USER GUIDE

Revelo mRNA-Seq for MagicPrep™ NGS

REF 30186621, 30186622, 30186623

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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

Contents

1 Introduction

1 Introduction

1.1 Overview

Intended Use

Revelo mRNA-Seq for MagicPrep NGS provides an automated end-to-end solution for stranded mRNA-Seq library preparation starting with total RNA. Revelo mRNA-Seq for MagicPrep NGS is intended for Research Use Only and not for use in diagnostic procedures.

Features

Revelo mRNA-Seq for MagicPrep NGS 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 with Unique Dual Index (UDI) adaptors.

Specifications

Input type:	Total RNA
Input amount:	10 ng–1 µg
# Reactions available:	32
Sample Indexes available:	Up to 96 UDI
Sequencing platforms:	Illumina NGS

1.2 Storage and Stability

Revelo mRNA-Seq for MagicPrep NGS 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 warrantied 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 for MagicPrep NGS kit (Part No. 30186621, 30186622, 30186623) is a bundle of:

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

Table 1. Revelo mRNA-Seq for MagicPrep NGS kit contents

Description	Reagent cartridge	Sample deck components	Index (UDI) numbers
Revelo mRNA-Seq for MagicPrep NGS A (PN 30186621)	30188834 (4)	30188830 30188831 30188832 30188833	1–8 9–16 17–24 25–32
Revelo mRNA-Seq for MagicPrep NGS B (PN 30186622)	30188834 (4)	30188844 30188845 30188846 30188847	33–40 41–48 49–56 57–64
Revelo mRNA-Seq for MagicPrep NGS C (PN 30186623)	30188834 (4)	30188852 30188853 30188854 30188855	65–72 73–80 81–88 89–96

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.

2 Contents

- **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).

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 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 workflow is provided in Figure 1.

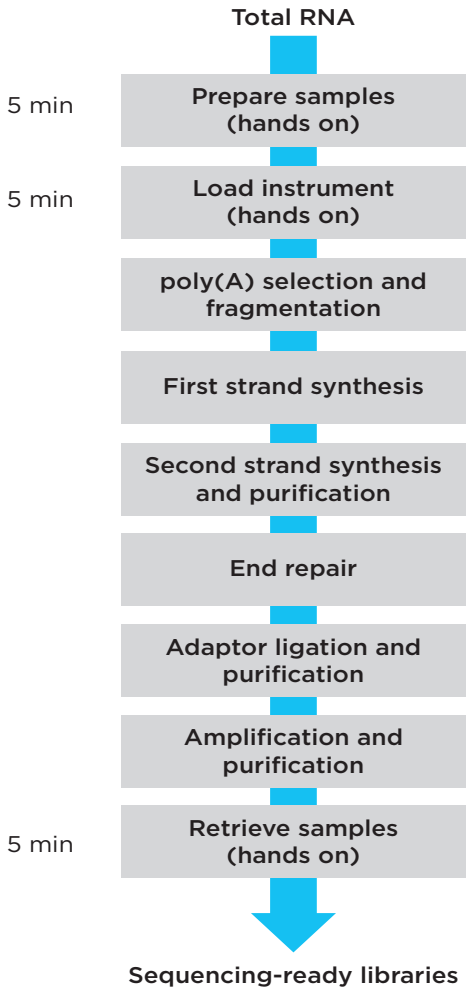

Figure 1. Revelo mRNA-Seq for MagicPrep NGS workflow.

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 for MagicPrep NGS produces mRNA-Seq libraries compatible with Illumina NGS platforms. These libraries should be sequenced using the Illumina protocol for multiplex sequencing, following the recommendations for the specific sequencer.

