



ZYMO RESEARCH

RNA - Seq  
Made Simple

# Zymo-Seq RiboFree® Total RNA Library Kit

Any Organism. One rRNA Depletion Solution.

## Highlights

- **The Fastest & Easiest Kit:** Prepare stranded, RiboFree® libraries from total RNA in 3.5 hours.
- **Compatible with Any Organism:** Novel probe-free technology depletes rRNA from any RNA source.
- **The Most Accurate:** Eliminate bias from rRNA depletion.

Catalog Numbers:  
R3000, R3003



Scan with your smart-phone camera to  
view the online protocol/video.



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# Product Contents

Zymo-Seq RiboFree® Total RNA Library Kit	R3000 (12 prep)	R3003 (96 prep)	Storage Temperature
R1 Reagent	24 µL	8 x 24 µL	-80°C
R2 Reagent	120 µL	8 x 120 µL	-80°C
D1 Reagent	120 µL	8 x 120 µL	-80°C
D2 Reagent	120 µL	8 x 120 µL	-80°C
D3 Stop Reagent	120 µL	8 x 120 µL	-80°C
L1 Reagent	120 µL	8 x 120 µL	-80°C
L2 Reagent	240 µL	8 x 240 µL	-80°C
L3 Reagent	120 µL	8 x 120 µL	-80°C
Zymo Taq™ PreMix	300 µL	8 x 300 µL	-80°C
Zymo-Seq™ UDI Primer Set (1-12) <sup>1</sup>	20 µL/Index	-	-80°C
Zymo-Seq™ UDI Primer Plate (1-96) <sup>2</sup>	-	10 µL/Index	-80°C
Select-a-Size MagBeads	10 mL	50 mL	4°C
Zymo-Seq™ Wash Buffer	6 mL	48 mL	Room Temp.
DNA Elution Buffer	10 mL	50 mL	Room Temp.
DNase/RNase-Free Water	1 mL	10 mL	Room Temp.
Instruction Manual	1	1	-

1 The provided **Zymo-Seq™ UDI Primer Set** (Indexes 1-12) (D3008) contains 12 pre-mixed unique dual-index barcode primers in 1.5 mL tubes. See **Appendix D** for primer specifications and index sequences.

2 The provided **Zymo-Seq™ UDI Primer Plate** (Indexes 1-96) (D3096) contains 96 pre-mixed unique dual-index barcode primers in a 96-well plate format. See **Appendix D** for primer specifications and index sequences.

# Specifications

- **Sample Input Material:** RNA
- **Recommended Input:** 500 ng<sup>1</sup>
- **Minimum Input:** 100 ng<sup>1</sup>
- **Maximum Input:** 5 µg
- **Input Quality:** Ensure RNA A260/A280 and A260/A230 ratios are **≥ 1.8**, **DNA-free**, and **PCR inhibitor-free** for high-fidelity cDNA transcription and depletion. For optimal results, please use high-integrity RNA as input whenever possible. For degraded RNA, refer to **Appendix E** for recommendations.
- **Equipment Required** (user provided): Thermal cycler, magnetic stand for 0.2-mL PCR tubes, and microcentrifuge
- **Processing Time:** As little as 3.5 hours (RNA to indexed library)<sup>2</sup>.
- **Sequencing:** Libraries are stranded and compatible with all Illumina® sequencing platforms. The Read 1 sequence will be antisense to the RNA molecule of origin.
- **Barcode Sequences:** Available for download [here](#) (USA Only), or by visiting the Documents section of the D3008 and D3096 product pages at [www.zymoresearch.com](http://www.zymoresearch.com).

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<sup>1</sup> See **Appendix B** for recommended rRNA depletion incubation times. Lower input will require longer incubation times and may show reduced rRNA depletion efficiency.

<sup>2</sup> Time may increase when performing longer incubation time during rRNA depletion.

