

Preparing Samples for ChIP Sequencing of DNA

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Introduction

This protocol explains how to prepare libraries of chromatin-immunoprecipitated DNA for analysis on the Illumina Cluster Station and Genome Analyzer. You will add adapter sequences onto the ends of DNA fragments to generate the following template format:

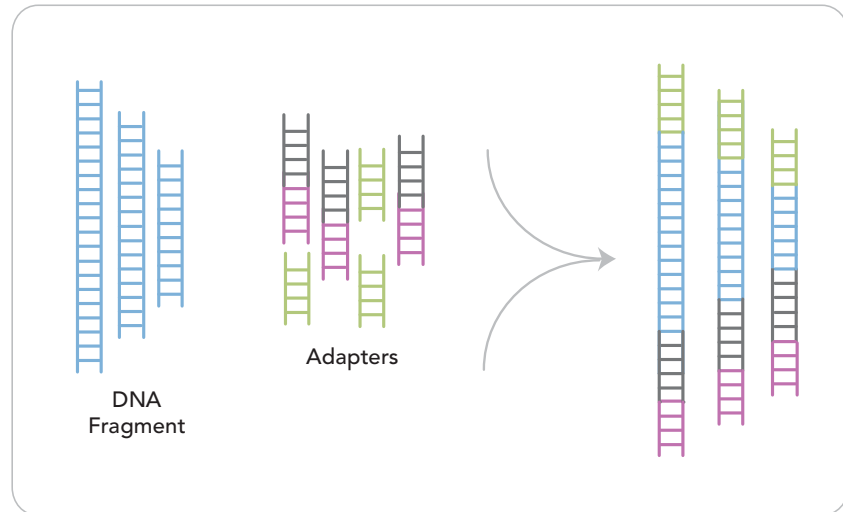


Figure 1 Fragments after Sample Preparation

The adapter sequences correspond to the two surface-bound oligos on the flow cells used in the Cluster Station.

Workflow

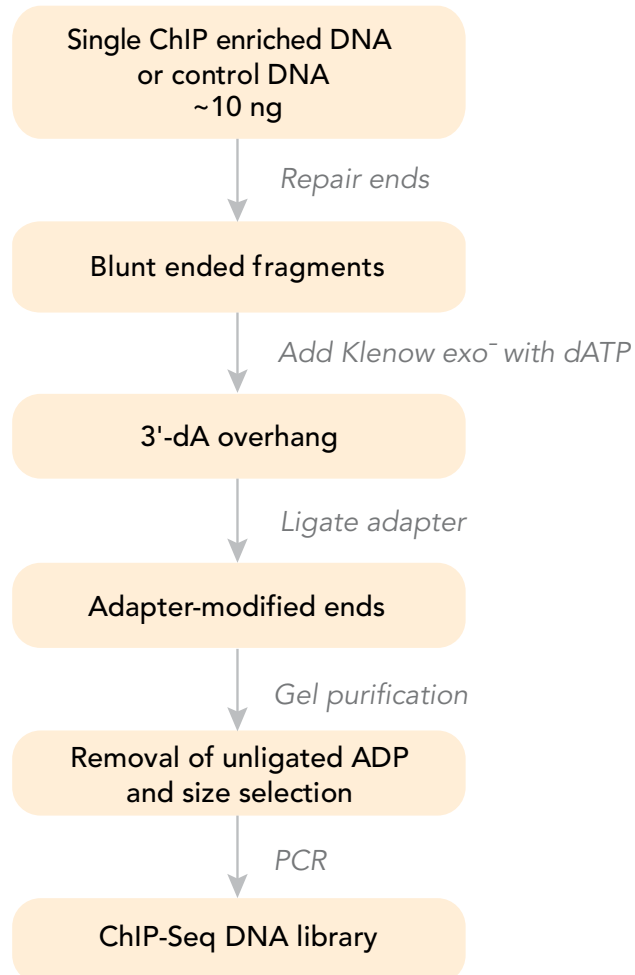


Figure 2 Sample Preparation Workflow

Kit Contents and Equipment Checklist

Check to ensure that you have all of the reagents identified in this section before proceeding to sample preparation.

ChIP-Seq Sample Prep Kit

Store at -20°C

This box is shipped at -80°C. As soon as you receive it, store the following components at -20°C.