Index Read Recommendations

Revelo mRNA-Seq for MagicPrep NGS libraries contain 8 base Unique Dual Indexes (UDI) for sample multiplexing. Both index 1 (i7) and index 2 (i5) should be sequenced for the detection of "index (barcode) hopping". Tecan UDI sequences differ from the sequences used by Illumina and can be found in **Appendix 6.1.**

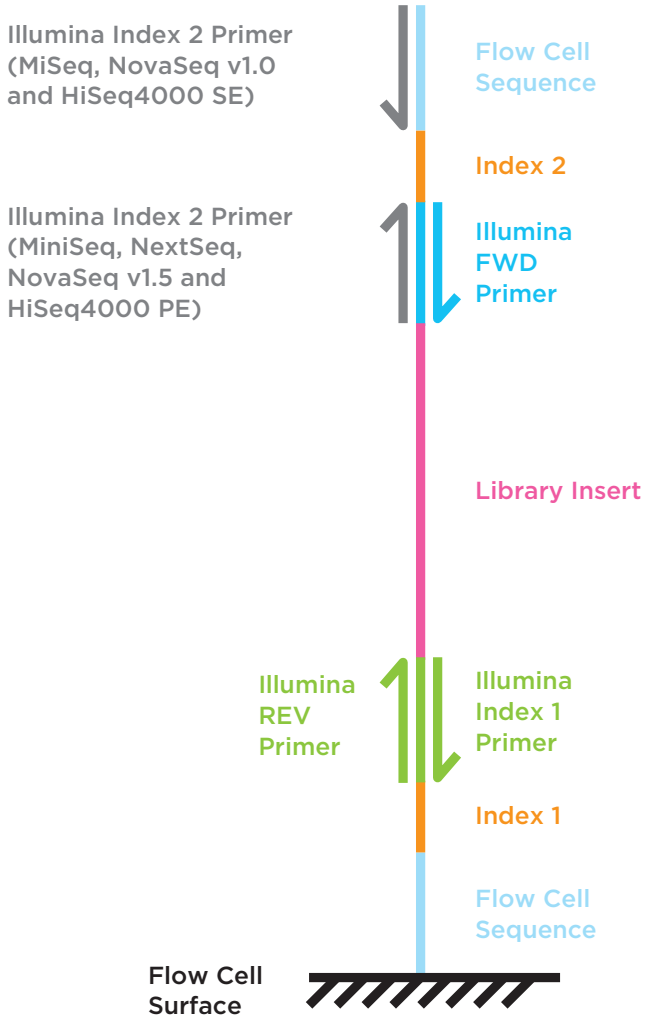

Figure 2. Revelo mRNA-Seq for MagicPrep NGS library structure.

3 Planning the Experiment

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 °C in a freezer without a defrost cycle.

