

## Library Preparation for Illumina ChIP-Seq [Annotated by the core]

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 ul ChIP DNA to be end-repaired (i.e. however much DNA was band isolated)

**[Annotation: or sonicated genomic or other fragmented ds DNA in water, up to 5 ug]**

5 ul 10X End-Repair Buffer

5 ul 2.5 mM dNTP Mix

5 ul 10 mM ATP

x ul sterile water to bring reaction volume to 49 ul

1 ul End-Repair Enzyme Mix

50 ul 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 ul of EB.

### STEP 2: Addition of 'A' base to 3' Ends

Use Klenow (3' to 5' exo-) from NEB Cat# M0212s

**\*\*Before starting, make up stocks of 1 mM dATP using NEB 100 mM dATP, e.g. add 5 ul of 100 mM dATP to 495 ul Qiagen Buffer EB; then make 50 ul 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 ul

Klenow buffer = NEB Buffer 2 5 ul

1 mM dATP (will have to make this up) 10 ul

Klenow fragment (3' to 5' exo minus) 1 ul

50 ul 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 ul 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.**

**[Annotation: Another good source of the Klenow and ligase enzyme is Enzymatics, [www.enzymatics.com](http://www.enzymatics.com). Their ligase in particular is recommended by the Sanger Institute for library construction]**

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

DNA from Step 2 10 ul

DNA ligase buffer 15 ul

RNase, DNase-free water 2 ul

Adapter oligo mix(1:10 to 1:50) >see note above 1 ul

**[Annotation: There are specific adapter primers for single read and paired read**

**libraries—make sure you use the correct ones!]**

DNA ligase 2 ul

30 ul 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 ul 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.

**[Annotation: for most libraries you will want to cut out a very specific size fragment, not a range of sizes. Also, for most genomic and related libraries you should see your material easily]**

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.

**[Annotation: We like to use Nucleotrap for fragment purification, but whatever works for you should be fine]**

**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.*

**[Annotation: The paired end primer 1.0 and the single read primer 1.1 are interchangeable. The paired end primer 2.0 and the single read primer 2.1 are NOT THE SAME! Make sure you use the correct primers for your purposes]**

a) Combine and mix the following components in a PCR plate  
DNA from Step 3 (optional: use all 30 ul from above step) 23 ul

**[Annotation: you may be able to go even lower than 23 ul, if you started with a lot of material following gel excision. We have gone as low as a couple of ul]**

Phusion DNA polymerase 25 ul

PCR primer 1.1 >see note above 1 ul

PCR primer 2.1 >see note above 1 ul

50 ul 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

**[Annotation: No one’s PCR machine should be set to 4 degrees. 10 degrees is plenty cold enough, you’re instruments will last much longer if you do this and you’ll save energy too]**

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

**[Annotation: The Sanger group uses SPRI beads for this, it's supposedly better at eliminating the primer dimers that form]**

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.

**[Annotation: for most libraries no gel excision is required following PCR, just the column or the SPRI beads. If the primer band is particularly intense following purification you may have to re-purify the library band by gel. Keep in mind the expected sizes of your library—the size band you cut out following adapter ligation plus about 30. Depending on how narrow a slice you took following adapter ligation the library will be sharp or more dispersed. Primer dimers are about 80-85, adapter dimers are about 120. Bands larger than your library indicate over PCR amplification. You don't want to see any of these!]**

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.

**[Annotation: the nanodrop is not necessarily accurate enough. We recommend bioanalyzer and/or qPCR quantitation, see the library quantitation section on the sequencing web page]**