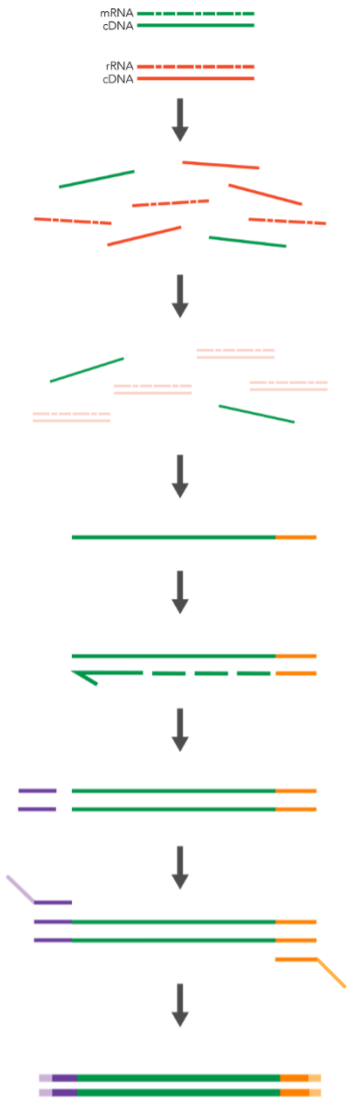
# Product Description

Ribosomal RNAs (rRNA) comprise approximately 90% of the total RNA and represent an obstacle to transcriptome enrichment of RNA molecules of interest. **Zymo-Seq RiboFree® Total RNA Library Kit** effectively removes rRNA with a probe-free strategy that is compatible with total RNA from any organism. RiboFree® Universal Depletion uses the input RNA as templates to drive the depletion of the reverse transcribed cDNA from the highly abundant sequences. This eliminates the need for organism-specific probes that are often a source of bias due to off-target hybridization.

In contrast to poly-A RNA sequencing, total RNA-Seq captures long-noncoding RNAs (lncRNA), intronic RNAs, nucleolar RNAs, and mRNAs with degraded poly(A) tails. Thus, **Zymo-Seq RiboFree® Total RNA Library Kit** allows the capture of any sample's full transcriptome (both coding and non-coding).

**Zymo-Seq RiboFree® Total RNA Library Kit** is all-inclusive and cost-effective, with most of the reagents in premixed formats ready for use. The streamlined workflow from total RNA to stranded NGS libraries includes: (1) total RNA is reverse transcribed and cDNA from rRNA is depleted; (2) Partial P7 and P5 adapters are ligated; (3) Index PCR amplification adds full-length, unique dual indexes to the stranded library. See the next page for a detailed Library Preparation Overview.

# Library Preparation Overview



## Section 1.1: First-Strand cDNA Synthesis

Reverse Transcription

## Section 1.2: RiboFree® Universal Depletion

Denature

Re-nature and Deplete

## Section 2.1: P7 Adapter Ligation

Add Partial P7

Second-Strand Synthesis

## Section 2.2: P5 Adapter Ligation

Add Partial P5

## Section 2.3: Library Index PCR

Index PCR

Stranded Library

# Protocol

## Buffer Preparation

- ✓ Add 24 mL of 100% ethanol (26 mL of 95% ethanol) to the 6 mL **Zymo-Seq™ Wash Buffer** concentrate (R3000), or 192 mL of 100% ethanol (208 mL of 95% ethanol) to the 48 mL **Zymo-Seq™ Wash Buffer** concentrate (R3003).

