January 2016

QIAseq 1-Step Amplicon Library Preparation Handbook

For the construction of Illumina-compatible libraries from multiplexed PCR amplicons

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAseq 1-Step Amplicon Library Kit (12)	
Catalog no.	**180412**
4x 1-Step Amplicon Buffer	150 µl
1-Step Amplicon Enzyme Mix	24 µl
HiFi PCR Master Mix, 2x	300 µl
Primer Mix Illumina® Library Amp	20 µl
RNase-Free Water	1.9 ml
Quick-Start Protocol	1

QIAseq 1-Step Amplicon Library Kit (96)	
Catalog no.	**180415**
4x 1-Step Amplicon Buffer	1.2 ml
1-Step Amplicon Enzyme Mix	192 µl
Adapter Plate 96-plex Illumina Barcode*	1 plate
HiFi PCR Master Mix, 2x	1.25 ml
Primer Mix Illumina Library Amp	150 µl
RNase-Free Water	5 x 1.9 ml
Quick-Start Protocol	1

* For adapter sequences, refer to Appendix D.

Storage

The QIAseq 1-Step Amplicon Library Kit is shipped on dry ice and should be stored at –15°C to –30°C upon arrival. When stored correctly at –15°C to –30°C, all reagents are stable for at least 6 months after delivery.

Intended Use

The QIAseq 1-Step Amplicon Library Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq 1-Step Amplicon Library Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

While next-generation sequencing (NGS) has become a vital tool for life sciences and medical research, library preparation remains a key bottleneck in the NGS workflow. The QIAseq 1-Step Amplicon Library Kit is designed for the preparation of Illumina-compatible NGS libraries from multiplexed PCR or gene panel products, and employs an optimized 30-minute one-step library preparation protocol that reduces workflow duration, sample loss and the potential for handling errors and cross contamination. The kit accepts multiplexed PCR products from a variety of sources including the QIAGEN GeneRead™ v2 panels, custom or lab-developed panels or multiplexed PCR assays, as well as other commercial panels. Optimized enzyme and buffer compositions ensure efficient library construction with a wide range of input amounts, and the entire protocol can optionally be performed at room temperature, enabling easy automation.

Principle and procedure

Purified amplicons from gene panels or multiplex PCR are converted to Illumina-compatible NGS libraries using a single, enzymatic library construction step. During this reaction, amplicons are simultaneously prepared for ligation and barcoded adapters are ligated to both ends of the DNA inserts. The adapters contain sequences required for the PCR enrichment of the subsequent library, for flow-cell-binding during bridge amplification and for sequencing primer binding sites for paired-end and multiplexed sequencing.

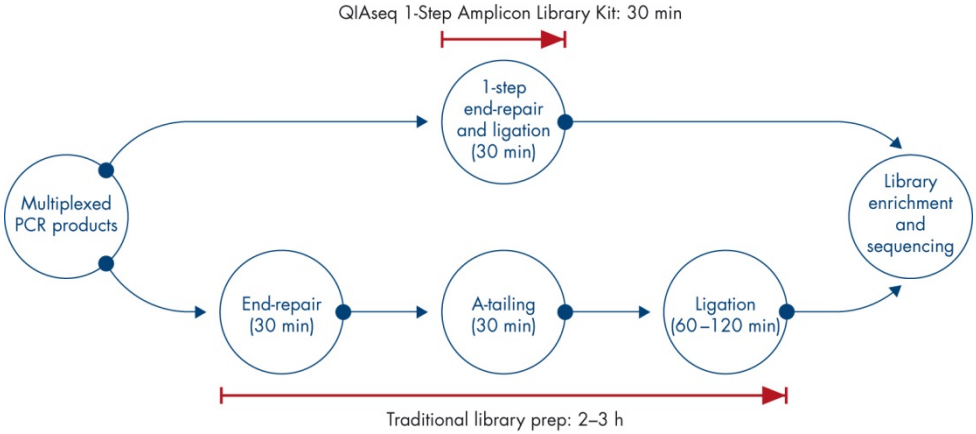

Figure 1. Scheme of optimized one-step amplicon library construction. Purified amplicons from multiplexed PCR or gene panels are converted to sequencing libraries by employing a 30-minute, one-tube library construction step. The libraries are purified with an easy and automatable size selection protocol, and the entire procedure can be performed at room temperature. For low-input applications, libraries can be amplified using the included HiFi PCR Master Mix.

