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ChIP-Seq Library Production (Based on Illumina Protocol and U. Penn. Functional Genomics Facility Protocol)
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Reagents:

ChIP Seq DNA Sample Prep Kit from Illumina: IP-102-1001

The following NEB reagents are used to replace the ones from the kit:

Quick Ligation Kit: M2200S
T4 DNA Polymerase: M0203S
Klenow(3' -> 5' exo): M0212S
T4 Polynucleotide Kinase: M0201S
Phusion High Fidelity Polymerase: F-530S
DNA Polymerase I, Large (Klenow) Fragment: M0210S
T4 DNA Ligase Buffer with 10mM ATP: B0202S

Illumina Oligos/Adapters (Genomic DNA Sample Prep Oligo Only Kit): FC-102-1003

Step 1: End Repair

1. Dilute Klenow DNA polymerase 1:5 with water for a final Klenow concentration of 1U/μL.
2. Prepare the end repair reaction using the following recipe; if making multiple libraries, make a master mix and then aliquot into ChIP samples. The final volume should be 50μL.

    10μL Water
    5μL T4 DNA ligase buffer with 10mM ATP
    2μL dNTP mix (10mM)
    1μL T4 DNA polymerase
    1μL Klenow (diluted to 1U/μL)
    1μL T4 PNK

3. Incubate in a thermal mixer for 30 minutes at 20°C.
4. Purify DNA using the QIAquick PCR Purification Kit (QIAGEN Cat# 28104); elute in 36μL EB.

Step 2: Add 'A' Bases to the 3' End of the DNA Fragments

1. Prepare the reaction to add "A" bases using the following recipe; if making multiple libraries, make a master mix and then aliquot into samples. The final volume should be 50µL.

    5µL NEB Buffer 2
    10µL dATP (1mM)
    1µL Klenow exo (3' to 5' exo minus)

2. Incubate in a thermal mixer for 30 minutes at 37°C.
3. Purify the DNA using the MinElute PCR Purification Kit (QIAGEN Cat# 28004); elute in 12µL EB.

Step 3: Ligate Adapters to DNA Fragments

1. Dilute Illumina-supplied adapters 1:10 in water.
2. Prepare the ligation reaction using the following recipe; if making multiple libraries, make a master mix and then aliquot into samples. The final volume should be 30µL.

    15µL Quick ligase buffer
    1µL Diluted adapter oligo mix
    3µL Water
    1µL Quick DNA ligase

3. Incubate at room temperature for 15 minutes.
4. Purify the DNA using the MinElute PCR Purification Kit; elute in 10µL EB.

Step 4: Size Select the Library

Ensure that the Illumina-recommended loading buffer is used, to prevent sample from escaping the well!

Loading Buffer
50mM Tris-HCl, pH 8.0
40mM EDTA
40% (w/v) Sucrose
Extremely small pinch xylene cyanol or bromophenol blue

1. Prepare a 50mL, 2% agarose gel in 1xTAE; allow agarose to cool, then add EtBr to final concentration of 0.5mg/mL. Pour into mini gel tray.
   Note: If preparing many libraries, adjust volumes accordingly for use with larger gel trays.
2. Load 1µg 100bp DNA ladder (NEB Cat# N3231) into first lane of gel (mix 2µL 0.5mg/mL ladder with 3µL loading buffer).
3. Add 3µL loading buffer to DNA samples from Step 3; load into gel, leaving at least one empty lane between samples, including the ladder.
4. Run gel at 120V for 1 hour.
5. View gel on UV gel box, and cut out the region of the gel corresponding to 250±25bp for each library using a clean scalpel or razor blade.
   Note: Only the DNA ladder is visible on the gel; the library samples do not contain enough DNA for visualization. To ensure that the proper size is selected for each library and to avoid prolonged exposure to UV light, make a cut into the lane with ladder about 1/3 of the way above the 200bp fragment. Then make a second cut, just below the 300bp fragment. These two cuts will mark the boundaries of the region that should be cut out for each DNA sample. [The purpose of cutting above the 200bp band is to ensure that the average library size does not go below 200bp, which is below the desired size threshold for efficient sequencing; the ladder appears to run slightly faster than the DNA samples.] Remove the gel from the UV box and cut out the appropriate regions by following the location of the initial cuts.
6. Cut a slice of the same size from an empty well on the gel and take this sample through the remainder of this protocol as a negative control.
7. Extract and purify the DNA from the gel slices using a QIAGEN Gel Extraction Kit (QIAGEN Cat# 28704); elute in 36µL EB.

Step 5: Enrich Adapter-Modified DNA Fragments by PCR

1. Prepare the PCR reaction using the following recipe; if making multiple libraries, prepare a master mix and then aliquot into adapter-modified DNA from Step 4. The final volume should be 50µL.

   10µL 5x Phusion buffer
   1.5µL 10mM dNTP mix
   1µL PCR primer 1.1
   1µL PCR primer 2.1
   0.5µL Phusion polymerase

2. Amplify DNA using the following PCR protocol:

   18 cycles of:
   10 seconds at 98°C
   30 seconds at 65°C
   30 seconds at 72°C
   5 minutes at 72°C
   Hold at 4°C

3. Purify amplified DNA using MinElute PCR Purification Kit; elute in 15µL EB.

Libraries are now ready for sequencing. Determine average size, DNA concentration, and general purity by running on Bioanalyzer machine (1µL needed for analysis).