

USER GUIDE

Revelo[®] mRNA-Seq for MagicPrep[™] NGS (Singular Genomics[®])

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Symbol	Meaning
	Catalog Number
	Consult instructions for use
	Contains sufficient for <n> tests
	Warning
	Important
	Expiration Date
	Temperature limitation
	Optional stopping point
	Note
	Manufacturer

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1 Introduction

1 Introduction

1.1 Overview

Intended Use

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) provides an automated end-to-end solution for stranded mRNA-Seq library preparation for the Singular Genomics G4™ sequencing platform. Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) is intended for Research Use Only and not for use in diagnostic procedures.

Features

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) kit provides a walk-away solution to produce high-quality mRNA-Seq libraries from total RNA. This kit is compatible with high quality total RNA obtained from a broad range of tissues or cell lines. This product is intended for use with the MagicPrep NGS system and includes all the reagents and consumables necessary for poly(A) selection followed by library construction using Singular Genomics Unique Dual Index (UDI) adaptors.

Specifications

Input type:	Total RNA
Input amount:	10 ng-1 µg
# Reactions available:	32
Sample Indexes available:	32 UDI
Sequencing platforms:	Singular Genomics G4

1.2 Storage and Stability

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) kit is shipped in two packages. Reagent cartridges are shipped on dry ice while the Sample deck components and magnetic beads are shipped at ambient temperature.



This product contains components with multiple storage temperature requirements. All shipments should be unpacked immediately upon receipt and contents stored appropriately.



Reagent cartridges should be stored at -20 °C in a freezer without a defrost cycle.



Magnetic bead solution should be stored at 2-8 °C.



Sample deck components should be stored at room temperature.



Kits handled and stored according to the above guidelines are warranted to perform through the labeled expiration date. Do not use kits that have passed the expiration date.

1 Introduction

1.3 Warnings and Precautions

1. Unpack and inspect the kits immediately upon receiving. In case of severe kit package damage, no dry ice left in the package or ice pack melted, and/or missing components, please contact Tecan Genomics Technical Support immediately (see **Section 5**). Please provide Tecan Genomics with the kit(s) and/or component(s) part number, and lot number. Do not use damaged components.
2. Follow your institution's safety procedures for working with chemicals and handling of biological samples. Follow good laboratory practices and safety guidelines. Wear a lab coat, disposable gloves and protective glasses where necessary. Changing gloves between handling samples is recommended to avoid cross-contamination between samples or reagents.
3. Consult your institution's environmental waste personnel on proper disposal of unused reagents. Check state and local regulations as they may differ from federal disposal regulations. This material may exhibit characteristics of hazardous waste requiring specific disposal requirements. Institutions should check their country hazardous waste disposal requirements.
4. An SDS for this product is available on the Tecan Genomics website at tecan.com/magicprep-ngs
5. Thoroughly review the MagicPrep NGS System Operating Manual for guidance on system installation, operating and setup procedures and troubleshooting. Ensure you are familiar with MagicPrep NGS system operation before performing this workflow for the first time.
6. MagicPrep NGS utilizes consumable parts that the user inserts into and retrieves from within the instrument. Never insert your hand or anything else besides MagicPrep NGS components into the instrument.

1.4 Before You Start



Please review the MagicPrep NGS System Operating Manual and this User Guide before using this kit for the first time, including the “Kit Contents”, “Planning the Experiment”, “Overview”, “Protocol” and “FAQ” sections. For more information, visit the product page (tecan.com/magicprep-ngs).

New to NGS? Contact Tecan NGS Technical Support at techserv-gn@tecan.com for tips and tricks on getting started.

2 Contents

2 Contents

2.1 Kit Contents

 This kit contains sufficient materials to prepare 32 mRNA-Seq libraries.

Each Revelo mRNA-Seq SG for MagicPrep NGS (Singular Genomics) kit (Part No. 30227692) is a bundle of:

- 4 x Reagent Cartridges
- 4 x Sample Deck Components
- 1 x Magnetic Beads (PN 30188835)

Table 1. Revelo mRNA-Seq SG for MagicPrep NGS (Singular Genomics) kit contents

Description	Reagent cartridge	Sample deck components	Singular Genomics Index (UDI) numbers
Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) A (PN 30227692)	30188834	30227695	1-8
		30227696	9-16
		30227697	17-24
		30227698	25-32

2.2 Additional Equipment, Reagents and Labware

Required Materials

- **Equipment**
 - Micropipettes: 0.5–10 µL, 2–20 µL, 20–200 µL, 200–1000 µL.
 - Microcentrifuge for 0.2 mL tubes or plates.
 - Qubit® 2.0, 3.0 or 4 Fluorometer (Thermo Fisher Scientific) or other appropriate fluorometer and accessories for quantification of total RNA and final libraries.
 - 5200 Fragment Analyzer System, Agilent 2100 Bioanalyzer, or equivalent for electrophoretic analysis of nucleic acids.
- **Reagents**
 - Low-EDTA TE Buffer, 1X, pH 8.0 (Fisher Scientific, Cat. #75793), for diluting nucleic acids.
 - Nuclease-free water (Fisher Scientific, Cat. #AAJ75793AP), for diluting nucleic acids.
 - Agilent High Sensitivity DNA Kit for Bioanalyzer (Agilent, Cat. #5067-4626), HS NGS Fragment Kit (1-6000bp) for Fragment Analyzer (Agilent, Cat. #DNF-474-0500) or equivalent.
- **Supplies and Labware**
 - Barrier (filtered) pipette tips, nuclease-free.
 - 0.2 mL PCR strip tubes or 0.2 mL thin-wall PCR plates.
 - Cleaning solutions such as RNaseZap® RNase Decontamination Solution (Thermo Fisher Scientific, Cat. #AM9780) and DNA OFF™ (MP Biomedicals, Cat. #11QD0500).

