Protocol for auto ChIPmentation for TFs

Using the iDeal ChIP-seq kit for TF (Diagenode; Cat. No. C01010055), TAG kit for ChIPmentation (Diagenode, Cat. No. C01011030) and the IP-Star Compact Automated System.

For chromatin preparation please follow the user manual of iDeal ChIP-seq kit for TFs.

For immunoprecipitation and tagmentation steps follow the guidelines below:

1. Switch ON the IP-Star® Compact.
2. Select "**Protocols**" icon and then "**ChIPmentation**" category.
3. Select "**ChIPmentation_08_D**" if you plan to run between 1 and 8 samples or "**ChIPmentation_16_D**" if you plan to run between 9 and 16 samples.
4. Setup the exact number of samples for your experiment including the positive and negative control IPs. Each IP has to be counted as a sample. Input is not a sample.

   Note: The Peltier block is now cooling down to 4°C to keep your samples cold.

5. Setup the parameters for your ChIPmentation experiment and press "Next".

   Recommended parameters:
   - Setup the "Ab coating" step to 3 hours
   - Setup the "IP reaction" step to 13 hours (overnight)
   - Setup the "Washes" step to 5 min
   - Setup the "Tagmentation" step to 10 min

   Note: The tagmentation time may depend on the target and the antibody used. As a general rule, we recommend using a tagmentation time of 10 minutes but it may be decreased to 5 or 2 minutes when the antibody-epitope binding is weak.

6. 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 **Reagent Racks 1 & 2** with reagent containers according to the screen.
   - Fill **Peltier Block 1** (and 2 if processing more than 8 samples) with 8-tube strips according to the screen.
7. Fill the strips with your samples and the reagents from the kit as described below and make sure that the liquid is at the bottom of each well.
   - Distribute 30 µl of **DiaMag Protein A-coated magnetic beads** in each well of row 3.

Version 1 - 10_2021

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- Prepare **ChIP Buffer** as described in the table below. The volumes are in µl and contain an excess.

	1 IP	*2 IPs*	*3 IPs*	*4 IPs*	*5 IPs*	*6 IPs*	*7 IPs*	*8 IPs*	*9 IPs*	*10 IPs*	*11 IPs*	*12 IPs*	*13 IPs*	*14 IPs*	*15 IPs*	*16 IPs*
5x ChIP Buffer iC1b	*60*	*180*	*240*	*300*	*360*	*420*	*480*	*540*	*780*	*840*	*900*	*960*	*1020*	*1080*	*1140*	*1200*
ChIP-seq grade Water	*234*	*702*	*936*	*1170*	*1404*	*1638*	*1872*	*2106*	*3042*	*3276*	*3510*	*3744*	*3978*	*4212*	*4446*	*4680*
5% BSA (DNA free)	*6*	*18*	*24*	*30*	*36*	*42*	*48*	*54*	*78*	*84*	*90*	*96*	*102*	*108*	*114*	*120*
TOTAL ChIP Buffer	*300*	*900*	*1200*	*1500*	*1800*	*2100*	*2400*	*2700*	*3900*	*4200*	*4500*	*4800*	*5100*	*5400*	*5700*	*6000*

- Prepare the **Ab coating mix** as described in the table below and distribute 100 µl in each well of row 6.

Antibody	x µl
ChIP Buffer	100 – x µl
200x Protease Inhibitor cocktail	0.5 µl

Note: The required amount of antibody per IP varies. Check the supplier's recommendation or perform a titration curve using different amounts of antibody.

Use 1 µg of IgG (negative control antibody) for the negative control IP. If a positive control IP is included, use 1 µg of the CTCF positive control antibody.

- Prepare the **Immunoprecipitation mix** as described in the table below and distribute 200 µl in each well of row 7.

Sheared chromatin	200 µl
BSA 5%	4 µl
200x Protease Inhibitor cocktail	1 µl

Keep aside **2 µl of the sheared chromatin** at 4°C to be used as an **INPUT** starting from step 2 of the TAG kit manual.

Note: If required, NaBu (HDAC inhibitor, 20 mM final concentration) or other inhibitors can also be added to the chromatin sample.

- Distribute **1 µl of Tagmentation enzyme** in each well of row 2.

8. Fill **Reagent Racks 1 & 2** with reagents according to the screen instructions and press "Next".

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9. Check the selected parameters, check the proper insertion of the racks and the consumables, close the door, and press "Run" to start.
10. ChIPmentation is running. The "Remaining time" calculation will give you an estimation of the processing time of your experiment.
11. The next morning, after the overnight incubation, recover the sample tubes in row 12, briefly spin the strip and place it on DiaMag02 magnetic rack (Cat. No. B04000001). Press "OK" and "Back" until the homepage appears on the screen.
12. Wait until supernatant is clear and discard the supernatant.

For the rest of library preparation follow the user manual of the TAG kit starting from STEP 2.

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