

June 2018

GeneRead™ QIAact Actionable Insights Tumor Panel Handbook

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

181910



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Kit Contents

| | |
|--|---------------------------------|
| GeneRead QIAact Actionable Insights Tumor Panel | |
| Catalog no. | 181910 and GRTP-101X-12* |
| Number of primer mix tubes | 4 |
| GeneRead QIAact Actionable Insights Tumor Panel pools with 773 variant positions in 12 genes for 12 samples† | |

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

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

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

The GeneRead QIAact Actionable Insights Tumor Panel (cat. no. 181910) is 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 delivery.

Intended Use

The GeneRead QIAact Actionable Insights Tumor Panel 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 Actionable Insights Tumor Panel 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. 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 Actionable Insights Tumor 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, page 5). Adjacent and potentially interacting primer pairs are separated into different pools for optimal performance. GeneRead QIAact Actionable Insights Tumor Panel is designed to analyze a panel of genes and can 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.

The GeneRead QIAact Actionable Insights Tumor Panel has 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 GeneRead QIAact Actionable Insights Tumor Panel 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 GeneRead QIAact Actionable Insights Tumor Panel covers specific hotspots designed to detect single-nucleotide variants (SNVs) and small (<20 bp) indels with the provided 4-pool set. GeneRead QIAact Actionable Insights Tumor Panel can enrich selected regions using as little as 40 ng 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 thermal cycler. Upon completion of the PCR run, 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.

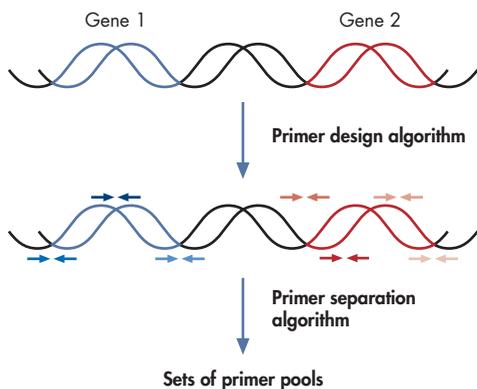


Figure 1. Multiplex PCR-based target enrichment scheme. The GeneRead QIAact Actionable Insights Tumor Panel uses multiplex PCR-based target-enrichment technology in combination with a sophisticated primer-design algorithm to maximize design coverage and minimize nonspecific amplification. The adjacent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

GeneRead QIAact Actionable Insights Tumor Panel

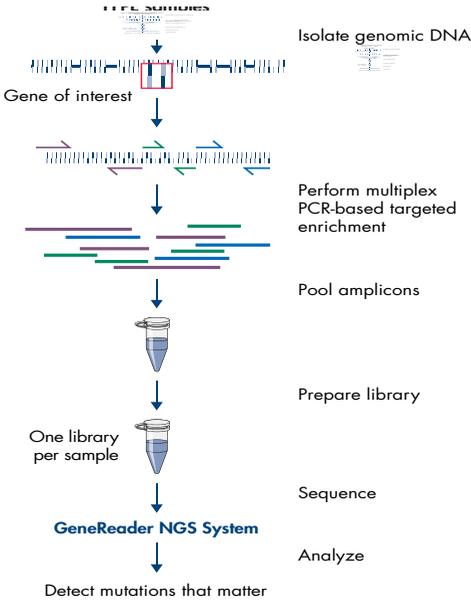


Figure 2. The GeneRead QIAact Actionable Insights Tumor Panel procedure.

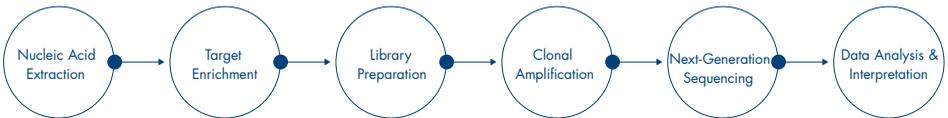


Figure 3. Overview of the complete NGS workflow with the GeneRead QIAact Actionable Insights Tumor Panel. The complete sample-to-insight procedure begins with DNA extraction, followed by target enrichment with the GeneRead QIAact Actionable Insights Tumor Panel, NGS library construction, sequencing and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

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 Actionable Insights Tumor Panel (GRTP-101X) and the sequencing kit used, we recommend using multiplex samples, as indicated in Table 1.