2 Contents

To Order:

- Agilent, www.agilent.com
- Fisher Scientific, www.fishersci.com
- MP Biomedicals, www.mpbio.com
- Thermo Fisher Scientific, www.thermofisher.com

3 Planning the Experiment

3 Planning the Experiment

3.1 Workflow and Time Required

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) is a completely automated mRNA-Seq library preparation workflow. Each instrument run will take approximately 10.5 hours, though workflow time can vary according to input RNA mass. A diagram of the Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) workflow is provided in Figure 1.

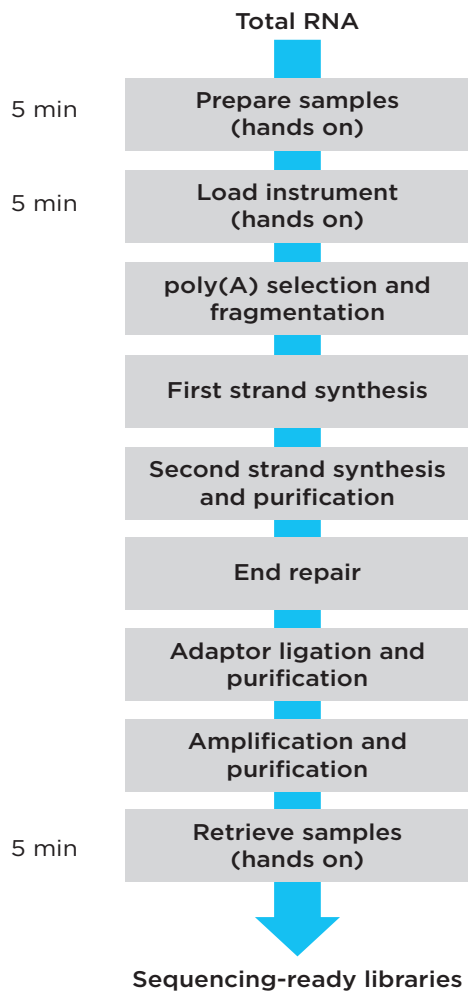


Figure 1. Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) workflow.

3 Planning the Experiment

3.2 Input RNA Requirements

RNA Quantity

This kit is compatible with a total RNA input between 10 ng and 1 µg per sample. Accurate quantification of total RNA is essential to ensure the minimum input requirement is met. Each set of 8 samples should be normalized to the same input amount to ensure sufficient amplification for each sample.

RNA Purity

RNA samples must be free of contaminating proteins and other cellular material, organic solvents (including phenol and ethanol), and salts used in many RNA isolation methods. If using an RNA isolation method based on organic solvents, such as TRIzol, we recommend column purification after isolation.

One measure of RNA purity is the ratio of absorbance readings. The A260:A280 ratio for RNA samples should be in excess of 1.8 and A260:A230 should be in excess of 2.0.

RNA Integrity

RNA samples of high molecular weight with little or no evidence of degradation will perform very well with this product. Revelo mRNA-Seq for MagicPrep NGS has not been tested with degraded samples. RNA integrity can be determined using the Agilent 5200 Fragment Analyzer System or Agilent 2100 Bioanalyzer.

DNase Treatment

Thorough DNase treatment of RNA is required prior to use with this system. The presence of genomic DNA in the RNA sample will adversely affect assay performance and data quality.

Guidance for Library Amplification

The user will select the input range that corresponds to their samples during run setup, establishing the default number of PCR cycles for that run based on input (Table 2). The number of amplification cycles can optionally be adjusted during run configuration to better optimize instrument performance for specific samples.

Table 2. Input ranges and PCR cycles

RNA input range (ng)	PCR cycles (default)
10-49	20
50-99	18
100-299	17
300-499	16
500-1,000	14



Important: The default number of PCR cycles are appropriate for accurately measured, high-quality, DNase-treated RNA inputs and are expected to produce sufficient library yield for sequencing.

3 Planning the Experiment

3.3 Sequencing Recommendations and Guidelines

Revelo mRNA-Seq SG for MagicPrep NGS (Singular Genomics) produces mRNA-Seq libraries compatible with the Singular Genomics G4 platform. These libraries should be sequenced using the Singular Genomics protocol for multiplex sequencing, following the manufacturer recommendations.

Index Read Recommendations

Revelo mRNA-Seq SG for MagicPrep NGS (Singular Genomics) libraries contain 12 base Unique Dual Indexes (UDI) for sample multiplexing. Both index 1 and index 2 should be sequenced for the detection of “index (barcode) hopping”. Singular Genomics UDI sequences can be found in **Appendix 6.1**.

