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LIBRARY PREPARATION AND QUANTIFICATION FOR CHROMATIN IMMUNOPRECIPITATION FOLLOWED BY HIGH-THROUGHPUT SEQUENCING (CHIP-SEQ)

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Abstract:-

Properly generating robust, high-quality libraries for ChIP-Seq will prepare your for Illumina sequencing and an overall successful ChIP-Seq experiment. It is often challenging to prepare DNA for a next-gen ChIP-Seq library because it's difficult to obtain a large amount of DNA after ChIP. In this paper, the library construction method is suitable for low concentration DNA in woody samples.

INTRODUCTION

ChIP-sequencing (or ChIP-Seq) is a technique used to analyze protein interactions with DNA. It combines chromatin immunoprecipitation (ChIP) with next generation sequencing in order to identify binding sites of DNA-associated proteins (M.L. Metzker et al., 2010). These binding sites are mapped throughout the entire genome for any protein of interest (Wang et al., 2009). ChIP-Seq has quickly become the standard method for regulation and has been further developed into a number of modified protocols that allow detection from plants (Dijk EL et al., 2014).

To meet the need for low-input library preparation for ChIP-Seq, several techniqu es have been developed and modified (Adli M et al., 2011), allowing inputs DNA d own to 10 pg (Shanka et al., 2011). To the best of our knowledge this is the study of low-input library construction techniques in woody plants.

1. Library Construction Protocol

A total amount of 50 ng DNA per sample was used as input material for the ChIP sample preparations. Sequencing libraries were generated using DNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. From the sample to the final data acquisition, each link of sample detection, database building and sequencing will have an impact on the quality and quantity of data, and the quality of data will directly affect the results of subsequent information analysis. In order to ensure the accuracy and reliability of sequencing data from the source, every step of sample detection, database building and sequencing should be strictly controlled to ensure the output of high-quality data fundamentally. The flow chart is ChIP-Seq library sequencing flow (Figure 1).

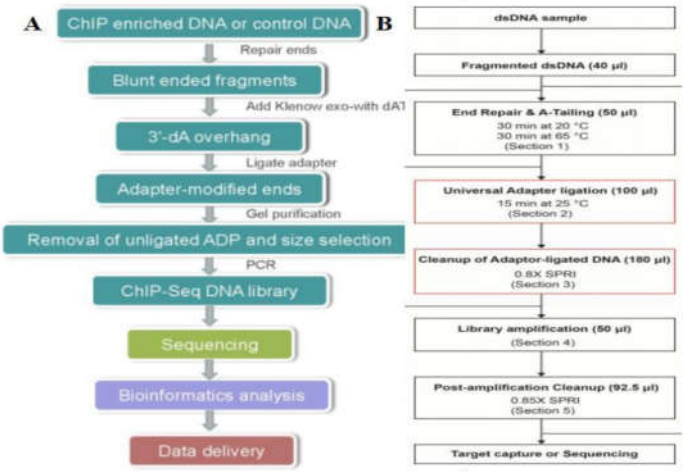

Fig.1 Process Workflow library of Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

2.1 The purification of DNA

Gnomic DNA degradation and contamination were monitored on 1.2% agarose gels. DNA purity was checked using the Nano-Photometer® spectrophotometer (IMPLEN, CA, USA). The DNA concentration was measured using a Qubit® DNA Assay Kit in a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). A total amount of 50 ng DNA per sample was used as input material for the ChIP sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample.

2.2 End repair add A-Tailing

1) Mix the following components in a sterile nuclease-free plate/tube: Fragmented DNA, end prep reaction buffer and enzyme mix.
2) Pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.
3) Place in a thermocycler,with the heated lid set to >75℃， and run the following program: 20℃ 30mins, and 4℃ hold.

2.3 Adapter Ligation

1) Add adaptor to each sample. Mix thoroughly by pipetting.Add 50 µl of Ligation Master Mix to each reaction tube. Pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.
2) Place the tubes in a thermocycler and run the following program with Lid heating off.

2.4 Clean-up of Adaptor-ligated DNA

1) Add adapter ligation reaction product and XP reagent together and mix well by pipetting up and down at least to 10 times.
2) Incubate for 5-15 minutes at room temperature.

