

The background of the page is decorated with several translucent blue spheres of various sizes, some of which are connected by thin, light blue lines, creating a molecular or network-like structure. The overall aesthetic is clean and scientific.

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Poly(A) RNA Selection Kit User Guide

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158-163 (Version 1: CORALL mRNA-Seq Library Prep Kits with UDIs)

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LITERATURE CITATION

For any publication using this product, please refer to it as the Lexogen's Poly(A)RNA Selection Kit V1.5.

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1. Overview

Lexogen's Poly(A) RNA Selection Kit V1.5 provides rapid and highly specific isolation of polyadenylated RNA from total RNA samples.

Total RNA is briefly denatured and the polyadenylated 3' ends present in most mRNAs are hybridized to oligo(dT) beads. Any RNA without poly(A) stretches, such as 28S and 18S ribosomal RNAs and tRNAs, will not be captured by the oligo(dT) beads and hence be removed during subsequent washing steps.

A typical downstream application for polyadenylated RNAs is Next Generation Sequencing (NGS). We recommend preparing libraries for RNA-Seq using Lexogen's CORALL Library Prep Kits (see front and back covers for product numbers). It is necessary to elute the poly(A) RNA from the beads before continuing with the CORALL RNA-Seq Library Prep protocols.

This Poly(A) Selection protocol is validated for use with input amounts ranging from 1 ng - 5 µg of total RNA: The input amount depends on the intended application and also determines the amount of oligo(dT) beads required. In general, use 2 µl oligo(dT) Magnetic Beads (**MB** ●) for up to 5 µg of total RNA input. With this kit a total of 480 µg total RNA (96 x 5 µg) can be purified.

When preparing RNA samples for downstream use with Lexogen's CORALL RNA-Seq Library Prep Kits, we recommend an input of 200 ng total RNA. This will enable full quantification capability of poly(A) selected RNA.

ATTENTION: Input amounts between 1 ng - 1 µg can be used for poly(A) selection prior to CORALL library preparation. Adjustments to elution volume are advised (see Appendix B, p.14) and we recommended to proceed directly to library preparation.

POLY(A) RNA SELECTION

70 min

25 min

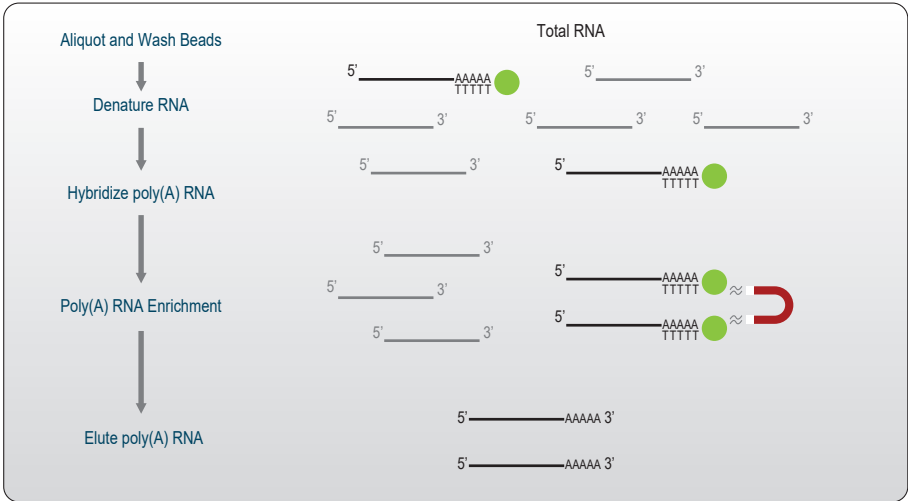


Figure 1. Schematic overview of the poly(A) RNA selection.

2. Kit Components and Storage Conditions

Poly(A) RNA Selection Kit V1.5, (+4 °C)

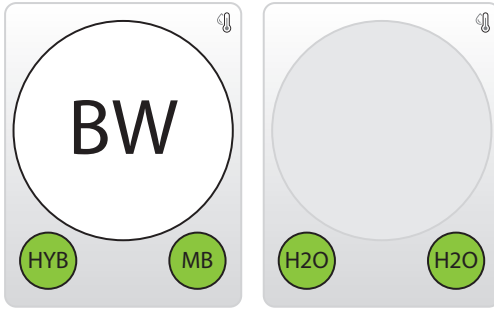


Figure 2. Location of kit components.

