IP-Star® Compact

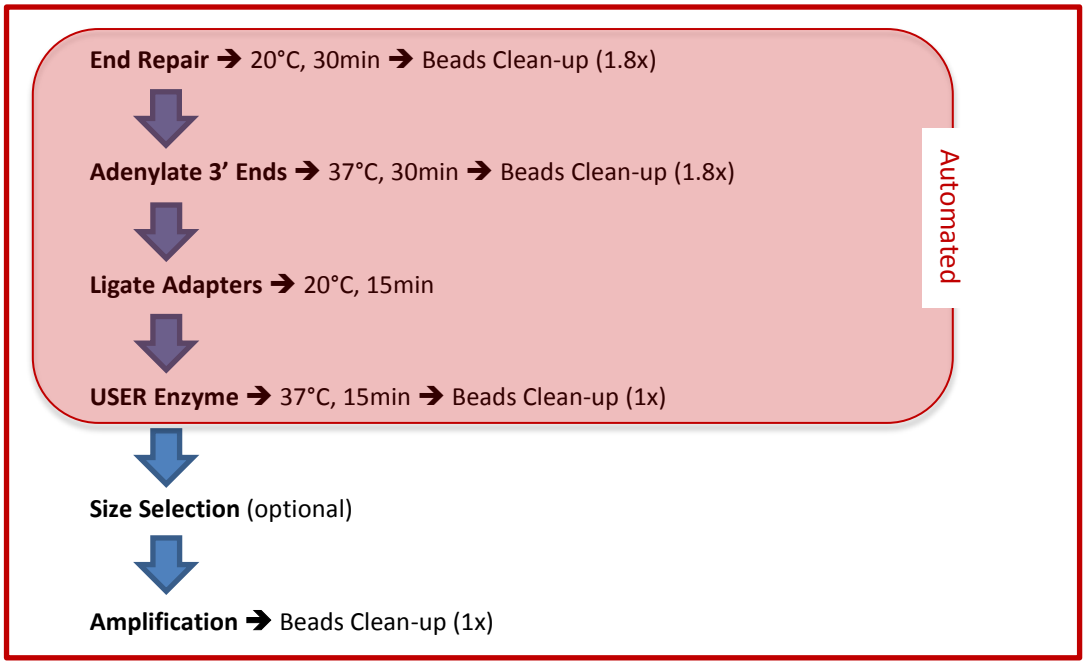
NEBNext® ChIP-Seq Library Prep for Illumina

1. About the protocol

The "NEBNext_ChIP-Seq_Illumina" protocol on the IP-Star® is using the standard "NEBNext® ChIP-Seq Library Prep for Illumina" kit and reagents from NEB®. This kit is compatible with Illumina® sequencers.

It provides flexibility to prepare 1 to 16 libraries in one run starting with **5-10ng** of DNA. The whole protocol takes approximately 3h40 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
NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina®	NEB®
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

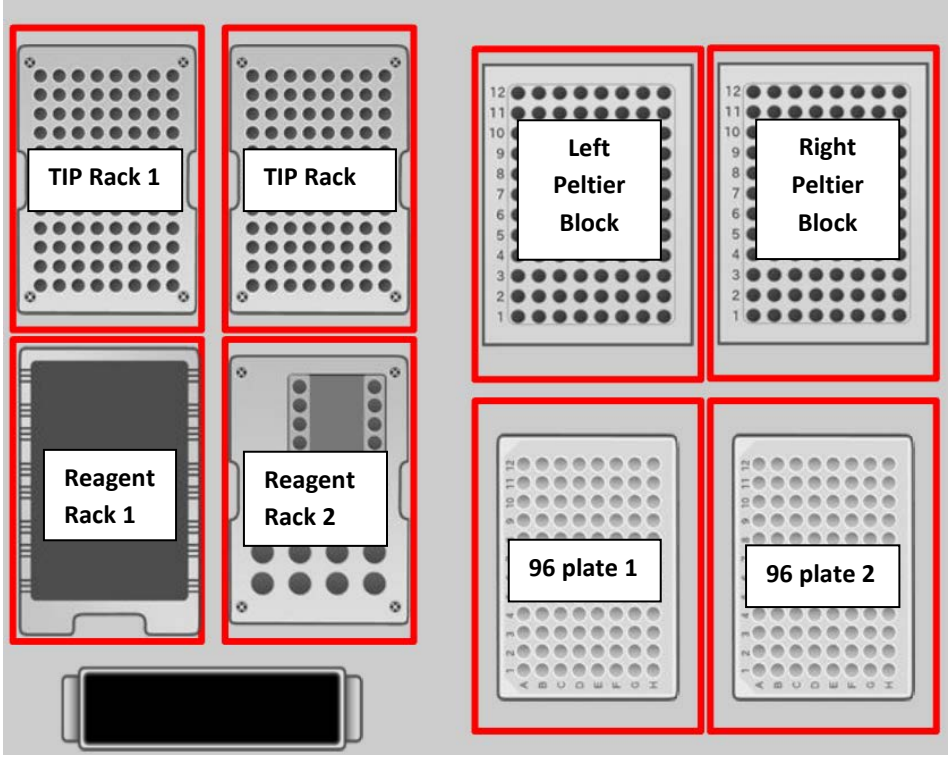
- Switch ON the IP-Star.
- Select "**Protocols**" icon and then click on "**Library prep**".
- Under "**Library prep**", select "**NEBNext_ChIP-Seq_Illumina**".

