Ren Lab ENCODE2 ChIP Library Preparation Protocol
This protocol contains instructions for preparing ChIP samples for sequencing on the Illumina platform.

Methods and Materials:
Pushion Hot Start High-fidelity DNA Polymerase(Thermo Fisher Cat.F-540L)
Quick Ligation Kit (NEB Cat.M2200L)
End-it DNA End-repair Kit (Epicentre Technologies Cat. No. ER0720)
Klenow (3'-5' exo-) (5U/ul) (NEB)
Dark Reader (Iso BioExpress U-2235-1)
MinElute Purification Kit (Qiagen)
Adapter Oligo mix (1:10 in H2O) (From Illumina)
Solexa PCR primer lo (From Illumina)
Solexa PCR primer up (From Illumina)
Certified Low Range Ultra Agarose (Bio-Rad)
50X TAE Buffer
Loading Buffer
100 bp DNA Ladder (Invitrogen)
QIAquick Gel Extraction Kit (QIAGEN, part # 28704 or 28706)
SYBR Safe (Invitrigen S33110)

Procedure:

1. **End-Repair**
    1. Use 1-10 ng as starting materials in 34ul of TE or 10mM Tris (EB). (Do not quantitate ChIP samles.) DNA ends are repaired to blunt ends by T4 DNA polymerase and phosphorylated at 3' ends by T4 Polynucleotide Kinase.

		ml	8
	DNA+H2O	34	
	10X End-repair Buffer	5	40
	2.5mM dNTPs	5	40
	10mM ATP	5	40
	END-IT enzyme mix	1	8
	Total vol.	50	16/per rxn

    2. Incubate for 45 min at room temperature.
    3. Follow the instructions in the QIAquick PCR Purification Kit to purify on one QIAquick MinElute column, eluting in 32 µl of QIAGEN EB.
        - If you do not plan to proceed to *Adenylate 3' Ends* immediately, the protocol can be safely stopped here. If you are stopping, store the samples at -15° to -25°C overnight or longer. When proceeding, thaw the samples on ice.
2. **Addition of an 'A' Base to the 3' End of the DNA fragments**

		ml	8
	DNA from section 1	32	
	10X NEB Buffer 2	5	40
	1mM dATP	10	80
	Klenow (NEB exo-) (5U/ul)	3	24
	Total vol.	50	18/per rxn

    1. Incubate for 30min. at 37C.
    2. Follow the instructions in the MinElute PCR Purification Kit to purify on one QIAquick MinElute column, eluting in 20 µl of QIAGEN EB.
    3. Speed-vac down to 4µl (approximately 8 minutes)

3. **Ligation of Adapters to the Ends of the DNA Fragments**

15ng-50ng starting DNA scale		ml	8
	DNA from section 2	4	
	2X Ligase Buffer	5	40
	Adapter Oligo mix	0.5	4
	DNA Ligase (1U/ml)	0.5	4
	Total vol.	10	9ul/per rxn

1. Incubate for 15min. at room temperature.
2. Follow the instructions in the QIAquick PCR Purification Kit to purify on one QIAquick MinElute column, eluting in 30 µl of QIAGEN EB.
    - If you do not plan to proceed to *Purify Ligation Products* immediately, the protocol can be safely stopped here. If you are stopping, store the samples at -15° to -25°C overnight or longer. When proceeding, thaw the samples on ice.
3. Speed-vac your samples to remove ethanol residue before loading onto your gel (30 sec – 5 min).

