

June 2017

# GeneRead™ QIAact Lung All-in-One Assay Handbook



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

**REF**

181930 and 181935



QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden

**MAT**

1107207

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

# Contents

Assay Components .....	3
Storage .....	8
Intended Use .....	9
Safety Information .....	9
Quality Control .....	9
Introduction .....	10
Principles of the Procedure .....	10
Equipment and Reagents to Be Supplied by User .....	14
Important Notes .....	15
Nucleic acid preparation .....	15
Nucleic acid quantification .....	16
Protocol: GeneRead QIAact Lung DNA Panel .....	18
Protocol: GeneRead QIAact Lung RNA Panel .....	20
Protocol: Sample Pooling for Clonal Amplification .....	20
Troubleshooting Guide .....	21
Symbols .....	23
Appendix A: Analyze the Library Using QIAxcel Advanced - GeneRead QIAact Lung DNA Panel .....	24
Appendix B: Analyze the Library Using QIAxcel Advanced - GeneRead QIAact Lung RNA Panel .....	25
Ordering Information .....	26

# Assay Components

<b>GeneRead QIAact Lung All-in-One Assay</b>	
<b>Catalog nos.</b>	<b>181930</b>
	<b>181935</b>
<b>Number of reactions</b>	<b>24</b>
GeneRead QIAact Lung DNA Panel (catalog no. 181930)	
GeneRead QIAact Lung RNA Panel (catalog no. 181935)	

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

## GeneRead QIAact Lung DNA Panel

<b>GeneRead QIAact Lung DNA Panel</b>	
<b>Catalog no.</b>	<b>181930</b>
<b>Number of reactions</b>	<b>24</b>
GeneRead QIAact Library Preparation and Target Enrichment Reagents (Kit Box 1)*	
QIAact Target Enrichment Panel and QIAact Adapters (Kit Box 2)*	

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

<b>GeneRead QIAact Library Preparation and Target Enrichment Reagents*</b>	
<b>Number of reactions</b>	<b>24</b>
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 QIAseq Beads	34 ml

\* Not for individual sale; to order reagents, see cat. no. 181930.

**QIAact Target Enrichment Panel and QIAact Adapters \***

<b>Number of reactions</b>	<b>24</b>
GeneRead™ QIAact Lung DNA Panel Forward Primers	130 µl
GeneRead QIAact Lung DNA Panel Reverse Primers	130 µl
QIAact Adapters 1-12 (contains 12 tubes with each tube corresponding to one sample-specific bar code; each tube can process up to 2 samples)	10 µl
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. 181930.

## GeneRead QIAact Lung RNA Panel

<b>GeneRead QIAact Lung RNA Panel</b>	
<b>Catalog no.</b>	<b>181935</b>
<b>Number of reactions</b>	<b>24</b>
GeneRead QIAact RNA Library Preparation and Target Enrichment Reagents (Kit Box 1)*	
QIAact RNA Target Enrichment Panel and QIAact Adapters (Kit Box 2)*	

\* Kit boxes 1 and 2 are components of the GeneRead QIAact Lung RNA Panel.

<b>GeneRead QIAact RNA Library Preparation and Target Enrichment Reagents *</b>	
<b>Number of reactions</b>	<b>24</b>
RP Primer	26 $\mu$ l
EZ Reverse Transcriptase	26 $\mu$ l
BC3 buffer, 5x	52 $\mu$ l
Rnase inhibitor	26 $\mu$ l
RH RNase	26 $\mu$ l
dNTP	26 $\mu$ l
XC buffer, 10x	52 $\mu$ l
BX enzyme	26 $\mu$ l
ERA enzyme	260 $\mu$ l
ERA buffer, 10x	130 $\mu$ l
DNA ligase	260 $\mu$ l
Ligation buffer, 5x	520 $\mu$ l
QIAseq RNA buffer	312 $\mu$ l
Nuclease-free water	2 x 2ml
HotStarTaq® DNA Polymerase	2 x 50 $\mu$ l
One bottle containing QIAseq Beads	34 ml

\* Not for individual sale; to order reagents, see cat. no. 181935.

