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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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ZYMO RESEARCH

RNA - Seq Made Simple™

Zymo-Seq™ miRNA Library Kit

Innovative gel-free workflow to simplify small RNA discovery.

Highlights

- **Accurate miRNA profiling**: A unique single adapter and circularization strategy reduces ligation bias and increases miRNA detection.
- **Direct detection:** Generates small RNA libraries without the need for small RNA enrichment.
- **Gel-free:** Removes adapter dimers from any input without lengthy gel excision.

Catalog Numbers:
R3006, R3007

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

Zymo-Seq™ miRNA Library Kit	R3006 (12 prep)	R3007 (96 prep)	Storage Temperature
miRNA Control (+)	10 µL	10 µL	**-80°C**
miRNA Adapter	12 µL	96 µL	**-80°C**
Ligation Enhancer	48 µL	384 µL	-20°C
RNase Inhibitor	24 µL	192 µL	-20°C
miRNA Ligation Buffer	18 µL	144 µL	-20°C
miRNA Ligase	12 µL	96 µL	-20°C
Blocking Agent	12 µL	96 µL	-20°C
Blocking Enzyme	12 µL	96 µL	-20°C
Circularization Enzyme	12 µL	96 µL	-20°C
Circularization Buffer	12 µL	96 µL	**-80°C**
RT Primer Mix	48 µL	384 µL	-20°C
RT Buffer	36 µL	288 µL	-20°C
RT Enzyme	18 µL	144 µL	-20°C
Zymo*Taq*™ PreMix	625 µL	6 x 625 µL	-20°C
miRNA UDI Primer Set (1-12)	15 µL / index	-	-20°C
miRNA UDI Primer Plate (1-96)	-	10 µL / index	-20°C
Dimer Removal Agent	12 µL	96 µL	-20°C
Dimer Removal Beads	240 µL	2 mL	4°C
Select-a-Size MagBead Concentrate	300 µL	2 x 300 µL	4°C
Select-a-Size MagBead Buffer	10 mL	2 x 10 mL	4°C
DNA Wash Buffer	6 mL	24 mL	Room Temp.
DNase/RNase-Free Water	1 mL	2 x 1 mL	Room Temp.
Instruction Manual	1	1	-

Specifications

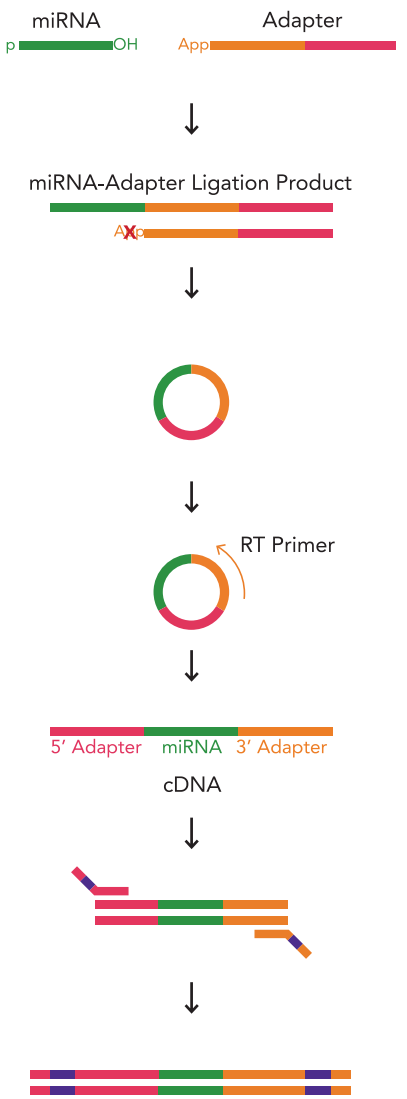
- **Sample Type:** High integrity total RNA (RIN ≥ 7) containing miRNA, or cell-free RNA extracted from plasma/serum samples not preserved with heparin. Small RNA enrichment is not necessary.
- **Recommended Input:** 100 ng of total RNA, or RNA extracted from ≥ 200 µL of plasma or serum.
- **Minimum Input:** 1 ng of total RNA, or RNA extracted from 50 µL of plasma or serum.
- **Maximum Input:** 200 ng of total RNA, or RNA extracted from 200 µL of plasma or serum.
- **Processing Time:** 2 hours hands-on, 8 hours total
- **Sequencing Platform Compatibility:** Libraries are compatible with all Illumina sequencing platforms.
- **Required Equipment:** Thermal cycler, magnetic stand for 0.2 mL PCR tubes or 96-well plate, mini centrifuge, and micro centrifuge.
- **Recommended Equipment:** A 96-well Aluminum Cooler Block for PCR tubes or a 96-well PCR tube rack may be placed on ice for ease of reagent assembly and maintaining samples at 4°C. To process > 12 samples at once, a multichannel pipette with low-retention tips is recommended for mixing and the timely addition of pre-aliquoted reagents.
- **Additional Components Required (Not Provided):** Molecular biology grade pure ethanol (≥ 95%)

