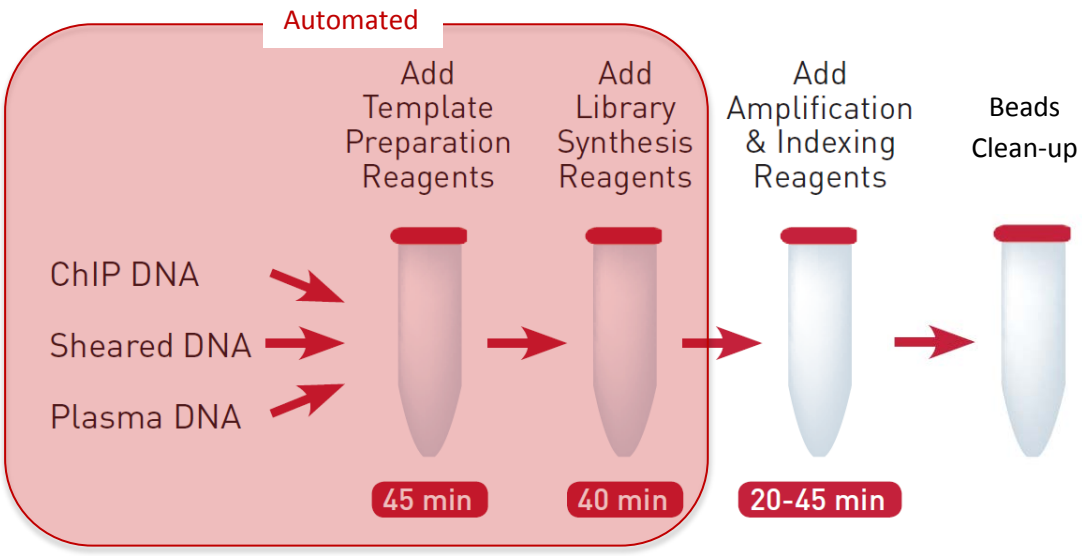
IP-Star® Compact

MicroPlex Library Preparation kit™ v2

1. About the protocol

The "**MicroPlex_Library_Preparation**" protocol on the IP-Star® is using the standard "**MicroPlex Library Preparation™ kit**" and reagents from **Diagenode**. The **MicroPlex Library Preparation™ kit** is the only kit on the market which is validated for ChIP-seq and which allows the preparation of indexed libraries from just **picogram inputs**.
It provides flexibility to prepare 1 to 48 libraries in one run starting with **50pg-50ng of DNA**. The whole protocol takes approximately 1h30. It allows you to prepare up to 96 libraries per day with 2 runs. At the end, you recover ligated products ready for amplification.

2. Workflow



3. Material required

a. Reagents & kits

Item	Supplier	Catalogue #
MicroPlex Library Preparation™ kit v2 x12	Diagenode	C05010012
MicroPlex Library Preparation™ kit v2 x48	Diagenode	C05010014
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
Tips (box)	Diagenode	C30040021
Tips (bulk)	Diagenode	C30040020

