IP-Star® Compact

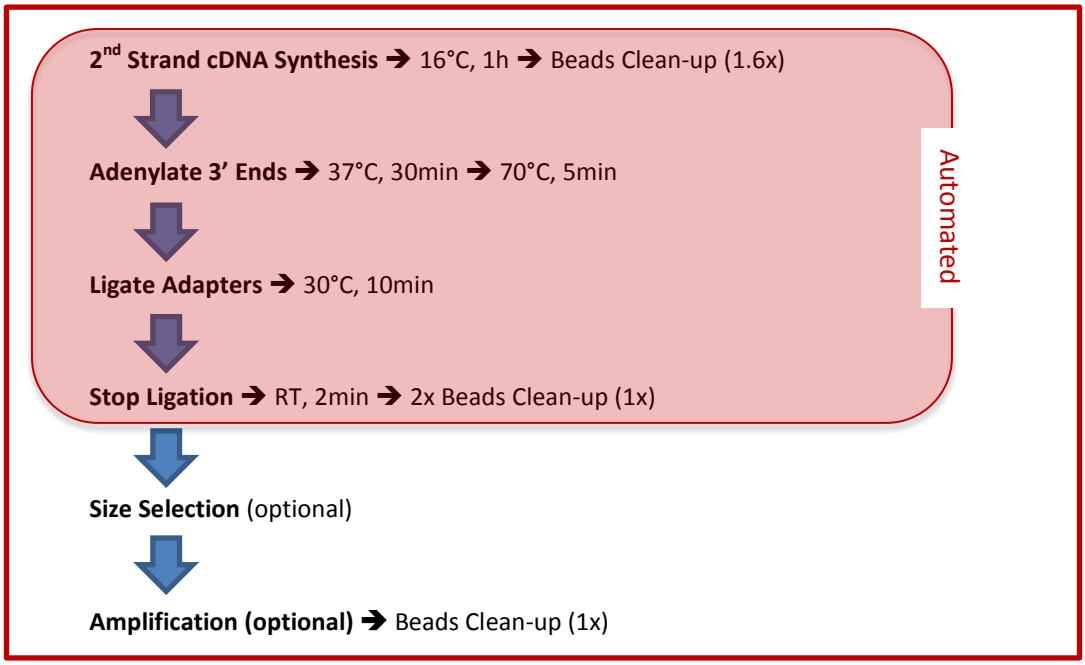
TruSeq™ Stranded RNA Sample Preparation

1. About the protocol

The "TruSeq_Stranded_RNA_SamplePrep" protocol on the IP-Star® is using the standard "TruSeq™ Stranded Total RNA Sample Preparation" kit or "TruSeq™ Stranded mRNA Sample Preparation" kit and reagents from Illumina®.

It provides flexibility to prepare 1 to 16 libraries in one run starting from **1st stranded cDNA**. The whole protocol takes approximately 3h30 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® Stranded Total RNA Sample Preparation	Illumina®
TruSeq® Stranded mRNA Sample Preparation	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

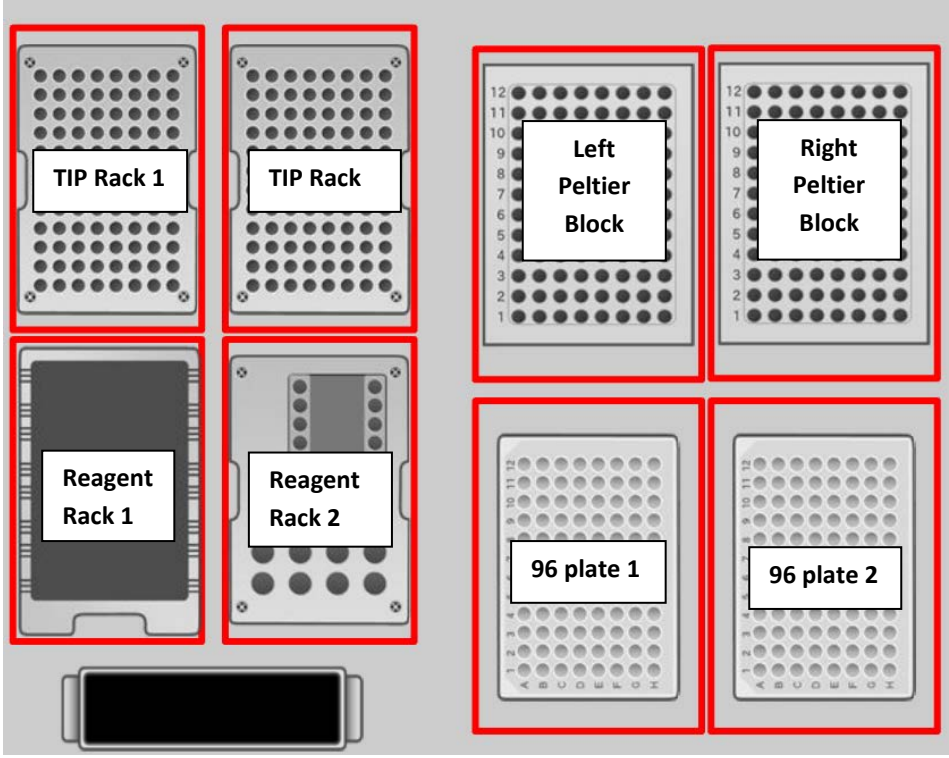
- Switch ON the IP-Star.
- Select "**Protocols**" icon and then click on "**Library prep**".
- Under "**Library prep**", select "**TruSeq_Stranded_RNA_SamplePrep**".

