SEQuoia RiboDepletion Kit

Catalog #	Description
17006487	**SEQuoia RiboDepletion Kit**, 24 reactions

For research purposes only.

Introduction

The SEQuoia RiboDepletion Kit is a post–library prep ribodepletion kit that eliminates fragments derived from ribosomal RNA (rRNA) and mitochondrial ribosomal RNA (mt rRNA) from RNA-Seq libraries. This kit contains the components needed to minimize the proportion of rRNA or mt rRNA reads and preserve sequencing reads for relevant RNA species, using a wide range of input library fragments (0.1–20 ng), without compromising the quality of the library. The kit does not include the SPRISelect Beads (Beckman Coulter, Inc.) needed for the purification step.

Ribosomal RNA is extremely abundant, constituting 80–90% of total RNA. In most applications, this large fraction is irrelevant. While it is possible to filter out those reads mapping to rRNA genes using bioinformatics, these fragments take up unnecessary sequencing space, needlessly inflate the screening scale and cost, and can reduce the overall sensitivity of detection. Therefore, efficient removal of rRNA is critical for cost-effective sequencing of RNA samples.

This kit uses a proprietary blend of synthetic biotinylated oligonucleotides that are homologous to complementary DNA (cDNA) produced from human, mouse, and rat cytoplasmic RNA (5S rRNA, 5.8S rRNA, 18S rRNA, and 28S rRNA) and mt rRNA (12S rRNA and 16S rRNA). Target cDNA sequences hybridize to complementary biotinylated oligonucleotides through a PCR-mediated reaction. These complexes are captured using streptavidin-coated paramagnetic beads and removed from the library.

Storage and Stability

The SEQuoia RiboDepletion Kit is guaranteed for 12 months after the shipping date if stored properly (see table below).

Table 1. Kit contents.

Component	Cap Color	Volume	Storage Temperature, °C
Probe Mix	Yellow	110 µl	–20
Primers	Blue	65 µl	
Amplification Mix	Green	880 µl	
Purification Beads*	Clear	2.5 ml	4

* Purification Beads are shipped separately and are stored separately from the rest of the kit. Bead appearance may vary between batches. This variation has no impact on performance.

Required Materials Not Provided

The following materials are required but not supplied. They have been validated with this protocol. Substitutions may not produce ideal results.

- 0.2 ml RNase- and DNase-free, low-binding PCR tube strips and cap strips (Bio-Rad #TBC0802 or #TBC1202)
- RNase-free filtered pipet tips: 10, 20, 200, and 1,000 µl
- SPRISelect Reagent (Beckman Coulter, Inc.)
- 10 mM Tris-HCl, pH 8.0 (RNase- and DNase-free)
- 80% ethanol (prepared fresh)
- Double-distilled water (ddH_2O)

Equipment List

- C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module, or equivalent, for accurate incubation temperatures
- Calibrated single-channel and multichannel pipets: 10, 20, 200, and 1,000 µl
- Magnetic rack for small-scale separation of magnetic beads in 0.2 ml tubes
- Vortexer
- Microcentrifuge for 0.2 ml tubes
- 96-well PCR cooling rack
- Ice bucket

Important Considerations – Please Read Before Starting

For maximum depletion efficiency, consider the following:

Average Library Fragment Size

The efficiency of a hybridization and capture depletion method depends on library fragment size. Larger fragments hybridize to the probes more efficiently, resulting in greater depletion. For maximum depletion efficiency of SEQuoia Complete Library Preps, the average library fragment size should be greater than 380 bp. Depleting a library composed of smaller fragments may result in a higher percentage of sequencing reads mapping to rRNA.

Multiplexing

One of the benefits of a post–library preparation depletion strategy is that indexed libraries can be pooled and depleted in a single reaction. To maximize depletion efficiency for each indexed library, combine equimolar ratios of the indexed libraries so as to not exceed 20 ng of cDNA (combined) per ribodepletion reaction. Libraries constructed using different library preparation kits is possible. However, ensure that they are generated using consistent indexing strategies. Do not multiplex single- and dual-indexed libraries.

Compatibility

The SEQuoia RiboDepletion Kit can deplete rRNA- and mt rRNA–derived library fragments constructed from most commercial RNA library preparation kits. To obtain optimal ribodepletion results when depleting rRNA from a library prepared using a kit from another vendor, follow the vendor's recommendations for amplification cycles if the protocol assumes the input sample type is total RNA. If the protocol assumes the input sample type is predepleted or enriched RNA, then reduce the number of recommended amplification cycles by three cycles.

