Product Profile

QIAseq® FastSelect™ –5S/16S/23S Kits

For fast and efficient bacterial rRNA removal during RNA-seq library prep

Bacterial rRNA removal is challenging, particularly for metatranscriptomic studies. The incredible diversity of the microbiome – with nearly 600,000 different 16S sequences and nearly 170,000 23S sequences – renders existing rRNA removal methods inefficient and insufficient. Current procedures such as hybridization/capture methods optimized only on a few species and species-specific enzymatic removal fail to remove appreciable amounts of rRNA from community samples. These methods waste a lot of reads on rRNA, which leads to insufficient gene detection and distortion of transcriptomic profiles. Furthermore, these methods are time-consuming, labor-intensive and ignore the 5S rRNA sequence.

QIAseq FastSelect –5S/16S/23S Kits provide:

- Compatibility with QIAGEN®, Illumina®, NEB® and other stranded RNA-seq library kits
- Flexibility in input RNA quantity (20 ng to 1 µg) and quality (fresh and high-quality RNA, FFPE RNA or degraded RNA)
- Convenience with independent fragmentation and rRNA removal modules
- Highly efficient rRNA removal in just one hour

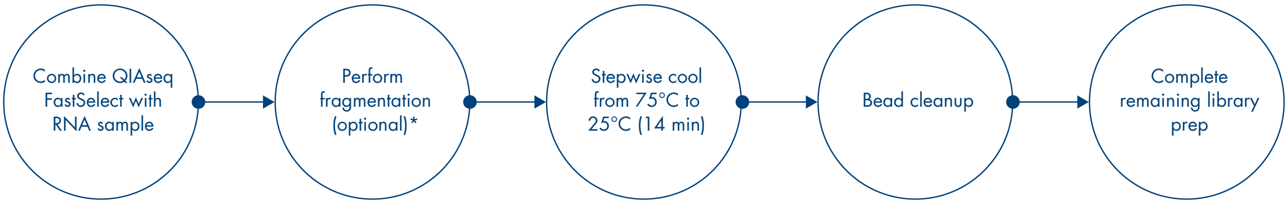


Figure 1. The QIAseq FastSelect –5S/16S/23S workflow. QIAseq FastSelect –5S/16S/23S is a one-step rRNA removal solution. Simply add QIAseq FastSelect reagent to the RNA sample, perform fragmentation (if required), stepwise cool the reaction from 75°C to 25°C over 14 minutes, perform a bead-based cleanup step and complete the remaining library prep steps. QIAseq FastSelect works with or without RNA fragmentation, providing the flexibility to use RNA from FFPE samples, degraded RNA samples or high-quality RNA as part of a standard RNA-seq library construction workflow.

* Absolute requirement for fragmentation, as well as the duration of the fragmentation, depends on the library prep kit and sample type. Fragmentation is not required for QIAseq FastSelect's performance.

A fast and simple process that easily integrates with your existing workflow

Most RNA removal or depletion strategies associated with RNA-seq library construction are sample pre-treatment strategies involving hybrid capture or enzymatic removal of unwanted RNA. Our unique QIAseq FastSelect procedure seamlessly integrates with QIAGEN, Illumina, NEB and other RNA-seq stranded library preparation kits, removing rRNA in a single step during reverse transcription (Figure 1). This is dramatically faster than competing RNA depletion kits, which involve laborious (>25 steps) and time-consuming (~2h) pre-treatment protocols. Furthermore, QIAseq FastSelect accommodates fresh, high-quality RNA, as well as degraded RNA from environmental samples.

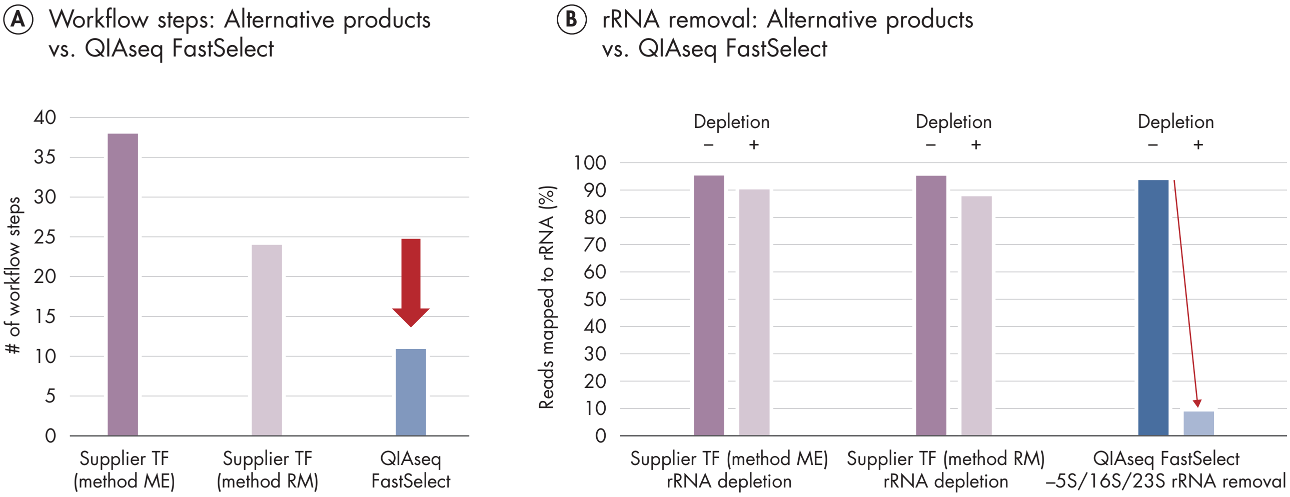
QIAseq FastSelect is compatible with all stranded RNA-seq library preparation kits, including QIAseq Stranded Total RNA Lib Kit (QIAGEN), TruSeq® Stranded (Illumina) and NEBNext® Ultra II Directional (New England Biolabs®) library preparation kits. The duration and other specific requirements of an optional fragmentation step (which is not a requirement for QIAseq FastSelect's performance) depend on the library preparation kit and sample type.

