Production Center for Global Mapping of Regulatory Elements

Preparation of ChIP DNA Library for Illumina Sequencing

Materials:

End-It DNA End Repair Kit" (Epicentre, Cat# ER0720)
QIAquick PCR Purification Kit
Klenow (3′→5′ exo-) (NEB Cat# M0212s)
100mM dATP
RNase DNase free water
LigaFast (Promega, Cat#M8221)
Illumina Adapter Oligo
QIAquick MinElute column
Phusion DNA polymerase (NEB, Cat# F-531)
Illumina PCR Primers
QIAGEN Gel Extraction Kit.
2% E-gel (invitrogen)

Kits are stored at -20^{0}C.

Protocol

1. End Repair

Use ChIP DNA prepared from 5x10^7 cells

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 you 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 35 µl of EB.

2. Addition of 'A' base to 3′ Ends

a) Prepare stocks of 1 mM dATP using NEB 100 mM dATP,
e.g. add 5 µl of 100 mM dATP to 495 µl sterile RNase DNase free Gibco water; then make 50 µl aliquots of this and freeze at -20^{0}C.

b) Combine and mix the following components

DNA from Step	1-34 µl
Klenow buffer (NEB2)	5 µl
1 mM dATP	10 µl

Klenow (3' to 5' exo minus)	1 µl
Total reaction volume	**50ul**

c) Incubate for 30 min at 37^0C.

d) Purify on one QIAquick **MinElute** column using the MinElute PCR Purification Kit and protocol, and eluting in 10 µl of EB.

3. Adapter ligation

a) Dilute the Illumina adapters 1:10 with water for gel purified DNA. Do not reuse diluted adapters.

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

DNA purified using MinElute	10 µl
2xDNA ligase buffer	15 µl
Adapter oligo mix(1:10)	1 µl
DNA ligase	2 µl
Total reaction volume	**30 µl**

c) Incubate for 15 min at room temperature.

d) Purify on QIAquick **MinElute** column using the MinElute PCR Purification Kit and protocol, and eluting in 17 µl of EB.

e) Run adapter ligated DNA on a 2% Agarose E-gel (SYBR SAFE).

e) Excise gel in the range 150-300bp with a clean scalpel. Be sure to take photos of the gel before and after the gel slices are excised.

f) Purify the DNA from the agarose slices using QIAGEN Gel Extraction Kit. Elute in 25 ul EB.

4. PCR Amplification and Size Selection

a)Dilute PCR primers (1.1 and 2.1) 1:1 with water and use 1 ul of each primer in a 50 ul reaction,

b) Combine and mix the following components-

DNA from Step 3	23 µl
Phusion DNA polymerase	25 µl
Diluted PCR primer 1.1	1 µl
Diluted PCR primer 2.1	1 µl
Total reaction volume	**50 µl**

c) Amplify using the following PCR protocol:

• 30 sec at 98 ^{o}C
• [10 sec at 98 ^{o}C, 30 sec at 65 ^{o}C, 30 sec at 72 ^{o}C]
(GOTO STEP2),14 more cycles
• 5 min at 72 ^{o}C
• Hold at 4 ^{o}C

d) Purify on one QIAquick MinElute column using the MinElute PCR Purification Kit and protocol, eluting in 17 µl of EB.

e) Run PCR amplified DNA on a 2% Agarose E-gel (SYBR SAFE).

e) Excise DNA of 150-300bp from the gel with a clean scalpel. Be sure to take photos of the gel both before and after the slices are excised.

f) Purify the DNA from the agarose slices using a QIAGEN Gel Extraction Kit. Elute in 25 ul EB.

5) Measure the DNA concentration (ng/ul) using Nanodrop spec.