Ren Lab ChIP-Seq Library Protocol

Purpose: Prepare ChIP-Seq Library for running Illumina Genome Analyzer

Methods and Materials:
Nanosep MF Filter tube(VWR Cat.29300-642)
Pushion Hot Start High-fidelity DNA Polymerase(NEB 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) Invitrigen
Dark Reader (Iso BioExpress U-2235-1)
Minelute purification (Qiagen)
Adapter Oligo mix (1:10 in H2O) (From Illumina's Kit)
PCR primer 1.1(From Illumina's Kit)
PCR primer 2.1(From Illumina's Kit)
SYBR Gold (Invitrigen S11494)

Procedure:

1. **End-Repair**

- Use 1-10 ng as starting materials in 34ul of TE or 10mM Tris (EB). DNA ends are repaired to blunt ends by T4 DNA polymerase and phosphorylated at 3' ends by T4 Polynucleotide Kinase.

		ml	x 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

- Incubate for 45 min at room temperature.
- Minelute purification (Qiagen). Elute once in 20ul of EB, the second time elute in 12uL.
- Total elute volume is 32uL.

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

		ml	x 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

- Incubate for 30min. at 37C.
- Minelute purification. Elute **twice** in 10ul of EB. Total elute volume is 20uL.
- Speedvac to 0ul.(Dry)

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

15ng-50ng starting DNA scale		ml	x8
	DNA from section 2	4	32
	2X Ligase Buffer	5	40
Adapter Oligo mix (1:10 in H2O)		0.5	4
	DNA Ligase (1U/ml)	0.5	4
	Total vol.	10	10/per rxn

- Incubate for 15min. at room temperature.
- Minelute purification. Elute **twice** in 15ul of EB. Total elute volume is 30uL.
- You can store in -80degree for days.

4. Gel Purification of the Products From the Ligation Reaction

- Prepare 8 % polyacrymide gel or buy:

	75ml	125ml
7 ml of 40 % acryl (29:1) in 4 degree fridge,	15ml	25ml
3.5ml of 10X TBE,	7.5ml	12.5ml
24.5 ml of ddH20,	51.7ml	86.1ml
900ul of 10% APS, in 4 degree fridge	750ul	1.25ml
90ul of TEMED (inflammable cabinet)	75ul	125ul

  Running buffer was 1X TBE(1L) .

- Mark the lines on the glass.
- Gel: Ladder//1//2//3//4//6//8//13//14////Ladder
- Ladder//5//7//9//10//11//12//4(1/13/09)//8(1/13/09)//Ladder////
- Flush each well .
- **Spin-vac for 30''-1min to get rid of ETOH.**
- Add ?ul of 6x Bromo phenol blue/xylene cyanol loading dye.(Based on RXN vol)
- Choose 100bps ladder, load 5uL into the first line and the last line,.
- Load all samples into 8% polyacrymide gel **every other** line.
- Run at 150volt--180volt for 50min, when the blue dye reached the 2/3 way of the gel.
- Gel Staining
  Use a glass container for staining
  Staining Buffer**(Prepare in Glassware bottle)**
  (100ml 1X TAE, 12uL 10,000 x SYBR Gold (Invitrigen S11494)
  Put gel into staining buffer for 15-20 min, shake the plate every 5 min. Cover the plate by foil to avoid the light.
- Excise bands around 200bps(200bps-400bps) with a clean scalpel.
- (Optional: Save the band around 400bp-800bp to do Chip-hyb.)
- Minced the gel piece by using a 0.5ml tube with many holes .Put the 0.5mL tube into a 2mL tube, and centrifuge at 14k for 2 min. Make holes with a needle.
- Add 2X volume EB Buffer(about 500uL EB buffer), Shake for O/N in the cold room.
- The next day, Quick spin the tubes.
- 50°C for 15 min on thermomixer. Spin 2 min @ 14k.
- Transfer the **supernatant** to Nanoseq column, spin 2 min @ 14k.
- Transfer the rest of things in the sample tube to the column, spin 2 min @ 14k.
- Add EB Buffer to bring to a total volume of 300ul.
- Add 1/10 volume(50ul) 3M NaOAC(ph 5.2) and vortex to mix.
- Add 4ul glycogen(20mg/ml) and 470 ul cold 100% ETOH(2.5volumn), vortex.
- Freeze at -80°C for 30 min (O/N),spin 13.8K for 30min in cold room. Remove supernatant.