## Before Starting

- ✓ Set the thermal cycler lid temperature to > 98°C for each program.
- ✓ Thaw and maintain -80°C components on ice during use. Mix reagents by flicking and centrifuge briefly.
- ✓ Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for ≥30 minutes prior to use.
- ✓ Resuspend the magnetic particles immediately before each use by vigorously inverting and vortexing the **Select-a-Size MagBeads** until homogenous.
- ✓ Avoid multiple freeze-thaws of -80°C components. Make aliquots as necessary.
- ✓ Using a multichannel pipette to transfer reagents will minimize hands-on time when processing multiple samples.
- ✓ **For new users, please read Appendix A: Select-a-Size MagBead Clean-up Protocol carefully before performing the protocol.**

## Section 1.1: First-Strand cDNA Synthesis (Yellow Caps)

1. Create the following thermal cycler program for a total reaction volume of 20  $\mu\text{L}$ :

Program	Step	Temperature	Time
Primer Annealing	1	98°C	3 min
	2	4°C	Hold
Reverse Transcription	3	25°C	5 min
	4	48°C	15 min
	5	4°C	Hold

2. Use **DNase/RNase-Free Water** to raise the volume of each RNA input sample to 8  $\mu\text{L}$  in a 0.2 mL PCR tube on ice.
3. Add 2  $\mu\text{L}$  of the **R1 Reagent** to each sample for a total of 10  $\mu\text{L}$ . Mix thoroughly by flicking or pipetting. Centrifuge briefly. (For inputs < 100 ng, 1  $\mu\text{L}$  of the **R1 Reagent** should be used. Supplement with 1  $\mu\text{L}$  of **DNase/RNase-Free Water**.)
4. Place the tube in the thermal cycler and run **Steps 1-2 (Primer Annealing)** of the program.
5. Add 10  $\mu\text{L}$  of the **R2 Reagent** to each sample during the 4°C hold (**Step 2**) or on ice. Mix thoroughly by pipetting. Centrifuge briefly.
6. Close the thermal cycler lid and continue **Steps 3-5 (Reverse Transcription)** of the program.
7. Proceed directly to **Section 1.2: RiboFree® Universal Depletion** for the depletion of ribosomal RNA.<sup>1</sup>

<sup>1</sup> To skip depletion, add 30  $\mu\text{L}$  of **DNase/RNase-Free Water** to raise the sample volume to 50  $\mu\text{L}$  and skip to Step 8 of **Section 1.2** and add the indicated volume of ethanol to continue.



## Section 1.2: RiboFree® Universal Depletion (Red Caps)

### Before Starting:

- ✓ Refer to **Appendix B** for additional RiboFree® Universal Depletion recommendations.
  - ✓ For degraded RNA, refer to **Appendix E** for recommendations.
  - ✓ This section involves adding reagents to tubes inside a thermal cycler.
1. Create the following thermal cycler program for a total reaction volume of 50  $\mu$ L:

Program	Step	Temperature	Time	Input
Pre-Depletion Incubation	1	98°C	3 min	
	2	68°C	5 min	
	3	68°C	Hold	
Depletion Reaction			30 min	(>1 $\mu$ g)
	4	68°C	1 hr	(500 ng - 1 $\mu$ g)
			2 hrs	(250 - 499 ng)
			4 hrs	(100 - 249 ng)
5	68°C	Hold		
Stop Depletion	6	98°C	2 min	
	7	25°C	Hold	

2. Add 10  $\mu$ L of the **D1 Reagent** directly to each 20  $\mu$ L sample on ice for a total of 30  $\mu$ L. Mix thoroughly by pipetting. Centrifuge briefly.
3. Transfer the sample tube to the thermal cycler and run **Steps 1-3 (Pre-Depletion Incubation)** of the program. **DO NOT** remove the sample tube from the thermal cycler at the **Step 3** hold.
4. Without removing the tube, add 10  $\mu$ L of the **D2 Reagent** to each 30  $\mu$ L sample for a total of 40  $\mu$ L. Mix thoroughly by pipetting.
5. Close the thermal cycler lid and continue **Step 4 (Depletion Reaction)** of the program. **DO NOT** remove the tube from the thermal cycler at the **Step 5** hold.