Choose from a variety of QIAseq FastSelect options

QIAseq FastSelect RNA Removal Kits are available in a variety of different formats and sizes to suit your specific applications. QIAGEN also offers kits for removing human, mouse and rat RNA for transcriptomic analysis from the gut and other host samples. Additionally, products can be customized to fit the species and RNA of your choice (Table 1).

A rapid protocol for robust rRNA removal from community samples

QIAseq FastSelect –5S/16S/23S provides a simpler workflow with dramatically higher rRNA removal compared to alternative rRNA removal products. QIAseq FastSelect –5S/16S/23S Kit was compared to two alternative methods – Supplier TF (method ME) and Supplier TF (method RM) – for rRNA removal from gut samples. QIAseq FastSelect –5S/16S/23S offers a much faster workflow (Figure 2A)



Ⓐ Workflow steps: Alternative products vs. QIAseq FastSelect

Ⓑ rRNA removal: Alternative products vs. QIAseq FastSelect

Figure 2. QIAseq FastSelect –5S/16S/23S Kits offer a rapid protocol that robustly removes rRNA from community samples. Stranded transcriptome libraries were prepared from total RNA isolated from Gut Microbiome Whole Cell Mix (ATCC) using the QIAseq Stranded Total RNA Lib Kit. For rRNA removal, three methods were used: Supplier TF (method ME) and Supplier TF (method RM), which used a hybrid capture workflow and QIAseq FastSelect –5S/16S/23S Kits. Compared to method ME and method RM from Supplier TF, QIAseq FastSelect –5S/16S/23S offers [A] a dramatically faster workflow with less handling and [B] a much higher percentage of rRNA-removed.

Table 1. A wide selection of QIAseq FastSelect RNA removal products

Product	Species	Targets	Number of samples	Catalog number
QIAseq FastSelect –rRNA HMR Kit	Human, mouse and rat	rRNA or globin mRNA	24, 96 or 384	Varies
QIAseq FastSelect –5S/16S/23S Kit	Any bacteria	Bacterial 5S, 16S, 23S rRNA	24, 96 or 384	Varies
QIAseq FastSelect Custom Kit	Your choice	Any RNA	1156	Varies