Ready-to-use barcoded adapters are available at concentrations optimized for amplicon sequencing. Single-use adapters are included with the QIAseq 1-Step Amplicon Library Kit (96) for ease-of-use and to reduce the potential for adapter cross-contamination. Adapters for the QIAseq 1-Step Amplicon Library Kit (12) are available separately. Each adapter contains two unique index sequences comprising eight nucleotides each, allowing up to 96 barcoded libraries to be pooled and sequenced in the same run.

Following library construction, excess adapters, adapter dimers and other reaction components are removed via precipitation onto Agencourt® AMPure® XP beads. This procedure is carried out at room temperature, and can be easily automated on various liquid-handling platforms for high-throughput applications.

Following library purification, a high-fidelity library enrichment step can be performed to generate sufficient library from low amounts of starting material. This reaction relies on a

high-fidelity DNA polymerase and optimized buffer conditions that ensure minimum GC bias and extremely low error rates.

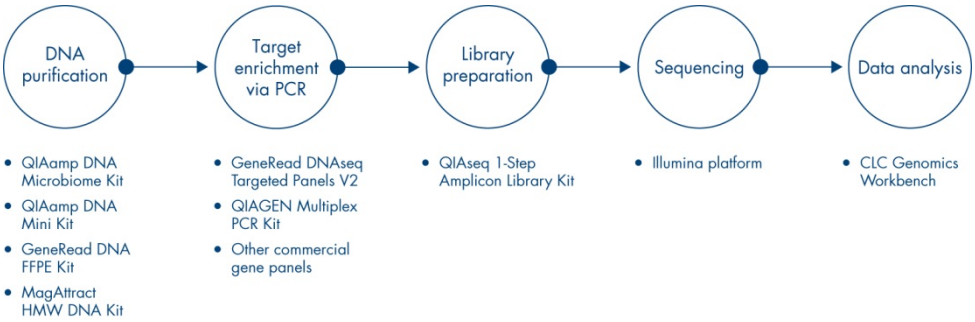

Figure 2. Overview of a complete targeted resequencing NGS workflow including the QIAseq 1-Step Amplicon Library Kit. After DNA extraction and purification with an appropriate kit, target enrichment is performed with the QIAGEN Multiplex PCR Kit, GeneRead DNAseq Targeted Panels v2 or other gene panels or PCR products. NGS library construction is performed with the QIAseq 1-Step Amplicon Library Kit and data is analyzed with the CLC Genomics or Biomedical Workbench.

Compatible sequencing platforms

- Illumina HiSeq®
- Illumina MiSeq®
- Illumina NextSeq®
- Illumina MiniSeq™

Starting materials

- PCR products generated with the GeneRead v2 DNAseq Targeted Panels
- PCR products generated with other custom or commercial gene panels
- PCR products generated with the QIAGEN Multiplex PCR Kit or other QIAGEN PCR reagents
- Multiplexed PCR amplicons generated with *Taq* or *Taq* derivatives

- Multiplexed PCR amplicons generated with Family B polymerases (see Appendix C)

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.

For NGS library construction:

- Agencourt AMPure XP Beads (cat. no. A63880, A63881)
- 80% ethanol
- PCR tubes or plates
- Pipet tips and pipets
- DNA LoBind tubes (from Axygene or Eppendorf)
- Vortexer
- Microcentrifuge
- Thermal cycler
- Magnetic racks for magnetic beads separation (e.g., Thermo Fisher Scientific/Life Technologies, DynaMag™-2 Magnet, cat.no. 12321D)

If using the QIAseq 1-Step Amplicon Library Kit (12), (cat.no. 180412), the following are required in addition:

- GeneRead Adapters (QIAGEN, cat. no. 180985 or 180986).

  Note: Adapters are included with QIAseq 1-Step Amplicon Library Kit (96) (cat.no. 180415).

For NGS library QC:

- QIAxcel® Advanced Instrument (QIAGEN, cat. no. 9001941) or similar capillary electrophoresis instrument
- GeneRead Library Quant Kit (QIAGEN, cat. no. 180612)

- Real-time PCR machine for library quantification

Important Notes

PCR products should be free of contaminating primers, primer-dimers or other PCR artifacts prior to library preparation.

Recommended library quantification method

QIAGEN's GeneRead Library Quant Kit (cat. no. 180612), which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library.