4 Protocol

4 Protocol

Protocol Notes

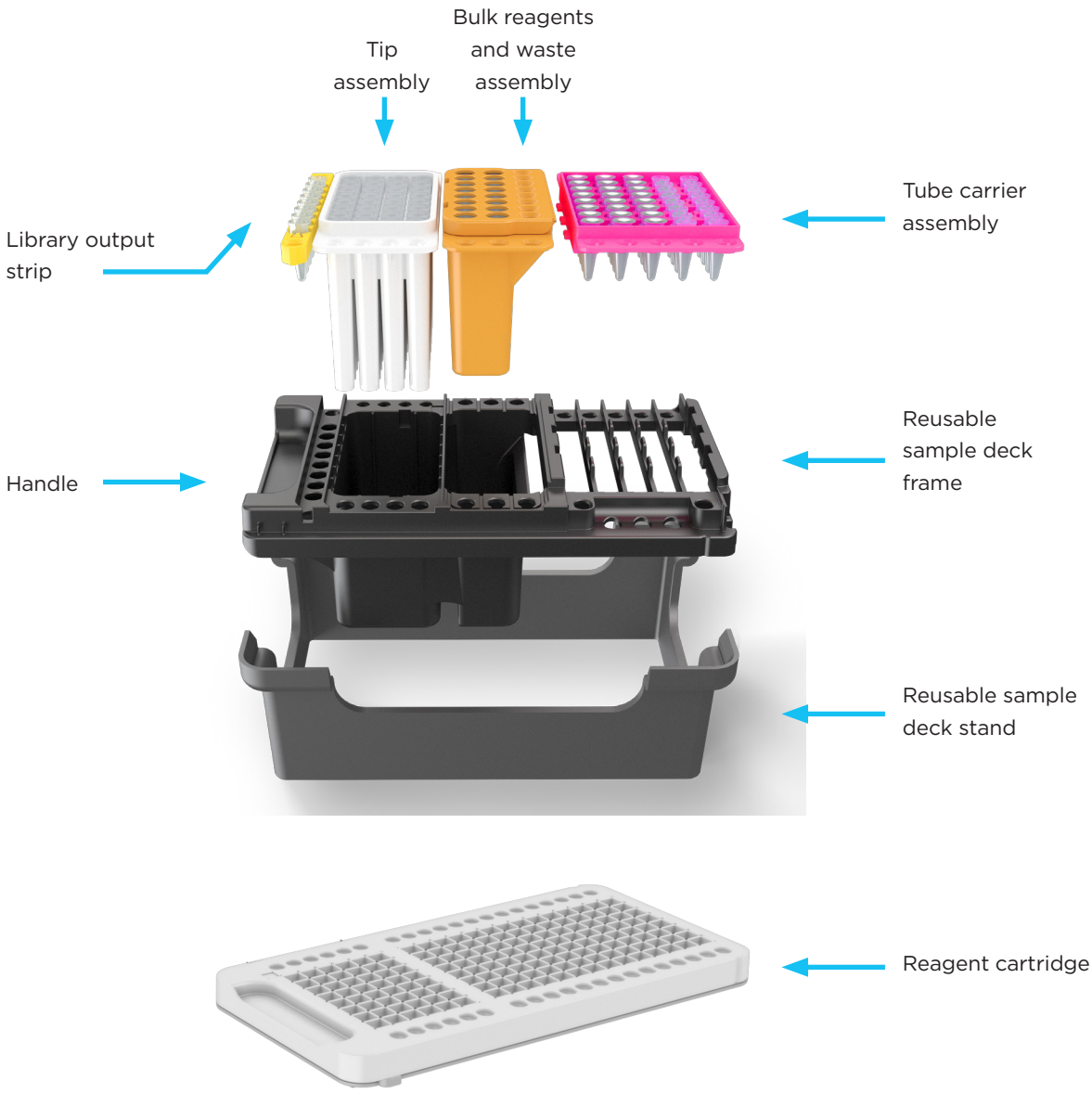

Figure 3. Sample deck components and reagent cartridge.

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 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.

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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 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).

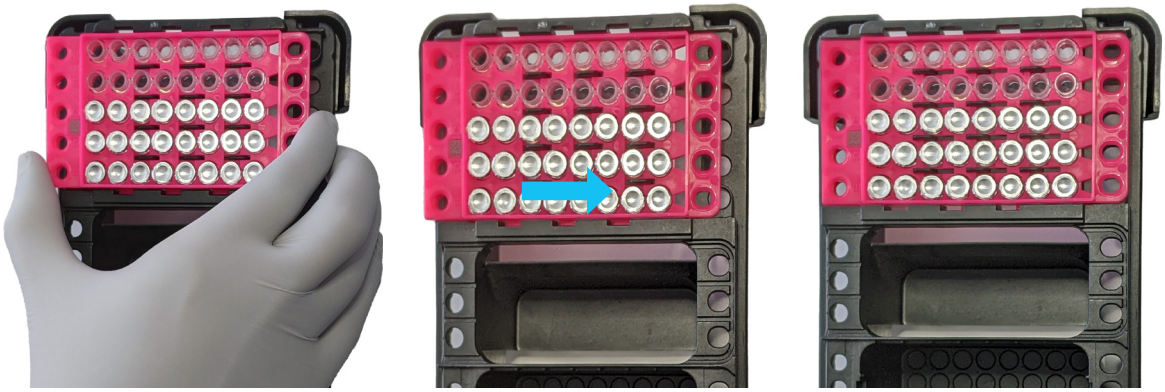

Figure 4. Inserting the tube carrier assembly. Align the tabs on the tube carrier assembly with the slots in the sample deck frame and push the tube carrier assembly down fully. Slide the tube carrier assembly so that rounded edges of the tube carrier assembly are flush with the side of the sample deck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

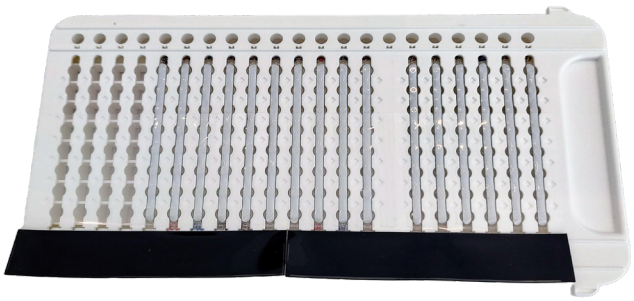

Figure 7. Bottom side of reagent cartridge with common seal attached (black strip).

