illumina®

TruSeq® Targeted RNA Expression Reference Guide

For Research Use Only. Not for use in diagnostic procedures.

ILLUMINA PROPRIETARY

Document # 15034665 v01
January 2016

Customize a short end-to-end workflow guide with the Custom Protocol Selector
support.illumina.com/custom-protocol-selector.html

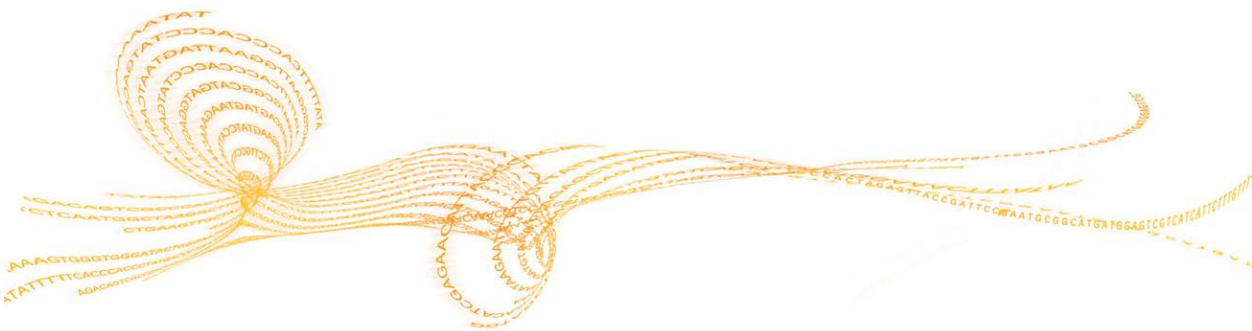
Revision History

Document	Date	Description of Change
Document # 15034665 v01	January 2016	• Changed title of this document to Reference Guide. • Updated design of workflow diagram. • Renamed and combined some procedures as needed to improve continuity. • Simplified consumables information at the beginning of each section. • Revised step-by-step instructions to be more succinct. • Added catalog numbers for fixed panel kits and contents of fixed panel boxes. • Removed box and tube part numbers from Kit Contents. • Removed reference to obsolete Experienced User Cards and added references to Custom Protocol Selector and new protocol guide and checklist.
Part # 15034665 Rev. C	December 2014	• Modified the PCR cycle ranges based on the number of amplicons in the TOP and the type of RNA input for *Amplify Libraries*. • Changed references from 'sample' prep to 'library' prep. • Removed use of plate name (eg, CDP plate), except for first instance and last instance in each procedure. • Updated *Additional Resources*. • Removed list of tables. • Updated SDS link to support.illumina.com/sds.html.
Part # 15034665 Rev. B	March 2014	• Modified recommended input amount of degraded RNA to ≥ 200 ng. • Added Advanced Analytical Technologies Fragment Analyzer option to check total RNA integrity, for degraded RNA pre-qualification, and to determine library concentration. • Added Advanced Analytical Technologies Fragment Analyzer and kits to *Consumables*. • Added reference to the *Expression Analysis of FFPE Samples* tech note. • Modified *Synthesize cDNA* procedures for degraded RNA. • Increased the PCR cycle range for FFPE input in the range of 201–1000 amplicons in the TOP. • Defined a region of 100–300 bp for molarity calculation. • Modified dilution of pooled libraries to 4 nM. • Specified a cluster concentration based on Instrument reagent kit used. • Removed R703 and R706 Index Adapters from and added R709 and R712 Index Adapters to the TruSeq Targeted RNA Index Kit, 48 Indices (192 Samples). • Modified name of *Experience User Card* to *Experienced User Card and Lab Tracking Form*.
Part # 15034665 Rev. A	July 2013	Initial release.

Table of Contents

Overview

Chapter 1



Introduction

This protocol explains how to prepare total RNA using the Illumina® TruSeq® Targeted RNA Expression kits to perform multiplexed gene expression profiling for 12–1000 targets per sample, up to 384 samples in a sequencing run. The goal of this protocol is to prepare samples for 3 types of experiments:

- **Custom Panels**—Select up to 1000 targets from a database of over 400,000 predesigned assays targeting individual exons, splice junctions, coding SNPs (cSNPs), gene fusions, and noncoding RNA transcripts.
- **Fixed Panels**—Use prepared, validated oligo pools covering various biological pathways and disease states.
- **Add-on**—Add a second oligo pool of up to 1000 targets to a fixed panel or previously designed custom panel.

The TruSeq Targeted RNA Expression protocol offers:

- Fast and easy sample preparation
  - Prepare samples and generate data in less than 2 days, with less than 4 hours of hands-on time.
  - Liquid-handling robotics are not necessary.
- An accurate method for validating gene expression arrays and RNA-Seq studies.

NOTE

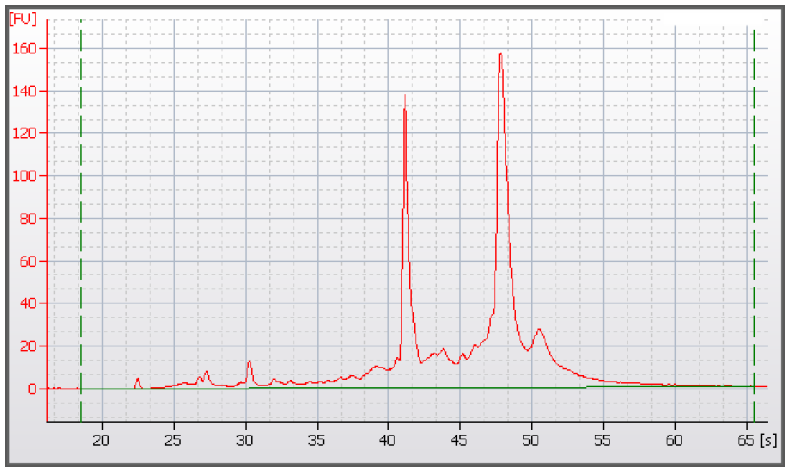
Completing the TruSeq Targeted RNA Expression protocol requires at least 1 TruSeq Targeted RNA Index Kit and at least 1 TruSeq Targeted RNA Fixed or Custom Panel kit. For details, see *Kit Contents* on page 29.

RNA Input Recommendations

Total RNA Input

- The TruSeq Targeted RNA Expression protocol is optimized for 50 ng of total RNA.
- Knowing the quality of the RNA starting material is important.
  - RNA with DNA contamination results in underestimating the amount of RNA used.
  - For samples with an RNA Quality Number (RQN) or RNA Integrity Number (RIN) ≥ 8, check the total RNA integrity after isolation. Use 1 of the following methods:
    - Advanced Analytical Technologies Fragment Analyzer using a Standard Sensitivity RNA Analysis Kit.
    - Agilent Technologies 2100 Bioanalyzer using an RNA 6000 Nano Kit.
  - Include a DNase step with the RNA isolation method.

Figure 1 Universal Human Reference (UHR) Starting RNA Bioanalyzer Trace



Degraded RNA

Depending on the quality of the sample, use ≥ 200 ng of RNA for degraded or FFPE samples.

