July 2018

GeneRead™ QIAact Lung DNA UMI Panel Handbook

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

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

REF 181931

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Sample to Insight

QIAGEN

Contents

Kit Contents

GeneRead QIAact Lung DNA UMI Panel	
Catalog no.	**181931**
Number of reactions	**24**
GeneRead QIAact DNA Library Prep and Target Enrichment Reagents (Kit Box 1)*	
GeneRead QIAact Lung DNA UMI Panel and Adapter Kit(Kit Box 2)*	

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

GeneRead QIAact DNA Library Prep 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 2 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. 181931.

QIAact Lung DNA UMI Panel and Adapter Kit *	
Number of reactions	**24**
GeneRead QIAact Lung DNA UMI Panel Forward Primers	130 µl
GeneRead QIAact Lung DNA UMI Panel Reverse Primers	130 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)	12µl
GeneReader™ TE-PCR Primer	40 µl
GeneReader Universal PCR Primer A	40 µl
GeneReader Universal PCR Primer B	40 µl

* Not for individual sale; to order products, see cat. no. 181931.

Storage

The GeneRead QIAact Library Prep and Target Enrichment Reagents (except QIAact Beads) are shipped on dry ice and should be stored at –15°C to –30°C upon arrival. QIAact Beads are shipped on cold packs and should be stored at 4°C. When stored properly, all reagents are stable for up to 3 months after delivery. GeneRead QIAact Lung DNA Panel is shipped on dry ice and should be stored at –15°C to –30°C upon arrival.

When stored properly, all reagents are stable for up to 3 months after delivery.

Intended Use

The GeneRead QIAact Lung DNA UMI Panel and GeneRead QIAact Library Prep and Target Enrichment Reagents are intended for Research Use Only and are not intended for the diagnosis, prevention or treatment of a disease.

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 Lung 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), copy number variation (CNVs) 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 artefactual errors that greatly limit the detection of true low-frequency variants in heterogeneous samples such as tumors.

The GeneRead QIAact Lung 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 GeneRead QIAact Lung 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 GeneRead QIAact Lung 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 eight (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).

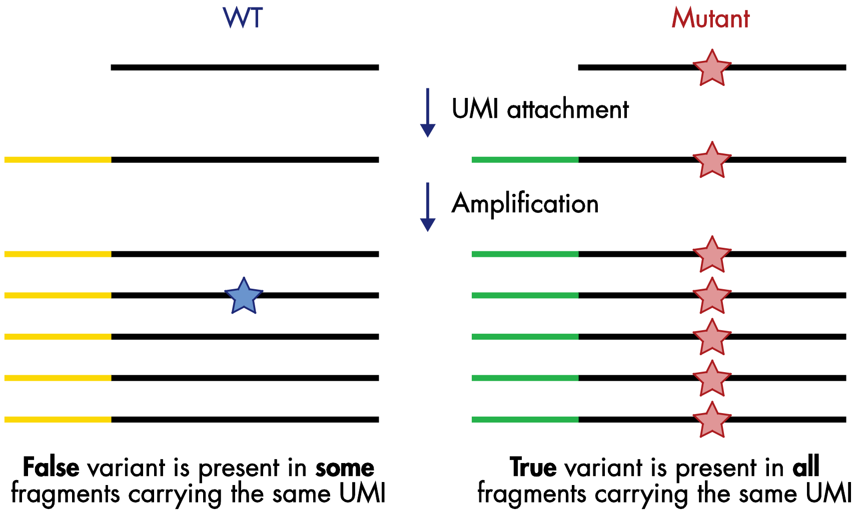

Figure 1. Principle of variant detection with UMI. Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while errors are mutations present in only one or a few reads within a UMI. Description of the variant calling algorithm can be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

Procedure

The GeneRead QIAact Lung DNA UMI Panel is provided as two primer mix tubes, with up to 200 primers per tube. The GeneRead QIAact Lung DNA UMI Panel is designed to enrich specific target regions in select genes (AKT1, ALK, BRAF, DDR2, EGFR, ERBB2/HER2, ESR1, FGFR1, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, PDGFRA, PIK3CA, PTEN, RICTOR, ROS1) using 40–100 ng of DNA.