We recommend purification of the PCR products prior to library preparation with Agencourt AMPure XP beads (see Appendix B).

Protocol: Library Construction Using the QIAseq 1-Step Amplicon Library Kit for Illumina Sequencing

This protocol describes library construction for sequencing on Illumina platforms.

Procedure

One-step end-repair and adapter ligation

1. Prepare a reaction mix for adapter ligation according to Table 1, adding the components to the PCR tube or plate containing purified PCR products.

   Note: When using barcode adapters, open one adapter tube at a time and change gloves between pipetting the different barcode adapters to avoid cross-contamination.

Table 1. Reagents for adapter ligation of PCR product

Component	Volume/reaction (µl)
Purified PCR amplicons from gene panel or multiplex PCR product	Variable; 10–100 ng*
4x 1-Step Amplicon Library Buffer	12.5
Adapter Plate 96-plex Illumina **or**	4
GeneRead 12-plex adapter (cat. nos. 180985 or 180986)†	2
1-Step Amplicon Enzyme Mix	2
DNase-free water	Variable
Total	**50**

* We recommend the quantification of PCR products via a microfluidics or capillary electrophoresis platform.

† If using adapters from another supplier, add the adapter to 1 µM final concentration, or according to supplier's directions.

2. Mix the components by pipetting up and down several times.
3. Program a thermal cycler to incubate at 25°C for 30 min. Optionally, the reaction can be incubated at room temperature.

   IMPORTANT: Do not use a thermal cycler with a heated lid.
4. After the reaction is complete, place the reactions on ice and proceed with purification using Agencourt AMPure XP beads.

Cleanup of adapter-ligated DNA with Agencourt AMPure XP beads

5. Prepare 1.5 ml LoBind tubes for each ligation reaction and label tubes or prepare a 96-well plate (depending on availability of magnetic rack and individual preferences).
6. Transfer the 50 µl ligation reaction from step 4 to the prepared 1.5 ml LoBind tube or 96-well plate. Add 50 µl nuclease-free water and 40 µl (0.4x volume) Agencourt AMPure XP beads to each ligation reaction (50 µl) and mix well by pipetting.

   Note: The addition of water ensures correct binding conditions.
7. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) for 5 min.
8. Prepare a new LoBind tube for each ligation reaction or a new 96-well plate.
9. Carefully transfer 133 µl supernatant to the new tubes without disturbing the beads. This will leave behind about 7 µl supernatant. Discard the beads, which contain unwanted large DNA fragments. The large DNA fragments are generated by ligation of the adapter to non-specific multiplex PCR products.

   Note: Do not discard the supernatant.
10. Add 40 µl of resuspended Agencourt AMPure XP Beads slurry to the supernatant and mix well by pipetting.
11. Incubate the mixture for 5 min at room temperature.
12. Pulse-spin the tube or plate. Pellet the beads on a magnetic stand (e.g., DynaMag) for 5 min, then carefully remove and discard the supernatant. Be careful not to disturb the beads, as they contain the library.

Note: Do not discard the beads.

13. Wash the beads by adding 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once for a total of 2 ethanol washes.
14. Try to remove the residual ethanol as much as possible without disturbing the beads. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. Remove from the magnetic stand.
15. Elute by resuspending in 26 µl of nuclease-free water. Mix well by pipetting and incubate the tube or plate at room temperature for 2 min to elute the DNA from the beads.
16. Place the tube or plate back on the magnetic rack to pellet the beads. Incubate until the liquid is clear.
17. Use 23.5 µl of the eluate in the library amplification procedure, or quantify and sequence directly if the amount of input amplicon was sufficient.

PCR amplification of purified library

18. Program a thermal cycler with a heated lid according to Table 2.

Table 2. Cycling conditions for the amplification of the DNA library

Time	Temperature	Number of cycles
Initial denaturation		
2 min	98°C	1
Annealing		
20 s	98°C	
30 s	60°C	4–10*
30 s	72°C	
Final extension		
1 min	72°C	1
∞	4°C	Hold

* **Note**: Cycle number depends on the amount and quality of input amplicon. In general, 4 cycles are sufficient for 20–500 ng of input PCR product and 10 cycles are sufficient for 1–20 ng of input PCR product. If input DNA is sufficient (>500 ng), library amplification can be omitted.