- After isolation, check the total RNA integrity to make sure that the average fragment size ≥ 200 bp. Use 1 of the following methods:
  - Advanced Analytical Technologies Fragment Analyzer using a Standard Sensitivity RNA Analysis Kit.
  - Agilent Technologies 2100 Bioanalyzer using an RNA 6000 Nano Kit.
- Degraded or FFPE RNA is shorter than full length RNA.
  - RNA with DNA contamination results in underestimating the amount of RNA used.
  - If starting with FFPE RNA, the sample input amount is based on sample quality. Use the percentage of RNA fragments > 200 nt fragment distribution value (DV_{200}) as a reliable determinant of FFPE RNA quality.

Table 1 FFPE RNA Input Recommendations

Quality	DV_{200}	Input Requirement Per Reaction
High	> 70%	200 ng
Medium	50–70%	400 ng
Low	30–50%	> 400 ng
Too Degraded	< 30%	Not recommended

Positive Control

Assay dropouts, where the number of counts is close to 0, might occur in highly degraded FFPE samples. Run FFPE samples in duplicate and use a positive control in each batch of samples. The control enables troubleshooting, if needed.

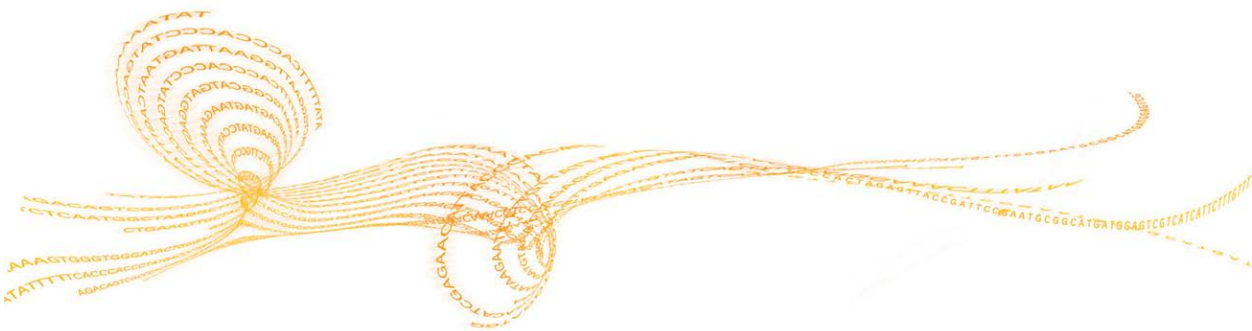
Use Agilent Technologies Human UHR total RNA (catalog # 740000) as a positive control sample for this protocol.

Additional Resources

Visit the TruSeq Targeted RNA Expression support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

Resource	Description
Custom Protocol Selector	http://support.illumina.com/custom-protocol-selector.html A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
TruSeq Targeted RNA Expression Protocol Guide (document # 1000000005002)	Provides only protocol instructions. The protocol guide is intended for experienced users. For new or less experienced users, see the TruSeq Targeted RNA Expression Reference Guide.
TruSeq Targeted RNA Expression Checklist (document # 1000000005012)	Provides a checklist of the protocol steps. The checklist is intended for experienced users. For new or less experienced users, see the TruSeq Targeted RNA Expression Reference Guide.
Expression Analysis of FFPE Samples tech note	Provides effectivity profiles for FFPE RNA.

Protocol



Introduction

This chapter describes the TruSeq Targeted RNA Expression protocol.

- Follow the protocol in the order shown, using the specified volumes and incubation parameters.
- Review Best Practices from the TruSeq Targeted RNA Expression support page on the Illumina website.
- Process samples in batches of no fewer than 16 samples.

Prepare for Pooling

If you plan to pool libraries, record information about your samples before beginning library prep. Different methods are available depending on the sequencing instrument you are using. See the TruSeq Targeted RNA Expression support page for more information.

Tips and Techniques

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Avoiding Cross-Contamination

- When adding or transferring samples, change tips between ***each sample***.
- When adding adapters or primers, change tips between ***each row*** and ***each column***.
- Remove unused index adapter tubes from the working area.

Sealing the Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Vortexing steps
  - Centrifuge steps
  - Thermal cycling steps
- Apply the adhesive seal to cover the plate and seal with a rubber roller.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates. Use Microseal 'B' for shaking, centrifuging, and long-term storage.
- Microseal 'A' adhesive film is effective for thermal cycling and easy to cut when using fewer than 96 wells.

Plate Transfers

- When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.

Centrifugation

- Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.

Handling Beads

- Pipette bead suspension slowly.
- When mixing, mix thoroughly.
- If beads are aspirated into the pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).
- When washing beads:
  - Use the appropriate magnet for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Keep the plate on the magnet until the instructions specify to remove it.
  - Do not agitate the plate while on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

The following diagram illustrates the workflow using a TruSeq Targeted RNA Expression kit. Safe stopping points are marked between steps.

Figure 2 TruSeq Targeted RNA Expression Workflow

Synthesize cDNA

This process reverse transcribes RNA into first strand cDNA using reverse transcriptase and random primers.

Consumables

- RCS1 (Reverse Transcription cDNA Synthesis Master Mix 1)
- 1.7 ml microcentrifuge tubes
- 96-well HSP plates (2)
- Microseal 'B' adhesive seals (4)
- Nuclease-free water
- RNase/DNase-free 8-tube strips and caps
- ProtoScript II Reverse Transcriptase (1.1 µl for intact total RNA or 2.1 µl for degraded RNA, per reaction)
- RNA
  - Intact total (50 ng per reaction) or
  - Degraded (≥ 200 ng per reaction)

About Reagents

- You can add intact total RNA and degraded RNA samples to the same plate.

Preparation

1. Prepare the following consumables:

Item	Storage	Instructions
ProtoScript II	-25°C to -15°C	Thaw at room temperature.
RCS1	-25°C to -15°C	Thaw at room temperature.

2. Save 1 of the following programs on a thermal cycler:
   - For intact total RNA, save the CDNASYN1 program:
     - Choose the preheat lid option and set to 100°C
     - 25°C for 5 minutes
     - 42°C for 15 minutes
     - 95°C for 10 minutes
     - Hold at 4°C
   - For degraded RNA or both RNA input types, save the CDNASYN2 program:
     - Choose the preheat lid option and set to 100°C
     - 25°C for 10 minutes
     - 42°C for 30 minutes
     - 95°C for 10 minutes
     - Hold at 4°C
3. Label a new 96-well HSP plate according to the input RNA:
   - CDP1 for intact total RNA.
   - CPD for degraded RNA.

Procedure

1. Vortex RCS1 for 5 seconds.

2 Centrifuge at 600 × g for 5 seconds.

3 Dilute according to your input RNA:
- Dilute 50 ng intact total RNA with nuclease-free water to 5 µl.
- Dilute ≥ 200 ng degraded RNA with nuclease-free water to 3 µl.

4 Add diluted RNA to the appropriate plate:
- Add 5 µl diluted intact total RNA to each well of the CDP1 plate.
- Add 3 µl diluted degraded RNA to each well of the CDP plate.

