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Chromatin analysis in yeast using NP-40 permeabilised sphaeroplasts

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Yeast (*Saccharomyces cerevisiae*), is a focus for research into the modulation of transcription by the components of chromatin (1, 2). We have been working on a yeast transcription factor which can function to exclude nucleosomes from the DNA associated with its binding motif (3). Previously the analysis of nucleosome position on chromosomal DNA in yeast has involved the preparation of nuclei followed by digestion with probe enzymes such as micrococcal nuclease or restriction enzymes (2). Such procedures typically take about 3 hours to complete and involve subjecting the nuclei to treatments that may be damaging to chromatin such as hypotonic cell lysis. In order to speed up and simplify such analyses, we developed the procedure reported here. In essence osmotically buffered yeast sphaeroplasts are gently permeabalised using trace amounts of the detergent NP-40. These permeable sphaeroplasts are briefly incubated in the presence of high concentrations of probe nucleases and the digested DNA then purified for indirect end label analysis. A similar approach has been adopted by Stewart *et al.* (4) for nuclease digestion of chicken fibroblast chromatin.

Yeast are grown overnight to a density of 1×10^7 cells/ml. 6×10^8 cells are harvested by centrifugation at 3 K rpm for 5 min, and washed in 20 ml 1 M Sorbitol. The cells are then spun again and resuspended in 10 ml 1 M Sorbitol/2% Glusulase (Du Pont) followed by gentle shaking at 30°C until the cells just become sphaeroplasted (see below). The sphaeroplasts are then spun at 2.5 K rpm for 5 min and washed in 20 ml 1 M Sorbitol. This is repeated to dilute out the Glusulase. The cells are resuspended each time with a large bore pipette or by gentle agitation. After a final spin, the cells are drained well and resuspended in 600 μl of cold Buffer A (1 M Sorbitol, 50 mM NaCl, 10 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM β-Mercaptoethanol and 0.5 mM Spermidine). A 1 ml micropipette tip is used at this stage to ensure that the sphaeroplasts are not clumped. Care however should be taken not to lyse the cells.

Six 100 μl aliquots (each containing 1×10^8 cells) are transferred to 1.5 ml Eppendorf tubes containing probe nuclease in 100 μl of cold Buffer A + 0.15% NP-40. This makes a 200 μl reaction with NP-40 at a final concentration of 0.075% which we find to be optimal for our yeast strains. For micrococcal nuclease digestion we use the enzyme (Pharmacia LKB) at a final concentration of 30–300 u/ml, see Figure 1. The reactions are mixed gently and incubated at 37°C for 3–5 minutes. The solution should remain turbid throughout the incubation. If it goes clear then the cells have lysed and a shorter sphaeroplasting or less NP-40 should be used. Using the conditions described above our sphaeroplasts remain intact (monitored by microscopy) for at least 20 minutes. The nuclease digestion is stopped by adding 20 μl of 250 mM EDTA/5% SDS followed by rapid mixing. The solution should clear indicating lysis of the cells. If not, a longer sphaeroplasting is needed. We therefore recommend that a set of pilot experiments be carried out to determine the optimal sphaeroplasting conditions. The solution is then treated with Proteinase K and RNaseA and extracted three times with Phenol/Chloroform (1:1). DNA is precipitated with 40 μl 5 M NH_4Ac and 200 μl Isopropanol and resuspended in 20 μl TE. Figure 1 shows a typical micrococcal nuclease digestion course using 2 μl of the digested DNA.

The DNA is digested to completion with an appropriate restriction enzyme, run on a 1–1.5% agarose gel and blotted for indirect end label analysis. DNA isolated from an aliquot of sphaeroplasts untreated with MNase should be used for a naked DNA control. We digest the DNA in 500 ul of Buffer A + 0.075% NP-40 with 30 u/ml MNase for 1 min at 37°C. The reaction is stopped as before and DNA repurified by a single Phenol/Chloroform extraction and precipitation.

