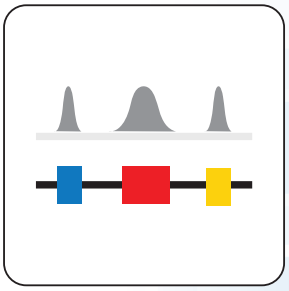
USER GUIDE



Universal RNA-Seq with NuQuant® + UDI

Catalog Numbers: 0537, 0538, 0539, 0540

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Revision: v2

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I. Introduction

A. Background

The Universal RNA-Seq with NuQuant + UDI kit is an end-to-end solution for strand-specific RNA-Seq library construction using nanogram amounts of total RNA obtained from a broad range of tissues or cell lines. The workflow is compatible with both high quality and degraded RNA derived from FFPE samples.

This kit includes AnyDeplete technology for depletion of unwanted transcript sequences. AnyDeplete can be applied to a wide variety of transcripts to reduce uninformative sequencing reads for more efficient use of sequencing resources. This kit also features NuQuant, a novel fluorescent-based method of quantifying library molarity in minutes. NuQuant reduces the time and error associated with standard library QC.

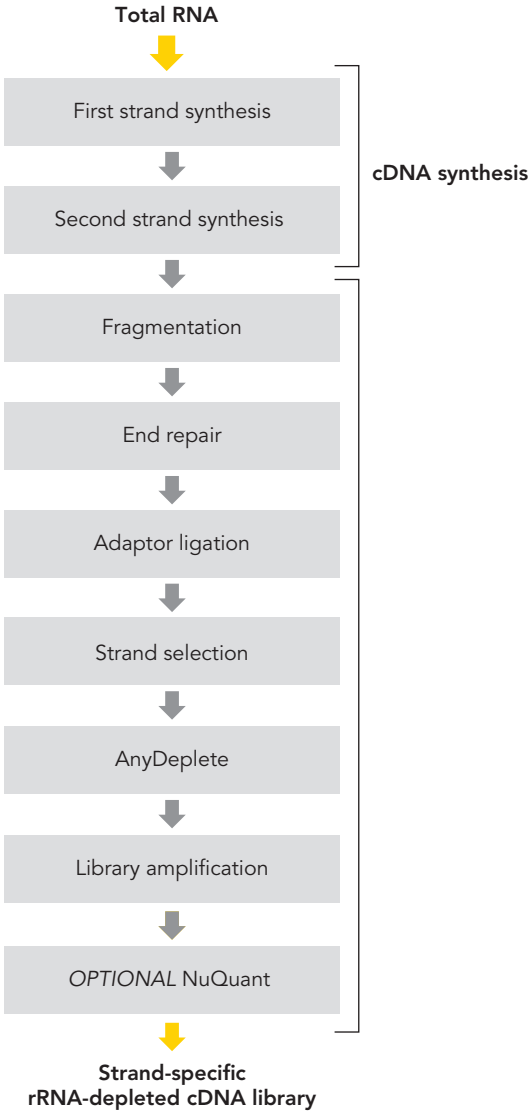
The Universal RNA-Seq with NuQuant + UDI kit provides up to 192 Unique Dual Index (UDI) adaptors with molecular tags enabling multiplex sequencing to further optimize efficiencies and cost savings during transcriptome analysis.

I. Introduction

B. Workflow

As shown in Figure 1, the streamlined workflow consists of double-stranded cDNA generation using a mixture of random and poly(T) priming, optional fragmentation of double-stranded cDNA, end repair, adaptor ligation, strand selection, targeted transcript depletion with AnyDeplete, and PCR amplification to produce the final library. The entire workflow, including fragmentation, can be completed in as few as 7 hours, and yields DNA libraries ready for either single read or paired-end sequencing on Illumina sequencing platforms.

Figure 1. Universal RNA-Seq with NuQuant + UDI workflow.



I. Introduction

C. Perfomance Specifications

The Universal RNA-Seq with NuQuant + UDI kit is designed to generate RNA-Seq libraries suitable for either single read or paired-end sequencing on Illumina NGS platforms. The Universal RNA-Seq with NuQuant + UDI kit provides a fast, simple and robust workflow for generation of Illumina compatible libraries from inputs of as little as 10 ng of high quality total RNA or 100 ng degraded RNA.

D. Quality Control

Every lot of the reagents in Universal RNA-Seq with NuQuant + UDI kit undergoes functional testing to meet specifications for library generation performance.

E. Storage and Stability

The Universal RNA-Seq with NuQuant + UDI kit is shipped on dry ice and should be unpacked immediately upon receipt. All components should be stored at –20 °C on internal shelves of a freezer without a defrost cycle.

The kit has been tested to perform to specifications after as many as six freeze/thaw cycles. Kits handled and stored according to the above guidelines will perform to specifications for at least six months.

F. Safety Data Sheet (SDS)

An SDS for this product is available on the NuGEN website at www.nugen.com/products/universal-rna-seq-library-preparation-kit-nuquant.

G. Before You Start

Please review this User Guide before using this kit for the first time, including the "Kit Components", "Planning the Experiment", "Overview", "Protocol" and "FAQ" sections. For more information, visit the Universal RNA-Seq with NuQuant page at www.nugen.com/products/universal-rna-seq-library-preparation-kit-nuquant.

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

II. Components

A. Reagents Provided

Universal RNA-Seq with NuQuant + UDI is a bundle of the core kit (Part No. 0368) and an Universal AnyDeplete Probe Mix. The core kit can be bundled with AnyDeplete Probe Mix targeting human rRNA (Part Nos. 0537 and 0537B), mouse rRNA+globin (Part Nos. 0538 and 0538B), *Drosophila* rRNA (Part Nos. 0539 and 0539B) or custom AnyDeplete probe mix (Part No. 0540 and 0540B). Universal RNA-Seq with NuQuant + UDI bundles are available with either UDI adaptor plate A (barcodes 1-96) or UDI adaptor plate B (barcodes 97-192).

Table 1. Universal RNA-Seq with NuQuant + UDI (Part No. 0368)

COMPONENT	0368-A01 PART NUMBER	VIAL LABEL	VIAL NUMBER
First Strand Primer Mix	S02680	Blue	A1 VER 13
First Strand Buffer Mix	S02052	Blue	A2 VER 8
First Strand Enzyme Mix	S02053	Blue	A3 VER 4
Second Strand Buffer Mix	S02054 (2)	Yellow	B1 VER 6
Second Strand Enzyme Mix	S02055	Yellow	B2 VER 4
Second Strand Stop Buffer	S02266 (2)	Yellow	B3 VER 2
End Repair Buffer Mix	S02056	Blue	ER1 VER 7
End Repair Enzyme Mix	S01687	Blue	ER2 VER 4
End Repair Enhancer	S02057	Blue	ER3 VER 2
Ligation Buffer Mix	S01689	Yellow	L1 VER 4
Universal Plus UDI 96-plex Adaptor Plate (Part No. 0537, 0538, 0539 and 0540 only)	S02480	—	—
Universal Plus UDI-B 96-plex Adaptor Plate (Part No. 0537B, 0538B, 0539B and 0540B only)	S02690	—	—
Ligation Enzyme Mix	S01690	Yellow	L3 VER 4
Strand Selection Buffer Mix I	S01710 (3)	Purple	SS1
Strand Selection Enzyme Mix I	S02058	Purple	SS2
AnyDeplete Buffer Mix	S02427	Purple	AD1 VER 1
Strand Selection Enzyme Mix II	S02060	Purple	SS4
AnyDeplete Enzyme Mix I	S02428	Purple	AD2 VER 1
AnyDeplete Enzyme Mix II	S02471	Purple	AD3 VER 1

II. Components

Table 1 Ovation Universal RNA-Seq System (Part No. 0343), *continued*

COMPONENT	0358-A01 PART NUMBER	VIAL LABEL	VIAL NUMBER
Amplification Reagent I	S02627	Red	AR1 VER 1
Amplification Reagent II	S02610	Red	AR2 VER 1
Amplification Enzyme Mix	S02629	Red	AR3 VER 1
Nuclease-free Water	S01113 (2)	Green	D1
NuQuant Buffer	S02517	Clear	—
NuQuant Standard	S02512	Clear	—

Table 2. AnyDeplete Probe Mixes (provided in the kit box)

COMPONENT	PART NUMBER	VIAL LABEL	VIAL NUMBER
Human rRNA	ADU001	Purple	Human ADU001
Mouse rRNA + Globin	ADU002	Purple	Mouse ADU002
Drosophila	ADU003	Purple	Drosophila ADU003
Custom AnyDeplete Probe Mixes	Contact your local sales representative or Tecan NGS Technical Support for a list of available AnyDeplete designs or for a new custom AnyDeplete design.		