Table 1. Possible multiplexing of samples

| GeneRead kit | Sample type | Multiplexing degree |
|---|---------------|---------------------|
| GeneRead Sequencing Q Kit (1), cat. no. 185200 | FFPE | ≤ 10 |
| GeneRead Sequencing Q Kit (4), cat. no. 185201 | Liquid biopsy | ≤ 6 |
| GeneRead Advanced Sequencing Q Kit (3), cat. no. 185231 | FFPE | ≤ 16* |
| | Liquid biopsy | ≤ 10 |

* Currently only 12 bar codes are available

After target enrichment and library preparation, use 400 pg pooled library DNA in the clonal amplification process (see the “Preparing Libraries for Emulsion Making” procedure section in the *GeneRead Clonal Amp Q Handbook* for more information). The sequencing should be set up to run for 100 cycles.

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

For DNA isolation

See page 10 for specific recommendations:

- “Recommended FFPE DNA preparation methods”
- “Recommended circulating cell-free DNA preparation method for liquid biopsy samples”

For target enrichment

- GeneRead DNAseq Panel PCR Kit V2 (cat. no. 181940, for 12 samples, or cat. no. 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)

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

-
- 0.2 ml PCR tubes, 96-well reaction plates or PCR strips and caps
 - Thermal cycler*
 - Pipet and pipet tips
 - Qubit® 2.0 Fluorometer* (Life Technologies, cat. no. Q32866) or comparable
 - Qubit dsDNA HS Assay Kit (Life Technologies, cat. no. Q32851/Q32854)
 - 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) (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)

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

Important Notes

DNA preparation

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 FFPE DNA preparation methods

QIAGEN's GeneRead DNA FFPE Kit (cat. no. 180134) is highly recommended for manual and semi-automated (QIAcube) 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. 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).

Recommended circulating cell-free DNA preparation method for liquid biopsy samples*

Optimal extraction of circulating cell-free DNA (ccfDNA) from plasma samples can be safeguarded by drawing blood into sample collection tubes that provide efficient stabilization of plasma samples (e.g., PAXgene® Blood ccfDNA Tube, cat.no. 768115). For preparation

* If genomic DNA samples are to be harvested from biological samples for which kits are not available, please contact QIAGEN Technical Services for suggestions.

of ccfDNA from liquid biopsy samples, use the QIAamp® Circulating Nucleic Acid Kit (cat. no. 55114) for manual and semi-automated (QIAcube) preparation. For higher throughputs, the ccfDNA extraction can be automated on the QIASymphony instrument using the QIASymphony DSP Circulating DNA Kit (cat. no. 937556). To maximize DNA yields, we recommend extraction from 4–5 ml plasma and elution in the lowest volume stated in the *QIAamp Circulating Nucleic Acid Kit Handbook*. Low DNA yields can present challenges, such as providing insufficient input material for detecting low frequency variants.

DNA quantification

Concentration determined by fluorometric quantitation

The concentration of DNA should be determined by fluorometric quantitation using the Qubit Fluorometer and the Qubit dsDNA HS Assay Kit.

Note: For FFPE samples, Qubit quantification might underestimate the amount of amplifiable DNA.

We recommend using a DNA concentration of 2.5 ng/μl for target enrichment from FFPE and liquid biopsy samples; however, for liquid biopsy samples, concentrations as low as 1 ng/μl can be used. Use of less than 1 ng/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 15.

Procedure

1. Remove PCR reagents (QIAact Panel primer mix and DNaseq Panel PCR kit) from the freezer, and thaw at room temperature (15–25°C). Do not remove the polymerase from the freezer until directly before use.
2. The GeneRead QIAact 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). Prepare PCR strips or a PCR plate according to the number of reactions and samples. Label with sample names and primer pool numbers.
3. Prepare PCR mixes according to Table 2. 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 GeneRead QIAact Actionable Insights Tumor Panel (GRTP-101X).

Table 2. Preparation of PCR mix for each primer mix pool

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

* 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 3 for an example layout).

Table 3. Layout example for sample positions in strips and columns

| Strip | Column | | | | | | | | | | | | |
|-------|---------|----|----|----|----|----|----|----|----|----|----|----|----|
| | 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: Four 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.

Note: Liquid biopsy samples with concentrations less than 2.5 ng/ μl can be used for target enrichment PCR but might lead to low amplification concentrations. If samples with a concentration of less than 2.5 ng/ μl are used, use 4 μl undiluted sample for each PCR tube.

7. Seal the wells. Place the strips or plate in the thermal cycler, and set up reaction parameters according to Table 4.

Table 4. Cycling conditions

| Time | Temperature | Number of cycles |
|------------|-------------|------------------|
| 15 minutes | 95°C | 1 |
| 15 seconds | 95°C | 26 |
| 4 minutes | 60°C | |
| 10 minutes | 72°C | 1 |
| Hold | 4°C | ∞ |

8. 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 hours.

Protocol: Sample Pooling and Purification

This protocol describes the procedure for sample pooling and purification. For PCR setup, see “Protocol: PCR Setup”, page 12.