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 Lung 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 21). 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* for more information). Based on the total number of amplicons that are produced by the GeneRead QIAact Lung DNA UMI Panel, we recommend a multiplex maximum of 12 samples for FFPE or a maximum of 6 samples for liquid biopsy samples.

When using the DNA libraries as part of the GeneRead QIAact Lung All-in-One Assay, we recommend a maximum multiplex of 6 samples for FFPE samples (see *GeneRead QIAact Lung All-in-One Assay Handbook* for more information).

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).

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 Prep and Target Enrichment Reagents and GeneRead QIAact Lung DNA UMI Panel, the following supplies are required:

For DNA isolation:

- See "Recommended 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 pipet tips and tubes
- QIAxcel® Advanced instrument (for information, visit **www.qiagen.com**)
- QIAxcel DNA High Resolution Kit (QIAGEN cat. no. 929002)
- QX DNA Size Marker 50–800 bp (50 µl) (QIAGEN cat. no. 929561)

- QX Alignment Marker 15 bp/3 kb (1.5 ml) (QIAGEN cat. no. 929522)
- QX Nitrogen Cylinder (x 6) (QIAGEN cat. no. 929705)
- Qubit® 3.0 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® System (for information, visit **www.qiagen.com**)
- 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 Mini Kit (cat. no. 51304), GeneRead DNA FFPE Kit (cat. no. 180134), QIAamp Circulating Nucleic Acid Kit (cat. no. 55114) 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. **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.

Recommended circulating-free DNA preparation method for liquid biopsy samples

Optimal extraction of circulating-free DNA (cfDNA) 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, QIAGEN cat. no. 768115). 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

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 Fluorometer (Thermo Fisher Scientific cat. no. Q33216). A minimum DNA input of 40 ng is recommended for the GeneRead QIAact Lung DNA UMI Panel†.

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† For unknown or lower quality FFPE DNA, an input range of 40–100 ng should be used for optimal performance.

Automation

The manual cleanup steps described in these instructions can be automated on the QIAcube instrument when FFPE samples are used. Three protocols for automating cleanup are provided in the GeneRead QIAact Panel Cleanup Kit (cat. no. 185446)

- “Protocol: GeneRead QIAact Panel Cleanup 1” describes the cleanup procedure after fragmentation, end repair and adapter ligation.
- “Protocol: GeneRead QIAact Panel Cleanup 2” describes the cleanup procedure after target enrichment PCR.
- “Protocol: GeneRead QIAact Panel Cleanup 3” describes the cleanup procedure after universal library amplification.

Protocol: Fragmentation, End-repair and A-addition

Important points before starting

- Ensure input DNA is in 10 mM Tris; for example, Buffer EB or Buffer ATE from QIAGEN or low TE (0.1x 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 (see Table 1).

   Note: If using 100 ng of DNA as input, dilute DNA to 25 ng/µl with nuclease-free water in a LoBind tube and add 4 µl of diluted DNA.
3. Prepare a reaction mixture for fragmentation, end-repair and A-addition according to Table 1. Mix the components well by pipetting up and down 10 times using a pipet set to 50 µl volume.
4. Add 16 µl master mix to each 0.2 ml 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 pipet set to 10 µl volume.

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Table 1. Preparation of mixture for fragmentation, end-repair and A-addition

	Volume (µl) for number of samples		
Reagent	**1**	**6**	**12**
DNA	4.00	–	–
Fragmentation Buffer, 10x	2.50	16.2	31.2
FERA Solution	0.75	4.9	9.4
Nuclease-Free Water	12.75	82.9	159.4
Total volume	**20.0**	**104.0**	**200.0**

5. Add 5 µl Fragmentation Enzyme Mix to each reaction. Mix well by pipetting up and down 10 times using a pipet 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 2. Be sure to use the instrument's heated lid (e.g., set to 103°C).

