Quick-Start Protocol September 2017

QIAseq™ Stranded Total RNA Library Kit

Part 3: CleanStart library amplification and data analysis recommendations

Immediately upon receipt, store the QIAseq Stranded Total RNA Kit (cat. nos. 180753 and 180755) at –30°C to –15°C. QIAseq Beads (cat. nos. 1107149, 1107460) should be stored at 4°C (**do not freeze**). If stored under these conditions, kits are stable until the date indicated on the QC label.

Further information

- *QIAseq Stranded Total RNA Library Handbook*: **www.qiagen.com/HB-2465**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- To maximize output yields and minimize adapter dimer formation during bead handling, use 2 ml tubes.
- **Important**: Ensure reactions are thoroughly mixed by pipetting up and down, prepared and incubated at recommended temperatures.
- Use a thermal cycler with a heated lid to incubate reactions.
- QIAseq CleanStart PCR reagents use a proprietary PCR reaction, in conjunction with modification enzymes, to ensure that previously constructed NGS libraries are removed. If a previously amplified CleanStart library needs to be re-amplified, for instance, if an additional library is needed to replace a failed NGS run, omit the decontamination step of the PCR protocol to disable selective degradation.

CleanStart amplification

1. Add 1.5 µl CleanStart PCR Primer Mix, Illumina® and 25 µl CleanStart PCR Mix 2X to each sample (23.5 µl) and perform amplification as described in Table 1. Recommended PCR cycles are listed in Table 2.

Table 1. CleanStart library amplification conditions

Step	Time	Temperature	Number of cycles
CleanStart decontamination*	15 min	37°C	1
Initial denaturation	2 min	98°C	1
PCR	20 s 30 s 30 s	98°C 60°C 72°C	7–16*
Final extension	1 min	72°C	1
Hold	∞	4°C	Hold

* Low-quality RNA (e.g., FFPE RNA) could require additional PCR cycles.

Table 2. Recommended cycle numbers

Total RNA		mRNA enrichment protocol	
Input†	Cycles	Input‡	Cycles
1 ng	14–16	100 ng	14–16
10 ng	11–13	500 ng	11–13
50 ng	9–11	1 µg	9–11
100 ng	7–9	5 µg	7–9

† Indicates amount of RNA previously enriched for mRNA or depleted for rRNA.

‡ Indicates amount of total RNA prior to mRNA enrichment.

2. After amplification, add 60 µl QIAseq Beads. Mix well by pipetting up and down 10 times.
3. Incubate for 5 min at room temperature.

4. Place the tubes on a magnetic rack. After the solution has cleared (~10 min or longer), carefully remove and discard the supernatant.

   Important: Do not discard the beads as they contain the DNA of interest.

5. With the tubes still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tube (2 to 3 times) to wash the beads. Carefully remove and discard the wash.

6. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol wash after this second wash. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.

7. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 5 to 10 min.

   Note: Visually inspect that the pellet is completely dry, but avoid over-drying.

8. Remove the tubes from the magnetic stand, and elute the DNA from the beads by adding 22 µl nuclease-free water. Mix well by pipetting.

9. Return the tubes to the magnetic rack until the solution has cleared.

10. Transfer 20 µl to clean tubes/plate. Alternatively, the samples can be stored at –20°C in a constant-temperature freezer.

    Note: Final QC measurements can be performed with the QIAxpert®, Agilent® Bioanalyzer/TapeStation (library size distribution, yields) or qPCR with primers complementary to the adapter sequences and DNA standards (with more accurate yield calculations) (see the QIAseq Library Quant Array Kit, cat. no. 333304). Accurate library yield measurements, especially with qPCR, ensure correct clustering of NGS flow cells. Refer to the kit handbook at **www.qiagen.com** for QC measurement details.

    Important: If excessive adapter dimers are visible after QC measurements with ~130 bp sizes (>1–2% of total library yields), perform a second purification with QIAseq Beads. This can be accomplished by bringing the sample to a final volume of 55 µl and repeating steps 2–10.

Recommendations for data analysis – RNA-seq alignment

Downstream NGS data can be analyzed with CLC (Biomedical) Genomics Workbench (Desktop or Server versions available). For further information, see **www.qiagen.com/products/bioinformatics/rnaseq-analysis**. (CLC) Biomedical Genomics Workbench is a comprehensive analysis package for the analysis and visualization of data from all major NGS platforms. The workbench supports and seamlessly integrates into a typical NGS workflow. (CLC) Biomedical Genomics Workbench is available for Windows®, Mac OS X and Linux platforms. Incorporating cutting-edge technology and algorithms, (CLC) Biomedical Genomics Workbench supports key NGS features within genomics, transcriptomics and epigenomics research fields. Additionally, it includes all the classical analysis tools of CLC Main Workbench.

Recommendations for data analysis – gene expression interpretation

Ingenuity® Pathway Analysis (IPA®) is an all-in-one, web-based software application that enables analysis, integration and understanding of data from gene expression, miRNA and SNP microarrays, as well as metabolomics, proteomics and RNA-seq experiments and is the ideal tool to interpret data generated using the QIAseq Stranded Total RNA Library / mRNA Select Kit. IPA is the market leader in gene expression analysis, and is cited in over 18,000 scientific publications to date. IPA's data analysis and search capabilities can be used to understand the significance of data, specific targets or candidate biomarkers in the context of larger biological or chemical systems. The software is backed by the Ingenuity Knowledge Base of highly structured, detail-rich biological and chemical findings.

For information on IPA, see **www.qiagen.com/ products/bioinformatics/rnaseq-analysis**

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.