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QIAact Myeloid DNA UMI Panel Handbook

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

181950

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

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAact Myeloid DNA UMI Panel	
Catalog no.	**181950**
Number of reactions	**24**
GeneRead QIAact Library Prep and Target Enrichment Reagents (Kit Box 1)*	
QIAact Beads (Kit Box 2)*	
GeneRead QIAact Myeloid DNA UMI Kit (Kit Box 3)*	

* The three Kit boxes are components of the QIAact Myeloid 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 1.9 ml
TEPCR Buffer, 5x	220 µl
UPCR Buffer, 5x	220 µl
HotStarTaq® DNA Polymerase	2 x 50 µl

* Not for individual sale; to order reagents, see cat. no. 181950.

GeneRead QIAact Myeloid DNA UMI Kit*	
Number of reactions	**24**
QIAact Myeloid DNA UMI Panel Forward Primers	130 µl
QIAact Myeloid DNA UMI 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 3 samples)	12 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. 181950.

Storage

The GeneRead QIAact Library Preparation 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. QIAact Myeloid DNA UMI Panel is shipped on dry ice and should be stored at –15°C to –30°C upon arrival. If stored under these conditions, the kit is stable until the date indicated on the QC label inside the kit lid.

Intended Use

The QIAact Myeloid DNA UMI Panel and GeneRead QIAact Library Preparation 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 QIAact Myeloid 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) and 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. The resultant background artefactual errors greatly limit the detection of true low-frequency variants in heterogeneous cancer samples.

The QIAact Myeloid 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 QIAact Myeloid 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 QIAact Myeloid 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 can 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. To reduce the number of false-positive variants, variants are called across all reads within a UMI versus only calling variants from the original sequencing read (Figure 1).

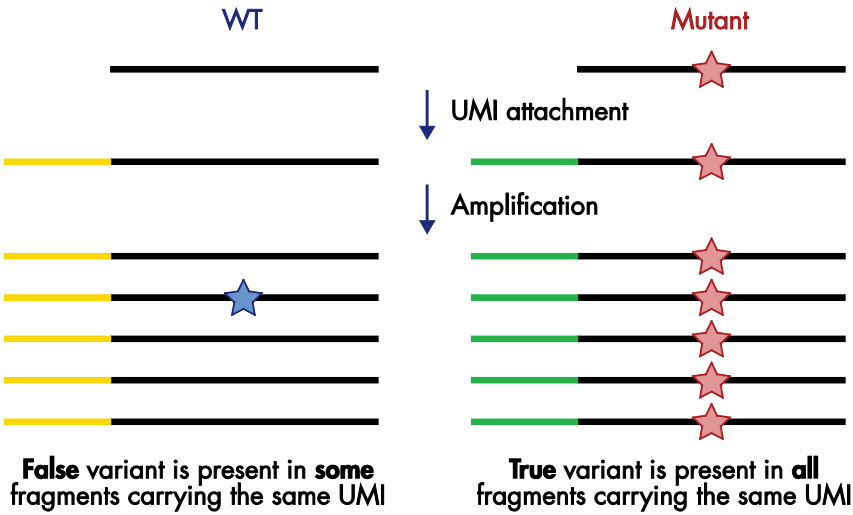

Figure 1. Principle of variant detection with UMI. Each original molecule is tagged by a UMI. True variants are those mutations present in the majority of reads within a UMI, while errors are mutations present in only one or a few reads within a UMI. Description of the variant calling algorithm can be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

Procedure

The QIAact Myeloid DNA UMI Panel is provided as two primer mix tubes, with approximately 400 primers per tube. The QIAact Myeloid DNA UMI Panel is designed to enrich specific target regions in select genes (ASXL1, CALR, CBL, CEBPα*, CSF3R, DNMT3A, EZH2, FLT3*, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NPM1, NRAS, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, TET2, TP53, U2AF1, ZRSR2) using 40 ng of DNA.