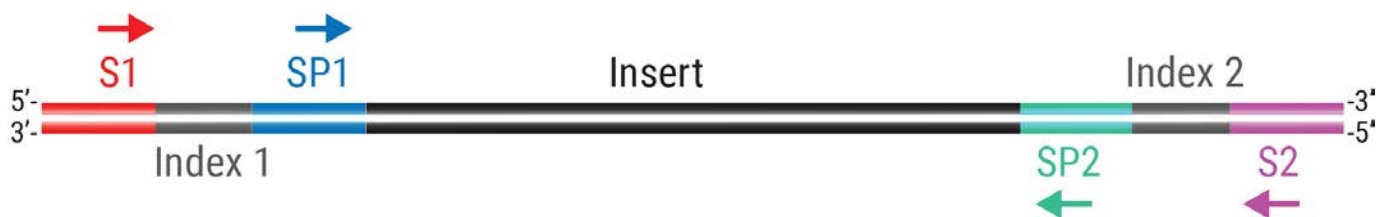


Figure 2. Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) library structure. S1 and S2 represent Index 1 and Index 2 sequencing primers, while SP1 and SP2 represent Read 1 and Read 2 sequencing primers, respectively (image from <https://singulargenomics.com/docs/sequences-of-singular-adapters/>)

3.4 Data Analysis

Once the data have been parsed according to sample, additional sample-specific data analyses may be employed according to the requirements of the experiment.



Note: The forward read from MagicPrep NGS mRNA-Seq libraries represents the sense strand. Contact Tecan NGS Technical Support for more information.

For details regarding index (barcode) sequences, please see **Appendix 6.1**.

3.5 Library Storage



Libraries prepared on MagicPrep NGS should be stored at $-20\text{ }^{\circ}\text{C}$ in a freezer without a defrost cycle.

4 Protocol

4 Protocol

Protocol Notes

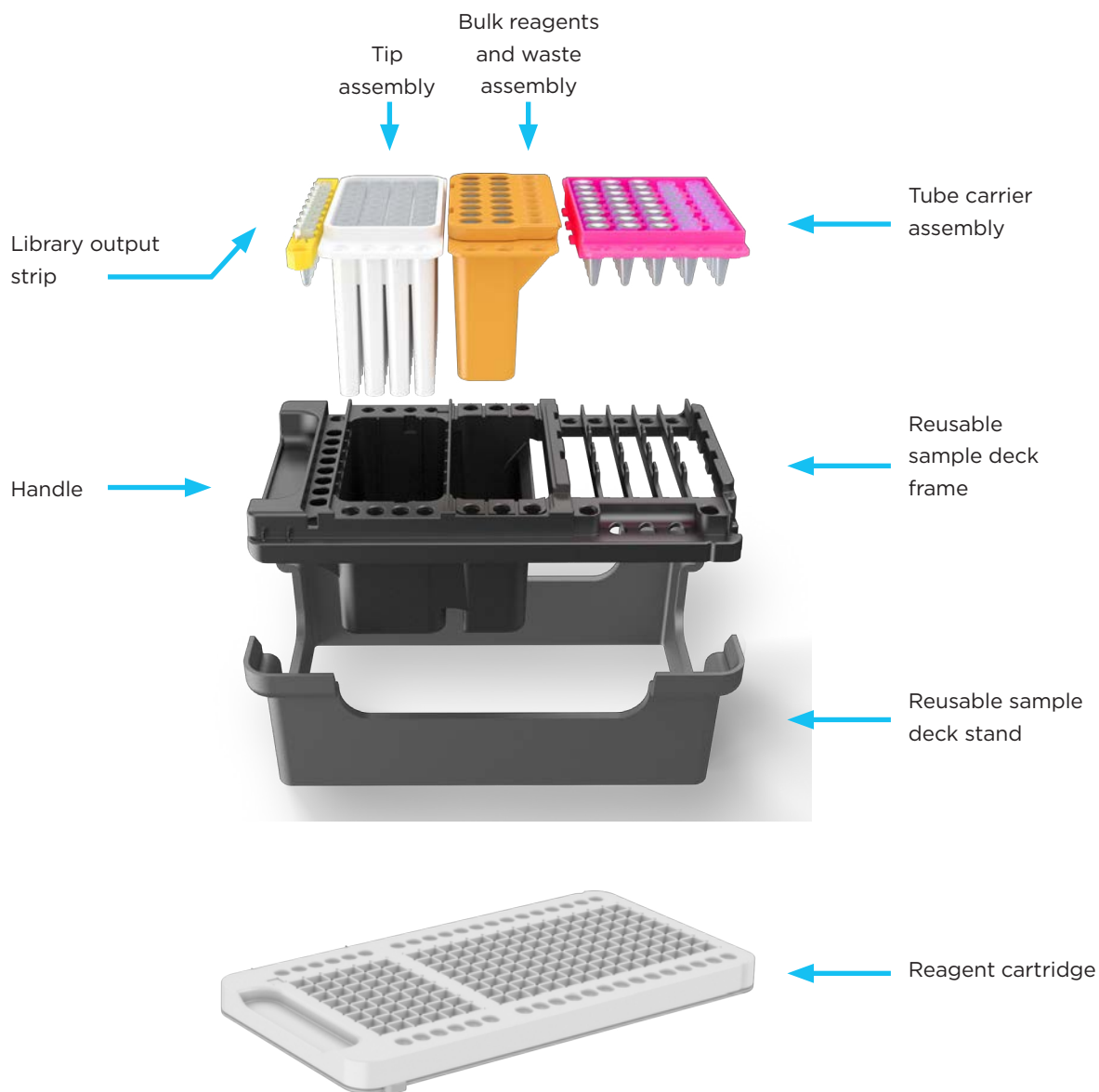


Figure 3. Sample deck components and reagent cartridge.

4 Protocol

Controls

- Tecan recommends the routine use of K562 RNA as a positive control, especially for first-time users. The use of positive control RNA will establish a baseline of performance for a given instrument run.
- Routine use of a no template control (NTC) is recommended to monitor the work environment for potential laboratory contamination.
- When a positive or negative control is included in a run, it will occupy one of the available sample positions for that run.