5 Combine the following volumes in a new 1.7 ml microcentrifuge tube. Multiply each volume by the number of samples being prepared.
Volumes include 10% extra reagent for multiple pools.

Reagent	Intact Total RNA Volume (µl)	Degraded RNA Volume (µl)
RCS1	4.4	4.4
ProtoScript II Reverse Transcriptase	1.1	2.2
10X DTT (0.1M)*	0	1.1
Total volume per pool	5.5	7.7

* Included with ProtoScript II Reverse Transcriptase reagent.

6 Invert to mix.

7 Centrifuge at 600 × g for 5 seconds.

8 Distribute evenly into each well of an 8-tube strip.

9 Add the volume appropriate for your plate:
- Add 5 µl to each sample well of the CDP1 plate.
- Add 7 µl to each sample well of the CDP plate.

10 Shake at 1600 rpm for 20 seconds.

11 Centrifuge at 280 × g for 1 minute.

12 Place on the preprogrammed thermal cycler and run the CDNASYN1 or CDNASYN2 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 7 days. Alternatively, leave on the thermal cycler overnight.

Hybridize Oligo Pool

This process hybridizes an oligo pool that contains upstream and downstream oligos specific to targeted regions of interest and then binds them to paramagnetic streptavidin beads. Combine up to 2 targeted oligo pools.

Consumables

- OB1 (Paramagnetic Streptavidin Beads)
- TOP (Targeted Oligo Pool)
- Microseal 'B' adhesive seals (2)
- 1.7 ml microcentrifuge tube
- [Optional] TE buffer (5 µl per reaction)

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region. For environmental, health, and safety information, see the SDS for this kit at support.illumina.com/sds.html.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
OB1	-25°C to -15°C	Thaw at room temperature.
TOP	-25°C to -15°C	Thaw at room temperature. Vortex for 5 seconds and then centrifuge briefly.
CDP or CDP1 plate	-25°C to -15°C	Thaw at room temperature. Centrifuge at 280 × g for 1 minute.

2 Save the following program as ANNEAL on a thermal cycler:
- Choose the preheat lid option and set to 100°C
- 70°C for 5 minutes
- 68°C for 1 minute
- 65°C for 2.5 minutes
- 60°C for 2.5 minutes
- 55°C for 4 minutes
- 50°C for 4 minutes
- 45°C for 4 minutes
- 40°C for 4 minutes
- 35°C for 4 minutes
- 30°C for 4 minutes
- Hold at 30°C

3 Unseal the CDP or CDP1 plate.

Procedure

1 Combine the following volumes in a new 1.7 ml microcentrifuge tube. Multiply each volume by the number of reactions being prepared.
Volumes include 10% extra reagent for multiple reactions.

Reagent	Volume (µl)
TOP	5.5
Additional TOP or TE buffer	5.5
Total volume per reaction	11

2 Vortex for 5 seconds.

3 Centrifuge at 600 × g for 5 seconds.

4 Distribute evenly into each well of an 8-tube strip.

5 Add 10 µl to each well of the CDP or CDP1 plate.

6 Shake at 1600 rpm for 20 seconds.

7 Incubate at room temperature for 1 minute.

8 Vortex OB1 for 5 seconds. Make sure volumes are thoroughly mixed.

9 Add 30 µl OB1 to each well of the CDP or CDP1 plate.

10 Shake at 1600 rpm for 1 minute.

11 Place on the preprogrammed thermal cycler and run the ANNEAL program.

12 Centrifuge briefly.

Wash, Extend, and Ligate Bound Oligos

This process removes unbound oligos from cDNA and adds sequences required for amplification. An AM1 wash removes unbound oligos from hybridized cDNA. A subsequent UB1 wash removes any residual AM1. A DNA polymerase extends from the upstream oligo through the targeted region, followed by ligation to the 5′ end of the downstream oligo.

Consumables

- AM1 (Wash 1 Buffer)
- ELM4 (Extension and Ligation Mix 4)
- RSB (Resuspension Buffer)
- UB1 (Wash 2 Buffer)
- 96-well midi plate
- Microseal 'B' adhesive seals (3)
- [Optional] RNase/DNase-free reagent reservoirs (4)

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region. For environmental, health, and safety information, see the SDS for this kit at support.illumina.com/sds.html.

About Reagents

- You can add intact total RNA and degraded RNA from different plates to the same HYP plate.
- UB1 is used 2 times during the process.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
AM1	-25°C to -15°C	Thaw at room temperature. Store at 2°C to 8°C. AM1 can be stored at 2°C to 8°C after the initial thaw.
RSB	-25°C to -15°C	Thaw at room temperature. RSB can be stored at 2°C to 8°C after the initial thaw.
UB1	-25°C to -15°C	Thaw at room temperature. Store at 2°C to 8°C. UB1 can be stored at 2°C to 8°C after the initial thaw.
ELM4	-25°C to -15°C	Thaw on ice.

2 Place the midi-plate insert inside the microheating system and preheat to 37°C.

3 Label a new 96-well midi plate HYP.

4 Unseal the CDP or CDP1 plate.

Procedure

1 Transfer all supernatant to the corresponding well of the HYP plate.

2 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

3 Remove and discard all of the supernatant from each well.
4 Move from the magnetic stand to a bench.
5 Add 100 µl AM1 to each well to resuspend the pellet.
6 Shake at 1800 rpm for 2 minutes.
7 Centrifuge at 280 × g for 5 seconds.
8 Unseal and place on a magnetic stand until the liquid is clear (~2 minutes).
9 Remove and discard all supernatant from each well.
10 Move from the magnetic stand to a bench.
11 Add 175 µl UB1 to each well.
12 Shake at 1800 rpm for 2 minutes.
13 Centrifuge at 280 × g for 5 seconds.
14 Unseal and place on a magnetic stand until the liquid is clear (~2 minutes).
15 Invert ELM4 to mix.
16 Remove and discard all supernatant from each well.
17 Move from the magnetic stand to a bench.
18 Add 40 of ELM4 to each well.
19 Shake at 1800 rpm for 2 minutes.
20 Centrifuge at 280 × g for 5 seconds.
21 Place on the 37°C preheated microheating system and incubate for 45 minutes.
22 Remove the adhesive seal from the plate.
23 Unseal and place on a magnetic stand until the liquid is clear (~2 minutes).
24 Remove and discard all supernatant from each well.
25 Add 50 µl of UB1 to each well.

Amplify Libraries

This process amplifies the extension-ligation product and adds Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for cluster formation.