Figure 2 shows a typical indirect end label analysis of the *MET25* gene. This technique gives identical nuclease digestion patterns to those obtained from nuclei (3) and the protocol can be completed in 40–50 minutes with the cells being in the digestion buffer for just 5 minutes. By sphaeroplasting with higher concentrations of Glusulase and washing the cells by filtration, the analysis (from growing cells to addition of Stop solution) can be performed in 20 minutes allowing 'snap-shots' to be taken of chromatin changes (N.A.K. unpublished data).

As well as micrococcal nuclease, this digestion buffer works with DNaseI and the restriction enzymes BamHI, EcoRI, DraI, XmnI and HaeII (data not shown). The presence of NP-40 in the buffer does not appear to affect chromatin structure as extended incubation of the cells at 37°C for up to an hour before addition of probe enzyme gives identical results on indirect end label analysis (LEB unpublished data). We have also used this technique successfully with *Schizosaccharomyces pombe* using NovoZym 234 (Novo BioLabs) as a sphaeroplasting enzyme. It should therefore be possible to extend the use of this technique to other yeasts and fungi that will grow in liquid culture.

REFERENCES

1. Winston,F. and Carlson,M. (1992) *Trends Genet.* **8**, 387–391.
2. Fascher,K.-D., Schmitz,J. and Hörz,W. (1990) *EMBO J.* **9**, 2523–2528.
3. Mellor,J., Jiang,W., Funk,M., Rathjen,J., Barnes,C.A., Hinz,T., Hegemann,J.H. and Philippsen,P. (1990) *EMBO J.* **9**, 4017–4026.
4. Stewart,A.F., Reik,A. and Schutz,G. (1991) *Nucleic Acids Res.* **19**. 3157.

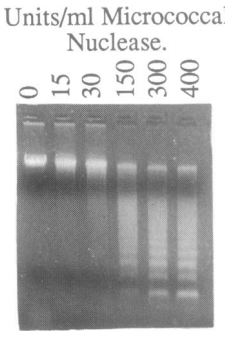

Figure 1. 1% agarose gel showing *Saccharomyces cerevisiae* DNA digested with an increasing concentration of micrococcal nuclease in NP-40 permeabilised sphearoplasts.

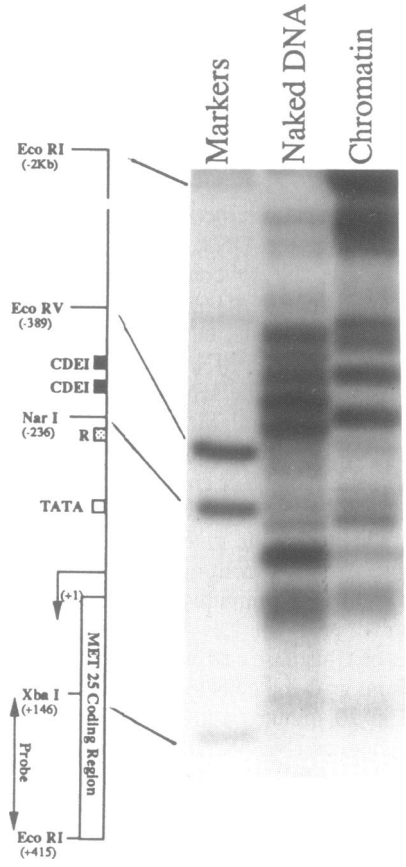

Figure 2. Southern blot indirect-end-label analysis of the *Saccharomyces cerevisiae* (strain DBY745) *MET25* gene 5′ region. Chromatin and naked DNA digests were performed as above. DNA was digested to completion with EcoRI and separated on a 1.5% agarose gel. Markers were prepared by digesting genomic DNA with EcoRV, NarI and XbaI followed by EcoRI. The blot was probed with the indirect-end-label shown in the diagram. CDEI, R and TATA are regulatory elements in the *MET25* 5′ region.