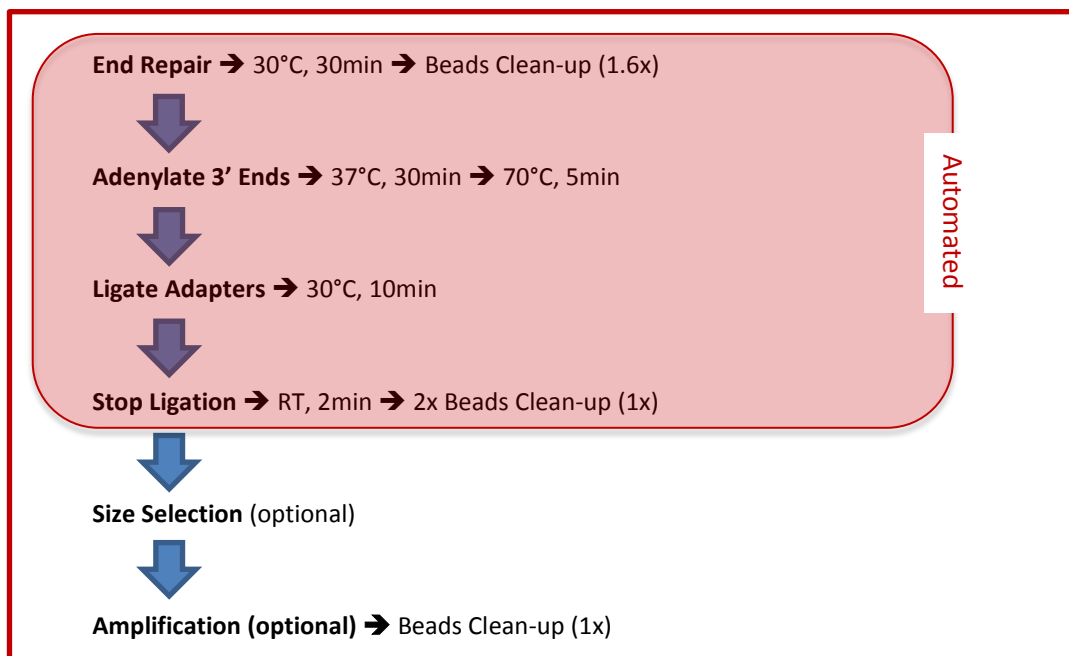


1. About the protocol

The “TruSeq_RNA_SamplePrep” protocol on the IP-Star[®] is using the standard “TruSeq[™] RNA Sample Preparation” kit v2 and reagents from Illumina[®].

It provides flexibility to prepare 1 to 16 libraries in one run starting from **cdNA**. The whole protocol takes approximately 3h for 8 samples. It allows you to prepare up to 32 libraries per day with 2 runs. At the end, you recover ligated products ready for size selection (if required) and amplification.

2. Workflow



3. Material required

a. Reagents & kits

Item	Supplier
TruSeq [™] RNA Sample Preparation Kit v2	Illumina [®]
Agencourt [®] AMPure [®] XP Beads	Beckman Coulter [®]
Fresh Ethanol 80%	Lab supplier

b. Consumables

Item	Supplier	Catalogue #
200 µl tube strips (8 tubes/strip) + cap strips	Diagenode	C30020002
2 ml microtube	Diagenode	C30010014
Medium reagent container	Diagenode	C30020003
Large reagent container	Diagenode	C30020004
96 well microplates	Diagenode	C30080030
Tips (box)	Diagenode	C30040021
Tips (bulk)	Diagenode	C30040020

4. IP-Star setup

- Switch ON the IP-Star.
- Select “**Protocols**” icon and then click on “**Library prep**”.
- Under “**Library prep**”, select “**TruSeq_RNA_SamplePrep**”.

