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QIAseq® FastSelect™ –rRNA Plant Handbook

Removal of cytoplasmic, mitochondrial, and chloroplast rRNA from plant samples for RNA-seq applications

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAseq FastSelect –rRNA Plant Kit	(24)	(96)	(384)
Catalog no.	**334315**	**334317**	**334319**
Number of reactions	**24**	**96**	**384**
QIAseq FastSelect –rRNA Plant	3 x 8 µl	96 µl	4 x 96 µl

Shipping and Storage

QIAseq FastSelect is shipped on blue ice. Upon receipt, all components should immediately be stored in a constant-temperature freezer at –30 to –15°C. Under these conditions, the components are stable, without showing any reduction in performance and quality, until the date indicated on the box label.

Intended Use

The QIAseq FastSelect –rRNA Plant 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 QIAseq FastSelect is tested against predetermined specifications to ensure consistent product quality.

Introduction

RNA-focused next-generation sequencing (NGS) enables a thorough investigation of both coding and noncoding RNAs. While performing stranded library preparation, significantly overrepresented RNAs such as ribosomal RNA (rRNA) must be avoided to facilitate optimal read allocation. In fact, cytoplasmic, mitochondrial, and chloroplast rRNA comprise more than 90% of the total RNA in common plant samples. QIAseq FastSelect –rRNA Plant is a breakthrough technology that rapidly and efficiently removes cytoplasmic, mitochondrial, and chloroplast rRNA during NGS library preparation from 1 ng – 1 µg of plant RNA samples, in just 14 minutes.

QIAseq FastSelect works with existing RNA-seq workflows during the reverse-transcription step for the removal of up to 99% of unwanted plant cytoplasmic, mitochondrial, and chloroplast rRNAs.

Library preparation kits from QIAGEN, Illumina®, Roche®, New England Biolabs® (NEB®), and others are compatible with the QIAseq FastSelect method for RNA removal.

Principle and procedure

QIAseq FastSelect is designed for fast and selective removal of cytoplasmic, mitochondrial, and chloroplast rRNA from total RNA during NGS RNA library preparation. The simple 1-step protocol calls for the FastSelect reagent to be directly combined with total RNA (1 ng – 1 µg) and any reaction buffers necessary for RNA heat fragmentation. After the optional heat fragmentation is performed, the reaction is gradually cooled to 25°C. The remaining library-preparation-specific steps then follow without the need for any additional enzymatic cleanups or RNA depletion steps. QIAseq FastSelect works by preventing the cDNA synthesis of rRNA. QIAseq FastSelect has been designed to work equally well with high-quality RNA or highly fragmented samples.

QIAseq FastSelect –rRNA Plant: Types of rRNAs that are removed and species

QIAseq FastSelect –rRNA Plant has been designed to remove cytoplasmic (5.8S, 18S, and 25S), mitochondrial (5S, 18S, and 26S), and chloroplast (4.5S, 5S, 16S, and 23S) rRNA from *Arabidopsis thaliana* and *Arabidopsis lyrata*. In addition to *Arabidopsis* total RNA, QIAseq FastSelect –rRNA Plant has been tested with a variety of other RNA samples (Table 1). Depending on rRNA sequence homology, FastSelect –rRNA Plant will work on additional plant species.

Table 1. Summary of rRNA removal using QIAseq FastSelect –rRNA Plant

Species	% rRNA reads without FastSelect	% rRNA reads with FastSelect	% knockdown
Arabidopsis	96.2	0.8	99
Barley	96.0	7.9	92
Corn	94.7	9.8	90
Cotton	94.1	1.3	99
Flaxseed	95.9	12.6	87
Maple	93.5	10.5	89
Oat	94.8	0.7	99
Potato	95.1	6.5	93
Rice	94.7	8.1	91
Rye	96.2	0.8	99
Sorghum	96.2	20.2	79
Soybean	96.0	6.3	93
Wheat	95.8	5.5	94

QIAseq FastSelect: sample type and stranded library kit compatibility

QIAseq FastSelect has been designed to be compatible with total RNA and mRNA-enriched samples isolated from cells, fresh/frozen tissue, FFPE tissue, whole blood, and serum/plasma samples, including exosomes. The QIAseq FastSelect reagent and protocol has been tested with a variety of commercially available stranded RNA library preparation kits from QIAGEN, Illumina, NEB, and Roche. For questions regarding specific protocols for kits that are not detailed in the handbook, please contact QIAGEN Technical Services at **support.qiagen.com**.

