SOP Title: Preparing Samples for ChIP Sequencing of DNA	Version 1.1, Page 1
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Staff Able to Perform Procedure: Research Technician and higher

Principle of the Method:
This protocol explains how to prepare libraries of chromatin-immunoprecipitated DNA for analysis on the Illumina Cluster Station and Genome Analyzer.

Sample Type: approx. 10 ng in 30 µl water

Equipment Requirements:
- Agilent 2100 Bioanalyzer (Agilent)
- PicoTiter Plate Shaker (Fischer)
- Bioanalyzer Chip Vortex (IKA)
- Thermal cycler (Applied Biosystems)
- Dark Reader transilluminator
- Electrophoresis Unit
- Gel trays and tank (Thermo Scientific)

Reagents & Material Requirements:
- ChIP-Seq Sample Prep Kit (Illumina)
- QIAquick PCR Purification Kit (QIAGEN)
- MinElute PCR Purification Kit (QIAGEN)
- Ethidium bromide
- 2% agarose gel
- 100 bp DNA ladder
- TAE buffer
- Loading buffer (50 mM Tris pH 8.0, 40 mM EDTA, 40% (w/v) sucrose)
- QIAquick Gel Extraction Kit (QIAGEN)
- RNAse- free centrifuge tubes 1.5ml, 200 ul (USA Scientific)
- Pipette Man 2ul, 20ul, 200ul, 1000ul (Rainin)
- Filter Pipette Tips (Rainin)
- Razor blades (VWR)

Perform End Repair
- Dilute Klenow DNA polymerase 1:5 with water.
- Prepare the following reaction mix:
  - 30 µl ChIP enriched DNA
  - 10 µl Water
  - 5 µl T4 DNA ligase buffer with 10 mM ATP
  - 2 µl dNTP mix
  - 1 µl T4 DNA polymerase
  - 1 µl Klenow DNA polymerase
  - 1 µl T4 PNK
- Mix well using pipettor. Avoid bubbles and foam.
- Incubate in the thermal cycler for 30 minutes at 20°C.
- Follow the instructions in the QIAquick PCR Purification Kit to purify on one QIAquick column, eluting in 34 µl of EB.

Add 'A' Bases to the 3' End of the DNA Fragments
- Prepare the following reaction mix:
  - 34 µl DNA Sample
  - 5 µl Klenow buffer

- 10 µl dATP
- 1 µl Klenow exo (3' to 5' exo minus)

- Mix well using pipettor.
- Incubate for 30 minutes at 37°C.
- Follow the instructions in the MinElute PCR Purification Kit to purify on one MinElute column, eluting in 10 µl of EB.

Ligate Adapters to DNA Fragments

- Dilute the Adapter oligo mix 1:10 with water.
- Prepare the following reaction mix:
  - 10 µl DNA sample
  - 15 µl DNA ligase buffer
  - 1 µl Diluted adapter oligo mix
  - 4 µl DNA ligase
- Mix well using pipettor.
- Incubate for 15 minutes at room temperature.
- Follow the instructions in the MinElute PCR Purification Kit to purify on one MinElute column, eluting in 10 µl of EB.

Size Select the Library

- **It is not recommended to purify multiple samples on a single gel due to cross contamination.
- Prepare a 50 ml, 2% agarose gel with 1X TE Buffer.
- Add ethidium bromide (EtBr) after the TE-agarose has cooled. Final concentration of EtBr should be 400 ng/ml
- Load 8 µl ladder and 3 µl loading buffer to one lane of the gel.
- Add 4 µl of loading buffer to 10 µl of DNA.
- Load the entire sample in another lane of the gel, leaving at least one empty lane between ladder and sample.
- Run gel at about 70V until samples are out of the wells, and then 120 V for about 60 minutes.
- View the gel on a Dark Reader transilluminator.
- Excise a region of gel with a clean razor blade. Cut the gel in the 150-300 bp range. Photograph the gel before and after the slice is excised.
- Cut a slice of the same size from and empty well on the same gel and take this sample through gel purification and PCR. No visible PCR product should be present.
- Use a QIAGEN Gel Extraction Kit to purify the DNA from the agarose slices using these steps:
  - 6X Buffer QG, no heat
  - 2X Isopropanol
  - Elute in 50 µl of EB if you saw DNA in the gel
  - Elute in 36 µl of EB if you did not see DNA in the gel

Enrich the Adapter-Modified DNA Fragments by PCR

- Prepare the following PCR reaction mix:
  - 36 µl DNA (34 µl DNA + 2 µl water if you saw DNA on the gel)
  - 10 µl 5X Phusion buffer
  - 1.5 µl dNTP mix
  - 1 µl PCR primer 1.1
  - 1 µl PCR primer 2.1
  - 0.5 µl Phusion polymerase
- Amplify using the following PCR protocol:
  - 98°C for 30 seconds
  - 18 cycles of:
    - 98°C for 10 seconds

- 65°C for 30 seconds
- 72°C for 30 seconds

- 72°C for 5 minutes
- Hold at 4°C

- Follow the instructions in the MinElute PCR Purification Kit to purify on a MinElute column, eluting in 15 µl of EB.

Validate the Library-Bioanalyzer Method

- Load 1 µl of the resuspended construct and 1 µl of the negative control on an Agilent 2100 Bioanalyzer.
- Check the size, purity, and concentration of the sample.