Product Description

Micro RNA sequencing (miRNA-seq) is a powerful approach used to profile the microRNAs present in total RNA or cell-free RNA extracted from biofluids. Conventional methods for capturing microRNAs for sequencing are cumbersome and prone to ligation biases that reduce the accuracy of microRNA detection. The **Zymo-Seq™ miRNA Library Kit** utilizes a single adapter and circularization strategy to reduce ligation bias. This streamlined workflow minimizes hands-on time and uses gel-free removal of adapter dimers to generate stranded libraries in a single day.

To prepare Zymo-Seq™ miRNA libraries, RNA is ligated to the miRNA adapter. Excess adapter is blocked to prevent interference with subsequent steps. The miRNA-adapter ligation product is circularized, and the blocked adapter dimer is removed. The circularized miRNA-adapter product is then reverse transcribed. The resulting cDNA is amplified using Indexing PCR primers which add on Illumina-compatible adapter and unique dual index (UDI) sequences. The PCR product is then purified and ready for sequencing.

Library Preparation Workflow



- **Adapter Ligation** (orange caps)
  RNase Inhibitor
  miRNA Adapter
  Ligation Enhancer
  miRNA Ligation Buffer
  miRNA Ligase
  DNase/RNase-Free Water
- **Adapter Blocking** (red caps)
  Blocking Agent
  Blocking Enzyme
- **Circularization** (yellow caps)
  Circularization Enzyme
  Circularization Buffer
- **Dimer Removal** (clear caps)
  Dimer Removal Agent
  Dimer Removal Beads
- **Reverse Transcription** (brown caps)
  RT Primer Mix
  RT Buffer
  RT Enzyme
  RNase Inhibitor
- **Index PCR** (green caps)
  Zymo*Taq*™ PreMix
  miRNA UDI Primer Set/Plate
- **Library Purification** (clear caps)
  Select-a-Size MagBeads
  DNA Wash Buffer
  DNase/RNase-Free Water

* Circle colors correspond to component cap colors and the associated workflow steps. The components used in each step are listed for convenience.

Tips and Techniques

Read carefully before proceeding.

Best Practices:

- ✓ Avoid multiple freeze thaws of all components, including input RNA. Aliquot components as necessary to minimize freeze-thaw cycles.
- ✓ Remove enzymes from -20°C storage just before use. Return enzymes to -20°C storage immediately after use.
- ✓ Thaw and maintain all components on ice unless otherwise noted.
- ✓ Flick to mix thawed components and briefly centrifuge prior to use (e.g. ≥ 10,000 x g on a microcentrifuge for 1.5 mL tubes, or pulse spin at max speed using a mini centrifuge).
- ✓ After adding each component, thoroughly mix by pipetting up and down 15-20 times. Briefly centrifuge at a speed sufficient to ensure all components are collected at the bottom of the tube.
- ✓ Pre-program thermal cycler with lid heating ON, set to ≥ 95°C for a continuous workflow. See **Appendix A (page 13)** for a summary of thermal cycler programs.

