ChIPettor™ PROTOCOL

ChIPettor™ SYSTEM FOR HISTONES AND TRANSCRIPTION FACTORS

For use with: ChIPettor™ System for Histones x10 (Cat. No. C01010160)
ChIPettor™ System for Histones x24 (Cat. No. C01010162)
ChIPettor™ System for Transcription Factors x10 (Cat. No. C01010161)
ChIPettor™ System for Transcription Factors x24 (Cat. No. C01010163)

Immunoprecipitation

These protocols have been optimised for **1M cells per IP for Histones and 4 M cells per IP for Transcription Factors**, although it is possible to reduce or increase the amount of cells. For using lower amounts of cells, simply dilute the chromation in shearing buffer before adding it to the IP reaction. For higher cell numbers you can increase the cell concentration in the shearing buffer, although this may require an additional optimization of the shearing conditions. Therefore we recommend performing separate ChIP's and pool the samples before purification of the DNA

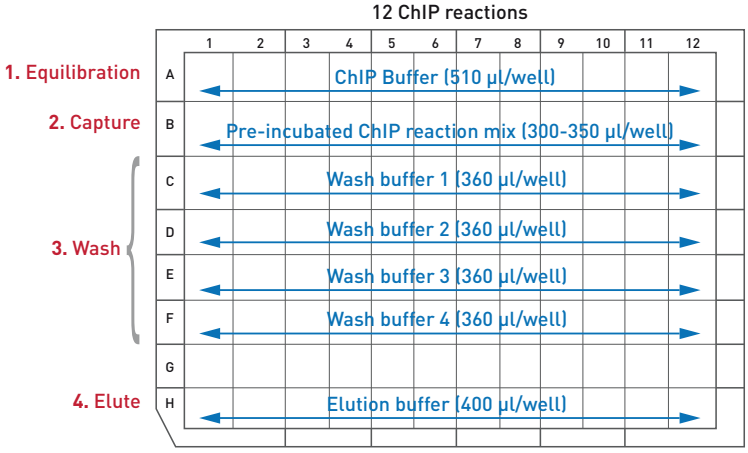
Determine the total number of IP's in the experiment. Please note that we recommend to include one negative control in each experiment (IP with the IgG negative control)

1. Prepare the **ChIP reaction mix** according to the tables here below. If required, NaBu (20 mM final concentration) or other inhibitors can also be added. **Use 1-2 µl of the rabbit IgG control antibody for the negative control IP.** If a positive control IP is included in the experiment, **use 1 µl of the H3K4me3 ChIP-seq grade control antibody** (for Histones) or **0.5 µl of the CTCF positive control antibody** (for Transcription Factors). When preparing the reaction mix, **place 1 µl of the sheared chromatin (for Histones)** or **2.5 µl of the sheared chromatin (for Transcription Factors)** aside to be used as an **input** the next day. x is the amount of antibody needed.

iDeal ChIP-Seq kit for Histones		iDeal ChIP-seq kit for Transcription Factors	
ChIP reaction mix per 1 IP		**ChIP reaction mix per 1 IP**	
Sheared Chromatin	100 µl	Sheared Chromatin	250 µl
5x iC1 buffer	60 µl	5x iC1b buffer	20 µl
5 % BSA	6 µl	5 % BSA	6 µl
200x Protease Inhibitor Cocktail	1.5 µl	200x Protease Inhibitor Cocktail	1.5 µl
Antibody	x µl	Antibody	x µl
H2O	132.5-x	H2O	72.5-x
	300 µl		350 µl

2. Incubate the tubes with ChIP reaction mix overnight at 4°C under constant rotation at 40 rpm on a rotating wheel.
3. The next morning, after the overnight incubation, briefly spin the tubes and load the 96-deep well plate with the **ChIP reaction mix (Row B)** and the reagents from the **iDeal ChIP-seq kit (except the Elution Buffer iE1)** according to the graph below.

NOTE: Elution Buffer iE1 must be added to the wells just before elution at room temperature. Before use, make sure the buffer is free of precipitates, if not pre-warm it at 37°C.



⚠ **NOTE:** From this step, put the plate at 4°c in a cold room or in a fridge.

4. Start the **ChIPettor™ System**. With the joystick you can navigate up, down, left and right by clicking on the respective arrow. Selection of the option of choice can be done by clicking in the middle of the joystick or the left button when SELECT or OK is shown on the screen.
5. Go to the **PURESPEED** Menu, select tip type e.g. **iDeal ChIP** and select the **Default** protocol. The protocol setup menu appears - click on **NEXT**, then on **RUN**.
6. Upon the message "**Install ProA 20 µl Tip(s) and Press CONT or CANCEL to exit**" install the necessary number of ProA resin tips on the pipette and make sure they fit tightly to it. Place the adapters to every second tip to increase the stability and **press CONT** to continue (or CANCEL to exit).
7. Upon the message "**EQUILIBRATION Start: Place tip(s) in equilibration solution**" place the pipette in row A making sure it sits stably on the plate and **press CONT** to start the equilibration.
8. Upon the message "**CAPTURE Start: Place tip(s) in Capture solution**" move the pipette to the next row (B) and **press CONT** to start the capture.
9. Upon the message "**Wash Start: Place tip(s) in Wash solution**" move the pipette to the next row (C) and **press CONT** to start wash 1.
10. Upon the message "**NEXT Step: Press OK when ready**" move the pipette to the next row (D) to start wash 2 and **press CONT**. Do the same for washes 3 and 4, pressing OK after each wash and moving the pipette to rows E and F respectively.
11. Upon the message "**ELUTE Start: Place tip(s) in Elution solution, press ON**" - Get the plate out of the cold room and keep it at room temperature for the Elution step. Fill the necessary number of wells in row H with **400 µl Elution Buffer iE1 at room temperature**, move the pipette to the row (H) and **press CONT** to start Elution.
12. Upon the message "**Press CONT to expel the eluate and discard tip(s)**" keep the pipette in the Elution row and **press CONT** to press out the remaining liquid from the tips into the wells. Then the tips can be discarded.
13. Transfer the supernatant to a new 1.5 ml tube and add **16 µl of iE2 buffer**
14. Setup the inputs:

    Histones: add **399 µl buffer iE1** and **16 µl buffer iE2** to **1 µl of the input** sample kept aside the day before

    Transcription Factors: add **397,5 µl buffer iE1** and **16 µl buffer iE2** to **2.5 µl** of the input sample kept aside the day before.
15. Incubate the samples and Inputs for **4 hours** in a thermomixer at 1,300 rpm and **65°C**. If required, the incubation at 65°C can be performed overnight.
16. Switch to the manual to "**STEP 4. Elution, decross-linking and DNA isolation**". Start to the step "Add 2 µl of carrier to each IP and input sample"

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