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Small RNA Library Prep Kit for Illumina

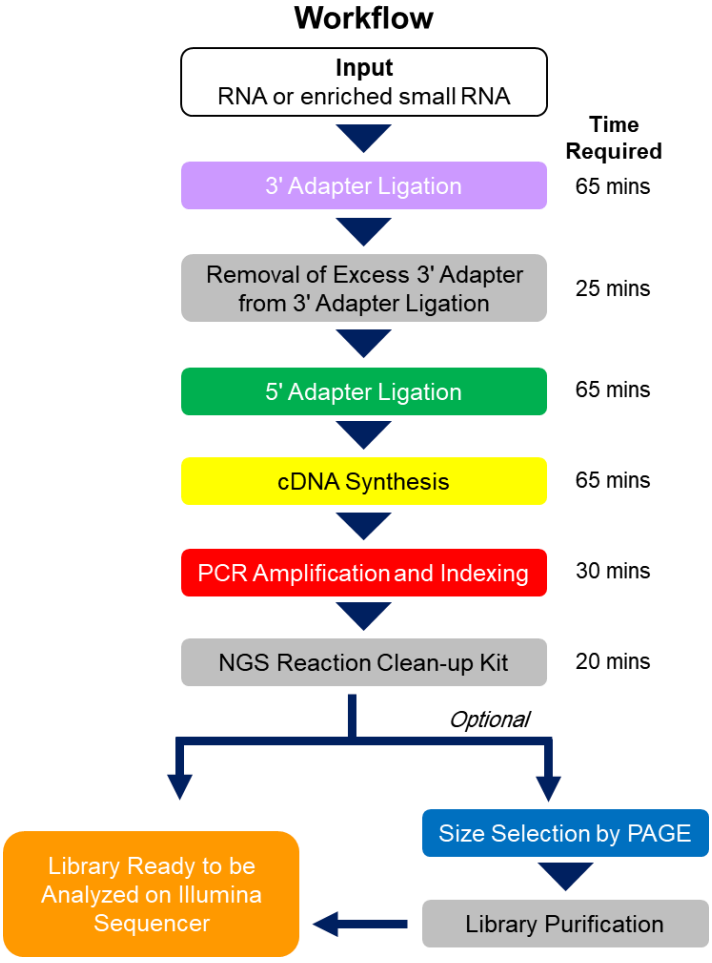
Product Insert

Product #63600, 63620

The Small RNA Library Prep Kit for Illumina consists of all the reagents and components required to generate small RNA libraries to be used for next-generation sequencing on an Illumina platform. All molecular reagents including adaptors, primers, enzyme mixes and buffers are provided. A purification module is also provided for rapid purification of nucleic acid products generated at various steps of the workflow. The purification module utilizes Norgen's patented resin technology, which enhances recovery of desired library intermediates or final products. The library prep workflow could be used for different forms of input including purified total RNA or enriched small RNA, as well as RNA from low content inputs such as plasma, serum and urine.

The Small RNA Library Prep Kit for Illumina has a streamlined procedure that reduces the handling time such that the entire library prep procedure could be completed in less than 5 hours (see diagram below). Input RNA (Total RNA or enriched Small RNA) is first subjected to 3' Adaptor ligation. Excess amount of 3' Adaptor is then removed. This is followed by 5' Adaptor ligation. The input RNA, flanked by 5' and 3' Adaptors is then converted into cDNA. DNA-based barcodes (indices) are then added using a limited-cycle PCR. The library product is then subjected to a clean-up and concentration step. In most cases, particularly when the input RNA is enriched in microRNA, the prepared library could be used directly for analysis on an Illumina sequencer. Alternatively, a procedure for PAGE-based size selection and subsequent purification is provided.

Workflow



Kit Components

Norgen's Small RNA Library Prep Kit for Illumina is shipped as two sub-component kits and each kit should be stored at the appropriate temperature upon arrival as indicated below:

	Small RNA Library Prep Kit for Illumina (Indexes 1-24) Product # 63600 (24 preps)	Small RNA Library Prep Kit for Illumina (Indexes 25-48) Product # 63620 (24 preps)	Storage
Small RNA Library Prep Reagent Kit	Product # 64600		-20°C
Small RNA Library Prep Forward Index Primers	Product # 64640	Product # 64610	-20°C
NGS Reaction Clean-Up Kit	Product # 63500		15°C to 25°C

Quality Control
In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Small RNA Library Prep Kit for Illumina is tested against predetermined specifications to ensure consistent product quality.