Important Notes

- We highly recommend DNase treatment (on-column and in-solution) of total RNA samples.
- It is not possible to test the efficiency of the FastSelect reaction by running a portion of the eluate from the bead cleanup on a Bioanalyzer®, TapeStation®, Fragment Analyzer™, etc. FastSelect works by inhibiting reverse transcription of bacterial rRNA, which does not occur until the first-strand synthesis reaction during library prep.
- QIAseq FastSelect is an inline solution for the removal of unwanted plant rRNAs during NGS library preparation. The total RNA input is defined by the range of the RNA library kit used. For example, the QIAseq Stranded Total RNA Lib Kit (cat. no. 180743 or 180745) has a total RNA input range of 100 ng – 1 µg. As a result, you would start with 100 ng – 1 µg into the FastSelect reaction.
- QIAseq FastSelect –rRNA Plant is compatible with other QIAseq FastSelect Kits and can be combined to remove additional RNAs. For example, when working with mixed plant/bacterial samples, QIAseq FastSelect –rRNA Plant is fully compatible with QIAseq FastSelect –5S/16S/23S (which is used to remove bacterial rRNA). For relevant protocols, please refer to the *QIAseq FastSelect –5S/16S/23S Handbook*, **www.qiagen.com/HB-2695**.
- The rRNA removal imparted by QIAseq FastSelect is extremely robust, especially when compared to other methods. We recommend to prepare libraries and use the standard protocol for library preparation unless specifically noted in the handbook.
- If the yield of the library is less than other methods, this is often caused by the increased removal of RNA imparted by the QIAseq FastSelect method and is normal. In our experience, adding 2 cycles of library amplification is usually sufficient to increase library yield for all downstream quantification and sequencing applications.
- Depending on the RNA-seq kit and RNA input amounts, adapter–dimers may be observed. If this happens, we recommend that you perform a second bead-based cleanup reaction of the final library.

Protocol: QIAseq FastSelect –rRNA Plant with the QIAseq Stranded Total RNA Lib Kit

Important points before starting

- The QIAseq Stranded Total RNA Lib Kit (cat. no. 180743 or 180745) is required for use with this protocol.
- This protocol has been tested with 100 ng – 1 µg of total RNA.
- Refer to the *QIAseq Stranded Total RNA Lib Kit Handbook* available at **www.qiagen.com/HB-2465**

Procedure

1. Thaw total RNA on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and return to ice.
2. Prepare the reagents required for the RNA fragmentation and QIAseq FastSelect –rRNA Plant.
   2a. Thaw 5x RT Buffer and nuclease-free water from the QIAseq Stranded Kit at room temperature.
   2b. Vortex 5x RT Buffer, nuclease-free water, and the tube(s) from the QIAseq FastSelect Kit, and then briefly centrifuge.
3. On ice, prepare the fragmentation/RNA removal reaction according to Table 2. Briefly centrifuge, mix by pipetting up and down 10 times, and centrifuge briefly again.
   Note: If setting up more than one reaction, prepare a volume of Master Mix that is 10% greater than what is required for the total number of reactions.

Table 2. Setup of fragmentation/RNA removal reactions

Component	Volume/reaction
Total RNA (100 ng – 1 µg)	Variable
RT Buffer, 5x*	8 µl
QIAseq FastSelect –rRNA Plant	1 µl
ERCC Control†	Optional
Nuclease-free water	Bring total reaction volume to 37 µl
Total volume	**37 µl**

* From the QIAseq Stranded Total RNA Lib Kit.