B. Additional Equipment, Reagents and Labware

Required Materials

- **Equipment**
  - Covaris Sonication System
  - Agilent 2100 Bioanalyzer or other materials and equipment for electrophoretic analysis of nucleic acids
  - Microcentrifuge for individual 1.5 mL and 0.5 mL tubes
  - 0.5–10 µL pipette, 2–20 µL pipette, 20–200 µL pipette, 200–1000 µL pipette
  - 2–20 µL or 5–50 µL multichannel pipette and 20–200 µL or 20–300 µL multichannel pipette, for sample mixing
  - Vortexer
  - Thermal cycler with 0.2 mL tube heat block, heated lid, and 100 µL reaction capacity
  - Qubit® 2.0, 3.0 or 4 Fluorometer (Thermo Fisher Scientific) or appropriate fluorometer and accessories for quantification of fragmented DNA and amplified libraries

II. Components

- **Reagents**
  - Agencourt RNAClean XP Beads or AMPure XP Beads (Beckman Coulter, Cat. #A63987 or A63881)
  - Low-EDTA TE Buffer, 1X, pH 8.0 (Alfa Aesar, Cat. #J75793) optional; for diluting nucleic acids
  - For DNase treatment:
    - 1 M Tris-HCl (Invitrogen, Cat. #15567-027)
    - 0.1 M DTT (dithiothreitol) (Affymetrix, Cat. #70726 150 UL)
    - HL-dsDNase (ArcticZymes, Cat. #70800-201)
  - Ethanol (Sigma-Aldrich, Cat. #E7023), for purification steps
  - Nuclease-free water (Alfa Aesar, Cat. #71786), for purification steps
  - Agilent High Sensitivity DNA Kit (Agilent, Cat. #5067-4626) or equivalent
  - EvaGreen®, 20X (Biotium, Cat. #31000) optional; for optimizing Library Amplification with qPCR
- **Supplies and Labware**
  - Nuclease-free pipette tips
  - 1.5 mL and 0.5 mL RNase-free microcentrifuge tubes
  - 0.2 mL PCR strip tubes or 0.2 mL thin-wall PCR plates
  - Low-retention microcentrifuge tubes (DNA LoBind Tubes, Eppendorf Cat.# 0030108035 or 0030108051)
  - Magnetic stand for 0.2 mL strip tubes or plates. (Beckman Coulter Cat. #A29164 or A32782; Thermo Fisher Scientific Cat. #12331D, 12027, or 12332D; Promega Cat. #V8351; others). Other magnetic stands may be used as well, although their performance has not been validated by Tecan.
  - Cleaning solutions such as RNaseZap® RNase Decontamination Solution (Thermo Fisher Scientific, Cat. #AM9780) and DNA OFF™ (MP Biomedicals, Cat. #11QD0500)
  - 96-well plate sealing foil (Thermo Fisher Scientific, Cat. #AB1720)
  - Disposable gloves
  - Kimwipes
  - Ice bucket

To Order:

- Agilent, www.agilent.com
- Alfa Aesar, www.alfa.com
- Beckman Coulter, www.beckmancoulter.com
- Biotium, www.biotium.com
- Covaris, www.covaris.com
- Eppendorf, www.eppendorf.com
- MP Biomedicals, www.mpbio.com
- Qiagen, www.qiagen.com
- Promega, www.promega.com
- Sigma-Aldrich, Inc., www.sigmaaldrich.com
- Thermo Fisher Scientific, www.thermofisher.com

III. Planning the Experiment

A. Input RNA Requirements

RNA Quantity

Total RNA input must be between 10–100 ng for high quality RNA, and 100–250 ng for degraded RNA. Inputs outside of these ranges may affect reaction stoichiometry, resulting in sub-optimal libraries. Lower input amounts will potentially result in insufficient yields depending on the requirements of the analytical platform. We strongly recommend quantification of total RNA to ensure the minimum input requirement is met.

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, we recommend column purification after isolation. One measure of RNA purity is the ratio of absorbance readings at 260 and 280 nm. The A260:A280 ratio for RNA samples should be in excess of 1.8 and the A260:A230 ratio should be in excess of 2.0.

RNA Integrity

When using degraded total RNA, we recommend using somewhat higher inputs in order to achieve yield and data quality similar to that of more intact RNA samples. RNA integrity can be determined using the Agilent 2100 Bioanalyzer and the RNA 6000 Nano LabChip®. The instrument provides a sensitive and rapid way of confirming RNA integrity prior to processing.

DNase Treatment

Thorough DNase treatment of RNA is required with this system. The presence of genomic DNA in the RNA sample will have adverse effects on assay performance and data quality. For samples that have NOT been DNase-treated, an integrated DNase treatment workflow is included in section **V.A.** of this user guide. For samples that have already been DNase-treated, follow the workflow in section **V.B.** of this user guide.

B. Working with the 96-Plex Adaptor Plates

The Adaptor Plate included with the 96 reaction Universal RNA-Seq with NuQuant + UDI kits contain adaptor mixes, each with a unique eight-base barcode. Each well contains sufficient volume for preparation of a single library. The Adaptor Plates are sealed with a foil seal designed to provide airtight storage.

Thaw adaptor plate on ice and spin down. Do NOT warm above room temperature. Make sure all adaptor mixes are collected at the bottom of the wells and place the adaptor plate on ice after centrifuging. When pipetting the adaptor mixes, puncture the seal for each well you wish to use with a fresh pipet tip, and transfer the appropriate volume of adaptor into your sample. The remaining wells of the plate should remain sealed for use at a later date. Cover used wells with a new foil seal (e.g., AlumaSeal II) to prevent any remaining adaptor-containing liquid from contaminating future reactions.

For barcodes sequence information, please see **Appendix A** on page 30.

III. Planning the Experiment

C. NuQuant

NuQuant® is a novel method to accurately measure molar concentrations of NGS libraries without the need for separate fragment size analysis. NuQuant is a proprietary method by which a specific number of fluorescent labels are incorporated into the library molecules during library preparation. Consequently, each library molecule has an equivalent number of labels incorporated, regardless of the size of the library fragment. The library molar concentration can be directly measured using fluorometers or standard plate readers.

NuQuant is compatible with Qubit 2.0, 3.0 or 4 as well as a wide variety of fluorescent plate readers.

For Qubit-based quantification, an app is required. The apps and installation instructions are available on GitHub: https://nugentechnologies.github.io/NuQuant/

D. Preparation and Storage of NuQuant Standard

Universal RNA-Seq with NuQuant includes a 50X NuQuant Standard stock solution. The fluorescence of this stock corresponds to a 32.3 µM Universal RNA-Seq with NuQuant library. A protocol for preparing the diluted standard for Qubit is provided below. For other fluorometers, a standard curve must be prepared. The diluted NuQuant Standard corresponds to a 644 nM library.

Preparation of Diluted NuQuant Standard for Qubit Fluorometers

Note:

- Concentrated and diluted NuQuant Standards should always be protected from light.
- Diluted NuQuant Standard may be stored at 4 °C for up to two months. Do not freeze diluted NuQuant Standard.

1. Remove concentrated 50X NuQuant Standard stock solution and NuQuant Buffer from storage.
2. Thaw concentrated standard on ice. Mix by vortexing, spin down and place on ice.
3. Prepare diluted NuQuant Standard in a DNA LoBind tube by combining 2 µL of 50X NuQuant Standard stock solution and 98 µL of NuQuant Buffer. Mix thoroughly by vortexing, spin down and store at 4 °C.

E. Amplified Library Storage

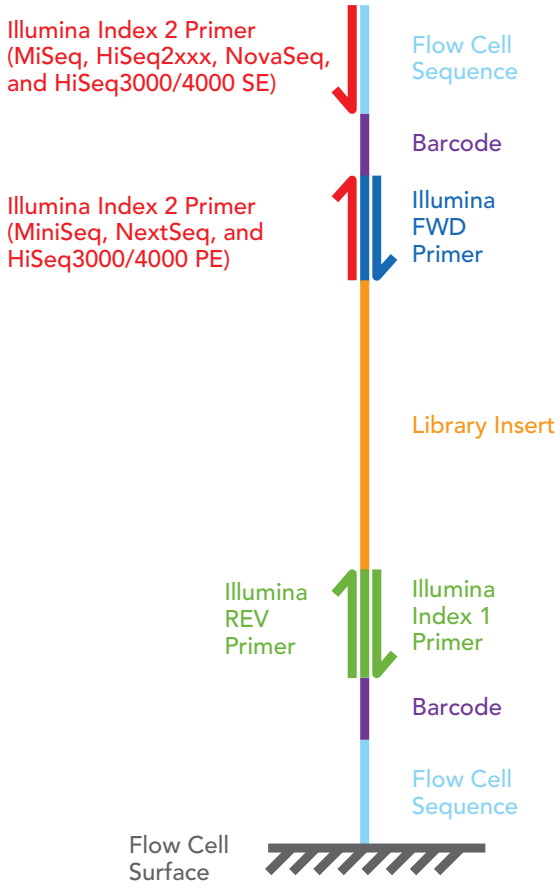
Amplified libraries may be stored at –20 °C.

F. Sequencing Recommendations and Guidelines

The Universal RNA-Seq with NuQuant + UDI kit produces RNA-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. The Universal RNA-Seq libraries contain 8 base Unique Dual Index (UDI) adaptors. Both index 1 (i7) and index 2 (i5) should be sequenced for the detection of "index (barcode) hopping". Additionally, a molecular tag is present in the i7 adaptor to allow detection of PCR duplication events. Index 1 should be sequenced to 16 bases to obtain the index and molecular tag information. Tecan barcodes differ from the sequences used by Illumina and can be found in **Appendix A**.

III. Planning the Experiment

Figure 2. Universal RNA-Seq with NuQuant + UDI Library Structure.



G. Data Analysis

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

Note: The forward read from Universal RNA-Seq with NuQuant + UDI libraries represents the sense strand. This may be opposite to stranded data from other library preparation kits and may require slight modification of the data analysis workflow. Contact Tecan NGS Technical Support (techserv-gn@tecan.com) for more information.

IV. Overview

A. Overview

The library preparation process used in the Universal RNA-Seq with NuQuant + UDI kit is performed in the following stages:

1.	DNase treatment (optional)	0.25 hours
2.	cDNA generation	1.5 hours
3.	Fragmentation and concentration	1.25 hours
4.	End repair	0.75 hours
5.	Adaptor ligation	0.5 hours
6.	Strand selection and AnyDeplete	1.5 hours
7.	Library amplification and purification	1.5 hours
	Total time to prepare amplified library	**7.25 hours**

Components in the Universal RNA-Seq with NuQuant + UDI kit are color coded, with each color linked to a specific stage of the process. Each stage requires making a master mix then adding it to the reaction, followed by incubation.

B. Protocol Notes

Controls

- We recommend the routine use of a positive control RNA, especially the first time a reaction is set up. The use of a positive control RNA will establish a baseline of performance and provide the opportunity to become familiar with the bead purification step. This step may be unfamiliar to many users and can be especially prone to handling variability in using the magnet plate, so a practice run with the plate is highly recommended.
- Routine use of a no template control (NTC) is recommended to monitor the work environment for potential carryover contamination of previous libraries.