* See Notes section page 38

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 nine (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 QIAact Myeloid 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 sample-specific bar code that is added during library preparation (see “Protocol: Adapter Ligation”, page 18). 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 for the QIAact Myeloid DNA UMI Panel” for more information). Based on the total

number of amplicons that are produced by the QIAact Myeloid DNA UMI Panel, we recommend a multiplex of eight (8) samples for either peripheral blood and/or bone marrow samples.

After target enrichment and library preparation, use 625 pg pooled DNA in the clonal amplification process (see "Preparing Libraries for Emulsion Making" in the *QIAGEN GeneRead Clonal Amp Q Handbook* for more information).

Equipment and Reagents to Be Supplied by User

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

In addition to the QIAact Myeloid DNA UMI Panel, the following supplies are required:

For DNA isolation:

- See "Recommended genomic DNA preparation methods", page 14

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 pipet tips and tubes
- Thermomixer, heated orbital incubator, heating block, or water bat
- 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 genomic DNA preparation methods

The QIAGEN QIAamp DNA Blood Mini Kit (Cat No. 51104) and QIAamp DSP DNA blood Mini kit (Cat. No. 61104), are recommended for manual preparation of genomic DNA samples from bone marrow and peripheral blood sample types. QIAamp DNA Blood Mini Qiacube Kit (cat. no. 51126), and QIAsymphony DSP DNA Mini Kit (cat. no. 937236), are recommended for the automated preparation of genomic DNA samples from bone marrow and peripheral blood sample types.

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.

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

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 equipment such as the Qubit 3.0 Fluorometer (Thermo Fisher Scientific cat. no. Q33216). DNA input of 40 ng is recommended.

> **Note**: If available DNA concentration is below 10 ng/µl, dilute DNA to 2.5 ng/µl and add 16.75 µl to the fragmentation reaction. If using this approach, the larger DNA volume replaces the nuclease free water.

* 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: Fragmentation, End-repair and A-addition

Important points before starting

- Ensure input DNA is in 10mM Tris, for example QIAGEN's Buffer EB, QIAGEN's Buffer ATE or low TE (0.1x TE, 0.1mM EDTA).*
- Pre-chill thermal cycler to 4°C.
- It is critical to keep the PCR tubes or plate on ice for the entire time during reaction setup.

Procedure

1. To use 40 ng of DNA as input, dilute DNA to 2.5 ng/µl with nuclease-free water in a LoBind tube.
2. For each sample add 16 µl of diluted DNA (40 ng total) to a separate 0.2 ml PCR tube.
3. Prepare a reaction mixture for fragmentation, end-repair and A-addition according to Table 1. 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 20 µl volume.
4. Add 4 µl master mix to each 0.2 ml PCR tube containing a separate DNA sample (from step 2).

* 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.

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

Component	1x Volume (µl)	8x Volume (µl)*
DNA	16	–
Fragmentation Buffer, 10x	2.5	21.25
FERA Solution	0.75	6.4
Nuclease-Free Water	0.75	6.4
Total volume	**20**	**34.05**

* Take care when pipetting as the solution is viscous.

5. Add 5 µl Fragmentation Enzyme Mix to each reaction and ensure that the reaction solution is mixed well by pipetting up and down 10 times using a pipet set to 15 µl volume.

   IMPORTANT: Do not vortex.

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 the next protocol, "Adapter Ligation", page18.

Protocol: Adapter Ligation

Procedure

1. Prepare the following ligation reaction master mix on ice and mix well by pipetting up and down 10 times using a pipet set to 50 µl volume. (Table 3).

Table 3. Reaction setup for adapter ligation

Component	1x Volume (µl)	8x Volume (µl)*
Ligation buffer, 5x	10	85
DNA Ligase	5	42.5
Ligation Solution (PEG6000, 50%)	7.2	61.2
Total volume	**22.2**	**188.7**

* Take care when pipetting as the solution is viscous.

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 10 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 the next protocol, "Cleanup of Adapter-ligated DNA with QIAact Beads".

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 for at least 30 minutes and vortex thoroughly before use.

   Recommendation: vortex for 1 minute at maximum speed.

2. Transfer 50 µl ligation reaction from "Protocol: Adapter Ligation", 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 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, keep tube lid open and 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 no longer appear shiny.