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Storage Conditions and Product Stability
All components of the Small RNA Library Prep Reagent Kit and the Small RNA Library Prep Forward Index Primers should be stored at -20°C upon arrival. The NGS Reaction Clean-Up Kit should be stored at room temperature (15-25°C). These reagents should remain stable for at least 1 year when stored at the specified conditions.

Small RNA Library Prep Reagent Kit

Product # 64650, 64600

The Small RNA Library Prep Reagent Kit includes all reagents required for each step of the small RNA library preparation workflow.

Step	Component	Product # 64650 (6 preps)	Product # 64600 (24 preps)	Tube cap color
3' Adaptor Ligation to Template RNA	3' Adaptor	8 µL	30 µL	
	3’ Adaptor Ligation Master Mix	80 µL	320 µL	
	T4 RNA Ligase 2 (Truncated)	8 µL	35 µL	
5' Adaptor Ligation	5' Adaptor	8 µL	30 µL	
	5’ Adaptor Ligation Master Mix	75 µL	300 µL	
	T4 RNA Ligase 1	8 µL	35 µL	
cDNA Synthesis from Ligated RNA Product	Reverse Primer	8 µL	30 µL	
	cDNA Synthesis Master Mix	56 µL	220 µL	
	TruScript Reverse Transcriptase	8 µL	35 µL	
PCR Amplification	2x NGS PCR Master Mix	330 µL	1.32 mL	
	PCR Reverse Primer	21 µL	81 µL	
	Forward Index Primer	10 µL (6 Indices Total)	Included in Small RNA Library Prep Forward Index Primers (# 64640 or # 64610)	Clear cap
Size Selection	NGS MW Ladder	50 µL	50 µL	
	NGS Control Ladder	50 µL	50 µL	
	Loading Dye	300 µL	300 µL	
	Nuclease-Free Water	1.25 mL	1.25 mL	White cap

Small RNA Library Prep Forward Index Primers

Product # 64640, 64610

The Small RNA Library Prep Forward Index Primers are included only with product # 63600 and 63620. They include 24 forward index primers required for the PCR Amplification step of the small RNA library preparation workflow.

Component	Product # 64640 (24 preps)	Product # 64610 (24 preps)
Small RNA Library Prep Forward Index Primers	10 µL of each forward index primer (Indexes 1-24), 24 Indices Total	10 µL of each forward index primer (Indexes 25-48), 24 Indices Total

NGS Reaction Clean-Up Kit

Product # 63400, 63500

The NGS Reaction Cleanup Kit includes all the reagents and columns for cleanup of three reaction steps of the workflow:
(1) Removal of excess 3' adaptor from 3' adaptor ligation
(2) Cleanup of the final library PCR product
(3) Optional cleanup of the gel-purified final library PCR product.

The cleanup procedure utilizes Norgen's proprietary resin technology.

Component	Product # 63400 (18 preps)	Product # 63500 (75 preps)
Buffer RL	5 mL	40 mL
Wash Solution A	3 x 2.4 mL (Reconstituted each bottle by adding 5.6 mL of 96-100% ethanol for a final volume of 8 mL)	38 mL (Reconstituted by adding 90 mL of 96-100% ethanol for a final volume of 128 mL)
Elution Solution A	1 mL	6 mL
Columns	18	75
Gel Filtration Columns	6	24
Collection Tubes	18	75
Elution Tubes	18	75
Product Insert	1	1

Customer-Supplied Reagents and Equipment

- Nuclease-Free PCR Tubes
- Nuclease-Free Microcentrifuge Tubes
- 96 – 100 % Ethanol
- Thermocycler
- Ice or Cold Block
- Microcentrifuge
- ***For Size Selection***
  - 6% Novex® TBE PAGE gel 1.0 mM 10-well (Life Technologies, Inc. #EC6265BOX)
  - SYBR® Gold Nucleic Acid Gel Stain (Life Technologies, Inc. #S-11494)
  - (Optional) Gel Breaker Tubes (IST Engineering #3388-100)

Small RNA Library Prep Procedure

Input Consideration

1. Ensure that RNA is isolated using a product that can recover small RNA (including microRNA). It should be noted that many products using silica-based purification technology may not recover any small RNA. Please consult manufacturer's specification.

