

# Automated Illumina® TruSeq® Stranded Total RNA library construction with the *epMotion*® 5075t/TMX

## Introduction

This protocol describes the configuration and preprogrammed methods for the automated construction of 8/16 or 24 sequencing ready libraries from 100-1000 ng total RNA with the Illumina TruSeq Stranded Total RNA kit. The overall hands on time is less than 1 hour, the total run time of the entire procedure is ~11.5 hours for 24 samples.

The three methods (\*.dws files) from the download archive need to be unzipped prior to importing into the epBlue™

Software. The labware archive (Top Update\_TS-stranded-TRNA.zip) needs to be imported into the epBlue Software as well.

The entire library construction procedure is carried out by 3 sub-methods that need to be run subsequently on the *epMotion*. Intermediate products from the individual sub-methods can be stored at -20 °C for up to 7 days, according to Illumina's kit user manual.

## Material and Methods

### Required equipment

- > *epMotion* 5075 TMX or *epMotion* 5075t
- > additional thermal module (Position C2)
- > Gripper
- > TS50 pipetting tool
- > TS300 pipetting tool
- > TM50-8 pipetting tool
- > TM300-8 pipetting tool
- > 4x thermoadapter for PCR plates, 96-well
- > Reservoir rack
- > 3x RR Module TC Safe Lock
- > PCR Cycler, e.g. Eppendorf Mastercycler® Pro S
- > Alpaqua® LE magnet plate (low elution volume magnet, Alpaqua order no. A000350)
  - ➔ this magnet is the only one known to work with the low elution volumes required in some steps of the procedure - don't use an other one!

### Required consumables

- > epT.I.P.S.® Motion 50 µL Filter
- > epT.I.P.S. Motion 300 µL Filter
- > Eppendorf twin.tec® PCR plates, 96-well, semi skirted
- > Eppendorf twin.tec PCR plates, 96-well, skirted (for the Index Adapters)
- > Eppendorf Safe-Lock Tube 1.5 mL
- > Eppendorf Safe-Lock Tube 2.0 mL
- > *epMotion* reservoir 30 mL
- > Eppendorf 400 mL reservoir
- > Agencourt® AMPure® XP beads (Beckman Coulter®, order nos. A63880, A63881, A63882)
- > Agencourt RNAClean® XP beads (Beckman Coulter, order nos. A63987, A66514)
- > 70 and 80 % Ethanol
- > RNase free water
- > mineral oil, PCR/molecular biology grade (Sigma-Aldrich®, order no. M5904-500ML)
- > SuperScript® II Reverse Transcriptase (Life Technologies®, order no. 18064-014)
- > Illumina TruSeq Stranded Total RNA kit (either Ribo-Zero™ or Ribo-Zero Gold)

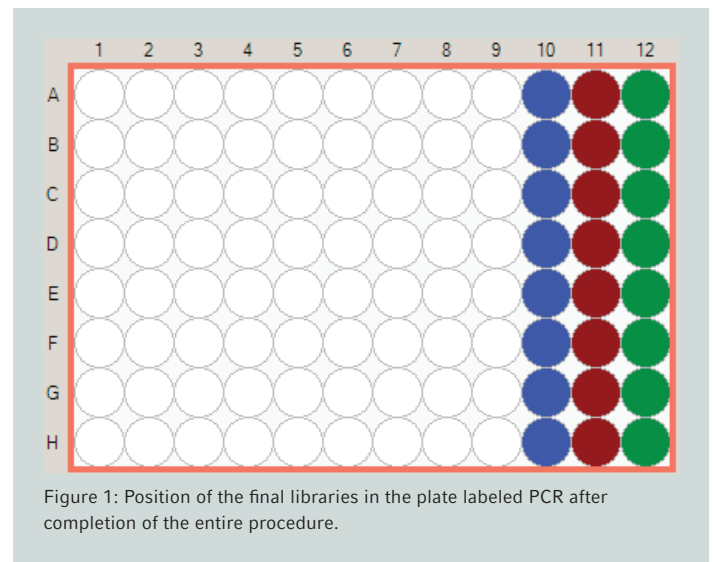
**Method**

Method Name	approx. Runtime (24 samples)
XXYYZZ-01-TS-STRNA.dws	5.5hrs
XXYYZZ-02-TS-STRNA.dws	4hrs
XXYYZZ-03-TS-STRNA.dws	2hrs, including external PCR