Table 2. Thermal cycler conditions

Time	**Temperature**	**Number of cycles**
1 minute	4°C	1
24 minutes	32°C	1
30 minutes	72°C	1
∞*	4°C	Hold

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

7. Start the program, then pause it when the thermal cycler block reaches 4°C.
8. Transfer the PCR tube to the pre-chilled 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 3. Mix well by pipetting up and down 10 times using a pipet set to 50 µl volume.

Table 3. Reaction setup for adapter ligation

	Volume (µl) for number of samples		
Reagent	**1**	**6**	**12**
Ligation Buffer, 5x	10.0	65.0	125.0
DNA Ligase	5.0	32.5	62.5
Ligation Solution (PEG6000, 50%)	7.2	46.8	90.0
Total volume	**22.2**	**144.3**	**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 only 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 pipet set to 25 µl. Centrifuge briefly (10–15 seconds), and then place on ice.
5. Program the thermal cycler to 20°C.

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

   IMPORTANT: Do not use the heated lid. If it is not possible to disable the heated lid on the thermocycler then leave the lid open.

7. After the reaction is complete, place the reactions on ice and proceed to "Protocol: Cleanup of Adapter-ligated DNA with QIAact Beads", page 18.

   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 minutes, and vortex thoroughly before use.

   Recommendation: Vortex beads for 1 minute 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 (1.0x volume) QIAact Beads to 100 µl diluted DNA solution. Mix well by pipetting up and down 10 times using a pipet set to 100 µl. Use a fresh tip for every sample.
4. Incubate for 5 minutes at room temperature.
5. Place the tube on the magnetic rack for 10 minutes 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° on the magnetic rack four times, shifting the position of the pellet, to wash the beads. Once complete wait until solution is clear (2–3 minutes). Completely remove ethanol.

8. Repeat previous step once.
9. After completely removing the ethanol close the tube lid.
10. Remove tube from the magnetic rack and centrifuge briefly (10–15 seconds).
11. Replace the tube on the magnetic rack and wait until solution is clear (2–3 minutes). Open 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 minutes on the rack.

    Note: Avoid over drying the beads. As drying depends on temperature and air flow, the drying time may vary. Adapt the drying time until the beads are no longer shiny but do not appear cracked.

    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 over drying.
12. Elute DNA from beads into 52 µl nuclease-free water. Mix well by pipetting up and down 10 times using a pipet set to 25 µl. Use a fresh tip for every sample. Place the tube on the rack until solution is clear (5–10 minutes).
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 previous step. Mix well by pipetting up and down 10 times using a pipet set to 50 µl. Use a fresh tip for every sample.
15. Incubate for 5 minutes at room temperature.
16. Place the tube on the magnetic rack for 10 minutes 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° on the magnetic rack four times, shifting the position of the pellet,

to wash the beads. Once complete, wait until the solution is clear (2–3 minutes). Completely remove ethanol.

19. Repeat 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 seconds).
22. Replace the tube on the magnetic rack and wait until solution is clear (2–3 minutes). 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 minutes on the rack.

    Note: Avoid over drying the beads. As drying depends on temperature and air flow, the drying time may vary. Adapt the drying time until the beads are no longer shiny but do not appear cracked. 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 tube lid to avoid over drying.
23. Elute DNA from beads in 22 µl nuclease-free water. Mix well by pipetting up and down 10 times using a pipet set to 15 µl. Use a fresh tip for every sample. Place the tube on the magnetic rack until solution is clear (5–10 minutes).
24. Prepare two 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 two PCR tubes: one for the forward target enrichment PCR and one for the reverse target enrichment PCR. Proceed to "Protocol: Target Enrichment PCR", page 23.

    Note: If reactions are to be stored following the QIAact Beads cleanup protocol, transfer them to a –20°C freezer. Samples are stable for up to 3 days.