4. IP-Star setup

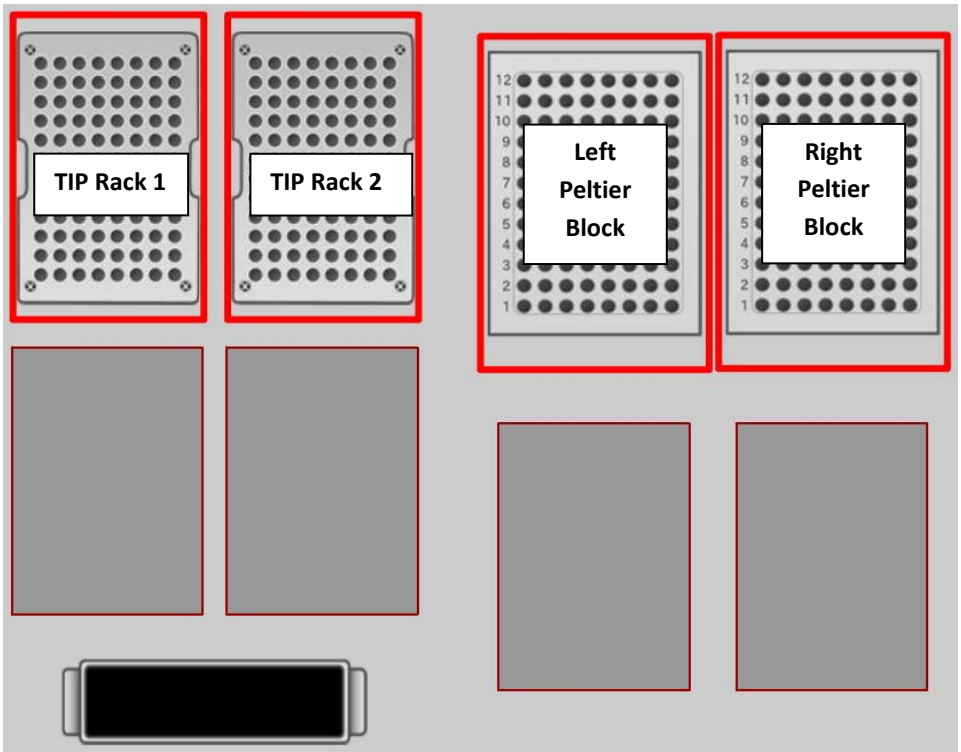
- Switch ON the IP-Star.
- Select "**Protocols**" icon and then click on "**Library prep**".
- Under "**Library prep**", select "**MicroPlex_Library_Preparation**".

Note:
If you plan to run between 1 and 8 samples, chose "**MicroPlex_Library_Preparation_08**"
If you plan to run between 9 and 16 samples, chose "**MicroPlex_Library_Preparation_16**"
If you plan to run between 17 and 24 samples, chose "**MicroPlex_Library_Preparation_24**"
If you plan to run between 25 and 32 samples, chose "**MicroPlex_Library_Preparation_32**"
If you plan to run between 33 and 40 samples, chose "**MicroPlex_Library_Preparation_40**"
If you plan to run between 41 and 48 samples, chose "**MicroPlex_Library_Preparation_48**"

- 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 the enzymes and reagents cold.

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



- Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
- Fill **Left and Right Peltier Blocks** with 200 µl tube strips according to the screen.

5. Reagents & Samples setup

Note:
Allow the reagent from "**MicroPlex Library Preparation™ kit**" to come at 4°C.
Work on ice from this point.

- Prepare the following mixes.

  - **Template Preparation pre-mix:**

	# 1	# 8	# 16	# 24	# 32	# 40	# 48
Template Preparation Buffer (red cap)	2 µl	16 µl	32 µl	48 µl	64 µl	80 µl	96 µl
Template Preparation Enzyme (red cap)	1 µl	8 µl	16 µl	24 µl	32 µl	40 µl	48 µl
TOTAL	**3 µl**	**24 µl**	**48 µl**	**72 µl**	**96 µl**	**120 µl**	**144 µl**

Note:
10 µl of DNA will be added later for each sample.