Consumables

- Index 1 (i7) adapters and white tube caps
- Index 2 (i5) adapters and orange tube caps
- HP3 (2N NaOH)
- PMM2 (PCR Master Mix 2)
- TDP1 (TruSeq DNA Polymerase 1)
- 1.7 ml microcentrifuge tube (1 more than the number of adapters)
- 5 ml microcentrifuge tube
- 96-well HSP plate
- Microseal 'A' film
- Microseal 'B' adhesive seals (2)
- Nuclease-free water (1.3 ml)
- TruSeq Index Plate Fixture Kit
- [Optional] RNase/DNase-free reagent reservoirs (4)
- [Optional] RNase/DNase-free 8-tube strips and caps (3)

About Reagents

- A fresh dilution of HP3 (2 N NaOH) is critical for successful denaturing.
- Maintaining an accurate pH is critical for elution of cDNA from the beads.
- After thawing, you can store undiluted HP3 at -25°C to -15°C.
- Add TDP1 to PMM2 immediately before use only, and do not store.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
Index adapters (i5 and i7)	-25°C to -15°C	Only remove adapters being used. Thaw at room temperature. Vortex each tube to mix. Centrifuge 600 × g for 5 seconds using 1.7 ml microcentrifuge tubes.
HP3	-25°C to -15°C	Thaw at room temperature. Dilute 1:40 with nuclease-free water in a new 5 ml microcentrifuge tube. Calculate the volume as 25 µl per sample, and add 10% when preparing multiple samples. Invert to mix.
PMM2	-25°C to -15°C	Thaw at room temperature.
TDP1	-25°C to -15°C	Thaw at room temperature.

2 Label a new 96-well HSP plate IAP.

3 Determine the required number (X) of PCR cycles using the following table:

RNA Input	Number of PCR Cycles (X)	
	High Quality (RIN ≥ 8.0)	Degraded (RIN < 8.0)
12–48 amplicons	32–36	34–38
49–96 amplicons	30–32	32–34

RNA Input	Number of PCR Cycles (X)	
	High Quality (RIN ≥ 8.0)	Degraded (RIN < 8.0)
97–200 amplicons	27–30	30–32
201–384 amplicons	25–27	28–30
385–1000 amplicons	23–25	26–28

4 Save the following program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 95°C for 2 minutes
- X cycles of:
  - 98°C for 30 seconds
  - 62°C for 30 seconds
  - 72°C for 60 seconds
- 72°C for 5 minutes
- Hold at 10°C

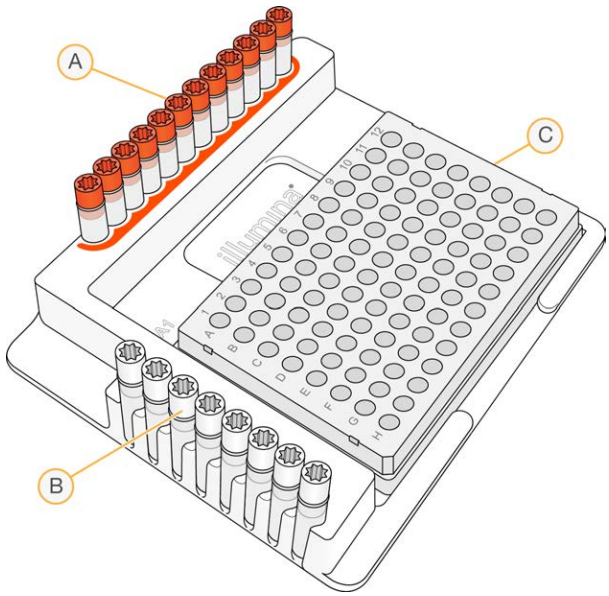
Procedure

1 Arrange Index 1 (i7) adapters in columns 1–12 of the TruSeq Index Plate Fixture.

2 Arrange Index 2 (i5) adapters in rows A–H of the TruSeq Index Plate Fixture.

3 Place the plate on the TruSeq Index Plate Fixture.

Figure 3 TruSeq Index Plate Fixture (96 libraries)



A Columns 1–12: Index 1 (i7) adapters (orange caps)
B Rows A–H: Index 2 (i5) adapters (white caps)
C 96-well plate

4 Using a multichannel pipette, add 4 µl of each Index 1 (i7) adapter down each column. Replace the cap on each i7 adapter tube with a new orange cap.

5 Using a multichannel pipette, add 4 µl of each Index 2 (i5) adapter across each row. Replace the cap on each i5 adapter tube with a new white cap.

6 Remove and discard all supernatant from the HYP plate.

7 Remove from the magnetic stand.

8 Add 22.5 µl diluted HP3 to each well.

9 Shake at 1800 rpm for 30 seconds.

10 Incubate at room temperature for at least 5 minutes.

11 While the plate is incubating, create the amplification mix:
- **96 libraries**—Add 56 µl TDP1 to 2.8 ml of PMM2.
- **48 libraries**—Combine 28 µl TDP1 and 1.4 ml PMM2 in a new 1.7 ml microcentrifuge tube.
- **16 libraries**—Combine 9.2 µl TDP1 and 460 µl PMM2 in a new 1.7 ml microcentrifuge tube.

12 Invert to mix.

13 Add 22 µl to each well of the IAP plate that contains index adapters.

14 Unseal the HYP plate.

15 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

16 Transfer 20 µl supernatant from each well of the HYP plate to the corresponding well of the IAP plate.

17 Shake at 1600 rpm for 30 seconds.

18 Centrifuge at 280 × g for 1 minute.

19 Place on the preprogrammed thermal cycler and run the program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Libraries

This process uses AMPure XP beads to purify the PCR products from the other reaction components.

Consumables

- RSB (Resuspension Buffer)
- 96-well HSP plate
- 96-well midi plate
- AMPure XP beads (85 µl per reaction)
- Freshly prepared 80% ethanol (EtOH) (400 µl per reaction)
- Microseal 'B' adhesive seals (3)
- [Optional] RNase/DNase-free reagent reservoirs (3)

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
AMPure XP beads	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
IAP plate	2°C to 8°C	If stored, let stand for 30 minutes to bring to room temperature. Centrifuge at 280 × g for 1 minute, and then unseal.

2 Prepare fresh 80% EtOH from absolute ethanol.

3 Vortex the AMPure XP Beads to disperse.

4 Label a new 96-well HSP plate LNP.

5 Label a new 96-well midi plate CLP.

Procedure

1 Add 85 µl AMPure XP Beads to each well of the CLP plate.

2 Centrifuge the IAP plate at 280 × g for 1 minute.

3 Unseal the IAP plate.

4 Transfer all supernatant from each well of the IAP plate to the corresponding well of the CLP plate.

5 Shake at 1800 rpm for 2 minutes.

6 Centrifuge the plate at 280 × g for 5 seconds.

7 Incubate room temperature for 15 minutes

8 Unseal and place on a magnetic stand until the liquid is clear (~5 minutes).

9 Remove and discard 135 µl supernatant from each well.

10 Wash 2 times as follows.

a Add 200 µl 80% EtOH to each well without disturbing the beads.
b Incubate at room temperature for 30 seconds.
c Remove and discard all supernatant from each well.

11 Air dry on the magnetic stand for 15 minutes.

12 Remove from the magnetic stand.

13 Add 15 µl RSB to each well to resuspend the dried pellet.

14 Shake at 1800 rpm for 2 minutes.

15 Centrifuge at 280 × g for 5 seconds.

16 Return RSB to 2°C to 8°C storage.

17 Incubate the CLP plate at room temperature for 2 minutes.

18 Unseal and place on a magnetic stand until the liquid is clear (~5 minutes).

19 Transfer 12.5 µl supernatant from each well of the CLP plate to the corresponding well of the LNP plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Pool and Quantify Libraries

This process combines, quantifies, denatures, and dilutes equal volumes of library in hybridization buffer. For help determining which libraries to pool, see *Pooling Guidelines* on page 40.