Protocol: Target Enrichment PCR

Procedure

1. Prepare the master mixes according to Table 4 and Table 5 in separate 1.5 ml LoBind tubes. Mix well by pipetting up and down 10 times using a pipet set to 30 µl volume.

Table 4. Preparing the reaction for forward target enrichment PCR

	Volume (µl) for number of samples		
Reagent	**1**	**6**	**12**
DNA library (from Adapter-ligated DNA QIAact Bead cleanup)	9.4	–	–
TEPCR Buffer, 5x	4.0	26.0	50.0
GeneRead QIAact Lung DNA UMI Panel Forward Primers	5.0	32.5	62.5
GeneReader TE-PCR Primer	0.8	5.2	10.0
HotStarTaq DNA Polymerase	0.8	5.2	10.0
Total volume	**20.0**	68.9	132.5

Table 5. Preparing the reaction for reverse target enrichment PCR

	Volume (µl) for number of samples		
Reagent	**1**	**6**	**12**
DNA library (from Adapter-ligated DNA QIAact Bead cleanup)	9.4	–	–
TEPCR Buffer, 5x	4.0	26.0	50.0
GeneRead QIAact Lung DNA UMI Panel Reverse Primers	5.0	32.5	62.5
GeneReader TE-PCR Primer	0.8	5.2.0	10.0
HotStarTaq DNA Polymerase	0.8	5.2.0	10.0
Total volume	**20.0**	68.9	132.5

2. Add 10.6 µl of master mix from either Table 4 or Table 5 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 pipet set to 10 µl, centrifuge briefly (10–15 seconds).
3. Set up the thermal cycler using the cycling conditions provided in Table 6.

   IMPORTANT: Ensure the heated lid on the thermal cycler is turned on for the PCR.

Table 6. Cycling conditions for target enrichment PCR

Time	Temperature	Number of cycles
13 minutes	95°C	1 (initial denaturation)
2 minutes	98°C	
15 seconds	98°C	8
10 minutes	68°C	
5 minutes	72°C	1
5 minutes*	4°C	1
∞	4°C	Hold

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

4. When the reaction is complete, place the reactions on ice and proceed to "Protocol: Cleanup of Target Enrichment PCR with QIAact Beads", page 25.

   Note: If reactions are to be stored after target enrichment, 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 the QIAact Beads come to room temperature (15–25°C) for at least 30 minutes and vortex thoroughly before use.

   Recommendation: Vortex beads for 1 minute at maximum speed.

2. Pulse-centrifuge the Forward and Reverse PCRs from “Protocol: Target Enrichment PCR”, page 23, 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 volume may be less than 40 µl due to evaporation. It is important to measure the combined PCR 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 pipet set to 100 µl. Use a fresh tip for every sample. Incubate for 5 minutes at room temperature.

4. Place the tube on the magnetic rack for 10 minutes 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° on the magnetic rack four times, shifting the position of the pellet, to wash the beads. Once complete wait, until the solution is clear (2–3 minutes). Completely remove ethanol with a 200 µl pipet tip.

7. Repeat 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 seconds).
10. Replace the tube on the magnetic rack and wait until the solution is clear (2–3 minutes). 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 minutes on the rack.

    Note: Avoid over drying the beads. As drying depends on temperature and air flow, the drying time may vary. Adapt the drying time until the beads are no longer shiny but do not appear cracked. 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 tube the lid to avoid over drying.
11. Elute DNA from beads in 16 µl nuclease-free water. Mix well by pipetting up and down 10 times using a pipet set to 8 µl. Use a fresh tip for every sample. Place on the magnetic rack until the solution is clear (5–10 minutes). Transfer 13.4 µl supernatant to a clean 0.2 ml PCR tube. Proceed to "Protocol: Universal PCR Amplification", page 27.

    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 master mix in a 1.5 ml LoBind tube according to Table 7.

