Modified TruSeq DNA Sample Preparation Protocol
for ChIP-Seq

Measure Concentration of DNA using the PicoGreen DNA Kit

Sample #	Name	Concentration (ng/µl)	DNA (µl)	EB Buffer (µl)	Volume (µl)	amount of DNA (ng)

Perform End Repair (**p**. 43 **TruSeq DNA Sample Preparation v**2 **Guide**)

Preparation

Remove from -20°C and thaw at room temperature

- Resuspension buffer
- End Repair Mix

Procedure

Add to 50 µl of fragmented DNA containig 10 ng of ChIP'ed DNA in 8-tube PCR strips

- 10 µl Resuspension buffer
- 40 µl End Repair Mix
- mix by pipetting, adjust the pipette to 100 µl, gently pipette the entire volume of each library up and down 10 times.

Incubation

- Preheat the cycler and incubate the End Repair Reaction for 30 **min at 30°C**

Clean Up

Follow the instructions of the MinElute Protocol from Qiagen

- Let the PB Buffer-DNA Mixture sit for 1 min on the Column
- Let the Wash buffer sit for 1 min on the column

Elute with 15 µl EB Buffer

Adenylate 3‘Ends

Preparation

Remove from -20°C and thaw at room temperature

- Resuspension buffer
- A-Tailing Mix

Procedure

Add to 15 µl of blunted DNA in 8-tube PCR strips

- 2,5 µl Resuspension buffer
- 12,5 µl A-Taling Mix
- mix by pipetting, adjust the pipette to 30 µl, gently pipette the entire volume of each library up and down 10 times.

Incubation

- Preheat the cycler and incubate the End Repair Reaction for 30 **min at** 37**°C**

Clean Up

- **no clean up**; **Immediately proceed to ligation of adapters**

Ligation of indexed DNA Adapters

Preparation

Remove from -20°C and thaw at room temperature

- appropriate DNA Adapter Indeces
- Stop Ligation Buffer
- Resuspension buffer

Procedure

Briefly centrifuge the thawed DNA Adapters and the Stop Ligation buffer at 600 x g

Dilute the DNA Adapters 1:50 with water to adjust for the smaller quantity of DNA

Add to 30 µl of adenylated DNA in 8-tube PCR strips

- 2,5 µl Resuspension buffer
- 2,5 µl Liagtion Mix (return the ligation Mix immediately after use to -20°C)
- 2,5 µl of the desired DNA Adapter
- mix by pipetting, adjust the pipette to 37,5 µl, gently pipette the entire volume of each library up and down 10 times

Incubation

- Preheat the cycler and incubate the End Repair Reaction for 10 **min at 30°C**
- **Remove the Ligation Mix from the thermal cycler**
- **Add 5 µl of Stop Ligation Buffer**
- mix by pipetting, adjust the pipette to 42,5 µl, gently pipette the entire volume of each library up and down 10 times

Clean up

Follow the instructions of the MinElute Protocol from Qiagen

- Let the PB Buffer-DNA Mixture sit for 1 min on the Column
- Let the Wash buffer sit for 1 min on the column

Elute with 20 µl EB Buffer

Enrich DNA Fragments by PCR

This process uses PCR to selectively amplify DNA fragments that have adapter molecules on both ends.

Preparation

Remove from -20°C and thaw at room temperature

- PCR Master Mix
- PCR Primer
- briefly centrifuge the PCR Master Mix and PCR Primer Cocktail to 600 x g for 5 sec.

Procedure

Add to 20 µl of size selcted DNA in 8-tube PCR strips

- 5 µl thawed Primer Cocktail
- 25 µl thawed PCR Master Mix

mix by pipetting, adjust the pipette to 40 µl, gently pipette the entire volume of each library up and down 10 times

Incubation

98°C for 30 sec

18 cycles

98°C for 10 sec

60°C for 30 sec

72 °C for 30 sec

72 °C for 5 min

Hold at 4°

Size Selection

Prepare 100 ml 2% agarose with SYBR Gold gel using 1X TAE buffer.

1. Add 2 g agarose to 100 ml 1X TAE buffer
2. Microwave the gel buffer until agarose is completely dissolved (3min)
3. Cool the gel buffer on the bench and add 10 µl SYBR Gold (SYBR Safe)
4. Pour the entire gel buffer to the tray
5. Let first polymerize at RT
6. Put into the cooling room (for several hours or over night)

Prepare the samples

1. Add 4µl of 6X Gel Loading Dye to each sample
2. Add 10µl DNA ladder
3. Set the agarose gel into the electrophoresis tank
4. Fill the tank with 1X TAE buffer
5. Load the Ladder
6. Load the samples, leave at least one well free
7. Run the gel:
    1. 60-120 min 80V

Gel out

1. cut according to the DNA-marker bands: 300-400 bp (Insert size 200-300bp)

Clean up:

- Follow the instructions of the Qiagen **MinElute** Gel extraction Kit

Validate library