Kit Component	Tube Label	Volume*	Storage
Magnetic Beads	MB ●	212 µl	🧊 +4 °C
RNA Hybridization Buffer	HYB ●	1,056 µl	🧊 +4 °C
Molecular Biology Grade Water	H ₂ O ●	2 x 1,320 µl	🧊 +4 °C
Bead Wash Buffer	BW ○	36,960 µl	🧊 +4 °C

*including ≥10 % surplus.

The kit is shipped at room temperature, upon receipt please store it at +4 °C. Before use, check the contents of **BW** ○ which may precipitate during shipping. If a precipitate is visible or the content appears turbid, incubate at 37 °C until buffer components dissolve completely.

3. User-supplied Consumables and Equipment

Check to ensure that you have all of the necessary materials and equipment before beginning library preparation. All reagents, equipment, and labware must be free of nucleases and nucleic acid contamination.

ATTENTION: Before starting this protocol, please read the [General Guidelines for Lexogen Kits](#), which are available online. These provide a detailed overview of RNA and kit component handling, as well as general RNA input requirements.

Equipment

- Magnetic rack / plate e.g., for 1.5 ml tubes: BcMag Magnetic separator-24, article# MS-03 from Bioclone; for 96-well plates: 96S Super Magnet Plate, article# A001322 from Alpaqua, Dynamag™-96 Side/Side Skirted. Magnets, article # 12331D/12027 from Thermo Fisher Scientific.
- Benchtop centrifuge (rotor compatible with 1.5 ml tubes or 96-well plates).
- Calibrated single-channel pipettes for handling 1 µl to 1,000 µl volumes.
- Thermomixer for 1.5 ml tubes or 96-well plates (dry bath incubator with shaking function).
- UV-spectrophotometer to quantify RNA.

Labware

- Suitable low binding pipette tips (pipette tips with aerosol barriers recommended).
- 1.5 ml tubes or PCR plate / tubes, low binding, certified ribonuclease-free.
- Vortex mixer.

Optional Equipment

- Automated microfluidic electrophoresis station (Agilent Technologies 2100 Bioanalyzer).
- Benchtop fluorometer and appropriate assays (for RNA quality control).
- Agarose gels, dyes, and electrophoresis rig (for RNA quality control).

The complete set of materials, reagents, and labware necessary for RNA selection and quality control is not listed. Consult Appendix A, p.12 and Appendix B, p.14 for more information on RNA quality and quantity. Consult Appendix C, p.15 for information on RNA quantification methods.

4. Detailed Protocol

Preparation

Aliquot and Wash Beads	Denature RNA	Hybridize Poly(A) RNA	Elute Poly(A) RNA
MB ● – stored at +4 °C HYB ● – stored at +4 °C BW ○ – stored at +4 °C	Total RNA – thawed on ice H₂O ● – stored at +4 °C	BW ○ – stored at +4 °C	H₂O ● – stored at +4 °C
Magnetic rack / plate	Thermocycler 60 °C, 1 min 25 °C, ∞	Thermomixer 1,250 rpm, set to 25 °C	Magnetic rack / plate Thermomixer / Thermo- cycler 70 °C, 1 min

Aliquot and Wash Beads

Magnetic Beads (**MB** ●) must be washed before use. All steps are performed at room temperature.

ATTENTION: Volume modifications apply for step 12 when using ≤500 ng total RNA and when poly(A) selected RNA is used for downstream CORALL library prep (see Appendix B, p.14).

- 1 Mix the Magnetic Beads (**MB** ●) well. Transfer 2 µl of the resuspended beads per poly(A) selection for up to 5 µg total RNA into a new 1.5 ml tube. **MB** ● can be washed as a batch if multiple poly(A) selections are required.
- 2 Place the tube on a magnet and let the beads collect for 5 minutes or until the supernatant is completely clear (depends on the magnet strength). Remove and discard the supernatant with a pipette while the tube remains in contact with the magnet.
- 3 Remove the tube from the magnetic rack and add 75 µl Bead Wash Buffer (**BW** ○) per poly(A) selection. Resuspend the beads and transfer the tube to the magnetic rack. Let the beads collect for 5 minutes; remove and discard the supernatant.
- 4 Repeat this washing step once (for a total of two washes).
- 5 Resuspend the beads in 10 µl RNA Hybridization Buffer (**HYB** ●) per sample. Pipette and mix carefully to avoid introducing air bubbles.

