

## ChIP Protocol: Yeast

### DAY 1:

inoculate 150ml medium with 5ml o/n culture  
grow to O.D.<sub>600</sub> 0.5 –0.7

crosslink (exactly) 150ml culture with 16.7ml 10% EM grade formaldehyde for 15' at RT (in a bottle, under the fume hood)

quench with 8.5ml 2.5M Glycine for 5', RT

pellet cells by centrifugation: JLA16-250-Rotor, 5000rpm, 5'

resuspend pellet in ~ 30ml PBS, transfer into 50ml-Falcon, wash centrifuge bucket with 20ml PBS, add to Falcon tube

pellet cells again: 5000rpm, 5'

resuspend pellet in 50ml PBS, centrifuge again

discard supernatant as complete as possible  
freeze cells at –20°C (or continue with day 2-protocol)

### DAY 2:

suspend pellet in 1ml FA-1 + CPI

Break open the cells:

split cells in 3 1.5ml-tubes containing 300µl glass beads  
shake on disruptor in the cold room for 40'

poke a hole in the bottom of each tube (hot needle) and place them in 2ml-tubes  
centrifuge 1' at 1000rpm

collect flow-through in a 15ml-Falcon tube

wash glass beads with 1ml FA-1/CPI (a total of 1ml for all 3 tubes!)

centrifuge again, add flow-through to Falcon-tube

rinse the 2ml-tubes with 1ml FA-1/CPI

Sonicate lysate to shear chromatin

**settings will change eventually!**

6', 15" on, 15" off, 30% intensity, on ice

centrifuge 5', 5000rpm, transfer supernatant in new 15ml-Falcon tube

**Preclearing with 4CL-B sepharose beads:**

add 200µl of a 50% slurry in FA-1

(if you take beads fresh from the bottle: wash them with FA-1)

rotate 1h at 4°C

centrifuge 2', 5000rpm

## **Immunoprecipitation**

use 700µl of cleared lysate for each IP

### A) IP using antibodies

add appropriate amount of antibody (info see below) to 700µl lysate  
rotate 2-3h at 4°C

add 55µl  $\mu$ -bind beads  
rotate for 1h at 4°C  
<negative control: unspecific Ab>

### B) IP with TAP-tag

add 55µl IgG-agarose beads  
rotate 3h at 4°C  
<negative control: 4CL-B sepharose>

## **Washing the IP**

Washing repeats the following steps:

centrifuge at 4°C and 1000rpm for 20'' – withdraw supernatant – add fresh buffer to beads and mix carefully

**After pelleting the first time: Transfer the supernatant of the unspecific Ab-control to a fresh tube. This is your supernatant/input/positive sample!!!**

Wash: 3x 1µl FA-1  
1x 1µl FA-2  
1x 1µl FA-3

add 900µl TE and transfer suspension to fresh tube  
centrifuge, remove TE, centrifuge again, remove residual TE  
add 250µl TE/1%SDS  
incubate at 65°C o/n

add 20µl supernatant and 200µl TE/SDS to a fresh tube and incubate at 65°C as well

## **DAY 3:**

centrifuge the tubes and transfer supernatant to a fresh tube (not the input control)  
add 10µl Proteinase K to every tube (or add before, so you only need 1 tip), incubate 2h at 55°C

## **DNA purification using the Qiagen PCR purification kit:**

add 1.12µl buffer PB to every sample, mix  
add solution in two portions to the column (max 700µl each time)  
centrifuge 1', 13.000rpm, discard flow-through  
wash with 750µl PE-buffer  
centrifuge 1', 13.000rpm, discard flow-through  
centrifuge 1', 13.000rpm again

place columns into fresh 1.5ml-tubes  
add 100µl EB+RNase, let sit for 1'  
centrifuge at 8000rpm for 5 min



**2.5M Glycine:**

Glycine 37.55g  
 H<sub>2</sub>O up to 200ml  
 \*Autoclave/store at RT

**Complete Protease Inhibitor (CPI):**

use CPI tablet from Roche#1697498  
 1 tablet into 20ml H<sub>2</sub>O = 25X stock  
 store at -20°C

**Proteinase K ( 20 mg/ml ) : 40 mg Proteinase K+2 ml buffer**

<u>Buffer:</u>	<u>Stock</u>	
10mM Tris pH7.5	1M	20µl
1mM CaCl <sub>2</sub>	1M	2µl
5% glycerol	80%	125µl
H <sub>2</sub> O		ad 20ml

**1M HEPES-KOH pH7.5:** Store at -20°C

HEPES (acid) 23.8g  
 H<sub>2</sub>O 80mL  
 KOH to pH 7.5  
 H<sub>2</sub>O ad 100ml

**Primers for ChIP Assay:**

Stock: make 100pmol/µL stocks of each primer with TE  
 working solution: 1:10

Ab Info:

<u>Ab</u>	<u>use</u>	<u>Ab usage</u>
mouse IgG	8µg	no Ab control
12CA5	8µg	□-HA mouse mAb
8WG16	8-9µg	□-PolII mouse mAb
9E10	25µL (5µg)	□-c-myc mouse mAb

beads

	<u>usage</u>
Sepharose Cl-4B	clearing of lysate (Sigma)
Gamma-bind G seph	pull down mouse IgG Ab (Amersham)
Rabbit IgG agarose	pull down TAP-tags (Sigma)