General Workflow

- MagicPrep NGS is designed to prepare 8 libraries at a time. While generating less than 8 libraries is possible, the unused reagents cannot be used for a second run. All consumables are single use only.
- Samples must be accurately quantified and correctly diluted to ensure equivalent amplification of each sample (see Table 2).
- Use the QR code below to watch a tutorial video on how to set up a MagicPrep NGS run.



Watch a MagicPrep
NGS run setup video.



Note: Prior to starting a run, ensure that the MagicPrep NGS instrument is powered on and fully initialized. The MagicPrep NGS initialization and hardware test takes approximately 10-15 minutes. For detailed information, refer to the MagicPrep NGS System Operating Manual.

4.1 Sample Preparation

1. Remove MagicPrep NGS mRNA-Seq - magnetic beads from 4 °C storage and leave at room temperature for at least 30 minutes prior to use. Vortex to fully resuspend the beads. Gently tap the tube against bench to collect contents to the bottom and keep at room temperature.
2. Remove the Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) reagent cartridge from -20 °C storage and place at room temperature. Keep the reagent cartridge at room temperature for 10 minutes.
3. Aliquot each total RNA input sample according to standardized mass ranges in Table 2 into a 0.2 mL strip tube or 96-well plate.
4. Dilute each RNA sample with nuclease-free water or 1X low-EDTA TE to a final volume of 50 µL.
5. Vortex beads thoroughly to mix and carefully pipette 60 µL of the bead solution into each sample. Pipette mix the sample and beads thoroughly.

4 Protocol

4.2 Setting up the Sample Deck

Use only new sample deck components and reagent cartridges for each run. Previously used components will not be accepted by MagicPrep NGS.



Note: Document the information listed on the sample deck box label for future reference. The sample deck box label identifies the specific UDI adaptors provided in that sample deck. For example, a label indicating “UDI Adaptor Set: A1” corresponds to kit A, sample deck 1 (indexes 01 - 08) as shown in Table 3 (see Appendix 6.1.).

1. Place empty reusable sample deck frame into the sample deck stand for assembly.
2. Place the box containing Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) sample deck components (Figure 3; kept at room temperature) on a laboratory bench and open it.
3. Remove the clear hygiene seal covering the sample deck components.



Important: Firmly tap down both the tube carrier assembly (pink) and bulk reagents and waste assembly (orange) against the bench to collect reagents at the bottom of the tubes and remove any bubbles. It is **essential** that there are no bubbles at the bottom of the tubes.

4. Transfer the tube carrier assembly, bulk reagents and waste assembly, tip assembly and library output strip from the sample deck box into correct positions in sample deck. The tube carrier assembly is slotted and must be inserted slightly offset and slid into position (Figure 4). Other components can be inserted directly and are keyed so that they will only insert in the correct orientation. All components should be flush when properly assembled (Figure 5).



Figure 4. Inserting the tube carrier assembly. Align the tabs on the tube carrier assembly with the slots in the sample deck frame and push the tube carrier assembly down fully. Slide the tube carrier assembly so that rounded edges of the tube carrier assembly are flush with the side of the sample deck frame.

4 Protocol



Figure 5. Fully assembled sample deck and sample deck components within the sample deck stand (handle facing towards you).

5. Orient the sample deck so that the handle faces toward you on the laboratory bench.
6. Using a pipette, transfer the RNA samples prepared in **Section 4.1** (110 μ L) to the first row A1 (Figure 6) of the tube carrier assembly. Make sure there are no bubbles at the bottom of the tube.



Figure 6. Top view of assembled sample deck. The tip assembly (white) is shown covered with the tip seal. When the sample deck handle is towards you, row A1 of the tube carrier assembly is on the left (blue arrow).



Note: When the sample deck is oriented with the handle facing toward you, the first sample should be delivered to the left-most well (A1).

7. While holding sample deck firmly in place, carefully remove the tip seal from the tip assembly (Figure 6).

4 Protocol

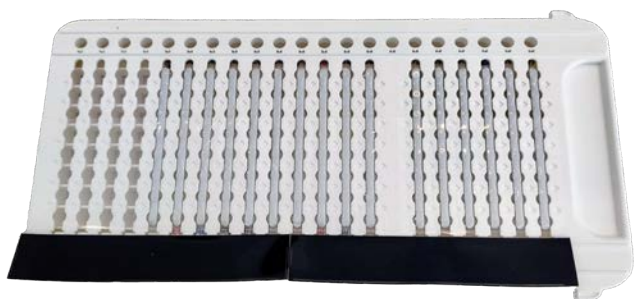


Figure 7. Bottom side of reagent cartridge with common seal attached (black strip).

8. After incubation at room temperature for 5-10 minutes, remove the common seal from the underside of the reagent cartridge by grasping the black strip and carefully peeling backwards. Ensure that no residual seal material remains at the nozzles and proceed immediately to starting the run.

4.3 Starting the Run



Note: Prior to starting a run, ensure the MagicPrep NGS instrument is powered on and fully initialized. For detailed information, refer to the MagicPrep NGS System Operating Manual.