- Wash with 1mL 70% EtOH, vortex, spin in cold room for 5min @13.8k rpm, remove all traces of EtOH, air dry 5 minutes (pellet turns clear).
- Resuspend precipitated DNA in 20ul EB. (on thermomixer 37°C for 2-5 min)

5. Enrichment of adapter-modified DNA fragments by PCR

Two steps' PCR

Step1. PCR mix without primers

		ml	x 8
	DNA from section 4	20	
	5x Phusion HF Buffer(NEB)	10	80
	10mM dNTPs	1	8
	H2O	13.5	108
	HotStart Phusion(NEB)	0.5	4
	Total vol.	45	25/per rxn

- Start PCR machine and start program \\MAIN\-PMRSEQ
  Step 1: 98°C for 30sec;
  Step 2: 65°C for 20sec;
  Step 3: 72°C for 20sec;
  Step 4: 98°C for 10sec;
  Step 5: 65°C for 20sec;
  Step 6: 72°C for 20sec;
  Step 7: 98°C for 10sec;
  Step 8: 4°C forever;

Step2. Primers' mix

		ml	x 8
	10uM Solexa_PCR_up	2.5	20
	10uM Solexa_PCR_lo	2.5	20
	Total vol.	5	5/per rxn

- Wait the PCR machine cold down to 4°C,add the primers' mix to each tube, mix well.
- Wait the other PCR Block heat up to 98°C, pause, transfer the tubes to it.
- Amplify using the following PCR protocol, \\MAIN\chipseq
  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
  Step 6: 72°C for 5min;
  Step 7: 4°C forever;

- Minelute purification. Elute twice in 20ul of EB.Total elute volume is 40uL.
- You can store in -80degree for days.

6. Gel Purification of the Products From the Ligation Reaction

- Prepare 8 % polyacrymide gel or buy: 75ml 125ml
  7 ml of 40 % acryl (29:1) in 4 degree fridge, 15ml 25ml
  3.5ml of 10X TBE, 7.5ml 12.5ml

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- Minced the gel piece by using a 0.5ml tube with many holes .Put the 0.5mL tube into a 1.5mL or 2mL tube, and centrifuge at 14k for 2 min. Make holes with a needle.
- Add 2X volume EB Buffer, Shake for O/N in the cold room.
- The next day, 50°C for 15 min on thermomixer. Spin 2 min @ 14k.
- Transfer the supernatant to Nanoseq column , spin 2 min @ 14k.
- Transfer the rest of things in the sample tube to the colume, spin 2 min @ 14k.
- Add EB Buffer to bring to a total volume of 300ul.
- Add 1/10 volume(50ul) 3M NaOAC(ph 5.2) and vortex to mix.
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- Freeze at -80°C for 30 min (O/N), spin 13.8K for 30min in cold room. Remove supernatant.
- Wash with 1mL 70% EtOH, vortex, spin in cold room for 5min @13.8k rpm, remove all traces of EtOH, air dry 5 minutes (pellet turns clear).
- Resuspend precipitated DNA in 10ul EB. (on thermomixer 37°C for 2-5 min)
- OD the samples by using Qubit.

7. Confirm DNA Library's quality by running in flash agarose Gel

- Based on Qubit reading, run 1uL into 1.2% Flash gel. Use 3ul 100bps ladder and 6x loading buffer which come with the flash gel.
- Find a previous accuracy sample as a control marker. Load the same amount.
- Take a picture, based on the band's brightness to confirm the accuracy of current samples.
- Load 1-1.2ng DNA library to run solexa.