# Mapping nucleosome positions in S. cerevisiae by quantitative PCR

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# Abstract

# Introduction

This protocol describes a method of assaying nucleosome positions in _S. cerevisiae_ by nuclease protection and quantitative PCR \(Q-PCR). Briefly, yeast cells are spheroplasted, treated with micrococcal nuclease, and single-nucleosome length DNA is gel-isolated and quantitated by Q-PCR using overlapping primer pairs tiling the length of a region of interest. In the accompanying _Nature_ paper, this technique is used to map the promoter nucleosome positions of several genes in the phosphate starvation response \(_PHO_) pathway in order to determine the accessibility of Pho4 \(transcription factor) binding sites. While this protocol has been used extensively with _S. cerevisiae_, it should, in principle, be readily adaptable for use in other yeast species.

# Reagents

**1 M sorbitol** - At room temperature. **1 M sorbitol** - Chilled to 4°C. **Tris-EDTA \(TE)** - 10 mM Tris, pH 7.5; 1 mM EDTA. **β-ME buffer \(11 ml)** - 20 mM EDTA; 0.7 M β-ME. Prepare fresh at room temperature before each experiment. **Lyticase buffer \(11 ml)** - 1 M sorbitol; 50 mM Tris, pH 7.8; 5 mM β-ME. Prepare fresh at room temperature before each experiment. **Buffer A \(4 ml)** - 1 M sorbitol; 10 mM Tris, pH 7.5; 50 mM NaCl; 5 mM $MgCl_2$; 1 mM $CaCl_2$; 1 mM β-ME. Prepare fresh on ice for each experiment. **Buffer B \(1.25 ml)** - Buffer A with 0.15% NP-40. Prepare fresh on ice for each experiment. **Lyticase** \(from _Arthrobacter luteus_; catalog# L2524, Sigma) - Dilute powder to make a 20,000 U/ml stock in $ddH_2O$. Store at -20°C. **Micrococcal nuclease \(MNase)** \(catalog# N5386, Sigma) - Dilute powder to make a 2 U/µl stock in 10 mM HEPES, pH 7.5; 100 mM NaCl; 1 mM $CaCl_2$; 50% glycerol. Store at -20°C. **MNase stop buffer \(0.5 ml)** - 5% SDS; 250 mM EDTA. Store at room temperature. Heat to 37°C or higher to dissolve SDS before using. **Proteinase K \(0.5 ml)** - 20 µg/µl in $ddH_2O$. Store at -20°C. **RNase A \(1 ml)** - 0.1 µg/µl in TE. Store at -20°C. **Phenol, buffer saturated solution, pH 7.5-7.9** **Chloroform** **4 M NaCl** **Ethanol \(EtOH), 100%** **Ethanol \(EtOH), 70%** **Eppendorf Phase Lock Gel Heavy, 2 ml** \(catalog# 0032-005.152, Eppendorf) **QIAquick Gel Extraction Kit** \(catalog# 28704, QIAGEN) **10x Taq buffer** - 100 mM Tris, pH 8.3; 500 mM KCl; 20 mM $MgCl_2$ **dNTP mix** - 8 mM total dNTPs \(i.e., 2 mM each d\(A|C|G|T)TP) **SYBR Green I** \(catalog# S-7567, Invitrogen) - Dilute 10,000x concentrate to a 100x stock using DMSO. Store protected from light at -20°C. **Taq polymerase** **Q-PCR primer sets** - Design primer pairs \(e.g., using "Primer3":http://frodo.wi.mit.edu from the Whitehead Institute) to produce overlapping ==$_{\text{== 100 bp products tiling a particular region of the genome, centered every 30-50 bp, and with T}}$m~'s as close as possible to 60°C. **Yeast genomic DNA** - Prepare from the same yeast strain where nucleosome positions will be assayed. Used for Q-PCR quantitation standards.