3) Place the plate/tube on magnetic stand until the solution clears and a pellet is formed.
4) While leaving the plate/tube on the magnet, remove and discard the supernatant without disturbing the pellet.
5) Add 200 µl of freshly prepared 80% ethanol solution to the pellet while it is still on the magnet. Incubate for 30 seconds, and then carefully remove the 80% ethanol solution.
6) Repeat Step 5 once. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.
7) Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open, watching the pellet to avoid over-drying.
8) Remove the tube/plate from the magnetic stand, resuspend the beads.
9) Mix well by pipetting up and down 10 times, or on a vortex mixer. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.
10) Incubate the plate/tube at room temperature for 2 minutes to elute DNA off the beads.
11) Place the plate/tube on the magnet until the liquid is clear.
12) Transfer the clear supernatant to a new plate/tube, transfer 20 µl to proceed with library amplification.

2.5 Library Amplification

To achieve the highest amplification efficiency and avoid primer depletion, it is critical to use an optimal concentration of high-quality primers. Primers should be used at a final concentration of 3µM each.

1) Add adapter-ligated DNA, HiFi HotStart ReadyMix, index Primer, Pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.
2) Amplify using the following cycling protocol:

Step	Temp	Time	Cycles
Initial Denaturation	98°C	3min	1
Denaturation	98°C	45sec	
Annealing	65°C	45sec	15 cycles
Extension	72°C	30sec	
Final extension	72°C	1min	1
Hold	4°C	∞	1

3) Move the plate/tube to post-PCR area before opening the plate/tube for Post-amplification Clean up

2.6 Post-amplification Clean up

1) Add library amplification reaction product and AMpure reagent together, mix well by pipetting up and down at least 10 times.
2) Incubate for 5-15 minutes at room temperature.
3) Place the plate/tube on magnetic stand until the solution clears and a pellet is formed.
4) While leaving the plate/tube on the magnet, remove and discard the supernatant without disturbing the pellet.
5) Add 200 µl of freshly prepared 80% ethanol solution to the pellet while it is still on the magnet. Incubate for 30 seconds, and then carefully remove the 80% ethanol solution.
6) Repeat Step 5 once, and ensure all ethanol has been removed.
7) Dry the pellet at room temperature with the lid open until all of the ethanol has evaporated, watching the pellet to avoid over-drying.
8) Thoroughly resuspend the beads in an appropriate volume of 0.1X TE. Always use PCR-grade water if proceeding to target capture.
9) Quickly spin the plate/tube and incubate at room temperature for 2-5 minutes.
10) Place the plate/tube on the magnet to capture the beads. Incubate until the liquid is clear.
11) Transfer the clear supernatant to a new plate/tube and store the libraries at -20°C

The total DNA content of a good library should be more than 200 ng (Qubit assay), with a balance of 200-500bp in fragment size and no primer dimer (bioanalyzer 2100 identification). After passing the library inspection, Illumina HiSeq was sequenced according to the effective concentration and the demand of the target offline data. Figure 2 shows the library detection results of Agilent 2100 of *Betula platyphylla Suk*.

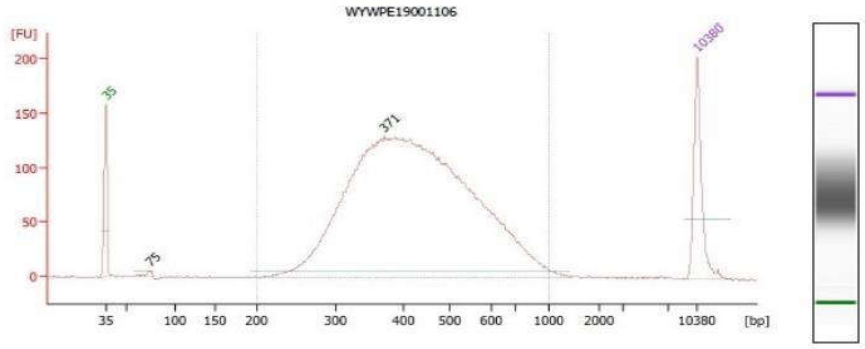

Fig.2 The library prepared with 50ng of fragmented DNA with size selection of *Betula platyphylla Suk*

Discussion

It is well understood that the quality of sequencing data depends highly upon the quality of the library. A major bottleneck in RNA sequencing is library construction. Therefore, the library construction process should guarantee a high molecular recover y of the original fragments with useful information in order to achieve the most geno mic coverage with the least amount of sequencing.

The Library preparation was performed following the ChIP-Seq Library Preparatio n Kit for Manual with the thess steps. The size of the target DNA fragments in the fi nal library is a key parameter for RNA-Seq library construction. Fragmentation is essential factor and most library preparation protocols.

After cDNA synthesis it was further amplified through PCR usually 12 to 15 cyc les is often performed to generate sufficient quantities of template DNA to allow accu rate quantification and to enrich for successfully adaptered fragments.

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