

Demonstrated Protocol: Bacterial Ribosomal RNA (rRNA) Depletion Workflow for RNA-Seq

Pub. No. MAN0009661 Rev. A.0

Overview

Purpose

The following workflow uses biotinylated probes to selectively and efficiently capture and remove the highly abundant 16S and 23S ribosomal RNA (rRNA) species from total RNA isolated from a spectrum of bacterial organisms. The resulting rRNA-depleted RNA can then be used in a variety of downstream applications, including the generation of whole transcriptome RNA-Seq libraries for sequencing with the Ion PGM™ or Ion Proton™ Sequencing System.

Life Technologies Demonstrated Protocols

Life Technologies Demonstrated Protocols have been successfully demonstrated by Life Technologies research and development but not formally validated. There are no technical specifications for Life Technologies demonstrated protocols. Users assume all risk when using these protocols, and recognize that support for Life Technologies demonstrated protocols occurs through community discussion. All customers are encouraged to discuss and contribute via the Ion Community at <http://ioncommunity.lifetechnologies.com>.

Required materials

Unless otherwise indicated, materials are available from Life Technologies.

Item description	Cat. no.
RiboMinus™ Eukaryote System v2 <i>or</i>	A15026
RiboMinus™ Eukaryote Kit v2	A15020
RiboMinus™ Bacterial Probe Mix ^[1]	—
Eppendorf LoBind® Microcentrifuge Tubes, 1.5-mL	Eppendorf 022431021
Nuclease-free Water	AM9938

^[1] See "Obtaining the RiboMinus Bacterial Probe Mix" on page 1.

Obtaining the RiboMinus™ Bacterial Probe Mix

Send requests for the RiboMinus™ Bacterial Probe Mix to your field application specialist (FAS) or techsupport@lifetech.com and include your name, institution and mailing address. Bacterial probes for 16S and 23S rRNA in a ready-to-use pool sufficient for 12 depletion reactions will be sent by mail.

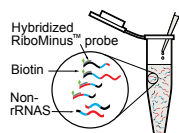
The following table lists bacterial species compatible with the RiboMinus™ Bacterial Probe Mix. To check probe compatibility with an organism of interest not listed in the table, email the FASTA sequence in .txt file format for the ribosomal sequence of interest to techsupport@lifetech.com. Include in the subject line: "RiboMinus™ for [your organism]".

Species compatible with the RiboMinus™ Bacterial rRNA Probe Mix	
<i>Bacillus anthracis</i>	<i>Neisseria meningitidis</i>
<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>
<i>Bacillus thuringiensis</i>	<i>Pseudomonas syringae</i>
<i>Burkholderia mallei</i>	<i>Salmonella enterica</i>
<i>Burkholderia pseudomallei</i>	<i>Shewanella oneidensis</i>
<i>Caulobacter crescentus</i>	<i>Streptococcus mutans</i>
<i>Enterococcus faecalis</i>	<i>Streptococcus pneumoniae</i>
<i>Escherichia coli</i>	<i>Streptococcus pyogenes</i>
<i>Klebsiella pneumoniae</i>	<i>Thermotoga maritima</i>
<i>Lactococcus lactis</i>	<i>Vibrio cholerae</i>
<i>Listeria innocua</i>	<i>Yersinia enterocolitica</i>
<i>Listeria monocytogenes</i>	<i>Yersinia pestis</i>
<i>Neisseria gonorrhoeae</i>	

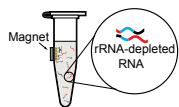
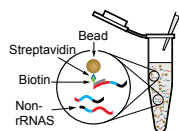
Workflow

Refer to the *RiboMinus™ Eukaryote System v2 User Guide* (Pub. no. MAN0007159) for detailed experimental procedures for preparing rRNA-depleted bacterial RNA with the RiboMinus™ Eukaryote System v2 or RiboMinus™ Eukaryote Kit v2. Simply substitute the RiboMinus™ Bacterial Probe Mix for the RiboMinus™ Eukaryote Probe Mix v2 in the first step of the RiboMinus™ procedure and perform the remaining steps as described in the user guide.