# Equipment

**Stratagene Mx3000P** or equivalent real-time fluorescence quantitative PCR system

# Procedure

**Spheroplasting** 1. Grow ==$_{\text{== 250 ml of yeast to OD}}$600 $_{\text{of ==}}$== 0.5 \(mid-log phase) under your condition of interest. 2. Pellet cells at 3800 g, 5 min at room temperature. 3. Decant pellet and resuspend in 10 ml β-ME buffer. Incubate at 30°C for 30 min. Shake gently every 10 min to prevent cells from settling. 4. Transfer sample to 15 ml conical tube, and pellet at 2000 g, 5 min at room temperature. 5. Wash pellet: resuspend in 15 ml room temperature 1 M sorbitol. Pellet at 2000 g, 5 min at room temperature, and decant. 6. Resuspend pellet in 9.5 ml lyticase buffer. Add 0.5 ml lyticase for a final concentration of 1000 U/ml. Incubate at 30°C for 30 min* on roller drum, or invert tube gently every 10 min to prevent cells from settling. * Incubation time is strain specific\! During a pilot run, determine an appropriate incubation time by monitoring spheroplasts lyse in water under a light microscope. 7. Pellet spheroplasts at 2000 g, 5 min at room temperature. Pipet off supernatant \(spheroplasts are fragile and do not pack as tightly as yeast). 8. Wash spheroplasts: resuspend gently in 15 ml 4°C 1 M sorbitol. Pellet, and pipet off supernatant. **Micrococcal nuclease \(MNase) titration** 9. Assuming a 4 point MNase titration series, resuspend spheroplasts in 0.8 ml buffer A. On ice, aliquot 0.2 ml spheroplasts to 4 microfuges tubes labeled "2 U", "1 U", "0.5 U", and "0 U". 10. Prepare 3 two-fold serial dilutions of MNase enzyme in buffer B on ice. To tube labeled "2 U MNase", add 2.1 µl of MNase stock \(2 U/µl) to 408 µl buffer B. To tube labeled "1 U MNase", add 205 µl of "2 U MNase" to 205 µl buffer B, etc. 11. On ice, add 200 µl of the "2 U MNase", "1 U MNase", and "0.5 U MNase" enzyme dilutions to the correspondingly labeled 200 µl aliquots of spheroplasts. Add 200 µl of buffer B to the "0 U" spheroplast aliquot. 12. Incubate at 37°C for 20 min. 13. Stop digestions by adding 40 µl of MNase stop buffer. Vortex well. 14. Add 12 µl of proteinase K to each sample. Vortex well, and incubate at 37°C for 1-5 hrs. **Purify mono-nucleosome length DNA** 15. Add 20 µl of 4 M NaCl and 1 volume \(450 µl) of phenol to all samples. Vortex thoroughly \(==$_{\text{== 20 sec).}}$ Add 1 volume \(450 µl) of chloroform to all samples. Vortex thoroughly \(==$_{\text{==}}$== 20 sec). 16. Spin 13-16,000 g for 10 min at room temperature to separate phases. 17. Transfer aqueous phase to pre-spun Eppendorf Phase Lock tubes. Add 1 volume of 1:1 phenol:chloroform \(250 µl of phenol, 250 µl of chloroform). Invert mixture several times to mix. Spin 13-16,000 g for 5 min at room temperature. 18. Add 1 volume \(450 µl) of chloroform. Mix and spin 13-16,000 g for 5 min at room temperature. 19. Transfer aqueous phase to new microfuge tubes. Add 1 ml of 100% EtOH. Mix well and precipitate DNA at -20°C for at least 30 min. 20. Spin samples 13-16,000 g for 20 min at room temperature. Aspirate off supernatant. 21. Resuspend DNA pellets in 200 µl of RNase A. Incubate at 37°C for 1-2 hrs. 22. Add 10 µl of 4 M NaCl and 0.5 ml of 100% EtOH. Precipitate DNA at -20°C for at least 30 min. 23. Spin samples 13-16,000 g for 20 min at room temperature. Aspirate off supernatant. 24. Wash pellets once with 1 ml of 70% EtOH. Spin and aspirate off EtOH. 25. Thoroughly air dry DNA pellets, and resuspend in 50 µl TE. 26. Run all samples \(3 µl each) on a 2% agarose gel. You should see ladders showing DNA fragment sizes corresponding to integral multiples of ==~== 150 bp. Ideally, you have will at least one sample enriched in 150 bp / mono-nucleosome length fragments that does not show any sign of over-digestion. Since the extent of digestion is dependent on such factors such as the amount of cells harvested, efficiency of

spheroplasting, effective activity of your MNase stock, etc., you may need to revise steps 9-12 and consider a higher-fold dilution series of MNase, more titration points, and/or varying the digestion time at 37°C. 27. Choose one or two samples with the greatest fraction of mono-nucleosome length fragments, and run 20-25 µl on a 1.5% agarose gel. Excise the ==~== 150 bp band, and extract DNA using the QIAquick Gel Extraction Kit or equivalent method. **Tiling Q-PCR** 28. Combine forward and reverse primer pairs \(5 µM each) to make 10 µM working dilutions. 29. Prepare a 3 or 4 point ten-fold serial dilution of yeast genomic DNA for use as Q-PCR quantitation standards. 30. To prepare your Q-PCR template, start with a 1:10 dilution of the gel-purified mono-nucleosome length DNA. Depending on the resulting quantitated values, you may need to adjust the amount of template DNA used per reaction. 31. Prepare a working solution of 7.5x SYBR green by diluting the 100x stock with $H_2O$. SYBR green is unstable in $H_2O$ and should be diluted fresh for each experiment. 32. Design the layout of your Q-PCR 96 well plate or strips, and determine the amounts necessary to assemble the following per-well Q-PCR reaction: 1 µl mono-nucleosome DNA, diluted 1:10; 5 µl 10x Taq buffer; 5 µl 2 mM dNTPs; 0.5 µl 7.5x SYBR green; 0.5 µl Taq; 5 µl 10 µM primer mix; 33 µl $H_2O$. 33. Use the following thermal cycling program: 1 cycle of \{94°C for 5 min}; 40 cycles of \{95°C for 30 sec, 57°C for 30 sec, 72°C for 1 min, 75°C for 10 sec, plate read}; 1 cycle of \{72°C for 5 min}. Include a 70-95°C melting curve at the end of the Q-PCR program, reading all points or every 0.2°C. 34. Be sure all quantitated nucleosome DNA values fall within the interpolated linear range defined by the genomic DNA serial dilution; if not, adjust the amount of template DNA as appropriate. Check to make sure all reactions are yielding homogeneous PCR products \(i.e., single peaks in the melting curve); if not, it may be necessary to design alternate primer pairs.

# Timing

2-3 days

# Troubleshooting

If you do not observe significant differences in nucleosome profiles under inducing and repressing conditions, it is possible that your promoters are becoming remodeled during the steps preceding MNase treatment. To test this possibility, you can try replicating your condition of interest in the β-ME and lyticase buffers, and the two 1 M sorbitol washes. For example, add 10 mM $KH_2PO_4$ if you are studying promoter chromatin structure under phosphate-repressing conditions and your yeast cultures were originally grown under these conditions.

# Anticipated Results

See Fig. 2a, b in the accompanying paper.

# References

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