Library Preparation for Chromatin-Immunoprecipitation-based Sequencing (ChIP-Seq)

Note: For safety and biohazard guidelines, *Applied Biosystems SOLiD™ System 2.0 User Guide* preface and "Safety" appendix. For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DNA derived from ChIP procedure for SOLiD™ sequencing can range from 100 bp to 2 kb in size with typical yield in the nanogram range. Therefore, the starting point of sample preparation for SOLiD™ System sequencing varies depending on the input DNA size range. Guidelines are provided in the following table:

ChIP Input DNA Size Range	Starting Point
<200 bp	Start from "Repair DNA Ends and Purify Sample"
≥200 bp	Start from "Shear and Purify the DNA"[1]

Shear and Purify the DNA

1. Dilute DNA to 5% w/w 2-µm borosilicate glass dry spheres:

Component	Volume (µL)
2-µm glass dry spheres	25 mg
20 ng to 2 µg DNA	X
1 M Tris, pH 8.0	5
Nuclease-free water	Variable
Total	**500**

2. Shear DNA using Covaris™ S2 System and transfer sheared DNA into a 1.5-mL LoBind tube
3. Separate DNA from glass spheres by centrifuging the tube at ≥10,000 × *g* (13,000 rpm) for 5 minutes and transfer the supernatant to a 15-mL conical propylene tube.
4. Purify sheared DNA with Qiagen MinElute® Reaction Cleanup Kit (≤5 µg/column).
    a. Add 3 volumes Buffer ERC to sheared DNA.
    b. Apply 700 µL sheared DNA solution to a MinElute® column and let stand for 2 minutes at room temperature.
    c. Centrifuge at ≥10,000 × *g* (13,000 rpm) for 1 minute and discard flow-through.
    d. Repeat steps b-c until the entire sample has been loaded onto the column.
    e. Add 750 µL Buffer PE.
    f. Centrifuge at ≥10,000 × *g* (13,000 rpm) for 2 minutes. Discard flow-through. Repeat this step to remove residual wash buffer.
    g. Air-dry the column for 2 minutes and transfer the column to a clean 1.5-mL LoBind tube.
    h. Add 20 µL Buffer EB to the column and let stand for 2 minutes.
    i. Centrifuge at ≥10,000 × *g* (13,000 rpm) for 1 minute.
    j. Repeat steps h and i.
5. Quantitate DNA on the NanoDrop™ ND-1000 Spectrophotometer if starting DNA > 500 ng. Otherwise assume 70% recovery rate and continue.

[1] Starting point at "Repair DNA Ends and Purify Sample" is also possible for fragments ≥ 200 bp.

Repair DNA Ends and Purify Sample

1. Mix the following components in a LoBind tube:

Component	Volume (μL)
Sheared DNA	38 or 40
End-Repair 10X Buffer (Epicentre® End-It™)	10
ATP (10 mM) (Epicentre® End-It™)	10
dNTPs (2.5 mM each) (Epicentre® End-It™)	10
End-Repair Enzyme Mix (Epicentre® End-It™)	2
Nuclease-free water	Variable
Total	**100**

2. Incubate the tube at room temperature for 30 minutes.
3. Purify sheared DNA with Qiagen MinElute® Reaction Cleanup Kit (≤5 μg/column). Elute with 20 μL Buffer EB twice (40 μL total). (Refer to step 4 of "Shear and Purify the DNA" for procedure.)
4. Quantitate DNA on the NanoDrop ND-1000 Spectrophotometer if starting DNA > 500 ng. Otherwise, assume 70% recovery rate and continue.

Ligate P1(ds) and P2(ds) Adaptors to End-Repaired DNA

1. Calculate the amount of adaptor needed for the reaction:
   (# μL adaptor needed) = (# μg DNA) × (10^6 pg/μg) × (1/660 pmol/pg DNA) × (1/insert size) × 30 ÷ (50 pmol/μL adaptor)

2. Mix the components below. Adjust volumes as necessary.

Component	Volume (μL)
P1 (ds) Adaptor (50 pmol/μL)	As determined in Step 1
P2 (ds) Adaptor (50 pmol/μL)	As determined in Step 1
2X Quick Ligase Buffer (NEB)	100
Quick Ligase Enzyme (NEB)	5.0
DNA	38-40
Nuclease-free water	Variable
Total	**200**