Protocol

First Hybridization Step

Recommendation: Quantify and assess the average fragment size of the library preparation reaction prior to starting this protocol. For best results, the average fragment size should be greater than 380 bp and the input amount should be 0.1–20 ng of library cDNA. If depleting multiple libraries in a single ribodepletion reaction, combine equimolar ratios of the indexed libraries so that the final quantity of total cDNA library fragments does not exceed 20 ng per ribodepletion reaction.

Prior to starting this step, program the thermal cycler according to Table 2.

Table 2. Thermal cycler hybridization protocol.*

Step	Temperature, °C	Time	PCR Cycles
1	98	1 min	1
2	62	10 min	1
3	72	1 min	1
4	20	Hold	1

* Use a heated lid set to 105°C. Set the sample volume to 50 µl.

Note: For optimal results, reactions should be assembled on ice using sterile and nuclease-free tubes, tube strips, or plates. Preheat the thermal cycler to 98°C prior to adding the hybridization reactions to ensure optimal results.

1. Thaw all components and mix thoroughly. Keep reagents on ice.
2. Prepare the reactions according to Table 3 by adding the following components to the reaction vessels.

Table 3. First hybridization reaction.

Reagent	Cap Color	Volume per Reaction, µl
Amplification Mix	Green	13.5
Probe Mix	Yellow	2
RNA-Seq library prep (0.1–20 ng of cDNA)*	—	Variable
ddH_2O	—	Variable
Total		**20**

* **Important:** Quantify and assess the average fragment size of the RNA-Seq library prior to starting this protocol.

3. Mix the reactions thoroughly by vortexing 5–10 sec.
4. Centrifuge briefly to collect the reactions at the bottom of the reaction vessels.
5. Incubate the reactions in a thermal cycler using the hybridization protocol (Table 2).

First Depletion Step

Allow the purification beads to come to room temperature before use.

1. Vortex Purification Beads 30 sec to resuspend them in solution.
2. For each reaction, aliquot 50 µl of the Purification Beads suspension into a fresh reaction vessel.
3. Place the reaction vessels on a magnetic strip at room temperature.

   Important: Do not proceed to the next step until the first hybridization step thermal cycling protocol has been completed.
4. Aspirate the solution from the beads and discard it.
5. Briefly centrifuge the reaction vessels that contain the hybridization reactions.
6. Transfer 20 µl of the hybridization reaction to a reaction vessel that contains Purification Beads.
7. Resuspend beads by pipetting up and down several times.
8. Incubate at room temperature for 15 min, gently mixing every 5 min.
9. Centrifuge briefly to collect the reactions at the bottom of the tubes.
10. Place the reaction vessels on a magnetic strip for 1–2 min until the solution is clear.
11. Transfer 18 µl of the supernatant from each reaction to a fresh reaction vessel.

Second Hybridization Step

Note: Preheat thermal cycler to 98°C.

1. Prepare the reactions according to Table 4 by adding the following components to the reaction vessel.

Table 4. Second hybridization reaction.

Reagent	Cap Color	Volume per Reaction, µl
Amplification Mix	Green	8
Probe Mix	Yellow	2
RNA-Seq library prep (from step 11)	—	18
ddH_2O	—	2
Total		**30**

2. Mix the reactions thoroughly by vortexing 5–10 sec.
3. Centrifuge briefly to collect the reactions at the bottom of the reaction vessels.
4. Incubate the reactions in a thermal cycler using the hybridization protocol (Table 2).

Second Depletion Step

1. Vortex Purification Beads 30 sec to resuspend the beads in solution.
2. For each reaction, aliquot 50 µl of the Purification Beads suspension into a fresh reaction vessel.
3. Place the reaction vessels on a magnetic strip at room temperature.

   Important: Do not proceed to the next step until the second hybridization step thermal cycling protocol has been completed.
4. Aspirate the solution from the beads and discard it.
5. Briefly centrifuge reaction vessels that contain the hybridization reactions.
6. Transfer 30 µl of the hybridization reaction to a reaction vessel that contains Purification Beads.
7. Resuspend beads by pipetting up and down several times.
8. Incubate at room temperature for 15 min, mixing every 5 min.
9. Briefly centrifuge reaction vessels that contain the hybridization reactions.
10. Place the reaction vessels on a magnetic strip for 1–2 min until the solution is clear.
11. Transfer 27 µl of the supernatant to a fresh reaction vessel.

