Sample Preparation for ChIP - Kit Protocol (version 1.0)

Introduction:

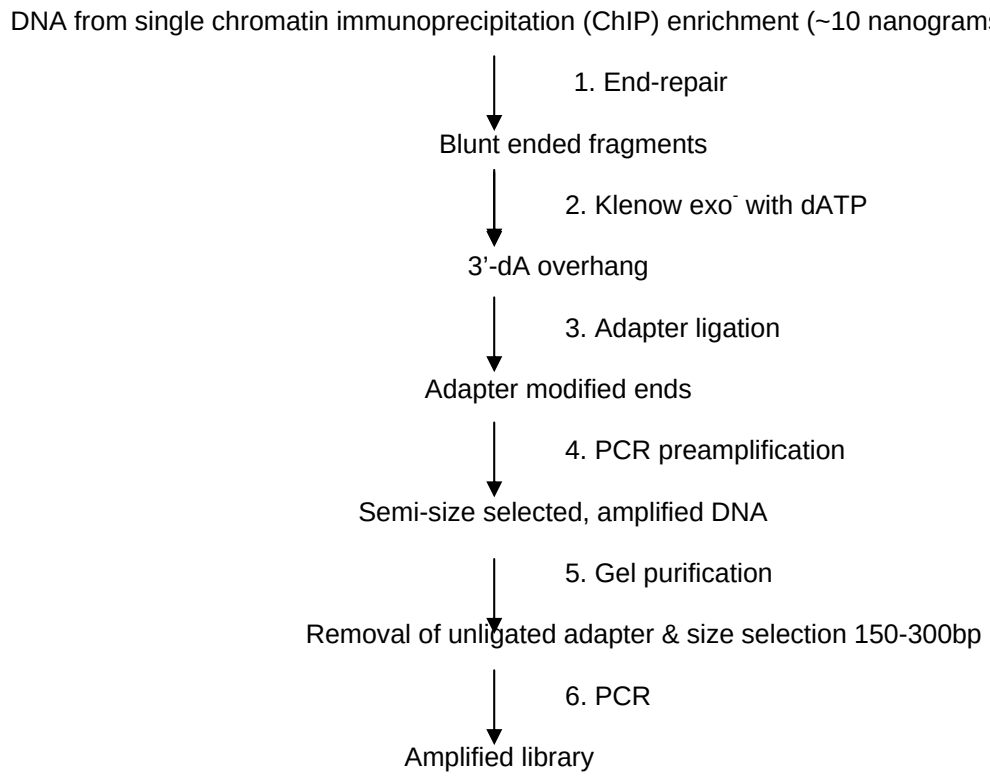
This protocol describes the preparation of libraries of chromatin-immuno-precipitated DNA in a format compatible with the Illumina's cluster amplification and sequencing platforms. The objective of the protocol is to add adapter sequences onto the ends of DNA fragments to generate the following construct:

Adapter 1	Sequencing primer	Genomic DNA fragment	Adapter 2

The 'Adapter 1' and 'Adapter 2' sequences correspond to the two surface-bound oligos on the flowcells used in the cluster amplification platform, and the 'Sequencing primer' corresponds to the primer used in the SBS sequencing reaction.

This protocol requires the same reagents used in the Sample Preparation Kit for Genomic DNA (p/n 0801-0303). Following sample preparation, samples can be hybridized and amplified onto a flowcell using the same reagents and Cluster Station protocol.

Overview of protocol:



Process:

1. End-repair

Introduction:

ChIP DNA contains double-stranded DNA fragments that are blunt-ended or comprised of 3' or 5' overhangs. The overhangs are converted to phosphorylated blunt ends in this step of the protocol by using a combination of T4 DNA polymerase, E. coli DNA Pol I large fragment (Klenow polymerase) and T4 polynucleotide kinase. The 3' to 5' exonuclease activity of these enzymes removes 3' overhangs and the polymerase activity fills in 5' overhangs. A more detailed explanation of the procedure can be found in Sambrook et al., 1989.

IMPORTANT! It is important that E. coli DNA Pol I large fragment (Klenow polymerase) is used and not the Klenow exo minus polymerase that is provided for the A-tailing step (step 3).

Materials:

- DNA from ChIP enrichment (~10 nanograms in 30 μl in water)
- T4 DNA ligase buffer with 10mM ATP
- dNTPs mix (10 mM each)
- T4 DNA pol. (3 U/μl)
- Klenow DNA pol. (5 U/μl)
- T4 PNK (10 U/μl)
- QIAquick PCR Purification Kit (QIAGEN, catalog number #28104)

Procedure:

1. Dilute Klenow DNA polymerase 1:5 with water at a concentration of 1U/ul.
2. Prepare the following reaction mix:

10 ng of ChIP Enriched DNA	30 μl
Water	10 μl
T4 DNA ligase buffer with 10mM ATP	5 μl
dNTP mix	2 μl
T4 DNA pol.	1 μl
Klenow DNA pol (1U/ul)	1 μl
T4 PNK	1 μl
Total	50 ul

3. Incubate for 30 min at 20°C in thermocylcer.
4. Purify on one QIAquick column using the QIAquick PCR Purification Kit and protocol, eluting in 34 μl of EB.

2. Addition of an ‘A’ base to the 3’ end of the DNA fragments

Introduction:

The adapters to be added to the ends of the blunt phosphorylated DNA fragments have a single ‘T’ base overhang at their 3’ end. These can only be added if a complementary single ‘A’ base is added to the 3’ end of the templates first. This can be done by employing the polymerase activity of Klenow fragment (3’ to 5’ exo minus) which adds an ‘A’ base to the ends of double-stranded DNA molecules.

