ChEC in budding yeast
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Modified from Schmid et al, Mol Cell, 2004
Size selection step modified 1-31-17 by GEZ

Setup

-Prior to harvesting cells, prepare Buffer A as described in Solutions

-For each strain, prepare 6 microfuge tubes for collecting time points. To each tube, add 90 µL stop buffer and 10 µL 10% SDS

Protocol

1. The day before the experiment, inoculate 3 mL YPD or SC medium with a single colony. Grow overnight at 30°C

2. In the morning, dilute the overnight culture to OD<sub>600</sub> = 0.2-0.3 in 50 mL YPD or SC medium in a 300 mL flask. Grow 50 mL culture until OD<sub>600</sub> = 0.5-0.7

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

4. 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

5. 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 6

6. Add 1.1 µL 1 M CaCl<sub>2</sub> (~2 mM final), mix, and incubate at 30°C

7. 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

8. Add 4 µL 20 mg/mL proteinase K. Digest protein at 55°C for 20 min

9. 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 (10 µg) linear acrylamide and 500 µL 100% ethanol, mix, and precipitate at -80°C for ≥30 min

10. Spin at max speed and 4°C for 10 min

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

12. Briefly air-dry pellets and resuspend in 29 µL Qiagen EB or comparable buffer + 1 µL 10 mg/mL RNase A and incubate at 37°C for 10 min

13. Run 5 µL RNase-treated DNA on a 1.5% agarose gel to check DNA fragmentation if desired

14. Dilute RNase-treated DNA to 200 µL with Qiagen EB or comparable buffer
15. Add 160 µL Ampure beads (0.8:1 beads:sample ratio) and pipet up and down 10X to mix. Incubate 5 min at RT

16. Collect beads on magnetic rack for 2 min

17. Remove the supernatant (~400 µL) to a new tube containing 16 µL 5 M NaCl (~200 mM final)

18. Extract DNA from the unbound fraction. Add 400 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 (10 µg) linear acrylamide and 1 mL 100% ethanol, mix, and precipitate at -80°C for ≥30 min

19. Spin at max speed and 4°C for 10 min

20. Wash pellets with 1 mL 70% ethanol and remove ethanol with vacuum

21. Briefly air-dry pellets and resuspend in 25 µL Qiagen EB or comparable buffer. Recovered DNA can be quantified by Qubit and the size distribution analyzed via TapeStation using a high-sensitivity tape

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**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
- 8 mL 1 M KCl
- 50 µL 0.2 M 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
- 4 mL 0.5 M EDTA
- 2 mL 0.2 M EGTA

- 400 mM NaCl
- 20 mM EDTA
- 4 mM EGTA