Amplification Step

Prior to starting this step, program the thermal cycler according to Table 5. Preheat thermal cycler to 98°C.

Table 5. Thermal cycler amplification protocol.*

Step	Temperature, °C	Time, sec	PCR Cycles
Denaturation	98	60	1
Primers	98	10	See Table 7
	61	15	
	72	20	
Final extension	72	120	1
Hold	4	Hold	1

* Use a heated lid set to 105°C. Set the sample volume to 50 µl.

1. Prepare the reactions by adding the components to the reaction vessels according to Table 6.

Table 6. Amplification reaction.

Reagent	Cap Color	Volume per Reaction, µl
Amplification Mix	Green	12
Primers	Blue	2.5
Supernatant from step 11 of 2nd depletion step	—	27
ddH_2O	—	8.5
Total		**50**

2. Mix the reactions thoroughly by vortexing 5–10 sec.
3. Briefly centrifuge to collect the reactions at the bottom of the reaction vessels.
4. Incubate the reactions in a thermal cycler using the amplification protocol (Table 5).

Table 7. PCR cycle recommendation.

Input cDNA, ng	Number of PCR Cycles
0.1	15
0.5	13
1.0	12
5.0	9
10	8
20	7

Postamplification Cleanup

1. Add an optimized volume of SPRISelect Beads to 50 µl of the Amplification Mix.

   Important: Use the SPRISelect Beads ratio that has been optimized for and recommended by the library prep kit vendor in the final purification step of the library preparation protocol (see Table 8).
2. Mix well to resuspend beads by pipetting up and down several times.

Table 8. SPRISelect Beads ratio recommended by vendors.

	SPRISelect Beads	
Library Preparation Kit	Ratio	Volume, µl
SEQuoia Complete Stranded RNA	1.2x	60
NEB NEBNext Ultra II Directional RNA	0.9x	45
Illumina® TruSeq® Total RNA	1.0x	50
Kapa HyperPrep Stranded RNA-Seq	1.0x	50

3. Incubate at room temperature for 1 min.
4. Briefly centrifuge to collect the reactions at the bottom of the tubes.
5. Place the tubes on a magnetic strip for 1–2 min until the solution is clear.

   Note: Keep the reaction vessels on the magnetic strip through step 11.
6. Aspirate the cleared solution and discard it.
7. Wash the beads: dispense 200 µl of freshly prepared 80% ethanol to each reaction vessel and incubate for 30 sec.
8. Carefully aspirate the ethanol and discard it.
9. Repeat wash step: Dispense 200 µl of freshly prepared 80% ethanol to each reaction vessel and incubate for 30 sec.
10. Carefully aspirate the ethanol and discard it.

11. Allow beads to air dry at room temperature by leaving them on the benchtop with the lids open for 1–4 min.

    Caution: Do not let beads overdry and do not dry at elevated temperatures. Doing so will result in poor sample recovery.

12. Add 20 µl of 10 mM Tris, pH 8.0 to each reaction vessel.
13. Mix to suspend the beads.
14. Incubate the reaction vessels at room temperature for 1 min.
15. Briefly centrifuge to collect the reactions at the bottom of the tubes.
16. Place the tubes on a magnetic strip for 1–2 min until the solution is clear.
17. Transfer 18 µl of supernatant to a fresh reaction vessel.
18. Store reactions at 4°C overnight or at –20°C if storing for longer than 24 hr.

Quality Control

The SEQuoia RiboDepletion Kit undergoes extensive quality control testing to ensure functionality and lot-to-lot consistency. This product is free of detectable DNase and RNase activity.

Bio-Rad Technical Support

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

Phone: 1-800-424-6723, option 2

Email: Support@Bio-Rad.com (U.S./Canada only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at bio-rad.com.

Related Products

Catalog #	Description
Consumables	
17005726	**SEQuoia Complete Stranded RNA Library Prep Kit**, 24 reactions
17005710	**SEQuoia Complete Stranded RNA Library Prep Kit**, 96 reactions
TBC0802	**0.2 ml 8-Tube PCR Strips and Domed Cap Strips**, high profile, clear
TBC1202	**0.2 ml 12-Tube PCR Strips and Domed Cap Strips**, high profile, clear
12011928	**SEQuoia Dual Indexed Primers Set**
12011930	**SEQuoia Dual Indexed Primers Plate**
Instruments	
1851197	**C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module**
1854095	**CFX96 Touch Deep Well Real-Time PCR System**

Visit bio-rad.com/SEQuoiaRiboDepletion for more information.

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