19. Mix the components in Table 3 in a 0.2 ml PCR tube or 96-well PCR plate.

Table 3. Reaction components for PCR amplification

Component	Volume (µl)
HiFi PCR Master Mix, 2x	25
Primer Mix (10 µM each)	1.5
Library DNA (from previous step)	23.5
Total	**50**

20. Transfer the PCR plate to the thermal cycler and start the program.
21. Once PCR is complete, add 50 µl of resuspended Agencourt AMPure XP Beads to each reaction (50 µl) and pipet up and down thoroughly to mix the beads and PCR mix.
22. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.

23. Wash the beads by adding 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once for a total of two ethanol washes. Remove from the magnetic stand.
24. Incubate on the magnetic stand for 5–10 min of until the beads are dry. Over-drying of beads may result in lower DNA recovery. Remove from the magnetic stand.
25. Elute by resuspending in 30 µl nuclease-free water. Mix well by pipetting. Pellet the beads on the magnetic stand. Carefully transfer 28 µl supernatant to a clean LoBind 1.5 ml tube or PCR plate.
26. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the expected size distribution of library fragments, for the absence of adapters, amplification primers, adapter-dimers or high-molecular-weight overamplification artifacts.

    Note: The library should show a distribution reflecting the size of the input PCR amplicons plus 120 bp. The increase in library length reflects the addition of the sequencing adapters to the PCR amplicons.

    Note: The median fragment size can be used in subsequent qPCR-based quantification methods to calculate library concentration.
27. Quantify the library using the GeneRead Library Quant Kit (cat. no. 180612 [not provided]) or a comparable method.
28. The purified library can be safely stored at –20°C in a DNA LoBind tube until ready to sequence.

Typical results

When high-quality, artifact-free PCR products are used; QIAseq 1-Step Amplicon libraries are typically free of adapter dimers, library amplification primers, excess adapters and high-molecular-weight amplification artifacts. When an appropriate cycle number for the input amount is chosen, library yield should be approximately 5 nM after purification, and the volume should be sufficient for quality control, library quantification and sequencing on most NGS platforms.

In the example experiment, as shown in figure 3, reference DNA was amplified with the GeneRead DNAseq V2 Human Comprehensive Cancer Panel (cat. no. 181901, 06/2015 version). This panel consists of 4 separate PCR reactions, each including 1988 primer pairs. Amplicons are designed to capture 160 cancer-related genes comprising approximately 745 kb of the genome. After PCR, products were pooled and analyzed for yield and length distribution (Figure 3).

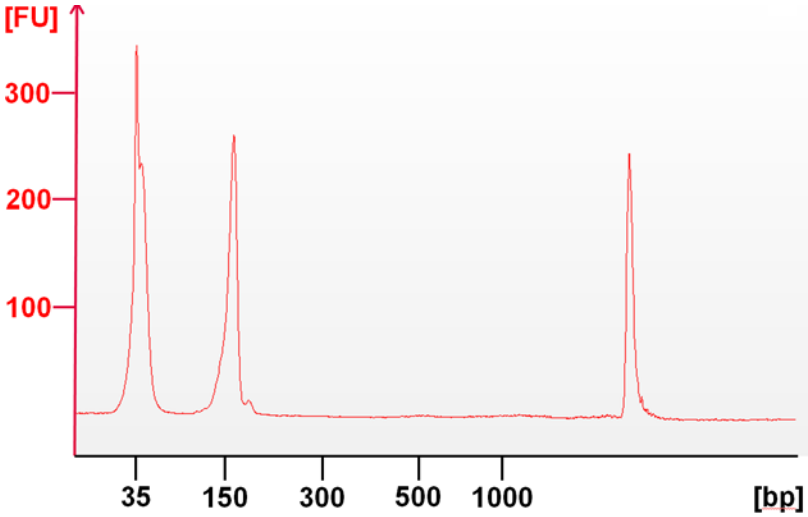

Figure 3. Electrophoresis trace of pooled multiplexed PCR amplicons. After amplification and pooling, a peak of expected size (160 bp), which represents the mean amplicon size of this set of PCR primers is seen. A second large peak centered at 35 bp is comprised of the electrophoresis marker, unextended primers and extended primer-dimers. During purification, these shorter products are removed, leaving only the desired PCR amplicons.