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 7).

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 8).

4 Protocol

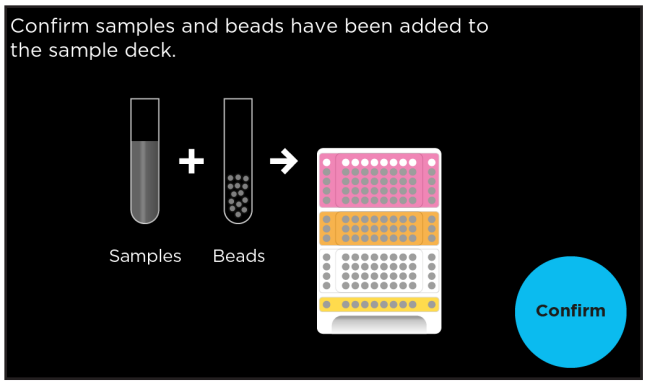

Figure 9. Sample confirmation.

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 9). 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.

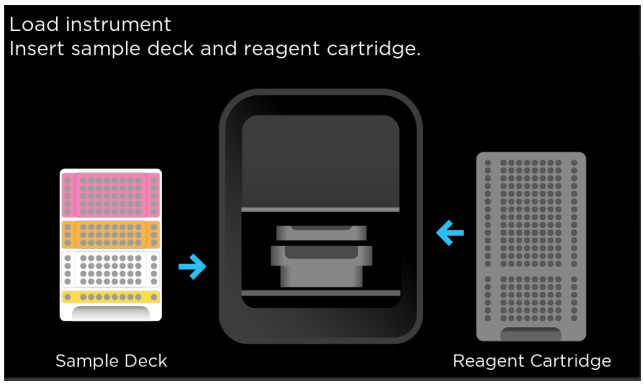

Figure 10. Insert sample deck and reagent cartridge.

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 10).

4 Protocol

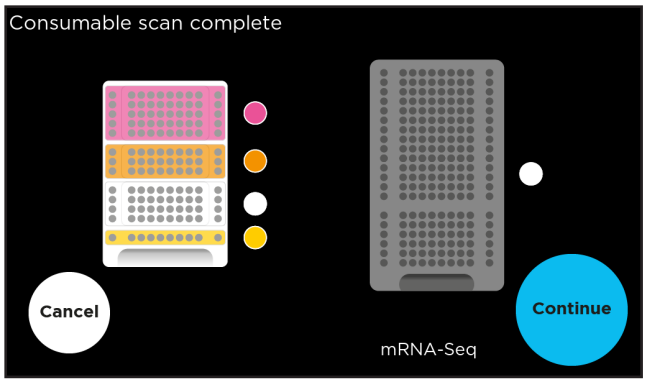

Figure 11. The consumable barcodes are scanned by the MagicPrep NGS instrument.

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 11).

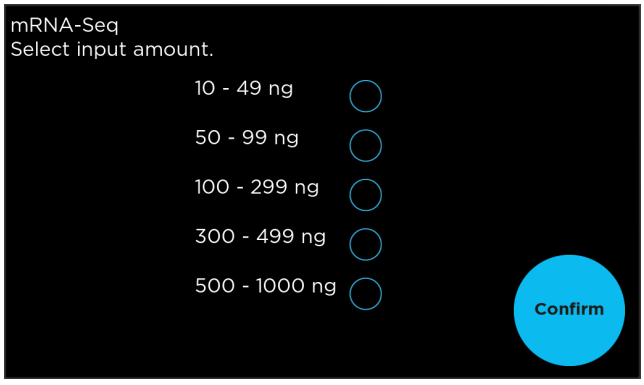

Figure 12. Input range definition.

