BISHOP LAB DMC1 CHIP PROTOCOL
Note: This protocol is optimized for meiotic yeast cells sporulating in liquid medium.

CROSS-LINKING

1. Harvest 100mLs of cells from sporulating culture (only half of these cells will be cross-linked; the other half is reserved for the no cross-linking control – to process these cells, skip cross-linking steps and start at step 7)
2. To cross-link, aliquot 50mLs of cells into an Erlenmeyer flask
3. Add formaldehyde to 1%
4. Shake gently at room temperature for 15 minutes (swirl flask occasionally because the yeast will settle to the bottom)
5. To quench cross-linking, add glycine to final concentration of 125mM
6. Shake gently at room temperature for 5 minutes
7. Pour cross-linked or non-cross-linked cells into 50mL conical tubes
8. Spin down 5 min at 3000 rpm in tabletop centrifuge
9. Pour off supernatant and resuspend cells in 1x PBS
10. Repeat spin
11. Pour off supernatant and resuspend cell pellet in 1-2 mLs 1x PBS
12. Divide each sample evenly into 2 microcentrifuge tubes
13. Quick spin, aspirate supernatant
14. Freeze pellet on dry ice
15. Store at –70ºC

Yield: 2 cross-linked pellets and 2 non-cross-linked pellets per strain

LYSIS AND SONICATION

1. Thaw cell pellet on ice for ~15 minutes
2. Add protease inhibitors (PI) fresh to 1M NaCl Lysis Buffer
3. Resuspend thawed cell pellets in 500μL buffer + PI
4. Add cold glass beads to 500μL mark on tube
5. Vortex at top speed for 5-10 minutes at 4ºC
6. Separate cell lysate from glass beads - poke a hole in the bottom of the tube using a hot 20-guage needle, place tube over a fresh microcentrifuge tube, and spin in for 1 minute at 3000 rpm at 4ºC
7. Sonicate lysate to final shear size of 1Kb (on a Branson Sonifier 450: output control=1, duty cycle=50%, count 20 pulses. Repeat this 3 times for each sample, with at least 1 minute on ice in between sonications)
8. Spin sonicated lysate for 10 minutes at top speed in a microcentrifuge at 4ºC
9. Transfer supernatant (lysate) to a fresh tube, noting volume – store on ice.
10. If necessary to increase amount of protein, resuspend cell pellet from step 8 in 500μL 1M NaCl lysis buffer + PI and repeat steps 3-9 (In step 9, combine lysate with lysate from first round, noting final volume)
11. Measure concentration of protein in lysate using Bradford Assay (average yield is about 5μg/μL protein per sample)
12. Reserve 30-40μL lysate for input DNA sample (2 rounds of phenol/chloroform extractions followed by EtOH precipication of DNA)

IMMUNOPRECIPITATION

(Note: These conditions are optimized for 2mg input protein for IP with Guinea Pig Dmc1 Antibody)

1. For each sample, calculate amount of lysate for 2mg protein
2. Aliquot this volume of lysate into microcentrifuge tube
3. If volume is less than 500μL, add 1M NaCl lysis buffer to adjust volume to 500μL
4. Add 1μL Dmc1 Guinea Pig antibody
5. Rotate samples overnight at 4ºC
6. Add 25μL Protein G magnetic beads (washed 2 times 1M NaCl lysis buffer) and 0.6μL BSA (10mg/mL stock)
7. Rotate samples for 2 hours at 4ºC

WASHING BEADS AND COLLECTING CHIP EXTRACT

1. Separate beads from lysate on magnet (this will take 1-2 minutes)
2. Remove supernatant from beads, discard
3. Resuspend beads in 1mL 1M NaCl lysis buffer
4. Rotate tubes at room temperature for 5 minutes (This is critical! Skimping on wash time yields dirty IPs!)
5. Repeat wash 4 more times with 1M NaCl lysis buffer
6. Repeat wash 3 times with 0.5M NaCl Buffer
7. Repeat wash 1 time with LiCl Wash Solution
8. Repeat wash 1 time with 1x TE, pH 8.0
9. After final wash, remove all traces of wash solution from the beads
10. Resuspend beads in 100μL 1x TE, pH 8.0
11. To reverse cross-links, heat samples at 65ºC overnight (at minimum, samples must be at 65ºC for six hours to reverse cross-links)
12. Transfer each sample carefully to a PCR tube
13. Heat to 95ºC for 30 minutes in PCR machine (keeps better constant temperature than a water bath)
14. Touch spin tubes
15. Separate supernatant from beads on magnet for at least 2 minutes
16. Transfer supernatant (ChIP extract) to fresh tube, being careful to not transfer beads
17. Sample is now ready for analysis
18. For a standard PCR reaction, use 2μL ChIP extract

SOLUTIONS

1M NaCl Lysis Buffer

50mM HEPES/KOH, pH 7.5
1M NaCl
5mM EDTA
1% Triton X-100
0.1% Na Deoxycholate

For 10mLs 1M NaCl lysis buffer, add following protease inhibitors fresh before performing lysis step:
10μL 1mg/mL Leupeptin (in H_20)
50μL 0.2M PMSF (in EtOH)
10μL 1mg/mL Pepstatin (in MeOH)

Alternatively, add 100x Protease Inhibitor cocktail to final concentration of 1x

0.5M NaCl Buffer

50mM HEPES/KOH, pH 7.5
0.5M NaCl
5mM EDTA
1% Triton X-100
0.1% Na Deoxycholate

LiCl Wash Buffer

10mM Tris-HCl, pH 8
250mM LiCl
0.5% NP-40
0.5% Na Deoxycholate
5mM EDTA