General Workflow

- Set up no fewer than or 8 reactions at a time (96-reaction kit) to ensure that you are not pipetting very small volumes and to ensure sufficient reagent recoveries for the full nominal number of samples from the kit. Making master mixes for fewer than 8 samples at a time may affect reagent recovery volumes.
- Thaw components used in each step and immediately place them on ice. Always keep thawed reagents and reaction tubes on ice unless otherwise instructed. Do not thaw all reagents at once.
- After thawing and mixing buffer mixes, if any precipitate is observed, re-dissolve the precipitate completely prior to use. You may gently warm the buffer mix for 2 minutes at room temperature followed by brief vortexing.
- Keep enzyme mixes on ice after briefly spinning to collect the contents. Do not vortex enzyme mixes nor warm any enzyme, adaptor or primer mixes.
- When preparing master mixes, use the minimal amount of extra material to ensure you are able to run the maximum number of reactions using the components provided in the kit.
- When placing small amounts of reagents into the reaction mix, pipet up and down several times to ensure complete transfer from the pipet tip to the reaction mix.

IV. Overview

- When instructed to mix via pipetting, gently aspirate and dispense a volume that is at least half of the total volume of the reaction mix.
- Always allow the thermal cycler to reach the initial incubation temperature prior to placing the tubes or plates in the block.

Reagents

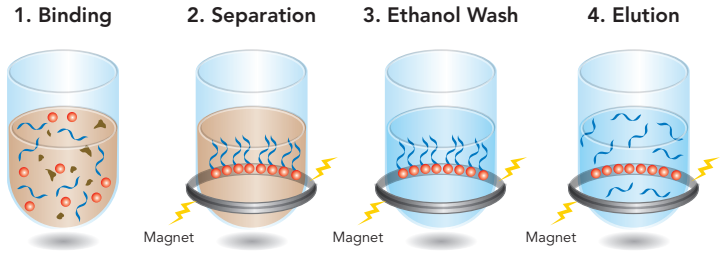
- Use the water provided with the kit (green: D1) or an alternate source of nuclease-free water. We do not recommend the use of DEPC-treated water with this protocol.
- Components and reagents from other Tecan products should not be used with this product.
- Use only fresh ethanol stocks to make ethanol for washes in the purification protocols.
- Make the ethanol mixes fresh, carefully measuring both the ethanol and water with pipettes. Lower concentrations of ethanol in wash solutions will result in loss of yield as the higher aqueous content will dissolve the cDNA and wash it off the beads or column.
- Ensure that all NuQuant reagents are at room temperature before use. Mix diluted libraries and standards thoroughly before performing NuQuant assay.

C. Agencourt® Beads

There are modifications to the Agencourt beads' standard procedure; therefore, you must follow the protocols outlined in this user guide for the use of these beads. The bead purification process consists of:

- Binding of DNA to Agencourt beads
- Magnetic separation of beads from supernatant
- Ethanol wash of bound beads to remove contaminants
- Elution

Figure 3. Agencourt bead purification



Reproduced from original picture from Agencourt/Beckman Coulter Genomics

Tips and Notes

- Remove beads from 4 °C and leave at room temperature for at least 30 minutes prior to use. Cold beads and buffer will result in reduced recovery.
- Prior to use, ensure beads are fully resuspended by vortexing or inverting and tapping the tube.
- Note that our recommendations in the bead protocols may differ from the standard Beckman Coulter protocols. Please follow the protocol as written in this guide.
- If using strip tubes or partial plates, ensure they are firmly placed on the magnetic plate. The use of individual tubes is not advised as they are not very stable on the magnetic plates.
- It is critical to let the beads separate on the magnet for the full time indicated at each step. Removing the binding buffer before the beads have completely separated will impact yields.
- After the binding step has been completed, take care to minimize bead loss when removing the binding buffer. Loss of beads at this step may impact yields. With the samples placed on the mag-

netic plate, carefully remove the specified quantity of binding buffer from each sample to avoid disturbing the beads.

- Ensure that the 70% ethanol wash is freshly prepared from fresh ethanol stocks. Lower percent ethanol mixes will reduce recovery.
- During the ethanol washes, do not allow the beads to disperse. Keep the samples on the magnet in order to keep the beads on the walls of the plate wells or tubes.
- It is critical that all residual ethanol be removed prior to elution. Therefore, when removing the final ethanol wash, first remove most of the ethanol, then allow the excess to collect at the bottom of the tube before removing the remaining ethanol. This also reduces the required bead air-drying time.
- After drying the beads, inspect each tube carefully and make certain that all the ethanol has evaporated before proceeding with the amplification step.

D. Programming the Thermal Cycler

Use a thermal cycler with a heat block designed for 0.2 mL tubes, equipped with a heated lid, and with a capacity of 100 µL reaction volume. Prepare the programs shown in Table 3 following the operating instructions provided by the manufacturer. For thermal cyclers with an adjustable heated lid, set the lid temperature at 100 °C. For thermal cyclers with a fixed-temperature heated lid use the default settings (typically 100 to 105 °C).

Table 3. Thermal Cycler Programming

cDNA SYNTHESIS		VOLUME
Program 1 Primer Annealing	65 °C – 1 min	4 µL or 7 µL
Program 2 DNase Treatment	37 °C – 10 min, 55 °C – 10 min, hold at 4 °C	7 µL
Program 3 First Strand Synthesis	25 °C – 5 min, 40 °C – 5 min, 70 °C – 15 min, hold at 4 °C	10 µL
Program 4 Second Strand Synthesis	16 °C – 60 min, hold at 4 °C	75 µL
END REPAIR		**VOLUME**
Program 5 End Repair	25 °C – 30 min, 70 °C – 10 min, hold at 4 °C	15 µL
LIGATION		**VOLUME**
Program 6 Adaptor Ligation	25 °C – 30 min, hold at 4 °C	30 µL
STRAND SELECTION		**VOLUME**
Program 7 Strand Selection I	72 °C – 10 min, hold at 4 °C	100 µL

IV. Overview

Table 3 Thermal Cycler Programming, *continued*

ANYDEPLETE		VOLUME
Program 8 Probe Binding	37 °C – 10 min, 95 °C – 2 min, 50 °C – 30 s, 65 °C – 5 min, hold at 4 °C	25 µL
Program 9 Targeted Depletion	60 °C – 30 min, 95 °C – 5 min, hold at 4 °C	50 µL
AMPLIFICATION		**VOLUME**
Program 10 Library Amplification	95 °C – 2 min, 2x(95 °C – 30 s, 60 °C – 90 s), 18x*(95 °C – 30 s, 65 °C – 90 s), 65 °C – 5 min, hold at 4 °C	100 µL

*The precise number of PCR cycles required depends on a number of factors including sample type, quality and input amount. The number of PCR cycles may be decreased or increased based on the requirements for a given sample. See **Appendix B** for a method to determine the appropriate number of cycles.

V. Protocol

For each section of the protocol, remove reagents from storage as listed. Thaw and place reagents at room temperature or on ice as instructed. After each section, continue immediately to the next section of the protocol unless otherwise directed.

Return all reagents to their appropriate storage conditions promptly after use unless otherwise instructed.

Important: Several reagents in this kit have similar names. Please verify reagent names before use.

Note: For samples in which RNA has not been DNase treated, start with First Strand cDNA Synthesis with Integrated DNase Treatment (section **V.A.**); for samples in which the RNA has been DNase treated, proceed to First Strand cDNA Synthesis Using DNase-treated RNA (section **V.B.**).

A. First Strand cDNA Synthesis with Integrated DNase Treatment

Important: Thorough DNase treatment of RNA is required with this system. The presence of genomic DNA in the RNA sample will have adverse effects on assay performance and data quality. This workflow is for samples that have NOT been DNase treated. For samples that have already been DNase treated, proceed directly to section **V.B. First Strand cDNA Synthesis Using DNase-treated RNA**.

For this protocol you will need the following additional reagents not included with the kit:

- 1 M Tris-HCl (pH 7.5)
- 0.1 M DTT
- HL-dsDNase
- 20 mM Nuclease-free $MgCl_2$

Table 4. HL-dsDNase Reaction Buffer

REAGENT	1 M TRIS-HCL	0.1 M DTT	NUCLEASE-FREE WATER (GREEN: D1)
STORAGE	—	–20 °C	—
1X REACTION VOLUME	7 µL	7 µL	86 µL

1. Remove Nuclease-free Water (green: D1) from –20 °C storage and place at room temperature.
2. Prepare the HL-dsDNase Reaction Buffer by combining 1 M Tris-HCl, 0.1 M DTT and D1 in a 0.5 mL capped tube according to the volumes shown in Table 4. Mix well by pipetting, spin down, and set aside for use in the DNase Master Mix (Table 5).
3. Remove First Strand Primer Mix (blue: A1 VER 13), First Strand Buffer Mix (blue: A2 VER 8), First Strand Enzyme Mix (blue: A3 VER 4) and HL-dsDNase from –20 °C storage for use in Tables 6 and 7.
4. Spin down A3 and HL-dsDNase and place on ice.
5. Thaw A1 and A2 at room temperature. Mix by vortexing, spin down and place on ice.

V. Protocol

6. Add 2 µL of total RNA (10–100 ng high quality total RNA or 100–250 ng degraded total RNA) to a 0.2 mL PCR tube and place on ice.
7. Add 2 µL of First Strand Primer Mix (A1) to each tube for a total of 4 µL. Mix by pipetting, spin, and place on ice.
8. Place the tubes in a pre-warmed thermal cycler programmed run Program 1 (Primer Annealing; see Table 3):

   65 °C – 1 min
9. Immediately remove the tubes from the thermal cycler and snap chill on ice.

