

# Illumina RNA Prep with Enrichment (L) Tagmentation

## Reference Guide



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## Revision History

Document	Date	Description of Change
Document # 1000000124435 v02	October 2020	Added Respiratory Pathogen ID/AMR panel information and Target Enrichment Kit catalog numbers.
Document # 1000000124435 v01	August 2020	Added Respiratory Virus Oligos Panel v2. Added dilution and pooling instructions in the normalization procedure for 3-plex Respiratory Virus Panel libraries Updated workflow diagram description to include the number of samples used to calculate processing times. Corrected formatting of index kit names.
Document # 1000000124435 v00	June 2020	Initial release.

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# Chapter 1 Overview

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## Introduction

The Illumina® RNA Prep, Tagmentation (L) with Enrichment kit pairs with an index kit and enrichment panel to generate enriched libraries for dual-indexed, paired-end sequencing. Reverse transcription converts RNA into complementary DNA (cDNA), which is then tagmented and amplified to add indexes and other adapters. The resulting libraries are normalized for one- or three-plex enrichment and further amplification.

Sequence-specific biotinylated probes combine with magnetic beads to capture regions of interest. The captured sequences are washed, eluted, and amplified to generate copies of the enriched library. A limited-cycle PCR program exponentially amplifies the enriched fragments, copying each fragment to increase the amount of library.

The kit offers the following features:

- ▶ High-quality sequencing data from a range of input, 10–100 ng total RNA
- ▶ Tagmentation with Enrichment Bead-Linked Transposomes (EBLTL) to create larger inserts
- ▶ Unique dual (UD) indexing with the IDT for Illumina DNA/RNA UD Indexes

## Input Recommendations

The protocol is optimized for 10–100 ng of purified total RNA or 20–100 ng RNA input from degraded or FFPE (DV200 ≥ 36.5) samples. Lower input amounts and lesser quality can reduce library yield.

Include a DNase treatment with the RNA isolation method. The DNase treatment ensures sample purity and accurate quantification. Before starting the protocol, quantify the total RNA using standard methods and assess quality using a fragment analysis method.

For RVOP and RPIP panel users only: The Respiratory Virus Oligo Panel and the Respiratory Pathogen ID/AMR Panel includes both DNA and RNA targets and therefore uses an input of either total nucleic acid or an equal pool by volume of separately eluted DNA and RNA from the same sample. An input of 10-100 ng of total nucleic acid is recommended. If DNA is included as part of the input, no DNase step should be performed as part of the protocol.

## Additional Resources

The following resources provide instructions and guidelines for preparing Illumina RNA Prep with Enrichment libraries. Visit the Illumina RNA Prep with Enrichment, (L) Tagmentation support pages for additional information:

- ▶ Compatible products and requirements for recording sample information, sequencing libraries, and analyzing data
- ▶ Questions and answers about using the kit
- ▶ Training videos about the kit and courses for related products and subjects
- ▶ The latest versions of the kit documentation

Resource	Description
<a href="#">Custom Protocol Selector</a>	A tool for generating end-to-end instructions tailored to your library prep method, run parameters, and analysis method, with options to refine the level of detail.
<i>Illumina RNA Prep with Enrichment, (L) Tagmentation Checklist (document # 1000000124436)</i>	Provides a checklist of steps for the experienced user.
<i>Illumina RNA Prep with Enrichment, (L) Tagmentation Consumables &amp; Equipment (document # 1000000124437)</i>	Provides an interactive checklist of user-provided consumables and equipment.
<i>Index Adapters Pooling Guide (document # 1000000041074)</i>	Provides guidelines for preparing dual-indexed libraries with balanced index combinations for sequencing on Illumina systems.
<i>Illumina Adapter Sequences (document # 1000000002694)</i>	Provides the nucleotide sequences that comprise Illumina oligonucleotides used in Illumina sequencing technologies.

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## Introduction

This section describes the Illumina RNA Prep with Enrichment, (L) Tagmentation protocol with step-by-step instructions to prepare and enrich libraries.

- ▶ Review the complete sequencing workflow, from sample through analysis, to ensure compatibility of products and experiment parameters.
- ▶ Confirm kit contents and make sure that you have the required consumables and equipment, including library prep and enrichment reagents, an enrichment panel, and index adapters. For a complete list, see *Supporting Information on page 23*.

## Pooling Preparation

When pooling libraries, record information about your samples before starting library prep. Use a recording tool compatible with your sequencing system and libraries. For compatibility information, see the Illumina RNA Prep with Enrichment support pages or the support pages for your system.

The protocol uses IDT for Illumina DNA/RNA UD Indexes to index libraries. These index primers add distinct Index 1 (i7) and Index 2 (i5) sequences to each end of a fragment. Each index sequence is 10 bp long.

- ▶ For strategies on forming low-plex, color-balanced pools, see the *Index Adapters Pooling Guide (document # 1000000041074)*.
- ▶ For index adapter sequences and how to record them, see *Illumina Adapter Sequences (document # 11000000002694)*.

## Handling Beads

The protocol uses three types of beads: AMPure XP, EBLTL, and SMB. Each bead has a specific technical application. Do not substitute one bead for another.

Apply the following techniques when handling beads:

- ▶ Use all beads at room temperature.
- ▶ Never use AMPure XP or SMB that have been stored < 2°C.
- ▶ Aspirate and dispense beads slowly due to viscosity.

- ▶ Vortex beads frequently throughout the protocol to resuspend. Resuspended beads are evenly distributed and homogenous in color.
- ▶ Dispense liquid so that beads on the side of the wells are wetted.
- ▶ Dispense liquid directly onto bead pellets.
- ▶ When the plate is on the magnetic stand, do not agitate the plate or disturb the bead pellet.
- ▶ If beads are aspirated into pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).
- ▶ If beads adhere to well walls, centrifuge at  $280 \times g$  for 3 seconds, and then pipette to resuspend.

## Tips and Techniques

### Protocol Continuity

- ▶ Follow the protocol in the order described using the specified parameters.
- ▶ Avoid extended pauses until RNA is converted into double-stranded cDNA.
- ▶ 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 well*.
- ▶ Remove unused index adapter plates from the working area.

### Handling Reagents and RNA

- ▶ Avoid multiple freeze-thaw cycles of input RNA.
  - ▶ You can store RNA in RNase-free water or TE buffer at  $-85^{\circ}\text{C}$  to  $-65^{\circ}\text{C}$  for up to 1 year.
  - ▶ If you must reuse the sample, aliquot into separate tubes for single-use.
- ▶ Keep thawed reagents on ice until needed. Promptly return all reagents to storage after use.
- ▶ When not in use, seal plates and close lids to limit contamination.

### Sealing the Plate

- ▶ Use Microseal 'B' adhesive seals throughout the protocol. The seals are effective at  $-40^{\circ}\text{C}$  to  $110^{\circ}\text{C}$ .
- ▶ Cover the plate with the seal, and seal with a rubber roller or wedge.
- ▶ After each use, discard seals from plates.

### Plate Transfers

- ▶ When transferring volumes between plates, transfer the specified volume from each well of the first plate to the corresponding well of the second plate.