**Continue Section 1.2 protocol on the next page.**

6. Without removing the tube, add 10  $\mu\text{L}$  of the **D3 Stop Reagent** to each 40  $\mu\text{L}$  sample for a total of 50  $\mu\text{L}$ . Mix thoroughly by pipetting.
7. Close the thermal cycler lid and continue through **Steps 6-7 (Stop Depletion)** of the program.
8. Remove the tube from the thermal cycler and centrifuge briefly<sup>1</sup>. Add 25  $\mu\text{L}$  of 95% ethanol to each 50  $\mu\text{L}$  sample for a total of 75  $\mu\text{L}$ . Mix thoroughly by pipetting.
9. Create a thermal cycler incubation program for 5 min at 95 °C.
10. Follow the clean-up protocol (**Appendix A**) using 150  $\mu\text{L}$  of **Select-a-Size MagBeads**. For elution, resuspend the beads in 10  $\mu\text{L}$  of **DNA Elution Buffer** and incubate at 95°C for 5 minutes. Once incubation is completed, remove the tube from the thermal cycler and let it cool to room temperature (about 2 minutes). Centrifuge briefly to collect all the eluate before placing the tube on the magnetic stand to elute.

***This is a safe stopping point. Cleaned-up cDNA can be safely stored at  $\leq 4^{\circ}\text{C}$  overnight or  $\leq -20^{\circ}\text{C}$  for up to one week.***

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<sup>1</sup> Refer to **Appendix E** for alternate cleanup recommendations for degraded RNA samples.

## Section 2.1: P7 Adapter Ligation (Green Caps)

1. Create the following thermal cycler program for a total reaction volume of 40  $\mu\text{L}$ :

Program	Step	Temperature	Time
P7 Ligation Reaction	1	37°C	15 min
	2	95°C	2 min
	3	4°C	Hold
Second Strand Synthesis	4	95°C	10 min
	5	63°C	30 sec
	6	72°C	7 min
	7	4°C	Hold

2. Add 10  $\mu\text{L}$  of **L1 Reagent** to each 10  $\mu\text{L}$  cDNA sample in a 0.2 mL PCR tube on ice for a total of 20  $\mu\text{L}$ . Mix by gently pipetting up and down. Centrifuge briefly.
3. Place the tube in the thermal cycler and run **Steps 1-3 (P7 Ligation Reaction)** of the program.
4. Add 20  $\mu\text{L}$  of **L2 Reagent** to each 20  $\mu\text{L}$  sample for a total of 40  $\mu\text{L}$ . Mix by gently pipetting up and down. Centrifuge briefly.
5. Place the tube in the thermal cycler and continue through **Steps 4-7 (Second Strand Synthesis)** of the program.
6. Remove the tube from the thermal cycler and centrifuge briefly. Follow the clean-up protocol (**Appendix A**) using 60  $\mu\text{L}$  of **Select-a-Size MagBeads**. For elution, resuspend the beads in 10  $\mu\text{L}$  of **DNA Elution Buffer** at room temperature (no need to incubate).

***This is a safe stopping point. Cleaned-up DNA can be safely stored at  $\leq 4^\circ\text{C}$  overnight or  $\leq -20^\circ\text{C}$  for up to one week.***

## Section 2.2: P5 Adapter Ligation (Green Caps)

1. Create the following thermal cycler program for a total reaction volume of 20  $\mu\text{L}$ :

Program	Step	Temperature	Time
P5 Ligation	1	25°C	15 min
Reaction	2	4°C	Hold

2. Add 10  $\mu\text{L}$  of **L3 Reagent** to each 10  $\mu\text{L}$  sample in a 0.2 mL PCR tube on ice for a total of 20  $\mu\text{L}$ . Mix by gently pipetting up and down. Centrifuge briefly.
3. Place the tube in the thermal cycler and run **Steps 1-2 (P5 Ligation Reaction)** of the program.
4. Remove the tube from the thermal cycler and centrifuge briefly. Add 80  $\mu\text{L}$  of **DNA Elution Buffer** to the tube to raise the volume to 100  $\mu\text{L}$ .
5. Follow the clean-up protocol (**Appendix A**) using 100  $\mu\text{L}$  of **Select-a-Size MagBeads**. For elution, resuspend the beads in 20  $\mu\text{L}$  of **DNA Elution Buffer** at room temperature (no need to incubate).