PCR products were purified with Agencourt AMPure XP Beads according to the *GeneRead DNAseq Targeted Panels V2 Handbook*, and 25 ng was used to generate libraries with the QIAseq 1-Step Amplicon Library Kit. Libraries were amplified with 4 cycles of PCR, and yielded a total of 28 µl of 5 nM library after purification (Figure 4, 1:10 dilution shown). While sequencing quality, read length and the number of reads obtained will vary,

depending on the sequencing platform, using a library quantification method such as the QIAGEN GeneRead DNAseq Library Quant Array enables more accurate clustering, optimizing data yield and quality.

Typical data generated with the QIAseq 1-Step Amplicon Library Kit contains minimal reads arising from adapter dimers, and when paired with a high-quality panel, often has >95% on-target reads, with even read distributions over target amplicons.

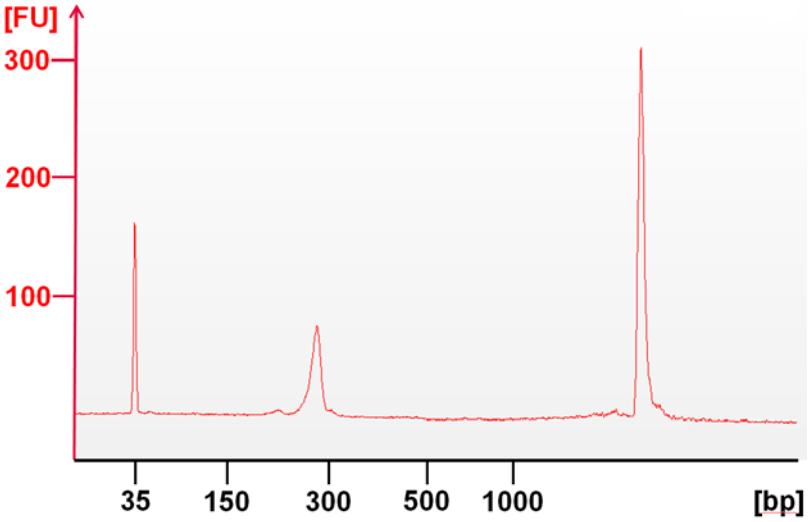

Figure 4. Electrophoresis trace of a purified library prepared using the QIAseq 1-Step Amplicon Library Kit. A characteristic size shift of 120 bp is observed due to the additional sequence added by the adapters. Typical libraries should be free of shorter peaks composed of excess primers, adapters or adapter-dimers and high-molecular-weight peaks, which can be introduced by the gene panel or through overamplification of the completed libraries.

Troubleshooting Guide

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

	Comments and suggestions
Low library yields	
a) Suboptimal reaction conditions due to impurity with multiple PCR products	Make sure to use PCR purification methods that effectively remove impurities that could potentially inhibit QIAseq 1-Step Amplicon Library Kit enzymes.
b) Insufficient amount of starting DNA	Make sure at least 2 ng multiplex PCR products and correct cycle numbers for library amplification PCR are used for library prep. Consider increasing the amount of PCR products used or the number of cycles of library amplification
c) Over-drying of Agencourt AMPure XP beads	Over-drying Agencourt AMPure XP beads could decrease elution efficiency. Ensure that the beads are not dried for more than 10 min at room temperature.
Unexpected signal peaks in capillary electrophoresis device traces	
a) Presence of shorter peaks between 60 and 160 bp	These peaks represent library adapters (about 60 bp), adapter dimers (about 120 bp) or adapter-ligated PCR primer dimers (about 150–160 bp) that occur when there is no, or insufficient, adapter depletion after library preparation. As adapter dimers can form clusters on the flow cell and will be sequenced, this will reduce the capacity of the flow cell for the library fragments, even though a low ratio of adapter-dimers versus library will not be a problem. If necessary, repeat the protocol 'Cleanup of adapter-ligated DNA with Agencourt AMPure XP Beads', page 13, to remove the residual adapters and adapter dimers.
b) Presence of larger library fragments	If the fragment population shifts higher than expected after adapter ligation and PCR enrichment (e.g., more than the expected 120 bp shift), this can be due to insufficient depletion of the larger nonspecific fragments. If necessary, repeat the protocol 'Cleanup of adapter-ligated DNA with Agencourt AMPure XP Beads', page 13, to remove the residual large fragments.

