ChEC in budding yeast

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Modified from Schmid et al, *Mol Cell*, 2004

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1. Grow 50 mL cells to OD_{600} = 0.5-0.7 in a 300 mL flask

2. Harvest cells in a 50 mL conical tube at 1,500 x *g* for 1 min

3. Wash cells 3 x 1 mL Buffer A. Transfer cells to a 1.5 mL tube with the first wash and spin as above between washes

4. Permeabilize cells. Resuspend pellet in 600 µL Buffer A + 0.1% digitonin (add 30 µL 2% digitonin in DMSO to 570 µL Buffer A) and incubate at 30°C for 5 min. Remove 100 µL as zero timepoint prior to step 5

5. Add 1.1 µL 1 M $CaCl_2$ (~2 mM final), mix, and incubate at 30°C

6. At each desired time point, remove a 100 µL aliquot of the digestion to a tube containing 90 µL stop solution and 10 µL 10% SDS and vortex to mix

7. Add 80 µg proteinase K. Digest protein at 55°C for 30 min

8. Extract nucleic acids. Add 200 µL phenol/chloroform/isoamyl alcohol, mix well, and spin at max. speed for 5 min in a microfuge. Transfer aqueous phases to new tubes, add 2 µL (30 µg) Glycoblue and 500 µL 100% ethanol, mix, and precipitate at -80^{o}C for ≥30 min

9. Spin at max speed and 4^{o}C for 10 min

10. Wash pellets with 1 mL 100% ethanol and aspirate ethanol

11. Briefly air-dry pellets and resuspend in 30 µL Qiagen EB or 0.1X TE buffer, pH 8.0

12. Add 10 µg RNase A and incubate at 37°C for 20 min. Run 5 µL RNase-treated DNA on a 1.5% agarose gel to check DNA fragmentation if desired

13. Enrich small DNA fragments. Add 75 µL Ampure XP beads to RNase-treated DNA and pipet up and down 10X to mix. Incubate 5 min at RT and collect beads on magnetic rack. Reserve supernatant (unbound fraction, ~105 µL, containing smaller DNA fragments) to a new tube containing 96 µL Qiagen EB or 0.1X TE buffer and 4 µL 5 M NaCl

14. Extract DNA from the unbound fraction. Add 200 µL phenol/chloroform/isoamyl alcohol, mix well, and spin at max. speed for 5 min in a microfuge. Transfer aqueous phases to new tubes, add 2 µL (30 µg) Glycoblue and 500 µL 100% ethanol, mix, and precipitate at -80^{o}C for ≥30 min

15. Spin at max speed and 4^{o}C for 10 min

16. Wash pellets with 1 mL 100% ethanol and remove ethanol with vacuum

17. Briefly air-dry pellets and resuspend in 25 µL 0.1X TE buffer, pH 8.0 or Qiagen EB (10 mM Tris pH 8)

Solutions

2% digitonin

Add 20 mg high-purity digitonin to 1 mL DMSO. Vortex ~30 sec to dissolve and store 100 µL aliquots at -20°C.

Buffer A (100 mL)

1.5 mL 1 M Tris pH 7.5	-	15 mM Tris
8 mL 1 M KCl	-	80 mM KCl
50 µL 0.2 M EGTA	-	0.1 mM EGTA

For 10 mL Buffer A (2 samples), add the following before use:
10 µL 10 mg/mL LPC (10 µg/mL final) or 1 cOmplete EDTA-free mini tablet
100 µL 100 mM PMSF (1 mM final)
10 µL 200 mM spermine (0.2 mM final)
5 µL 1 M spermidine (0.5 mM final)

2X Stop buffer (100 mL)

8 mL 5 M NaCl	-	400 mM NaCl
4 mL 0.5 M EDTA	-	20 mM EDTA
2 mL 0.2 M EGTA	-	4 mM EGTA