Sample Considerations:

This kit may be used on high integrity total RNA without the need for additional small RNA enrichment. Using partially degraded RNA will result in a higher proportion of short sequencing reads (<15 nt) that correspond to degraded rRNAs. Cell-free RNA extracted from plasma or serum samples not preserved with heparin may also be used.

Not all plasma/serum RNA extraction and total RNA purification kits isolate miRNAs with the same efficiency. Users should confirm that the method used isolates miRNAs effectively. We recommend extraction with ***Quick*-cfRNA Serum & Plasma Kit (cat. R1059)** for plasma and serum samples and ***Quick*-RNA Microprep Kit (cat. R1050)** for low input/easy-to-lyse samples. For additional recommendations and guidance with choosing an RNA extraction kit suitable for a specific sample, contact us at tech@zymoresearch.com

When preparing libraries for the first time, we recommend preparing a control library using 1 µL of the included miRNA Control with 16 cycles of amplification. See **Appendix D (page 16)** for an example library profile.

Protocol

Section 1: Adapter Ligation and Blocking

Before Starting:

- ✓ Review **Best Practices (page 5)** for important handling information.
- ✓ Create the following thermal cycler program with lid heating ON (≥ 95ºC) for a total volume of 17 µL.

Program	Step	Temperature	Time
Adapter Prep	1	70°C	2 min
	2	4ºC [1]	Hold (≥ 2 min)
Adapter Ligation	3	25°C	1 hour
	4	65°C	5 min
	5	65°C	Hold
Blocking Agent	6	65°C	5 min
	7	65°C to 37°C	Ramp down[2] (0.1ºC/s)
	8	37°C	Hold
Blocking Enzyme	9	37°C	1 hour
	10	65°C	20 min
	11	4°C	Hold

1. Thaw components with **orange** caps and **red** caps on ice. Thaw **Ligation Enhancer** at room temperature. Once thawed, flick to mix and briefly centrifuge before use.
2. Adjust each RNA sample volume to 6.5 µL with **DNase/RNase-Free Water** in a 0.2 mL PCR tube.
3. Add 1 µL of **miRNA Adapter** to each sample on ice.
4. Prior to use, pre-heat **Ligation Enhancer** at 37°C for 5 minutes to improve ease of pipetting this viscous component[3]. Place at room temperature after pre-heating and while in use.

Continue Section 1 protocol on the next page.

[1] Alternatively, place samples on ice for the ≥ 2-minute incubation instead of 4ºC on the thermal cycler.

[2] Ramp down from 65°C to 37°C at a rate of 0.1°C per second (approximately ~6 minutes).

[3] Recommended methods of pre-heating include: heat block/digital dry bath, incubator, or thermal cycler (if **Ligation Enhancer** is aliquoted into 0.2 mL PCR tube(s).

5. Add 4 µL of **Ligation Enhancer** to each sample *slowly*. **Ligation Enhancer** is very viscous. Set pipette to 10 µL and mix by pipetting up and down slowly **15 to 20 times**. Briefly centrifuge, then place on ice.
6. Place the sample(s) in the pre-heated thermal cycler and run **Steps 1-2 (Adapter Prep)**. After incubation is complete, transfer the sample(s) to ice for the next step[1].
7. Add 1 µL of **RNase Inhibitor** to each sample on ice.
8. Add 1.5 µL of **miRNA Ligation Buffer** to each sample on ice.
9. Add 1 µL of **miRNA Ligase** to each sample on ice. Set a pipette to 10 µL and mix by pipetting up and down 15 to 20 times. Briefly centrifuge.
10. Place the sample(s) in the thermal cycler and run **Steps 3-5 (Adapter Ligation). DO NOT REMOVE** the samples from the thermal cycler at the **Step 5** hold.
11. Add 1 µL of **Blocking Agent** to each sample for a total of 16 µL. Mix in the thermal cycler by pipetting[2].
12. Close the thermal cycler lid and proceed past the hold to run **Steps 6-8 (Blocking Agent). DO NOT REMOVE** the sample(s) from the thermal cycler at the **Step 8** hold.
13. Without removing the sample(s) from the thermal cycler, add 1 µL of **Blocking Enzyme** for a total of 17 µL. Mix in the thermal cycler by pipetting[2].
14. Close the thermal cycler lid and proceed past the hold to run **Steps 9-11 (Blocking Enzyme)** of the program.
15. Remove sample(s) from the thermal cycler and place on ice. Proceed to **Section 2.**