		Comments and suggestions
c)	Incorrect library fragment size after adapter ligation	During library preparation, adapters of approximately 60 bp are ligated to both ends of the DNA library fragments. This should be reflected on a capillary electrophoresis device by a shift in size of all library fragments of 120 bp. If using library adapters from other suppliers, please refer to the size information given in the respective documentation. The absence of a clear size shift may indicate no, or only low, adapter ligation efficiency. Make sure to use the parameters and incubation times described in the handbook, as well as the correct amount of starting DNA.

Appendix A: DNA Isolation and Quality Control

High-quality DNA is essential for obtaining reliable sequencing results, and proper sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, extraction reagents or other contaminants can degrade DNA, interfere with DNA quantification or inhibit downstream PCR.

For the isolation and purification of high-quality genomic DNA, we recommend the following QIAGEN kits:

- QIAamp® DNA Mini Kit (cat. no. 51304) for the preparation of genomic DNA samples from fresh tissues
- GeneRead DNA FFPE Kit (cat. no. 180134) for the preparation of NGS-ready genomic DNA FFPE tissue samples
- MagAttract® HMW DNA Kit (cat. no. 67563) for isolation of high-molecular-weight genomic DNA
- QIAamp DNA Microbiome Kit (cat. no. 51704) for isolation of bacterial microbiome DNA from mixed samples
- QIAamp Circulating Nucleic Acid Kit (cat. no. 55114)

For accurate DNA quantification, we recommend the QIAxpert® (cat. no. 9002340).

Note: When using a UV-vis spectrophotometer to quantify DNA, ensure that samples have been treated to remove RNA, since the absorption spectra of RNA and DNA overlap significantly. For best results, DNA should be re-suspended in DNase-free water or DNase-free 10 mM Tris buffer pH 8.0. Do not use DEPC-treated water.

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

Concentration and purity determined by UV spectrophotometry

The concentration and purity of DNA should be determined with a UV-vis spectrophotometer. Prepare dilutions and measure absorbance in 10 mM Tris buffer pH 8.0, since the absorption spectra of nucleic acids is dependent on pH.

The A_{260}:A_{280} ratio should be greater than 1.8.

The concentration determined by A_{260} should ideally be >2.5 µg/ml DNA.

For accurate DNA quantification, we recommend the QIAxpert (cat. no. 9002340).

DNA integrity

For best results, genomic DNA should be greater than 2 kb in length, with many fragments greater than 10 kb. This can be checked by running a fraction of each DNA sample on a 1% agarose gel.

FFPE DNA

If FFPE DNA will be used, PCR conditions and cycle number for target enrichment may need to be optimized. This can be done with standard qPCR reagents, or for the GeneRead V2 DNAseq panels, the QIAGEN GeneRead DNA QuantiMIZE assay.

Appendix B: Amplicon Preparation and Quality Control

Amplicon preparation and quality control

The QIAseq 1-Step Amplicon Library Kit accepts amplicons generated from a wide variety of multiplex PCR reactions or gene panels. Regardless of the PCR system used, high-quality amplicons are key to the production of high-quality NGS libraries.

When conducting target enrichment via multiplex PCR, avoid overamplification into the plateau phase. When overamplified, amplicons produced in earlier cycles of PCR serve as both primer and template for further amplification, generating long, chimeric molecules. These are visible as high-molecular-weight products with a broad range of lengths, and can be visible on the QIAxcel or Agilent® Bioanalyzer trace as a second peak much larger than the amplicons of interest, or as a high-molecular-weight smear sometimes extending into the wells on an agarose gel. These chimeric products can interfere with library preparation and sequencing, and if present, cycle number should be decreased appropriately.

Following PCR, amplicons should be purified with Agencourt AMPure XP Beads or a similar cleanup technology to remove excess primers, primer dimers and buffer components. As the QIAseq 1-Step Amplicon Library Kit accepts a wide range of DNA inputs, amplicon quantification after purification is not strictly necessary if a robust DNA extraction and PCR system is available, but is recommended. Amplicons can be quantified on a QIAxcel, agarose gel, Agilent Bioanalyzer, Qubit® or Nanodrop.

Low-diversity libraries

In contrast to a standard whole genome library, the base composition at the beginning of the reads in an amplicon-sequencing library reflects the base composition of the primers used to produce the PCR amplicons. In experiments involving hundreds or thousands of primer pairs,

the sequence diversity at the beginning of each read is high; however in low-multiplexing experiments, where only 8–12 primer pairs are used, sequence diversity is low.