**QIAact RNA Target Enrichment Panel and QIAact Adapters \***

<b>Number of reactions</b>	<b>24</b>
GeneRead™ QIAact Lung RNA Panel Forward Primers	130 µl
GeneRead QIAact Lung RNA Panel Reverse Primers	130 µl
QIAact Adapters 13-24 (contains 12 tubes with each tube corresponding to one sample-specific bar code; each tube can process up to 2 samples)	10 µl
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. 181935.

---

## Storage

The GeneRead QIAact Library Preparation and Target Enrichment Reagents (except QIAseq Beads) are shipped on dry ice and should be stored at  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  upon arrival. QIAseq Beads are shipped on cold packs and should be stored at  $4^{\circ}\text{C}$ . When stored properly, all reagents are stable for up to 3 months after delivery.

GeneRead QIAact Lung DNA Panel and GeneRead QIAact Lung RNA Panel are shipped on dry ice and should be stored at  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  upon arrival. When stored properly, components are stable for up to 3 months after delivery.

---

## Intended Use

For use with DNA and RNA extracted from formalin-fixed paraffin embedded (FFPE) tissue only. The GeneRead QIAact Lung All-in-One Assay is intended for Research Use Only and is 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](http://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 Panel and GeneRead QIAact Lung RNA Panel that comprise the GeneRead QIAact Lung All-in-One Assay are tested against predetermined specifications, to ensure consistent product quality.

---

# Introduction

Next Generation sequencing is a useful tool to detect genetic variations, including somatic mutations, single nucleotide variants (SNVs), copy number variation (CNVs), small insertions and deletions (inDels) from DNA, plus translocations/fusions and exon skipping events from RNA. Targeted enrichment technology enables next-generation sequencing (NGS) platform users to sequence specific regions of interest from both DNA and RNA, effectively increasing sequencing depth and throughput with lower cost.

The GeneRead QIAact Lung All-in-One Assay is designed to detect both DNA (SNV, CNV and inDels) and RNA (fusions and exon skipping events) mutations from the same patient sample.

The GeneRead QIAact Lung All-in-One Assay integrates unique molecular index (UMI) technology and target-specific, primer-based target enrichment, which enables sensitive mutation detection by targeting just regions of interest for NGS on the GeneReader system.

The GeneRead QIAact Lung All-in-One Assay 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.

## Principles of the Procedure

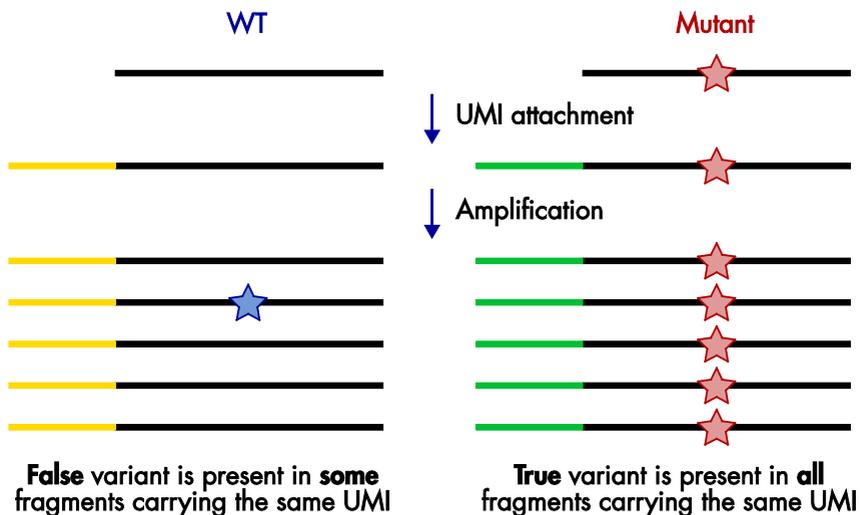
The GeneRead QIAact Lung All-in-One Assay relies on target-specific primer enrichment and UMIs for uniform coverage and sensitive fusion detection.