1. Touch the “Start” button on the home screen (Figure 8).

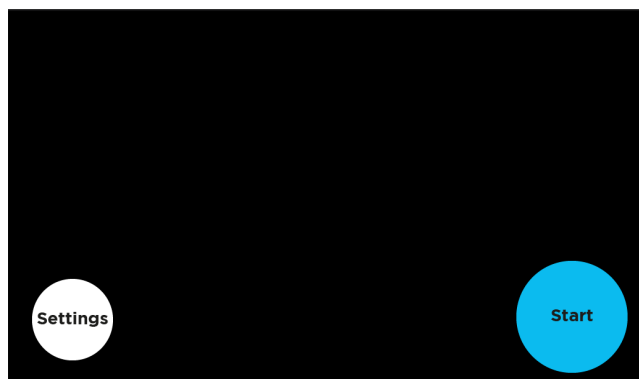


Figure 8. The home screen.

2. Confirm that samples and beads have been correctly added to Row A1 of the tube carrier assembly in the sample deck (Figure 9).

4 Protocol

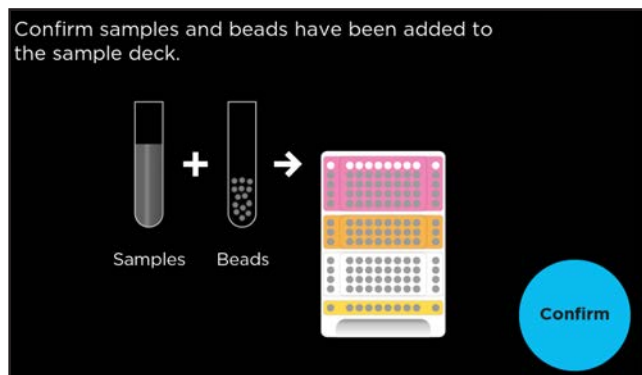


Figure 9. Sample confirmation.

3. Upon touching the “Confirm” button, the door will pop open. Once the door has been unlocked, pull the door open completely. Do not insert your hand(s) into the instrument.
4. Insert the reagent cartridge, followed by the sample deck (without the sample deck stand) into the MagicPrep NGS instrument according to the prompt (Figure 10). Insert the sample deck and the reagent cartridge completely until the part ‘clicks’ in place and the image disappear from the screen. Close the door.

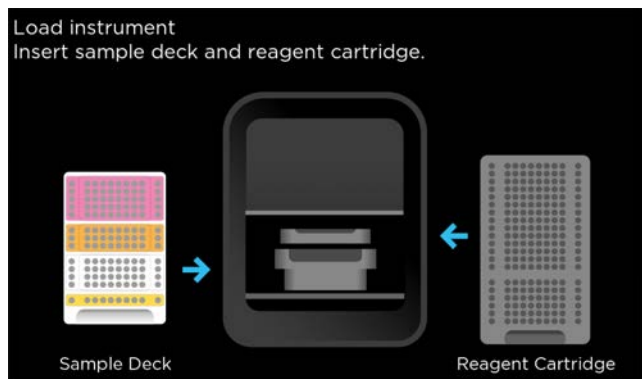


Figure 10. Insert sample deck and reagent cartridge.

5. Once the door is closed, the reagent cartridge and sample deck barcodes are scanned to ensure all consumable components are present and determine the protocol to run (Figure 11).

4 Protocol

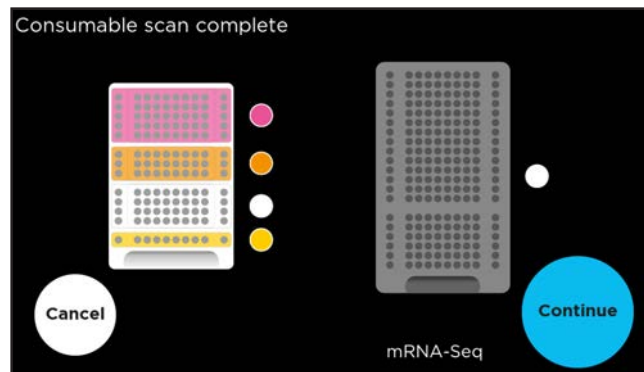


Figure 11. The consumable barcodes are scanned by the MagicPrep NGS instrument.



Note: The user can elect to use expired sample deck and reagent cartridge components but run performance cannot be guaranteed by Tecan. Previously used components will not be accepted by MagicPrep NGS. For other errors, consult the MagicPrep NGS Instrument manual.

6. Select the input range that corresponds to your sample input amount and touch “Confirm” (Figure 12).

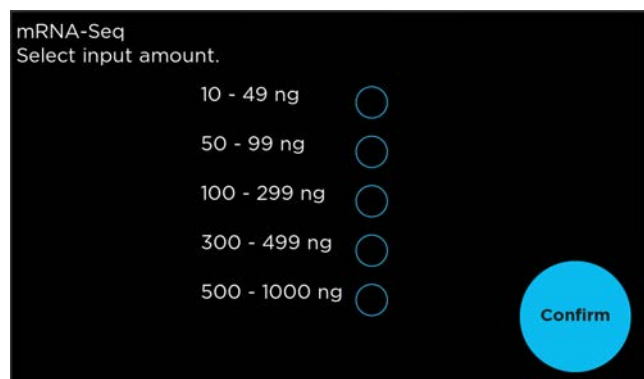


Figure 12. Input range definition.

7. Select the optimal number of PCR cycles for your input. The recommended number of PCR cycles will be populated based on your sample input quantities (Table 2). You may adjust the number of PCR cycles to suit your particular samples (Figure 13).



Note: Document the number of PCR cycles used for each run for your records.