† ERCC Control RNA can be added according to the concentrations specified by the manufacturer. If added, the total fragmentation/RNA removal reaction volume should remain 37 µl.

4. Incubate as described in Table 3, according to input RNA quality and desired insert size.

   Important: Regardless of the time and temperature chosen in step 1, steps 2–9 must be performed.

Table 3. Combined QIAseq fragmentation and FastSelect hybridization protocol

Input RNA quality	Step	Insert size ~150–250 bp	Insert size ~350 bp
High quality (RIN >9)	1*	15 min at 95°C	3 min at 95°C
Moderate quality (RIN 5–6)	1*	10 min at 95°C	3 min at 95°C
FFPE or degraded sample (RIN <3)	1*	No fragmentation†	No fragmentation†
Steps 2–9 are performed regardless of input RNA quality. They need to be performed whether the RNA is high quality, moderate quality, FFPE, or degraded.	2	2 min at 75°C	2 min at 75°C
	3	2 min at 70°C	2 min at 70°C
	4	2 min at 65°C	2 min at 65°C
	5	2 min at 60°C	2 min at 60°C
	6	2 min at 55°C	2 min at 55°C
	7	2 min at 37°C	2 min at 37°C
	8	2 min at 25°C	2 min at 25°C
	9	Hold at 4°C	Hold at 4°C

* Choose one option for the time on step 1 according to the input RNA quality and desired insert size.

† Also suitable for exosomal RNA or RNA of other origin with a size of 80–500 bp.

5. Refer to the *QIAseq Stranded Total RNA Lib Kit Handbook* and immediately proceed to "Protocol: First-strand Synthesis".
6. Follow the *QIAseq Stranded Total RNA Lib Kit Handbook* to perform all remaining library construction steps.

Protocol: QIAseq FastSelect –rRNA Plant with TruSeq Stranded Library Preparation

Important points before starting

- The TruSeq® Stranded mRNA Library Prep (Illumina cat. no. 20020594 or 20020595) is required for use with this protocol.
  Note: With this protocol, do not perform mRNA purification. Instead, follow the steps outlined below before proceeding to "Synthesize First Strand cDNA" in the *TruSeq Stranded mRNA Reference Guide*. By doing this, a stranded, total RNA library preparation will be performed.
- This protocol has been tested with 100 ng – 1 µg of total RNA.
- **Important**: It is highly recommended to dilute the Illumina adapters 2-fold compared to what is suggested in the default Illumina protocol.
- Refer to the *TruSeq Stranded mRNA Reference Guide* (1000000040498).

Procedure

1. Vortex the tube(s) from the QIAseq FastSelect Kit, and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. To 100 ng – 1 µg of total RNA, which is required to be in a maximum volume of 5 µl, add 1 µl of QIAseq FastSelect –rRNA Plant.
3. From the TruSeq Stranded mRNA Library Prep, add 14.5 µl FPF to bring the volume of the reaction to 20.5 µl.
4. Mix thoroughly by pipetting up and down several times, and then briefly centrifuge to collect residual liquid from the sides of the tubes.

5. Incubate in a thermal cycler with a heated lid, as described in Table 4.

Important: Table 5 can be consulted to adjust RNA insert size. Irrespective of time at 94°C, steps 2–9 listed in Table 4 must be performed.

Table 4. Combined TruSeq Stranded fragmentation and FastSelect hybridization protocol

Step	Time and temperature
1*	8 min at 94°C*
2	2 min at 75°C
3	2 min at 70°C
4	2 min at 65°C
5	2 min at 60°C
6	2 min at 55°C
7	2 min at 37°C
8	2 min at 25°C
9	Hold at 4°C

Note: The remaining steps 2–9 are performed regardless of the time at 94°C.

* The initial step at 94°C can be modified to permit longer RNA insert sizes. Refer to Table 5 for recommendations.

