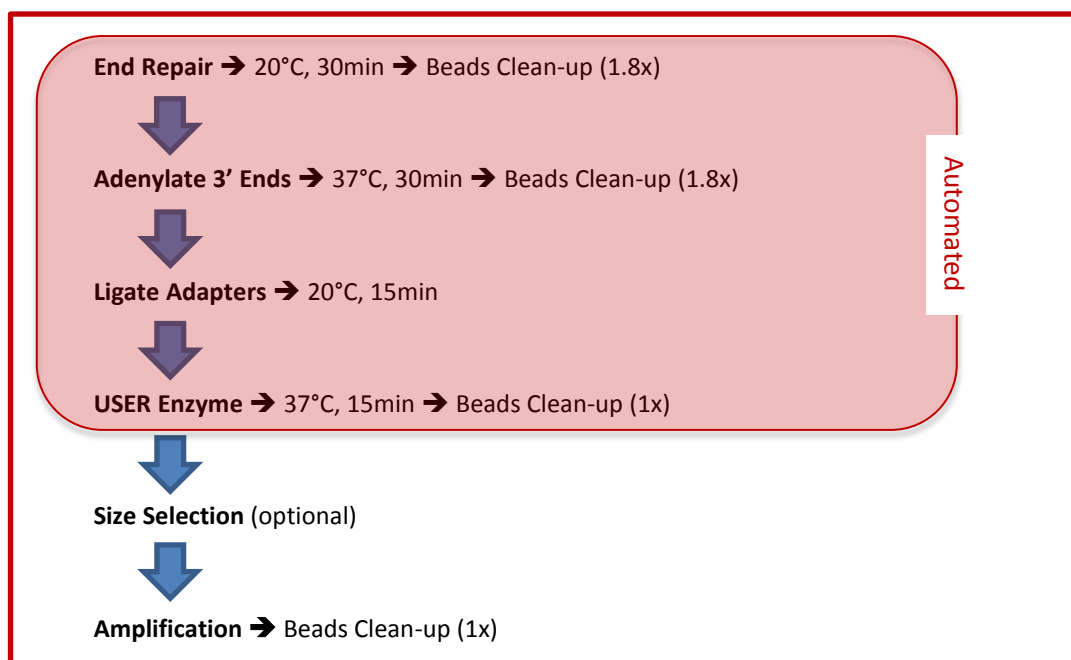


1. About the protocol

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**”

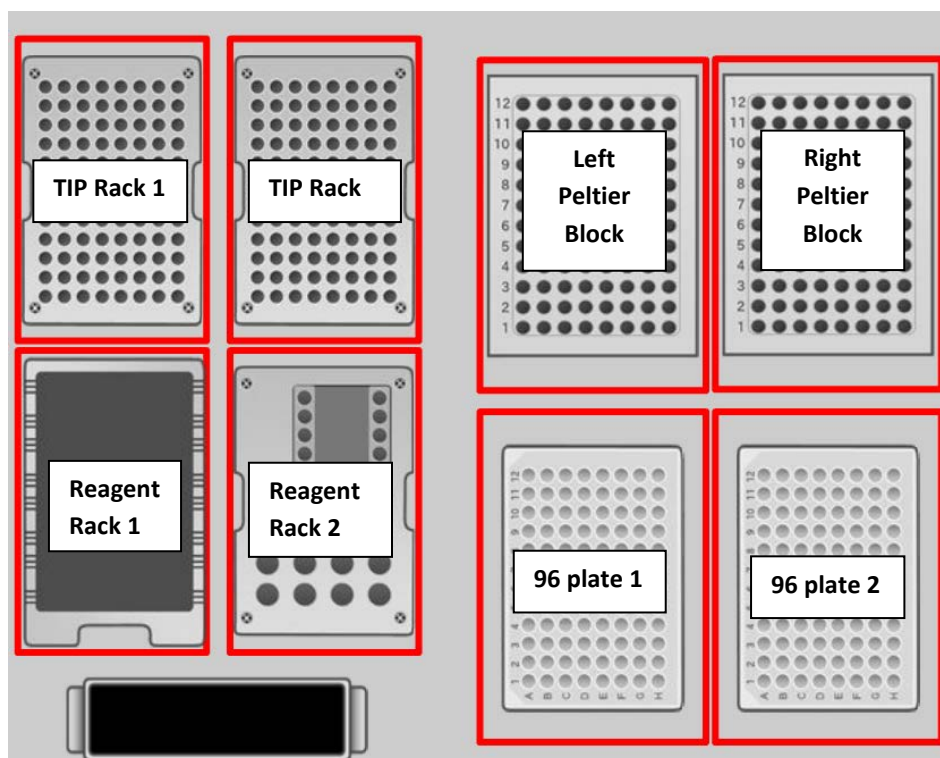
If you plan to run between 9 and 16 samples, chose “**NEBNext_ChIP-Seq_Illumina_16**”