4 Protocol

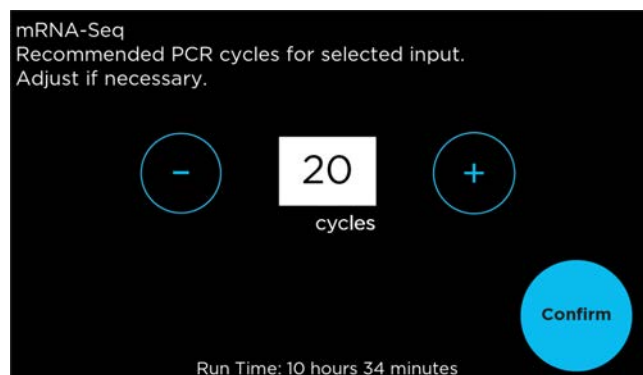


Figure 13. Confirm or adjust the number of PCR cycles for your run.

8. A summary of the run parameters is displayed (Figure 14). If you need to revise these parameters, touch “Open door” and repeat the run setup procedure. If the displayed run parameters are correct, touch “Start” to start the run.

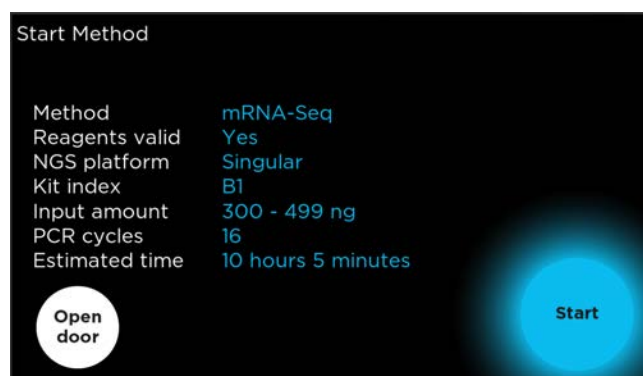


Figure 14. Start method screen.



Note: Once a run is started, the instrument screen will display the estimated time for completion of each step in the library preparation process. The LED strip provides the progress of the complete run. No further user intervention is required until the run completes.

4.4 Recovering the Libraries



Note: Upon run completion, libraries should be retrieved within 65 hours.

1. When a run completes, “mRNA-Seq complete” will be displayed (Figure 15). Touch the “Retrieve libraries” button when ready to retrieve your completed libraries.

4 Protocol

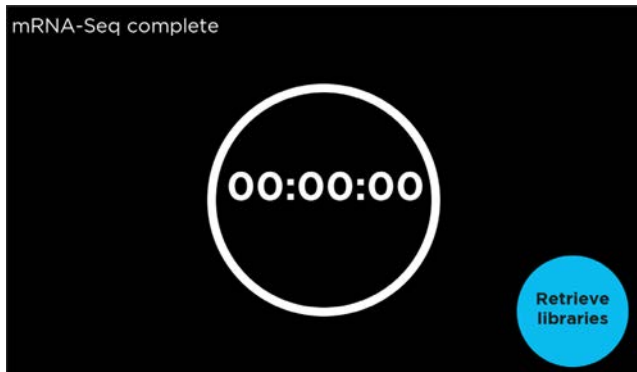


Figure 15. Run complete screen.

2. Wait for MagicPrep NGS to prepare for library retrieval. A countdown timer will display until the door can be opened.
3. When the instrument is ready, the door will open automatically. Push the door all the way down to completely open it.
4. Remove sample deck and reagent cartridge (Figure 16), placing the sample deck into the sample deck stand.

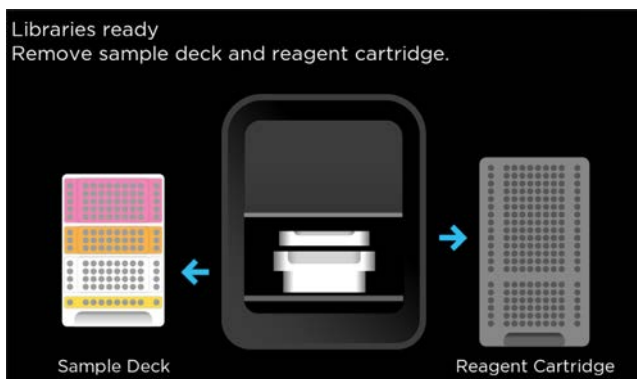


Figure 16. Libraries ready screen.

5. Completed libraries are in the library output strip (Figure 3). Seal library output strip tubes with the included strip caps before removing them from the sample deck. The left-most tube (with the handle facing towards you) contains the library representative of your first sample. See **Appendix 6.1** for library index sequence positioning.
6. Close the door completely. MagicPrep NGS is now ready to start a new run.
7. Store sequencing libraries at $-20\text{ }^{\circ}\text{C}$ until ready for assessment and sequencing.

4 Protocol

4.5 Disposal of reagent waste and plastics



Note: Review the SDS for hazardous chemical information. Follow the chemical waste and plastic disposal guidelines for your institution.

1. Remove the strip tube and tip assemblies from the sample deck and discard. Do not discard the sample deck frame or sample deck stand.
2. Remove the bulk reagent and waste assembly from the sample deck and open the lid. Remove the 1.2 mL tubes individually and empty any remaining buffer into the bulk reagent and waste assembly. Tap the tube to remove any liquid and discard the tubes.
3. Empty the reagent waste from the bulk reagent and waste assembly into aqueous laboratory waste. Discard the bulk reagent and waste assembly.

