# Illumina Sequencing Library Construction from ChIP DNA

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**[Abstract]** The Illumina sequencing platform is very popular among next-generation sequencing platforms. However, the DNA sequencing library construction kit provided by Illumina is considerably expensive. The protocol described here can be used to construct high-quality sequencing libraries from chromatin immunoprecipitated DNA. It uses key reagents from third-party vendors and greatly reduces the cost in library construction for Illumina sequencing.

## Materials and Reagents

1. QIAquick PCR purification kit (QIAGEN, catalog number: 28104)
2. QIAquick gel extraction kit (QIAGEN, catalog number: 28704)
3. MinElute PCR purification kit (QIAGEN, catalog number: 28004), store the columns at 4 °C.
4. Gibco UltraPure water (Life Technologies, Gibco®, catalog number: 10977-015)
5. End-it DNA End repair kit (Epicentre®, catalog number: ER0720)
6. Klenow fragment (3’ ≥ 5’ exo minus) (New England Biolabs, catalog number: M0212S)
7. 100 mM dATP (Life Technologies, Invitrogen™, catalog number: 10216-018 or VWR International, catalog number: PAU1201)
8. LigaFast ligation kit (Promega corporation, catalog number: M8221)
9. Ethanol
10. Elution buffer
11. End repair buffer
12. Klenow buffer
13. Cyan/orange loading buffer
14. 2% E-gel, precast agarose gel (Life Technologies, Invitrogen™) (can be replaced by house made 2% agarose gel)
15. Phusion HF PCR master mix (New England Biolabs, catalog number: F531S or F531L)
16. Illumina Adapter oligo mix from Illumina sequencing kit, but can be replaced by house made oligo mix using fast denaturation and slow reannealing as described in reference 1. The adapter oligo sequences are:
    5' P-GATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG

5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT

17. Illumina PCR primers 1.1 and 2.1 from Illumina sequencing kit, but can be replaced by ordinary synthesized oligos with the following sequences:
    Illum1.1:
    5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCC GATCT
    Illum2.1:
    5' CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT

## Equipment

1. BECKMAN centrifuges and rotor (Beckman Coulter)
2. PCR thermocycler (PerkinElmer or F. Hoffmann-La Roche)
3. NanoDrop Micro-Volume UV-Vis Spectrophotometer

## Procedure

1. Purification from ethanol precipitated ChIP DNA. Use QIAquick PCR purification kit, and elute in 50 µl elution buffer. Use 34 µl for library construction and the rest for qPCR verification.
2. Input DNA needs to be diluted before making library. After purification, measure the concentration of input DNA by Nanodrop. If it reads 20 ng/µl, dilute 5 fold, if close to 40 ng/µl dilute 10 fold.
3. End-repair:
    Mix the following components in a 1.5 ml Eppendorf tube:
    34 µl purified ChIP DNA (or add Gibco water up to 34 µl)
    5 µl 10x end repair buffer
    5 µl 2.5 mM dNTP mix
    5 µl 10 mM ATP
    1 µl end-repair enzyme mix
    50 µl total
    Incubate for 45 min at room temperature (RT).
    Purify using QIAquick PCR purification column, elute in 34 µl.
4. Addition of ‘A’ base to the 3’ ends:
    Mixing the following components in a 1.5 ml Eppendorf tube:
    34 µl ChIP DNA from step 3
    5 µl Klenow buffer = NEB buffer 2

10 µl 1 mM dATP

1 µl Klenow fragment (3'-5' exo minus)

50 µl total reaction volume

(1 mM dATP is diluted from 100 mM dATP stock and aliquoted in 25 µl, freeze-thaw only once)

Incubate 30 min at 37 °C.

Purify using MinElute PCR purification column, elute in 12 µl.

5. Adaptor ligation:

   Mix the following components in a 1.5 ml Eppendorf tube:

   11 µl ChIP DNA from step 4

   15 µl DNA ligase buffer

   1 µl 1: 20 diluted adaptor oligo

   3 µl DNA ligase

   30 µl total

   (If doing multiplexing, make sure to add in each reaction with different adaptor, and label clearly.)

   Incubate 15 min at RT.

6. Gel selection to get rid of excessive adaptors:

   Dilute cyan/orange loading buffer 10 fold, add 6 µl to the 30 µl reactions from step 5, load in two wells of an E-gel. Also load 20 µl 10-fold diluted 50 bp ladder. Run E-gel for 20 min. Cut between 150 and 450 bp. Purify the DNA using QIAquick gel extraction kit, elute in 30 µl buffer.

7. PCR with Illumina primers:

   1:1 dilute Illumina PCR primers 1.1 and 2.1 with Gibco water.

   Mixing the following components in PCR stripe tubes:

   30 µl ChIP DNA from step 4

   28 µl Phusion PCR mastermix

   1 µl diluted primer 1.1

   1 µl diluted primer 2.1

   60 µl total reaction volume

   Run PCR cycle:

   98 °C 30 sec

   98 °C 10 sec

   65 °C 30 sec

   72 °C 30 sec

   GOTO step 2 for 15 times

   72 °C 5 min

4 °C hold.

8. Size selection on 2% agarose gel:
   Add 1 µl undiluted cyan/orange loading dye to the PCR reaction, load all in 3 wells on E-gel, run for 30 min alongside with 20 µl 1:10 diluted 50 bp ladder and 20 µl 1:10 diluted 100 bp ladder.
   Cut between 150 and 450 bp. Take pictures before and after the slice is excised. Make sure to avoid the ~100 bp adaptor band. A good library should have a smear centering at ~200 bp. Strong band at ~100 bp indicates over amplification of adaptors and the library may not be good enough quality.
   Purify the DNA using QIAquick gel extraction kit and elute in 30 µl buffer.
9. Measure DNA concentration:
   Use NanoDrop to measure the DNA concentration. A good library should be relatively concentrated (*e.g.,* > 10 ng/µl).

## Acknowledgments

The protocol has been tested and optimized by different researchers in the Snyder lab, Stanford University (Lefrancois *et al.,* 2009).

## References

1. Lefrancois, P., Euskirchen, G. M., Auerbach, R. K., Rozowsky, J., Gibson, T., Yellman, C. M., Gerstein, M. and Snyder, M. (2009). Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing. *BMC Genomics* 10: 37.
2. Lefrancois, P., Zheng, W. and Snyder, M. (2010). ChIP-Seq using high-throughput DNA sequencing for genome-wide identification of transcription factor binding sites. *Methods Enzymol* 470: 77-104.

 