Note:
If you plan to run between 1 and 8 samples, chose "**TruSeq_Stranded_RNA_SamplePrep_08**"
If you plan to run between 9 and 16 samples, chose "**TruSeq_Stranded_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 Stranded Total RNA/mRNA Sample Preparation kit**" 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.

  - **2nd Strand Synthesis Mix:**

	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8
Second Strand Marking Master 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
Second Strand Marking Master 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**

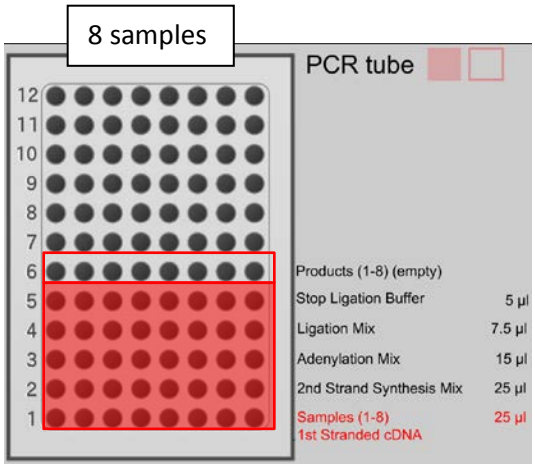
	# 9	# 10	# 11	# 12	# 13	# 14	# 15	# 16
A-Tailing Mix	112.5 µl	125 µl	137.5 µl	150 µl	167.5 µl	175 µl	187.5 µl	200 µl
Resuspension Buffer	22.5 µl	25 µl	27.5 µl	30 µl	32.5 µl	35 µl	37.5 µl	40 µl
TOTAL	**135 µl**	**150 µl**	**165 µl**	**180 µl**	**195 µl**	**210 µl**	**225 µl**	**240 µl**

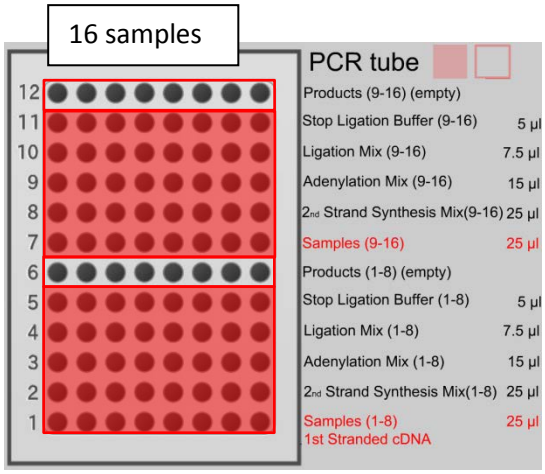
  - **Ligation Mix:**

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

Ligation Mix	2.5 µl
Resuspension Buffer	2.5 µl
Appropriate Adapter Index	2.5 µl
TOTAL	**7.5 µl**

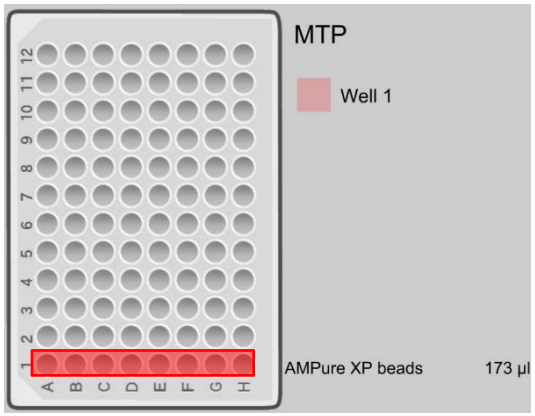
- Fill the **Left Peltier Block** with the mixes according to the screen layout.
- Fill **25 µl of 1st stranded cDNA** in lane 1 (and 7 if processing more than 8 samples).





- 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).