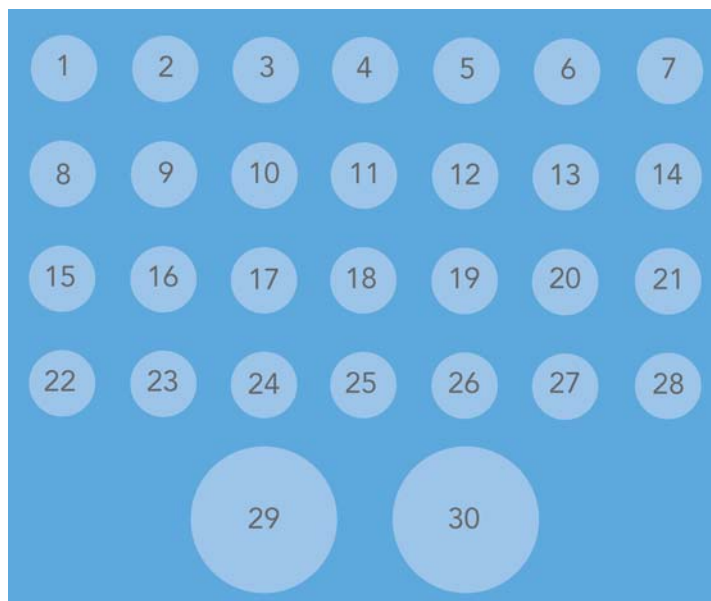


Figure 3 ChIP-Seq Sample Prep Kit

1. T4 DNA Ligase Buffer with 10 mM ATP, part # 1000534
2. 10 mM dNTPs Mix, part # 1000564
3. T4 DNA Polymerase, part # 1000514
4. Klenow DNA Polymerase, part # 1000515
5. T4 PNK, part # 1000519
6. Klenow Buffer, part # 1000535
7. 1 mM dATP, part # 1000520
8. Klenow Fragment (3' to 5' exo minus), part # 1000536
9. 2X DNA Ligase Buffer, part # 1000523
10. Adapter Oligo Mix, part # 1000521
11. DNA Ligase, part # 1000522
12. 5x Phusion* buffer, part # 1000585
13. Phusion* Polymerase (Finnzymes Oy), part # 1000584
14. Ultra Pure Water, part # 1001913

15. PCR Primer 1.1, part # 1000537
16. PCR Primer 1.1, part # 1000537
17. PCR Primer 2.1, part # 1000538
18. PCR Primer 2.1, part # 1000538
19. - 30. Empty

Equipment Checklist

Check to ensure that you have all of the necessary user-supplied equipment before proceeding to sample preparation.

- ▶ Benchtop microcentrifuge
- ▶ Benchtop centrifuge
- ▶ Dark Reader transilluminator
- ▶ Disposable scalpels
- ▶ Electrophoresis unit
- ▶ Gel trays and tank
- ▶ Thermal cycler or heat block

Perform End Repair

This protocol converts the overhangs into phosphorylated blunt ends, using T4 DNA polymerase, E. coli DNA Pol I large fragment (Klenow polymerase), and T4 polynucleotide kinase (PNK). The 3' to 5' exonuclease activity of these enzymes removes 3' overhangs and the polymerase activity fills in the 5' overhangs.

Consumables

Illustrina-Supplied

- ▶ T4 DNA ligase buffer with 10mM ATP
- ▶ dNTPs mix
- ▶ T4 DNA polymerase
- ▶ Klenow DNA polymerase
- ▶ T4 PNK
- ▶ Water

User-Supplied

- ▶ ChIP enriched, QPCR verified DNA (approx. 10 ng in 30 μ l water)
- ▶ QIAquick PCR Purification Kit (QIAGEN, part # 28104)

Procedure

1. Dilute Klenow DNA polymerase 1:5 with water for a final Klenow concentration of 1U/ μ l.
2. Prepare the following reaction mix:
 - ChIP enriched DNA (30 μ l)
 - Water (10 μ l)
 - T4 DNA ligase buffer with 10mM ATP (5 μ l)
 - dNTP mix (2 μ l)
 - T4 DNA polymerase (1 μ l)
 - Klenow DNA polymerase (1 μ l)
 - T4 PNK (1 μ l)The total volume should be 50 μ l.
3. Incubate in the thermal cycler for 30 minutes at 20°C.
4. Follow the instructions in the QIAquick PCR Purification Kit to purify on one QIAquick column, eluting in 34 μ l of EB.

Add 'A' Bases to the 3' End of the DNA Fragments

This protocol adds an 'A' base to the 3' end of the blunt phosphorylated DNA fragments, using the polymerase activity of Klenow fragment (3' to 5' exo minus). This prepares the DNA fragments for ligation to the adapters, which have a single 'T' base overhang at their 3' end.

Consumables **Illumina-Supplied**

- ▶ Klenow buffer
- ▶ dATP
- ▶ Klenow fragment (3' to 5' exo minus)

User-Supplied

- ▶ MinElute PCR Purification Kit (QIAGEN, part # 28004)



NOTE

This protocol requires a MinElute column rather than a normal QIAquick column.

Procedure

1. Prepare the following reaction mix:
 - DNA sample (34 μ l)
 - Klenow buffer (5 μ l)
 - dATP (10 μ l)
 - Klenow exo (3' to 5' exo minus) (1 μ l)The total volume should be 50 μ l.
2. Incubate for 30 minutes at 37°C.
3. Follow the instructions in the MinElute PCR Purification Kit to purify on one MinElute column, eluting in 10 μ l of EB.

Ligate Adapters to DNA Fragments

This protocol ligates adapters to the ends of the DNA fragments, preparing them to be hybridized to a flow cell.