Low sequence diversity can interfere with normal cluster calling, phasing and quality matrix calculation on Illumina platforms. We recommend users to follow all guidelines set forth by the instrument manufacturer for the sequencing of low-diversity libraries if lower-complexity amplicons are used. These may include the intentional underclustering of the flow cell or mixing amplicon libraries with the PhiX standard or barcoded high-diversity whole genome or RNAseq libraries.

Appendix C: Optional A-Tailing Protocol

While the QIAseq 1-step Amplicon Library Kit accepts PCR products from a wide range of sources, some care must be taken to confirm polymerase compatibility. The novel one-step reaction requires that PCR amplicons contain 3′ A-overhangs for efficient ligation. *Taq* polymerase, the most commonly used thermostable DNA polymerase, and its derivatives, by default carry out this non-templated A-addition during the PCR reaction. *Taq* and *Taq*-derivatives have attributes that make them amenable to multiplex PCR, and many commercial gene panels employ a *Taq*-based enzyme.

In contrast to *Taq*, other polymerases with strong 3′–5′ exonuclease activities do not carry out this reaction. While these enzymes are not commonly used for multiplexed PCR, amplicons produced with such enzymes are still compatible with the QIAseq 1-Step Amplicon Library Kit, but require A-tailing prior to ligation. A suggested A-tailing protocol is given below.

Materials required for A-tailing

- MinElute® PCR Purification Kit (QIAGEN, cat. no. 28004 or 28006), or Agencourt AMPure XP Beads (Beckman Coulter, cat. no. A63880)

- Klenow (3′→ 5′ exo-) Low Concentration (Enzymatics, P7010-LC-L)
- 10X Blue Buffer (Enzymatics, B0110)
- 100 mM dATP Solution (Enzymatics, N2010-A-L)
- Pipet tips and pipets

Procedure

C1. Mix the components in Table 4 and add to the PCR tube or plate containing purified PCR products.

Table 4. Reaction components for A-tailing

Component	Volume (µl)
Purified PCR product	20
10x Blue Buffer	5
100 mM dATP	1
Klenow (3′→ 5′ exo-) Low Concentration	1
DNase-free water	23
Total	**50**

C2. Incubate in a thermal cycler or heating block for 30 minutes at 37°C.

C3. Purify with the MinElute PCR Purification Kit or Agencourt AMPure XP Beads as per the manufacturer's directions.

Appendix D: Adapter Indices for the Adapter Plate 96-plex Illumina Barcode

The index sequences used in Adapter Plate 96-plex Illumina Barcode are listed in Table 5. Indices 501–508 and 701–712 correspond to the respective Illumina adapter indices. The indices i5 and i7 are combined to generate 96 individual index combinations. The layout of the 96-plex single-use adapter plate is displayed in Figure 5.

Table 5. Adapter barcodes in the QIAseq 1-Step Amplicon Library Kit (96)

Codes for entry on sample sheet			
D50X barcode name	**i5 bases for entry on sample sheet**	**D50X barcode name**	**i7 bases for entry on sample sheet**
D501	TATAGCCT	D701	ATTACTCG
D502	ATAGAGGC	D702	TCCGGAGA
D503	CCTATCCT	D703	CGCTCATT
D504	GGCTCTGA	D704	GAGATTCC
D505	AGGCGAAG	D705	ATTCAGAA
D506	TAATCTTA	D706	GAATTCGT
D507	CAGGACGT	D707	CTGAAGCT
D508	GTACTGAC	D708	TAATGCGC
		D709	CGGCTATG
		D710	TCCGCGAA
		D711	TCTCGCGC
		D712	AGCGATAG

	1	2	3	4	5	6	7	8	9	10	11	12
A	501/701	501/702	501/703	501/704	501/705	501/706	501/707	501/708	501/709	501/710	501/711	501/712
B	502/701	502/702	502/703	502/704	502/705	502/706	502/707	502/708	502/709	502/710	502/711	502/712
C	503/701	503/702	503/703	503/704	503/705	503/706	503/707	503/708	503/709	503/710	503/711	503/712
D	504/701	504/702	504/703	504/704	504/705	504/706	504/707	504/708	504/709	504/710	504/711	504/712
E	505/701	505/702	505/703	505/704	505/705	505/706	505/707	505/708	505/709	505/710	505/711	505/712
F	506/701	506/702	506/703	506/704	506/705	506/706	506/707	506/708	506/709	506/710	506/711	506/712
G	507/701	507/702	507/703	507/704	507/705	507/706	507/707	507/708	507/709	507/710	507/711	507/712
H	508/701	508/702	508/703	508/704	508/705	508/706	508/707	508/708	508/709	508/710	508/711	508/712

Figure 5. Adapter Plate 96-plex Illumina Barcode Plate layout.