Denature RNA

RNA samples are briefly heated to resolve secondary structures and promote efficient hybridization.

- 6 Dilute up to 5 µg of total RNA to a volume of 10 µl with RNase-free water. **REMARK:** For CORALL library prep the recommended total RNA input is 1 ng -1 µg (see Appendix B, p.14).

- 7 Denature RNA samples using a thermocycler at 60 °C for 1 minute and then hold at 25 °C. **ATTENTION:** Proceed directly to step 8. Do not cool samples excessively or place denatured RNA on ice.

Hybridize Poly(A) RNA

The denatured total RNA is incubated with the washed beads, which specifically bind polyadenylated RNAs. RNAs lacking a poly(A) tail are then washed away, leaving only purified poly(A) RNA hybridized to the beads.

- 8 Add the 10 µl of denatured RNA to 10 µl of washed beads and incubate in a thermomixer at 25 °C for 20 minutes with 1,250 rpm agitation. **ATTENTION:** Do not cool samples excessively or place denatured RNA on ice. If no thermomixer is available, incubate in a thermocycler (with vortex & spin down) every 10 minutes.

- 9 Transfer the tube onto a magnetic rack and let the beads collect for 5 minutes (or until the supernatant is clear). Remove and discard the supernatant.

- 10 Remove the tube from the magnetic rack and add 100 µl Bead Wash Buffer (**BW** ○). Resuspend the magnetic beads and mix well. Incubate using a thermomixer at 25 °C for 5 minutes with 1,250 rpm agitation. Collect the magnetic beads by placing the tube onto a magnetic stand for 5 minutes or until the supernatant is completely clear. Remove and discard the supernatant.

- 11 Repeat this washing step once (for a total of two washes).


RNA Elution

The poly(A) RNA is eluted from the Magnetic Beads by heating in water.

- 12 Completely remove the supernatant from the last wash (step 11) and resuspend the beads in 25 μl of Molecular Biology Grade Water (H_2O). **REMARK:** For CORALL library prep, elute in 12 μl and transfer 10 μl to avoid bead carryover. The 10 μl can then be directly inserted into CORALL. For other applications where total input amount was ≤ 500 ng, or if a higher mRNA concentration is desired, reduce the elution volume to 12 μl .

- 13 Incubate for 1 minute at 70 $^{\circ}\text{C}$.

- 14 Immediately transfer the tube onto a magnet and let the beads collect for 5 minutes (or until the supernatant is clear).

- 15 Transfer the clear supernatant into a fresh tube. The supernatant now contains the poly(A) selected RNA that can be used for quality control, concentration determination, and further downstream applications. **ATTENTION:** Avoid bead carryover.  Safe stopping point. The poly(A) selected RNA can be stored at -20 $^{\circ}\text{C}$ at this point.

- 16 For long term storage we recommend adding Tris, pH 7.0 to the isolated poly(A) RNA to final concentration of 10 mM (i.e., add 2.5 μl of 100 mM Tris pH 7.0 if the RNA was eluted in 25 μl). Furthermore, addition of RNase inhibitor (e.g., RNasin) can be recommended for long-term storage of poly(A) RNA (see Appendix A, p.12).

5. Short Procedure

ATTENTION: Spin down solutions before opening tubes or plates!

70 min

Poly(A) Selection

Standard Input (>500 ng - 5 µg)	Lower Input (≤500 ng) & CORALL Library Prep (1 ng - 1 µg)
Aliquot and Wash Magnetic Beads	
<input type="checkbox"/> Wash 2 µl MB ● twice with 75 µl BW ○. <input type="checkbox"/> Withdraw and discard supernatant. <input type="checkbox"/> Resuspend washed beads in 10 µl HYB ●.	
Denature RNA	
<input type="checkbox"/> Prepare total RNA in a volume of 10 µl. <input type="checkbox"/> Incubate for 1 min at 60 °C, hold at 25 °C.	
Hybridize Poly(A) RNA	
<input type="checkbox"/> Add denatured RNA (10 µl) to 10 µl of washed beads. <input type="checkbox"/> Incubate for 20 min at 25 °C / 1,250 rpm. <input type="checkbox"/> Wash 2 x for 5 min at 25 °C / 1,250 rpm with 100 µl BW ○. <input type="checkbox"/> Withdraw and discard supernatant.	
Elute Poly(A) RNA	
<input type="checkbox"/> Add 25 µl H₂O ●.	<input type="checkbox"/> For ≤500 ng or CORALL lib prep: Add 12 µl H₂O ●.
<input type="checkbox"/> Incubate for 1 min at 70 °C. <input type="checkbox"/> Immediately place on magnet for 5 min or until supernatant is clear. <input type="checkbox"/> Transfer the clear supernatant into a fresh tube. ➡ Safe stopping point.	