### Unique molecular index

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

The adaptor-ligated molecules are then amplified by PCR for target enrichment and library amplification. Due to intrinsic noise and sequence-dependent bias, 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 tumours. To reduce the number of false-positives, variants are called 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](http://www.qiagen.com).

---

When focusing on fusion detection from RNA accurate quantification of the number of fusion events in the original purified RNA can be achieved by retracing the RNA UMI tagged transcript fragments.

## Procedure

The GeneRead QIAact Lung All-in-One Assay is provided as two separate panels.

1. The GeneRead QIAact Lung DNA Panel is designed to enrich selected genes and regions using 40 to 100 ng of DNA.
2. The GeneRead QIAact Lung RNA Panel is designed to enrich selected fusion targets using 100 ng of total RNA.

### **GeneRead QIAact Lung DNA Panel**

Genomic DNA samples are first fragmented, end-repaired and A-tailed within a single, controlled multi-enzyme reaction. The prepared DNA fragments are then ligated at the 5' ends to a sequencing platform-specific adapter containing a UMI and a sample-specific bar code.

Ligated DNA molecules are subject to limited cycles of target enrichment PCR. 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.

### **GeneRead QIAact Lung RNA Panel**

Total RNA is first reverse-transcribed to first strand cDNA. A separate, second strand synthesis is used to generate double strand (ds)-cDNA. This (ds)-cDNA is then end-repaired and A-tailed in a single tube protocol. The prepared (ds)-cDNAs are then ligated at the 5' ends to a sequencing platform-specific adapter containing UMI and sample specific bar code.

---

Ligated (ds)-cDNA molecules are subject to limited cycles of target enrichment PCR. 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.

### Recommendation for combining DNA and RNA libraries for clonal amplification

The DNA and RNA libraries generated from more than one sample can be sequenced at the same time in a single flow cell. This is made possible by the addition of a sample-specific bar code that is added during library preparation. The sequencing libraries prepared from multiple samples must be pooled prior to clonal amplification to allow them to be sequenced together in a single flow cell (see “Protocol: Library Concentration Normalization and Pooling” in the *QIAGEN GeneRead Clonal Amp Q Handbook* for more information). Based on the total number of amplicons that are produced by the GeneRead QIAact Lung All-in-One Assay, we recommend a multiplex of 12 libraries, 6 libraries generated from DNA and 6 libraries generated from matched RNA.

### Data Analysis

Once the library has been sequenced, results from the DNA libraries and RNA libraries can be analyzed separately using the GeneRead QIAact Lung DNA and RNA workflows, respectively. The two workflows automatically perform all steps necessary to generate a report summarizing all of the DNA and RNA mutation information. All detected mutations can be further interpreted by QIAGEN Clinical Insight (QCI™) analysis.

# Equipment and Reagents to Be Supplied by User

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

In addition to the QIAact Library Preparation and Target Enrichment Reagents for the GeneRead QIAact Lung DNA Panel and GeneRead QIAact Lung RNA Panel, the following supplies are required:

## For nucleic acid isolation:

- DNA isolation, GeneRead DNA FFPE Kit (cat. no. 180134)
- RNA isolation, QIAGEN RNeasy® FFPE Kit (cat. no. 73504)

## For library construction and targeted enrichment:

- High-quality, nuclease-free water. **Do not use DEPC-treated water**
- 80% ethanol, made fresh
- Microcentrifuge
- 1.5 ml LoBind tubes (Eppendorf®)
- 0.2 ml PCR tubes, 96-well PCR plates or PCR strips and caps
- Thermal cycler (e.g., Bio-Rad® C1000™)
- Multichannel pipettor
- Single-channel pipettor
- DNase-free pipette tips and tubes
- QIAxcel® Advanced instrument (for information, visit [www.qiagen.com](http://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 RNA 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](http://www.qiagen.com))
- Agilent® 2100 Bioanalyzer®
- Agilent High Sensitivity DNA Kit (Agilent cat. no. 5067-4626)
- Agilent 2200 TapeStation®
- Agilent High Sensitivity D1000 Screentape (Agilent cat. no. 5067-5584) or equivalent
- Agilent High Sensitivity D1000 Reagents (Agilent cat. no. 5067-5585) or equivalent

## Important Notes

### Nucleic acid preparation

Maximizing nucleic acid quality is essential for obtaining good sequencing results

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

---

Residual traces of proteins, salts or other contaminants may either degrade the extracted nucleic acids or decrease the efficiency of (if not block completely) the enzyme activities necessary for optimal target enrichment.