Hybridize RiboMinus™ Bacterial Probe Mix and total RNA sample (~30 minutes)



Capture and remove rRNA-probe complexes (~6 minutes)

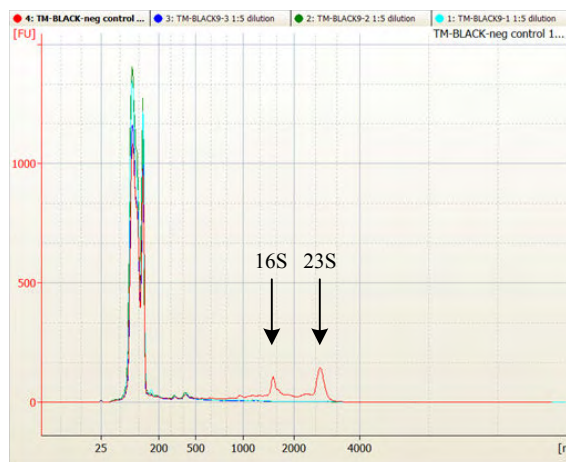


Concentrate the rRNA-depleted RNA (~25 minutes)



Example results and analysis

The following figure shows an example Agilent® 2100 Bioanalyzer® instrument analysis of rRNA-depleted RNA and mock-treated control total RNA. In the rRNA-depleted samples, peaks corresponding to bacterial rRNA species are no longer visible in the Bioanalyzer® trace. Using these procedures, over 99% of the 16S and 98% of the 23S rRNA were removed from *E. coli* DH10B total RNA.



E. coli DH10B cells were lysed using TRIzol® Reagent (Cat. no. 15596026). Total RNA was purified on a *mirVana™* column following the "Total RNA Isolation Procedure" in the *mirVana™* miRNA Isolation Kit protocol (Cat. no. AM1560). The total RNA was DNase-treated to remove residual DNA. Total RNA (3 µg) was depleted of rRNA species in triplicate using the described protocol. A negative (mock) control was processed using nuclease-free water in place of the Bacterial Probe Mix. The four samples were diluted 1:5 and analyzed using the Agilent® 2100 Bioanalyzer® RNA 6000 Pico Kit. The 16S and 23S rRNA species from *E. coli* are observed in the trace at around 1600 nt and 2900 nt, respectively, in the negative control (red trace).

RNA-Seq processing

RNA libraries can be constructed from the rRNA-depleted RNA using the Ion Total RNA-Seq Kit v2 (Cat. no. 4475936) and sequenced using the Ion PGM™ or Ion Proton™ System. Ordering information for additional kits, available from Life Technologies, for the Ion PGM™ and Ion Proton™ workflows is shown in the following table.

Ion Workflow	Kit	Cat. no.
Ion PGM™ System	Ion PGM™ Template OT2 200 Kit	4480974
	Ion PGM™ Template OT2 400 Kit	4479878
	Ion PGM™ Sequencing 200 Kit v2	4482006
	Ion PGM™ Sequencing 400 Kit	4482002
	Ion 314™, or Ion 316™, or Ion 318™ Chip Kit v2	4482261 or 4483188 or 4484354
Ion Proton™ System	Ion PI™ Template OT2 200 Kit v2	4485146
	Ion PI™ Template OT2 200 Kit v3	4488318
	Ion PI™ Sequencing 200 Kit v2	4485149
	Ion PI™ Sequencing 200 Kit v3	4488315
	Ion PI™ Chip Kit v2	4482321

Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Note: For the SDSs of chemicals not distributed by Life Technologies Corporation, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)

- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Ion contact information

Web site: lifetechnologies.com/iontorrent

Ion community: ioncommunity.lifetechnologies.com

Support email: ionsupport@lifetech.com

Phone numbers

In North America: 1-87-SEQUENCE (1-877-378-3623)

Outside of North America: +1-203-458-8552

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

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For support visit lifetechnologies.com/support or email techsupport@lifetech.com

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