Index Illumina Library Construction in 96 well plate

This protocol is based on Epigenomics Library Construction v8.3.
Sonicated chromatin was reserved while chromatin immunoprecipitation was performed. At the conclusion of ChIP, the reserved chromatin was subject to reverse cross linking, proteinase and RNase treatment in parallel with the ChIP DNA. The DNA recovered from SPRI purification was placed into library construction in parallel with ChIP DNA.

A. End-Repair

Reagent: End-It DNA End-Repair Kit (Epicenter Cat. No. ER0720)

1 Use ~0.5 ng (if you have enough DNA, ~50ng DNA could be used) as starting material in 34 µl of 10mM Tris pH8.0 (EB). Save remaining ChIP at -20°C Clearly label all tubes

	µl
DNA	34
10X End-repair Buffer	5
2.5mM dNTPs	5
10mM ATP	5
END-IT enzyme mix	1
Total vol.	50

2 Incubate for 45' at room temperature.

3 Add **exactly** 110λ SPRI beads to reaction, pipette ≥10x, incubate at RT 5'

4 AMPure XP bead purification:

a) Make sure that the SPRI beads are equilibrated to room temp and shaken gently to resuspend beads evenly
b) Place reaction on magnetic station ≥ 5'
c) Aspirate cleared solution from plate and discard
d) Wash beads with 200λ of 70% ethanol and incubate for ≥ 1'
e) Aspirate ethanol and discard

Note: using **freshly prepared and precise** 70% ethanol is critical. > than 70% ethanol results in inefficient washing of smaller molecules, < 70% ethanol can cause sample loss

f) Repeat 70% ethanol wash
g) Aspirate ethanol and quickly spin plate (1000 rpm, 1m)
h) Return to magnetic station and remove residual ethanol with pipet
i) Leave the plate open at RT for 2-3' to dry pellet: do not over dry

j) Add 32λ EB to each well, mix reaction by pipette 10x

B. Addition of an 'A' Base to the 3' End of the DNA fragments

Reagent: Klenow (3'-5' exo-) (New England Biolabs Cat. # M0212L)

	µl
DNA from section A	32
10X Klenow Buffer	5
1mM dATP	10
Klenow (3'-5' exo-) (5U/ul)	3
Total vol.	50

1 Incubate for 30' at 37°C.

2 Add **exactly** 110λ 2.5M NaCl/20% PEG solution, pipette ≥10x, incubate 5'RT

3 Place samples on magnet and remove supt.

4 Wash x2 with 70% ethanol

5 Air dry for ~2-3'

6 Add 10λ of EB, mix by pipette ≥ 10x

7 Place on magnet and transfer eluate to new wells for next step

C. Ligation of Adapters to the Ends of the DNA Fragments

Reagent: DNA ligase (New England Biolabs, M2200S)

Note: for indexing, each library has its own unique adapter. For accuracy, make master mix (enzyme and buffer) for 2 -3 samples per adapter instead of handling 0.5λ adapter

	µl
DNA from section B	10
2X Ligase Buffer	9
Adapter Oligo mix (1:10 in H_2O)	0.5
DNA Ligase (1U/µl)	0.5
Total vol.	20

1 Incubate 15' at room temperature

2 Raise ligation volume to 100λ w/ EB or low TE

3 Add 70λ beads to the surface of ligation reaction, pipette ≥ 10x and incubate for ≥ 5' at RT.
4 Follow AMPure XP bead purification as before
5 Add 25λ of EB, allow to sit for 5' RT, place on magnetic station for 5' and move cleared sample to new plate and proceed to enrichment step

D. Enrichment of adapter-modified DNA fragments by PCR

	µl
DNA from section C	24
PFU ULTRA II HS 2X MASTER	25
Index PCR F/R primer Mix [12.5 uM]	1
Total vol.	50

1. Amplify using the following PCR protocol:
   - 120 sec at 95°C
   - [30 sec at 95°C, 30sec at 55°C, 60 sec at 72°C] 14 cycles
   - 10 min at 72°C
   - Hold at 4°C

   Note: if you start with very small ChIP input concentrations, you may want to increase the amplification cycle number

E. Gel Purification of the Products From the PCR Reaction

Purpose: To remove remaining primers or adapter dimers and to size select your sample for sequencing. You can load up to 24λ per well and there are 10 lanes. We use 20 λ of 100 base pair ladder (at10ng/λ). Neither the ladder not the samples require loading buffer

Run 1-2% e-gels for ~ 16' until any adapter dimer is near the bottom of the gel.
Cut gel between 300-600bps and gel purify using Qiagen gel extraction kit.

Check concentration of sample and run on Agilent BioAnalyzer to check for quality