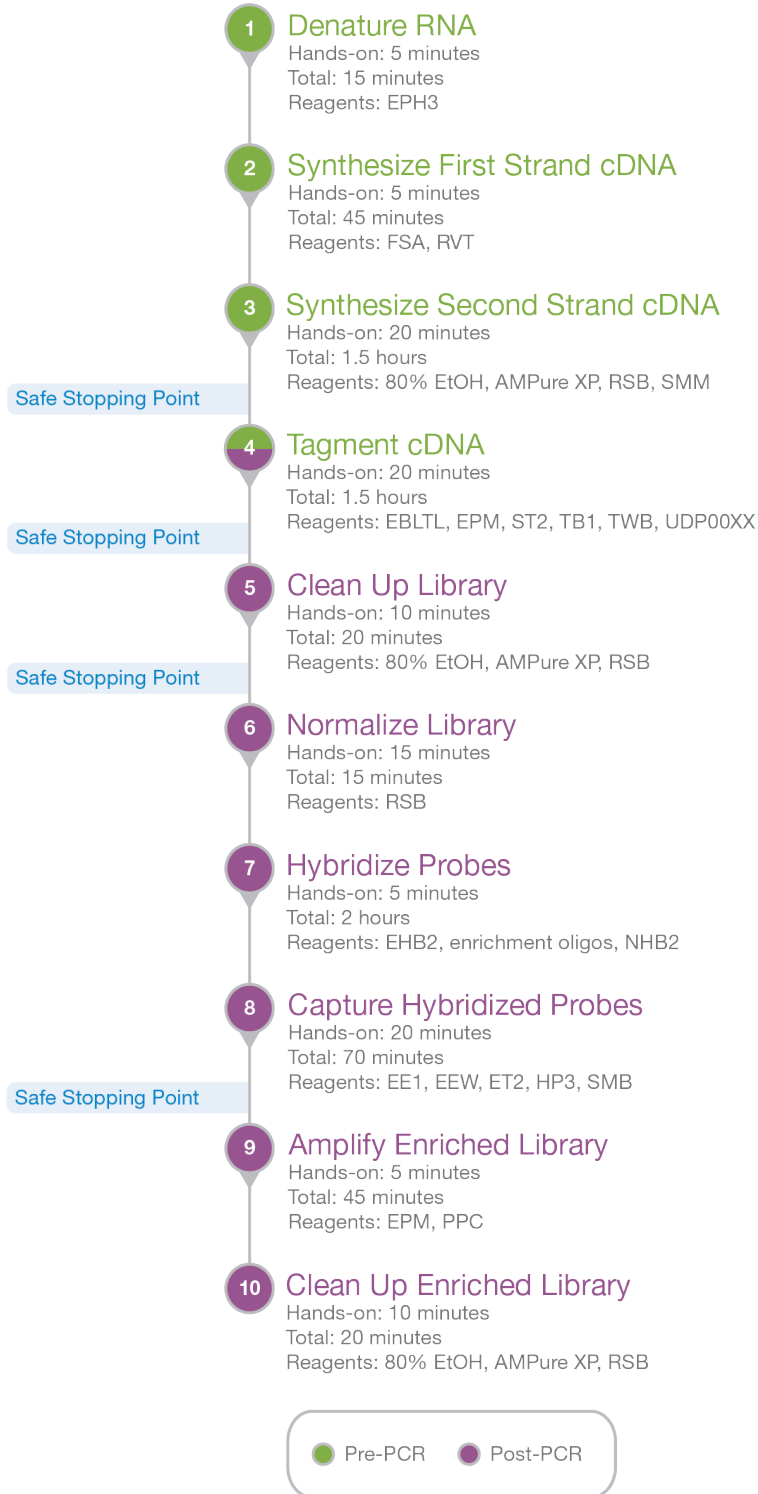
### Centrifugation

- ▶ At any step, centrifuge at  $280 \times g$  for 10 seconds to consolidate liquid or beads in the bottom of the well and prevent sample loss.



## Library Prep and Enrichment Diagram

The following diagram provides an overview of the Illumina RNA Prep with Enrichment protocol using a single sample. Safe stopping points are marked between steps.



## Denature RNA

This step denatures the total RNA and anneals random hexamers. The random hexamers prime the sample for cDNA synthesis.

### Consumables

- ▶ EPH3 (Elute, Prime, Fragment High Concentration Mix)
- ▶ Nuclease-free ultrapure water
- ▶ 96-well PCR plate, semiskirted
- ▶ Microseal 'B' adhesive film
- ▶ Prepare for a later procedure:
  - ▶ FSA (First Strand Synthesis Act D Mix)

### Preparation

- 1 Prepare the following consumables:

Reagent	Storage	Instructions
EPH3	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
FSA	-25°C to -15°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

- 2 Save the following DEN\_RNA program on the thermal cycler:
  - ▶ Choose the preheat lid option and set to 100°C
  - ▶ Reaction volume is 17  $\mu$ l
  - ▶ 65°C for 5 minutes
  - ▶ Hold at 4°C

### Procedure

- 1 In each well of a new PCR plate, dilute 10–100 ng total RNA in nuclease-free ultrapure water to a volume of 8.5  $\mu$ l.
- 2 Add 8.5  $\mu$ l EPH3 to each well.
- 3 Pipette 10 times to mix, and then seal.
- 4 Centrifuge at 280  $\times$  g for 3 seconds.
- 5 Place on the preprogrammed thermal cycler and run the DEN\_RNA program. Total program time is ~5 minutes.
- 6 Centrifuge at 280  $\times$  g for 10 seconds.
- 7 Immediately proceed to *Synthesize First Strand cDNA*. Each well contains 17  $\mu$ l denatured RNA bound with random hexamers.

## Synthesize First Strand cDNA

This step reverse transcribes the hexamer-primed RNA fragments to produce first strand complementary DNA (cDNA).

## Consumables

- ▶ FSA (First Strand Synthesis Act D Mix)
- ▶ RVT (Reverse Transcriptase)
- ▶ 1.7 ml microcentrifuge tube, RNase-free
- ▶ Microseal 'B' adhesive film
- ▶ Prepare for a later procedure:
  - ▶ Agencourt AMPure XP
  - ▶ RSB (Resuspension Buffer) (frozen)
  - ▶ SMM (Second Strand Marking Master Mix)



### WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at [support.illumina.com/sds.html](http://support.illumina.com/sds.html).

## Preparation

- 1 Prepare the following consumables:

Reagent	Storage	Instructions
AMPure XP	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.
RSB*	-25°C to -15°C	Thaw at room temperature. Vortex and invert to mix.
RVT	-25°C to -15°C	Store until needed. Flick to mix, and then centrifuge briefly.
SMM	-25°C to -15°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

\* The RSB in this protocol is stored at different temperatures. Use the specified storage temperature.

- 2 Save the following FSS program on the thermal cycler:
  - ▶ Choose the preheat lid option and set to 100°C
  - ▶ Reaction volume is 25 µl
  - ▶ 25°C for 10 minutes
  - ▶ 42°C for 15 minutes
  - ▶ 70°C for 15 minutes
  - ▶ Hold at 4°C

## Procedure

- 1 In a 1.7 ml tube on ice, combine exactly the following volumes to prepare First Strand Synthesis Master Mix. Multiply each volume by the number of samples.
  - ▶ FSA (9 µl)
  - ▶ RVT (1 µl)
 Volumes include reagent overage for accurate pipetting.
- 2 Thoroughly pipette First Strand Synthesis Master Mix to mix.
- 3 Add 8 µl First Strand Synthesis Master Mix to each well.