6. Appendix A: General RNA Requirements

RNA Integrity

The integrity of an RNA sample can be assessed with a variety of methods. We recommend the use of a microfluidics assay such as the RNA6000 series for the 2100 Bioanalyzer (Agilent Technologies Inc.). However, RNA quality can also be assessed with denaturing agarose gel electrophoresis if such a device is not available. Most microfluidics platforms will carry out an automated peak analysis and generate a quality score (RIN or RQN), and we recommend a RIN score of 8 or greater for optimal full-length poly(A) RNA selection. Typically such samples have easily detectable rRNA peaks and a comparatively low abundance of short RNAs, which can arise from both intact short transcripts as well as from RNA degradation. Poly(A) RNA can also be isolated from lower quality RNA, but this may lead to 3'-bias in subsequent reactions.

ATTENTION: RNA isolation methods that quantitatively co-isolate all RNAs below 200 nt (tRNAs, 5S rRNA) may result in lower RIN scores, despite intact 18S and 28 S rRNA peaks.

Potential Contaminants

RNA samples should be free of salts, metal ions, and organic solvents which can be carried over from RNA extraction. Several sources of contamination can be detected with an UV-Vis spectrophotometer. An acceptably pure RNA sample should have an A260/A280 ratio between 1.8 and 2.1. The A260/A230 ratio should also be greater than 1.8. Contaminants may have a negative impact on the efficiency of the protocol. For isolation of high quality total RNA we recommend Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008).

Genomic DNA Contamination

Depending on the RNA extraction protocol used, samples may contain significant amounts of genomic DNA (gDNA), which is indistinguishable from RNA on a spectrophotometer. Furthermore, as many of the dyes used in RNA microfluidics assays stain single-stranded nucleic acids much more intensely than double-stranded, low to moderate amounts of gDNA may not be readily visible with an RNA-specific microfluidics assay. We highly recommend examining all RNA samples on a denaturing agarose gel or using a fluorometric assay with DNA- and RNA-specific dyes to check samples for DNA contamination. On an agarose gel, gDNA can appear as either a dark mass which remains in the slot if relatively intact, or as a high molecular weight smear if it has been sheared during extraction.

The best way to avoid gDNA contamination is to use an RNA extraction protocol that DNase I treatment is generally not required when poly(A) selection is performed, as genomic DNA will not be captured by the magnetic beads and is therefore, efficiently removed during the washing steps. However, if desired, gDNA can optionally be removed from irreplaceable samples by acidic phenol extraction or DNase I digestion. If samples must be DNase I-treated, heat inactiva-

tion should be avoided, and the enzyme should be deactivated by other means such as phenol / chloroform extraction or silica column purification.

RNA Storage

If immediate RNA extraction is not possible, tissue samples can be either flash-frozen with liquid nitrogen or submerged in RNAlater (Life Technologies Inc.) and stored at -80 °C. After extraction, RNA can be stored at -20 °C or -80 °C in 10 mM Tris pH 7.0. Avoid frequent freeze-thaw cycles as RNA might be sheared. Addition of an RNase inhibitor, e.g., RNasin from Promega at a final concentration of 0.4 U/μl, is recommended.

RNA Spike-in Control Mixes

RNA spike-in control mixes such as the ERCC RNA Spike-in Controls (Ambion Inc.) and/or Lexogen's set of artificial spike in transcripts called SIRVs (Spike-in RNA Variant Control Mixes, Cat. No. 025, 050, 051, 141), should be spiked into total RNA before poly(A) selection. As both ERCC control RNAs and SIRVs are polyadenylated they will be isolated with the magnetic beads along with the mRNA. Such control mixes serve as a control and anchor set for the comparison of experiments. Also for some applications such as RNA-Seq they enable the hypothesis-neutral calculation of strandedness, isoform quantitation, and the validation of the performance of isoform-specific RNA-Seq workflows.

