Aberdeen ChIP-seq protocol for Invivo and invitro

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Reagent	catalog no	supplier
L15	11415049	Gibco
FBS	FB100/500	Biosera
Heparin	H3393-10KU	Sigma
Pen/Strep	P4333-100ML	Sigma
Percoll	10232919	GE Health care
10xHBSS	H4641-500ML	Sigma
Na_2Co_3	S7795-500G	Sigma
Molecular grade water	7732-18-5	Sigma
Sterile Syringe	Z683620	Sigma
Sterile 100um nylon mash	CC8111-0102	Starlab

Extraction media (500ml)	
Reagent	**volume**
L15	490 ml
2% FBS	9.95 ml
0.1% heparin	500 ul
Washing media (50ml)	
Reagent	**volume**
L15	49.9ml
0.1% FBS	50 ul
0.1% heparin	50 ul
Growth media (500ml)	
Reagent	**volume**
L15	445 ml
10% FBS	50 ml
1%Pen/ Strep	5 ml
51% Percoll (50ml)	

H2O	18.9 ml
Percoll	25.5 ml
10xHBSS	5 ml
Sodium bicarbonate (5M)	357 ul
Heparin (1ul/U)	50 ul

Tissue disruption and crosslinking

Tissue disruption

1. Dissect out the entire head kidney (using scalpel, tweezers and spatula) and put into a 50 mL tube (tube 1) containing 20 mL extraction media.
2. Take a 100 µm nylon mesh and fit it onto a new 50 mL tube
3. Using a sterile pipette or tweezers, transfer the head kidney from the tube 1 and place it on nylon mesh.
4. Working gently, squeeze the head kidney though the 100 µm nylon mesh using the rubber end of the plunger from a 2 mL sterile syringe. Continually apply **extraction media** from tube 3 to prevent cells from drying out and assist with their transit through the mesh. NOTE: Be very gentle, head kidney tissue will break up quite easily, but this may take 10 minutes.
5. Make the final volume of the disrupted cell suspension to 20 mL using additional **extraction media**

*At this stage disassociated cells can be used for ChIP and ATAC-seq protocol for complete head kidney cells not just leukocytes.

*For leukocytes extraction follow "Separate leukocytes using a percoll gradient"

Separate leukocytes from erythrocytes using a percoll gradient

This is a delicate procedure and may require some practice.

6. Pipette 5 mL of 51% percoll into a 50 mL tube.
7. VERY gently layer 20 mL of the cell suspension from Step 9 onto the top of the percoll without causing the layers to mix.
8. Spin the tube for 30 min at *400g* in a centrifuge pre-cooled to 4°C. NOTE: Do not use a break on centrifuge! It will take approximately 10 min to stop.
9. Handling the tube carefully so as not to disturb the stratification, use a 10 mL pipette or similar to carefully remove cells at the interface between Percoll and extraction media (these are the desired leukocytes) and transfer to a new 50 mL tube, expect to remove approx. 5 mL from the cell layer at interface. RBC will have pelleted to bottom of tube.
10. Adjust the volume of the collected leukocytes to 20 mL with **washing media.**
11. Decant the wash medium carefully, making sure not to discard the cell pellet. Repeat steps 10 and 11 once more to eliminate percoll completely.
12. Count the cells using haemocytometer and dilute to 1x106mL-1 and check viability with trypan blue (should be >90% viable). It is required to have a min 18x106 cells per fish for the 3 stimulations.

Crosslinking Head kidney tissue disassociated cells

13. Count cells from **step 5** and allocate (in 1.5ml tubes, safe lock tubes) the right number of cells needed the experiment (in this case 2 million)
14. Centrifuge 10 mins, 800 g, 4˚C. Remove and discard supernatant
15. To the pellet, add 1ml of formaldehyde solution 1% and resuspend the pellet by pipetting
16. Incubate under constant rotation at room temperature for 5 mins
17. Quench the reaction with 125 µl of glycine 1M (0.125M final). Incubate 10 mins, room temperature, under constant rotation
18. Centrifuge 5 mins, 1000 g, 4˚C. Remove and eliminate in appropriate trash the supernatant
19. Resuspend with 1ml of 1x PBS with PIC by pipetting (quick vortex if necessary). Centrifuge 5 mins, 1000 g, 4˚C. Remove the supernatant

Crosslinking Head kidney Leukocytes

- After **step 12**, plate right number of cells in each well and incubate at 24°C for 24 hours. Aim for 1 million cells in 1ml of growth media
- After 24 hours, using a cell scraper, scrap the bottom of each well making sure all the cells that are stuck on the bottom of the well are resuspended. Transfer the cells in a 2ml tube
- For crosslinking follow the **steps from 14-19**

After cross linking cell pellets were stored at -80°C until further use.

Instruction for the next step for ChIP libaray preparations were followed from the diagenode Chimpenation for histone kit (C01011009).