### Recommended genomic DNA preparation methods

The GeneRead DNA FFPE Kit (cat. no. 180134) or QS GeneRead DNA FFPE Treatment Kit (cat. no. 185306) in combination with the QIAasymplicity DSP DNA Mini Kit (cat. no. 937236) is highly recommended for the preparation of genomic DNA samples from FFPE tissue. Ensure that samples have been treated for the removal of RNA, as RNA contamination will cause inaccuracies in DNA concentration measurements. **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. **Do not use DEPC-treated water.**

### Recommended genomic RNA preparation methods

The QIAGEN RNeasy® FFPE Kit (cat. no. 73504) is highly recommended for the preparation of total RNA samples from FFPE tissue. **Do not** omit the recommended DNase treatment step to remove DNA.

For best results, all RNA samples should be resuspended in RNase-free water. **Do not use DEPC-treated water.**

## Nucleic acid quantification

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

---

## Purity determined by UV spectrophotometry

Nucleic acid purity 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.

Pure RNA has an  $A_{260}/A_{280}$  ratio of 1.9–2.1 in 10 mM Tris•HCl, pH 7.5.

Pure DNA has an  $A_{260}/A_{280}$  ratio of 1.8 in 10 mM Tris•HCl, pH 7.5.

## Concentration

The concentration of nucleic acid should be determined by fluorometric quantitation using equipment such as the Qubit 3.0 Fluorometer (Thermo Fisher Scientific cat. no. Q33216) and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific cat. no. Q32851) and the Qubit RNA HS Assay Kit (Thermo Fisher Scientific cat. no. Q32851) for DNA and RNA, respectively.

\* 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.

---

# Protocol: GeneRead QIAact Lung DNA Panel

The GeneRead QIAact Lung DNA Panel is designed to detect SNVs, inDel and CNV targets in key lung cancer genes. The kit is for a total of 24 reactions. The complete protocol is described in the GeneRead™ QIAact Lung DNA Panel Handbook. In brief:

1. DNA is first extracted from two 10 micron FFPE tissue sections.
2. The GeneRead QIAact Lung DNA Panel is provided as two primer mix tubes, targeting the sense and anti-sense DNA strands. The GeneRead QIAact Lung DNA Panel is designed to work with 40-100 ng of DNA.
3. Genomic DNA samples are first fragmented, end-repaired and A-tailed within a single, controlled multi-enzyme reaction. The prepared DNA fragments are then ligated at the 5' ends with a specific adapter containing a UMI and a sample-specific bar code (bar codes 1-12).
4. Ligated DNA molecules are subject to limited cycles of target enrichment PCR, with gene-specific primers and a universal forward primer complementary to an adapter sequence.
5. A universal PCR with GeneReader specific sequences is then carried out to amplify the targets and complete the library.
6. The libraries are then assessed for product size (bp) and yield (ng) using the QIAxcel Advanced instrument and the QIAxcel DNA High Resolution Kit (1200) (see Appendix A).
7. Typically, 3–20 ng/μl of PCR product are obtained after target enrichment and library preparation.