- 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 ₂ O	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 ₂ O	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

- **Adaptor Ligation Mix:**

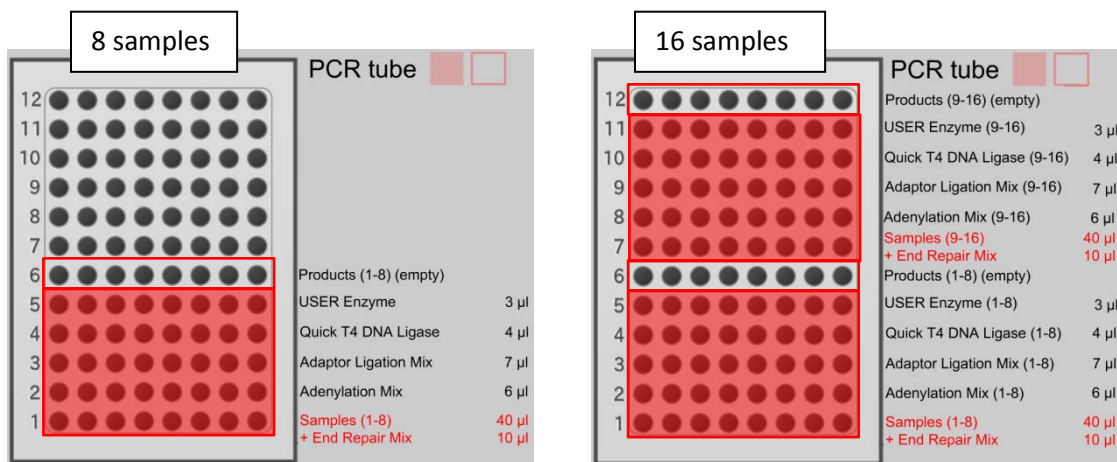
Note:

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
Diluted NEBNext Adaptor (1.5 µM)	1 µl
TOTAL	7 µl

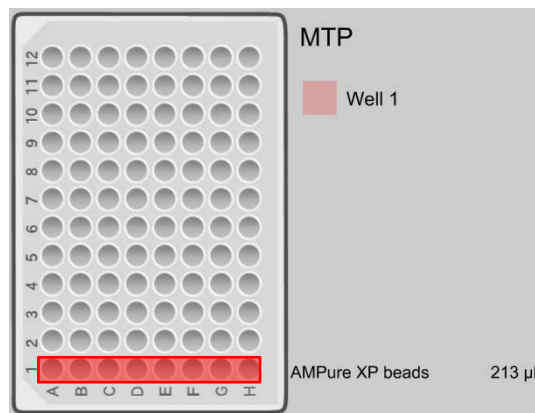
- Fill the **Left Peltier Block** with the mixes according to the screen layout.
- Add 4 μ l **T4 DNA Ligase** to each sample in **lane 3**
- Add 10 μ l of **End Repair Mix** to each samples in **lane 1**
- Add **40 μ l of Samples** in lane 1 (and 7 if processing more than 8 samples). If you have less than 40 μ l, complete with water.



- Fill 213 μ l 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).