Table 5. DNase Master Mix

REAGENT	$MGCL_2$ SOLUTION	HL-dsDNASE REACTION BUFFER (TABLE 4)	HL-dsDNASE
STORAGE	—	–20 °C	–20 °C
1X REACTION VOLUME	1 µL	1 µL	1 µL

10. Once primer annealing is complete, prepare DNase Master Mix by combining HL-dsDNase Reaction Buffer (Table 4), $MgCl_2$ Solution and HL-dsDNase according to the volumes shown in Table 5.
11. Add 3 µL of DNase Master Mix to each sample tube for a total of 7 µL.
12. Mix by pipetting, spin down and place in a pre-warmed thermal cycler programmed to run Program 2 (DNase Treatment; see Table 3):

    37 °C – 10 min, 55 °C – 10 min, hold at 4 °C

Table 6. First Strand Master Mix

REAGENT	FIRST STRAND BUFFER MIX (BLUE: A2 VER 8)	FIRST STRAND ENZYME MIX (BLUE: A3 VER 4)
STORAGE	—	–20 °C
1X REACTION VOLUME	2.5 µL	0.5 µL

13. Prepare First Strand Master Mix by combining A2 and A3 in a 0.5 mL capped tube according to the volumes shown in Table 6.
14. Add 3 µL of First Stand Master Mix to each sample tube for a total of 10 µL. Mix well by pipetting, spin down and place on ice.
15. Place the tubes in a pre-warmed thermal cycler programmed to run Program 3 (First Strand cDNA Synthesis; see Table 3):

    25 °C – 5 min, 40 °C – 5 min, 70 °C – 15 min, hold at 4 °C
16. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.
17. Continue immediately to section **V.C. Second Strand cDNA Synthesis**.

V. Protocol

B. First Strand cDNA Synthesis Using DNase-treated RNA

Important: Thorough DNase treatment of RNA is required with this system. The presence of genomic DNA in the RNA sample will have adverse effects on assay performance and data quality. This workflow is for samples that have been DNase treated prior to the Universal RNA-Seq with NuQuant protocol.

1. Remove Nuclease-free Water (green: D1) from –20 °C storage and place at room temperature.
2. Remove First Strand Primer Mix (blue: A1 VER 13), First Strand Buffer Mix (blue: A2 VER 8) and First Strand Enzyme Mix (blue: A3 VER 4) from –20 °C storage for use in Table 7.
3. Spin down A3 and place on ice.
4. Thaw A1 and A2 at room temperature. Mix by vortexing, spin down and place on ice.
5. Add 5 µL of total RNA (10–100 ng high quality total RNA or 100–250 ng degraded total RNA) to a 0.2 mL PCR tube.
6. Add 2 µL of First Strand Primer Mix (A1) to each tube for a total of 7 µL. Mix by pipetting, spin, and place on ice.
7. Place the tubes in a pre-warmed thermal cycler programmed run Program 1 (Primer Annealing; see Table 3):

   65 °C – 1 min
8. Immediately remove the tubes from the thermal cycler and snap chill on ice.

Table 7. First Strand Master Mix

REAGENT	FIRST STRAND BUFFER MIX (BLUE: A2 VER 8)	FIRST STRAND ENZYME MIX (BLUE: A3 VER 4)
STORAGE	—	–20 °C
1X REACTION VOLUME	2.5 µL	0.5 µL

9. Once Primer Annealing is complete, prepare First Strand Master Mix by combining A2 and A3 in a 0.5 mL capped tube according to the volumes shown in Table 7.
10. Add 3 µL of First Strand Master Mix to each sample tube for a total of 10 µL. Mix well by pipetting, spin down and place on ice.
11. Place the tubes in a pre-warmed thermal cycler programmed to run Program 3 (First Strand cDNA Synthesis; see Table 3):

    25 °C – 5 min, 40 °C – 5 min, 70 °C – 15 min, hold at 4 °C
12. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.
13. Continue immediately to section **V.C. Second Strand cDNA Synthesis**.

V. Protocol

C. Second Strand cDNA Synthesis

1. Remove Second Strand Stop Buffer (Yellow: B3 VER 2) from –20 °C for use after Program 4.

Table 8. Second Strand Master Mix

REAGENT	SECOND STRAND BUFFER MIX (YELLOW: B1 VER 6)	SECOND STRAND ENZYME MIX (YELLOW: B2 VER 4)
STORAGE	–20 °C	–20 °C
1X REACTION VOLUME	63 µL	2 µL

2. Spin down the contents of B2 and place on ice.
3. Thaw B1 and B3 at room temperature, mix by vortexing, spin and place on ice.
4. Prepare a master mix by combining B1 and B2 in a 0.5 mL capped tube according to the volumes shown in Table 8.
5. Add 65 µL of Second Strand Master Mix to each sample tube for a total of 75 µL. Mix well by pipetting, spin down and place on ice.
6. Place the tubes in a pre-cooled thermal cycler programmed to run Program 4 (Second Strand cDNA Synthesis; see Table 3):

   16 °C – 60 min, hold at 4 °C
7. Remove the tubes from the thermal cycler and spin to collect condensation.
8. Add 45 µL of B3 to each sample tube for a total of 120 µL. Mix well by pipetting and spin down.
9. Continue immediately to section **D. cDNA Fragmentation** or store samples at –20 °C.

Note: cDNA fragmentation may be omitted from the protocol†. To skip cDNA fragmentation, add 25 µl Nuclease-free Water (Green: D1) and continue to section **E. cDNA Concentration**.

Optional stopping point: Store samples at –20 °C.

† Note: Contact Tecan NGS Technical Support for additional information.

V. Protocol

D. cDNA Fragmentation

1. Remove Agencourt beads from 4 °C storage and Nuclease-free water (Green: D1) from –20 °C storage and place on the bench top to reach room temperature for use in the next step.
2. Fragment all DNA samples with Covaris Ultrasonication according to the manufacturer's recommendations.

   Note:

   - When processing cDNA derived from intact total RNA, a median fragment size of 200–500 bp is recommended.
   - Alternative fragmentation methods may be suitable for use with the Universal RNA-Seq kit, but have not been validated by Tecan.
3. After fragmentation, collect 100 µL fragmented cDNA from the Covaris tube. If the collected volume is lower than 100 µL, add nuclease-free water to bring the total volume to 100 µL.

E. cDNA Concentration

1. Ensure the Agencourt beads and D1 have completely reached room temperature before proceeding.
2. Prepare a 70% ethanol wash solution.

   Important:
   - It is critical that the ethanol solution in the purification steps be prepared fresh on the day of the experiment from a recently opened stock container.
   - Measure both the ethanol and the water carefully prior to mixing. Failure to do so can result in a higher than anticipated aqueous content, which may reduce amplification yield.
3. Resuspend the beads by inverting and tapping the tube. Ensure that the beads are fully resuspended before adding to the sample. After resuspending, do not spin the beads.
4. At room temperature, add 180 µL (1.8 volumes) of bead suspension to 100 µL fragmented cDNA†. Mix thoroughly by pipetting.

   Note: At this step, the reaction will be 280 µL. Pipet carefully to avoid spilling the sample.
5. Split each sample into two 140 µL aliquots.
6. Incubate at room temperature for 10 minutes.
7. Transfer the tubes to the magnet and let stand 5 minutes to completely clear the solution of beads.
8. Keeping the tubes on the magnet, carefully remove the binding buffer and discard it.
9. With the tubes still on the magnet, add 200 µL of freshly prepared 70% ethanol and allow to stand for 30 seconds.

   Note: The beads should not disperse; instead, they will stay on the walls of the tubes. Significant loss of beads at this stage will impact cDNA yields, so ensure beads are not removed with the binding buffer or the washes.

† If tubes have become noticably warped or plastic integrity compromised, transfer all of the sample to fresh tubes.

V. Protocol

10. Remove the 70% ethanol wash using a pipette.
11. Repeat the 70% ethanol wash one more time, for a total of two washes.

Note: It is critical to remove as much of the ethanol as possible. Use at least 2 pipetting steps and allow excess ethanol to collect at the bottom of the tubes after removing most of the ethanol in the first pipetting step.

12. Air-dry the beads on the magnet for 10 minutes. Inspect each tube carefully to ensure that all of the ethanol has evaporated.
13. Remove the tubes from the magnet.
14. Add 11 µL room temperature D1 to the first aliquot of dried beads. Mix thoroughly to ensure all beads are resuspended.
15. Transfer the first aliquot of resuspended beads to the second aliquot of dried beads. Mix thoroughly to ensure all of the beads are resuspended.
16. Incubate at room temperature for 3 minutes.
17. Transfer the tubes to the magnet and let stand for 3 minutes to completely clear the solution of beads.
18. Transfer 10 µL of eluate to a fresh 0.2 mL tube, ensuring as few beads as possible are carried over, and place on ice.

Optional stopping point: Store samples at –20 °C.

F. End Repair

Table 9. End Repair Master Mix

REAGENT	END REPAIR BUFFER MIX (BLUE: ER1 VER 7)	END REPAIR ENZYME MIX (BLUE: ER2 VER 4)	END REPAIR ENHANCER (BLUE: ER3 VER 2)
STORAGE	–20 °C	–20 °C	–20 °C
1X REACTION VOLUME	4 µL	0.5 µL	0.5 µL

1. Spin down the contents of ER2 and ER3 and place on ice.
2. Thaw ER1 at room temperature. Mix by vortexing, spin down and place on ice.
3. Prepare a master mix by combining ER1, ER2 and ER3 in a 0.5 mL capped tube according to the volumes shown in Table 9. Mix well by pipetting, spin down and place on ice.
4. Add 5 µL of End Repair Master Mix to each sample tube for a total of 15 µL. Mix well by pipetting, spin down and place on ice.

V. Protocol

5. Place the tubes in a thermal cycler programmed to run Program 5 (End Repair; see Table 3):

   25 °C – 30 min, 70 °C – 10 min, hold at 4 °C

6. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.