2. It is highly recommended that RNA isolation is performed using Norgen Biotek Corp's RNA purification products. Based on patented silicon carbide technology, all of Norgen's purification products will recover small RNA including microRNAs.

3. Quantification of RNA could be performed by standard procedures including spectrophotometry (such as NanoDrop™), capillary electrophoresis (such as Agilent bioanalyzer) or fluorescent-based detection (such as Qubit®).

4. For RNA concentration that is below the detection limit of the aforementioned methods (usually 5 ng/µL and below), it is highly recommended that the RNA be quantified using Norgen's **Low Abundance RNA Quantification Kit** (Cat# 58900).

Procedure for 3' Adaptor Ligation to Template RNA

3′ Adaptor Ligation

1. Adjust input RNA with Nuclease-Free Water to a final volume of 6 µL.
   - For Total RNA inputs, use a total of 100 ng to 1 µg.
   - For enriched Small RNA inputs or RNA isolated from low RNA abundance samples such as liquid biopsies (plasma, serum, urine etc), use a total of 50 pg to 500 ng.

2. Mix input RNA and **3' Adaptor** in a nuclease-free PCR tube as follows:

Component	Volume
3' Adaptor	1 µL
Input/Template RNA	6 µL
Total Volume	**7 µL**

   NOTE: For inputs of 100 ng or lower, it is recommended to use a diluted 3' Adaptor. Prepare a 3' Adaptor stock by diluting the 3' Adaptor in Nuclease-Free Water in a 1:1 fashion (1 µL of 3' Adaptor added to 1 µL of Nuclease-Free Water)

3. Incubate the mixture at 70°C in a pre-heated thermocycler for 2 minutes. Place the tube on ice.

4. Add the following components:

Component	Volume
3’ Adaptor Ligation Master Mix	12 µL
T4 RNA Ligase 2 (Truncated)	1 µL
Total Volume	**20 µL**

   NOTE: For more than one preparation, the above components could be prepared as a mixture and added to the mixture from Step 3.

5. Incubate the reaction at 28°C for 1 hour in a thermocycler.

6. Proceed to “Removal of Excess 3' Adaptor from 3' Adapter Ligation.

   Optional: If the reaction is not to be further processed immediately or is to be used in other workflows, heat inactivate the reaction at 70°C for 10 minutes.

Procedure for Removal of Excess 3' Adaptor from 3' Adaptor Ligation

Removal of Excess 3' Adaptor from 3' Adaptor Ligation

- For the NGS Reaction Clean-Up Kit (Product # 63400) of the Small RNA Library Prep Kit for Illumina (6 preps) (Product # 63610): Ensure that 5.6 mL of 96-100% Ethanol is added to each of the bottles of Wash Solution A.

NGS Reaction Clean-Up Kit

- For the NGS Reaction Clean-Up Kit (Product # 63500) of the Small RNA Library Prep Kit for Illumina (24 preps) (Products # 63600 & 63620): Ensure that 90 mL of 96-100% Ethanol is added to the bottle of Wash Solution A.

1. Transfer the 3' Adaptor Ligation reaction (20 µL) from the section "3' Adaptor Ligation to Template RNA" to a 1.5 mL microcentrifuge tube.

2. Add 300 µL of **Buffer RL**. Mix by Vortexing.

3. Add 100 µL of 96-100 % Ethanol. Mix by Vortexing.

4. Assemble a spin column with the provided collection tube. Transfer the mixture in Step 3 to the top reservoir of the spin column.