---

# Protocol: GeneRead QIAact Lung RNA Panel

The GeneRead QIAact Lung RNA Panel is designed to detect fusions and exon skipping events in key lung cancer genes. The kit is for a total of 24 reactions. The complete protocol is described in the GeneRead™ QIAact Lung RNA Panel Handbook. In brief:

1. RNA is first extracted from two 10 micron FFPE tissue sections.
2. The GeneRead QIAact Lung RNA Panel is provided as two primer mix tubes, targeting known translocations. The GeneRead QIAact Lung RNA Panel is designed to work with 100 ng of total RNA.
3. Total RNA samples are first reverse-transcribed to first-strand cDNA, prior to second strand synthesis. End-repair and A-tailing are performed in a multi-step single tube reaction. The prepared (ds)-cDNA fragments are then ligated at the 5' ends with a specific adapter containing a UMI and a sample-specific bar code (bar codes 13-24).
4. Ligated DNA molecules are subject to limited cycles of target enrichment PCR, with fusion-specific primers and a universal forward primer complementary to an adapter sequence.
5. A universal PCR with GeneReader specific sequences is then carried out to amplify the targets and complete the library.
6. The libraries are then assessed for product size (bp) and yield (ng) using the QIAxcel Advanced instrument and the QIAxcel DNA High Resolution Kit (1200) (see Appendix B).
7. Typically, 3–20 ng/μl of PCR product are obtained after target enrichment and library preparation.

---

# Protocol: Sample Pooling for Clonal Amplification

The GeneRead QIAact Lung All-in-One Assay is comprised of the GeneRead QIAact Lung DNA Panel and GeneRead QIAact Lung RNA Panel and is designed to detect both DNA and RNA mutations from the same patient sample. The assay allows testing of a total of 24 reactions. In brief:

1. Prepare independent DNA and RNA libraries, from sequential FFPE sections, using the GeneRead QIAact Lung DNA Panel and GeneRead QIAact Lung RNA Panel, respectively.
2. Combine DNA and RNA libraries, from a single sample, in a 4:1 ratio (DNA:RNA) based on the yield results (ng/ $\mu$ l) from the QIAxcel DNA High Resolution Kit analysis.
  - 2a. First dilute all libraries to a 100pg/ $\mu$ l working stock.
  - 2b. For each of the 6 samples to be sequenced combine 2 $\mu$ l of RNA library working stock with 8 $\mu$ l of DNA library working stock in a 1.5ml LoBind tube.
  - 2c. Close the tube and vortex for 5-10 seconds. Briefly centrifuge the tube.
  - 2d. Pooled libraries can be stored at -20°C for up to one month. Avoid multiple freeze-thaw cycles.
3. The sequencing libraries prepared from separate 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 *QIAGEN GeneRead Clonal Amp Q Handbook* for more information).
4. For the total number of amplicons targeted by the GeneRead QIAact Lung All-in-One Assay a multiplex of 12 libraries, 6 libraries generated from DNA and 6 libraries generated from matched RNA is recommended (1 sample = combined DNA/RNA library prepared from a single sample).
5. After sample pooling (combined DNA and RNA libraries) use 500 pg pooled samples in the clonal amplification process (see "Preparing Libraries for Emulsion Making" in the *QIAGEN GeneRead Clonal Amp Q Handbook* for more information).

# 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](http://www.qiagen.com/FAQ/FAQList.aspx). The scientists in QIAGEN Technical Services are always happy to answer any questions that you may have regarding the information and/or protocols in this handbook or for any sample and assay technologies (for contact information, visit [www.qiagen.com](http://www.qiagen.com)).

## Comments and suggestions

---

### Library preparation and target enrichment

- |   |  |
|---|--|
| a) No or low PCR product yield (<2ng/ $\mu$ l) after Universal PCR with the QIAact Lung DNA Panel | Concentration of DNA to be used for library preparation and target enrichment should be determined by fluorometric quantitation.   |
| b) No or low PCR product yield (<2ng/ $\mu$ l) after Universal PCR with the QIAact Lung DNA Panel | Ensure that all reaction components are thoroughly mixed as described in this handbook before use.<br>Ensure that all reaction components are correctly added at each stage and thoroughly mixed as described in this handbook.  |
| c) No or low PCR product yield (<2ng/ $\mu$ l) after Universal PCR with the QIAact Lung DNA Panel | When using DNA derived from FFPE samples, ensure that the DNA is of suitable quality. It is recommended to first assess the extracted DNA using the QIAseq DNA QuantiMIZE Array or Assay Kit (QC Score $\leq 0.04$ , proceed as the genomic DNA is of good quality; QC Score $> 0.04$ , proceed with caution as the DNA is highly fragmented/damaged). |
| d) No or low PCR product yield (<2ng/ $\mu$ l) after Universal PCR with the QIAact Lung DNA Panel | Increase DNA input used for library preparation. If 40ng was used initially increase input to 100ng. If 100ng was used initially increase input to 250ng.  |
| e) No or low PCR product yield (<2ng/ $\mu$ l) after Universal PCR with the QIAact Lung RNA Panel | Ensure that 100 ng of RNA is used as input for "Protocol: First strand cDNA synthesis". Concentration of RNA should be determined by fluorometric quantitation.  |