G. Adaptor Ligation

Table 10. Ligation Master Mix

REAGENT	NUCLEASE-FREE WATER (GREEN: D1)	LIGATION BUFFER MIX (YELLOW: L1 VER 4)	LIGATION ENZYME MIX (YELLOW: L3 VER 4)
STORAGE	–20 °C	–20 °C	–20 °C
1X REACTION VOLUME	4.5 µL	6.0 µL	1.5 µL

1. Spin down L3 and place on ice.

Note: Thaw adaptor plate on ice and spin down. Do NOT warm above room temperature. Make sure all adaptor mixes are collected at the bottom of the wells and place the adaptor plate on ice after centrifuging.

2. Thaw Adaptor Plate on ice, spin down, and return to ice.
3. Thaw L1 and D1 at room temperature. Mix by vortexing, spin down and place on ice.
4. Add the entire 15 µL of the sample to the appropriate adaptor well, mix well by pipetting, then transfer the entire 18 µL of sample to a PCR tube.
5. Prepare a master mix by combining D1, L1 and L3 in a 0.5 mL capped tube according to the volumes shown in Table 10. Mix by pipetting slowly, without introducing bubbles, spin down and place on ice. Use the master mix immediately.

Note: The L1 Ligation Buffer Mix is very viscous. Pipet this reagent slowly and mix thoroughly.

6. Add 12 µL of Ligation Master Mix to each sample tube for a total of 30 µL. Mix thoroughly by pipetting slowly and gently, spin down and place on ice. Proceed immediately with the incubation.
7. Place the tubes in a thermal cycler programmed to run Program 6 (Adaptor Ligation; see Table 3):

   25 °C – 30 min, hold at 4 °C

8. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.

V. Protocol

H. Strand Selection

1. Remove Agencourt beads from 4 °C storage and Nuclease-free water (Green: D1) from –20 °C storage and place on the bench top to reach room temperature for use in the next step.

Table 11. Strand Selection Master Mix

REAGENT	STRAND SELECTION BUFFER MIX I (PURPLE: SS1)	STRAND SELECTION ENZYME MIX I (PURPLE: SS2)
STORAGE	–20 °C	–20 °C
1X REACTION VOLUME	69 µL	1 µL

2. Thaw SS1 at room temperature. Mix by vortexing, spin down and place on ice.
3. Spin down SS2 and place on ice.
4. Prepare a master mix by combining SS1 and SS2 in a 0.5 mL capped tube according to the volumes shown in Table 11.
5. Add 70 µL of the Strand Selection I Master Mix to each sample for a total of 100 µL. Mix by pipetting, spin down and place on ice.
6. Place the tubes in a pre-warmed thermal cycler programmed to run Program 7 (Strand Selection I; see Table 3):

   72 °C – 10 min, hold at 4 °C
7. Remove the tubes from the thermal cycler and spin to collect condensation.

I. Strand Selection Purification

1. Ensure the Agencourt beads and D1 have completely reached room temperature before proceeding.
2. Resuspend the beads by inverting and tapping the tube. Ensure the beads are fully resuspended before adding to samples. After resuspending, do not spin the beads.
3. Add 80 µL (0.8 volumes) of the bead suspension to the Strand Selection reaction product. Mix thoroughly by pipetting.
4. Incubate at room temperature for 10 minutes.
5. Transfer the tubes to the magnet and let stand 5 minutes to completely clear the solution of beads.
6. Carefully remove the binding buffer and discard it.

Note: The beads should not disperse; instead, they will stay on the walls of the tubes. Significant loss of beads at this stage will impact the amount of DNA carried into subsequent steps of the protocol, so ensure beads are not removed with the binding buffer or the wash.

7. With the tubes still on the magnet, add 200 µL of freshly prepared 70% ethanol and allow to stand for 30 seconds.
8. Remove the 70% ethanol wash using a pipette.

V. Protocol

9. Repeat the 70% ethanol wash one more time, for a total of two washes.

Note: It is critical to remove as much of the ethanol as possible. Use at least two pipetting steps and allow excess ethanol to collect at the bottom of the tubes after removing most of the ethanol in the first pipetting step. Use at least two pipetting steps and allow excess ethanol to collect at the bottom of the tubes after removing most of the ethanol in the first pipetting step.

10. Air dry the beads on the magnet for 10 minutes. Inspect each tube carefully to ensure that all of the ethanol has evaporated. It is critical that all residual ethanol be removed prior to continuing.
11. Remove the tubes from the magnet.
12. Add 16 µL D1 to the dried beads. Mix thoroughly to ensure all beads are resuspended.
13. Transfer the tubes to the magnet and let stand for 3 minutes for the beads to clear the solution.
14. Carefully remove 15 µL of the eluate, ensuring as few beads as possible are carried over, transfer to a fresh set of PCR tubes and place on ice.

Optional stopping point: Store samples at –20 °C.

J. Probe Binding

Table 12. Probe Binding Master Mix

REAGENT	ANYDEPLETE BUFFER MIX (PURPLE: AD1 VER 1)	STRAND SELECTION ENZYME MIX II (PURPLE: SS4)	ANYDEPLETE PROBE MIX (PURPLE: ADXX)	ANYDEPLETE ENZYME MIX I (PURPLE: AD2 VER 1)
STORAGE	–20 °C	–20 °C	–20 °C	–20 °C
1X REACTION VOLUME	5 µL	0.5 µL	4 µL	0.5 µL

1. Spin down SS4 and AD2 and place on ice.
2. Thaw AD1 and AnyDeplete Probe Mix at room temperature. Mix by vortexing, spin down and place on ice.
3. Prepare a master mix by combining AD1, AnyDeplete probe mix, SS4 and AD2 in a 0.5 mL capped tube, according to the volumes shown in Table 12.
4. Add 10 µL of Probe Binding Master Mix to each sample for a total of 25 µL. Mix by pipetting, spin down and place on ice.
5. Place the tubes in a pre-warmed thermal cycler programmed to run Program 8 (Probe Binding; see Table 3):

   37 °C – 10 min, 95 °C – 2 min, 50 °C – 30 s, 65 °C – 5 min, hold at 4 °C

V. Protocol

6. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.

K. Targeted Depletion

Table 13. Targeted Depletion Master Mix

REAGENT	NUCLEASE-FREE WATER (GREEN: D1)	ANYDEPLETE BUFFER MIX (PURPLE: AD1 VER 1)	ANYDEPLETE ENZYME MIX II (PURPLE: AD3 VER 1)
STORAGE	—	–20 °C	–20 °C
1X REACTION VOLUME	16 µL	5 µL	4 µL

1. Spin down AD3 and place on ice.
2. Thaw D1 and AD1 at room temperature. Mix AD1 by vortexing, spin down and place on ice.
3. Prepare a master mix by combining AD1, AD3 and D1 in a 0.5 mL capped tube according the volumes in Table 13. Mix thoroughly by pipetting, spin down and place on ice.
4. Add 25 µL of Targeted Depletion Master Mix to each sample for a total of 50 µL. Mix by pipetting, spin down and place on ice.
5. Place the tubes in a pre-warmed thermal cycler programmed to run Program 9 (Targeted Depletion; see Table 3):

   60 °C – 30 min, 95 °C – 5 min, hold at 4 °C
6. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.

L. Library Amplification

Note: The number of cycles used for PCR amplification should be optimized via real-time PCR whenever using a sample for the first time with the kit, or using a new amount of input. See **Appendix B** for a protocol to determine the appropriate number of cycles for your sample.

1. Remove Agencourt beads from 4 °C storage and Nuclease-free water (Green: D1) from –20 °C storage and place on the bench top to reach room temperature for use in the next section.

V. Protocol

Table 14. Library Amplification Master Mix

REAGENT	NUCLEASE-FREE WATER (GREEN: D1)	AMPLIFICATION REAGENT I (RED: AR1 VER 1)	AMPLIFICATION REAGENT II (RED: AR2 VER 1)	AMPLIFICATION ENZYME MIX (RED: AR3 VER 1)
STORAGE	—	–20 °C	–20 °C	–20 °C
1X REACTION VOLUME	31.5 µL	10 µL	8 µL	0.5 µL

2. Spin down AR3 and place on ice.
3. Thaw AR1 and AR2 at room temperature. Mix by vortexing, spin down and place on ice.
4. Prepare a master mix by sequentially combining D1, AR1 and AR2 in an appropriately sized capped tube, according to the volumes shown in Table 14. Add AR3 at the last moment and mix well by pipetting, taking care to avoid bubbles. Spin down and place on ice.
5. On ice, add 50 µL of Library Amplification Master Mix to each sample for a total of 100 µL.
6. Place the tubes in a pre-warmed thermal cycler programmed to run Program 10 (Library Amplification; see Table 3):

   95 °C – 2 min, 2x(95 °C – 30 s, 60 °C – 90 s), 18x*(95 °C – 30 s, 65 °C – 90 s), 65 °C – 5 min, hold at 4 °C

   *The number of PCR cycles may be decreased or increased based on the requirements for a given sample.
7. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.

Optional stopping point: Store samples at –20 °C.

M. Amplified Library Purification

1. Ensure the Agencourt beads and D1 have completely reached room temperature before proceeding.
2. Resuspend the beads by inverting and tapping the tube. Ensure the beads are fully resuspended before adding to samples. After resuspending, do not spin the beads.
3. At room temperature, add 100 µL of the Library Amplification reaction product to 100 µL (1.0 volumes) of bead suspension. Mix thoroughly by pipetting.

   Note: At this step, the reaction will be 200 µL. Pipet carefully to avoid spilling the sample.
4. Incubate at room temperature for 10 minutes.
5. Transfer the tubes to the magnet and let stand 5 minutes to completely clear the solution of beads.
6. While the tubes are still on the magnet, carefully remove 200 µL of the binding buffer and discard it.

V. Protocol

Note: This is a modifiction to the standard protocol. Remove tubes from the magnet and resuspend beads in 70% ethanol during the wash steps rather than leaving the tubes on the magnet.

7. Remove the tubes from the magnet, add 200 µL of freshly prepared 70% ethanol, mix thoroughly by pipetting, transfer the tubes back to the magnet, and let stand 3–5 minutes to completely clear the solution of beads.
8. Remove the 70% ethanol wash using a pipette.
9. Repeat the 70% ethanol wash one more time for a total of two washes.