7. Appendix B: RNA Amount

High quality mRNA isolation relies on high quality input RNA. The poly(A) content of an RNA sample or tissue varies, but is usually between 1 and 3 % of the total RNA amount. This protocol was tested extensively with various mouse tissues and human reference RNA. Total RNA isolated from kidney, liver, and brain for instance has a higher poly(A) content than total RNA isolated from lung or heart. For Universal Human Reference RNA (UHRR) the amount of poly(A) RNA recovered is about 2 % of the total RNA input.

An important factor in determining the percentage of poly(A) RNA recovered is using the same method for concentration measurement before and after poly(A) enrichment. Determining the total RNA input by NanoDrop measurements and the recovered poly(A) RNA using Bioanalyzer may result in the impression of a low mRNA recovery as the two methods differ significantly in the concentration determination, with the NanoDrop usually giving much higher concentration values.

The lowest RNA input recommended to enable recovery of measurable amounts of poly(A) RNA is 200 ng total RNA. Reducing input below 500 ng requires reducing the elution volume at step 12 to 12 μ l. We typically recommend using up to 5 μ g of total RNA input with 2 μ l of Magnetic Beads (MB ●). Inputs larger than 5 μ g total RNA may also be used, by proportionally scaling up the reagent volumes. Please refer to the online Frequently Asked Questions for further guidelines (<https://www.lexogen.com/polya-rna-selection-kit/#polyafaq>).

ATTENTION: Inputs \leq 200 ng of total RNA will result in poly(A) RNA that is not quantifiable by usual quality control methods (e.g., Bioanalyzer, Qubit, NanoDrop etc). However, the recovered RNA can be used directly for downstream NGS library preparation.

Input for CORALL Library Prep

The recommended total RNA input range for poly(A) selection prior to CORALL library preps is 1 ng - 1 μ g.

ATTENTION: If using total RNA amounts $>$ 1 μ g for downstream use with CORALL RNA-Seq Library Prep Kits please ensure that the equivalent poly(A) selected RNA inserted into the library prep reaction does not exceed the equivalent of 1 μ g of total RNA. **EXAMPLE:** if 5 μ g of total RNA is used for Poly(A) selection, elute the poly(A) RNA in 25 μ l of water (H₂O) and take 1/5th, as input for CORALL library prep (i.e., 5 μ l as the equivalent of 1 μ g starting total RNA).

8. Appendix C: Quality Control

Quality Control Methods

Quality control of poly(A) RNA can only be reliably performed where the input amount is >200 ng and is best performed using microcapillary electrophoresis. For low- to medium-throughput applications, we recommend the Agilent Bioanalyzer 2100 and RNA 6000 Pico chips (Agilent Technologies, Inc.) or similar. Typically, 1 μ l of eluted poly(A) RNA produced according to the directions in this manual can be analyzed directly on a Pico RNA chip. For high-throughput applications instruments such as LabChip GX II (Perkin Elmer) or 2200 TapeStation (Agilent Technologies, Inc.) are recommended. Depending on the minimum sample loading requirements for each instrument, 1 μ l of the eluted poly(A) RNA may be diluted to the required volume (e.g., 2 μ l sample for TapeStation RNA 6000 Pico and High Sensitivity R6K kits and 10 μ l for LabChip GX II).

ATTENTION: Be advised that poly(A) RNA does not contain 18S and 28S rRNA peaks anymore, hence an RNA integrity number (RIN) or RNA Quality Number (RQN) cannot be calculated.

If microcapillary electrophoresis platforms are not available, very basic quality control can also be performed by separating a small aliquot of the RNA on a polyacrylamide or a denaturing agarose gel. RNA quantification can also be performed with an inexpensive benchtop fluorometer using one of several commercially available assays. We recommend using a Qubit HS RNA assay (Life Technologies, Inc.). Most UV-Vis spectrophotometers are not sensitive enough at these concentrations to accurately quantify low RNA amounts and should be avoided.