5. Centrifuge at **≥3,500 x *g* (6,000 RPM)** for 1 minute. Discard flowthrough

6. Apply 600 µL of **Wash Solution A** (with Ethanol added) to the column and centrifuge at **~14,000 x *g* (14,000 RPM)** for 1 minute. Discard flowthrough.

7. Perform a dry spin. Centrifuge at **~14,000 x *g* (14,000 RPM)** for 2 minutes.

8. Assemble the spin column with an Elution Tube. Apply 12 µL of **Elution Solution A**. Centrifuge at **~200 x *g* (2,000 RPM)** for 1 minute, followed by **~14,000 x *g* (14,000 RPM)** for 2 minutes.

9. Transfer the eluate (~10 - 12 µL of final volume) to a nuclease-free PCR tube. Proceed immediately to "5' Adaptor Ligation".

Procedure for 5' Adaptor Ligation

5′ Adaptor Ligation

1. Aliquot (n+1) x 1 µL of **5' Adaptor** into a nuclease-free PCR tube, where n = number of small RNA library preparation. For input of 100 ng or lower, make the same dilution as 3' Adaptor by diluting the **5' Adaptor** in Nuclease-Free Water in a 1:1 fashion (1 µL of **5' Adaptor** added to 1 µL of Nuclease-Free Water)

2. Incubate the **5' Adaptor** (Original or diluted) at 70°C in a pre-heated thermocycler for 2 minutes. Place the tube on ice.

3. Add the following components to the entire eluate (~10 - 12 µL of final volume) obtained from the previous section “Removal of Excess 3' Adaptor from 3' Adaptor Ligation”:

Component	Volume
5’ Ligation Adaptor Master Mix	11 µL
Denatured 5' Adaptor	1 µL
T4 RNA Ligase 1	1 µL
Total Volume	**~23 µL**

NOTE: For more than one preparation, the above components could be prepared as a mixture.

4. Incubate the reaction at 28°C for 1 hour in a thermocycler.

5. Proceed to “cDNA Synthesis from Ligated RNA Product”.

Optional: If the reaction is not to be further processed immediately or is to be used in other workflows, heat inactivate the reaction at 70°C for 10 minutes.

Procedure for cDNA Synthesis from Ligated RNA Product

cDNA Synthesis

1. Add 1 µL of **Reverse Primer** to the reaction from the ligated product (~23 µL) at the end of the section “5’ Adaptor Ligation”. For input of 100 ng or lower, make the same dilution as **3' Adaptor** by diluting the **Reverse Primer** in Nuclease-Free Water in a 1:1 fashion (1 µL of **Reverse Primer** added to 1 µL of Nuclease-Free Water)

2. Incubate the mixture at 70°C in a pre-heated thermocycler for 2 minutes. Place the tube on ice.

3. Add the following components for reverse transcription:

Component	Volume
cDNA Synthesis Master Mix	8 µL
TruScript Reverse Transcriptase	1 µL
Total Volume	**~ 33 µL**

   NOTE: For more than one preparation, the above components could be prepared as a mixture.

4. Incubate the reaction at 50°C for 1 hour in a thermocycler.

5. Proceed to “PCR Amplification and Indexing”.

   Optional: If the reaction is not to be used immediately or is to be used in other workflows, heat inactivate the reaction at 70°C for 15 minutes. The generated cDNA could be stored at -20°C

Procedure for PCR Amplification and Indexing

PCR Amplification and Indexing

1. Add the following components to the cDNA synthesis reaction from the section “cDNA Synthesis from Ligated RNA Product”. The volume of the cDNA Synthesis is 33 µL.

Component	Volume
Nuclease-Free Water	11 µL
2x NGS PCR Master Mix	50 µL
PCR Reverse Primer	3 µL
Index (X) Primer	3 µL
Final Volume	**100 µL**

NOTE: For more than one preparation, the above components (**minus the Index Primer**) could be prepared as a mixture and added to the mixture from the section “cDNA Synthesis from Ligated RNA Product”.
NOTE: For sequence of each index, please refer to the end of the manual.