Safe stopping point: Store samples at ≤ -20°C overnight.

[1]Alternatively, place samples on ice for the ≥ 2-minute incubation instead of 4ºC on the thermal cycler.

[2]**Optional:** Sample(s) may be removed briefly to centrifuge. Return sample(s) to the thermal cycler immediately.

Section 2: Circularization and Dimer Removal

Before Starting:

- ✓ Equilibrate **Dimer Removal Beads** to room temperature for 30 minutes. **Do not** place the **Dimer Removal Beads** on ice at any time.
- ✓ Create the following thermal cycler program with lid heating ON (≥ 95ºC) for a total reaction volume of 20 µL.

Program	Step	Temperature	Time
Circularization	1	37°C	1 hour
	2	37°C	Hold
Dimer Anneal	3	37°C	10 min
	4	37°C	Hold
Dimer Removal	5	37°C	10 min
	6	37°C	Hold

1. Thaw components with **yellow** caps and the **Dimer Removal Agent** on ice. Once thawed, flick to mix and briefly centrifuge before use. **DO NOT** place the **Dimer Removal Beads** on ice.
2. For each sample, prepare a master mix of the **Circularization** components as described below. Overage has already been included.

Circularization Component	Volume
Circularization Buffer	1.1 µL
Circularization Enzyme	1.1 µL
Total	2.2 µL

3. Add 2 µL of the master mix to each sample on ice. Pipet to mix and briefly centrifuge.
4. Place the sample(s) in the thermal cycler and run Steps **1-2 (Circularization). DO NOT REMOVE** the samples from the thermal cycler at the **Step 2** hold.
5. Add 1 µL of **Dimer Removal Agent** to each sample**.** Mix in the thermal cycler by pipetting[1].

Continue Section 2 protocol on the next page.

[1]**Optional:** Sample(s) may be removed briefly to centrifuge. Return sample(s) to the thermal cycler immediately.

6. Close the thermal cycler lid and proceed past the hold to run **Steps 3-4 (Dimer Anneal).**

7. Once the thermal cycler program has been initiated, proceed immediately to the next step to **Prepare the Dimer Removal Beads**.

Prepare the Dimer Removal Beads:

a. Vortex the **Dimer Removal Beads** for 30 seconds, or until homogenous.

b. For each sample, aliquot 20 µL of **Dimer Removal Beads** to a new 0.2 mL PCR tube.

c. Just before the **Step 3 (Dimer Anneal)** reaches completion, place tube(s) containing **Dimer Removal Beads** on a magnetic stand at room temperature for 1 minute, or until the supernatant is clear.

d. Remove and discard the cleared supernatant from the tube(s) containing **Dimer Removal Beads**

e. Transfer the tube(s) containing prepared **Dimer Removal Beads** from the magnet to a PCR tube rack at room temperature for the next steps.

8. Once the **Dimer Anneal** program has reached the **Step 4** hold, remove the sample(s) from the thermal cycler, and briefly centrifuge.

9. Place the sample(s) on a PCR tube rack at room temperature.

10. Transfer the entire sample reaction to a tube of prepared **Dimer Removal Beads** and pipet to mix until homogenous.

11. Immediately return the sample(s) to the thermal cycler and proceed past the hold to run **Steps 5-6 (Dimer Removal)**.