***This is a safe stopping point. Cleaned-up DNA can be safely stored at  $\leq 4^\circ\text{C}$  overnight or  $\leq -20^\circ\text{C}$  for up to one week.***

## Section 2.3: Library Index PCR (Green Caps)

1. Create the following thermal cycler program for a total reaction volume of 50  $\mu\text{L}$ . Adjust the total cycles based on the input total RNA amount as listed on the right side of the table.

Step	Temperature	Time	
1	95°C	10 min	
2	95°C	30 sec	} > 1 $\mu\text{g}$ = 10 cycles 500 ng – 1 $\mu\text{g}$ = 11-12 cycles 250 – 499 ng = 12-13 cycles 100 – 249 ng = 13-14 cycles < 100 ng = $\geq$ 14 cycles
3	60°C	30 sec	
4	72°C	1 min	
	-- Go to step 2 --		
5	72°C	7 min	
6	4°C	Hold	

2. Add 5  $\mu\text{L}$  of the appropriate pre-mixed **Zymo-Seq UDI Primers**<sup>1</sup> to each 20  $\mu\text{L}$  sample in a 0.2 mL PCR tube for a total of 25  $\mu\text{L}$ .
3. Add 25  $\mu\text{L}$  of **Zymo Taq™ PreMix** to the tube for a total of 50  $\mu\text{L}$ . Mix by gently pipetting up and down. Centrifuge briefly.
4. Place the tube in the thermal cycler and run the program above.
5. Remove the tube from the thermal cycler and centrifuge briefly. Add 50  $\mu\text{L}$  of **DNA Elution Buffer** to the tube to raise the volume to 100  $\mu\text{L}$ .
6. Follow the clean-up protocol (**Appendix A**) using 85  $\mu\text{L}$  of **Select-a-Size MagBeads**. For elution, resuspend the beads in 15-25  $\mu\text{L}$  of **DNA Elution Buffer** at room temperature (no need to incubate).

The eluate is your final RNA-Seq library<sup>2</sup>. Libraries may be stored at  $\leq 4^\circ\text{C}$  overnight or  $\leq -20^\circ\text{C}$  for long-term storage.

<sup>1</sup> See **Appendix D** for index primer sequences and barcodes.

<sup>2</sup> Recommended: Remove an aliquot (e.g. 5  $\mu\text{L}$ ) for analysis and quantitation.

# Appendices

## Appendix A: Select-a-Size MagBead Clean-up Protocol

### Before Starting:

- ✓ Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for  $\geq 30$  minutes prior to use.
  - ✓ Resuspend the magnetic particles immediately before each use by vigorously shaking or vortexing the **Select-a-Size MagBeads** until homogenous.
1. Add the indicated volume of **Select-a-Size MagBeads** to each sample. Mix thoroughly by pipetting until homogenous. Incubate for 5 minutes at room temperature.
  2. Place the sample on a magnetic stand for 5 minutes, or until the beads have fully separated from the solution. Without dislodging the bead pellet, aspirate slowly and discard the supernatant.
  3. While the sample is still on the magnetic stand, add 200  $\mu\text{L}$  of **Zymo-Seq™ Wash Buffer** without disturbing the bead pellet. Aspirate slowly and discard the supernatant without dislodging the bead pellet. Repeat this step for a total of 2 washes.
  4. While the sample is still on the magnetic stand, keep the tube cap open to air-dry the beads. After 1 minute, aspirate any residual **Zymo-Seq™ Wash Buffer** that has collected at the bottom of the tube. Continue to air-dry until the bead pellet appears matte without cracking. See footnote 1.
  5. Remove the sample from the magnetic stand. Add the indicated volume of **DNA Elution Buffer** to the beads and mix thoroughly by pipetting up and down until homogenous. Incubate at the indicated temperature.
  6. Place the sample on the magnetic stand for 1-2 minutes or until the eluate is clear. Transfer the eluate to a new 0.2-mL PCR tube for each sample.

