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APPLICATION NOTE

Use of the iDeal® chromatin immunoprecipitation (ChIP) kit for the identification of transcription factor binding sites in the pathogenic yeast *Candida glabrata*

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BACKGROUND

The iDeal ChIP-seq kit for Transcription Factors from Diagenode has been designed to allow efficient chromatin immunoprecipitation in mammals. The team of Frédéric Devaux at Sorbonne-Université in Paris has long standing experience in the genome-wide identification of transcription factors in different yeast species (1). In this application note, they describe the procedure they used and the results they obtained, for validating the use of the iDeal kit on *C. glabrata* cells. More specifically, they tested the kit on 4 different specific transcription factors involved in various stress responses and having different biochemical properties. They show that the iDeal ChIP-seq kit for Transcription Factors can be used for ChIP in yeast with high enrichment ratios, either measured by qPCR or by Illumina sequencing.

MATERIAL

Chromatin immunoprecipitation:

iDeal ChIP-seq kit for Transcription Factors (Diagenode, Cat. No. C01010055)

Additional supplies for yeast protocol:
Fastprep bead beater Bio101 (MP Biomedicals)
2 ml screw cap tubes for Fastprep bead beater (MP Biomedicals, Cat. No. 5076-200 and 5065-002)
Sterile glass beads 425-600 µm (Sigma Cat. No. G8772)
32% paraformaldehyde (Electron Microscopy Sciences)
20 mM Tris-HCl pH 7.4, 150 mM NaCl
Fixation buffer (2x50 ml) (Diagenode, Cat. No. C01019002)
1.25 M Glycine (2x50 ml) – (Diagenode, Cat. No. C01019019)
Fastprep bead beater Bio101 (MP Biomedicals)
2 ml screw cap tubes for Fastprep bead beater (MP Biomedicals, Cat. No. 5076-200 and 5065-002).
Sterile glass beads 425-600 µm (Sigma Cat. No. G8772)
32% paraformaldehyde (Electron Microscopy Sciences)
20 mM Tris-HCl pH 7.4, 150 mM NaCl

Library preparation:
MicroPlex Library Preparation Kit v3 (Diagenode, Cat. No. C05010001)
Primer indexes for MicroPlex v3 (Diagenode, Cat. No. C05010008)

PROCEDURE

1. Crosslinking Step

   - Grow an overnight culture (50 ml) of yeast cells in appropriate liquid medium until the A_{600} reaches 0.6 – 1
   - Mix 3.3 mL of iDeal Fixation Buffer with 1.7 mL of 32% paraformaldehyde and add these 5 mL of cross-linking reagent to the yeast culture.
   - Incubate at room temperature for 15 minutes.
   - Add 5.5 mL of glycine 2.5 M and incubate 5 min at room temperature to block the cross-linking reaction.
   - Centrifuge in a 50 mL falcon tube for 5 min at 4000 g and remove supernatant.
   - Resuspend the cell pellet in 25 mL cold (4°C) TBS buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl) and centrifuge again.

2. Cell lysis and chromatin shearing

   - Resuspend the pellet in 3 mL of iDeal Lysis Buffer IL1 and incubate 20 min at 4°C.
   - Centrifuge the cells at 4000 g for 5 min. Discard the supernatant.
   - Resuspend the pellet in 2 mL of iDeal Lysis Buffer IL2 and incubate 10 min at 4°C. Centrifuge again and remove supernatant.
   - Resuspend the pellet in 300 µL Shearing buffer (298.5 µL iDeal Shearing Buffer iS1b + 1.5 µL iDeal Protease inhibitor cocktail 200X). Transfer the liquid into a Fastprep tube (2 ml screw cap tubes for Fastprep bead beater) containing 300 µL of sterile glass beads (425-600 µm).
   - Break cells in a Bead beater Fastprep-24 device at 4°C (3 cycles of 30 sec at maximum speed. Put the tubes in ice for 2 min after each cycle).
   - Punch a hole at the bottom of the Fastprep tubes using a needle. Fix the Fastprep tubes at the top of 15 ml polyethylene Falcon tubes. Centrifuge at 900 g for 1 min at 4°C to collect the liquid in the 15 ml Falcon tubes. Transfer into a 1.5 mL Bioruptor sonication tube.
   - Fill the bath of the Bioruptor sonicator with cold water and some ice. Set the power at high intensity. Sonicate for 5 rounds of 30 sec on /30 sec off. Put the sample on ice for 2 min. Add some ice to the bath of the Bioruptor and repeat the sonication cycle.*