Materials:

- DNA from section 1 (34 μl)
- Klenow buffer (10×)
- dATP (1 mM)
- Klenow fragment (3’ to 5’ exo minus) (5 U/μl)
- Hot-block or PCR machine
- MinElute PCR Purification Kit (QIAGEN, catalog number #28004)

Note: A Qiaquick MinElute column is required for this step instead of a normal Qiaquick column.

Procedure:

1. Prepare the following reaction mix:

DNA from section 1	34 μl
Klenow buffer	5 μl
dATP	10 μl
Klenow fragment (3’ to 5’ exo minus)	1 μl
Total	50 ul

2. Incubate for 30 min at 37 ^{o}C.
3. Purify on one QIAquick MinElute column using the MinElute PCR Purification Kit and protocol, eluting in 10 μl of EB.

3. Ligation of adapters to the ends of the DNA fragments

Introduction:

The ligation reaction requires adapters supplied by Illumina.

Materials:

- DNA from section 2 (10μl)
- DNA ligase buffer (2×)
- Adapter oligo mix
- DNA ligase (1 U/μl)
- QIAquick PCR Purification Kit (QIAGEN, catalog number #28104)

Note: Dilute the Illumina adapters 1:10 with water to adjust for the smaller quantity of DNA.

Procedure:

1. Prepare the following reaction mix:

DNA from section 2	10 μl
DNA ligase buffer	15 μl
Adapter oligo mix(1:10)	1 μl
DNA ligase	4 μl
Total	30 ul

2. Incubate for 15 min at room temperature.
3. Purify on one Qiaquick column using the QIAquick PCR Purification Kit and protocol, eluting in 30 μl of EB.

4. Enrichment of adapter-modified DNA fragments by PCR

Introduction:

15 cycles are used to ensure adequate yield for the gel purification step. Note that 23/30 μl of the adapter-modified DNA is added to the PCR.

Materials:

- DNA from section 3 (30 μl)
- Water
- 2x PCR Master Mix
- PCR primer 1.1
- PCR primer 2.1
- PCR machine
- MinElute PCR Purification Kit (QIAGEN, catalog number #28004)

Procedure:

1. Prepare the following PCR reaction mix:

DNA	23 μl
Phusion DNA polymerase	25 μl
PCR primer 1.1	1 μl
PCR primer 2.1	1 μl
Total	50 ul

2. Amplify using the following PCR protocol:

    - 30 sec at 98 ^{o}C
    - [10 sec at 98 ^{o}C, 30 sec at 65 ^{o}C, 30 sec at 72 ^{o}C] 15 cycles
    - 5 min at 72 ^{o}C
    - Hold at 4 ^{o}C

3. Purify on one QIAquick MinElute column using the MinElute PCR Purification Kit and protocol, eluting in 10 μl of EB.

5. Gel purification of the products from the ligation reaction

Introduction:

The process selects a size-range of templates to go on the cluster generation platform.

Materials:

- DNA from section 4 (10μl)
- Electrophoresis apparatus
- QIAquick Gel Extraction Kit (QIAGEN, catalog number #28704)
- Ethidium Bromide
- 2% agarose gel
- 100 bp DNA ladder
- UV

Procedure:

1. Prepare a 2% agarose gel in a final volume of 50 ml 1 × TAE buffer. Add the ethidium bromide to the gel during its preparation at a concentration of 400 ng/ml.
2. Load 500ng of 100bp DNA ladder to one lane of the gel. Add 3 μl of loading buffer to the DNA from the cleaned-up PCR (10 μl). Load the entire sample in another lane of the gel leaving at least a gap of one empty lane between ladder and sample
3. Run gel at 120 V for 60 min. (these conditions depend on your gel rig setup)
4. View the gel using long-wave UV.
5. Excise a chunk of gel in the range 150-300bp with a clean scalpel. Be sure to take photos of the gel before and after the slice is excised.
6. Purify the DNA from the agarose slices using a QIAGEN Gel Extraction Kit.

6. Enrichment of adapter-modified DNA fragments by PCR

Introduction:

Do a second round of amplification using the gel-extracted DNA. You may want to start with different amount of template to decide on the optimal input amount. Example showing here uses 1 μl of DNA from section 5.

Materials:

- DNA from section 5 (30 μl)
- Water
- 2x PCR Master Mix
- PCR primer 1.1
- PCR primer 2.1
- PCR machine
- QIAquick PCR Purification Kit (QIAGEN, catalog number #28104)

Procedure:

1. Prepare the following PCR reaction mix:

DNA	1 μl
Phusion DNA polymerase	25 μl
PCR primer 1.1	1 μl
PCR primer 2.1	1 μl
Water	22 μl
Total	50 ul

2. Amplify using the following PCR protocol:
    - 30 sec at 98 ^{o}C
    - [10 sec at 98 ^{o}C, 30 sec at 65 ^{o}C, 30 sec at 72 ^{o}C] 15 cycles
    - 5 min at 72 ^{o}C
    - Hold at 4 ^{o}C
3. Purify on one QIAquick column using the QIAquick PCR Purification Kit and protocol, eluting in 30 μl of EB.
4. Measure concentration by running 1 μl of the product on a bioanalyzer, or 1 μl using Nanodrop,
5. Perform several qPCR QC experiments to evaluate if the library is properly enriched.