12. Once the incubation is complete, remove the sample(s) from the thermal cycler and centrifuge for **10 seconds**. Place the sample(s) on a magnetic rack for 1 minute, or until the supernatant is clear.

13. Transfer the entire cleared supernatant to a new 0.2 mL PCR tube and place on ice. Avoid bead carryover into subsequent steps.

Proceed to Section 3 promptly. Do not store overnight.

Section 3: Reverse Transcription

Before Starting:

✓ Create the following thermal cycler program with lid heating ON (≥ 95ºC) for a total reaction volume of 29 µL.

Program	Step	Temperature	Time
RT Primer Prep	1	65°C	5 min
	2	4ºC[1]	Hold (≥ 2 min)
Reverse Transcription	3	42°C	1 hour
	4	65°C	20 min
	5	4°C	Hold

1. Thaw the **RNase Inhibitor** (**orange**) and all components with **brown** caps on ice. Once thawed, flick to mix and briefly centrifuge before use.
2. Add 4 µL of **RT Primer Mix** to each sample on ice. Pipet to mix and briefly centrifuge.
3. Place the sample(s) in the thermal cycler and run **Steps 1-2 (RT Primer Prep)**. After incubation is complete, transfer the sample(s) to ice. Keep the sample(s) on ice[1].
4. For each sample, prepare a master mix of the **Reverse Transcription** components as described below. Overage has already been included.

Reverse Transcription Component	Volume
RT Buffer	3.3 µL
RNase Inhibitor	1.1 µL
RT Enzyme	1.65 µL
Total	6.05 µL

5. Add 5.5 µL of the master mix to each sample. Pipet to mix and briefly centrifuge.
6. Place the sample(s) in the thermal cycler and run **Steps 3-5 (Reverse Transcription).**

Safe stopping point: Store samples at ≤ -20°C overnight.

[1] Alternatively, place samples on ice for the ≥ 2-minute incubation instead of 4ºC on the thermal cycler.

Section 4: Index PCR

Before Starting:

✓ Create the following **Index PCR** program for a total reaction volume of 70 µL.

Step	Temperature	Time	
1	95°C	10 min	
2	95°C	30 sec	10-22 cycles[1]
3	62°C	30 sec	
4	72°C	30 sec	
5	72°C	7 min	
6	4°C	Hold	

1. Thaw the following components with **green** caps on ice: **Zymo*Taq*™ Premix** and **miRNA UDI Primers** (individual tubes or plate). Once thawed, flick to mix and briefly centrifuge prior to use.
2. Add 6 µL of a unique **miRNA UDI Primer** to each sample[2].
3. Add 35 µL of **Zymo*Taq*™ Premix** to each sample. Pipet to mix and briefly centrifuge.
4. Place the sample(s) in the thermal cycler and run the **Index PCR Program**.
5. Remove the sample(s) from the thermal cycler and place on ice. Proceed to **Section 5.**

Safe stopping point: Store samples at ≤ -20°C for up to one week.

[1] Refer to **Appendix B (page 14)** for the recommended PCR cycles based on sample input.

[2] Refer to **Appendix C (page 15)** for information about the included barcodes.

Section 5: Library Purification

Before Starting:

- ✓ Add 300 µL of ***Select-a-Size*** **Magbead Concentrate** to 10 mL ***Select-a-Size*** **Magbead Buffer** for first time usage.
- ✓ Allow ***Select-a-Size*** **MagBeads** to equilibrate to room temperature for at least 30 minutes prior to use.
- ✓ Add 24 mL of 100% ethanol (26 mL of 95% ethanol) to the 6 mL **DNA Wash Buffer** concentrate (Cat. No. D4003-2-6), or 96 mL of 100% ethanol (104 mL of 95% ethanol) to the 24 mL **DNA Wash Buffer** concentrate (Cat. No. D4003-2-24).