Note: With the final wash, it is critical to remove as much of the ethanol as possible. Use at least two pipetting steps and allow excess ethanol to collect at the bottom of the tubes after removing most of the ethanol in the first pipetting step.

10. Air dry the beads on the magnet for a minimum of 10 minutes. Inspect each tube carefully to ensure that all of the ethanol has evaporated. It is critical that all residual ethanol be removed prior to continuing.
11. Remove the tubes from the magnet.
12. Add 26 µL D1 to the dried beads. Mix thoroughly to ensure all beads are resuspended.
13. Incubate at room temperature for 5 minutes.
14. Transfer the tubes to the magnet and let stand for 2 minutes for the beads to completely clear the solution.
15. Carefully remove 25 µL of the eluate, ensuring as few beads as possible are carried over; transfer to a fresh set of PCR tubes and place on ice.

Optional stopping point: Store samples at –20 °C.

N. Quantitative and Qualitative Assessment of the Library

Library Quantification with NuQuant†

Note: A detailed protocol for the Tecan NuQuant Qubit assay is provided below. For information on using NuQuant with other fluorometers and fluorescent plate readers, please contact Tecan NGS Technical Support.

1. Ensure NuQuant Buffer and diluted NuQuant Standard (prepared in section **III. D.**) have reached room temperature before proceeding.
2. Aliquot 200 µL NuQuant Buffer into a new thin-wall, clear, 0.5 mL tube. Label this tube S1 (blank).

† For quantification of libraries without use of NuQuant, a qPCR-based method is recommended. Contact Tecan NGS Technical Support for more information.

V. Protocol

3. Aliquot 195 µL NuQuant Buffer into thin-wall, clear, 0.5-mL tubes. Prepare one tube for the diluted standard and one tube for each library. Label the diluted standard tube S2.

Note: The S2 Standard represents a Universal RNA-Seq with NuQuant library with a concentration of 16.1 nM.

4. Add 5 µL of diluted NuQuant Standard or library to each tube containing NuQuant Buffer from Step 3. Close lids securely, mix thoroughly by vortexing, and spin briefly to collect all liquid into the bottom of the tube.

Important
- The diluted NuQuant Standard may settle over time. Mix thoroughly before aliquoting.
- If standards and libraries are not thoroughly mixed, inaccurate results may occur.

5. Measure samples as directed for your specific quantification platform:
   a. Qubit 2.0: Select "Univ. Plus" from the Qubit home screen. Follow the on screen prompts to read S1, S2, and samples.

Note: For Qubit 2.0, the "Assay Concentration" is displayed after reading each sample. To calculate the stock concentration of each library, select the "Calculate Stock Conc." button, and change the "Volume of Sample Used" to 5 µL. The correct stock concentration of your library will be displayed.

   b. Qubit 3.0 and 4: Navigate to the saved location of Tecan NuQuant app and select "Univ. Plus". Follow the on screen prompts to read S1, S2, and samples.

Note: For Qubit 3.0 and 4, enter the original sample volume (5 µL) after reading standard 2. The result for each sample will be displayed as the "Original Calculated Sample Concentration" and the "Qubit Tube Concentration."

V. Protocol

Library Pooling and Assessment

1. Pool the libraries according to NuQuant concentration. An example using the calculation $V_1=(C_2/N*V_2)/C_1$, where C= concentration, N = number of libraries in the pool and V=volume, is provided in Table 15 below.

Table 15. Example Library Pooling Calculation using NuQuant Concentration

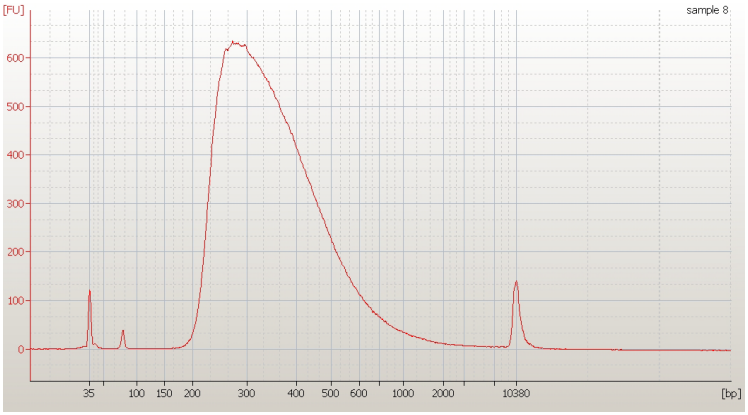
LIBRARY	NUQUANT CONCENTRATION (C_1)	TARGET CONCENTRATION OF POOL (C_2)	TARGET VOLUME OF POOL (V_2)	VOLUME OF LIBRARY TO ADD TO POOL (V_1)
1	250 nM	10 nM	200 µL	2.0 µL
2	220 nM			2.3 µL
3	280 nM			1.8 µL
4	240 nM			2.1 µL
Volume of low-EDTA or nuclease-free water to add				191.8 µL
Total volume of library pool				200 µL

V. Protocol

2. Validate the library pool for sequencer loading following the Illumina guidelines, "Best practices for manually normalizing library concentrations" for your specific sequencer. See **Appendix A**. of this guide for guidelines on color balancing and multiplexing of Tecan libraries. A typical fragment distribution for Universal RNA-Seq with NuQuant libraries prepared with high quality input RNA is shown in Figure 4.

> **Important:** As with any library quantitation method, individual operators, lab equipment and instruments will influence the optimal loading quantitation. Validation of the library pool should be determined empirically for each new library type and sequencer platform.

Figure 4. Library size distribution from total RNA sample. 100 ng of Universal Human Reference total RNA was processed using a 200 bp fragmentation target size. The resulting library was run on an Agilent Bioanalyzer using the High Sensitivity DNA assay. 15 cycles of PCR were performed.



3. Prepare libraries for sequencing following the Illumina "Denature and Dilute Libraries Guide" for your specific sequencer.

VI. 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 contact Tecan NGS Technical Support at +31.13.5780215 (Phone) or email europe-gn@tecan.com.

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

VII. Appendix

A. Barcode Sequences and Guidelines for Multiplex Experiments

Unique Dual Index (UDI) barcode sequences for the 96-plex UDI Adaptor Plates are provided below.

Table 16. Barcode sequences for Universal Plus UDI 96-Plex Adaptor Plate (Indexes 1-96; S02480)

PLATE POSITION	INDEX 1 (i7) SEQUENCE	INDEX 2 (i5) SEQUENCE	PLATE POSITION	INDEX 1 (i7) SEQUENCE	INDEX 2 (i5) SEQUENCE	PLATE POSITION	INDEX 1 (i7) SEQUENCE	INDEX 2 (i5) SEQUENCE
A01	CGCTACAT	AACCTACG	A05	AGGTTCCT	TCGAACCT	A09	GCCTTAAC	CCGTTATG
B01	AATCCAGC	GCATCCTA	B05	GAACCTTC	CAAGGTAC	B09	ATTCCGCT	TGTCGACT
C01	CGTCTAAC	CAACGAGT	C05	AAGTCCTC	AGCTACCA	C09	ATCGTGGT	CTCTATCG
D01	AACTCGGA	TGCAAGAC	D05	CCACAACA	CATCCAAG	D09	GCTACAAC	ACTGCTTG
E01	GTCGAGAA	CTTACAGC	E05	ATAACGCC	CTCACCAA	E09	TCTACGCA	CGCCTTAT
F01	ACAACAGC	ACCGACAA	F05	CCGGAATA	TCAGTAGG	F09	CTCCAATC	ATAGGTCC
G01	ATGACAGG	ACATGCCA	G05	CCAAGTAG	GAACGTGA	G09	ACTCTCCA	TGATCACG
H01	GCACACAA	GAGCAATC	H05	AAGGACCA	AGGAACAC	H09	GTCTCATC	CGGATCAA
A02	CTCCTAGT	CCTCATCT	A06	ACGCTTCT	CCTAAGTC	A10	GCCAGAAT	TACTAGCG
B02	TCTTCGAC	TACTGCTC	B06	CTATCCAC	AACGCACA	B10	AATGACGC	TGGACCAT
C02	GACTACGA	TTACCGAC	C06	TGACAACC	GTCAACAG	C10	GTACCACA	GCGCATAT
D02	ACTCCTAC	CCGTAACT	D06	CAGTGCTT	ACACCTCA	D10	ACGATCAG	ATCGCAAC
E02	CTTCCTTC	TTCCAGGT	E06	TCACTCGA	TATGGCAC	E10	TAACGTCG	TCAGCCTT
F02	ACCATCCT	CCATGAAC	F06	CTGACTAC	CGCAATGT	F10	CGCAACTA	CATTGACG
G02	CGTCCATT	TTCCTCCT	G06	GTGATCCA	ACTCAACG	G10	AACACTGG	ACAGGCAT
H02	AACTTGCC	CCAACTTC	H06	ACAGCAAG	GTCTGCAA	H10	CCTGTCAA	AGGTCTGT
A03	GTACACCT	GAGACCAA	A07	TGCTGTGA	CACGATTC	A11	TCCTGGTA	CAGATCCT
B03	ACGAGAAC	ACAGTTCG	B07	CAACACAG	AGAAGCCT	B11	CATCAACC	CTCCTGAA
C03	CGACCTAA	CTAACCTG	C07	CCACATTG	TACTCCAG	C11	AGCAGACA	AGAGGATG
D03	TACATCGG	TCCGATCA	D07	TAGTGCCA	CGTCAAGA	D11	GAAGACTG	CACCATGA
E03	ATCGTCTC	AGAAGGAC	E07	TCGTGCAT	CTGTACCA	E11	TCTAGTCC	CGGTAATC
F03	CCAACACT	GACGAACT	F07	CTACATCC	TCACCTAG	F11	CTCGACTT	GAGTGTGT
G03	TCTAGGAG	TTGCAACG	G07	CATACGGA	AACACCAC	G11	CTAGCTCA	AACTGAGG
H03	CTCGAACA	CCAACGAA	H07	TGCGTAAC	CGTCTTCA	H11	TCCAACTG	TGTGTCAG
A04	ACGGACTT	ATCGGAGA	A08	CAGGTTCA	AACGTAGC	A12	GACATCTC	TGTCACAC
B04	CTAAGACC	CCTAACAG	B08	AGAACCAG	GCAACCAT	B12	ACTGCACT	AGATCGTC
C04	AACCGAAC	CATACTCG	C08	GAATGGCA	GATCCACT	C12	GTTCCATG	CAATGCGA
D04	CCTTAGGT	TGCCTCAA	D08	AGGCAATG	ACCTAGAC	D12	ACCAAGCA	TGCTTGCT
E04	CCTATACC	TACAGAGC	E08	TAGGAGCT	CTAGCAGT	E12	CTCTCAGA	AATGGTCG
F04	AACGCCTT	CGAGAGAA	F08	CGAACAAC	TCGATGAC	F12	ACTCTGAG	AGTTGTGC
G04	TCCATTGC	AGGTAGGA	G08	CATTCGTC	TTGGTGCA	G12	GCTCAGTT	GTATCGAG
H04	CAAGCCAA	GAACGAAG	H08	AGCCAACT	AGTGCATC	H12	ATCTGACC	GTACGATC

