Chromatin Shearing in yeast cells using Bioruptor™

Diagenode - June 2008

50 ml of yeast cells (O.D.600 = 0.7):

1. Fixation: Incubate cells with 1.25 ml of 37% Formaldehyde during 20 min at 30°C
2. To stop fixation incubate with 6.25 ml of 3M Glycine for 20 min at 30°C
3. Collect 50ml of fixed cells
4. Wash 2 times with TBS (1x)
5. Resuspend the pellet in 1ml FA Lysis buffer + 1mM PMSF
6. Add 1ml of glass beads
7. Vortex for 6 times for 3 min (with 3 min breaks on ice between each run)
8. Collect the lysate (should be 1ml)
9. Sonicate the lysate with Bioruptor™
   Total time: 25min
   Cycles: 30sec ON/30sec OFF
   Power: High
   Tubes: TPX 15ml (Diagenode)
   Min vol. 1ml/ max vol. 2ml
10. Spin the sonicated sample for 30min at 16.000g at 4°C
11. Collect the supernatant for subsequent Immunoprecipiation

FA Lysis buffer:

- 50mM Hepes-KOH (pH 7,5)
- 150mM NaCl
- 1mM EDTA
- 1% Triton-X 100
- 0,1% Sodium deoxycholate
- 0,1% SDS

Ref: Chromatin_Shearing_Bioruptor_0609V3

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