Library Preparation for Illumina ChIP-Seq

Kits are stored at -20C.

STEP 1: End Repair using 'End-It DNA End Repair Kit' from Epicentre, Cat# ER0720

Recommendation: After band isolation of ChIP DNA , follow the 'End-It DNA End Repair Kit' protocol as written below.

a) Combine and mix the following components in a microfuge tube

1-34 μl ChIP DNA to be end-repaired (i.e. however much DNA was band isolated)
5 μl 10X End-Repair Buffer
5 μl 2.5 mM dNTP Mix
5 μl 10 mM ATP
x μl sterile water to bring reaction volume to 49 μl
1 μl End-Repair Enzyme Mix
50 μl Total reaction volume

b) Incubate at **room temperature** for 45 minutes.
c) Purify on one QIAquick column using the QIAquick PCR Purification Kit and protocol, eluting in 34 μl of EB.

STEP 2: Addition of 'A' base to 3′ Ends
Use Klenow (3′→5′ exo-) from NEB Cat# M0212s

**Before starting, make up stocks of 1 mM dATP using NEB 100 mM dATP, e.g. add 5 μl of 100 mM dATP to 495 μl Qiagen Buffer EB; then make 50 μl aliquots and freeze at -20C. Defrost aliquots only once.

a) Combine and mix the following components in a PCR plate

DNA from Step 1	34 μl
Klenow buffer = NEB Buffer 2	5 μl
1 mM dATP (will have to make this up)	10 μl
Klenow fragment (3' to 5' exo minus)	1 μl
	50 μl Total reaction volume

b) Incubate for 30 min at 37 °C.
c) Purify on one QIAquick MinElute column using the MinElute PCR Purification Kit and protocol. Elute in 10 μl EB.

STEP 3: Adapter ligation
Use LigaFast from Promega Cat#M8221 and the adapter mix from the Illumina.

****Note: Dilute the Illumina adapters 1:30 with water to adjust for the smaller quantity of DNA. Excess adapters can interfere with sequencing. The adapters may have to be titrated relative to starting material.***

a) Combine and mix the following components in a microfuge tube

Component	Volume
DNA from Step 2	10 μl
DNA ligase buffer	15 μl
RNase, DNase-free water	2 μl
Adapter oligo mix(1:10 to 1:50) >see note above	1 μl
DNA ligase	2 μl
	30 μl Total reaction volume

b) Incubate for 15 min at room temperature.
c) Purify on one Qiaquick MinElute column using the MinElute PCR Purification Kit and protocol. Elute in 10 μl EB.
d) Band isolate the DNA (using either a 2% Invitrogen E-gel (Invitrogen Cat# G5018-02) or a poured 2% agarose TAE gel) by cutting a gel slice that does NOT include any DNA from an adapter-adapter band migrating at ~120 bp. The intended ligation product may not be visible at this stage. Isolate DNA in the 150-300 bp range.
e) Purify the DNA from the agarose slice using a QIAGEN Gel Extraction Kit. Elute in 30 ul EB. If making multiple libraries be very careful to avoid cross-contamination by either leaving many empty lanes between samples or by using one gel per sample.

STEP 4: PCR and Size Selection
Use Phusion DNA polymerase, NEB Cat# F-531 and Illumina primers

- "PCR primer 1.1" which is part of the Illumina kit
- "PCR primer 2.1" which is part of the Illumina kit

****Dilute kit primers 1:1 with water and use 1 ul of this in a 50 ul reaction, i.e. use the primers at half the concentration as shipped to save reagent costs.***

a) Combine and mix the following components in a PCR plate

Component	Volume
DNA from Step 3 (optional: use all 30 ul from above step)	23 ul
Phusion DNA polymerase	25 μl
PCR primer 1.1 >see note above	1 μl
PCR primer 2.1 >see note above	1 μl
	50 μl Total reaction volume

b) Amplify using the following PCR protocol:

- 30 sec at 98 °C
- [10 sec at 98 °C, 30 sec at 65 °C, 30 sec at 72 °C] 15 cycles total (GOTO =14)
- 5 min at 72 °C
- Hold at 4 °C

c) Purify on one QIAquick MinElute column using the MinElute PCR Purification Kit and protocol. Elute in 10 μl EB.

d) Run product on a 2% agarose gel.

e) Excise a large band in the range 150-300bp with a clean scalpel. Be sure to take photos of the gel both before and after the slice is excised. Estimate and record the median product size.

f) Purify the DNA from the agarose slice using a QIAGEN Gel Extraction Kit. Elute in 30 ul EB. If making multiple libraries be very careful to avoid cross-contamination by either leaving many empty lanes between samples or by using one gel per sample.

g) Measure the DNA concentration (ng/ul) and A_{260}/A_{280} by Nanodrop spectrophotometer. The DNA is now ready for sequencing.