ChIP-Seq Library Prep

Version 1.0

Date:
Starting Materials

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

End Repair (NEB Next Kit)
30ul DNA
5ul Neb Next Buffer
2.5ul Enzyme Mix
12.5ul H20
50ul Total

Incubate 30 minutes at 20 degrees in PCR machine
Purify with 1.8 volumes ampure beads (90ul) - elute in 43ul H20

A-tail
42ul DNA
5ul NEB #2
1ul dATP
2ul Klenow exo-
50uL Total

Incubate 30 minutes at 37 degrees
Purify with 1.8 volumes ampure beads (90uL) - elute 35ul H20

Adaptor Ligation (NEBNext Kit)
34ul DNA
1ul adapter mix (1uM if <10ng starting material or 1.5uM if <50ng)
10ul 5X ligase buffer
5ul Ligase
50ul Total

Incubate 30 minutes at 20 degrees
Purify 2X with ampure beads
1st round - 1 volume ampure; elute in 50ul
2nd round - 0.8 volume ampure; elute in 51ul

Check Linearity with qPCR - use 1/20 of sample with MC703-MC704
Use threshold value at midpt of amplification to calculate final number of cycles to use

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PCR Enrichment with NEB Next High Fidelity
DNA
2X NebNext High Fidelity Mix 25ul
20uM PE primer 1(MC703) 1ul
20uM PE primer 2(MC704) 1ul
H20
Total 50ul

Cycling Parameters
98X 30"
98X 10"
65X 30"
72X 30"
72X 5'
Hold at 4 degrees

Purify Samples with 50ul Ampure Beads and elute with 20ul H20

Run 1ul of this on 2% agarose gel - stained with sybr gold (30 minutes)

Spec 1ul on Qubit