     **Note: It is recommended to use the Bioruptor with cooling system allowing for efficient control of the temperature in the sonication bath. In that case there is no need of ice. When using the Bioruptor Pico and 1.5 ml Bioruptor Microtubes (Cat. No. C30010016), sonicate for 5 -12 cycles 30 '' ON/30'' OFF. When using the Bioruptor Plus and 1.5 ml TPX Bioruptor Microtubes (Cat. No. C30010010-300), sonicate for 10-30 cycles 30 '' ON/30'' OFF at High Power.*

   - Centrifuge 15 sec at 16,000 g and 4°C, then transfer the supernatant in a new 1.5 mL tube.
   - Centrifuge 10 min at 16,000 g and 4°C, then transfer the supernatant in a new 1.5 mL tube. *Note that at this step the sample can be stored at -80°C for several weeks*

3. Preparation of anti-myc antibody coated magnetic beads

   - Prepare the 1x ChIP buffer iC1b by mixing 3.2 mL of iDeal ChIP-seq grade water, 0.8 mL of iDeal 5x ChIP Buffer iC1b and 80 µL of iDeal 5% BSA. Keep this mix on ice.
   - Transfer n*30 µL of iDeal Protein A-coated magnetic beads (n being the number of ChIP to be

performed) in one 1.5 mL tube.

- Add 1 mL of 1x ChIP buffer iC1b and incubate 5 min at 4°C on a rotating wheel. Do a short spin of centrifuge and put the tube on a magnetic bench, wait for 1 min and then remove the supernatant. Repeat this washing step two more times.
- Resuspend the beads in n*30 µL of 1x ChIP buffer iC1b, n being the number of ChIP reactions to be performed. Then aliquot 30 µL of beads in n tubes.
- Add to each tube 70 µL of the following mix: 6 µL of iDeal 5% BSA, 1.8 µL of iDeal protease inhibitor cocktail 200X, 5 µL of anti-myc antibody (1 µg/µL), 20 µL of iDeal 5x ChIP Buffer iC1b and 37.2 µL of iDeal ChIP-seq grade water. Incubate for a minimum of 2 hours at 4°C on a rotating wheel. Then the beads are ready to use.

4. Chromatin immunoprecipitation

The sheared chromatin is shared between three aliquots: 2.5 µL are kept at 4°C to be used as the INPUT sample (see section 5 of the procedure), 250 µL are mixed with the beads prepared in the previous section (IP sample), the rest of the chromatin is stored at -80°C.

- Add 250 µL of sheared chromatin to one tube (100 µL) of beads. Incubate at 4°C on a rotating wheel overnight.
- Do a short spin of centrifuge and put the tube on a magnetic bench, wait for 1 min and then remove the supernatant.
- Add 350 µL of iDeal Wash Buffer iW1, incubate 5 min at 4°C on the rotating wheel. Do a short spin of centrifuge and put the tube on a magnetic bench, wait for 1 min and then remove the supernatant.
- Proceed the same way with iDeal Wash Buffers iW2, iW3 and iW4.
- Resuspend the beads in 100 µL of iDeal Elution Buffer iE1 and incubate 30 min at room temperature on a rotating wheel.
- Do a short spin of centrifuge and put the tube on a magnetic bench, wait for 1 min and then transfer the supernatant in a new 1.5 mL tube. Add 4 µL of iDeal Elution Buffer iE2. In parallel, add to the 2.5 µL of INPUT sample mentioned at the beginning of this section 97.5 µL of Elution Buffer iE1 and 4 µL of Elution Buffer iE2.

5. Crosslink reversal and DNA purification

DNA purification was performed using the IPure DNA purification kit from Diagenode (included in the kit iDeal ChIP-seq for TF).