---

### Comments and suggestions

---

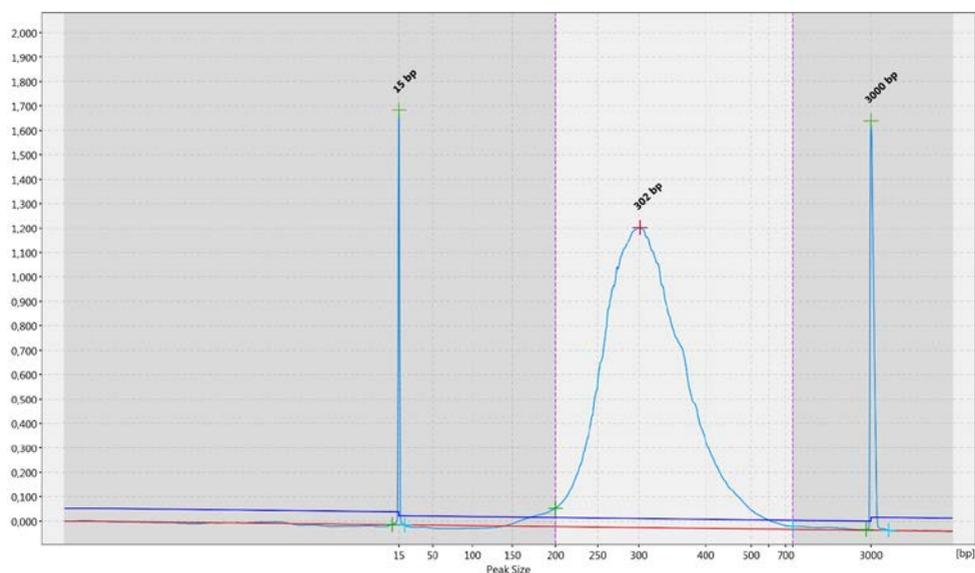
- |   |   |
|---|---|
| f) No or low PCR product yield (<2ng/ $\mu$ l) after Universal PCR with the QIAact Lung RNA Panel | Ensure that all reaction components are thoroughly mixed as described in this handbook before use.<br>Ensure that all reaction components are correctly added at each stage and thoroughly mixed as described in this handbook. |
| g) No or low PCR product yield (<2ng/ $\mu$ l) after Universal PCR with the QIAact Lung RNA Panel | Increase RNA input used for library preparation. If 100ng was used initially increase input to 200ng of RNA.  |

# Symbols

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

# Appendix A: Analyze the Library Using QIAxcel Advanced - GeneRead QIAact Lung DNA Panel

After the library is constructed and purified, QIAxcel Advanced, in combination with the QIAxcel DNA High Resolution Kit (cat. no. 929002), can be used to check the fragment size and concentration. The majority of the library fragments are between 200–800 bp in size (Figure 2). Amounts of DNA under the peak can be used to quantify libraries.



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

## Appendix B: Analyze the Library Using QIAxcel Advanced - GeneRead QIAact Lung RNA Panel

After the library is constructed and purified, QIAxcel Advanced, in combination with the QIAxcel DNA High Resolution Kit (cat. no. 929002), can be used to check the fragment size and concentration. The library fragments to be quantified are between 200–400 bp in size (Figure 3). Amounts of DNA under the peak can be used to quantify libraries. Additional peaks are observed at approximately 150bp and 600bp, however, these do not impact quantification and sequencing results.