Typical Results

The poly(A) content of an RNA sample or tissue varies, but is usually between 1 and 3 % of the total RNA amount. From 5 μ g Universal Human Reference RNA (UHRR) around 100 ng poly(A) RNA can be isolated (~2 %).



Figure 3. Bioanalyzer traces of two independent poly(A) selections from 5 μ g (red trace) and 500 ng (blue trace) Universal Human Reference RNA (UHRR). Poly(A) RNA was eluted in 25 μ l for 5 μ g or 12 μ l for 500 ng of molecular biology grade water and 1 μ l was loaded onto an RNA 6000 Pico chip (Agilent Technologies, Inc.). The concentration of the samples was 3.60 ng/ μ l and 0.48 ng/ μ l respectively.

When quantifying RNA recovery, the same instrument, or type of measurement should be used for the starting total RNA and the final selected poly(A) RNA. For example, use a Bioanalyzer, or a NanoDrop for both measurements. Different instruments or measurement assays will have different sensitivity that can affect the accuracy of the yield comparison).

The total RNA input can be compared to the poly(A) selected RNA for example using a Bioanalyzer or similar assay. Here, we recommend diluting the total RNA to a level compatible with the assay sensitivity range, in order to obtain a meaningful comparison of the peak profile of the total RNA with the poly(A) selected product (Figure 4).

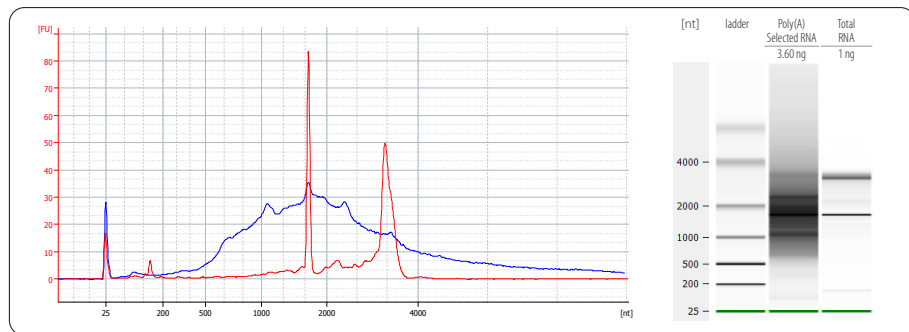


Figure 4. Bioanalyzer traces of Universal Human Reference Total RNA (red trace) and poly(A) selected RNA from 5 µg Universal Human Reference RNA (blue trace). The total amount of RNA loaded onto the Bioanalyzer RNA 6000 Pico chip (Agilent Technologies, Inc.) is indicated for each sample.

9. Appendix D: Revision History

Publication No. / Revision Date	Change	Page
157UG069V0101 Dec. 13, 2022	Updated Legal Disclaimer text.	2
	Updated Kit Components Figure 2 and Table to reflect current packaging and storage requirements.	6
	Added link to General Guidelines.	7
	Removed General section.	8
157UG069V0100 Nov. 11, 2020	Kit upgrade to V1.5, new oligo(dT) Magnetic Beads were included, Kit Catalog Number changed to 157.96.	1, 4, 6, 10 - 13, 15
	Legal terms and conditions statements updated.	2
	Volume of Magnetic Beads to add per sample was changed to 2 µl in Detailed Protocol and Short Procedure.	10 - 13
	RNA input amount recommendations updated.	12, 16
	Figures 3 and 4 updated.	17 - 18
039UG069V104 Jun. 07, 2019	Added notes for use with CORALL Library Prep Kits.	4, 11, 15
	Updated input recommendations.	4, 15
	Short Procedure reformatted.	13
039UG069V103 Dec. 14, 2017	Added note at step 7.	11
	Added safe stopping points.	11-12
	Added adjusted volumes for Bead Wash Buffer.	15
039UG069V102 Dec. 12, 2016	Restructuring of Appendices.	13 - 19
	Figure 4: Comparison of total RNA and poly(A) selected RNA.	17
039UG069V101 Nov. 26, 2015	Initial Release.	

Associated Products:

008 (SPLIT RNA Extraction Kit)

025, 050, 051, 141 (Spike-In RNA Variant Controls: SIRV-Sets 1 - 4)

171 - 176 (CORALL RNA-Seq V2 with UDIs)

Poly(A) RNA Selection Kit V1.5 · User Guide

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