VII. Appendix

Table 17. Barcode sequences for Universal Plus UDI-B 96-Plex Adaptor Plate (Indexes 97-192; S02690)

PLATE POSITION	INDEX 1 (i7) SEQUENCE	INDEX 2 (i5) SEQUENCE	PLATE POSITION	INDEX 1 (i7) SEQUENCE	INDEX 2 (i5) SEQUENCE	PLATE POSITION	INDEX 1 (i7) SEQUENCE	INDEX 2 (i5) SEQUENCE
A01	ACCGGATT	TGGCATGA	A05	CCTTCACA	GTAGAGCA	A09	AACTTCGC	GATGTTCG
B01	CAACCTAG	ATGACCGA	B05	ATGTCGTG	AGGATGTG	B09	ATGGAACG	CATTGCAC
C01	CAATTGGC	TCTGTCCA	C05	GCAGACTT	TCCTCTGA	C09	CGATGAAG	TCGATTCG
D01	CTAATGCC	AACCAAGG	D05	GAACAGAC	GTCTACCT	D09	TTCGCACA	TATGGCTG
E01	TAACTGCG	GTAGTCAC	E05	GTCAAGTC	AAGAGGAG	E09	AACCGGTA	TACTGGAC
F01	CACAACGT	GATCGTAC	F05	TGTTAGCG	AGACGGTT	F09	ATCGACGT	CAGATACG
G01	GACAGCTT	GATACGTG	G05	AGCAGCAA	GTAGAAGC	G09	ATCGAGTC	TTCTGTGC
H01	AACACCGA	CTAGGAAG	H05	GAAGGATC	AGGCTCTT	H09	TGCTGTCT	AGGTCATC
A02	CATAAGCC	CAATGCAG	A06	ATCCTGTC	ACGAGATG	A10	CCACCAAT	ACCACCAA
B02	ACGAACGA	CCACATAC	B06	GCACTACA	GAAGCACA	B10	CCAAGCTA	TGCCATGT
C02	ACGCCAAT	CACACTTC	C06	AACAGTCC	CTTCTCAG	C10	TAGCACCA	CTAGCCTA
D02	ACCGCAAT	GCTTCGAA	D06	ACGTGACT	ACTCGTTC	D10	CCAATGTC	CGCAATCA
E02	CGCACATA	ACCACTGA	E06	ACTCGACA	GCACAAGA	E10	CGATAACG	CGACAACT
F02	AGTGGAAC	AGCTACGT	F06	CACCGATA	GAGTAACC	F10	GCAAGAAC	AGAGCCTA
G02	ACAGATCC	AGCGTACA	G06	GAACAAGC	ACGACCAT	G10	GCTACACA	CCTCACAT
H02	CGTCATAC	TCCAACGA	H06	AGGAAGCT	CAATCACC	H10	CATCACTC	GAACAGCA
A03	GTTACACC	AGAAGTGC	A07	CTGTACTC	CTAGATGC	A11	TACGTTCC	GATGTGGA
B03	TGAGACAG	CTGGTATC	B07	TACCGCTA	CGTAAGGT	B11	AAGGATCG	AAGTCGCA
C03	GCGTAGAT	AATCGCAC	C07	TGTATGGC	CTACCACT	C11	ACAGCAGA	TGAGAGAC
D03	CCTGTATG	GCTCTATG	D07	GAGCCTAT	CTCTGTAC	D11	TCAGCTCT	CCAGCATT
E03	GCAGCATA	GTCATCGA	E07	TCACTCTG	CTGTTACG	E11	TAGGAAGC	GACAATGC
F03	CCTTGTAC	CTCCTACA	F07	ACGCGTTA	CCACCATA	F11	AGGAGTAC	GTCATTGC
G03	GTCAGGAA	TCTTCGGA	G07	ACATTCGG	TCACTGTC	G11	TCGATACC	CAGTACTG
H03	CAATGTGG	TGTCAACG	H07	TAGCACGT	AAGAGTCG	H11	CTATGTCG	CGCATATG
A04	ACCGAGAT	CACCACAA	A08	TCACGACA	ACGAGCAA	A12	CCTACCTA	ACCACAAC
B04	CACAGTCA	TCAACGCT	B08	CCATATCG	TACGGAAC	B12	CGAAGCAA	CCACAATC
C04	ATACGAGC	GCCAATCA	C08	CACGCAAT	TCGTAGAC	C12	GACATGAG	CAGAGAGT
D04	TTCACGCA	ATCTCGCA	D08	AGATTCGC	ACACACGT	D12	CTGAACAC	ATGAAGCC
E04	AAGATGCC	ACTCCAAG	E08	CTGACACA	TCACCGTA	E12	CGAAGGAT	AGTCGCAA
F04	AACCACGT	ACTTCCAC	F08	GTATACCG	TAGTTGCC	F12	GAAGACCA	ACTGCCAT
G04	ACAGAACG	CTCCTAAG	G08	GTGCCTAA	CACTCACT	G12	CAAGCGTA	TGACGTCA
H04	ACGCACAA	CACTGCAA	H08	CCATCTAC	CGACACTA	H12	TCGCACTA	AATCCGCA

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 on the sample sheet. This is not required if the Illumina Experiment Manager (IEM) or BaseSpace Prep tab is used.

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B. Library Amplification Optimization with qPCR

When using the kit for the first time, or working with a new sample type or input amount, we recommend performing a qPCR step prior to Library Amplification to determine the optimum number of cycles needed and ensure there is no excess amplification.

Perform a 1/10th scale qPCR reaction as follows:

Table 18. Library Amplification qPCR Master Mix

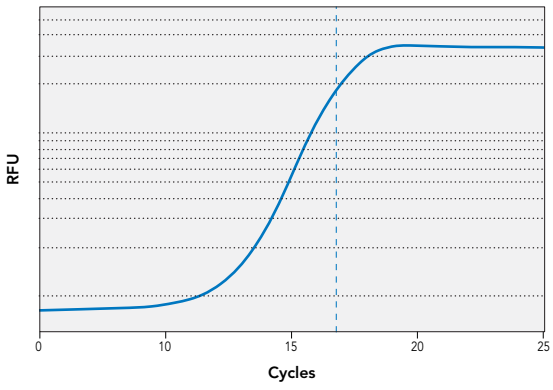
REAGENT	NUCLEASE-FREE WATER (GREEN: D1)	AMPLIFICATION REAGENT I (RED: AR1 VER 1)	AMPLIFICATION REAGENT II (RED: AR2 VER 1)	AMPLIFICATION ENZYME MIX (RED: AR3 VER 1)	20X EvaGreen
STORAGE	—	–20 °C	–20 °C	–20 °C	–20 °C
1X REACTION VOLUME	2.65 µL	1 µL	0.8 µL	0.05 µL	0.5 µL

1. Prepare a PCR master mix according to the volumes shown in Table 17.
2. Aliquot 5.0 µL of PCR master mix per sample into a 0.2 mL qPCR strip or plate.
3. Add 5.0 µL of library (after Targeted Depletion) for a total qPCR volume of 10 µL. Mix well by pipetting, spin and place on ice.
4. Perform real-time qPCR with the following cycling conditions:

   95 °C – 2 min, 2x(95 °C – 30 s, 60 °C – 90 s), 35x(95 °C – 30 s, 65 °C – 90 s),

   The cycle number used for subsequent library amplification should be near the top of the exponential phase of the amplification plot (17 cycles in Figure 5 as an example).

Figure 5. Stylized qPCR Amplification Plot



For PCR, choose a cycle number within the exponential phase of amplification. For PCR, choose a cycle number within the exponential phase of amplification. Follow the steps below to amplify 45 µL of library..

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Table 19. Library Amplification Master Mix

REAGENT	NUCLEASE-FREE WATER (GREEN: D1)	AMPLIFICATION REAGENT I (RED: AR1 VER 1)	AMPLIFICATION REAGENT II (RED: AR2 VER 1)	AMPLIFICATION ENZYME MIX (RED: AR3 VER 1)
STORAGE	—	–20 °C	–20 °C	–20 °C
1X REACTION VOLUME	28.35 µL	9 µL	7.2 µL	0.45 µL

5. Spin down AR3 and place on ice.
6. Thaw D1, AR1 and AR2 at room temperature. Mix by vortexing, spin down and place on ice.
7. Prepare a master mix by sequentially combining D1, AR1 and AR2 in an appropriately sized capped tube, according to the volumes shown in Table 18. Add AR3 at the last moment and mix well by pipetting, taking care to avoid bubbles. Spin down and place on ice.
8. On ice, add 45 µL of Library Amplification Master Mix to each sample for a total of 90 µL.
9. Place the tubes in a pre-warmed thermal cycler programmed to run Program 11 (Library Amplification; see Table 3), where N is the number of cycles determined from the above real-time qPCR assay:

   95 °C – 2 min, 2x(95 °C – 30 s, 60 °C – 90 s), N*(95 °C – 30 s, 65 °C – 90 s), 65 °C – 5 min, hold at 4 °C

   *The number of PCR cycles may be decreased or increased based on the requirements for a given sample.
10. Remove the tubes from the thermal cycler, spin to collect condensation and place on ice.
11. Proceed with section **M. Amplified Library Purification**.

