Ren Lab ENCODE ChIP Library Preparation Protocol

This protocol contains instructions for preparing ChIP samples for sequencing on the Illumina platform using a ChIP-Seq DNA Sample Prep Kit (Illumina, Cat#15026486).

Repair DNA fragments to blunt ends using the End-It™ DNA End-Repair Kit (Epicentre, Cat#ER81050):

1. Use 1-10 ng of starting material in 34 µL of TE or 10 mM Tris.
2. Add the following reagents to each sample.

Reagent	Volume per Sample
DNA	34 µL
10x End-It Buffer	5 µL
2.5mM dNTPs	5 µL
10mM ATP	5 µL
End-It enzyme mix	1 µL
Total Volume	*50 µL*

3. Mix tube gently, spin briefly and incubate at room temperature for 45 min.
4. Use Qiagen MinElute PCR Purification kit (Qiagen, Cat#28006) to purify the samples. Elute each sample twice with 16 µL EB.

A-tailing 3' End

5. Add the following reagents to each sample.

Reagent	Volume per Sample
DNA	32 µL
Klenow Buffer (10x NEBuffer 2)	5 µL
1mM dATP	10 µL
Klenow Fragment (3'→5' exo-) (5U/ul) (NEB, Cat#M0212L)	3 µL
Total Volume	*50 µL*

6. Mix tube gently, spin briefly and incubate at 37°C for 30 min.
7. Use Qiagen MinElute PCR Purification kit to purify the samples. Elute each sample twice with 10 µL EB.
8. Use a centrifugal evaporator to decrease the volume of the eluted samples down to 4 µL.

Ligate Adapters to Fragments

9. Add the following reagents to each sample.

Reagent	Volume per Sample
DNA	4 µL
TruSeq Adapters diluted 1:10	0.5 µL
2x Quick Ligase Buffer	5 µL
Quick T4 DNA Ligase (1U/ml) (NEB, Cat#M2200L)	0.5 µL
Total Volume	*10 µL*

10. Mix tube gently, spin briefly and incubate at room temperature for 15 min.
11. Heat inactivate the ligase by incubating at 65°C for 10 min.

Size Selection and Gel Purification

12. Prepare a 2% high-resolution agarose gel.
13. Add gel loading buffer and load the samples on the gel, skipping every other row to avoid contamination between samples.
14. Run the gel at 90V for 100 min.
15. Cut out each 300-500bp fragment into separate 2 mL tubes.
16. Use Qiagen MinElute Gel Extraction kit (Qiagen, Cat#28606) to purify the samples. Elute each sample twice with 10.5 µL EB.

PCR Amplification

17. Before performing full-scale PCR amplification, determine optimal cycle number by qPCR using KAPA DNA Quantification kit (KAPA Biosystems, Cat#KK4953).
18. Perform full-scale PCR using KAPA Library Amplification kit (KAPA Biosystems, Cat#KK2620). Set up reactions as follows:

Reagent	**Volume per Sample**
DNA	20 µL
2x KAPA PCR master mix	23 µL
KAPA PCR Primer Mix	1 µL
Total Volume	44 µL

19. Amplify using the following PCR protocol:

    Step 1: 98°C for 45sec;
    Step 2: 98°C for 15sec;
    Step 3: 65°C for 30sec;
    Step 4: 72°C for 30sec;
    (Repeat steps 2-4 for the optimal number of cycles as determined above)
    Step 6: 72°C for 5min;

Size Selection and Gel Purification

20. Prepare a 2% high-resolution (APEX) agarose gel.
21. Add 6 µL of 6x loading buffer to your samples.
22. Load the samples on the gel, skipping every other row to avoid contamination between samples.
23. Run the gel at 90V for 100 min.
24. Cut out each 300-500bp fragment into separate 2 mL tubes.
25. Use Qiagen MinElute Gel Extraction kit to purify the samples. Elute each sample once with 12 µL EB.
26. Measure DNA concentration of samples (e.g. using Qubit Fluorometer).
27. Samples are now ready to be sequenced on Illumina platform.