- 4 Pipette 10 times, and then seal.
- 5 Centrifuge at  $280 \times g$  for 10 seconds.
- 6 Place on the preprogrammed thermal cycler and run the FSS program.  
Total program time is ~43 minutes and each well contains a volume of 25  $\mu$ l.

## Synthesize Second Strand cDNA

This step removes the RNA template and synthesizes a replacement strand to generate blunt-ended, double-stranded cDNA fragments. Magnetic beads then separate the cDNA from the Second Strand Synthesis Master Mix.

### Consumables

- ▶ RSB (Resuspension Buffer)
- ▶ SMM (Second Strand Marking Master Mix)
- ▶ Agencourt AMPure XP
- ▶ Freshly prepared 80% ethanol (EtOH)
- ▶ 96-well PCR plate, semiskirted
- ▶ Microseal 'B' adhesive film

### Preparation

- 1 Prepare 80% EtOH from absolute EtOH.
- 2 Save the following SSS program on the thermal cycler:
  - ▶ Choose the preheat lid option and set to 40°C
  - ▶ Reaction volume is 50  $\mu$ l
  - ▶ 16°C for 1 hour
  - ▶ Hold at 4°C

### Procedure

#### Generate cDNA

- 1 Centrifuge the sealed PCR plate at  $280 \times g$  for 10 seconds.
- 2 Invert SMM to mix, and then centrifuge briefly.
- 3 Add 25  $\mu$ l SMM to each well.
- 4 Pipette 10 times, and then seal.
- 5 Centrifuge at  $280 \times g$  for 10 seconds.
- 6 Place on the preprogrammed thermal cycler and run the SSS program.  
Total program time is ~1 hour and each well contains a volume of 50  $\mu$ l.

#### Clean Up cDNA

- 1 Centrifuge the sealed PCR plate at  $280 \times g$  for 10 seconds.
- 2 Vortex AMPure XP to resuspend.

- 3 Add 90  $\mu$ l AMPure XP to each well.
- 4 Seal and shake at 2200 rpm for 1 minute.
- 5 Incubate at room temperature for 5 minutes.
- 6 Centrifuge at  $280 \times g$  for 10 seconds, and then unseal.
- 7 Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
- 8 Remove and discard all supernatant.
- 9 Wash beads as follows.
  - a Keep on the magnetic stand and add 175  $\mu$ l fresh 80% EtOH to each well.
  - b Wait 30 seconds.
  - c Remove and discard all supernatant.
- 10 Repeat wash a **second** time.
- 11 With a 10  $\mu$ l pipette, remove all residual EtOH.
- 12 Air-dry on the magnetic stand for 2 minutes. Do not over-dry the beads.
- 13 Remove from the magnetic stand.
- 14 Add 19.5  $\mu$ l RSB to each well.
- 15 Seal and shake at 2700 rpm for 1 minute.
- 16 Incubate at room temperature for 2 minutes.
- 17 Centrifuge at  $280 \times g$  for 10 seconds, and then unseal.
- 18 Place on the magnetic stand and wait until the liquid is clear (~4 minutes).
- 19 Transfer 17.5  $\mu$ l supernatant from each well to a new PCR plate.  
Small amounts of bead carryover do not affect performance.

### SAFE STOPPING POINT

If you are stopping, seal the plate and store at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 7 days.

## Tagment cDNA

This step uses Enrichment Bead-Linked Transposomes to tagment double-stranded cDNA. The tagmentation process fragments cDNA and adds adapter sequences.

After tagmentation, the fragments are purified and amplified to add index adapter sequences for dual indexing and P7 and P5 sequences for clustering. For help selecting index adapters, see [Pooling Preparation on page 3](#).

### Consumables

- ▶ EBLTL (Enrichment Bead-Linked Transposomes)
- ▶ EPM (Enhanced PCR Mix)
- ▶ Index adapter plate (UDP0XXX)
- ▶ ST2 (Stop Tagment Buffer 2)
- ▶ TB1 (Tagmentation Buffer 1)

- ▶ TWB (Tagmentation Wash Buffer)
- ▶ Nuclease-free ultrapure water
- ▶ 1.7 ml microcentrifuge tube
- ▶ Microseal 'B' adhesive film

## About Reagents

- ▶ Each well of the index adapter plate is single-use and contains > 10  $\mu$ l UDPOXXX, which are premixed Index 1 (i7) and Index 2 (i5) adapters.
- ▶ The row and column labels are printed on the underside of the index adapter plate. Raise the plate overhead to check the labels.

## Preparation

- 1 Prepare the following consumables:

Reagents	Storage	Instructions
EBLTL	2°C to 8°C	Bring to room temperature, and then vortex for 30 seconds to mix. If beads remain at the bottom of the tube, vortex until beads are resuspended.
EPM	-25°C to -15°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
Index adapter plate	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge at 1000 $\times$ g for 1 minute.
ST2	Room temperature	Vortex to mix, and then centrifuge briefly.
TB1	-25°C to -15°C	Bring to room temperature, and then vortex to mix.
TWB	Room temperature	Vortex to mix.

- 2 If the ST2 tube has precipitate:
  - a Heat at 37°C for 10 minutes.
  - b Vortex until precipitate is dissolved.
  - c Return to room temperature.
- 3 Save the following TAG program on the thermal cycler:
  - ▶ Choose the preheat lid option and set to 100°C
  - ▶ Reaction volume is 50  $\mu$ l
  - ▶ 55°C for 5 minutes
  - ▶ Hold at 10°C
- 4 Save the following TAG\_PCR program on the thermal cycler:
  - ▶ Choose the preheat lid option and set to 100°C
  - ▶ Reaction volume is 50  $\mu$ l
  - ▶ 72°C for 3 minutes
  - ▶ 98°C for 3 minutes
  - ▶ X cycles of:
    - ▶ 98°C for 20 seconds
    - ▶ 60°C for 30 seconds
    - ▶ 72°C for 1 minute
  - ▶ 72°C for 3 minutes
  - ▶ Hold at 10°C

Input	Number of Cycles (X) <sup>1</sup>
High-quality RNA with DV200 > 80%	14
FFPE and RNA with DV200 < 80%	17
<b>[Respiratory Virus Panel]</b> Extracted viral RNA	17

<sup>1</sup> To achieve the desired library yield and specificity, optimize the number of PCR cycles for your sample type and input.

## Procedure

### Tagment With EBLTL

- 1 Centrifuge the sealed PCR plate at 280 × g for 10 seconds.
- 2 In a 1.7 ml tube, combine exactly the following volumes to prepare Tagmentation Master Mix. Multiply each volume by the number of samples.
  - ▶ TB1 (11.5 µl)
  - ▶ EBLTL (11.5 µl)
  - ▶ Nuclease-free ultrapure water (14.5 µl)
 Volumes include reagent overage for accurate pipetting.
- 3 Thoroughly vortex the Tagmentation Master Mix to resuspend.
- 4 Add 32.5 µl Tagmentation Master Mix to each well.
- 5 Pipette thoroughly, and then seal.
- 6 Place on the preprogrammed thermal cycler and run the TAG program.  
Total program time is ~5 minutes and each well contains a volume of 50 µl.