Procedure

1. Pulse-centrifuge the PCR strip or plate and 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 μ l.

2. Transfer 80 μ l from each sample to a 1.5 ml LoBind tube for purification.

Note: If the volume is not 80 μ l, adjust the volume by adding water to the sample.

3. Add 72 μ l (0.9x volume) Agencourt AMPure XP beads to 80 μ l 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 minutes at room temperature (15–25°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 minutes), carefully transfer 140 μ l 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 μ l supernatant will leave behind approximately 12 μ l 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 μ l Agencourt AMPure XP beads (this corresponds to 1.6x the original volume of PCR product, which was 80 μ l) to the supernatant, mix well and incubate for 5 minutes 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 a AMPure bead ratio of <1.6x volume 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 minutes).

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 μ l 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.

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 minutes while the tube is on the rack with the lid open.

13. Elute DNA target beads with 28 μ l nuclease-free water. Mix well by vortexing. Spin down briefly, and place the tube on the rack until the solution is clear. Transfer 25 μ l supernatant to a clean LoBind tube.

14. Determine the amount of PCR-enriched DNA using a Qubit Fluorometer and Qubit dsDNA HS Assay Kit.

15. Note: See “Target-enriched DNA Quality Control Methods”, page 19. 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 –20°C freezer for up to 72 hours.

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 the DNA integrity and amount of genomic DNA used for target enrichment (40 ng total; corresponding to 16 µl at 2.5 ng/µl, see page 12). 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 number 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. The sample(s) in question can be purified again by adjusting the volume of the eluate to 80 µl with water and repeating steps 3 to 14 of "Protocol: Sample Pooling and Purification" (page 17). Alternatively, if ethanol or salt carryover is suspected (low yields after library prep), the samples can be purified using the MinElute® PCR purification kit (cat. no.28004 or 28006). |
|--------------------------------------|---|

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 GeneRead QIAact 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 |
|--|-------------------|
|  | Catalog number |
|  | Manufacturer |



Appendix A: 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 on page 22. The amplicons should be in the correct size range (usually approximately 150 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.

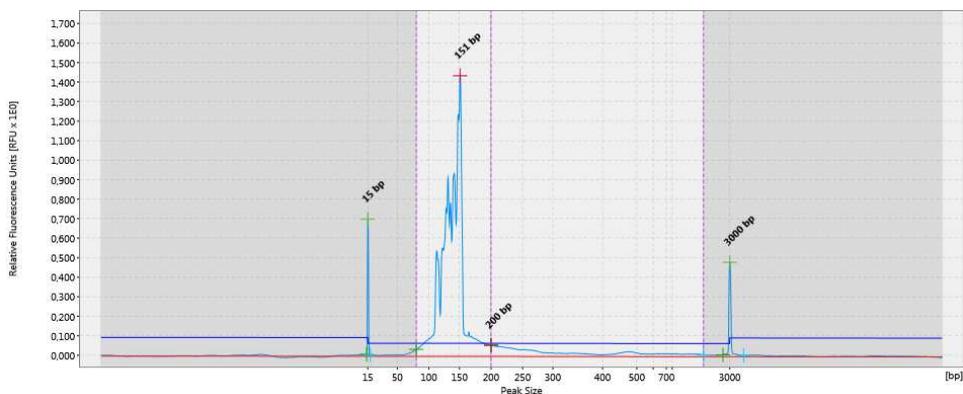


Figure 4. Trace data from the QIAxcel Advanced capillary electrophoresis instrument, showing the pooled and purified multiplex PCR product of the GeneRead QIAact Actionable Insights Tumor Panel (GRTP-101X). A peak observed at approximately 150 bp represents the amplicons.

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. 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 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.

Ordering Information

| Product | Contents | Cat. no. |
|---|--|-------------------------|
| GeneRead QIAact Actionable Insights Tumor Panel | Set of 4 pools containing wet-bench verified primer for target enrichment of a focused panel of 12 genes | 181910 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 |
| PAXgene Blood ccfDNA Tube | 100 blood collection tubes (10 ml) (For collection of whole blood samples and stabilization of ccfDNA) | 768115 |
| QIAsymphony PAXgene Blood ccfDNA Kit | Reagent cartridge, accessories and Proteinase K vials for 192 preps. | 768536 |
| QIAamp Circulating Nucleic Acid Kit | For 50 preps: QIAamp Mini Columns, Tube Extenders, QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors and Collection Tubes | 55114 |

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

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 | Updated the volume of PCR-enriched DNA to be used in analysis. Added revision history |
| R6 06/2018 | Updated to include change from QIAseq to QIAact beads. QuantIMIZE removed Qubit added as library quantification method |

Limited License Agreement for the GeneRead QIAact Actionable Insights Tumor Panel (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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