- Incubate the IP and INPUT samples for 4 hours at 65°C on an agitating thermoblock, with agitation set up at 300 rpm.
- Add 2 µL of IPure DNA carrier, 100 µL of isopropanol 100% and 10 µL of IPure Beads v2 (mix the beads before pipetting). Incubate 10 min at room temperature on a rotating wheel.
- Do a short spin of centrifuge and put the tube on a magnetic bench, wait for 1 min and then remove the supernatant. Add 100 µL of IPure Wash Buffer 1 (complemented with 1 volume of isopropanol before use). Incubate 30 sec at room temperature.
- Do a short spin of centrifuge and put the tube on a magnetic bench, wait for 1 min and then remove the supernatant. Add 100 µL of IPure Wash Buffer 2 (complemented with 1 volume of isopropanol before use). Incubate 30 sec at room temperature. Make a short pin of centrifuge

and put the tube on the magnetic bench. Make sure that you remove all the remaining wash buffer.
- Resuspend the beads with 25 µL of IPure Buffer C and incubate 15 min at room temperature on a rotating wheel.
- Do a short spin of centrifuge and put the tube on a magnetic bench, wait for 1 min and then transfer the supernatant in a new 1.5 mL tube.

Your IP and INPUT samples are now ready for downstream analyses. Alternatively, they can be stored at -20 °C for several weeks or at -80°C for several months.

6. Analyses of the IP and INPUT samples using Quantitative Real time PCR (Q-RT-PCR)

We used a Bio-Rad qPCR device and the Promega kit for qPCR but you may use any other qPCR set up.

- Prepare three dilutions (1/4, 1/8 and 1/16) for the IP and INPUT samples. In a Biorad qPCR 96-well plate, mix 2 µL of each dilution with 1 µL of primer mix (10 µL of forward primer at 100 pM + 10 µL of reverse primer at 100 pM + 80 µL water) and 10 µL of SYBRmix (Promega).
- Put the plate in the CFX96 real tyme system machine (Biorad). The PCR program is: 10 min 95°C followed by 40 PCR cycles (15 sec at 95 °C, 15 sec at 54 °C, 30 sec at 60 °C).

7. Analyses of the IP and INPUT samples using Illumina sequencing

- The libraries were prepared with the MicroPlex v2 kit from Diagenode using the supplier recommendations.
- The libraries were verified quantified and verified using the fragment analyzer and the Qubit devices.
- The sequencing was performed using a NextSeq 500 device.

RESULTS

Strategy

To validate the iDeal ChIP-seq kit on yeast, we performed series of IPs on transcription factors in the pathogenic yeast *Candida glabrata*, for which we knew the targets, using qPCR and Illumina sequencing to quantify the final IP/Input enrichments. We choose to apply iDeal to several transcription factors with different DNA binding properties, hence representing different challenges for the ChIP efficiency. More precisely, we targeted: Hap5, which is highly expressed and constitutively bound to its target gene *GRX4*; Pdr1 which has medium expression and constitutively interacts with the promoter of *CDR1* and with its own promoter; Mac1, which has medium expression and interacts with *CTR1* only in conditions of copper starvation; Zap1 which has high expression only when zinc is limiting and binds to its own promoter and to *ZRT2* only in zinc starvation conditions.

F. Devaux's group had built strains in which these factors had been tagged with 13 Myc epitopes and all the primers required for qPCR on the different target genes mentioned above were available. For all IPs, a mock IP using the untagged parental yeast strain grown in exactly the same conditions as the tagged strains was performed to assess the specificity of the observed enrichments. All experiments were performed twice.

qRT-PCR analyses

Figure 1 presents the relative enrichments obtained for the different gene targets of the corresponding transcription factors. Primers targeting the promoter of the *YHB1* gene (which is not bound by any of the studied transcription factors) were used to normalize all the enrichments. The values represented in the figures are then the IP/Input ratio of the gene of interest divided by the IP/Input ratio obtained for the *YHB1* promoter.

For all IPs, a significant enrichment was measured compared to the mock IP and to the *YHB1* control. The relative enrichments ranged from 7 fold for the Mac1 IP up to 90 fold for the Zap1 IP.

ChIP-seq analyses

Figure 2 represents the ChIP-seq patterns obtained for the IPs at the loci of the genes used for qPCR analyses. We observed ChIP peaks, clearly distinct from the mock IP and from the local background, for each of the tested genes. Hence, the ChIP-seq results confirmed the previous qPCR analyses. For most of the target genes, the enrichments observed in the ChIP-seq experiments were even higher than the enrichments measured by qPCR.