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1 The optimal air-dry time can vary depending on the humidity and temperature. **Optimally dried beads should appear matte without cracking**. Start with 5 minutes of air-dry time and adjust the time as needed to achieve optimally dried beads. Wash buffer carryover from insufficiently dried beads or overdried, cracked beads may reduce nucleic acid recovery.

## Appendix B: Depletion Incubation Guide

RiboFree® universal depletion of highly abundant transcripts is based on the enzymatic digestion of high concentration complementary sequences. The duration of the depletion incubation is inversely proportional to the input concentration. Lower inputs require longer incubation.

We have found that longer incubation times (up to 16 hours) do not adversely affect the sample. To obtain optimal depletion efficiency, especially for organisms or input amount not shown in the product data (see [Datashheet](#) and Q1 in [Product FAQ](#)), optimization of depletion incubation time is recommended.

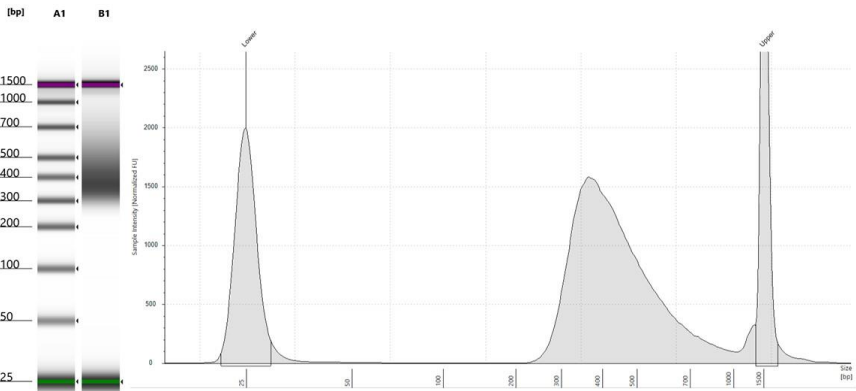
The PCR cycle number for the final Library Index Reaction will vary with RNA input. The cycle numbers provided are guidelines when depletion is performed. Users should determine the optimal cycle numbers empirically.

Recommended incubation time and PCR cycle number for standard applications:

RNA Input	Depletion Time	Index PCR Cycles
> 1 µg	30 min	10
500 ng - 1 µg	1 hour	11-12
250 ng – 499 ng	2 hours	12-13
100 ng – 249 ng	4 hours	13-14
< 100 ng	4-16 hours	≥14

## Appendix C: Library Characterization

Libraries should be visualized by running an agarose gel or using an automated electrophoresis instrument (i.e. Agilent TapeStation, Agilent Bioanalyzer, etc) to determine that the correct library size is present. Below is an example of a library prepared with this kit and characterized using the D1000 ScreenTape® on Agilent TapeStation® 4150 (Figure 1). Yields will vary depending on the total quantity and quality of sample input RNA and the number of PCR cycles.



**Figure 1.** Agilent 4150 TapeStation® D1000 electropherogram of a typical Zymo-Seq RiboFree® Total RNA Library using 500 ng of Universal Human Reference RNA and indexed using 11 PCR cycles. Fragment sizes mainly range from 200 to 600 bp for libraries prepared from intact RNA (RIN > 9.0).