### Wash Tagmented cDNA

- 1 Centrifuge the sealed PCR plate at 280 × g for 10 seconds.
- 2 Incubate at room temperature for 2 minutes.
- 3 Add 10 µl ST2 to each well.
- 4 Seal and shake at 2200 rpm for 1 minute.
- 5 Incubate at room temperature for 5 minutes.
- 6 Centrifuge at 280 × g for 10 seconds, and then unseal.
- 7 Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
- 8 Remove and discard all supernatant.
- 9 Wash beads as follows.
  - a Remove from the magnetic stand.
  - b Add 100 µl TWB to each well.
  - c Seal and shake at 2000 rpm for 1 minute.
  - d Centrifuge at 280 × g for 3 seconds.
  - e Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
  - f Remove and discard all supernatant.
- 10 Wash beads a **second** time.

- 11 Wash beads a **third** time, *skipping step f*.  
TWB remains in the wells to prevent over-drying.
- 12 Keep on the magnetic stand and immediately proceed to *Amplify Tagmented DNA*.  
Each well contains 100 µl beads with tagmented cDNA.

## Amplify Tagmented DNA

- 1 Combine exactly the following volumes to prepare PCR Master Mix. Multiply each volume by the number of samples.
  - ▶ EPM (23 µl)
  - ▶ Nuclease-free ultrapure water (23 µl)Volumes include reagent overage for accurate pipetting.
- 2 Thoroughly vortex PCR Master Mix to mix.
- 3 Keeping the plate on the magnetic stand, remove and discard all TWB supernatant.
- 4 With a 20 µl pipette, remove all residual TWB.  
Foam is normal and does not affect the library.
- 5 Remove from the magnetic stand.
- 6 Add 40 µl PCR Master Mix to each well.
- 7 Using a new pipette tip for each well, pierce the foil covering the index adapter plate wells that you intend to use.
- 8 Add 10 µl UDP0XXX to each well. Transfer volumes from the index adapter plate to the PCR plate.
- 9 Seal and shake at 2000 rpm for 1 minute.
- 10 Centrifuge at 280 × g for 3 seconds.
- 11 Place on the preprogrammed thermal cycler and run the TAG\_PCR program.  
Total program time is ~50–60 minutes and each well contains 50 µl beads with DNA attached.

### SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days. Alternatively, leave on the thermal cycler for up to 24 hours.

## Clean Up Library

This step uses magnetic beads to purify the tagmented library.

### Consumables

- ▶ RSB (Resuspension Buffer) (refrigerated)
- ▶ Agencourt AMPure XP
- ▶ Freshly prepared 80% ethanol (EtOH)
- ▶ 96-well PCR plate, semiskirted
- ▶ Microseal 'B' adhesive film



## Preparation

- 1 Prepare the following consumables:

Reagent	Storage	Instructions
AMPure XP	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.
RSB*	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.

\* The RSB in this protocol is stored at different temperatures. Use the specified storage temperature.

- 2 Prepare 80% EtOH from absolute EtOH.

## Procedure

- 1 Centrifuge the sealed PCR plate at 280 × g for 10 seconds.
- 2 Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
- 3 Transfer 45 µl supernatant from each well to a new PCR plate.
- 4 Vortex AMPure XP to resuspend.
- 5 Add 81 µl AMPure XP to each well.
- 6 Seal and shake at 2200 rpm for 1 minute.
- 7 Incubate at room temperature for 5 minutes.
- 8 Centrifuge at 280 × g for 10 seconds, and then unseal.
- 9 Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
- 10 Remove and discard all supernatant.
- 11 Wash beads as follows.
  - a Keep on the magnetic stand and add 175 µl fresh 80% EtOH to each well.
  - b Wait 30 seconds.
  - c Remove and discard all supernatant.
- 12 Wash beads a **second** time.
- 13 With a 20 µl pipette, remove all residual EtOH.
- 14 Air-dry on the magnetic stand for 2 minutes. Do not over-dry the beads.
- 15 Remove from the magnetic stand.
- 16 Add 17 µl RSB to each well.
- 17 Seal and shake at 2700 rpm for 1 minute.
- 18 Incubate at room temperature for 2 minutes.
- 19 Centrifuge at 280 × g for 10 seconds, and then unseal.
- 20 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
- 21 Transfer 15 µl supernatant from each well to a new PCR plate.

### SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 30 days.

## Normalize Library

This step quantifies and normalizes libraries, then combines them into one pool for one- or three-plex enrichment. Results are optimized for 200 ng of each library.

### Consumables

- ▶ RSB (Resuspension Buffer) (refrigerated)
- ▶ Qubit dsDNA BR Assay Kit
- ▶ 96-well PCR plate, semiskirted
- ▶ **[Optional]** Agilent DNA 1000 Kit

### Preparation

- 1 If you are resuming the protocol after a safe stopping point, prepare the following consumable:

Reagent	Storage	Instructions
RSB*	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.

\* The RSB in this protocol is stored at different temperatures. Use the specified storage temperature.

### Procedure

- 1 Analyze 1 µl library with the Qubit dsDNA BR Assay Kit.  
Expected yield is ~200 ng.
- 2 **[Optional]** Analyze 1 µl library with the Agilent 2100 Bioanalyzer System and a DNA 1000 Kit.
- 3 **[RPIP Libraries]**
  - ▶ For one-plex enrichment, dilute one 200 ng library to a volume of 7.5 µl.
  - ▶ For three-plex enrichment, dilute three 200 ng libraries to a volume of 2.5 µl each.
- 4 **[Respiratory Virus Panel Libraries]**
  - ▶ For one-plex enrichment, transfer 7.5 µl undiluted library to one well of a new PCR plate.
  - ▶ For three-plex enrichment, dilute three 200 ng libraries to a volume of 2.5 µl each.
- 5 **[All Other Libraries]** Dilute libraries in RSB as follows.
  - ▶ For one-plex enrichment, dilute one 200 ng library to a volume of 7.5 µl.
  - ▶ For three-plex enrichment, dilute three 200 ng libraries to a volume of 2.5 µl each.
- 6 **[Diluted Libraries]** In one well of a new PCR plate, combine the applicable number of 200 ng libraries:

Number of 200 ng Libraries (Enrichment Plexity)	Total Mass (ng)	Total Volume (µl)
1	200	7.5
3	600	7.5

- ▶ If the total volume is > 7.5 µl, use a vacuum concentrator or Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) to concentrate the pooled sample to 7.5 µl.
- ▶ If you are using a vacuum concentrator, use a no heat setting and a medium drying rate.
- ▶ If you are using an Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa), you do not need to rinse the device before use. Most of the volume filters through in 5 minutes. Larger starting volumes can take up to 30 minutes to filter.

## Hybridize Probes

This step adds capture probes to pooled libraries to target regions of interest. The procedure uses enrichment reagents and oligos from an enrichment panel.

### Consumables

- ▶ EHB2 (Enrich Hyb Buffer 2)
- ▶ NHB2 (Hyb Buffer 2 + IDT NXT Blockers)
- ▶ Enrichment oligos
- ▶ 96-well PCR plate, semiskirted
- ▶ Microseal 'B' adhesive film

### About Reagents

- ▶ NHB2 can precipitate in storage.