Table 7. Preparing the reaction for universal PCR amplification

	Volume (µl) for number of samples		
Reagent	**1**	**6**	**12**
Enriched DNA (from "Protocol: Cleanup of Target Enrichment PCR with QIAact Beads")	13.4	–	–
UPCR Buffer, 5x	4.0	26.0	50.0
GeneReader Universal PCR Primer A	0.8	5.2	10.0
GeneReader Universal PCR Primer B	0.8	5.2	10.0
HotStarTaq DNA Polymerase	1.0	6.5	12.5
Total volume	**20.0**	**42.9**	**82.5**

2. Add 6.6 µl of the master mix from Table 7 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 pipet set to 10 µl, and centrifuge briefly (10–15 seconds).
3. Set up the thermal cycler using the cycling conditions provided in Table 8.

   IMPORTANT: Ensure the heated lid on the thermal cycler is turned on for the PCR.

Table 8. Cycling conditions for universal PCR amplification

Time	Temperature	Number of cycles
13 minutes 2 minutes	95°C 98°C	1 (initial denaturation)
15 seconds 2 minutes	98°C 60°C	21
5 minutes	72°C	1
5 minutes*	4°C	1
∞	4°C	Hold

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

4. When the reaction is complete, place the reactions on ice and proceed to "Protocol: Cleanup of Universal PCR with QIAact Beads", page 29.

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

Protocol: Cleanup of Universal PCR with QIAact Beads

Procedure

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

   Recommendation: Vortex for 1 minute at maximum speed.
2. Transfer 20 µl PCR from "Protocol: Universal PCR Amplification", page 27, to a 1.5 ml LoBind tube. Add nuclease-free water to bring the volume to 100 µl.

   Note: The PCR volume may be less than 20 µl due to evaporation. It is important to measure the PCR 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 pipet set to 100 µl. Use a fresh tip for every sample. Incubate for 5 minutes at room temperature.
4. Place the tube on the magnetic rack for 10 minutes 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 a10 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° on the magnetic rack four times, shifting the position of the pellet, to wash the beads. Once complete, wait until the solution is clear (2–3 minutes). Completely remove the ethanol with a 200 µl pipet tip.
7. Repeat 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 seconds).
10. Replace the tube on the magnetic rack and wait until solution is clear (2–3 minutes). Open 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 minutes on the rack.

    Note: Avoid over drying the beads. As drying depends on temperature and air flow, the drying time may vary. Adapt the drying time until the beads are no longer shiny but do not appear cracked. 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 over drying.
11. Elute DNA from beads in 30 µl nuclease-free water. Mix well by pipetting up and down 10 times using a pipet 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 minutes). 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 A: Analyze the Library using the QIAxcel Advanced", page 33). 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 2 ng/µl as this may impact performance.

    Optional: The DNA library assessment could also be performed on the Agilent 2100 Bioanalyzer with the Agilent High Sensitivity DNA Kit (see "Appendix B: Analyze the Library using the Agilent 2100 Bioanalyzer", page 34).

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 (<2 ng/µl) after Universal PCR	Concentration of DNA to be used for library preparation and target enrichment should be determined by fluorometric quantitation. Ensure that all reaction components are thoroughly mixed as described in these instructions before use Ensure that all reaction components are correctly added at each stage and thoroughly mixed as described in these instructions. 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 250 ng.
d)	No or low PCR product yield (<2 ng/µl) after Universal PCR from FFPE sample-derived DNA	When using DNA derived from FFPE samples, ensure that the DNA is of suitable quality.

Symbols

Symbol	Symbol definition
Σ <N>	Contains reagents sufficient for x tests
REF	Catalog number
🏭	Manufacturer

Appendix A: Analyze the Library using the QIAxcel Advanced

After the library is constructed and purified, analyze it 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 on average 350 bp* for DNA derived from FFPE tissue and 300 bp* for cfDNA. The majority of the library fragments are between 200–800 bp in size (Figure 2).