> **Note:**
> If you plan to run between 1 and 8 samples, chose "**NEBNext_ChIP-Seq_Illumina _08**"
> If you plan to run between 9 and 16 samples, chose "**NEBNext_ChIP-Seq_Illumina_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 "**NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina®**" 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
NEBNext End Repair Reaction Buffer	5 µl	10 µl	15 µl	20 µl	25 µl	30 µl	35 µl	40 µl
NEBNext End Repair Enzyme Mix	1 µl	2 µl	3 µl	4 µl	5 µl	6 µl	7 µl	8 µl
Sterile H_2O	4 µl	8 µl	12 µl	16 µl	20 µl	24 µl	28 µl	32 µl
TOTAL	**10 µl**	**20 µl**	**30 µl**	**40 µl**	**50 µl**	**60 µl**	**70 µl**	**80 µl**

	# 9	# 10	# 11	# 12	# 13	# 14	# 15	# 16
NEBNext End Repair Reaction Buffer	45 µl	50 µl	55 µl	60 µl	65 µl	70 µl	75 µl	80 µl
NEBNext End Repair Enzyme Mix	9 µl	10 µl	11 µl	12 µl	13 µl	14 µl	15 µl	16 µl
Sterile H_2O	36 µl	40 µl	44 µl	48 µl	52 µl	56 µl	60 µl	64 µl
TOTAL	**90 µl**	**100 µl**	**110 µl**	**120 µl**	**130 µl**	**140 µl**	**150 µl**	**160 µl**

  - **Adenylation Mix:**

	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8
NEBNext dA-Tailing Reaction Buffer (10X)	5 µl	10 µl	15 µl	20 µl	25 µl	30 µl	35 µl	40 µl
Klenow Fragment (3´→ 5´ exo$^-$)	1 µl	2 µl	3 µl	4 µl	5 µl	6 µl	7 µl	8 µl
TOTAL	**6 µl**	**12 µl**	**18 µl**	**24 µl**	**30 µl**	**36 µl**	**42 µl**	**48 µl**

	# 9	# 10	# 11	# 12	# 13	# 14	# 15	# 16
A-Tailing Mix	45 µl	50 µl	55 µl	60 µl	65 µl	70 µl	75 µl	80 µl
Resuspension Buffer	9 µl	10 µl	11 µl	12 µl	13 µl	14 µl	15 µl	16 µl
TOTAL	**44 µl**	**60 µl**	**66 µl**	**72 µl**	**78 µl**	**84 µl**	**90 µl**	**96 µl**

  - **Ligation Mix:**

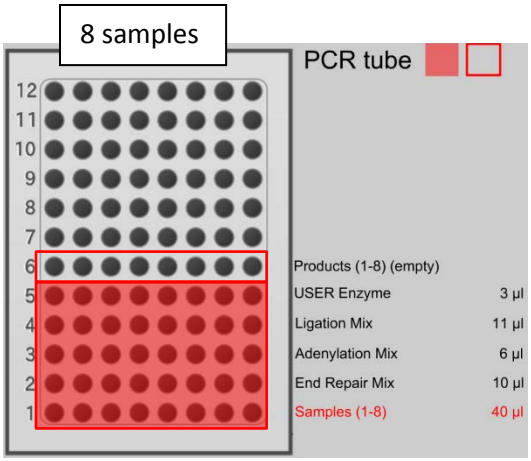
Note:

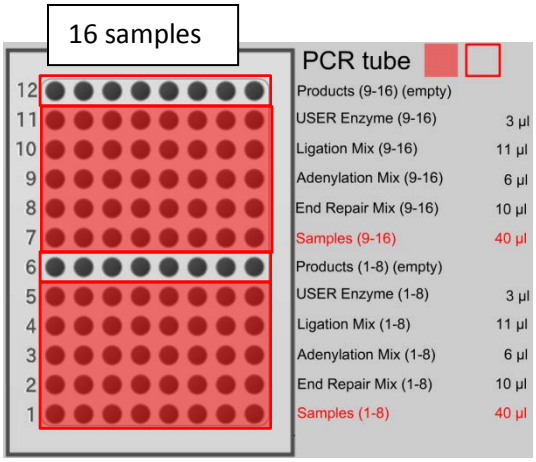
This mix is different for each sample because of the use of different Adaptor Index for each sample.

Dilute the NEBNext Adaptor (15 µM) to 1.5 µM in Nuclease Free Water for immediate use.

Quick Ligation Reaction Buffer (5X)	6 µl
Quick T4 DNA Ligase	4 µl
Diluted NEBNext Adaptor (1.5 µM)	1 µl
TOTAL	**11 µl**

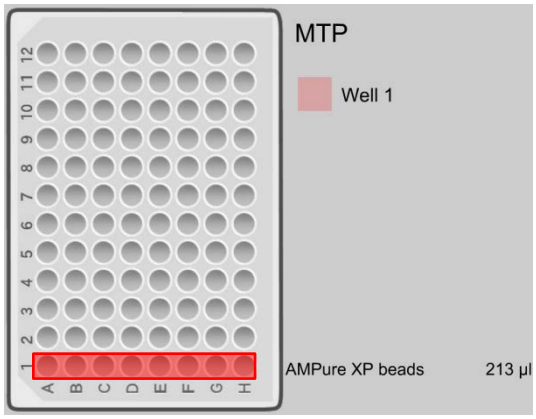
- Fill the **Left Peltier Block** with the mixes according to the screen layout.
- Fill **40 µl of Samples** in lane 1 (and 7 if processing more than 8 samples). If you have less than 40µl, complete with water.





- Fill 213 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 **fresh prepared Ethanol 80%** in the container on the **Reagent Rack 1**.
- Fill **0.1x TE 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).