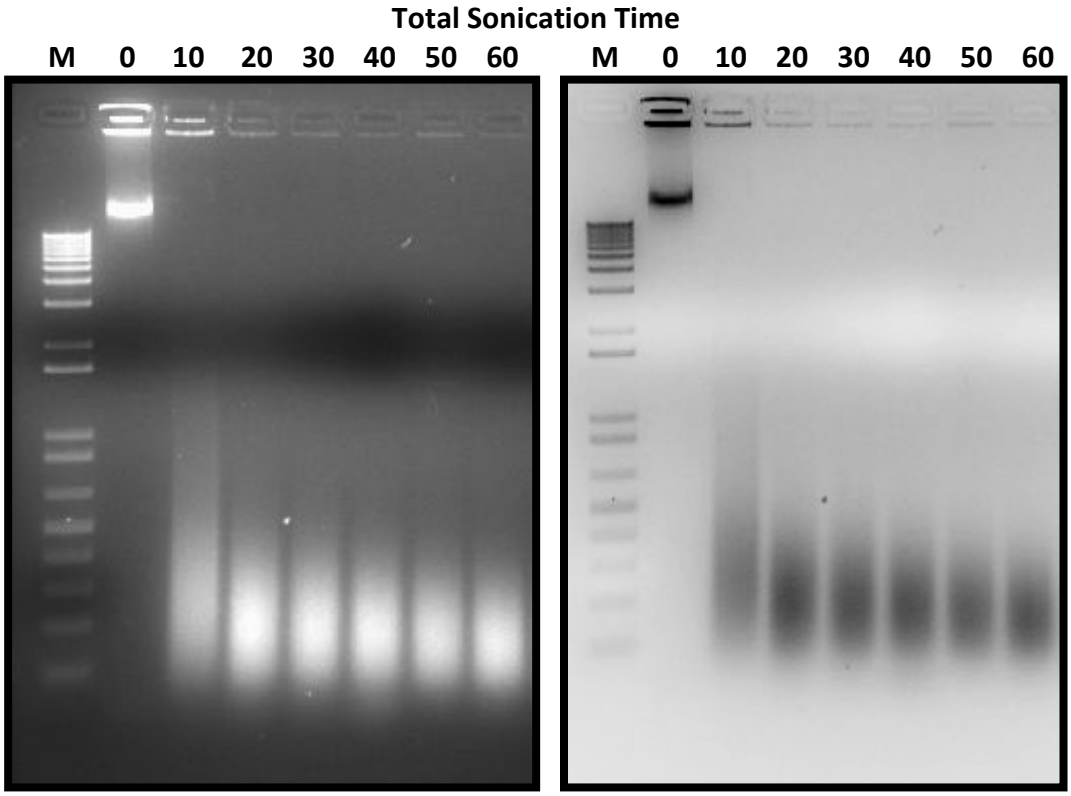
Yeast Chromatin Prep

Example protocols and results are based on customer feedback.



No clarification of sample *Zymolyase treated cells*

Protocol

Cell Type: Wild type *S. pombe* cells grown to an OD of 1.3-1.5 in YEA
Total Sample Volume: 250-300ul
Fixation Time: 1% Formaldehyde, 15 min
Sonicator Amplitude Setting: 70%
Sonication Pulse Rate: 20 seconds On, 40 seconds Off
Total Sonication On Time: 12 minutes
Sample Process Temperature: 4°C

Customer Notes

Use Zymolase for lysis

- Cross-link 50 - 200ml of S. pombe cells grown to a density of OD600 @ 1.3-1.5 with 1% formaldehyde for 15 minutes
- Quench cross-linking, wash and spin cells down to 0.16-0.18g per pellet
- Resuspend cells in 1.0ml of room temperature PEMS buffer and add 20-40ul of Zymolase per pellet
- Incubate at 32^0 for one hour in an end-over-end nutator
- Wash cells 2X in PEMS buffer
- Resuspend cells in 250ul of lysis buffer and transfer to the 0.5ml PCR tube (Brand Tech #781312)
- Sonication (settings listed above)

Zymolase concentration may require standardization for each new batch. Manufacturers report batch to batch variability for enzyme activity.

- DNA samples shown are prior to centrifugation and clearing of the lysate, the entire genome is represented.
- We use 10X less SDS in our lysis buffer, so 0.1% SDS.

Lower SDS concentration is hugely beneficial to us for 3 reasons:

1. We don't have to decrease SDS concentration afterwards, so our protein concentration remains high for the IP part of ChIP (especially relevant for rare proteins)
2. Some proteins' structures do not tolerate such high SDS levels, unfold and don't refold properly. This could affect their interactions with other proteins or DNA, thus affecting their localization. Also note that for the proteins that miss-fold and don't refold following lowering the SDS concentration, the antibody no longer recognizes the protein, even once the SDS level is reduced to the normal 0.1%.
3. High SDS concentration (1%) interferes with antibody antigen interaction, and thus must be lowered for the IP part of ChIP experiments.