4.6 Qualitative and Quantitative Assessment of Libraries

1. Assess the libraries by running 2 μL of 5 ng/ μL of each library on a Fragment Analyzer using the HS NGS Fragment Kit or 1 μL on a Bioanalyzer with the High Sensitivity DNA Kit (Agilent Technologies). A typical fragment distribution for high quality inputs is shown in Figure 17.

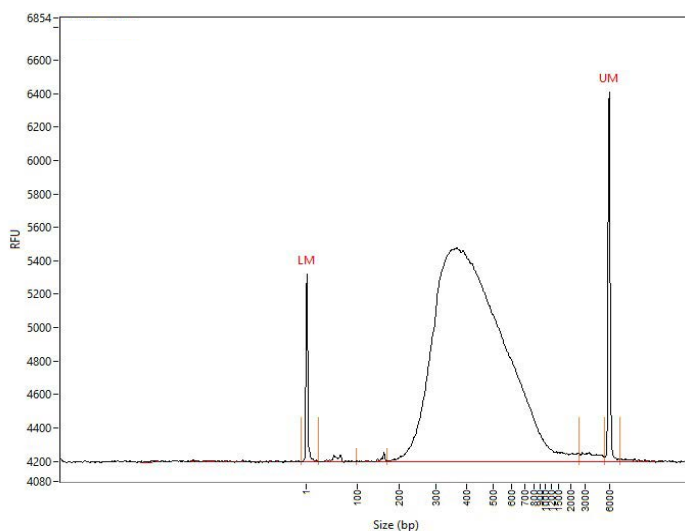


Figure 17. Fragment distribution when 2 μL of 50 ng/ μL library is separated on a Fragment Analyzer using the HS NGS Fragment Kit. Starting material is 100 ng K562 total RNA.

2. Quantify the libraries for pooling using a fluorescence or qPCR-based method.
3. Validate the library pool for sequencer loading following the Singular Genomics guidelines (https://techwriting.singulargenomics.com/G4-Documentation/G4-Best-Practices-Quality-Control_600011.pdf).

5 Technical Support

5 Technical Support

For help with any of our products, please contact Tecan NGS Technical Support at 650.590.3674 (direct) or 888.654.6544, option 2 (toll-free, U.S. only) or email techserv-gn@tecan.com

In Europe email Tecan NGS Technical Support at europe-gn@tecan.com

In all other locations, contact your Tecan NGS reagent distributor for technical support.

6 Appendix

6 Appendix

6.1 Index (UDI) Sequences and Guidelines for Multiplex Experiments

Index (barcode) sequences for Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) kit is given below in Table 3. .

Table 3. Index sequences for Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) UDI Adaptor set.

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) kit A (30227692)			
	Index number	Index 1 sequence	Index 2 sequence
Sample deck 1	01	TAAGACCCTACT	GGGACATATTGA
	02	CGAAGTACATCC	TAGGACGTAACG
	03	TAGCCTTCCAAA	AGTATGGCAAGA
	04	GCCTTCAAGTC	TAGAGTCGTCGT
	05	CAACGGTTCCGG	ACGTTTCGCTCG
	06	GTTGCATGGCCC	TAGGGAACGATG
	07	ATCGTTGCTATC	ATGACTCCGCAT
	08	CCTCGAATTCAT	GGTTGCTACCGG
Sample deck 2	09	TGAACGTCGGCC	TCCTCGATTGAA
	10	CATCTAGCAAGC	ATGTAGCGTCTC
	11	TATCGAGGCAAC	CATCATGCGTAC
	12	GAGACGTAGCAA	ACCTTGACCGGG
	13	ATCATGCGCCCG	TTGACGAGATCT
	14	AGGAGCTAGGGA	GGGCTAATGTCA
	15	ATCGACCATGCT	TTAGGAGCGAAC
	16	TGCGAATCGACA	GTACATCGAGTA
Sample deck 3	17	ATGTTCCCTCT	AGGCTTTGTCAT
	18	TCGCTCATCTAG	CGACGATATTTG
	19	CCTAAGGTAAAC	AAACTCCGTTGT
	20	GAATAGCGCTTA	ACGTACCAAGAC
	21	CGATGTACATCC	TCCGATGTCGGC
	22	CAAGTCGAAACC	AGGTTACCGCGT
	23	GTAACGGATAGC	ATGCCGAAACGT
	24	GAAGCTTGGTCA	ACATACGCGGGG
Sample deck 4	25	AACCCGTAACCA	GGATCTAGGACG
	26	AATGCTCCCCTA	TCGACTCTCCGT
	27	GTATGACGGATG	TACGCTAGACAA
	28	GCAAAGCTTGGA	ATTCGGGTAAG
	29	TCTAACCGGCTA	CTAGCCAACGCC
	30	ATTGGAGCCCGC	TGATAGCCGGTT
	31	TGCCGATCTAT	GCATGGTTCCTA
	32	ACATCGCATGTT	GACGGTAATGAG

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6.2 Frequently Asked Questions (FAQs)

Getting Started

Q1. What materials are provided with Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics)?

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) consists of sample deck components, reagent cartridges and a single tube of magnetic beads.

Q2. What materials or equipment are required or will be useful?

A comprehensive list of required and recommended equipment can be found in **Section 2.2**

Q3. How long can I expect my kit to perform to specification?

Tecan Genomics library preparation kits are warrantied through the labeled expiration date. Detailed warranty information can be found at the beginning of this User Guide.

Input Recommendations

Q4. What methods do you recommend for RNA isolation?