### Preparation

- 1 Preheat the microheating system to 50°C.
- 2 Prepare the following consumables:

Reagent	Storage	Instructions
EHB2	2°C to 8°C	Bring to room temperature, and then vortex to mix.
Enrichment oligos	-25°C to -15°C	Thaw at room temperature, and then vortex to mix.
NHB2	-25°C to -15°C	Thaw at room temperature, and then vortex to mix. Lay in the preheated microheating system and incubate for 5 minutes.

- 3 Mix preheated NHB2 as follows.
  - a Vortex three times for 10 seconds each time.
  - b Pipette to fully resuspend. Keep warm until use to prevent the reformation of precipitates.
- 4 If EHB2 or NHB2 appears crystallized or cloudy, vortex or pipette until clear.
- 5 Save the following HYB program on the thermal cycler:
  - ▶ Choose the preheat lid option and set to 100°C
  - ▶ Reaction volume is 25 µl
  - ▶ 95°C for 5 minutes
  - ▶ 18 cycles of 1 minute each:
    - ▶ 94°C for the first cycle
    - ▶ Decrease 2°C per subsequent cycle
  - ▶ 58°C for 90 minutes
  - ▶ Hold at 58°C for ≤ 24 hours

The minimum hybridization time at 58°C is 90 minutes. Extending the hold to up to 24 hours is optional for overnight hybridization. Without the extended hold, total program time is ~2 hours.

### Procedure

- 1 Add the following volumes *in the order listed* to each well of a new PCR plate.

- ▶ 200 ng library or 600 ng pool (7.5  $\mu$ l)
  - ▶ NHB2 (12.5  $\mu$ l)
  - ▶ Enrichment oligos (2.5  $\mu$ l)
  - ▶ EHB2 (2.5  $\mu$ l)
- 2 Pipette 10 times to mix, and then seal.
  - 3 Centrifuge at  $280 \times g$  for 3 seconds.  
EHB2 can make the reaction appear cloudy, which is normal.
  - 4 Place on the preprogrammed thermal cycler and run the HYB program.
  - 5 Allow to incubate at  $58^{\circ}\text{C}$  for 90 minutes to 24 hours.  
Each well contains a volume of 25  $\mu$ l.

## Capture Hybridized Probes

This step uses magnetic beads to capture probes hybridized to the targeted library fragments of interest. Heated washes remove nonspecific binding from the beads. The enriched library is then eluted from the beads.

### Consumables

- ▶ EE1 (Enrichment Elution Buffer 1)
- ▶ EEW (Enhanced Enrichment Wash)
- ▶ ET2 (Elute Target Buffer 2)
- ▶ HP3 (2 N NaOH)
- ▶ SMB (Streptavidin Magnetic Beads)
- ▶ 1.7 ml microcentrifuge tube
- ▶ 96-well PCR plate, semiskirted (2)
- ▶ Microseal 'B' adhesive film

### Preparation

- 1 Preheat the microheating system to  $58^{\circ}\text{C}$ .
- 2 Prepare the following consumables:

Reagent	Storage	Instructions
EE1	$-25^{\circ}\text{C}$ to $-15^{\circ}\text{C}$	Thaw at room temperature, and then vortex to mix.
EEW	$-25^{\circ}\text{C}$ to $-15^{\circ}\text{C}$	Thaw at room temperature, and then vortex to mix.
ET2	$2^{\circ}\text{C}$ to $8^{\circ}\text{C}$	Bring to room temperature, and then vortex to mix.
HP3	$-25^{\circ}\text{C}$ to $-15^{\circ}\text{C}$	Thaw at room temperature, and then vortex to mix.
SMB	$2^{\circ}\text{C}$ to $8^{\circ}\text{C}$	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.

- 3 Place EEW in the preheated microheating system. Keep heated.
- 4 Combine exactly the following volumes to prepare Elution Master Mix. Multiply each volume by the total number of samples.
  - ▶ EE1 (28.5  $\mu$ l)
  - ▶ HP3 (1.5  $\mu$ l)
 Volumes include reagent overage for accurate pipetting despite possible foam.

- 5 Thoroughly pipette Elution Master Mix to mix, and then set aside at room temperature.
- 6 Set the thermal cycler as follows.
  - ▶ If it has an incubation option, set it to 58°C.
  - ▶ If it does not have an incubation option, save the following Incubation program:
    - ▶ Choose the preheat lid option and set to 70°C
    - ▶ Reaction volume is 100 µl
    - ▶ Hold at 58°C

## Procedure

### Capture

- 1 Centrifuge the sealed PCR plate at 280 × g for 10 seconds.
- 2 Vortex SMB to resuspend.
- 3 Add 62.5 µl SMB to each well.
- 4 Slowly pipette until the beads are resuspended, and then seal.
- 5 Place in the 58°C thermal cycler and incubate for 15 minutes. Keep the program on. The thermal cycler runs continuously through the capture and four washes.
- 6 Immediately after the 15-minute incubation, do as follows.
  - a Centrifuge at 280 × g for 10 seconds.
  - b Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
- 7 Remove and discard all supernatant.

### First Wash

- 1 Remove from the magnetic stand.
- 2 Add 50 µl preheated EEW to each well.
- 3 Seal and shake at 2400 rpm for 4 minutes.
- 4 Return unused EEW to the microheating system and keep heated.
- 5 Return the plate to the 58°C thermal cycler and incubate for 5 minutes.
- 6 Immediately after the 5-minute incubation, do as follows.
  - a Centrifuge at 280 × g for 3 seconds.
  - b Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
- 7 Remove and discard all supernatant.

### Second and Third Washes

- 1 Remove from the magnetic stand.
- 2 Add 50 µl preheated EEW to each well.
- 3 Seal and shake at 2000 rpm for 1 minute.
- 4 Return unused EEW to the microheating system and keep heated.

- 5 Return the plate to the 58°C thermal cycler and incubate for 5 minutes.
- 6 Immediately after the 5-minute incubation, do as follows.
  - a Centrifuge at 280 × g for 3 seconds.
  - b Place on the magnetic stand and wait until the liquid is clear (~2 minutes). Keep the program on.
- 7 Remove and discard all supernatant.
- 8 Repeat steps 1–7 for the third wash.

## Transfer Wash

- 1 Remove from the magnetic stand.
- 2 Add 50 µl preheated EEW to each well.
- 3 Seal and shake at 2000 rpm for 1 minute.
- 4 Centrifuge at 280 × g for 3 seconds.
- 5 Transfer 50 µl resuspended bead solution from each well to a new PCR plate.  
The transfer minimizes residual reagents that can inhibit amplification.
- 6 Seal and centrifuge at 280 × g for 3 seconds.
- 7 Return to the 58°C thermal cycler and incubate for 5 minutes.
- 8 Immediately after the 5-minute incubation, place on the magnetic stand and wait until the liquid is clear (~2 minutes).
- 9 Remove and discard all supernatant.
- 10 With a 20 µl pipette, remove and discard all residual EEW.
- 11 Immediately proceed to *Elute* to prevent over-drying.