(XX = year, YY = month, ZZ = day)

This approach is programmed to provide as much automation as possible, thus only up to 24 samples can be processed. Please only process 8/16 or 24 samples, sample numbers non divisible by 8 are not supported. The entire workflow – from 100 to 1000 ng total RNA sample input to sequencing ready libraries – is divided into three *epMotion* methods (or sub-methods, see above). Each of the methods ends at a “Safe Stopping Point”, allowing to store the intermediate products at -20 °C to -15 °C for up to 7 days, as stated in the Kit’s user guide. In the default setup only the third sub-method requires a user intervention to perform the enrichment PCR in an external cycler. To avoid dead volumes, all Illumina Kit reagents are programmed either in 1.5 mL or 2 mL Tubes, only Agencourt Magnetic Beads and Ethanol for the washes are provided in 30 mL reservoirs to allow 8 channel pipetting. All liquid waste is collected in a 400 mL reservoir in B0. As most of the used volumes are very low, all reagents must be checked for foam, air bubbles etc. to ensure best performance prior to starting the runs. For some of the reagents, the beads and the mineral oil, it is mandatory to let them reach ambient temperature to ensure proper function and pipetting due to changes in viscosity. During the procedure no cooling of the reagents is required. All steps of the procedure are performed in 96-well twin.tec PCR plates (semi-skirted), for the multiple heat incubation steps above 37 °C, samples are overlaid with oil to avoid evaporation and allow temperature incubations on the *epMotion*. Only the enrichment PCR needs to be carried out in an external PCR cycler. The methods were developed on the *epMotion* 5075 TMX, but can also be transferred to the *epMotion* 5075t.

**Important:** The output plate containing the samples that need to be processed in the subsequent sub-method will always be placed on the C2 position (Temp) set to 10 °C at the end of the individual methods. Final libraries will be found in columns 10-12 of the plate labeled PCR. The volume is 17.5 µL:



**Sub-method 01**

Start with 100 – 1000 ng total RNA in a volume of 10 µL per sample. 8/16 or 24 samples have to be provided in the first three columns (Wells A1-H3) of a 96-well twin.tec semi skirted PCR plate (BRP+DFP), placed on the TMX position. The method ends with clean cDNA in the first three columns (A1-H3) of a second 96-well twin.tec semi skirted PCR plate (ALP). This plate needs to be used in the sub-method 02.