1. Just before use, vortex ***Select-a-Size*** **MagBeads** until homogenous.
2. Add 125 µL of ***Select-a-Size*** **MagBeads** to each sample. Mix thoroughly by pipetting until homogenous.
3. Incubate for 5 minutes at room temperature.
4. Place the sample(s) on a magnetic stand until the beads have fully separated from the solution, leaving a clear solution.
5. Without disturbing the beads, remove and discard the cleared supernatant. Leave the sample(s) on the magnetic stand.
6. Add 200 µL of **DNA Wash Buffer** to each sample. Without disturbing the beads, remove and discard the supernatant. Repeat this step for a total of 2 washes.
7. Keep the sample(s) on the magnetic stand with the tube caps open to air-dry the beads. After 1 minute, remove any residual **DNA Wash Buffer** that has collected at the bottom of the tube(s). Continue to air dry until the bead pellet is no longer glossy (~5-10 minutes)[1].
8. Remove the sample(s) from the magnetic stand and resuspend the beads with 12.5 µL of **DNase/RNase-Free Water.** Pipet to mix.
9. Incubate for 5 minutes at room temperature.
10. Place the sample(s) on the magnetic stand for 3 minutes, or until the solution is completely clear.
11. Transfer 10 µL of the eluate to a new tube. Avoid bead carryover.

This eluate is your final miRNA-seq library. Store at ≤ -20°C.

[1] The optimal air-dry time may vary depending on humidity and temperature. Optimally dried beads appear matte without cracking. Start with 3 minutes of air-dry time and increase the time as needed. **DNA Wash Buffer** carry over from insufficiently dried beads or over-dried, cracked beads may reduce nucleic acid recovery.

Appendices

Appendix A: Summary of Thermal Cycler Programs

Create the following programs with lid heating ON, and set to ≥ 95°C.

Section	Program	Step	Temperature	Time
Section 1 3 hours	Adapter Prep	1	70°C	2 min
		2	4°C[1]	Hold (≥ 2 min)
	Adapter Ligation	3	25°C	1 hour
		4	65°C	5 min
		5	65°C	Hold
	Blocking Agent	6	65°C	5 min
		7	65°C to 37°C	Ramp down[2] (0.1ºC/s)
		8	37°C	Hold
	Blocking Enzyme	9	37°C	1 hour
		10	65°C	20 min
		11	4°C	Hold
Safe Stopping Point: Store overnight at ≤ -20°C				
Section 2 1.5 hours	Circularization	1	37°C	1 hour
		2	37°C	Hold
	Dimer Anneal	3	37°C	10 min
		4	37°C	Hold
	Dimer Removal	5	37°C	10 min
		6	37°C	Hold
Continue to Section 3				
Section 3 1.5 hours	RT Primer Prep	1	65°C	5 min
		2	4°C[1]	Hold (≥ 2 min)
	Reverse Transcription	3	42°C	1 hour
		4	65°C	20 min
		5	4°C	Hold
Safe Stopping Point: Store overnight at ≤ -20°C				
Section 4 1 hour		1	95°C	10 min
	Index PCR (10-22 cycles) Steps 2-4	2	95°C	30 sec
		3	62°C	30 sec
		4	72°C	30 sec
		5	72°C	7 min
		6	4°C	Hold
Safe Stopping Point: Store at ≤ -20°C				

[1] Alternatively, place samples on ice for the ≥ 2-minute incubation instead of 4ºC on the thermal cycler.

[2] Ramp down from 65°C to 37°C at a rate of 0.1°C per second (approximately ~6 minutes).

Appendix B: PCR Cycle Guidelines

The number of PCR cycles should be selected based on the amount and type of RNA used to generate the library. The table below contains the recommended number of cycles relative to sample input. miRNA content is sample-dependent; equivalent input amounts may contain variable amounts of miRNA. For samples that are expected to contain lower levels of miRNA/small RNA, select the higher PCR cycle from the table.