    Note: As the beads in different tubes may dry at different rates, once beads are dry close 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 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 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 tube from the magnetic rack and centrifuge briefly (10-15 seconds).
22. Replace 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, keep tube lid open and 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 no longer appear shiny. Ethanol carryover can affect enrichment PCR efficiency in the next protocol, "Target Enrichment PCR".

    Note: As the beads in different tubes may dry at different rates, once beads are dry close 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 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 the next protocol,"Target Enrichment PCR".

    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 following master mixes as indicated in Tables 4 and 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. Reaction components for forward target enrichment PCR

Component	1x Volume (µl)	8x Volume (µl)*
DNA library (from "Protocol: Cleanup of Adapter-ligated DNA with QIAact Beads")	9.4	-
TEPCR buffer, 5x	4	34
QIAact Myeloid DNA UMI Panel Forward Primers	5	42.5
GeneReader TE-PCR Primer	0.8	6.8
HotStarTaq DNA Polymerase	0.8	6.8
Total volume	**20**	**90.1**

* Take care when pipetting as the solution is viscous.

Table 5. Reaction components for reverse target enrichment PCR

Component	1x Volume (µl)	8x Volume (µl)*
DNA library (from "Protocol: Cleanup of Adapter-ligated DNA with QIAact Beads")	9.4	-
TEPCR buffer, 5x	4	34
QIAact Myeloid DNA UMI Panel Reverse Primers	5	42.5
GeneReader TE-PCR Primer	0.8	6.8
HotStarTaq DNA Polymerase	0.8	6.8
Total volume	**20**	**90.1**

* Take care when pipetting as the solution is viscous.

2. Add 10.6 µl of master mix from either Table 4 or 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 10 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
2 minutes	98°C	1 (Initial denaturation)
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 the next protocol, "Cleanup of Target Enrichment PCR with QIAact Beads".

   Note: If reactions are to be stored after Target Enrichment PCR protocol, 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 for at least 30 minutes and vortex thoroughly before use.

   Recommendation: vortex for 1 minute at maximum speed.

2. Pulse-centrifuge the Forward and Reverse PCR reactions from "Protocol: Target Enrichment PCR", page 23, and combine them in a 1.5 ml LoBind tube and add nuclease-free water to bring the volume to 100 µl.

   Note: The combined PCR reaction volume may be less than 40 µl due to evaporation. It is important to measure the combined PCR reaction 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 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 tube from the magnetic rack and centrifuge briefly (10-15 seconds).
10. Replace 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, keep tube lid open and 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 no longer appear shiny. . Ethanol carryover can affect PCR efficiency in the next protocol, "Universal PCR Amplification".

    Note: As the beads in different tubes may dry at different rates, once beads are dry close tube 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 solution is clear (5–10 minutes). Transfer 13.4 µl supernatant to a clean 0.2 ml PCR tube. Proceed to the next protocol, "Universal PCR Amplification".

    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 following master mix as indicated in Table 7 in a 1.5 ml LoBind tube. Mix well by pipetting up and down 10 times using a pipet set to 30 µl volume.

Table 7. Reaction components for universal PCR amplification

Component	1 x Volume (µl)	8 x Volume (µl)*
Enriched DNA (from "Cleanup of Target Enrichment PCR with QIAact Beads")	13.4	-
UPCR buffer, 5x	4	34
GeneReader Universal PCR Primer A	0.8	6.8
GeneReader Universal PCR Primer B	0.8	6.8
HotStarTaq DNA Polymerase	1	8.5
Total volume	**20**	**56.1**

* Take care when pipetting as the solution is viscous.

2. Add 6.6 µl of 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 10 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 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	95°C	1
2 minutes	98°C	1 (Initial denaturation)
15 seconds	98°C	19
2 minutes	60°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 the next protocol, "Cleanup of Universal PCR with QIAact Beads".

   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 for at least 30 minutes and vortex thoroughly before use.

   Recommendation: vortex for 1 minute at maximum speed.

2. Transfer 20 µl PCR reaction from "Protocol: Universal PCR Amplification", to a 1.5 ml LoBind tube. Add nuclease-free water to bring the volume to 100 µl.