Note:

If you plan to run between 1 and 8 samples, chose “**TruSeq_RNA_SamplePrep_08**”

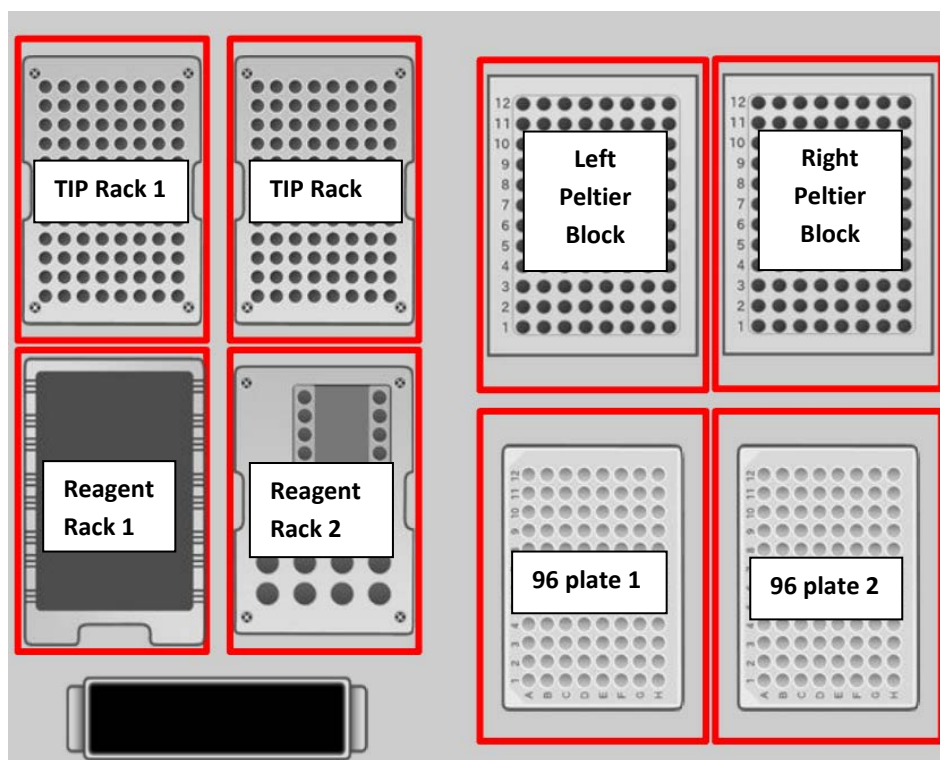
If you plan to run between 9 and 16 samples, chose “**TruSeq_RNA_SamplePrep_16**”

- Setup the exact number of samples that you want to process.

Note:

The **Left Peltier Block** is now cooling down to 4°C to keep your samples cold.

- Setup all the plastics on the platform according to the screen layout.



- Fill **TIP Rack 1** (and 2 if processing 16 protocol) with tips according to the screen.
- Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.
- Fill **96 plate 1** (and 2 if processing 16 protocol) with 96 well microplates.
- Fill **Left and Right Peltier Blocks** with 200 µl tube strips according to the screen.

5. Reagents & Samples setup

Note:

Allow the reagent from “TruSeq[™] RNA Sample Preparation kit v2” to come at 4°C.

Allow “Agencourt[®] AMPure[®] XP Beads” to come at room temperature.

Work on ice from this point.

- Prepare the following mixes.

- **End Repair Mix:**

	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8
End Repair Mix	20 µl	40 µl	60 µl	80 µl	100 µl	120 µl	140 µl	160 µl
Resuspension Buffer	5 µl	10 µl	15 µl	20 µl	25 µl	30 µl	35 µl	40 µl
TOTAL	25 µl	50 µl	75 µl	100 µl	125 µl	150 µl	175 µl	200 µl

	# 9	# 10	# 11	# 12	# 13	# 14	# 15	# 16
End Repair Mix	180 µl	200 µl	220 µl	240 µl	260 µl	280 µl	300 µl	320 µl
Resuspension Buffer	45 µl	50 µl	55 µl	60 µl	65 µl	70 µl	75 µl	80 µl
TOTAL	225 µl	250 µl	275 µl	300 µl	325 µl	350 µl	375 µl	400 µl

- **Adenylation Mix:**

	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8
A-Tailing Mix	12.5 µl	25 µl	37.5 µl	50 µl	67.5 µl	75 µl	87.5 µl	100 µl
Resuspension Buffer	2.5 µl	5 µl	7.5 µl	10 µl	12.5 µl	15 µl	17.5 µl	20 µl
TOTAL	15 µl	30 µl	45 µl	60 µl	75 µl	90 µl	105 µl	120 µl