Input Amount	PCR Cycles
RNA from 200 µL plasma or serum	17-20
RNA from 100 µL plasma or serum	18-21
RNA from 50 µL plasma or serum	19-22
100 ng Total RNA	13-16
10 ng Total RNA	16-19
1 ng Total RNA	19-22
1 µL miRNA Control (+)	16

PCR cycle recommendations for serum/plasma volumes assume the entire 6 µL of RNA eluate is used to prepare Zymo-Seq miRNA libraries when the indicated volume of serum/plasma has been processed using the ***Quick*-cfRNA Serum & Plasma Kit (Cat. No. R1059)**.

Appendix C: Index Primer Sequences

If multiplexing samples together, use an index primer set only once per lane. Index primers in the **miRNA UDI Primer Set (Indexes 1-12)** are dispensed in 1.5 mL tubes and have 8 nt barcodes (R3006). Index primers in the **miRNA UDP Primer Plate (Indexes 1-96)** are dispensed in a single-use 96-well plate and have 10 nt barcodes (R3007). Index primers are provided as pre-mixed forward and reverse primers at 10 µM total concentration (5 µM each).

The complete index sample sheet is available for download in the Documents section of the **Zymo-Seq™ miRNA Library Kit** product page at https://www.zymoresearch.com/products/zymo-seq-mirna-library-kit.

Primer Sequences:

Forward Primer Sequence (i5):
5′-AATGATACGGCGACCACCGAGATCTACAC**NNNNNNNN(NN)**ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′

Reverse Primer Sequence (i7):
5′-CAAGCAGAAGACGGCATACGAGAT**NNNNNNNN(NN)**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-3′

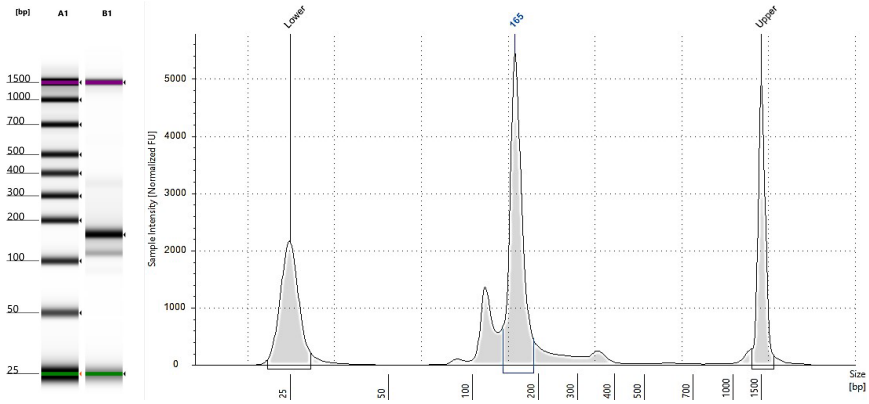
When preparing ≥ 2 libraries, select UDI primers sequentially down a column, not across a row.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Index 01	Index 09	Index 17	Index 25	Index 33	Index 41	Index 49	Index 57	Index 65	Index 73	Index 81	Index 89
B	Index 02	Index 10	Index 18	Index 26	Index 34	Index 42	Index 50	Index 58	Index 66	Index 74	Index 82	Index 90
C	Index 03	Index 11	Index 19	Index 27	Index 35	Index 43	Index 51	Index 59	Index 67	Index 75	Index 83	Index 91
D	Index 04	Index 12	Index 20	Index 28	Index 36	Index 44	Index 52	Index 60	Index 68	Index 76	Index 84	Index 92
E	Index 05	Index 13	Index 21	Index 29	Index 37	Index 45	Index 53	Index 61	Index 69	Index 77	Index 85	Index 93
F	Index 06	Index 14	Index 22	Index 30	Index 38	Index 46	Index 54	Index 62	Index 70	Index 78	Index 86	Index 94
G	Index 07	Index 15	Index 23	Index 31	Index 39	Index 47	Index 55	Index 63	Index 71	Index 79	Index 87	Index 95
H	Index 08	Index 16	Index 24	Index 32	Index 40	Index 48	Index 56	Index 64	Index 72	Index 80	Index 88	Index 96