   Note: The PCR reaction volume may be less than 20 µl due to evaporation. It is important to measure the PCR reaction 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 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 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 tube from the magnetic rack and centrifuge briefly (10-15 seconds).
10. Replace 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, keep tube lid open and 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 no longer appear shiny. Ethanol carryover may affect downstream processing and sample assessment.

    Note: As the beads in different tubes may dry at different rates, once beads are dry close 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 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 36). 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 37).

14. Proceed to "Protocol: Library Concentration Normalization and Pooling for the QIAact Myeloid DNA UMI Panel. This protocol is to be used in place of the "Protocol: Library Concentration Normalization and Pooling" from the GeneRead Clonal Amp Q Handbook.

Protocol: Library Concentration Normalization and Pooling for the QIAact Myeloid DNA UMI Panel

Important points before starting

- Pipettes should be used to measure correct volumes.
- The following instructions apply to pooling libraries that are equal in concentration and produced from the same target enrichment panel.

Things to do before starting

If the libraries to be used are frozen, thaw at room temperature (15–25°C).

Procedure

Dilution of libraries and normalization of concentration to prepare working stock solutions

1. Calculate the amount of library and dilution buffer (Buffer D) required to achieve a working stock solution concentration of 100 pg/µl for each library.

   Note: Pipetting errors due to inaccurate transfer of small volumes can lead to an imbalanced representation of individual libraries in the library pool, poor recovery of live beads after enrichment, or an excess of polyclonal beads in the sequencing run. We recommend adjusting sample dilution calculations so that a minimum of 2 µl sample is pipetted. An additional dilution step can be necessary when using highly concentrated libraries.

   Note: All libraries must be at the same concentration before pooling.

2. Transfer the appropriate volumes of library and Buffer D to a 1.5 ml tube.
3. Close each tube and vortex for 5–10 seconds, and then pulse-centrifuge the tube.

   Note: Diluted libraries can be stored at –20°C for up to one month. Avoid freeze–thaw cycles of stored library.

4. For each bar-coded library that is to be pooled (up to a maximum of 8), transfer 5µl of each working stock (see above) to a 1.5ml tube.
5. Close the tube and vortex for 5–10 seconds, and then pulse-centrifuge the tube.
6. Transfer 10µl of the library pool from step 5 into a 1.5ml tube and incubate at 95°C for 2 minutes on a thermomixer then immediately cool on ice.
7. Pulse-centrifuge the tube and transfer 6.25µl of the pre-denatured 100 pg/µl library pool into a 2 ml non-skirted Sarstedt tube and add 493.75 µl of Buffer D.
8. Close the tube, vortex for 5–10 seconds, and then pulse-centrifuge the tube.
9. Proceed with "Protocol: Emulsion Making" as described in the GeneRead Clonal Amp Q Handbook.

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**.

Library preparation and target enrichment	Comments and suggestions
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.
b) No or low PCR product yield (<2 ng/µl) after Universal PCR	Ensure that all reaction components are thoroughly mixed as described in this handbook before use. Ensure that all reaction components are correctly added at each stage and thoroughly mixed as described in this handbook.

Symbols

Symbol	Symbol definition
	Contains reagents sufficient for <N> tests
	Catalog number
	Manufacturer

Appendix A: Analyze the Library Using 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 blood and bone marrow. The majority of the library fragments are between 200–800 bp in size (Figure 3).