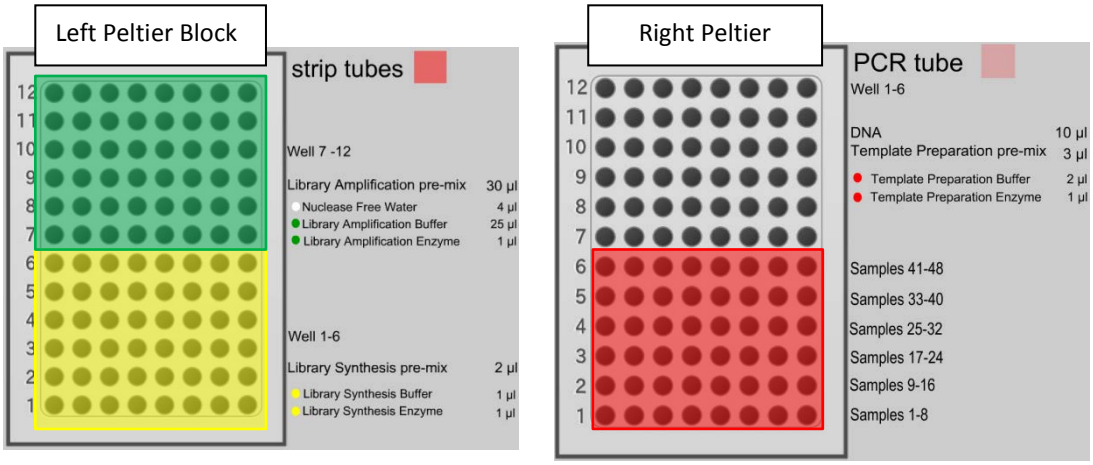
  - **Library Synthesis pre-mix:**

	# 1	# 8	# 16	# 24	# 32	# 40	# 48
Library Synthesis Buffer (yellow cap)	1 µl	8 µl	16 µl	24µl	32 µl	40 µl	48 µl
Library Synthesis Enzyme (yellow cap)	1 µl	8 µl	16 µl	24 µl	32 µl	40 µl	48 µl
TOTAL	**2 µl**	**16 µl**	**32 µl**	**48 µl**	**64 µl**	**80 µl**	**96 µl**

  - **Library Amplification pre-mix:**

	# 1	# 8	# 16	# 24	# 32	# 40	# 48
Nuclease-Free Water (clear cap)	4 µl	32 µl	64 µl	96 µl	128 µl	160 µl	192 µl
Library Amplification Buffer (green cap)	25 µl	200 µl	400 µl	600 µl	800 µl	1000 µl	1200 µl
Library Amplification Enzyme (green cap)	1 µl	8 µl	16 µl	24 µl	32 µl	40 µl	82 µl
TOTAL	**30 µl**	**240 µl**	**480 µl**	**720 µl**	**960 µl**	**1200 µl**	**1440 µl**

- Fill the **Left Peltier Block** with **Library Synthesis pre-mix** and **Library Amplification pre-mix** according to the screen layout.
- Fill the **Right Peltier Block** with the **Template Preparation pre-mix** according to the screen layout.
- Fill **10 µl of DNA** in each sample according to the screen layout.



- Close the door and Run.

6. Library Amplification

- Recover your samples on the **Left Peltier Block** in **lane 7** to **12** according to the screen layout.
- Add **5 µL of one Indexing Reagent** to each sample.
- Mix 4 times with a pipette set to 50 µL.
- Centrifuge the tubes or plate.
- Transfer tubes or plate to a pre-programmed real-time thermal cycler or non-real-time thermal cycler and incubate as follows (MicroPlex v2 Instruction Manual, 4.3 Library amplification step, page 12). **Caution**: Do not program a denaturing step until after Step 2 below.

Library Amplification Reaction

	Stage	Temperature	Time	Number of Cycles
Extension & Cleavage	1	72°C	3 min	1
	2	85°C	2 min	1
Denaturation	3	98°C	2 min	1
Addition of Indexes	4	98°C	20 s	4
		67°C	20 s	
		72°C	40 s	
Library Amplification	▲ 5	98°C	20 s	▲ 5 to16 (see table on right)
		*72°C	50 s	
	6	4°C	Hold	1

*Acquire fluorescence data at this step, if monitoring amplification in real-time.

▲ Stage 5 Amplification Guide

DNA Input (ng)	Number of Cycles
50	5
20	6
10	7
5	8
2	10
1	11
0.2	14
0.05	16