Appendix D: Library Validation and Quantification

Libraries should be visualized on a platform such as Agilent TapeStation® or Bioanalyzer® to determine if the correct library size is present. The expected library size is ~165 bp. This protocol has been optimized to eliminate adapter dimer products (~148 bp). Yields will vary depending on the total quantity and quality of sample input RNA. Below is an example of a completed Zymo-Seq™ miRNA library visualized on the Agilent TapeStation® 4150.

Libraries may be quantified using Nanodrop, Qubit, TapeStation, etc. However, quantitative PCR is the recommended method to accurately determine library concentration prior to loading on an Illumina sequencer.



Characterization of a completed control library. Agilent TapeStation® 4150 D1000 electropherogram of a typical **Zymo-Seq™ miRNA Library** using 1 µL of the included positive control (10 fmol of a random 21-mer) using 16 PCR cycles. 10 fmol of the control is approximately 67 pg/µL. **A1** is a molecular weight marker, and **B1** is the completed miRNA library.

Appendix E: Considerations for Sequencing and Data Analysis

Preparation for Clustering

The small library size (~165 bp) reflects the successful capture of miRNAs (~21 nt) and may be prone to over-clustering on the sequencer as smaller fragments bind to the flow cell with high efficiency. Thus, accurate quantification of the final library pool concentration is critical to achieve optimal clustering and sequencing results. For this, we recommend using quantitative PCR (e.g., KAPA® Library Quantification Kit).

Sequencing Parameters

Libraries generated with this workflow should be sequenced at a maximum of 75 bp read length. Longer sequencing will require greater amounts of adapter trimming and may exceed the insert.

- For most miRNA studies, single-end sequencing is appropriate.
- For isomiR detection, paired-end sequencing may be more suitable.

Adapter Trimming

Zymo-Seq™ miRNA libraries are compatible with bioinformatics tools designed for Illumina's TruSeq Small RNA libraries. Prior to sequence alignment, sequenced reads should undergo adapter trimming. Any commonly used trimming program may be used. For example, the following cutadapt (https://cutadapt.readthedocs.io/en/stable/) command will trim adapter sequences, and filter reads with inserts shorter than 15nt (Martin et al. 2011).

```
cutadapt -u 1 -a TGGAATTCTCGGGTGCCAAGG -m 15
```

Ordering Information

Product Description	Catalog No.	Size
Zymo-Seq™ miRNA Library Kit	R3006	12 preps
Zymo-Seq™ miRNA Library Kit	R3007	96 preps

Individual Kit Components	Catalog No.	Amount
Zymo*Taq*™ PreMix	E2003 E2004	50 reactions 200 reactions
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNA Wash Buffer	D4003-2-6 D4003-2-24 D4003-2-48	6 mL 24 mL 48 mL
DNA Elution Buffer	D3004-4-10 D3004-4-50	10 mL 50 mL
PCR Strip MagStand	3DP-1002	1 unit

Zymo-Seq™ UDI Primers (D3008/D3096) are **NOT** compatible with Zymo-Seq™ miRNA Library Kit (R3006/R3007).

At this time, only the miRNA UDI Primer Sets included in this kit may be used. For additional questions, please contact tech@zymoresearch.com.

Complete Your Workflow

Sample Collection

Prevent RNA Degradation

DNA/RNA Shield [Cat. No. R1100-50]

NGS-Grade RNA Extraction

RNA Isolation from Any Sample Type

Quick-RNA Microprep [Cat. No. R1050]
Quick-cfRNA Serum & Plasma [Cat. No. R1059]
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RNA-Seq Library Prep Kit

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Notes

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