2. Perform PCR Cycling in a thermocycler as follows:

Component	Temperature	Time	No. of Cycles
Initial Denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	12-15
Annealing	60°C	30 sec	
Extension	72°C	15 sec	
Final Extension	72°C	10 min	1
Hold	4°C	Infinite	-

NOTE: For RNA inputs lower than 100 ng, the No. of PCR Cycles could be increased up to 20.

3. Proceed to “Clean-up of Final Indexed PCR Product” using the NGS Reaction Cleanup Kit.

Optional: If the reaction is not to be used immediately, store at -20°C

Procedure for Clean-up of Final Indexed PCR Product

NGS Reaction Clean-Up Kit

- For the NGS Reaction Clean-Up Kit (Product # 63400) of the Small RNA Library Prep Kit for Illumina (6 preps) (Product # 63610): Ensure that 5.6 mL of 96-100% Ethanol is added to each of the bottles of Wash Solution A.

- For the NGS Reaction Clean-Up Kit (Product # 63500) of the Small RNA Library Prep Kit for Illumina (24 preps) (Products # 63600 & 63620): Ensure that 90 mL of 96-100% Ethanol is added to the bottle of Wash Solution A.

1. Transfer the PCR Product (100 µL) from the section “PCR Amplification and Indexing” to a 1.5 mL microcentrifuge tube.

2. Add 300 µL of **Buffer RL**. Mix by vortexing.

3. Add 50 µL of 96-100% Ethanol. Mix by vortexing.

4. Assemble a spin column with the provided collection tube. Transfer the mixture in Step 3 to the top reservoir of the spin column.

5. Centrifuge at **≥3,500 x *g* (6,000 RPM)** for 1 minute. Discard flowthrough

6. Apply 600 µL of **Wash Solution A** (with Ethanol added) to the column and centrifuge at **~14,000 x *g* (14,000 RPM)** for 1 minute. Discard flowthrough.

7. Perform a dry spin. Centrifuge at **~14,000 x *g* (14,000 RPM)** for 2 minutes.

8. Assemble the spin column with an Elution Tube. Apply 20 µL of **Elution Solution A**. Centrifuge at **~200 x *g* (2,000 RPM)** for 1 minute, followed by **~14,000 x *g* (14,000 RPM)** for 2 minutes.

9. Assess the quality and quantity of the cleaned library on an Agilent High Sensitivity DNA Chip. For optimal quality, a single peak of ~ 140 bp should be obtained. In such cases, the library could be used directly for analysis on an Illumina sequencing platform (Figure 1). If multiple peaks were observed or if a significant amount of adaptor-adaptor-ligation products (~120 bp) is present, it is recommended to carry out the optional size selection procedure below.