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 12).

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

4 Protocol

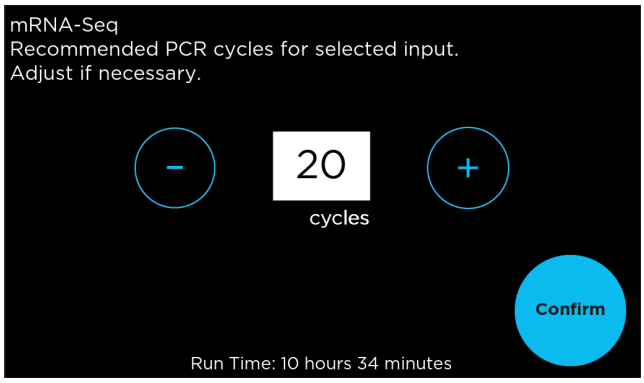

Figure 13. Confirm or adjust the number of PCR cycles for your run.

8. A summary of the run parameters is displayed (Figure 13). 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.

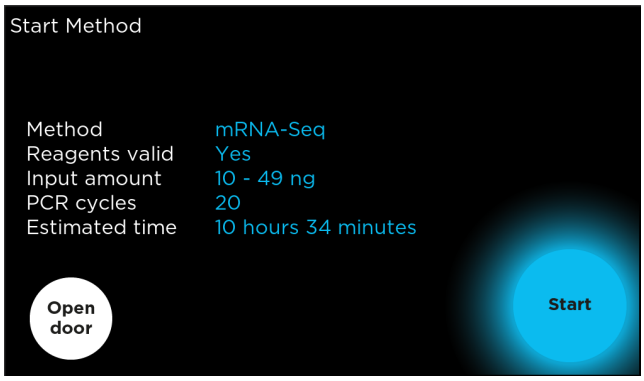

Figure 14. Start method screen.

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.

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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 14). Touch the “Retrieve libraries” button when ready to retrieve your completed libraries.

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 15), placing the sample deck into the sample deck stand.

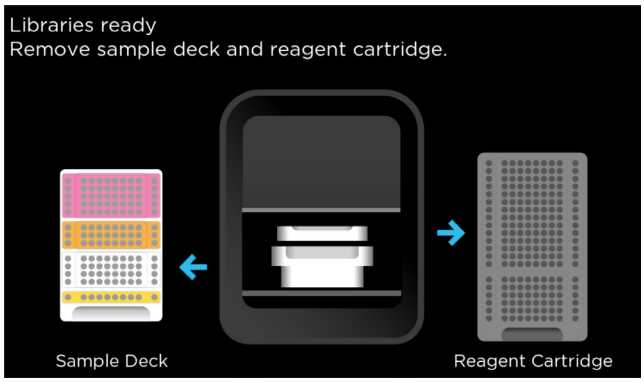

Figure 16. Libraries ready screen.

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 °C until ready for assessment and sequencing.

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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 16.

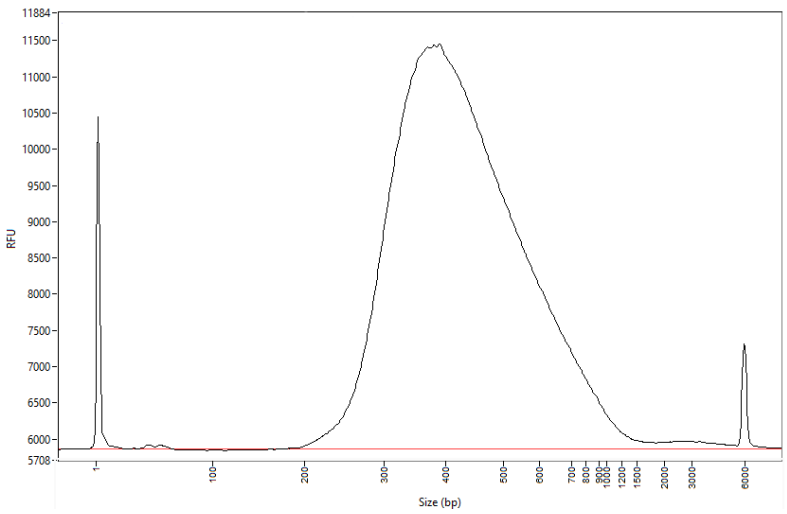

Figure 17. Fragment distribution when 2 µL of 50 ng/µL library is separated on a Fragment Analyzer using the HS NGS Fragment Kit. Starting material is 100 ng K562 total RNA.