	# 9	# 10	# 11	# 12	# 13	# 14	# 15	# 16
A-Tailing Mix	112.5 µl	125 µl	137.5 µl	150 µl	167.5 µl	175 µl	187.5 µl	200 µl
Resuspension Buffer	22.5 µl	25 µl	27.5 µl	30 µl	32.5 µl	35 µl	37.5 µl	40 µl
TOTAL	135 µl	150 µl	165 µl	180 µl	195 µl	210 µl	225 µl	240 µl

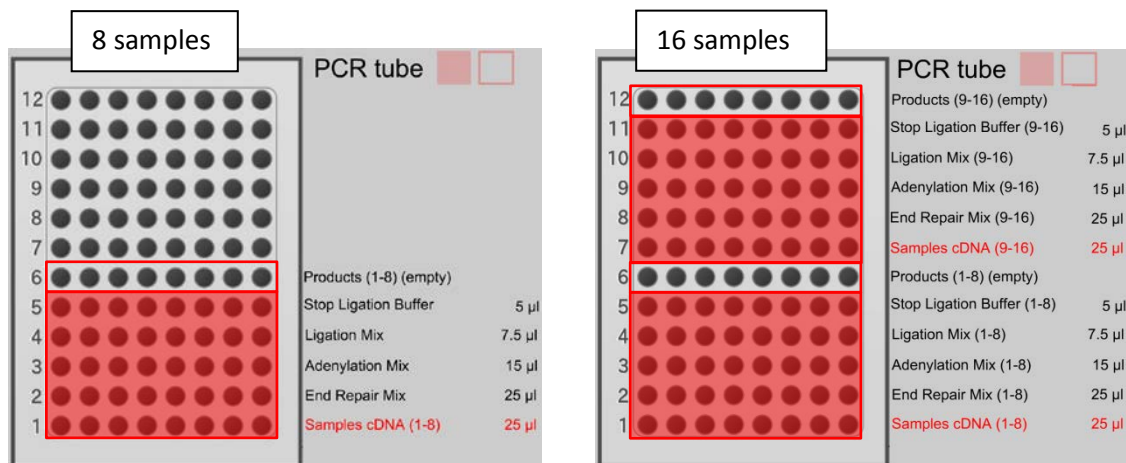
- **Ligation Mix:**

Note:

This mix is different for each sample because of the use of different Adapter Index for each sample

DNA Ligase Mix	2.5 µl
Resuspension Buffer	2.5 µl
Appropriate Adapter Index	2.5 µl
TOTAL	7.5 µl

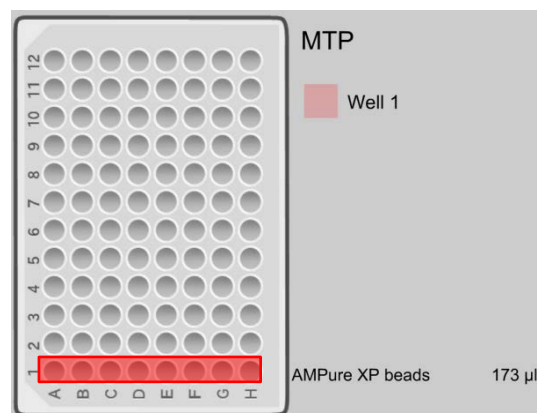
- Fill the **Left Peltier Block** with the mixes according to the screen layout.
- Fill **25 µl of cDNA Samples** in lane 1 (and 7 if processing more than 8 samples).



- Fill 173 µl of **Agencourt[®] AMPure[®] XP Beads** in lane 1 on **96 Plate 1** (and 2 if processing more than 8 samples).

Note:

Resuspend the beads with pipetting up and down several times before dispense them.



- Fill **freshly prepared Ethanol 80%** in the container on the **Reagent Rack 1**.
- Fill **Resuspension Buffer** in the container on **Reagent Rack 2**.
- Close the door and Run.

6. End

- Recover your samples on the **Left Peltier Block** in **lane 6** (1-8) and **lane 12** (9-16).

