TruSeq Targeted RNA Expression Checklist

Synthesize cDNA

- ☐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 a plate:
  - Add 5 µl diluted intact total RNA to the CDP1 plate.
  - Add 3 µl diluted degraded RNA to the CDP plate.
- ☐5 Combine the following volumes in a 1.7 ml microcentrifuge tube. Multiply each volume by the number of samples being prepared.

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 an 8-tube strip.
- ☐9 Add the volume for your plate:
  - Add 5 µl to the CDP1 plate.
  - Add 7 µl to the CDP plate.
- ☐10 Shake at 1600 rpm for 20 seconds.
- ☐11 Centrifuge at 280 × g for 1 minute.
- ☐12 Place on the 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

- ☐1 Combine the following volumes in a 1.7 ml microcentrifuge tube. Multiply each volume by the number of reactions being prepared.

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 into an 8-tube strip.
- ☐5 Add 10 µl to 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.
- ☐9 Add 30 µl OB1 to the CDP or CDP1 plate.
- ☐10 Shake at 1600 rpm for 1 minute.
- ☐11 Place on the thermal cycler and run the ANNEAL program.
- ☐12 Centrifuge briefly.

Wash, Extend, and Ligate Bound Oligos

- ☐1 Transfer all supernatant to the HYP plate.
- ☐2 Place on a magnetic stand until liquid is clear.
- ☐3 Remove and discard all of the supernatant.
- ☐4 Move from the magnetic stand to a bench.
- ☐5 Add 100 µl AM1 to each well.
- ☐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 liquid is clear.
- ☐9 Remove and discard all supernatant.
- ☐10 Move from the magnetic stand to a bench.
- ☐11 Add 175 µl UB1.
- ☐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 liquid is clear.
- ☐15 Invert ELM4.
- ☐16 Remove and discard all supernatant.
- ☐17 Move from the magnetic stand to a bench.
- ☐18 Add 40 of ELM4.
- ☐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 liquid is clear.
- ☐24 Remove and discard all supernatant.
- ☐25 Add 50 µl of UB1.

Amplify Libraries

- ☐1 Arrange Index 1 (i7) adapters in columns 1–12.
- ☐2 Arrange Index 2 (i5) adapters in rows A–H.
- ☐3 Place the plate on the TruSeq Index Plate Fixture.
- ☐4 Add 4 µl of each Index 1 adapter down each column.
- ☐5 Add 4 µl of each Index 2 adapter across each row.
- ☐6 Remove and discard all supernatant from the HYP plate.
- ☐7 Remove from the magnetic stand.
- ☐8 Add 22.5 µl diluted HP3.
- ☐9 Shake at 1800 rpm for 30 seconds.
- ☐10 Incubate at room temperature for at least 5 minutes.
- ☐11 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 1.7 ml microcentrifuge tube.
  - **16 libraries**—Combine 9.2 µl TDP1 and 460 µl PMM2 in a 1.7 ml microcentrifuge tube.
- ☐12 Invert to mix.
- ☐13 Add 22 µl to the IAP plate.
- ☐14 Unseal the HYP plate.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Transfer 20 µl supernatant from the HYP plate to the IAP plate.
- ☐17 Shake at 1600 rpm for 30 seconds.
- ☐18 Centrifuge at 280 × g for 1 minute.
- ☐19 Place on the 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

- ☐1 Add 85 µl AMPure XP Beads to the CLP plate.
- ☐2 Centrifuge the IAP plate at 280 × g for 1 minute.
- ☐3 Unseal the IAP plate.
- ☐4 Transfer all supernatant to 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 liquid is clear.
- ☐9 Remove and discard 135 µl supernatant.
- ☐10 Wash 2 times with 200 µl 80% EtOH.
- ☐11 Air dry on the magnetic stand for 15 minutes.
- ☐12 Remove from the magnetic stand.
- ☐13 Add 15 µl RSB.
- ☐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 plate at room temperature for 2 minutes.
- ☐18 Unseal and place on a magnetic stand until liquid is clear.
- ☐19 Transfer 12.5 µl supernatant to 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

- ☐1 Transfer 5 µl from the LNP plate to a 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.
- ☐6 Select the **Region Analysis** tab.
- ☐7 Drag the blue region lines to capture the 100–300 bp region.
- ☐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.

Acronyms

Acronym	Definition
ACD1	Amplicon Control DNA 1
ACP1	Amplicon Control Oligo Pool 1
TSO	TruSight Oligos
CLP	Clean-up Plate
EBT	Elution Buffer with Tris
ELM4	Extension Ligation Mix 4
FPU	Filter Plate Unit
HT1	Hybridization Buffer
HYP	Hybridization Plate
IAP	Index Amplification Plate
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNP	Library Normalization Plate
LNS2	Library Normalization Storage Buffer 2
LNW1	Library Normalization Wash 1
OHS2	Oligo Hybridization for Sequencing Reagent 2
PAL	Pooled Amplicon Library