CONCLUSION

Our experiments demonstrated that the iDeal ChIP-seq kit, including magnetic DNA purification by IPure technology, and the MicroPlex library preparation kits can be successfully combined to perform ChIP-qPCR and ChIP-seq experiments in yeasts. We obtained significant qPCR enrichments and very clear ChIP-seq enrichment patterns at the promoters of known targets for 4 very different transcription factors. This protocol is now routinely used in our laboratory to ChIP other transcription factors in the human pathogen *Candida glabrata* and in the model yeast *Saccharomyces cerevisiae*.

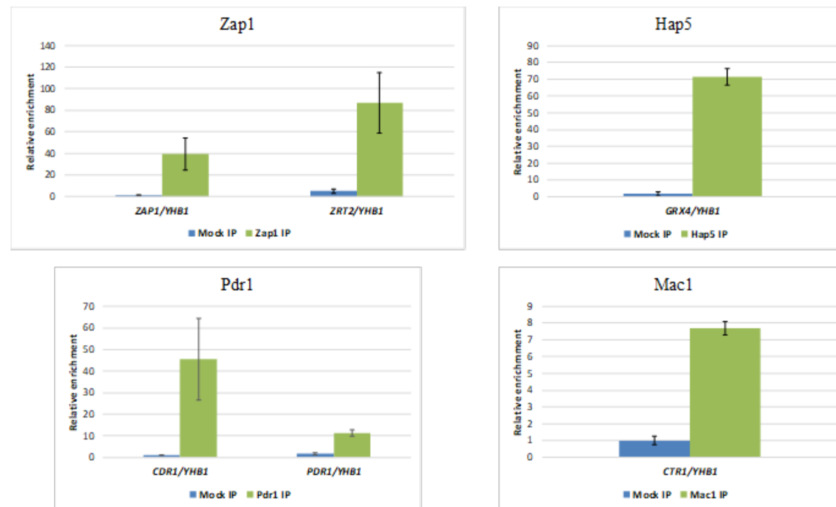

Figure 1: qPCR measurements of the IP/Input enrichments of the positive control targets for four different transcription factors (Zap1, upper left, Hap5, upper right, Pdr1, bottom left, Mac1 bottom right), relative to the values obtained for the YHB1 promoter used as a negative control.

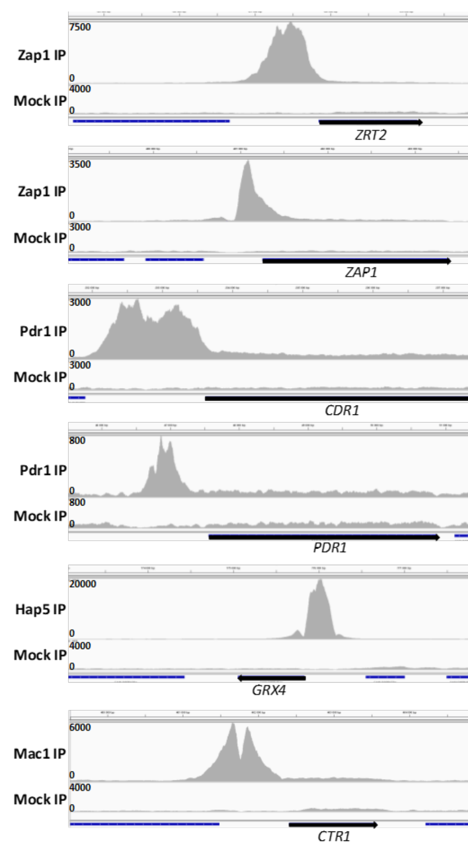

Figure 2: Selected genomic regions illustrating different patterns of ChIP-seq detection are presented using Integrative Genomics Viewer software (2). The y-axis represents the number of reads and the x-axis represents genomic coordinates at the locus of the positive control genes used in the qPCR experiments. Each frame represents a 5kb window on the C. glabrata genome. For each IP, the corresponding mock IP is represented.

REFERENCES

1. Merhej J, Thiebaut A, Blugeon C, Pouch J, Ali Chaouche Mel A, Camadro JM, Le Crom S, Lelandais G, Devaux F. (2016) A Network of Paralogous Stress Response Transcription Factors in the Human Pathogen Candida glabrata. *Front Microbiol*. 7:645.

2. Thorvaldsdottir, H., Robinson, J.T., and Mesirov, J.P. (2013) Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform* 14: 178-192.