Table 5. Fragmentation time at 94°C for alternative RNA insert sizes

Time at 94°C*	Range of insert length (bp)	Median insert length (bp)	Average final library size (Bioanalyzer bp)
0 min	130–350	200	467
1 min	130–310	190	439
2 min	130–290	185	410
3 min	125–250	165	366
4 min	120–225	160	326
8 min	120–210	155	309
12 min	115–180	140	272

* The remaining steps 2–9 from Table 4 must be performed regardless of the time at 94°C.

6. Using 17 µl of this fragmented/hybridized RNA, refer to the *TruSeq Stranded mRNA Reference Guide* and immediately proceed to "Synthesize First Strand cDNA."

   Note: From the *TruSeq Stranded mRNA Reference Guide*, the procedural step "Place the RBP plate on the magnetic stand and wait until the liquid is clear (~5 minutes)" is not applicable.

7. Follow the *TruSeq Stranded mRNA Reference Guide* to perform all remaining library construction steps.

IMPORTANT 	It is highly recommended to dilute the Illumina adapters 2-fold compared to what is suggested in the default Illumina protocol.

Protocol: QIAseq FastSelect –rRNA Plant with the NEBNext Ultra II Directional Library Prep Kit

Important points before starting

- The NEBNext® Ultra II Directional RNA Library Prep Kit for Illumina (NEB cat. no. E7760S or E7760L) is required for use with this protocol.
- This protocol has been tested with 5 ng – 1 µg of total RNA.
- Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual*.

Procedure

1. Vortex the tube(s) from the QIAseq FastSelect Kit, and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. Referring to Section 4 from the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual*, perform the following in place of steps 4.1.1 through 4.1.4:
   2a. Assemble the fragmentation and priming reaction described in Table 6 on ice in a nuclease-free tube.

Table 6. NEBNext Ultra II Stranded fragmentation and priming mix

Component	Volume/reaction
Total RNA (5 ng–1 µg)	4 µl
(lilac) NEBNext First Strand Synthesis Reaction Buffer*	4 µl
(lilac) Random primers*	1 µl
Total volume	**9 µl**

* From NEBNext Ultra II Directional Library Prep Kit.

2b. To the assembled fragmentation and priming mix, add 1 µl of QIAseq FastSelect –rRNA Plant.

2c. Mix thoroughly by pipetting up and down several times, and then briefly centrifuge to collect residual liquid from the sides of the tubes

2d. Incubate in a thermal cycler with a heated lid, as described in Table 7, according to your input RNA quality.

Important: Regardless of the time and temperature chosen in step 1, steps 2–9 must be performed.

Table 7. Combined NEBNext Ultra II fragmentation and FastSelect hybridization protocol

Step	Intact RNA (RIN >7)	Partially degraded RNA (RIN 2–6)
1	15 min at 94°C	7–8 min at 94°C
2	2 min at 75°C	2 min at 75°C
3	2 min at 70°C	2 min at 70°C
4	2 min at 65°C	2 min at 65°C
5	2 min at 60°C	2 min at 60°C
6	2 min at 55°C	2 min at 55°C
7	2 min at 37°C	2 min at 37°C
8	2 min at 25°C	2 min at 25°C
9	Hold at 4°C	Hold at 4°C

3. Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* and immediately proceed to "First Strand cDNA Synthesis Reaction".
   Note: "First Strand cDNA Synthesis Reaction" is chapter 4.2 in the instruction manual.
4. Follow the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* to perform all remaining library construction steps.

Protocol: QIAseq FastSelect –rRNA Plant with the KAPA RNA HyperPrep Kit

Important points before starting

- The KAPA® RNA HyperPrep Kit (Roche cat. no. KK8540 and KK8541) is required for use with this protocol.
- This protocol has been tested with 25 ng – 1 µg of total RNA.
- **Important**: It is highly recommended to dilute the KAPA adapters 1.5-fold compared to what is suggested in the default KAPA protocol.
- Refer to the *KAPA RNA HyperPrep Kit Technical Data Sheet*.