C. Frequently Asked Questions (FAQs)

Getting Started

Q1. What materials are provided with the Universal RNA-Seq with NuQuant + UDI kit?
The Universal RNA-Seq with NuQuant + UDI kit provides all necessary buffers, adaptors, primers and enzymes for library construction. The kit also provides nuclease-free water for purification elution steps. For custom AnyDeplete probes, please contact Tecan NGS Technical Support. Agencourt beads, HL-dsDNase for optional integrated DNase treatment and EvaGreen for optional qPCR must be purchased separately.

Q2. What equipment is required or will be useful?
A list of required and recommended equipment can be found in **section II.B.** of this user guide.

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Input Recommendations

Q3. 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 solvents 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.

Q4. Can I use the Universal RNA-Seq with NuQuant + UDI kit with RNA from any organism?
This system has been designed for use with a broad range of different organisms using organism-specific primers to target specific transcripts for depletion. AnyDeplete probe mixes for human (rRNA), mouse (rRNA plus globin) and *Drosophila* (rRNA) are available. Please contact Tecan NGS Technical Support for a list of available custom AnyDeplete designs or to design a new custom AnyDeplete probe mix.

Q5. Can I use carrier RNA during RNA isolation?
We do not recommend the use of carriers during RNA isolation. If a carrier is required, please contact Tecan NGS Technical Support for more information.

Q6. Do I need to use high-quality total RNA?
The Universal RNA-Seq with NuQuant + UDI kit is designed to work with purified total RNA. When using purified total RNA, samples should be of high molecular weight with little or no evidence of degradation. While it is impossible to guarantee the highest levels of performance when using RNA of lower quality, this system should allow the successful analysis of somewhat degraded samples. With such samples, users may experience lower yields and may encounter affected sequencing metrics.

Q7. Do I need to perform an rRNA depletion or poly(A) enrichment step before processing with the Universal RNA-Seq with NuQuant + UDI kit?
The system is designed to use total RNA as input. rRNA depletion or poly(A) enrichment are not necessary. rRNA, as well as other unwanted transcript types, can be targeted for depletion using AnyDeplete.

Q8. How much total RNA do I need as input?
Refer to section **III.A.** for a discussion of RNA input requirements.

Q9. Can contaminating genomic DNA interfere with the Universal RNA-Seq with NuQuant + UDI kit performance?
Contaminating genomic DNA can interfere with accurate RNA quantification and may be incorporated into libraries. For samples that have not undergone a thorough DNase treatment during RNA purification or is suspected of containing contaminating genomic DNA, we recommend starting the protocol at section **V.A. First Strand cDNA Synthesis with Integrated DNase Treatment**.

General Workflow

Q10. Does this system contain a SPIA®-based amplification?
No. SPIA-based amplification is not used in this kit.

Q11. Does Tecan provide reagents for performing the fragmentation step of the protocol?
We recommend using the Covaris instrument for cDNA fragmentation, as suggested in the "Materials" section of this user guide. Tecan does not provide the reagents used in the fragmentation steps.

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Q12. I don't have access to a Covaris instrument, can I use alternative fragmentation methods?
We have evaluated only Covaris fragmented DNA during the development of these systems. Other mechanical means of fragmentation, such as sonication or enzymatic fragmentation, may be suitable as long as the method generates a tight size distribution of DNA fragments with the required median size.

Q13. Can I skip mechanical fragmentation before cDNA concentration?
Yes, fragmentation can be omitted from the protocol. Note that with high quality input samples, RNA depletion may be less efficient and transcript coverage may be biased towards the 5' end. Contact Tecan NGS Technical Support for additional information.

Q14. Can this system be used with other library preparation workflows?
The Universal RNA-Seq with NuQuant + UDI kit is an end-to-end solution designed to generate libraries for Illumina sequencing starting from total RNA and have not been tested with alternative library preparation systems.

Q15. How does the protocol improve the efficiency of ligation and avoid adaptor dimer formation?
The Universal RNA-Seq with NuQuant + UDI kit utilizes optimized chemistries to increase the efficiency of blunt-end adaptor ligation and minimize the formation of adaptor dimer in the library.

Q16. How does your protocol enable strand retention?
The Universal RNA-Seq with NuQuant + UDI kit utilize targeted degradation of an incorporated modified nucleotide to ensure library inserts all carry the same directionality.

Q17. Where can I safely stop in the protocol?
Samples can be placed in short-term storage at –20°C after second strand synthesis, cDNA purification, end repair, strand selection or after any of the bead purification steps.

SPRI Bead Purifications

Q18. What is the difference between RNAClean XP and AMPure XP SPRI beads? Can both be used interchangeably?
RNAClean XP beads are certified to be RNase and DNase free. We have tested both RNAClean XP and AMPure XP beads in our kits and observe no difference in performance between products.

Q19. What magnetic separation devices do you recommend for the SPRI bead purifications?
Due to the large number of commercially available magnets, we do not have a comprehensive list of compatible products. However, many magnets are compatible. As long as the magnet is strong enough to clear the solution of magnetic beads, it can be applied to the system. We have the following guidelines for selecting a magnetic separation device:

a. Use of a magnet designed for 0.2 mL tubes (PCR tubes) can help improve performance. Compared to magnets that are designed for 1.5 mL tubes, these minimize loss that can occur when samples are transferred from one tube to another.

b. Prior to purchasing, check the manufacturer's specifications for minimum and maximum volumes that can be effectively treated. For the Ovation Universal RNA-Seq System, the minimum volume is 12 µL and the maximum is 200 µL.

c. Test the magnet with a mock purification to ensure the magnet will effectively clear the solution under the conditions in the NuGEN workflow. Performing this mock purification will also help you to gain familiarity with the purification workflow.

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Q20. How can I ensure maximum recovery of sample from the SPRI bead purification?

a. Allow the SPRI beads to reach room temperature before use; cold beads result in lower yields.

b. Ensure that the beads are fully resuspended in solution before adding to the sample.

c. Always use fresh ethanol during the washing steps. When preparing the ethanol, measure out the ethanol and water separately to ensure the desired ethanol concentration is obtained.

d. Mix the bead suspension and sample thoroughly to ensure maximum binding of the samples to the beads.

Library Quantification and Qualification

Q21. How do I measure my final library yield? Can I use an Agilent Bioanalyzer to evaluate the product?

We recommend using NuQuant to accurately quantify the final libraries for multiplex pooling using a Qubit instrument. The final library pool concentration should be determined using a qPCR-based method before loading onto an Illumina sequencer. Alternatively, use standard NGS library QC methods to quantify your library or refer to the Illumina "Denature and Dilute Libraries Guide" for your specific sequencer.

Q22. How many bases do the Universal RNA-Seq with NuQuant + UDI adaptors add to the library?

The adaptors add 138 bp to the size of the final library.

Sequencing Recommendations

Q23. How much material should I load into the sequencer?

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

Q24. What kind of error correction is used to minimize the impact of sequencing errors in the barcodes?

The barcodes provided in the Universal Plus UDI 96-Plex Adaptor Plate (S02480) are a minimum edit distance of 3 from other barcodes in the adaptor plate. This means that a minimum of three edits (replacement, insertion, or deletion) must occur before one barcode becomes a different barcode. The barcodes provided in the Universal Plus UDI-B 96-Plex Adaptor Plate (S02690) are a minimum edit distance of 2 from other barcodes in the adaptor plate. For further details on the barcode 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.

Q25. What sequencing parameters should I use with your libraries?

The Universal RNA-Seq with NuQuant + UDI kit is designed for use with the standard Illumina sequencing primers for both single-end and paired-end sequencing applications. The final libraries contain a unique 8 base index 1 and a unique 8 base index 2 suitable for detection of "index hopping". Both indexes must be sequended to enable detection of "index hopping". Additionally, 8 random bases are present adjacent to index 1 and can be used as a molecular tag to detect PCR duplicates. The index 1 read should be set to sequence 16 bases to capture the molecular tag information for detection of PCR duplicates.

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Q26. Can the Universal RNA-Seq with NuQuant + UDI libraries be used with paired-end sequencing?
Yes, they can be used for both single end and paired-end sequencing. Special consideration should be given to the expected insert size in the paired-end assay. Contact Tecan NGS Technical Support (techserv-gn@tecan.com) for additional information.

Q27. What guidelines do you recommend for sample multiplexing?
Color balancing of the barcodes is recommend when performing low multiplexed sequencing. Color balancing ensures that each flow cell cluster can be efficiently discriminated by having different bases at each cycle of the index read. The Universal Plus UDI adaptor plate (S02480) is color balanced by columns. Using all 8 barcodes from a column will provide a color balanced multiplexed sample. For multiplexing fewer than 8 samples, or when using the Universal Plus UDI-B adaptor plate (S02690) review the barcode sequences being used for appropriate color balance. Contact Tecan NGS Technical Support (techserv-gn@tecan.com) for additional information.

Data Analysis

Q28. Can I use standard alignment algorithms to analyze strand-specific sequencing data?
Yes. Strand-specific reads can be processed and mapped to reference sequences using the same methods used for other RNA-Seq libraries. Note that in libraries generated by the Universal RNA-Seq with NuQuant + UDI kit, the forward read corresponds to the sense strand.

Custom AnyDeplete

Custom depletion designs can be tailored to any transcript, any organism. Please contact Tecan NGS Technical Support at techserv-gn@tecan.com for more information on available existing designs or to develop a new custom design.

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