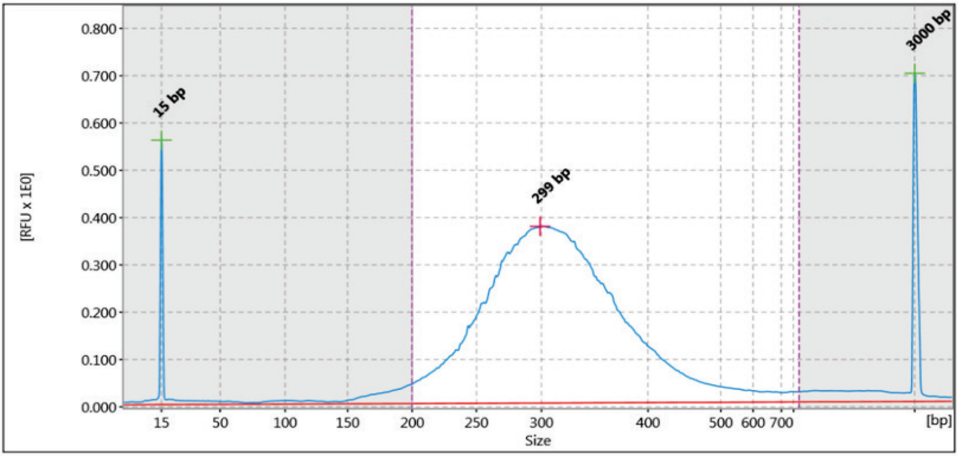

Figure 2. Sample QIAxcel Advanced image of a GeneRead QIAact Lung DNA UMI library. The majority of the library fragments are between 200–800 bp in size.

Note: for DNA derived from FFPE tissue, typically 60–70% of the peak will be between 200–400 bp in size, whereas for cfDNA, typically 80–90% of the peak will be between 200–400 bp in size.

* Data generated from DNA reference standards.

Appendix B: Analyze the Library using the Agilent 2100 Bioanalyzer

After the library is constructed and purified, a Bioanalyzer can be used to check the fragment size and concentration with the High Sensitivity DNA Kit. The majority of the library fragments are between 200–800 bp in size (Figure 3). Amounts of DNA under the appropriate peaks can be used to quantify libraries.

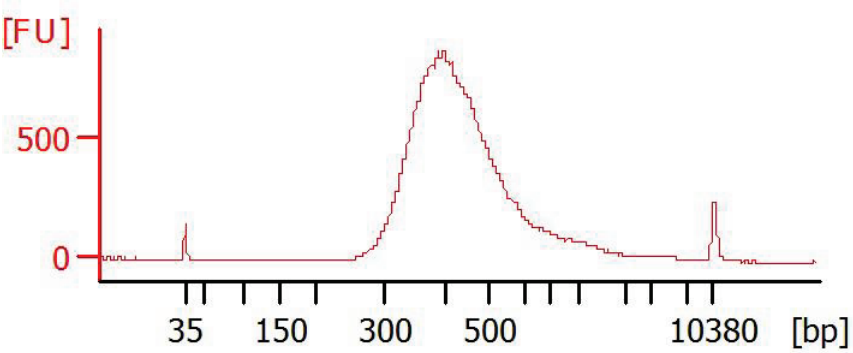

Figure 3. Sample Bioanalyzer image of a GeneRead QIAact Lung DNA UMI library. The majority of the library fragments are between 200–800 bp in size.

Ordering Information

Product	Contents	Cat. no.
GeneRead QIAact Lung DNA UMI Panel (24)	GeneRead QIAact Library Prep and Target Enrichment Reagents (24) and QIAact Target Enrichment Panel and QIAact Adapters (24)	181931
Related Products		
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Collection Tubes (2 ml), reagents and buffers	51304
GeneRead DNA FFPE Kit (50)	QIAamp MinElute® columns, Proteinase K, UNG, collection tubes (2 ml), buffers, Deparaffinization Solution, RNase A	180134

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Revision History

Revision Date	Contents	Rev. no.
04/2018	Updated kit contents for cleanup beads. Added Revision History.	R2
06/2018	Updated to include change from QIAseq to QIAact beads. QuantiMIZE was removed and Qubit was added as a library quantification method.	R3

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Notes