Appendix E: Data Analysis

After sequencing, multiplex data can be analyzed using QIAGEN's CLC Genomics or Biomedical Workbench or the cloud-based GeneRead DNAseq Sequence Variant Analysis Software. Our data analysis software will perform quality control, read trimming (removing primer sequences), mapping to a reference genome and variant identification. Please refer to the corresponding documentation for data analysis.

Ordering Information

Product	Contents	Cat. no.
QIAseq 1-Step Amplicon Library Kit (12)	Reagents for Illumina Amplicon Seq library preparation, and library amplification	180412
QIAseq 1-Step Amplicon Library Kit (96)	Reagents for Illumina Amplicon Seq library preparation and library amplification, including 96-plex Illumina Adapters	180415
QIAGEN library preparation accessories		
GeneRead Adapter I Set A 12-plex (144)	12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180985
GeneRead Adapter I Set B 12-plex (144)	12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180986
GeneRead DNAseq Library Quant Array	Reagents for NGS sample library quantification following targeted enrichment	180601
GeneRead qPCR SYBR® Green Mastermix	Master mix for use with the GeneRead Library Quant Arrays and Kit	Varies
GeneRead Size Selection Kit (50)	Spin columns and buffers for purification of PCR products >150 bp	180514
QIAquick® PCR Purification Kit (50)	QIAquick Spin Columns, Buffers, Collection Tubes (2 ml) for purification of PCR products <150 bp	28104
QIAGEN panels for target enrichment		
GeneRead DNAseq Targeted Panels V2	Sets of 1 or 4 pools containing wet-bench verified primer sets for targeted enrichment of a focused panel of <100 genes	181900
GeneRead DNAseq Targeted HC Panel V2	Sets of 4 pools containing wet-bench verified primer sets for targeted enrichment of a focused panel of >100 genes	181901

Product	Contents	Cat. no.
GeneRead DNAseq Custom Panel V2	Pools containing primer sets for targeted enrichment of a customized panel of genes or genomic regions	181902
GeneRead DNAseq Mix-n-Match Panel V2	Pools containing wet-bench verified primer sets for targeted enrichment of a custom panel of genes	181905
GeneRead DNAseq Panel PCR Kit V2	PCR chemistry for use with the GeneRead DNAseq Panel V2 System	Varies
GeneRead DNA QuantiMIZE Array Kit	qPCR arrays for optimizing the amount of input DNA and PCR cycling conditions for targeted enrichment of FFPE DNA	180642
QIAGEN PCR reagents for target enrichment		
QIAGEN Multiplex PCR Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 x 0.85 ml), 5x Q-Solution® (1 x 2.0 ml), RNase-Free Water (2 x 1.7 ml)	206143
QIAGEN Multiplex PCR *Plus* Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x Multiplex PCR Master Mix (3 x 0.85 ml), 5x Q-Solution (1 x 2 ml), RNase-Free Water (2 x 1.9 ml), 10x CoralLoad® Dye (1 x 1.2 ml)	206152
REPLI-g® whole genome amplification for sensitive applications		
REPLI-g Single Cell Kit (96)	Reagents for whole genome amplification from limited input materials or single cells	150345
REPLI-g FFPE Kit (100)	Reagents for whole genome amplification from FFPE samples	150245
QIAGEN kits for genomic DNA isolation and purification		
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

Product	Contents	Cat. no.
GeneRead DNA FFPE Kit (50)	QIAamp MinElute columns, Proteinase K, UNG, Collection Tubes (2 ml), Buffers, Deparaffinization Solution, RNase A	180134
MagAttract HMW DNA Kit (48)	For 48 DNA preps: MagAttract Suspension G, Buffer ATL, Buffer AL, Buffer MB, Buffer MW1, Buffer PE, Proteinase K, RNase A, Buffer AE, Nuclease-Free Water	67563
QIAamp DNA Microbiome Kit (50)	For 50 DNA preps: 50 QIAamp UCP Mini Columns, 50 Pathogen Lysis Tubes L, buffers, reagents, Collection Tubes (2 ml)	51704

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