## Appendix D: Unique Dual Index (UDI) Primer Sets

Indexes in the **Zymo-Seq™ UDI Primer Set (Indexes 1-12)** are dispensed in 1.5 mL tubes (D3008), and indexes in the **Zymo-Seq™ UDI Primer Plate (Indexes 1-96)** are dispensed in single-use foil-sealed 96-well plates (D3096). Indexes come as pre-mixes, and the forward and reverse primers are provided at 5 µM total concentration (2.5 µM each).

The complete [index sample sheet](#) is available for download [here](#) (USA Only), or by visiting the Documents section of the D3008 and D3096 product pages at [www.zymoresearch.com](http://www.zymoresearch.com).

### Primer Sequences:

Forward Primer Sequence (i5):

5'-AATGATACGGCGACCACCGAGATCTACAC**NNNNNNNN**ACACTC  
TTTCCTACACGACGCTCTTCCGATCT-3'

Reverse Primer Sequence (i7):

5'-CAAGCAGAAGACGGCATAACGAGAT**NNNNNNNN**GTGACTGGAG  
TTCAGACGTGTGCTCTTCCGATCT-3'

Note: **NNNNNNNN** correspond to the “Bases in Adapter” columns in the [index sample sheet](#) mentioned above.

### UDI Primer Plate (D3096) Setup:

To use UDI primers, choose  $\geq 2$  sets down a column not across a row.

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI_01	UDI_09	UDI_17	UDI_25	UDI_33	UDI_41	UDI_49	UDI_57	UDI_65	UDI_73	UDI_81	UDI_89
B	UDI_02	UDI_10	UDI_18	UDI_26	UDI_34	UDI_42	UDI_50	UDI_58	UDI_66	UDI_74	UDI_82	UDI_90
C	UDI_03	UDI_11	UDI_19	UDI_27	UDI_35	UDI_43	UDI_51	UDI_59	UDI_67	UDI_75	UDI_83	UDI_91
D	UDI_04	UDI_12	UDI_20	UDI_28	UDI_36	UDI_44	UDI_52	UDI_60	UDI_68	UDI_76	UDI_84	UDI_92
E	UDI_05	UDI_13	UDI_21	UDI_29	UDI_37	UDI_45	UDI_53	UDI_61	UDI_69	UDI_77	UDI_85	UDI_93
F	UDI_06	UDI_14	UDI_22	UDI_30	UDI_38	UDI_46	UDI_54	UDI_62	UDI_70	UDI_78	UDI_86	UDI_94
G	UDI_07	UDI_15	UDI_23	UDI_31	UDI_39	UDI_47	UDI_55	UDI_63	UDI_71	UDI_79	UDI_87	UDI_95
H	UDI_08	UDI_16	UDI_24	UDI_32	UDI_40	UDI_48	UDI_56	UDI_64	UDI_72	UDI_80	UDI_88	UDI_96

## Appendix E: Degraded RNA Recommendations

For optimal results when using degraded RNA with an RNA Integrity Number (RIN) of approximately 4 or lower as input, use the recommended modifications below:

- ✓ Use a higher amount as input whenever possible. The more severe the degradation of the RNA, the higher amount the input that should be used. For example, for RNA samples with a RIN ~4, use at least 250 ng of input RNA. For RNA samples with a RIN  $\leq 2$ , use at least 500 ng.
- ✓ Substitute the **Select-a-Size MagBead** cleanup in **Section 1.2** with [RNA Clean & Concentrator – 5](#) (R1013) cleanup:
  - Upon the completion of **Step 7** in **Section 1.2**, use **RNA Clean & Concentrator -5** for the cleanup according to the product [protocol](#), “Total RNA Clean-up”. Elute in 10  $\mu$ L of **DNA Elution Buffer**.
  - All subsequent **Select-a-Size MagBead** sample purifications should be followed as listed in the standard **Zymo-Seq RiboFree® Total RNA Library Kit** protocol.
- ✓ Refer to the modified depletion incubation time and PCR cycle number guidelines below:

RNA Input	Depletion Time	Index PCR Cycles
> 1 $\mu$ g	30 min	13
500 ng - 1 $\mu$ g	1 hour	14-15
250 ng – 499 ng	1-2 hours	15-16

## Appendix F: Considerations for Sequencing and Bioinformatics

### *Instrument Compatibility*

The libraries prepared with this kit are compatible with all Illumina® sequencing platforms. When sequencing libraries on the MiSeq®, please use the MiSeq Software Updater v2.2.0.2 (containing RTA v.1.17.28 rel. 3/18/2013) or later.