2. Quantify the libraries for pooling using a fluorescence or qPCR-based method.
3. Validate the library pool for sequencer loading following the Illumina guidelines, “Best practices for manually normalizing library concentrations” for your specific sequencer. See **Appendix 6.1.** of this guide for guidelines on color balancing and multiplexing of Tecan libraries.
4. Prepare libraries for sequencing following the Illumina “Denature and Dilute Libraries Guide” for your specific sequencer (see **support.illumina.com**).

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 kits are given below in Table 3. Indexes are color balanced in pairs (i.e. A01 + B01, C01 + D01, etc.) and in sets of 8 by column. Revelo mRNA-Seq for MagicPrep NGS index sequences correspond to those used in other Tecan kits utilizing adaptor plate UDI-A.

Table 3. Index sequences for Revelo mRNA-Seq for MagicPrep NGS UDI Adaptor sets.

	Revelo mRNA-Seq for MagicPrep NGS kit A (30186621)			Revelo mRNA-Seq for MagicPrep NGS kit B (30186622)			Revelo mRNA-Seq for MagicPrep NGS kit C (30186623)		
	Index number	Index 1 (i7) sequence	Index 2 (i5) sequence	Index number	Index 1 (i7) sequence	Index 2 (i5) sequence	Index number	Index 1 (i7) sequence	Index 2 (i5) sequence
Sample deck 1	01	CGCTACAT	AACCTACG	33	AGGTTCCT	TCGAACCT	65	GCCTTAAC	CCGTTATG
	02	AATCCAGC	GCATCCTA	34	GAACCTTC	CAAGGTAC	66	ATTCCGCT	TGTCGACT
	03	CGTCTAAC	CAACGAGT	35	AAGTCCTC	AGCTACCA	67	ATCGTGGT	CTCTATCG
	04	AACTCGGA	TGCAAGAC	36	CCACAACA	CATCCAAG	68	GCTACAAC	ACTGCTTG
	05	GTCGAGAA	CTTACAGC	37	ATAACGCC	CTCACCAA	69	TCTACGCA	CGCCTTAT
	06	ACAACAGC	ACCGACAA	38	CCGGAATA	TCAGTAGG	70	CTCCAATC	ATAGGTCC
	07	ATGACAGG	ACATGCCA	39	CCAAGTAG	GAACGTGA	71	ACTCTCCA	TGATCACG
	08	GCACACAA	GAGCAATC	40	AAGGACCA	AGGAACAC	72	GTCTCATC	CGGATCAA
Sample deck 2	09	CTCCTAGT	CCTCATCT	41	ACGCTTCT	CCTAAGTC	73	GCCAGAAT	TACTAGCG
	10	TCTTCGAC	TACTGCTC	42	CTATCCAC	AACGCACA	74	AATGACGC	TGGACCAT
	11	GACTACGA	TTACCGAC	43	TGACAACC	GTCAACAG	75	GTACCACA	GCGCATAT
	12	ACTCCTAC	CCGTAACT	44	CAGTGCTT	ACACCTCA	76	ACGATCAG	ATCGCAAC
	13	CTTCCTTC	TTCCAGGT	45	TCACTCGA	TATGGCAC	77	TAACGTCG	TCAGCCTT
	14	ACCATCCT	CCATGAAC	46	CTGACTAC	CGCAATGT	78	CGCAACTA	CATTGACG
	15	CGTCCATT	TTCCTCCT	47	GTGATCCA	ACTCAACG	79	AACACTGG	ACAGGCAT
	16	AACTTGCC	CCAACTTC	48	ACAGCAAG	GTCTGCAA	80	CCTGTCAA	AGGTCTGT
Sample deck 3	17	GTACACCT	GAGACCAA	49	TGCTGTGA	CACGATTC	81	TCCTGGTA	CAGATCCT
	18	ACGAGAAC	ACAGTTCG	50	CAACACAG	AGAAGCCT	82	CATCAACC	CTCCTGAA
	19	CGACCTAA	CTAACCTG	51	CCACATTG	TACTCCAG	83	AGCAGACA	AGAGGATG
	20	TACATCGG	TCCGATCA	52	TAGTGCCA	CGTCAAGA	84	GAAGACTG	CACCATGA
	21	ATCGTCTC	AGAAGGAC	53	TCGTGCAT	CTGTACCA	85	TCTAGTCC	CGGTAATC
	22	CCAACACT	GACGAACT	54	CTACATCC	TCACCTAG	86	CTCGACTT	GAGTGTGT
	23	TCTAGGAG	TTGCAACG	55	CATACGGA	AACACCAC	87	CTAGCTCA	AACTGAGG
	24	CTCGAACA	CCAACGAA	56	TGCGTAAC	CGTCTTCA	88	TCCAACTG	TGTGTCAG
Sample deck 4	25	ACGGACTT	ATCGGAGA	57	CAGGTTCA	AACGTAGC	89	GACATCTC	TGTCACAC
	26	CTAAGACC	CCTAACAG	58	AGAACCAG	GCAACCAT	90	ACTGCACT	AGATCGTC
	27	AACCGAAC	CATACTCG	59	GAATGGCA	GATCCACT	91	GTTCCATG	CAATGCGA
	28	CCTTAGGT	TGCCTCAA	60	AGGCAATG	ACCTAGAC	92	ACCAAGCA	TGCTTGCT
	29	CCTATACC	TACAGAGC	61	TAGGAGCT	CTAGCAGT	93	CTCTCAGA	AATGGTCG
	30	AACGCCTT	CGAGAGAA	62	CGAACAAC	TCGATGAC	94	ACTCTGAG	AGTTGTGC
	31	TCCATTGC	AGGTAGGA	63	CATTCGTC	TTGGTGCA	95	GCTCAGTT	GTATCGAG
	32	CAAGCCAA	GAACGAAG	64	AGCCAACT	AGTGCATC	96	ATCTGACC	GTACGATC