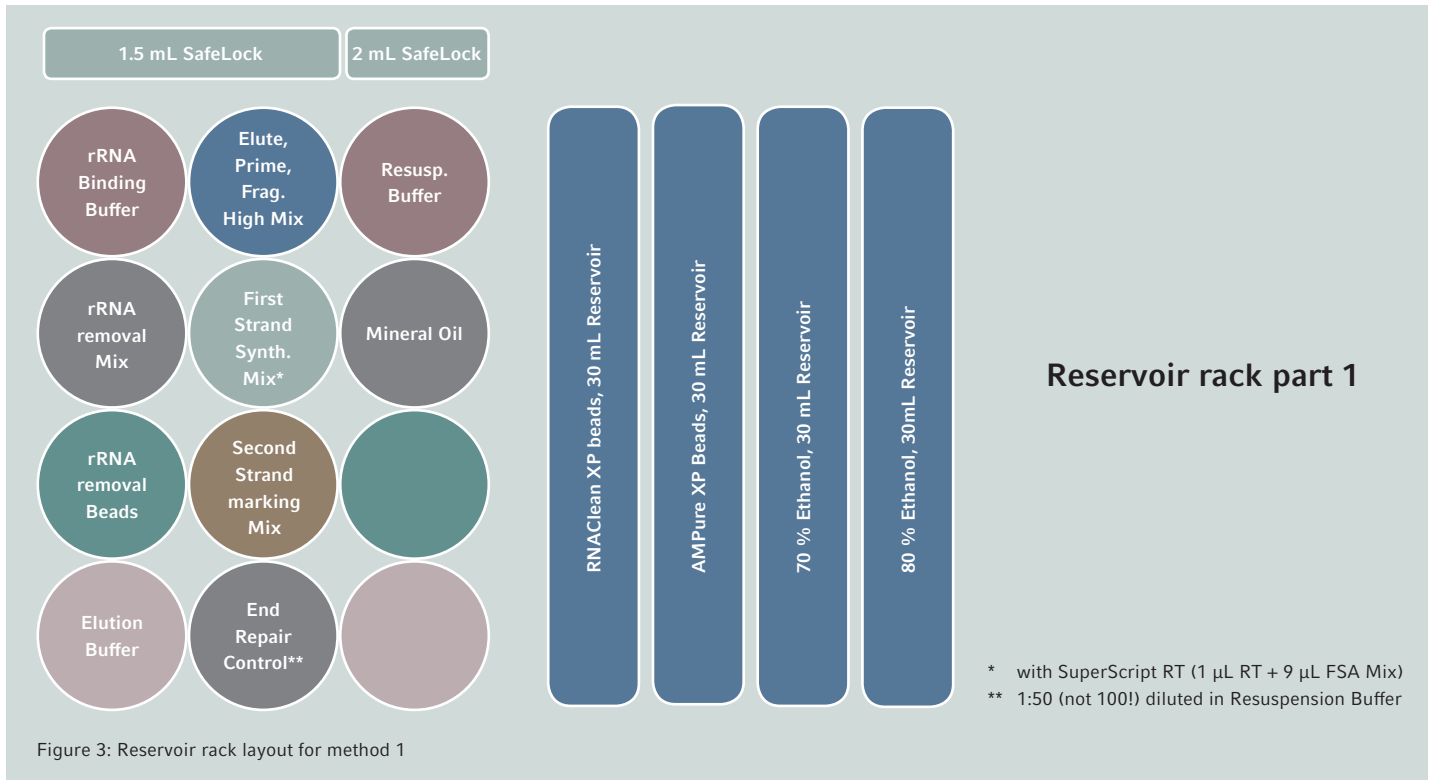
**Worktable Layout**

Position	Item	Position	Item
A2	50 µL Filtrertips	B3	50 µL Filtrertips
A3	50 µL Filtrertips	B4	Thermoadapter PCR 96
A4 (TMX)	Thermoadapter PCR 96 + PCR plate with RNA samples (labeled BRP+DFP)	C1	Thermoadapter PCR 96 + empty PCR plate (labeled ALP)
B0	400 mL tub for liquid waste	C2 (Temp)	Thermoadapter PCR 96
B1	300 µL Filtrertips	C3	Reservoir rack with 3x RR Module Safe Lock + 4x 30 mL reservoir for reagents
B2	300 µL Filtrertips	C4	Alpaqua LE magnet plate + empty PCR plate (labeled RRP-RCP)



Figure 2: epMotion worktable layout for method 1

Reservoir rack layout



**Attention:** If kit included controls are not going to be used, use plain resuspension buffer in the according reagent position.

**Sub-method 02**

Start with the PCR plate labeled ALP containing the cDNAs from sub-method 01 in Positions A1 – H3, placed on position B4. The method ends with A-tailed and Index Adapter ligated clean cDNA in a PCR plate labeled PCR. Depending on the sample number, sequencing setup, pooling scheme etc. the number, combination and labware of the Index Adapters (position B3) needs to be modified ➔ also review/adjust steps 26 and following + the worktable in the method. If the default setup is being used, a user intervention to refill 50  $\mu$ L tips is required.