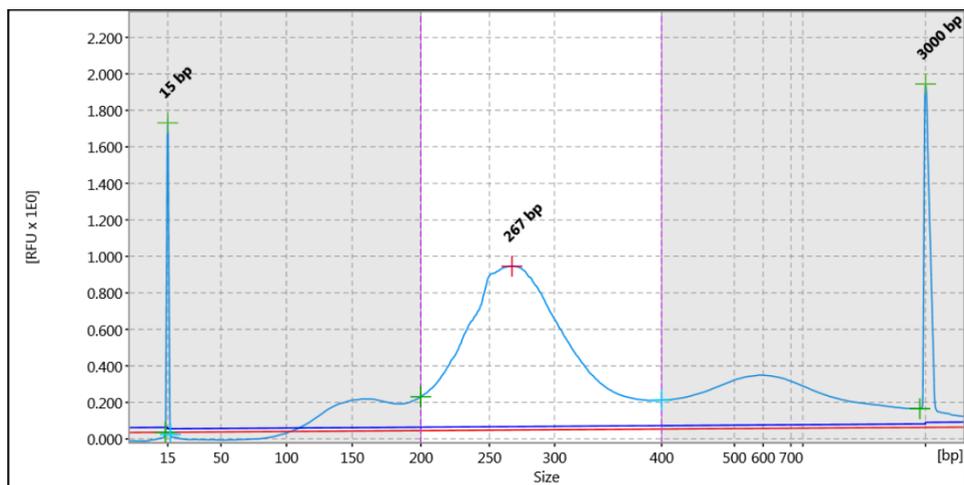


Figure 3. Sample QIAxcel Advanced image of a GeneRead QIAact Lung RNA library.

## Ordering Information

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
GeneRead QIAact Lung DNA Panel (24)	GeneRead QIAact Library Preparation and Target Enrichment Reagents (24) and QIAact Target Enrichment Panel and QIAact Adapters (24)	181930
GeneRead QIAact Lung RNA Panel (24)	GeneRead QIAact Library Preparation and Target Enrichment Reagents (24) and QIAact Target Enrichment Panel and QIAact Adapters (24)	181935
<b>Related Products</b>		
QIAseq DNA QuantiMIZE Array Kit	qPCR arrays for FFPE DNA quality assessment	333404
QIAseq DNA QuantiMIZE Assay Kit	qPCR assays for FFPE DNA quality assessment	333414
GeneRead DNA FFPE Kit (50)	QIAamp MinElute® columns, Proteinase K, UNG, collection tubes (2 ml), buffers, Deparaffinization Solution, RNaseA	180134
QIAGEN RNeasy® FFPE Kit (50)		73504

---

This page intentionally left blank.

---

This page intentionally left blank.

#### Limited License Agreement for GeneRead QIAact Lung All-in-One Assay

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

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

For updated license terms, see [www.qiagen.com](http://www.qiagen.com).

Trademarks: QIAGEN<sup>®</sup>, Sample to Insight<sup>®</sup>, QIAamp<sup>®</sup>, QIAxcel<sup>®</sup>, QIAxpert<sup>®</sup>, GeneRead™, HotStarTaq<sup>®</sup>, MinElute<sup>®</sup>, QCI™ (QIAGEN Group); GeneReader™ (Intelligent Bio-Systems, Inc.); Agilent<sup>®</sup>, Agilent Technologies<sup>®</sup>, Bioanalyzer<sup>®</sup> (Agilent Technologies, Inc.); Bio-Rad<sup>®</sup>, C1000™ (Bio-Rad Laboratories, Inc.); Eppendorf<sup>®</sup> (Eppendorf AG); PAXgene<sup>®</sup> (PreAnalytiX GmbH); DynaMag™, Qubit<sup>®</sup> (Thermo Fisher Scientific or its subsidiaries). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. 06/2017 HB-2369-001

© 2017 QIAGEN all rights reserved.

---

Ordering [www.qiagen.com/shop](http://www.qiagen.com/shop) | Technical Support [support.qiagen.com](http://support.qiagen.com) | Website [www.qiagen.com](http://www.qiagen.com)