## Elute

- 1 Thoroughly pipette Elution Master Mix to mix.
- 2 Remove the plate from the magnetic stand.
- 3 Add 23 µl Elution Master Mix to each well.
- 4 Seal and shake at 2600 rpm for 1 minute.
- 5 Incubate at room temperature for 2 minutes.
- 6 Centrifuge at 280 × g for 10 seconds, and then unseal.
- 7 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
- 8 Transfer 21 µl supernatant from each well to a new PCR plate.
- 9 Add 4 µl ET2 to each well.
- 10 Seal and shake at 2000 rpm for 1 minute.  
Each well contains a volume of 25 µl.

### SAFE STOPPING POINT

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

## Amplify Enriched Library

This step uses a 14-cycle PCR program to amplify the enriched library.

### Consumables

- ▶ EPM (Enhanced PCR Mix)
- ▶ PPC (PCR Primer Cocktail)
- ▶ Microseal 'B' adhesive film
- ▶ Prepare for a later procedure:
  - ▶ RSB (Resuspension Buffer) (refrigerated)
  - ▶ Agencourt AMPure XP

### Preparation

- 1 Prepare the following consumables:

Reagent	Storage	Instructions
AMPure XP	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.
EPM	-25°C to -15°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
PPC	-25°C to -15°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
RSB*	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.

\* The RSB in this protocol is stored at different temperatures. Use the specified storage temperature.

- 2 Save the following AMP program on the thermal cycler.
  - ▶ Choose the preheat lid option and set to 100°C
  - ▶ Reaction volume is 50 µl
  - ▶ 98°C for 30 seconds
  - ▶ 14 cycles of:
    - ▶ 98°C for 10 seconds
    - ▶ 60°C for 30 seconds
    - ▶ 72°C for 30 seconds
  - ▶ 72°C for 5 minutes
  - ▶ Hold at 10°C

### Procedure

- 1 Centrifuge the sealed plate at 280 × g for 10 seconds.
- 2 Add 5 µl PPC to each well of the PCR plate.
- 3 Add 20 µl EPM to each well.
- 4 Seal and shake at 2000 rpm for 1 minute.
- 5 Centrifuge at 280 × g for 10 seconds.
- 6 Place on the preprogrammed thermal cycler and run the AMP program.  
Total program time is ~35 minutes and each well contains a volume of 50 µl.

## SAFE STOPPING POINT

If you are stopping, store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler for up to 24 hours.

## Clean Up Enriched Library

This step uses magnetic beads to purify the enriched library.

### Consumables

- ▶ RSB (Resuspension Buffer)
- ▶ Agencourt AMPure XP
- ▶ Freshly prepared 80% ethanol (EtOH)
- ▶ 96-well PCR plate, semiskirted
- ▶ Microseal 'B' adhesive film

### Preparation

- 1 Prepare 80% EtOH from absolute EtOH.

### Procedure

- 1 Centrifuge the sealed plate at 280 × g for 10 seconds.
- 2 Vortex AMPure XP to resuspend.
- 3 Add 90 µl AMPure XP to each well.
- 4 Seal and shake at 2200 rpm for 1 minute.
- 5 Incubate at room temperature for 5 minutes.
- 6 Centrifuge at 280 × g for 10 seconds, and then unseal.
- 7 Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
- 8 Remove and discard all supernatant.
- 9 Wash beads as follows.
  - a Keep on the magnetic stand and add 175 µl fresh 80% EtOH to each well.
  - b Wait 30 seconds.
  - c Remove and discard all supernatant.
- 10 Wash beads a **second** time.
- 11 With a 20 µl pipette, remove all residual EtOH.
- 12 Air-dry on the magnetic stand for 2 minutes.
- 13 Remove from the magnetic stand.
- 14 Add 32 µl RSB to each well.
- 15 Seal and shake at 2600 rpm for 1 minute.
- 16 Incubate at room temperature for 2 minutes.



- 17 Centrifuge at  $280 \times g$  for 10 seconds, and then unseal.
- 18 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
- 19 Transfer 30  $\mu$ l supernatant from each well to a new PCR plate.

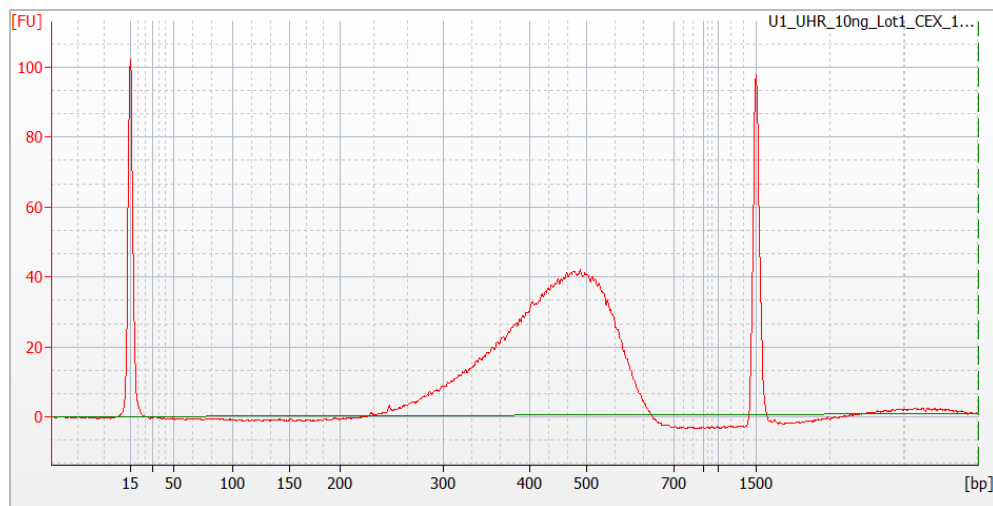
### SAFE STOPPING POINT

If you are stopping, seal the plate and store at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 7 days.

## Check Enriched Library

- 1 Check the enriched library using both of the following methods:
  - ▶ Analyze 1  $\mu$ l enriched library with the Qubit dsDNA HS Assay kit to quantify library concentration ( $\text{ng}/\mu\text{l}$ ).
  - ▶ Analyze 1  $\mu$ l enriched library with the Agilent 2100 Bioanalyzer System and a DNA 1000 Kit to qualify.

**Figure 1** Example Yield From Bioanalyzer



## Dilute Library to the Starting Concentration

This step dilutes libraries to the starting concentration for the NovaSeq 6000, NextSeq 550, or NextSeq 500 System. After diluting to the starting concentration, libraries are ready to be denatured and diluted to the final loading concentration. Illumina recommends paired-end runs for sequencing. The number of cycles per index read is 10, and the number of cycles per read varies depending on the sequencing system.

- 1 Obtain the molarity value of the library or pooled libraries using the applicable method:
  - ▶ For libraries quantified with a Bioanalyzer only, use the molarity value obtained for the library.
  - ▶ For libraries quantified with a Bioanalyzer and Qubit, use the following formula to calculate the molarity value. Apply the average size from the Bioanalyzer and the concentration from the Qubit.

$$\frac{\text{ng}/\mu\text{l}}{660 \text{ g/mol} \times \text{average library size}} \times 10^6 = \text{Molarity (nM)}$$

- 2 Using the molarity value, calculate the volumes of RSB and library needed to dilute libraries to the starting concentration for your system.