Procedure

1. Vortex the tube(s) from the QIAseq FastSelect Kit, and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. From the KAPA RNA HyperPrep Kit, prepare the fragmentation and priming mix described in Table 8 at room temperature in a nuclease-free tube.

Table 8. KAPA RNA HyperPrep fragmentation and priming mix

Component	Volume/reaction
Total RNA (25 ng–1 µg)	9 µl
Fragment, prime, and elute buffer (2X)*	10 µl
Total volume	**19 µl**

* From the KAPA RNA HyperPrep Kit.

3. To the assembled fragmentation and priming mix, add 1 µl of QIAseq FastSelect –rRNA Plant.
4. Mix thoroughly by gently pipetting the reaction up and down several times, and then briefly centrifuge to collect residual liquid from the sides of the tubes.
5. Incubate in a thermal cycler with a heated lid, as described in Table 9, according to your input RNA quality.

   Important: Regardless of the time and temperature chosen in step 1, steps 2–9 must be performed.

Table 9. Combined KAPA RNA HyperPrep fragmentation and FastSelect hybridization protocol

Input RNA type	Step	Time and temperature
Intact	1*	**Choose**: 8 min at 94°C *or* 6 min at 94°C *or* 6 min at 85°C
Partially degraded	1†	1–6 min at 85°C
Degraded (e.g., FFPE)	1‡	No fragmentation
Steps 2–9 are performed regardless of input RNA quality. They need to be performed whether the RNA is high quality, moderate quality, FFPE, or degraded.	2	2 min at 75°C
	3	2 min at 70°C
	4	2 min at 65°C
	5	2 min at 60°C
	6	2 min at 55°C
	7	2 min at 37°C
	8	2 min at 25°C
	9	Hold at 4°C

* Choose one option, depending on whether you want a desired mean library insert size of 100–200 bp (8 min at 94°C), 200–300 bp (6 min at 94°C), or 300–400 bp (6 min at 85°C).

† For a desired mean library insert size of 100–300 bp.

‡ For a desired mean library insert size of 100–200 bp.

6. Refer to the *KAPA RNA HyperPrep Kit Technical Data Sheet* and immediately proceed to "1st Strand Synthesis".
7. Follow the *KAPA RNA HyperPrep Kit Technical Data Sheet* to perform all remaining library construction steps.

IMPORTANT 	It is highly recommended to dilute the KAPA adapters 1.5-fold compared to what is suggested in the default KAPA protocol.

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
Adapter–dimer observed in final library QC	
Final library insufficiently cleaned	Perform a second cleanup of the final library using the same beads-to-sample ratio as in the first cleanup.

Ordering Information

Product	Contents	Cat. no.
QIAseq FastSelect –rRNA Plant Kit (24)	For 24 reactions: cytoplasmic, mitochondrial and chloroplast rRNA removal reagent; supports plant	334315
QIAseq FastSelect –rRNA Plant Kit (96)	For 96 reactions: cytoplasmic, mitochondrial and chloroplast rRNA removal reagent; supports plant	334317
QIAseq FastSelect –rRNA Plant Kit (384)	For 384 reactions: cytoplasmic, mitochondrial and chloroplast rRNA removal reagent; supports plant	334319
QIAseq Stranded Total RNA Lib Kit (24)	For 24 stranded RNA-seq sequencing library preparation reactions: fragmentation, reverse transcription, second-strand synthesis + end-repair + A-addition, adapter ligation, CleanStart® PCR enrichment, and QIAseq Beads for library cleanups	180743
QIAseq Stranded Total RNA Lib Kit (96)	For 96 stranded RNA-seq sequencing library preparation reactions: fragmentation, reverse transcription, second-strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment, and QIAseq Beads for library cleanups	180745

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.

Document Revision History

Date	Changes
06/2020	Initial release

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