We recommend a column-based method, including:

- Norgen Biotek Total RNA Purification Kit
- Zymo Research Quick-RNA™ Kits
- Arcturus PicoPure® RNA Isolation Kit
- Ambion PureLink® RNA Mini Kit
- Qiagen RNeasy Kits

Organic methods such as TRIzol® Reagent should be subsequently followed with a column-based clean-up method.

Q5. Can I use TRIzol® or other phenol-chloroform based extractions for RNA isolation?

We do not recommend the use of TRIzol® or similar methods as any carry-over of organic solvent may inhibit downstream enzyme activity. If using TRIzol extracted RNA, we recommend using a column-based purification of the RNA prior to input into the kit.

Q6. Can I use carrier RNA during RNA isolation?

No. Residual carrier RNA can result in poor data quality. For more information, contact Tecan NGS Technical Support at techserv-gn@tecan.com.

Q7. How much total RNA do I need for library generation?

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) can be used with 10 ng to 1 µg purified, high-quality (RIN ≥7.0) total RNA. Input amounts outside this range may affect reaction stoichiometry, resulting in sub-optimal libraries that produce variable and unsatisfactory results. Lower input amounts will potentially result in insufficient yields depending on the requirements of the analytical platform.

Q8. Do I need to perform an rRNA depletion step before processing samples with Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics)?

No rRNA depletion step is needed. The Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) workflow performs a poly(A) enrichment step to remove rRNA and non-polyadenylated transcripts.

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Q9. Do you recommend DNase treatment of purified total RNA samples?

Yes. When using purified total RNA samples, large amounts of contaminating genomic DNA may amplify during the process. For this reason DNase treatment is recommended prior to running Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics).

Q10. Can I use Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) with RNA from any organism?

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) has been designed and tested only with total RNA inputs isolated from human samples. However, any input RNA sample containing some fraction of 3'-poly(A) sequence should yield a sequenceable library. For more information, contact Tecan NGS Technical Support at techserv-gn@tecan.com.

General Workflow

Q11. Can I perform fewer than 8 preps at a time?

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) performs 8 preps with every run. Empty positions will not produce libraries. Reagent cartridges and sample deck components cannot be reused.

Q12. Does Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) deplete ribosomal RNA?

No. Revelo mRNA-Seq SG for MagicPrep NGS utilizes poly(A) selection to target poly-adenylated mRNA transcripts. This yields sequencing data with minimal rRNA contamination.

Q13. Can I modify the number of PCR amplification cycles recommended by Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics)?

Yes, the user will have the opportunity to adjust cycle number from the recommended value selected based upon sample input. Table 2 of the User Guide contains guidelines on the number of cycles to use, though some samples may perform better with a different number of cycles.

Q14. Are Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) libraries stranded?

Yes, libraries prepared with Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) are stranded. The first sequencing read represents the sense strand.

Q15. My reagents have expired. Can I still run Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics)?

Yes, although we do not recommend the use of expired reagents, the user will still have the option to run the protocol after the instrument detects the use of expired reagents.

Library quantification/qualification

Q16. How many bases do the Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) adaptors add to the library?

The adaptors add 151 bp to the library.

Q17. What is the expected library size?

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) libraries generated with high-quality human total RNA contain inserts that are between 300–350 bp on average. Total library size will be 400–500 bp.

Q18. What sequencers are compatible with your libraries?

Revelo mRNA-Seq for MagicPrep NGS (Singular Genomics) libraries are compatible with the Singular Genomics G4 sequencing platform utilizing standard on-board sequencing primers.

Q19. How much material should I load into the sequencer?

Please follow manufacturer's recommendations for library QC, quantification, color-balancing and loading of the amplified library on the sequencer.

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Q20. What kind of results should I expect from my library prep?

Results for your specific sample may vary depending on a range of factors (quantification accuracy, mRNA proportion, inhibitor content, etc.). For 100 ng input of high quality K562 RNA (RIN > 9.0) using the recommended number of PCR cycles, we expect the following results:

Library yield \geq 200 ng

Yield variability \leq 40 % by mass

Adaptor/primer artifact \leq 5 % by molarity

Q21. What kind of errors are anticipated when sequencing index reads?

One of the challenges of multiplexing is to avoid index mis-association, which means that some reads are assigned to the wrong samples based on their indexes. Index mis-association can have different sources, such as low-quality oligos, cross-contamination, or index hopping. Index hopping happens when free adapters and primers that are not bound to any sample interact with adapters from another sample during cluster formation. This creates a hybrid molecule that can initiate a cluster with a mismatched index in another well. Other factors that can cause index hopping are low sequence diversity or incomplete extension. Index hopping can affect some applications more than others, such as somatic mutation detection or differential expression analysis.

The reported levels of index hopping vary depending on the platform and the chemistry used, but they can range from less than 1% to 10%. Experiments performed by Singular Genomics across four sequencing runs with 24 Salmonella whole-genome samples per lane, each with a different pair of unique dual indexes averaged only 0.07% of reads having aberrant index pairs.

Costello M, Fleharty M, Abreu J, Farjoun Y, Ferriera S, Holmes L, Granger B, Green L, Howd T, Mason T, Vicente G, Dasilva M, Brodeur W, DeSmet T, Dodge S, Lennon NJ, Gabriel S., Characterization and remediation of sample index swaps by non-redundant dual indexing on massively parallel sequencing platforms. BMC Genomics. 2018; 19:332. doi: 10.1186/s12864-018-4703-0

Singular Genomics Reference Guide

Adapters and Indices for the G4 Sequencing Platform

<https://singulargenomics.com/wp-content/uploads/2022/10/Adapters-and-Indices-for-G4-600007-087.pdf>

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