4. <u>Purify Ligation Products</u>

1. Prepare a 120 ml for large size, 80ml for medium size, 40ml for small size, 2% agarose gel with distilled water and TAE according to the manufacturer's instructions. The final concentration of TAE should be 1X.
2. Add SYBR Safe after the TAE-agarose has cooled (1:10,000).
3. Cast the gel using a comb that can accommodate 56 µl in each well. Recommended well size: 1 mm (length) x 8 mm (width) x 7 mm (height).
4. Load 10 µl of the ladder solution to one lane of the gel.
5. Load the entire sample in another lane of the gel, leaving a gap of at least one empty lane between ladder and sample.
6. Run gel at 120 V for 60 minutes (6 V/cm).
7. View the gel on a Dark Reader transilluminator
8. Place a clean scalpel vertically above the sample in the gel at the desired size of the template.
9. Excise 200bp – 400bp region of gel with a clean scalpel.
    - Cutting a band of 400 bp will result in an insert size of approximately 300 bp, accounting for the influence of the adapters on the gel mobility. In order to avoid contamination with adapters that have ligated to each other, Illumina recommends that the excised fraction should 300 bp or greater.
10. Follow the instructions in the QIAquick Gel Extraction Kit to purify on one QIAquick column, eluting in 30 µl of QIAGEN EB.
    - If you do not plan to proceed to *Enrich DNA Fragments* immediately, the protocol can be safely stopped here. If you are stopping, store the samples at -15° to -25°C overnight or longer. When proceeding, thaw the samples on ice.

5. <u>Enrich DNA Fragments</u>

1. Prepare the reaction mix, in a 200 µl thin wall PCR tube using the appropriate PCR Index Primer for each separate sample:

	Volume (µl)
DNA from section 4	30
5x Phusion HF Buffer (NEB)	10
10mM dNTPs	1
H2O	3.5
Solexa PCR primer lo	2.5
Solexa PCR primer up	2.5
HotStart Phusion (NEB)	0.5
Total	50

	Volume (µl)
DNA from section 4	30
5x Phusion HF Buffer (NEB)	10
10mM dNTPs	1
H2O	5.5
PCR primer 1.1	1.0
PCR primer 2.1	1.0
PCR primer index #	1.0
HotStart Phusion (NEB)	0.5
Total	50

- Amplify using the following PCR protocol,
  - Step 1: 98°C for 30sec;
  - Step 2: 98°C for 10sec;
  - Step 3: 65°C for 30sec;
  - Step 4: 72°C for 30sec;
  - Step 5: go to step 2, 17 cycles (for Solexa); 22 cycles (for Illumina Kit)
  - Step 6: 72°C for 5min;
  - Step 7: 4°C forever;
- PCR purify. Elute twice with EB;Total elute volume is 25uL.
- You can store in -80degree for days.

6. <u>Purify Final Product</u>

1. Prepare a 120 ml for large size, 80ml for medium size, 40ml for small size, 2% agarose gel with distilled water and TAE according to the manufacturer's instructions. The final concentration of TAE should be 1X.

2. Add SYBR Safe after the TAE-agarose has cooled (1:10,000).
3. Cast the gel using a comb that can accommodate 56 μl in each well. Recommended well size: 1 mm (length) x 8 mm (width) x 7 mm (height).
4. Load 10 μl of the ladder solution to one lane of the gel.
5. Load the entire sample in another lane of the gel, leaving a gap of at least one empty lane between ladder and sample.
6. Run gel at 120 V for 60 minutes (6 V/cm).
7. View the gel on a Dark Reader transilluminator
8. Place a clean scalpel vertically above the sample in the gel at the desired size of the template.
9. Excise 200bp – 400bp region of gel with a clean scalpel.
   - Cutting a band of 400 bp will result in an insert size of approximately 300 bp, accounting for the influence of the adapters on the gel mobility. In order to avoid contamination with adapters that have ligated to each other, Illumina recommends that the excised fraction should 300 bp or greater.
10. Follow the instructions in the QIAquick Gel Extraction Kit to purify on one QIAquick column, eluting in 25 μl of QIAGEN EB.
    - If you do not plan to proceed to *Enrich DNA Fragments* immediately, the protocol can be safely stopped here. If you are stopping, store the samples at -15° to -25°C overnight or longer. When proceeding, thaw the samples on ice.
11. OD the samples by using Qubit and dilute the samples down to 10nM