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Note: Manual sample sheet creation for sequencing on the Illumina MiniSeq, NextSeq, or HiSeq 3000/4000 instruments requires inputting the reverse complement of the Index 2 (i5) sequences in the sample sheet. This is not required if Illumina Experiment Manager (IEM) or BaseSpace Prep tab is used.

6.2 Frequently Asked Questions (FAQs)

Getting Started

Q1. What materials are provided with Revelo mRNA-Seq for MagicPrep NGS?
Revelo mRNA-Seq for MagicPrep NGS 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 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.

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Q8. Do I need to perform an rRNA depletion step before processing samples with Revelo mRNA-Seq for MagicPrep NGS?
No rRNA depletion step is needed. The Revelo mRNA-Seq for MagicPrep NGS workflow performs a poly(A) enrichment step to remove rRNA and non-polyadenylated transcripts.

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.

Q10. Can I use Revelo mRNA-Seq for MagicPrep NGS with RNA from any organism?
Revelo mRNA-Seq for MagicPrep NGS 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 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 deplete ribosomal RNA?
No. Revelo mRNA-Seq 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?
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 libraries stranded?
Yes, libraries prepared with Revelo mRNA-Seq for MagicPrep NGS 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?
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 adaptors add to the library?
The adaptors add 133 bp to the library.

Q17. What is the expected library size?
Revelo mRNA-Seq for MagicPrep NGS 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 libraries are compatible with Illumina sequencing platforms utilizing standard on-board sequencing primers.

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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.

Q20. What kind of error correction is used to minimize the impact of sequencing errors during index reads?
Each index is a minimum edit distance of three from any other index. This allows error correction of one mismatch during demultiplexing. For further details on the index design strategy, please refer to Faircloth BC, Glenn TC (2012), Not All Sequence Tags Are Created Equal: Designing and Validating Sequence Identification Tags Robust to Indels. *PLoS ONE* 7(8): e42543. doi:10.1371/journal.pone.0042543.

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