Consumables

- HP3 (2N NaOH)
- RSB (Resuspension Buffer)
- 2 ml microcentrifuge tubes (2 per pooled library, plus 1)
- Nuclease-free water (90 µl)
- Agilent DNA 1000 Kit, or
- Advanced Analytical Standard Sensitivity NGS Fragment Analysis Kit (1 bp–6000 bp)
- [Optional] RNase/DNase-free reagent reservoirs

About Reagents

- A fresh dilution of HP3 is critical for successful denaturing.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
HP3	-25°C to -15°C	Thaw on ice. Combine 10 µl HP3 with 90 µl nuclease-free water to prepare a fresh dilution of 0.2 M HP3. Invert to mix.
RSB	2°C to 8°C	Let stand 30 minutes to bring to room temperature.
LNP plate	-25°C to -15°C	If stored, thaw at room temperature. Centrifuge at 280 × g for 1 minute, and then unseal.

Procedure

1 Transfer 5 µl from each well of the LNP plate to a new 2 ml microcentrifuge tube.

2 Vortex for 5 seconds.

3 Centrifuge at 600 × g for 5 seconds.

4 Load 1 µl pooled library onto the Standard Sensitivity NGS Fragment Analysis Kit or DNA 1000 Kit.

5 Determine the concentration of the pooled library using the Advanced Analytical Technologies Fragment Analyzer or Agilent Technologies 2100 Bioanalyzer.

6 Select the **Region Analysis** tab.

7 Drag the blue region lines to capture the 100–300 bp region.

NOTE
When using degraded RNA, a nonspecific peak at approximately 150 bp might be present. Include this peak. The peak clusters, but does not align to the manifest.

Figure 4 Example of an Intact RNA Library Size Distribution

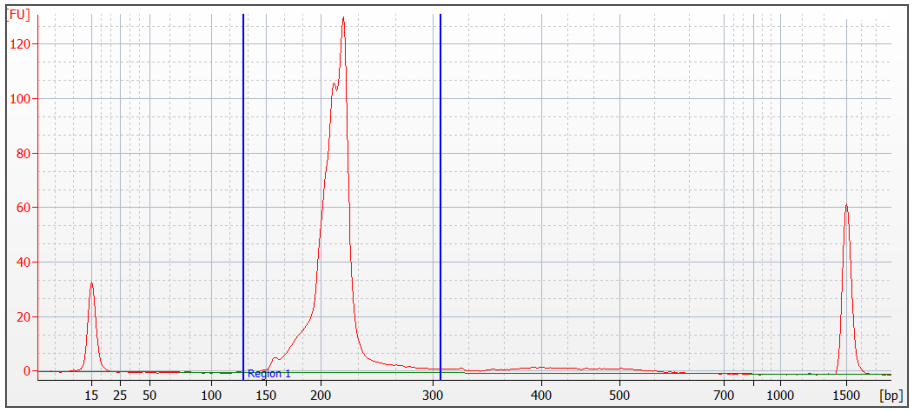
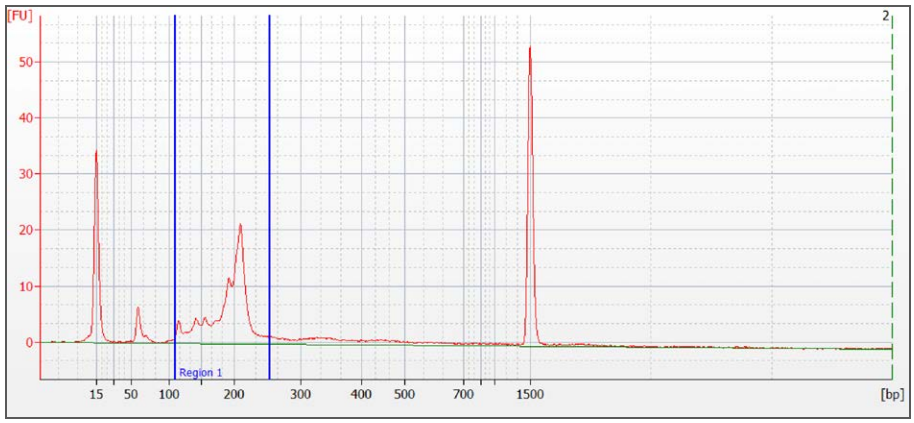


Figure 5 Example of an FFPE RNA Library Size Distribution



8 Dilute each pooled library to 4 nM using RSB.

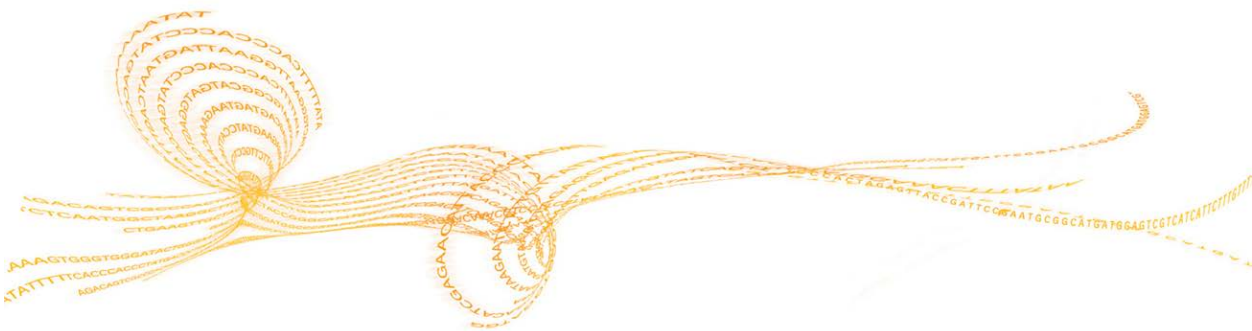
9 Denature and dilute the 4 nM library to the concentration for the sequencing instrument you are using. See the denature and dilute guide for your instrument.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C.

Supporting Information

Appendix A



Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all the required consumables and equipment.

Acronyms

Acronym	Definition
A50X	Adapter Index
AM1	Wash 1 Buffer
CDP	cDNA Plate
CDP1	cDNA Plate 1
CLP	Clean Up Plate
cSNP	Coding SNP
DLSO	Downstream Locus-Specific Oligo
DV	Fragment distribution value
ELM4	Extension and Ligation Mix 4
EUC	Experienced User Card
FFPE	Formalin-fixed, paraffin-embedded
HP3	2N NaOH
HYP	Hybridization Plate
IAP	Index Adapter Plate
LNP	Library Normalization Plate
OB1	Paramagnetic Streptavidin Beads
P5	Flow cell binding site
P7	Flow cell binding site
PCR	Polymerase Chain Reaction
PMM2	PCR Master Mix 2
R7XX	Adapter Index
RCS1	Reverse Transcription cDNA Synthesis Master Mix 1
RIN	RNA Integrity Number
RQN	RNA Quality Number
RSB	Resuspension Buffer
SBS3	Read 2 sequencing primer binding site
smRNA	Small RNA sequencing primer binding site
TDP1	TruSeq DNA Polymerase 1

Acronym	Definition
TOP	Targeted Oligo Pool
UB1	Wash 2 Buffer
ULSO	Upstream Locus-Specific Oligo

Kit Contents

Performing the TruSeq Targeted RNA Expression protocol requires at least 1 index kit and at least 1 fixed or custom panel kit. When additional TruSeq Targeted RNA kits are not necessary for additional assays, use supplemental kits.