Ⓐ Detected genes: No treatment vs. QIAseq FastSelect

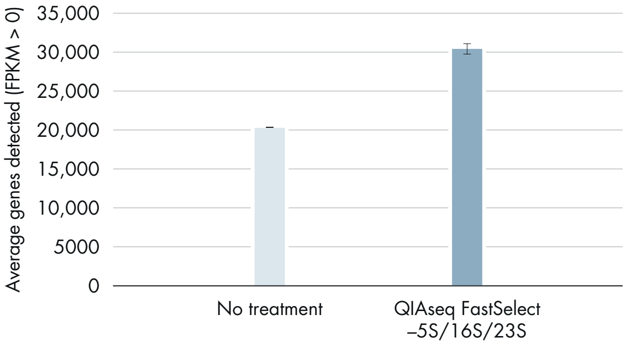
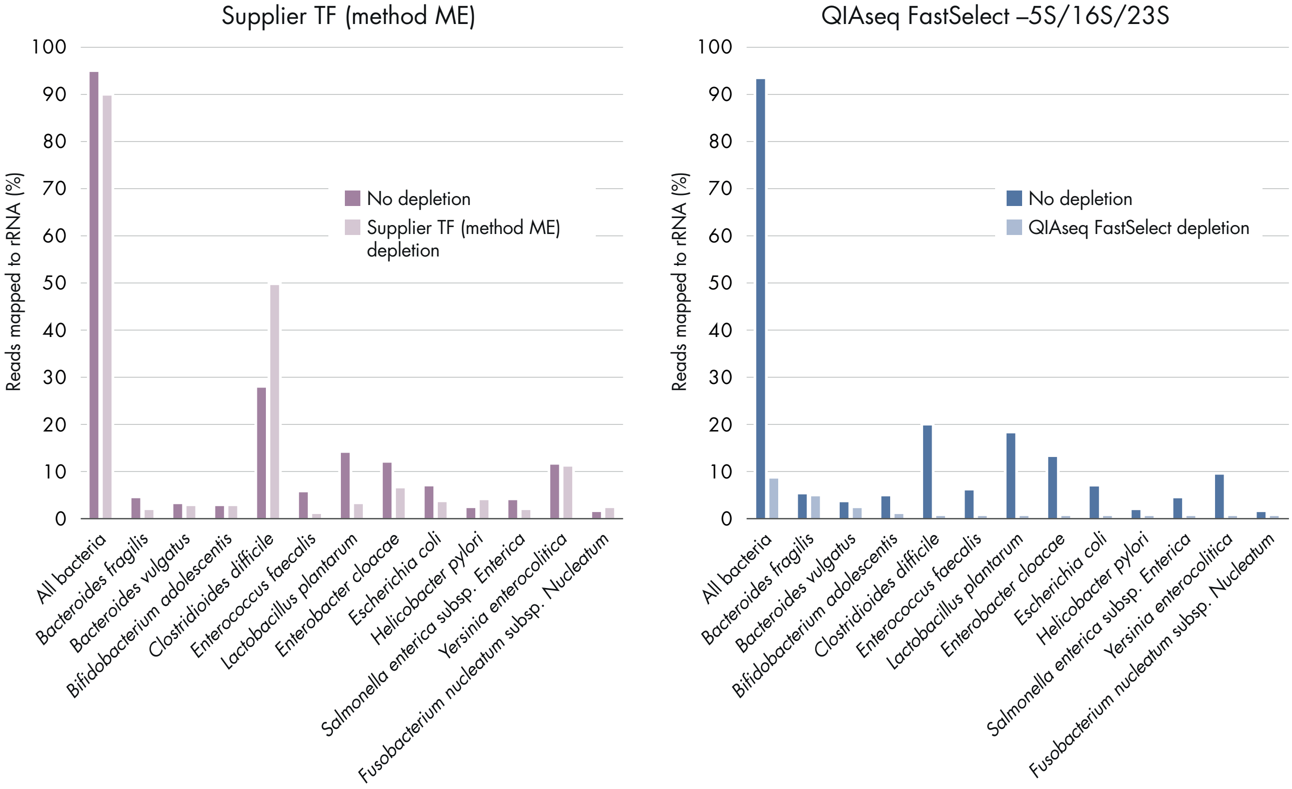


Figure 3. QIAseq FastSelect –5S/16S/23S robustly removes rRNA at the individual species level. Stranded transcriptome libraries were prepared from total RNA isolated from Gut Microbiome Whole Cell Mix (ATCC) using the QIAseq Stranded Total RNA Lib Kit. rRNA was depleted using Supplier TF (method ME) or the QIAseq FastSelect –5S/16S/23S Kit. [A] By robustly removing rRNA, QIAseq FastSelect –5S/16S/23S frees more than 85% of sequencing reads for non-rRNA genes, dramatically increasing the number of unique genes detected. [B] Compared to Supplier TF (method ME), QIAseq FastSelect –5S/16S/23S removes rRNA with greater efficiency, both at the total level and individual species level.

Ⓑ Individual species rRNA mapping: Supplier TF vs. QIAseq FastSelect



compared to both methods from Supplier TF. Moreover, QIAseq FastSelect –5S/16S/23S removed over 90% of the 5S/16S/23S rRNA, allowing more economical use of sequencing reads for gene expression analysis – while methods ME and RM removed less than 10% of the same rRNAs (Figure 2B).

Increased read budget and superior performance across species

By efficiently removing rRNA, QIAseq FastSelect –5S/16S/23S frees up a substantial read budget. This means more than 85% of the sequencing reads are made available for gene expression analysis (versus ~5% without rRNA removal). Increased read budget means a higher number of unique genes are detected. With the QIAseq FastSelect –5S/16S/23S, FPKM >0 increased 50% from approximately 20,000 to over 30,000, demonstrating the value of robust rRNA removal (Figure 3A).

QIAseq FastSelect delivers superior performance at the total species level and across individual species. The QIAseq FastSelect –5S/16S/23S Kit removes rRNA from a broad range of bacterial species with greater efficiency compared to Supplier TF (Figure 3B).

Ordering Information

Product	Contents	Cat. no.
QIAseq FastSelect –5S/16S/23S Kit (24)	Includes 3 tubes of QIAseq FastSelect –5S/16S/23S rRNA removal reagent, reaction buffer, QIAseq Beads with QIAseq bead binding buffer and nuclease-free water sufficient for 24 samples	335925
QIAseq FastSelect –5S/16S/23S Kit (96)	Includes 1 tube of QIAseq FastSelect –5S/16S/23S rRNA removal reagent, reaction buffer, QIAseq Beads with QIAseq bead binding buffer and nuclease-free water sufficient for 96 samples	335927
QIAseq FastSelect –5S/16S/23S Kit (384)	Includes 4 tubes of QIAseq FastSelect –5S/16S/23S rRNA removal reagent, reaction buffer, QIAseq Beads with QIAseq bead binding buffer and nuclease-free water sufficient for 384 samples	335929

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Learn more at **www.qiagen.com**.