3. Add 1 μL of Quick Ligase per 40 μL reaction volume and incubate at room temperature for 10 minutes.
4. Purify the DNA using the Agencourt® AMPure® Kit.
   a. Add 1.8 volumes Agencourt® AMPure® beads to the sample. Incubate for 10-15 minutes at room temperature on a rotator.
   b. Place the tube of sample in the magnetic rack. After the solution clears, remove the supernatant and discard.
   c. Dispense 200 μL freshly prepared 70% ethanol, vortex the tube, and incubate for 30 seconds at room temperature.
   d. If needed, pool beads from both tubes into one 1.5-mL LoBind tube.
   e. Place the tube of sample in the magnetic rack. After the solution clears, aspirate out ethanol and discard.
   f. Repeat steps c-e 2 more times.
   g. Place the tube of sample in the magnetic rack and remove the supernatant. Pulse-spin to remove the residual ethanol. Repeat this step 2 to 3 more times to remove residual ethanol and then dry the beads at room temperature for 5 minutes.
   h. Add 22 μL 10 mM Tris pH 8 to the sample and vortex for 10 seconds. Pipette the solution up and down several times to ensure homogeneity.
   i. Place the tube of sample in the magnetic rack. After the solution clears, transfer the eluted sample (supernatant) into a new 1.5-mL LoBind tube. Repeat this step twice.
5. (Optional) Save 2 μL purified ligated library DNA, in case a library QC needs to be run for troubleshooting purposes.

Size-Selection of the DNA

1. Set up a 6% DNA retardation PAGE gel (Invitrogen™) with **1X TBE buffer**.
2. Add 6 µL **Gel Loading Solution** to the sample.
3. Load 2 µL TrackIt™ 25 bp ladder. Load the dye-mixed sample (500 ng to 1 µg per well).
4. Run the gel at 160 V for approximately 30 minutes or until the marker is close to the edge of the gel.
5. Stain the gel with **Ethidium Bromide** for 5 minutes.
6. Destain with nuclease-free water for 2 minutes.
7. Excise the band at desired size range using a clean razor blade.
8. Make a hole in the bottom of a 0.5-mL tube using a 21 gauge needle and, place the gel piece in this tube.
9. Place the 0.5-mL tube with the gel in a 1.5-mL LoBind tube and centrifuge at ≥10,000 × *g* (13,000 rpm) for 3 minutes. Repeat with a new tube if gel remains in the 0.5-mL tube. Afterwards, pool the tubes.

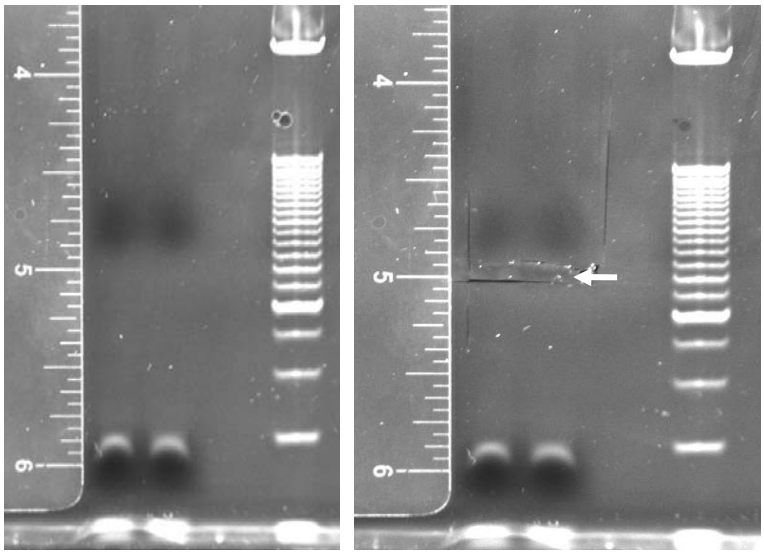

Figure 1. Excision of ChIP sample cut at desired size range invisible on PAGE gel.

Nick Translation and Library Amplification

1. Resuspend gel pieces in 100 µL Invitrogen™ SuperMix. Transfer the gel solution as follows:

If starting input DNA is…	Then…
>100 ng	Distribute as 25-µL aliquots in 4 PCR reaction tubes. Add 75 µL SuperMix to each aliquot.
≤100 ng	Distribute as 33-µL aliquots in 3 PCR reaction tubes. Add 67 µL SuperMix to each aliquot.