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
NextSeq 550 and NextSeq 500	4	0.8
NovaSeq 6000	0.6	120
MiniSeq [RPIP panel]	2	2
MiSeq [RPIP panel]	4	10

- 3 Dilute each library to the starting concentration for your system using RSB. Combine 10 µl each diluted library in a tube to pool libraries.
- 4 Follow the denature and dilute instructions for your system to dilute libraries to the final loading concentration.

# Supporting Information

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## Acronyms

Acronym	Definition
CEX	Coding Exome Oligos
dsDNA	Double-stranded DNA
EBLTL	Enrichment Bead-Linked Transposomes
EE1	Enrichment Elution Buffer 1
EEW	Enhanced Enrichment Wash
EHB2	Enrich Hyb Buffer 2
EPH3	Elute, Prime, Fragment High Concentration Mix
EPM	Enhanced PCR Mix
ET2	Elute Target Buffer 2
EtOH	Ethanol
HP3	2 N NaOH
NHB2	Hyb Buffer 2 + IDT NXT Blockers
PPC	PCR Primer Cocktail
RPIP	Respiratory Pathogen ID/AMR Panel
RSB	Resuspension Buffer
RVO	Respiratory Virus Oligos
SMB	Streptavidin Magnetic Beads
SMM	Second Strand Marking Master Mix
ST2	Stop Tagment Buffer 2
TB1	Tagmentation Buffer 1
TWB	Tagment Wash Buffer
UD	Unique dual

## Kit Contents and Storage

Make sure that you have all reagents identified in this section before starting library prep. The protocol requires one Illumina RNA Prep with Enrichment, (L) Tagmentation, one panel, and at least one IDT for Illumina DNA/RNA UD Indexes set. Combine all four sets to index 384 libraries.

Component	Name	Illumina Catalog #
Library Prep	Illumina RNA Prep with Enrichment, (L) Tagmentation (16 Samples)	20040536
	Illumina RNA Prep with Enrichment, (L) Tagmentation (96 Samples)	20040537

Component	Name	Illumina Catalog #
Indexes	IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)	20027213
	IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)	20027214
	IDT for Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes, 96 Samples)	20042666
	IDT for Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes, 96 Samples)	20042667
Panel	Illumina Exome Panel	20020183
	Respiratory Virus Oligos Panel v2	20044311
Respiratory Pathogen ID/AMR Enrichment Kits	Respiratory Pathogen ID/AMR PanelTarget Enrichment Kit (RUO) A	20047050
	Respiratory Pathogen ID/AMR PanelTarget Enrichment Kit (RUO) B	20046969
	Respiratory Pathogen ID/AMR PanelTarget Enrichment Kit (RUO) C	20047051
	Respiratory Pathogen ID/AMR PanelTarget Enrichment Kit (RUO) D	20047052

The required prep kits provide reagents for denaturing, cDNA synthesis, library prep, and enrichment in a 16- or 96-sample size, while the panels provide application-specific oligos. The index sets provide premixed Index 1 (i7) and Index 2 (i2) adapters.

The Illumina kits do not include Agencourt AMPure XP. For suppliers, see [Ancillary Consumables](#).

## Illumina RNA Prep with Enrichment, (L) Tagmentation (16 Samples) (20040536)

### Illumina cDNA Synthesis

Quantity	Reagent	Description	Cap	Shipment	Storage
1	EPH3	Elute, Prime, Fragment, High Concentration Mix	Clear	-25°C to -15°C	-25°C to -15°C
1	FSA	First Strand Synthesis Act D Mix	Amber	-25°C to -15°C	-25°C to -15°C
1	RSB	Resuspension Buffer	Clear	-25°C to -15°C	-25°C to -15°C
1	RVT	Reverse Transcriptase	Clear	-25°C to -15°C	-25°C to -15°C
1	SMM	Second Strand Marking Master Mix	Clear	-25°C to -15°C	-25°C to -15°C

### Illumina DNA/RNA Prep — Tagmentation Beads

Quantity	Reagent	Description	Cap	Shipment	Storage
1	EBLTL	Bead-Linked Transposomes	Yellow	2°C to 8°C	2°C to 8°C
2	RSB	Resuspension Buffer	Clear	2°C to 8°C	2°C to 8°C

### Illumina DNA/RNA Prep — Tagmentation Buffers

Quantity	Reagent	Description	Cap	Shipment	Storage
1	ST2	Stop Tagment Buffer 2	Red	2°C to 8°C	Room temperature
1	TWB	Tagment Wash Buffer	Clear	2°C to 8°C	Room temperature

## Illumina DNA/RNA Prep — Tagmentation PCR Reagents

Quantity	Reagent	Description	Cap	Shipment	Storage
2	EPM	Enhanced PCR Mix	Clear	2°C to 8°C	-25°C to -15°C
1	TB1	Tagmentation Buffer 1	Clear	2°C to 8°C	-25°C to -15°C

## Illumina RNA Fast Hyb Enrichment Beads + Buffers

Quantity	Reagent	Description	Cap	Shipment	Storage
1	EHB2	Enrich Hyb Buffer 2	Clear	2°C to 8°C	2°C to 8°C
1	ET2	Elute Target Buffer 2	Clear	2°C to 8°C	2°C to 8°C
1	RSB	Resuspension Buffer	Clear	2°C to 8°C	2°C to 8°C
1	SMB	Streptavidin Magnetic Beads	Clear	2°C to 8°C	2°C to 8°C

## Illumina RNA Fast Hyb Enrichment PCR + Buffers

Quantity	Reagent	Description	Cap	Shipment	Storage
1	EE1	Enrichment Elution Buffer 1	Clear	2°C to 8°C	-25°C to -15°C
1	EEW	Enhanced Enrichment Wash	Amber	2°C to 8°C	-25°C to -15°C
1	EPM	Enhanced PCR Mix	Clear	2°C to 8°C	-25°C to -15°C
1	HP3	2 N NaOH	Clear	2°C to 8°C	-25°C to -15°C
1	NHB2	Hyb Buffer 2 + IDT NXT Blockers	Blue	2°C to 8°C	-25°C to -15°C
1	PPC	PCR Primer Cocktail	Clear	2°C to 8°C	-25°C to -15°C

Illumina RNA Prep with Enrichment, (L) Tagmentation  
(96 Samples) (20040537)

## Illumina cDNA Synthesis

Quantity	Reagent	Description	Cap	Shipment	Storage
4	EPH3	Elute, Prime, Fragment, High Concentration Mix	Clear	-25°C to -15°C	-25°C to -15°C
4	FSA	First Strand Synthesis Act D Mix	Amber	-25°C to -15°C	-25°C to -15°C
2	RSB	Resuspension Buffer	Clear	-25°C to -15°C	-25°C to -15°C
1	RVT	Reverse Transcriptase	Clear	-25°C to -15°C	-25°C to -15°C
4	SMM	Second Strand Marking Master Mix	Clear	-25°C to -15°C	-25°C to -15°C

## Illumina DNA/RNA Prep — Tagmentation Beads

Quantity	Reagent	Description	Cap	Shipment	Storage
4	EBLTL	Bead-Linked Transposomes	Yellow	2°C to 8°C	2°C to 8°C
2	RSB	Resuspension Buffer	Clear	2°C to 8°C	2°C to 8°C