Make sure that you have all reagents identified in this section before proceeding to the library preparation procedures.

TruSeq Targeted RNA Index Kits

Each index kit contains 2 boxes: an indexes box and a replacement cap box.

- The 48 index kit contains 14 index tubes, 8 A50X indexes and 6 R7XX indexes, for 48 total index combinations. Each tube contains 4 reactions for a total of 192 reactions.
- Each 96 index kit contains 20 index tubes, 8 A50X indexes and 12 R7XX indexes, for 96 total index combinations. Each tube contains 4 reactions for a total of 384 reactions. These kits can be combined for a total of 384 indexes and 1536 reactions.

Index Box - 48 Indices (192 Samples), Store at 125°C to -15°C (RT-401-1001)

Slot	Reagent	Description
1	A501	A501 Index Adapter
2	A502	A502 Index Adapter
3	A503	A503 Index Adapter
4	A504	A504 Index Adapter
5	A505	A505 Index Adapter
6	A506	A506 Index Adapter
7	A507	A506 Index Adapter
8	A508	A506 Index Adapter
9	R701	R701 Index Adapter
10	R702	R702 Index Adapter
11	R704	R704 Index Adapter
12	R705	R705 Index Adapter
13	R709	R709 Index Adapter
14	R712	R712 Index Adapter

Index Box A - 96 Indices (384 Samples), Store at -25°C to -15°C (RT-402-1001)

Slot	Reagent	Description
1	A501	A501 Index Adapter
2	A502	A502 Index Adapter
3	A503	A503 Index Adapter
4	A504	A504 Index Adapter
5	A505	A505 Index Adapter
6	A506	A506 Index Adapter
7	A507	A507 Index Adapter
8	A508	A508 Index Adapter
9	R701	R701 Index Adapter

Slot	Reagent	Description
10	R702	R702 Index Adapter
11	R703	R703 Index Adapter
12	R704	R704 Index Adapter
13	R705	R705 Index Adapter
14	R706	R706 Index Adapter
15	R707	R707 Index Adapter
16	R708	R708 Index Adapter
17	R709	R709 Index Adapter
18	R710	R710 Index Adapter
19	R711	R711 Index Adapter
20	R712	R712 Index Adapter

Index Box B - 96 Indices (384 Samples), Store at -25°C to -15°C (RT-402-1002)

Slot	Reagent	Description
1	A501	A501 Index Adapter
2	A502	A502 Index Adapter
3	A503	A503 Index Adapter
4	A504	A504 Index Adapter
5	A505	A505 Index Adapter
6	A506	A506 Index Adapter
7	A507	A507 Index Adapter
8	A508	A508 Index Adapter
9	R713	R713 Index Adapter
10	R714	R714 Index Adapter
11	R715	R715 Index Adapter
12	R716	R716 Index Adapter
13	R717	R717 Index Adapter
14	R718	R718 Index Adapter
15	R719	R719 Index Adapter
16	R720	R720 Index Adapter
17	R721	R721 Index Adapter
18	R722	R722 Index Adapter
19	R723	R723 Index Adapter
20	R724	R724 Index Adapter

Index Box C - 96 Indices (384 Samples), Store at -25°C to -15°C (RT-402-1003)

Slot	Reagent	Description
1	A501	A501 Index Adapter
2	A502	A502 Index Adapter
3	A503	A503 Index Adapter
4	A504	A504 Index Adapter
5	A505	A505 Index Adapter
6	A506	A506 Index Adapter
7	A507	A507 Index Adapter
8	A508	A508 Index Adapter

Slot	Reagent	Description
9	R725	R725 Index Adapter
10	R726	R726 Index Adapter
11	R727	R727 Index Adapter
12	R728	R728 Index Adapter
13	R729	R729 Index Adapter
14	R730	R730 Index Adapter
15	R731	R731 Index Adapter
16	R732	R732 Index Adapter
17	R733	R733 Index Adapter
18	R734	R734 Index Adapter
19	R735	R735 Index Adapter
20	R736	R736 Index Adapter

Index Box D - 96 Indices (384 Samples), Store at -25°C to -15°C (RT-402-1004)

Slot	Reagent	Description
1	A501	A501 Index Adapter
2	A502	A502 Index Adapter
3	A503	A503 Index Adapter
4	A504	A504 Index Adapter
5	A505	A505 Index Adapter
6	A506	A506 Index Adapter
7	A507	A507 Index Adapter
8	A508	A508 Index Adapter
9	R737	R737 Index Adapter
10	R738	R738 Index Adapter
11	R739	R739 Index Adapter
12	R740	R740 Index Adapter
13	R741	R741 Index Adapter
14	R742	R742 Index Adapter
15	R743	R743 Index Adapter
16	R744	R744 Index Adapter
17	R745	R745 Index Adapter
18	R746	R746 Index Adapter
19	R747	R747 Index Adapter
20	R748	R748 Index Adapter

Index Adapter Replacement Caps, Store at 15°C to 30°C

Description
i7 Index Tube Caps, Orange
i5 Index Tube Caps, White

TruSeq Targeted RNA Fixed Panel Kits

Each of the following fixed panel kits contains 2 boxes: a fixed panel box and a core reagents box. The fixed panel box contains 1 or 2 fixed panel tubes, which correspond with the name and type of the kit.

Table 2 TruSeq Targeted RNAFixed Panel Kits

Kit Name	Number of Assays	Catalog #
TruSeq Targeted RNA Neuro Panel Kit (48 Samples)	78	RT-201-1001
TruSeq Targeted RNA Neuro Panel Kit (96 Samples)	78	RT-202-1001
TruSeq Targeted RNA Hedgehog Panel Kit (48 Samples)	—	RT-201-1002
TruSeq Targeted RNA Hedgehog Panel Kit (96 Samples)	—	RT-202-1002
TruSeq Targeted RNA Cell Cycle Panel Kit (48 Samples)	63	RT-201-1003
TruSeq Targeted RNA Cell Cycle Panel Kit (96 Samples)	63	RT-202-1003
TruSeq Targeted RNA Wnt Panel Kit (48 Samples)	—	RT-201-1004
TruSeq Targeted RNA Wnt Panel Kit (96 Samples)	—	RT-202-1004
TruSeq Targeted RNA Stem Cell Panel Kit (48 Samples)	100	RT-201-1005
TruSeq Targeted RNA Stem Cell Panel Kit (96 Samples)	100	RT-202-1005
TruSeq Targeted RNA P450 Panel Kit (48 Samples)	—	RT-201-1006
TruSeq Targeted RNA P450 Panel Kit (96 Samples)	—	RT-202-1006
TruSeq Targeted RNA P53 Panel Kit (48 Samples)	—	RT-201-1007
TruSeq Targeted RNA P53 Panel Kit (96 Samples)	—	RT-202-1007
TruSeq Targeted RNA NFkB Panel Kit (48 Samples)	—	RT-201-1008
TruSeq Targeted RNA NFkB Panel Kit (96 Samples)	—	RT-202-1008
TruSeq Targeted RNA Cardiotox Panel Kit (48 Samples)	—	RT-201-1009
TruSeq Targeted RNA Cardiotox Panel Kit (96 Samples)	—	RT-202-1009
TruSeq Targeted RNA Apoptosis Panel Kit (48 Samples)	120	RT-201-1010
TruSeq Targeted RNA Apoptosis Panel Kit (96 Samples)	120	RT-202-1010