2. Add 1.5 µL Library PCR Primer 1 and 1.5 µL Library PCR Primer 2 to each tube of gel mixture.
3. Add 0.2 µL AmpliTaq® and 0.25 µL Pfu polymerase.
4. Run the PCR cycling program:

Temp (ºC)	Time	Condition/note
72	20 min	Nick translation
95	5 min	Hold
95	15 sec	×8-16 cycles (see table on right)
62	15 sec	
70	1 min	
70	5 min	Hold
4	Forever	Hold

Starting Amount of DNA	Number of PCR Cycles
20 ng to 100 ng	13-16
100 ng to 1 µg	10-12
1 µg to 2 µg	8-10

5. Load and run 4 µL of sample on a 2.2% Lonza FlashGel® for 6 minutes at 275 V.

6. If robust amplification products are visible, proceed to step 9. Otherwise, run the PCR cycling program:

Temp (ºC)	Time	Condition
95	5 min	Hold
95	15 sec	×2-3 cycles
62	15 sec	
70	1 min	
70	5 min	Hold
4	Forever	Hold

7. Add 1 µL 5X FlashGel® Loading Dye to 4 µL of sample. Load and run the loading dye-sample mixture on a 2.2% Lonza FlashGel® for 6 minutes at 275 V.
8. If necessary, repeat steps 6 and 7 until robust library amplification products are observed.
9. Pool all PCR samples (including shredded gel pieces) into a 0.45-µm filter Nanosep® spin column with a 1.5-mL LoBind collection tube. Centrifuge at ≥10,000 × *g* (13,000 rpm) for 5 minutes.
10. Purify the DNA using the Agencourt® AMPure® Kit. Elute the DNA with 20 µL 10 mM Tris pH 8 solution. (Refer to step 4 of "Ligate P1(ds) and P2(ds) Adaptors to End-Repaired DNA" for procedure.)
11. If there is overamplification, proceed with gel purification and DNA elution. Otherwise, skip to library purity quality control and quantitation.

Library Gel Purification

1. Prepare a 3% or 4% agarose gel in **1X TAE Buffer**.
2. Add 2 µL **10X Gel Loading Solution** to the 20 µL fragment library.
3. Load 2 µL TrackIt™ 25 bp ladder. Load DNA sample in the remaining wells.
4. Run the gel at 120 V until the dye in the marker is close to the bottom of the gel.
5. If needed, stain the gel with **Ethidium Bromide** for 5 minutes.
6. Destain with nuclease-free water for 2 minutes.
7. Excise the band between 150-200 bp using a clean razor blade and weigh the gel slice in a 15-mL polypropylene conical colorless tube.
8. Add 6 volumes Buffer QG to one volume of gel.
9. Dissolve the gel slide by vortexing at room temperature for until the gel slice has dissolved completely.
10. Add one gel volume of isopropyl alcohol to the sample and mix by inverting the tube several times.
11. Apply the sample to MinElute® column(s) (up to 400 mg gel per column) and let the column(s) stand for 2 minutes at room temperature.
12. Centrifuge the column(s) at ≥10,000 × *g* (13,000 rpm) for 1 minute and discard flow-through. Repeat this step until the entire sample is loaded onto the column(s)
13. Add 500 µL Buffer QG to each column. Centrifuge at ≥10,000 × *g* (13,000 rpm) for 1 minute and discard flow-through.
14. Add 750 µL Buffer PE to each column. Centrifuge at ≥10,000 × *g* (13,000 rpm) for 2 minutes and discard flow-through. Repeat centrifugation at ≥10,000 × *g* (13,000 rpm) for 2 minutes and discard flow-through.
15. Air-dry the column(s) for 2 minutes and transfer the samples to clean 1.5-mL LoBind tube(s).
16. Add 10 µL Buffer EB to each column. Let the column(s) stand for 2 minutes at room temperature and then centrifuge at ≥10,000 × *g* (13,000 rpm) for 1 minute. Repeat this step.
17. If necessary, pool the eluted samples and store in a 1.5-mL LoBind tube.

Library Purity Quality Control (QC) and Quantitation

1. Run 1 µL of library DNA on a Bioanalyzer.
2. On a 2.2% Lonza FlashGel®, load 5 µL FlashGel® QuantLadder and 5 µL of 2 different amounts of library sample (5 to 20 ng total DNA per sample, in 5 µL total volume).
3. Run the gel at 275 V for 6 minutes.
4. Quantitate the DNA amount using the FlashGel® QuantLadder as a reference.

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