Consumables **Illumina-Supplied**

- ▶ DNA ligase buffer
- ▶ Adapter oligo mix
- ▶ DNA ligase
- ▶ Ultra pure water

User-Supplied

- ▶ MinElute PCR Purification Kit (QIAGEN, part # 28004)

Procedure

1. Dilute the Adapter oligo mix 1:10 with water to adjust for the smaller quantity of DNA.

2. Prepare the following reaction mix:

- DNA sample (10 μ l)
- DNA ligase buffer (15 μ l)
- Diluted adapter oligo mix (1 μ l)
- DNA ligase (4 μ l)

The total volume should be 30 μ l.

3. Incubate for 15 minutes at room temperature.
4. Follow the instructions in the MinElute PCR Purification Kit to purify on one MinElute column, eluting in 10 μ l of EB.

Size Select the Library

This protocol removes excess adaptors and selects a size range of templates to go on the Cluster Station.

Consumables

User-Supplied

- ▶ 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, part # 28704)

Procedure



CAUTION

Illumina does not recommend purifying multiple samples on a single gel due to the risk of cross-contamination between libraries.

1. Prepare a 50 ml, 2% agarose gel with TAE buffer. Final concentration of TAE should be 1X at 50 ml.
2. Add ethidium bromide (EtBr) after the TAE-agarose has cooled to avoid ethidium bromide inhalation.
Final concentration of EtBr should be 400 ng/ml (i.e., add 20 µg EtBr to 50 ml of 1X TAE).
3. Load 500 ng of 100 bp DNA ladder to one lane of the gel.
4. Add 3 µl of loading buffer to 10 µl of the DNA from the purified ligation reaction.
5. Load the entire sample in another lane of the gel, leaving at least one empty lane between ladder and sample.
6. Run gel at 120 V for 60 minutes.
7. View the gel on a Dark Reader transilluminator to avoid being exposed to UV light.



NOTE

Prolonged personnel exposure to UV light can damage your DNA.



NOTE

Illumina recommends using the 100 bp ladder to determine the correct position in which to excise the gel. This is due to the very small amount of starting material.

8. Excise a region of gel with a clean scalpel. The gel slice should contain the material in the 200 ± 25 bp range. Be sure to photograph the gel before and after the slice is excised.
9. Cut a slice of the same size from an empty well on the same gel and take this sample through gel purification and PCR. No visible PCR product should be present.
10. Use a QIAGEN Gel Extraction Kit (QIAGEN, part # 28704) to purify the DNA from the agarose slices and elute DNA in 36 μ l.

Enrich the Adapter-Modified DNA Fragments by PCR

In this protocol you will perform PCR amplification using the gel-extracted DNA. As a negative control, perform PCR amplification on the sample extracted from an empty well (step 9, *Size Select the Library*)

Consumables **Illumina-Supplied**

- ▶ Phusion* polymerase
- ▶ 5x Phusion* buffer
- ▶ dNTP mix
- ▶ PCR primer 1.1
- ▶ PCR primer 2.1

User-Supplied

- ▶ MinElute PCR Purification Kit (QIAGEN, part # 28004)

Procedure

1. Prepare the following PCR reaction mix:
 - DNA (36 μ l)
 - 5x Phusion* buffer (10 μ l)
 - dNTP mix (1.5 μ l)
 - PCR primer 1.1 (1 μ l)
 - PCR primer 2.1 (1 μ l)
 - Phusion* polymerase (0.5 μ l)The total volume should be 50 μ l.
2. Amplify using the following PCR protocol:
 - a. 30 seconds at 98°C
 - b. 18 cycles of:
 - 10 seconds at 98°C
 - 30 seconds at 65°C
 - 30 seconds at 72°C
 - c. 5 minutes at 72°C
 - d. Hold at 4°C
3. Follow the instructions in the MinElute PCR Purification Kit to purify on a MinElute column, eluting in 15 μ l of EB.

Validate the Library

The amount of starting material is very low (10 ng), and after 18 cycles of pcr, the yield could still be too low to see on a regular gel, even though it is enough for cluster generation. Illumina recommends performing the following, more sensitive quality control analysis on your sample library using an Agilent Technologies 2100 Bioanalyzer.

Bioanalyzer Method

1. Load 1 μ l of the resuspended construct and 1 μ l of the negative control on an Agilent Technologies 2100 Bioanalyzer.
2. Check the size, purity, and concentration of the sample.

Alternative Methods

For users who do not have access to an Agilent Technologies 2100 Bioanalyzer or similar instrument, you may try using a sensitive dsDNA measurement assay such as the Quant-iT dsDNA HS Assay Kit, 100 assays 0.2-100 ng for use with the Qubit fluorometer (Invitrogen). Note that this will not allow you to check the size and purity of your sample. Do not use an OD₂₆₀/280 ratio for concentration measurements, since this will not distinguish dsDNA from primers, and therefore cannot be used to validate the library.



Illumina, Inc.
9885 Towne Centre Drive
San Diego, CA 92121-1975
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

