

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 H2O

50ul Total

Incubate 30 minutes at 20 degrees in PCR machine
Purify with 1.8 volumes ampure beads (90ul) - elute in 43ul H2O

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 H2O

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