## Illumina DNA/RNA Prep — Tagmentation Buffers

Quantity	Reagent	Description	Cap	Shipment	Storage
4	ST2	Stop Tagment Buffer 2	Red	2°C to 8°C	Room temperature
1	TWB	Tagment Wash Buffer	Clear	2°C to 8°C	Room temperature

## Illumina DNA/RNA Prep — Tagmentation PCR Reagents

Quantity	Reagent	Description	Cap	Shipment	Storage
4	EPM	Enhanced PCR Mix	Clear	2°C to 8°C	-25°C to -15°C
4	TB1	Tagmentation Buffer 1	Clear	2°C to 8°C	-25°C to -15°C

## Illumina RNA Fast Hyb Enrichment Beads + Buffers

Quantity	Reagent	Description	Cap	Shipment	Storage
1	EHB2	Enrich Hyb Buffer 2	Clear	2°C to 8°C	2°C to 8°C
1	ET2	Elute Target Buffer 2	Clear	2°C to 8°C	2°C to 8°C
1	RSB	Resuspension Buffer	Clear	2°C to 8°C	2°C to 8°C
2	SMB	Streptavidin Magnetic Beads	Clear	2°C to 8°C	2°C to 8°C

## Illumina RNA Fast Hyb Enrichment PCR + Buffers

Quantity	Reagent	Description	Cap	Shipment	Storage
2	EE1	Enrichment Elution Buffer 1	Clear	2°C to 8°C	-25°C to -15°C
4	EEW	Enhanced Enrichment Wash	Amber	2°C to 8°C	-25°C to -15°C
2	EPM	Enhanced PCR Mix	Clear	2°C to 8°C	-25°C to -15°C
1	HP3	2 N NaOH	Clear	2°C to 8°C	-25°C to -15°C
1	NHB2	Hyb Buffer 2 + IDT NXT Blockers	Blue	2°C to 8°C	-25°C to -15°C
1	PPC	PCR Primer Cocktail	Clear	2°C to 8°C	-25°C to -15°C

### IDT for Illumina DNA/RNA UD Indexes, Store at -25°C to -15°C (96 Indexes, 96 Samples) (20027213, 20027214, 20042666, 20042667)

Quantity	Reagent	Description
1	UDP0001-UDP0096	Set A index adapter plate
1	UDP0097-UDP0192	Set B index adapter plate
1	UDP0193-UDP0288	Set C index adapter plate
1	UDP0289-UDP0384	Set D index adapter plate

## Enrichment Panels (20020183, 20042472, 20044312)

Panel Name	Quantity	Reagent	Description	Shipment	Storage
Illumina Exome Panel	1	CEX	Coding Exome Oligos	-25°C to -15°C	-25°C to -15°C
Respiratory Virus Panel v2	1	RVO	Respiratory Virus Oligos	-25°C to -15°C	-25°C to -15°C
Respiratory Pathogen ID/AMR Panel (TBD)	TBD	TBD	TBD	TBD	TBD

## Respiratory Pathogen ID/AMR Panel Target Enrichment Kits (RUO)

For the following kits, use the Explify software app in Basespace for analysis.

Respiratory Pathogen ID/AMR Panel Target Enrichment Kit (RUO) A (96 Indexes, 96 Samples) Catalog number: 20047050

Products included	Catalog number	Quantity
Illumina RNA Prep with Enrichment (L) Tagmentation 96 Samples	20040537	1
Respiratory Pathogen ID/AMR Panel + IDbyDNA; 32 enrichments in 3-plex format; 96 Samples	20046187	1
IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes)	20027213	1

Respiratory Pathogen ID/AMR Panel Target Enrichment Kit (RUO) B (96 Indexes, 96 Samples) Catalog number: 20046969

Products included	Catalog number	Quantity
Illumina RNA Prep with Enrichment (L) Tagmentation 96 Samples	20040537	1
Respiratory Pathogen ID/AMR Panel + IDbyDNA; 32 enrichments in 3-plex format; 96 Samples	20046187	1
IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes)	20027214	1

Respiratory Pathogen ID/AMR Panel Target Enrichment Kit (RUO) C (96 Indexes, 96 Samples) Catalog number: 20047051

Products included	Catalog number	Quantity
Illumina RNA Prep with Enrichment (L) Tagmentation 96 Samples	20040537	1
Respiratory Pathogen ID/AMR Panel + IDbyDNA; 32 enrichments in 3-plex format; 96 Samples	20046187	1
IDT for Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes)	20042666	1

## Respiratory Pathogen ID/AMR Panel Target Enrichment Kit (RUO) D (96 Indexes, 96 Samples) Catalog number: 20047052

Products included	Catalog number	Quantity
Illumina RNA Prep with Enrichment (L) Tagmentation 96 Samples	20040537	1
Respiratory Pathogen ID/AMR Panel + IDbyDNA; 32 enrichments in 3-plex format; 96 Samples	20046187	1
IDT for Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes)	20042667	1

### Consumables and Equipment

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

#### Ancillary Consumables

Consumable	Supplier
1.5 ml microcentrifuge tubes	General lab supplier
1.7 ml microcentrifuge tubes	General lab supplier
1.7 ml microcentrifuge tubes, RNase-free	General lab supplier
10 µl pipette tips	General lab supplier
20 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
1000 µl pipette tips	General lab supplier
96-well PCR plates, semiskirted	General lab supplier
Agilent DNA 1000 Kit	Agilent, catalog # 5067-1504
Agencourt AMPure XP, 5 ml	Beckman Coulter, catalog # A63880
Ethyl alcohol, pure (500 ml)	Sigma-Aldrich, catalog # E7023
Microseal 'B' PCR Plate Sealing Film	Bio-Rad, catalog # MSB1001
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, catalog # 89094-658
Qubit Assay Tubes	Thermo Fisher Scientific, catalog # Q32856
Qubit dsDNA BR Assay Kit	Thermo Fisher Scientific, catalog # Q32850 or Q32853
Ultrapure water, nuclease-free	General lab supplier

#### Ancillary Equipment

Equipment	Supplier
10 µl multichannel pipettes	General lab supplier
10 µl single-channel pipettes	General lab supplier
20 µl multichannel pipettes	General lab supplier
20 µl single-channel pipettes	General lab supplier



Equipment	Supplier
200 µl single-channel pipettes	General lab supplier
200 µl multichannel pipettes	General lab supplier
1000 µl single-channel pipettes	General lab supplier
2100 Bioanalyzer Desktop System	Agilent Technologies, catalog # G2940CA
Adhesive seal roller	General lab supplier
Bio-Rad C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	Bio-Rad, catalog # 1851197
Microsample incubator	General lab supplier
1.5 ml tube block for microsample incubator	General lab supplier
Magnetic Stand-96	Thermo Fisher Scientific, catalog # AM10027
Microplate centrifuge	General lab supplier
Vortexer	General lab supplier
One of the following shakers:	Q Instruments, catalog #:
• BioShake iQ High-Speed Thermal Mixer	• 1808-0506
• BioShake XP High-Speed Thermal Mixer	• 1808-0505

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: [www.illumina.com](http://www.illumina.com)  
Email: [techsupport@illumina.com](mailto:techsupport@illumina.com)

## Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
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Safety data sheets (SDSs)—Available on the Illumina website at [support.illumina.com/sds.html](http://support.illumina.com/sds.html).

Product documentation—Available for download from [support.illumina.com](http://support.illumina.com).



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