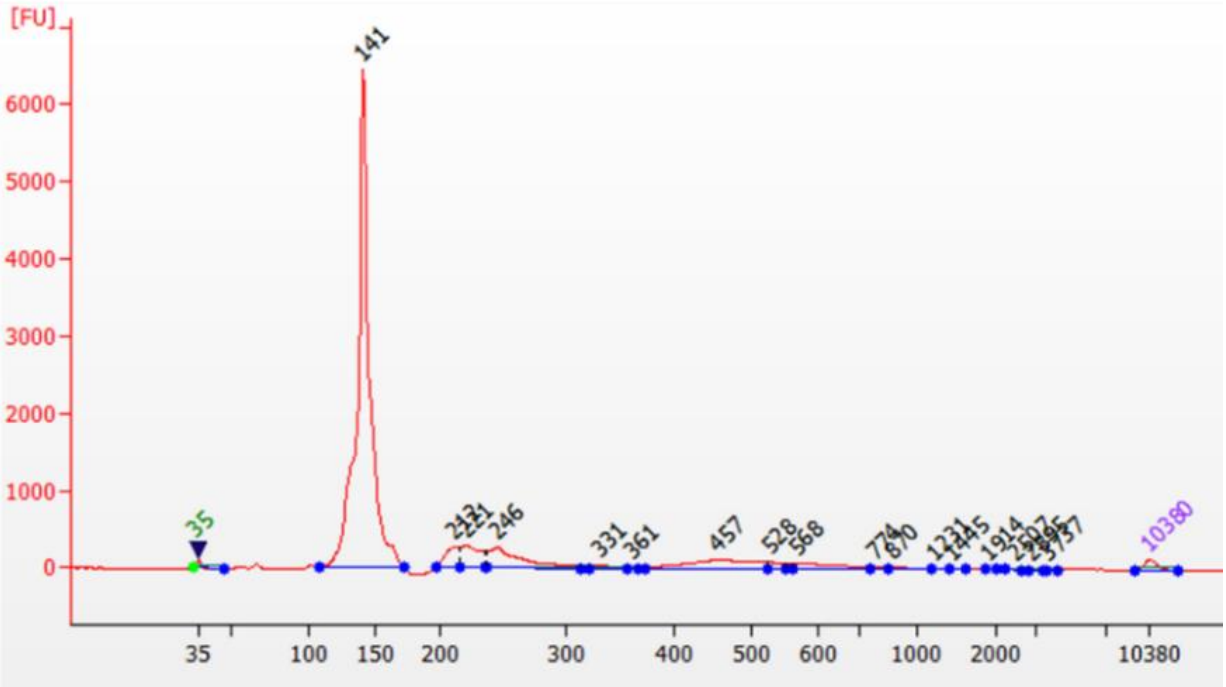


Figure 1. An example of a purified small RNA library on an Agilent 2100 Bioanalyzer using a High Sensitivity DNA Chip. The library was prepared using a mixture of synthetic microRNAs as an input. A single peak of ~ 141 bp was obtained and could be used directly for analysis on an Illumina next-generation sequencing platform.

Optional Procedure for Size Selection and Clean-up of Final Indexed PCR Product using Polyacrylamide Gel and NGS Reaction Cleanup Kit

Size Selection by PAGE

NGS Reaction Clean-Up Kit

Customer-Supplied Reagents and Equipment

- 6% Novex® TBE PAGE gel 1.0 mM 10-well (Life Technologies, Inc. #EC6265BOX)
- SYBR® Gold Nucleic Acid Gel Stain (Life Technologies, Inc. #S-11494)
- Gel Breaker Tubes (IST Engineering #3388-100)
- Microfuge Tubes

1. Set up a 6% Novex® TBE PAGE gel for electrophoresis according to manufacturer's instruction.

2. Mix 10 µL of **Loading Dye** with up to 20 µL of the remaining cleaned library obtained at the end of the section "Clean-up of Final Indexed PCR Product"

3. Load each of the library mixtures prepared in Step 2 in one well of the 6% PAGE 10-well gel. Up to 8 libraries could be loaded on each gel.

4. Load 10 µL of the **NGS MW Ladder** in one well of the 6% PAGE 10-well gel.

5. Load 10 µL of the **NGS Control Ladder** in one well of the 6% PAGE 10-well gel.

6. The **Loading Dye** consists of two dyes - a high MW dye with light blue color and a low MW dye with dark blue color. Run the gel for 1 hour at 120 V or until the dark blue dye reaches the bottom of the gel. Do not let the dark blue dye exit the gel.

7. Remove the gel from the apparatus. Place the gel in a clean container with 1x TBE spiked with either SYBR Gold or Ethidium Bromide for ~ 20 minutes. View the gel on a UV transilluminator.

8. The adaptor-ligated constructs containing the microRNAs (~21 nucleotides) are represented by a band ~ 140 bp (see Figure 2). The adaptor-ligated constructs containing the piRNA (~30 nucleotides) are represented by a band ~ 150 bp. Excise the desired band(s) accordingly. Avoid recovering the adaptor-adaptor-ligated constructs with no insert which is ~ 120 bp.

9. Homogenize the gel piece of each sample by either (i) crushing into small pieces inside a microfuge tube with a small pestle or (ii) placing the gel piece in a Gel Breaker Tube (IST Engineering) that is placed in a microfuge tube and centrifuge at **~14,000 x *g* (14,000 RPM)** for 2 minutes.