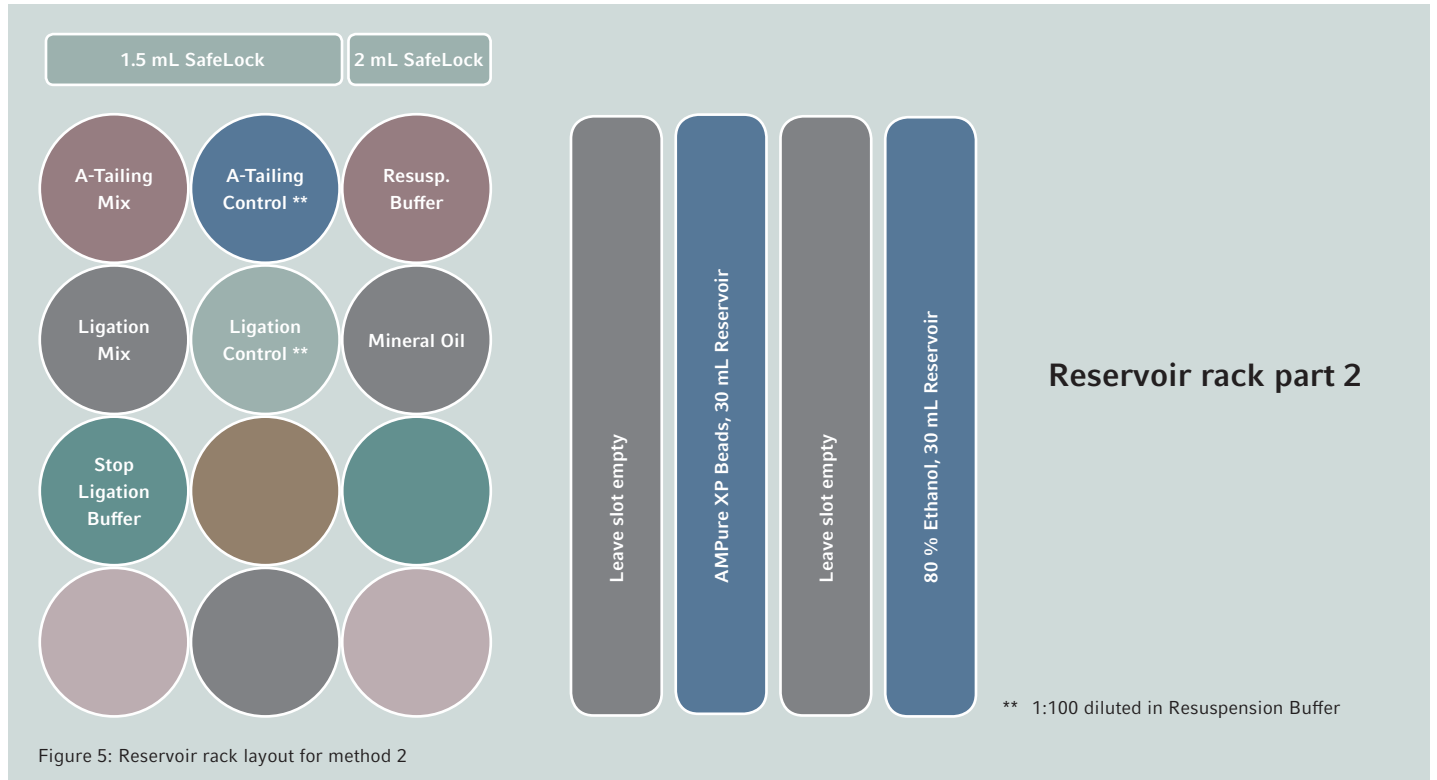
**Worktable Layout**

Position	Item	Position	Item
A2	50 $\mu$ L Filtrertips	C1	Thermoadapter PCR 96 + empty PCR plate (labeled PCR)
A3	50 $\mu$ L Filtrertips	C2 (Temp)	Thermoadapter PCR 96
A4 (TMX)	Thermoadapter PCR 96	C3	Reservoir rack with 3x RR Module SafeLock + 2x 30 mL Reservoir (pos.5 & 7)
B0	400 mL tub for liquid waste	C4	Alpaqua LE magnet plate
B1	300 $\mu$ L Filtrertips		
B2	300 $\mu$ L Filtrertips		
B3	skirted PCR plate with index adapters ➔ review method programming		
B4	Thermoadapter PCR 96 + PCR plate with cDNA (ALP) from sub-method 01		



Figure 4: epMotion worktable layout for method 2

Reservoir rack layout



**Attention:** If kit included controls are not going to be used, use plain resuspension buffer in the according reagent position.

**Sub-method 03**

Start with 20  $\mu$ L A-tailed and Index Adapter ligated samples in plate labeled PCR from sub-method 02 on TMX position. Only the PCR setup will be pipetted, which takes a couple of minutes, then a user intervention occurs where the PCR plate needs to be sealed and cycled in a PCR cycler to enrich the libraries. Reopen the plate after PCR and restore on the TMX position prior to continuing the method.