Fixed Panel Box - 48 Samples, Store at -25°C to -15°C

Acronym	Description
FP01	TruSeq Targeted RNA Neuro Panel
FP02	TruSeq Targeted RNA Hedgehog Panel
FP03	TruSeq Targeted RNA Cell Cycle Panel
FP04	TruSeq Targeted RNA Wnt Panel
FP05	TruSeq Targeted RNA Stem Cell Panel
FP06	TruSeq Targeted RNA P450 Panel
FP07	TruSeq Targeted RNA P53 Panel
FP08	TruSeq Targeted RNA NFkB Panel
FP09	TruSeq Targeted RNA Cardiotox Panel
FP10	TruSeq Targeted RNA Apoptosis Panel

Core Reagents Box - 48 Samples, Store at -25°C to -15°C

Slot	Reagent	Description
1	AM1	Wash 1 Buffer
2	UB1	Wash 2 Buffer
3	RSB	Resuspension Buffer
4	OB1	Paramagnetic Streptavidin Beads
5	ELM4	Extension and Ligation Mix 4
6	PMM2	PCR Master Mix 2
7	RCS1	Reverse Transcription cDNA Synthesis Master Mix 1
8	TDP1	TruSeq DNA Polymerase 1
9	HP3	2 N NaOH

Fixed Panel Box - 96 Samples, Store at -25°C to -15°C

Quantity	Acronym	Description
2	FP01	TruSeq Targeted RNA Neuro Panel
2	FP02	TruSeq Targeted RNA Hedgehog Panel
2	FP03	TruSeq Targeted RNA Cell Cycle Panel
2	FP04	TruSeq Targeted RNA Wnt Panel
2	FP05	TruSeq Targeted RNA Stem Cell Panel
2	FP06	TruSeq Targeted RNA P450 Panel
2	FP07	TruSeq Targeted RNA P53 Panel
2	FP08	TruSeq Targeted RNA NFkB Panel
2	FP09	TruSeq Targeted RNA Cardiotox Panel
2	FP10	TruSeq Targeted RNA Apoptosis Panel

Core Reagents Box - 96 Samples, Store at -25°C to -15°C

Slot	Reagent	Description
1	AM1	Wash 1 Buffer
2	UB1	Wash 2 Buffer
3	RSB	Resuspension Buffer
4	OB1	Paramagnetic Streptavidin Beads
5	ELM4	Extension and Ligation Mix 4
6	PMM2	PCR Master Mix 2
7–8	RCS1	Reverse Transcription cDNA Synthesis Master Mix 1
9	TDP1	TruSeq DNA Polymerase 1
10–11	HP3	2 N NaOH

TruSeq Targeted RNA Custom Panel Kits

The following custom panel kits each contain a custom panel box and a core reagents box.

Table 3 TruSeq Targeted RNA Custom Panel Kits

Kit	Catalog #
TruSeq Targeted RNA Custom Panel Kit, 48 Samples	RT-101-1001
TruSeq Targeted RNA Custom Panel Kit, 96 Samples	RT-102-1001

Custom Panel Box, Store at -25°C to -15°C

Each custom panel box provides a custom oligo pool:

- The 48-sample box contains 1 tube.
- The 96-sample box contains 2 tubes.

Core Reagents Box - 48 Samples, Store at -25°C to -15°C

Slot	Reagent	Description
1	AM1	Wash 1 Buffer
2	UB1	Wash 2 Buffer
3	RSB	Resuspension Buffer
4	OB1	Paramagnetic Streptavidin Beads
5	ELM4	Extension and Ligation Mix 4
6	PMM2	PCR Master Mix 2

Slot	Reagent	Description
7	RCS1	Reverse Transcription cDNA Synthesis Master Mix 1
8	TDP1	TruSeq DNA Polymerase 1
9	HP3	2 N NaOH

Core Reagents Box - 96 Samples, Store at -25°C to -15°C

Slot	Reagent	Description
1	AM1	Wash 1 Buffer
2	UB1	Wash 2 Buffer
3	RSB	Resuspension Buffer
4	OB1	Paramagnetic Streptavidin Beads
5	ELM4	Extension and Ligation Mix 4
6	PMM2	PCR Master Mix 2
7–8	RCS1	Reverse Transcription cDNA Synthesis Master Mix 1
9	TDP1	TruSeq DNA Polymerase 1
10–11	HP3	2 N NaOH

TruSeq Targeted RNA Supplemental Content Kits, Store at -25°C to -15°C

Kit	Reagent	Description	Quantity	Catalog #
Supplemental Box, 48 Samples	TOP	Targeted Oligo Pool	1 tube	RT-801-1001
Supplemental Box, 96 Samples	TOP	Targeted Oligo Pool	2 tubes	RT-802-1001

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol.

The protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Consumables

Consumable	Supplier
1.7 ml microcentrifuge tubes	General lab supplier
2 ml microcentrifuge tubes	General lab supplier
5 ml microcentrifuge tubes	General lab supplier
15 ml conical tubes	General lab supplier
96-well skirted PCR plates (HSP plates)	Bio-Rad, part # MSP-9601
96-well storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, part # AB-0859
Agencourt AMPure XP, 60 ml kit	Beckman Coulter, part # A63881/A63880
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
Ice bucket	General Lab Supplier
Microseal 'A' film	Bio-Rad, part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
ProtoScript II Reverse Transcriptase, 10,000 units	NEB, catalog # M0368L
Nuclease-free water	General lab supplier
RNase/DNase-free 8-tube strips and caps	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
One of the following (for total RNA integrity and degraded RNA pre-qualification): • Standard Sensitivity RNA Analysis Kit, 15 nt (500 samples) • RNA Nano 6000 Kit	• Advanced Analytical Technologies, part # DNF-471-0500 • Agilent Technologies, part # 5067-1511

Consumable	Supplier
One of the following (for library concentration): • Standard Sensitivity NGS Fragment Analysis Kit, 1–6000 bp (500 samples) • DNA 1000 Kit	• Advanced Analytical Technologies, part # DNF-473-0500 • Agilent Technologies, part # 5067-1504
TE Buffer	General lab supplier
TruSeq Index Plate Fixture Kit	Illumina, catalog # FC-130-1005
[Optional] Human UHR total RNA	Agilent Technologies, part # 740000

Equipment

Equipment	Supplier
Benchtop microcentrifuge	General lab supplier
One of the following: • Fragment Analyzer Automated CE System • 2100 Bioanalyzer Desktop System	• Advanced Analytical Technologies, part # FSv2-CE2 or FSv2-CE10 • Agilent Technologies, part # G2940CA
High-Speed Microplate Shaker	VWR, catalog # • 13500-890 (110 V/120 V) or • 14216-214 (230 V)
Magnetic stand-96	Life Technologies, part # AM10027
Microplate centrifuge	General lab supplier
Midi plate insert for heating system	Illumina, catalog # BD-60-601
One of the following: SciGene TruTemp Heating System Hybex Microsample Incubator	Illumina, catalog # • SC-60-503 (115 V) or • SC-60-504 (220 V) SciGene, catalog # • 1057-30-0 (115 V) or • 1057-30-2 (230 V)

Thermal Cyclers

The following table lists the recommended settings for the recommended thermal cycler, and other comparable models. If your lab has a thermal cycler that is not listed, validate the thermal cycler before performing the protocol.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, Constant at 100°C	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate

Index Sequences

TruSeq Targeted RNA Expression kits contain the following the index adapter sequences.