10. Add 200 µL of Nuclease-Free Water to the homogenized gel piece. Mix by vortexing briefly.

11. Rotate end-to-end for at least 2 hours to overnight at room temperature.

12. Briefly centrifuge the microfuge tubes to collect all liquid at the bottom of the tube. Transfer the entire content (eluate and the gel debris) to the top of a **Gel Filtration Column** (assembled with a microfuge tube, not provided)

13. Centrifuge the unit at **~14,000 x *g* (14,000 RPM)** for 2 minutes. Discard the **Gel Filtration Column** and retain the eluate in the microfuge tube.

14. Add 200 µL of **Buffer RL**. Mix by Vortexing.

15. Add 200 µL of 96-100 % Ethanol. Mix by Vortexing.

16. Assemble a spin column with the provided collection tube. Transfer the mixture in Step 15 to the top reservoir of the spin column.

17. Centrifuge at **≥3,500 x *g* (6,000 RPM)** for 1 minute. Discard flowthrough

18. Apply 600 µL of **Wash Solution A** (with Ethanol added) to the column and centrifuge at **~14,000 x *g* (14,000 RPM)** for 1 minute. Discard flowthrough.

19. Repeat Step 18 once.

20. Perform a dry spin. Centrifuge at **~14,000 x *g* (14,000 RPM)** for 2 minutes.

21. Assemble the spin column with an Elution Tube. Apply 10 – 20 µL of **Elution Solution A**. Centrifuge at **~200 x *g* (2,000 RPM)** for 1 minute, followed by **~14,000 x *g* (14,000 RPM)** for 2 minutes.

22. Assess the quality and quantity of the cleaned library on an Agilent High Sensitivity DNA Chip.

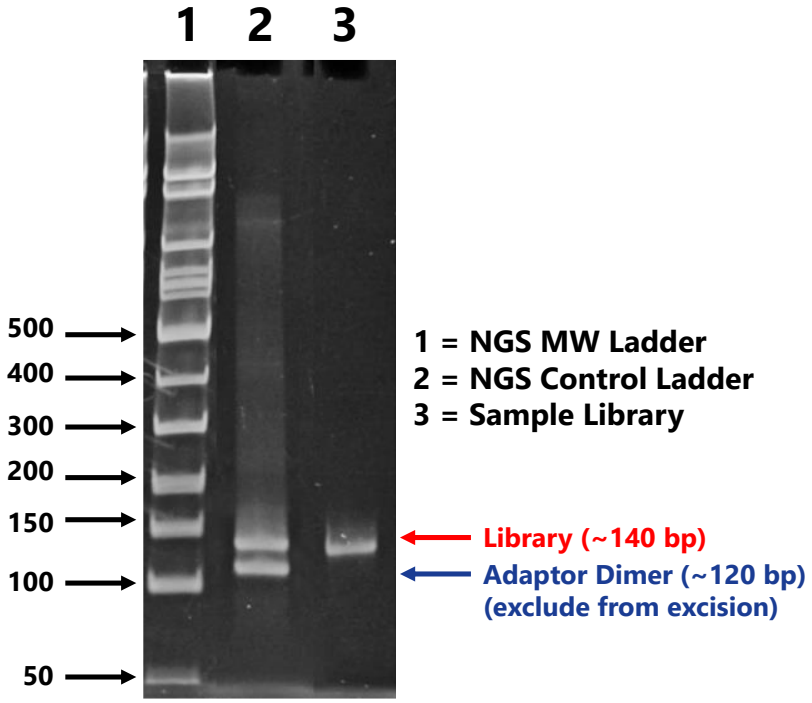


Figure 2. An example of a small RNA library on resolved on a 6% Novex® TBE PAGE gel. The library containing microRNAs resolved at around 140 bp and migrated just below the 150 bp marker of the provided **NGS MW Ladder**. The **NGS Control Ladder** contains two bands for alignment during band excision. The top band co-migrate with the expected size of library construct containing mainly microRNAs (~140 bp). The bottom band co-migrate with the expected size of an adaptor-adaptor-ligation product (~ 120 bp) that should be excluded.