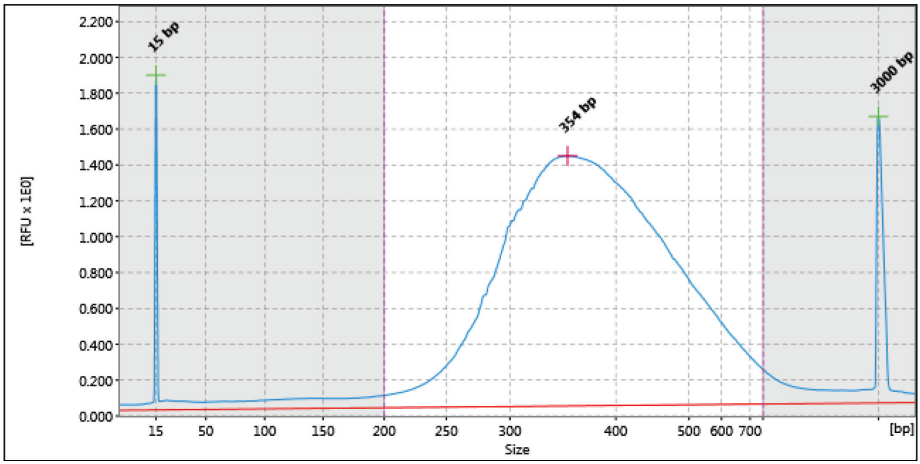

Figure 3. Sample QIAxcel Advanced image of a GeneRead QIAact Myeloid DNA UMI library. The majority of the library fragments are between 200–800 bp in size.

* 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 4). Amounts of DNA under the appropriate peaks can be used to quantify libraries.

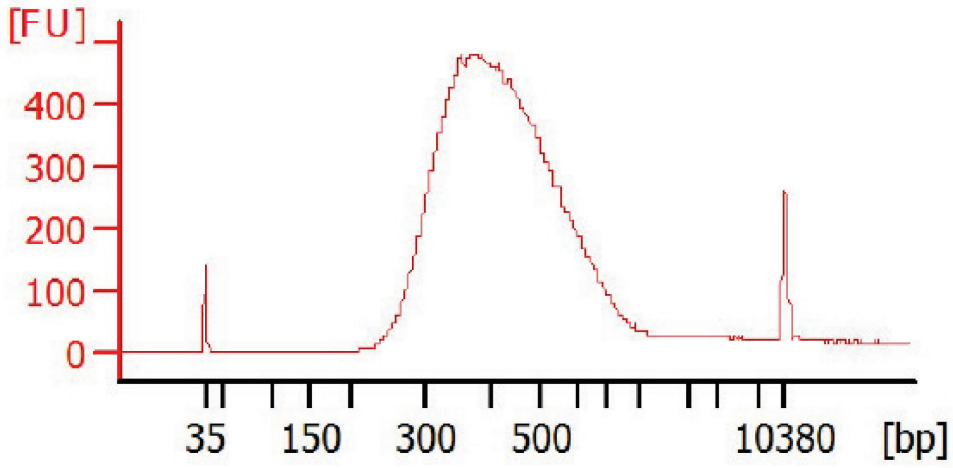

Figure 4. Sample Bioanalyzer image of a GeneRead QIAact Myeloid DNA UMI library. The majority of the library fragments are between 200–800 bp in size.

Notes

High GC content within the CEBPα gene (human Chromosome 19) makes it challenging to detect mutations within this region. Coverage of CEBPα can drop below 44x for any nucleotide located within the 28 bp interval from position 33792589 to 33792617.

Using the QIAact Myeloid DNA UMI Panel, it is possible to detect internal tandem duplication (ITD) in FLT3 of up to 204 bp. However, insertions of >177 bp may or may not be detected depending on the experimental conditions.

Ordering Information

Product	Contents	Cat. no.
QIAact Myeloid DNA UMI Panel (24)	GeneRead QIAact Library Prep and Target Enrichment Reagents (24) GeneRead QIAact Myeloid DNA UMI Kit (24) and QIAact Beads	181950
Related Products		
QIAamp DNA Blood Mini Kit	For 50 DNA minipreps: QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Tubes Collection (2 ml)	51104
QIAamp DSP DNA blood Mini kit	For 50 preps: QIAamp Mini Spin Columns, Buffers, Reagents, Tubes, VacConnectors	61104
QIAamp DNA Blood Mini Qiacube Kit	For 240 DNA minipreps: QIAamp Mini Rotor Adapters (preloaded with spin columns and elution tubes), QIAGEN Protease and buffers.	51126
QIAsymphony DSP DNA Mini Kit	For 192 µl preparations: 2 reagent cartridges, enzyme tube racks and accessories.	937236

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