Table 4 A501–A508 Indexes

Index	Sequence
A501	TGAACCTT
A502	TGCTAAGT
A503	TGTTCTCT
A504	TAAGACAC
A505	CTAATCGA
A506	CTAGAACA
A507	TAAGTTCC
A508	TAGACCTA

Table 5 TruSeq Targeted RNA Index Kit, 48 Indices - R7XX

Index	Sequence
R701	ATCACG
R702	CGATGT
R704	TGACCA
R705	ACAGTG
R709	GATCAG
R712	CTTGTA

Table 6 TruSeq Targeted RNA Index Kit A, 96 Indices - R701–R712

Index	Sequence	Index	Sequence
R701	ATCACG	R707	CAGATC
R702	CGATGT	R708	ACTTGA
R703	TTAGGC	R709	GATCAG
R704	TGACCA	R710	TAGCTT
R705	ACAGTG	R711	GGCTAC
R706	GCCAAT	R712	CTTGTA

Table 7 TruSeq Targeted RNA Index Kit B, 96 Indices - R713–R724

Index	Sequence	Index	Sequence
R713	AGTCAA	R719	GTGAAA
R714	AGTTCC	R720	GTGGCC
R715	ATGTCA	R721	GTTTCG
R716	CCGTCC	R722	CGTACG
R717	GTAGAG	R723	GAGTGG
R718	GTCCGC	R724	GGTAGC

Table 8 TruSeq Targeted RNA Index Kit C, 96 Indices - R725–R736

Index	Sequence	Index	Sequence
R725	ACTGAT	R731	CACGAT
R726	ATGAGC	R732	CACTCA
R727	ATTCCT	R733	CAGGCG
R728	CAAAAG	R734	CATGGC
R729	CAACTA	R735	CATTTT
R730	CACCGG	R736	CCAACA

Table 9 TruSeq Targeted RNA Index Kit D, 96 Indices - R737–R748

Index	Sequence		Index	Sequence
R737	CGGAAT		R743	TACAGC
R738	CTAGCT		R744	TATAAT
R739	CTATAC		R745	TCATTC
R740	CTCAGA		R746	TCCCGA
R741	GACGAC		R747	TCGAAG
R742	TAATCG		R748	TCGGCA

Pooling Guidelines

Use the TruSeq Targeted RNA Calculator on the Illumina website to determine the number of libraries to pool. The calculator accounts for the following factors:

- The assay plexity (TOP) for each library.
- The available read budget for a sequencing run.

NOTE

Increasing the plexity of your experiment decrease the number of libraries you can sequence. For more information, see the *Considerations for Designing a Successful TruSeq Targeted RNA Expression Experiment* tech note.

Additional pooling factors are as follows.

- Pool FFPE and intact RNA samples separately.
- The dynamic range for gene expression of assays in the TOP. If the pool spans a large dynamic range of expression (> 4 logs), decrease the number of libraries to pool. This decrease ensures adequate read budget for the low expressing targets. For custom pools, determine the number empirically.
- Expression levels vary between sample types. Unrelated libraries can have widely varying concentrations. To make sure that each library receives adequate reads, normalize and pool different library types in groups. In the following example, samples with the same shading are pooled.

Table 10 Library Pooling for Quantification

Index	R701	R702	R703	R704	Sample Type
A501	Sample 1 Rep 1	Sample 1 Rep 2	Sample 2 Rep 1	Sample 2 Rep 2	Cancer
A502	Sample 3 Rep 1	Sample 3 Rep 2	Sample 4 Rep 1	Sample 4 Rep 2	Normal
A503	Sample 5 Rep 1	Sample 5 Rep 2	Sample 6 Rep 1	Sample 6 Rep 2	Drug treatment
A504	Sample 7 Rep 1	Sample 7 Rep 2	Sample 8 Rep 1	Sample 8 Rep 2	No treatment

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 11 Illumina General Contact Information

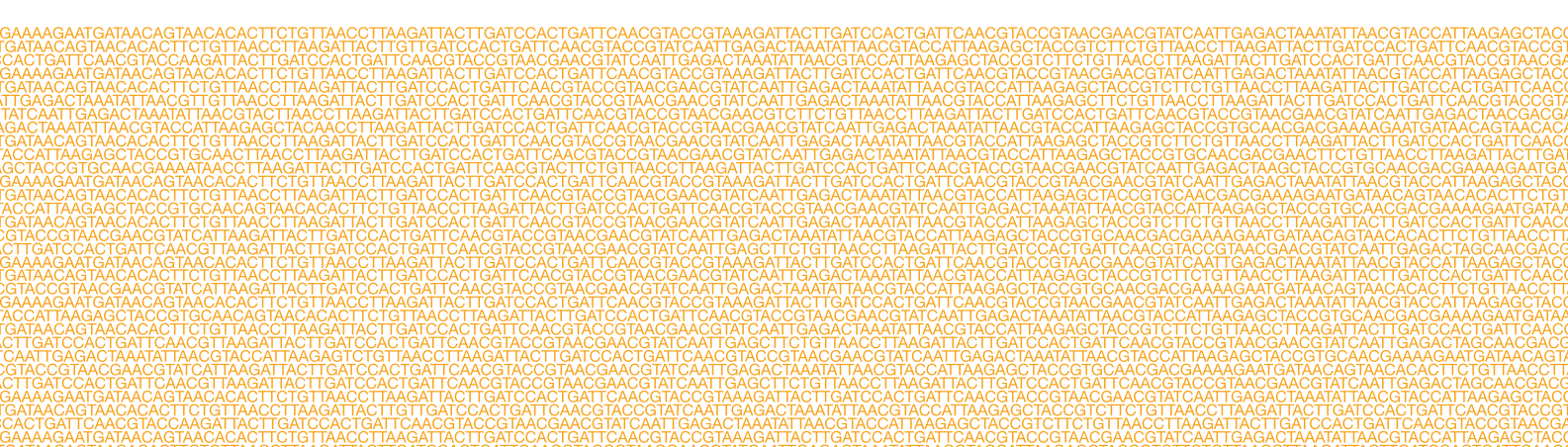
Website	www.illumina.com
Email	techsupport@illumina.com

Table 12 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com