Sequence Information for Forward Index Primers

Indexing Primers

- All Forward Index Primers utilize the same codes as Illumina TruSeq® Small RNA indices. The following primers are provided with the kit, with index sequences highlighted in red.
- For 24 Prep Kit # 63600, RPI1 to RPI24 are provided (Product# 64640)
- For 24 Prep Kit # 63620, RPI25 to RPI48 are provided (Product #64610)

Index Primer Name	Sequence
Forward Index Primer (Illumina RPI1)	CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI2)	CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI3)	CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI4)	CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI5)	CAAGCAGAAGACGGCATACGAGAT**CACTGT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI6)	CAAGCAGAAGACGGCATACGAGAT**ATTGGC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI7)	CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI8)	CAAGCAGAAGACGGCATACGAGAT**TCAAGT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI9)	CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI10)	CAAGCAGAAGACGGCATACGAGAT**AAGCTA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI11)	CAAGCAGAAGACGGCATACGAGAT**GTAGCC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI12)	CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI13)	CAAGCAGAAGACGGCATACGAGAT**TTGACT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI14)	CAAGCAGAAGACGGCATACGAGAT**GGAACT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI15)	CAAGCAGAAGACGGCATACGAGAT**TGACAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI16)	CAAGCAGAAGACGGCATACGAGAT**GGACGG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI17)	CAAGCAGAAGACGGCATACGAGAT**CTCTAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI18)	CAAGCAGAAGACGGCATACGAGAT**GCGGAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI19)	CAAGCAGAAGACGGCATACGAGAT**TTTCAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI20)	CAAGCAGAAGACGGCATACGAGAT**GGCCAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI21)	CAAGCAGAAGACGGCATACGAGAT**CGAAAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI22)	CAAGCAGAAGACGGCATACGAGAT**CGTACG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI23)	CAAGCAGAAGACGGCATACGAGAT**CCACTC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI24)	CAAGCAGAAGACGGCATACGAGAT**GCTACC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI25)	CAAGCAGAAGACGGCATACGAGAT**ATCAGT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI26)	CAAGCAGAAGACGGCATACGAGAT**GCTCAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI27)	CAAGCAGAAGACGGCATACGAGAT**AGGAAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI28)	CAAGCAGAAGACGGCATACGAGAT**CTTTTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI29)	CAAGCAGAAGACGGCATACGAGAT**TAGTTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA

Forward Index Primer (Illumina RPI30)	CAAGCAGAAGACGGCATACGAGAT**CCGGTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI31)	CAAGCAGAAGACGGCATACGAGAT**ATCGTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI32)	CAAGCAGAAGACGGCATACGAGAT**TGAGTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI33)	CAAGCAGAAGACGGCATACGAGAT**CGCCTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI34)	CAAGCAGAAGACGGCATACGAGAT**GCCATG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI35)	CAAGCAGAAGACGGCATACGAGAT**AAAATG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI36)	CAAGCAGAAGACGGCATACGAGAT**TGTTGG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI37)	CAAGCAGAAGACGGCATACGAGAT**ATTCCG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI38)	CAAGCAGAAGACGGCATACGAGAT**AGCTAG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI39)	CAAGCAGAAGACGGCATACGAGAT**GTATAG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI40)	CAAGCAGAAGACGGCATACGAGAT**TCTGAG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI41)	CAAGCAGAAGACGGCATACGAGAT**GTCGTC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI42)	CAAGCAGAAGACGGCATACGAGAT**CGATTA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI43)	CAAGCAGAAGACGGCATACGAGAT**GCTGTA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI44)	CAAGCAGAAGACGGCATACGAGAT**ATTATA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI45)	CAAGCAGAAGACGGCATACGAGAT**GAATGA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI46)	CAAGCAGAAGACGGCATACGAGAT**TCGGGA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI47)	CAAGCAGAAGACGGCATACGAGAT**CTTCGA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Forward Index Primer (Illumina RPI48)	CAAGCAGAAGACGGCATACGAGAT**TGCCGA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA

Related Products	**Product #**
Total RNA Purification Kit	17200
Plasma/Serum RNA Purification Mini Kit	55000
Low Abundance RNA Quantification Kit	58900
microRNA Clean-up and Concentration Kit	63420, 63520

Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology and NGS-associated technologies are patented and/or patent pending. See www.norgenbiotek.com/patents

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