### *Preparation for Clustering*

Accurate quantification of the final library concentration is critical to achieve optimal clustering and sequencing results. For this, we recommend using quantitative PCR (e.g., KAPA® Library Quantification Kit). Adding a high complexity spike-in such as PhiX to the final library pool is recommended.

### *Trimming Reads*

The **Zymo-Seq RiboFree® Total RNA Library Kit** employs a low-complexity bridge to ligate the Illumina® P7 adapter sequence to the library inserts (See the library structure below). This sequence can extend up to 10 nucleotides. QC analysis software (e.g., FastQC<sup>1</sup>) may raise flags such as “Per base sequence content” at the beginning of Read 2 due to this low complexity bridge sequence.



If desired, these 10 nucleotides can be removed in addition to adapter trimming. An example using Trim Galore!<sup>2</sup> for such trimming is as below:

```
trim_galore --paired --clip_R2 10 -a  
NNNNNNNNNAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC -  
a2 AGATCGGAAGAGCGTCGTGTAGGGAAAGA sample.R1.fastq.gz  
sample.R2.fastq.gz
```

### *Library Strand Information*

The Read 1 sequence will be antisense to the RNA transcript from which it originates. Therefore, the strandedness of the library should be set as reverse stranded for applicable bioinformatics tools.

1 FastQC is publicly available at <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.

2 Trim Galore! is a publicly available software and accessible at <https://github.com/FelixKrueger/TrimGalore>.

# Ordering Information

Product Description	Catalog No.	Size
Zymo-Seq RiboFree® Total RNA Library Kit	R3000	12 preps
Zymo-Seq RiboFree® Total RNA Library Kit	R3003	96 preps
Zymo-Seq RiboFree® Universal cDNA Kit	R3001	12 preps
Zymo-Seq™ UDI Primer Set (Indexes 1-12)	D3008	12 Indexes
Zymo-Seq™ UDI Primer Plate (Indexes 1-96)	D3096	96 Indexes

Individual Kit Components	Catalog No.	Amount
Zymo <i>Taq</i> ™ PreMix	E2003	50 reactions
	E2004	200 reactions
Select-a-Size MagBeads	D4084-4-10	10 ml
	D4084-4-50	50 ml
DNase/RNase-Free Water	W1001-1	1 ml
	W1001-4	4 ml
	W1001-6	6 ml
	W1001-10	10 ml
	W1001-30	30 ml
Zymo-Seq™ Wash Buffer	R3004-1-6	6 mL
	R3004-1-48	48 mL
DNA Elution Buffer	D3004-4-10	10 mL
	D3004-4-50	50 mL

# Complete Your Workflow

## RNA-Seq *Made Simple*<sup>®</sup>

Sample to Sequencer in a Single Day



### Sample Collection

Prevent RNA Degradation

DNA/RNA Shield™ [Cat. No. R1100-50]



### NGS-Grade RNA Extraction

RNA Isolation from Any Sample Type

Quick-RNA™ Miniprep Plus [Cat. No. R1057]



### RNA-Seq Library Prep Kit

The Easiest and Fastest Total RNA-Seq Library Prep Kit

Zymo-Seq RiboFree® Total RNA Library Kit [Cat. No. R3000]



### Stranded Libraries

In as little as 4 hours















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Integrity of kit components is guaranteed for up to six months from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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