**Worktable Layout**

Position	Item	Position	Item
A2	50 $\mu$ L Filtrertips	C1	Thermoadapter PCR 96
A3	empty	C2 (Temp)	Thermoadapter PCR 96
A4 (TMX)	Thermoadapter PCR 96 + PCR plate (PCR) with samples from sub-method 02	C3	Reservoir rack with 3x RR Module Safe Lock + 2x 30 mL Reservoir (pos.5 & 7)
B0	400 mL tub for liquid waste	C4	Alpaqua LE magnet plate
B1	300 $\mu$ L Filtrertips		
B2	300 $\mu$ L Filtrertips		
B3	empty		
B4	Thermoadapter PCR 96		

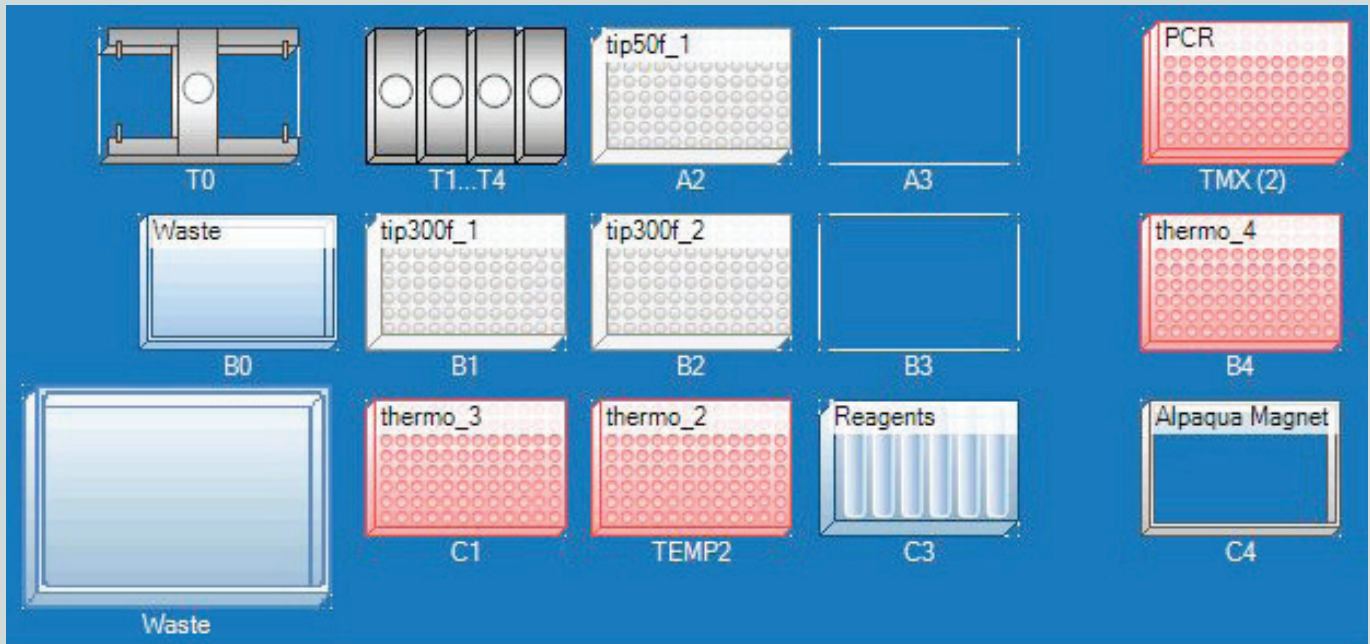


Figure 6: epMotion worktable layout for method 3

Reservoir rack layout

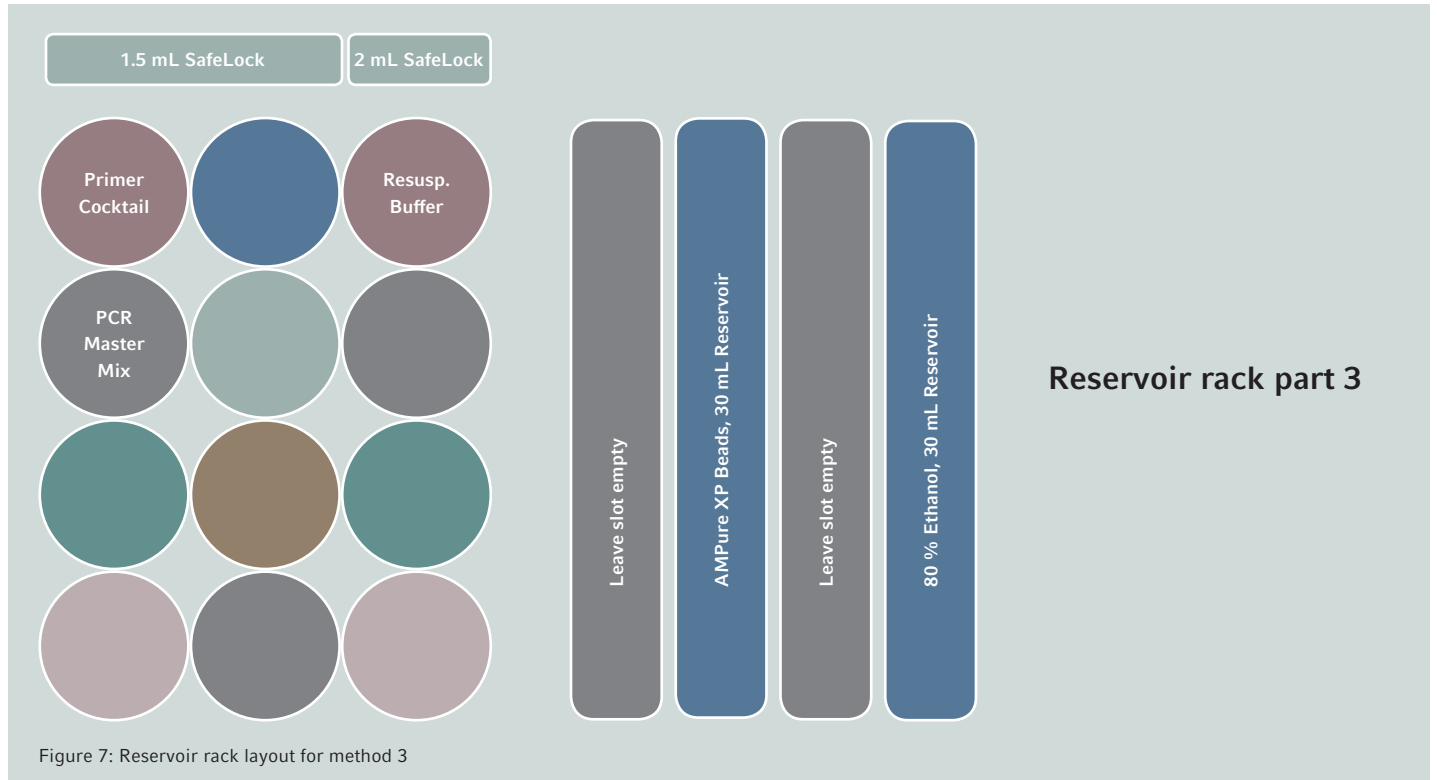


Figure 7: Reservoir rack layout for method 3

After all three sub-methods have been completed, the final libraries are in the plate labeled PCR on position C2 (Temp) at 10 °C.



## Ordering Information

Description	Order no. international
epMotion® 5075t	5075 000.302
Thermal module	5075 757.001
TS 50 dispensing tool	5280 000.010
TS 300 dispensing tool	5280 000.037
TM50-8 dispensing tool	5280 000.215
TM300-8 dispensing tool	5280 000.231
Gripper	5282 000.018
Thermoadapter PCR 96	5075 787.008
Reservoir rack	5075 754.002
Reservoir rack module TC Safe-Lock	5075 799.081
epT.I.P.S.® Motion, 50 µL, filtered	0030 014.413
epT.I.P.S.® Motion, 300 µL, filtered	0030 014.456
Reservoir 30 mL	0030 126.505
Reservoir 400 mL	5075 751.364
Eppendorf twin.tec® PCR Plate 96, semi-skirted	0030 128.575
Eppendorf twin.tec® PCR Plate 96, skirted	0030 128.648
Eppendorf Safe-Lock Tubes, 1.5 mL	0030 120.086
Eppendorf Safe-Lock Tubes, 2.0 mL	0030 120.094

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