to low DNA quality	Make sure to use high-quality DNA to ensure optimal amplification and enrichment of targeted region.
b) Low or undetectable levels of PCR-enriched DNA present after purification	Check that the DNA integrity and amount of genomic DNA used for targeted enrichment (40 ng total; corresponding to 16 µl at 2.5 ng/µl, see page 14). Make sure that correct PCR setup and PCR cycling was used. Check that purification of pooled PCR was performed using correct stoichiometry of Agencourt AMPure XP beads. Shake Agencourt AMPure XP beads bottle before use to resuspend settled particles. After the 80% ethanol wash, do not dry the Agencourt AMPure XP beads for longer than 15 minutes.
Unexpected signal peaks in capillary electrophoresis device traces	
Presence of peaks larger than 220 bp	Peaks from fragments >220 bp represent nonspecific amplification artifacts resulting from insufficient depletion of large fragments after multiplex PCR. The presence of large fragments in the PCR-enriched DNA will result in a significant amount of large fragments present after library preparation, which will affect sequencing specificity. A low ratio of artifacts to PCR-enriched DNA in a library will not affect sequencing results. 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.

	Comments and suggestions
Unexpected loss of coverage in subsequent GeneReader sequencing workflow	
Underrepresentation of specific parts of amplicons, monitored by subsequent GeneReader sequencing	Primer sets of the Actionable Insights Tumor Panel are distributed across 4 pools to minimize nonspecific amplification products. Make sure to perform 4 amplification reactions using primer mix pools A1–A4 for each genomic DNA sample. Check that all 4 amplification reactions of the same sample were pooled after multiplex PCR before proceeding with purification.

Symbols

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

Appendix A: PCR-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 on page 24. The amplicons should be in the correct size range (usually approximately 160 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 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. For sample preparation and data analysis, we recommend the setups described in the following sections.

QIAxcel Advanced instrument setup

- For final PCR-enriched DNA analysis, use 1 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 1 µl aliquot to 10 µl with Buffer EBA results in a final dilution of 1/10. Make sure to recalculate the dilution factor for final PCR-enriched DNA quantification results.

  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 sample. For final PCR-enriched DNA analysis, pre-dilute the sample by adjusting 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.
- Agilent High-Sensitivity DNA Kit (Agilent Technologies cat. no. 5067-4626) should be used for final PCR-enriched DNA analyses.

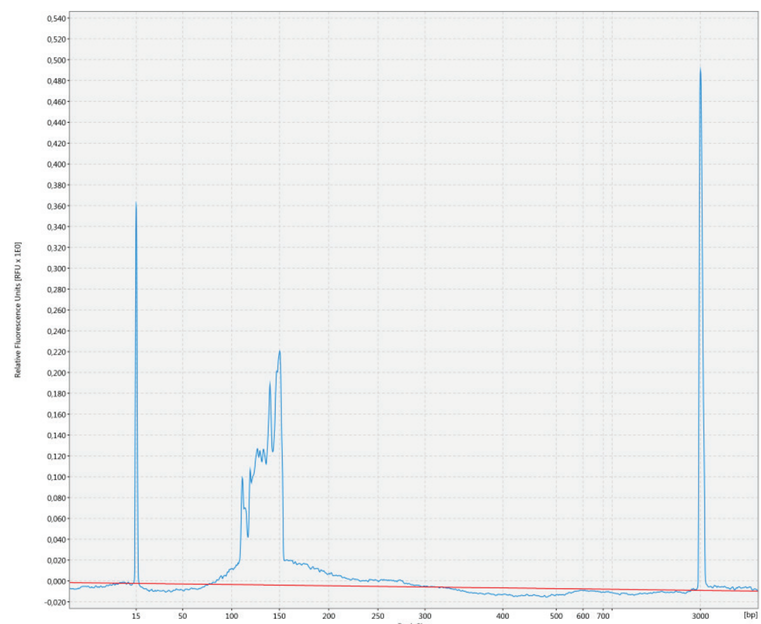

Figure 4. Trace data from the capillary electrophoresis instrument QIAxcel Advanced, showing the pooled and purified multiplex PCR product of Actionable Insights Tumor Panel (GRTP-101X). A peak observed at approximately 140 bp represents the amplicons.

Appendix B: FFPE-Purified DNA Quality and Quantity

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 targeted enrichment step in the NGS workflow. The GeneRead DNA QuantiMIZE System is a qPCR-based approach that determines the quantity and quality of DNA that is amenable to PCR-based targeted enrichment prior to NGS. The system provides a sensitive and accurate approach to qualify, quantify, and optimize DNA isolated from biological samples and is primarily for FFPE samples. Please refer to the corresponding kit handbook for determining FFPE sample-derived DNA quantity and quality with the GeneRead DNA QuantiMIZE System. If GeneRead DNA QuantiMIZE Kits are not used to adjust the number of PCR cycles, proceed with 26 cycles for all samples. The use of the GeneRead DNA QuantiMIZE Kits is recommended for quantification and quality control of the DNA.

Note: When using the QuantiMIZE Analysis Sheet (available online at **www.qiagen.com**), the following information should be provided:

- “Custom DNAseq Panel: # primers per pool”: set number to 75 for GRTP-101X
- “Custom DNAseq Panel: # pools”: set number to 4 for GRTP-101X

Table 4. 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.*

* This recommendation does not apply to UNG-treated genomic DNA samples.

Ordering Information

Product	Contents	Cat. no.
GeneRead QIAact Panels, Powered by QCI	Sets of pools containing wet-bench verified primer for targeted enrichment	181910
Panel – Actionable Insight Tumor Panel	Set of 4 pools containing wet-bench verified primer for targeted enrichment of a focused panel of 12 genes	GRTP-101X-12
GeneRead DNAseq Panel PCR Kit V2 (12)	For 12 reactions: PCR chemistry for use with the GeneRead DNAseq Panel V2 System	181940
GeneRead DNAseq Panel PCR Kit V2 (96)	For 96 reactions: PCR chemistry for use with the GeneRead DNAseq Panel V2 System	181942
Related products		
GeneRead DNA FFPE Kit (50)	QIAamp® MinElute® columns, Proteinase K, UNG, Collection Tubes (2 ml), Buffers, Deparaffinization Solution, RNase A	180134
GeneRead 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)	180654

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.

Notes

Notes

Limited License Agreement for the GeneRead QIAact Panels, Powered by QCI (GRTP-101X)

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

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