S. cerevisiae Δpho84 complementation: growth curves

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Abstract

Complementation of *a S. cerevisiae Δpho84* strain with putative phosphate transporter(s)

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Protocol

Step 1.

Synthesise ORF, codon-optimised for expression in *Saccharomyces cerevisiae*, with a C-terminal His_6 tag and clone into p416 GPD under the control of the constitutive GPD promoter (pXXX).

Step 2.

Transform *S. cerevisiae* strain BY2959 (*pho84*::HIS3) with either p416 GPD or pXXX

Step 3.

Transform BY5676 with p416 GPD (this will be your positive control).

Step 4.

Grow cells at 30°C, 200 rpm shaking for 16 hours in Sc-ura medium (0.79% yeast nitrogen base without (w/o) amino acids (Formedium), 700 mg L^{-1} complete supplement mix - ura (Formedium), 2% glucose).

N.B. Grow each strain in triplicate (3 independently obtained transformants).

Step 5.

Centrifuge cells at 1,810 x *g* for 3 minutes, and washed twice with low-phosphate (LPi) medium*, pH 4.5.

*Rubin, G.M., *Three forms of the 5.8-S ribosomal RNA species in Saccharomyces cerevisiae.* Eur J Biochem, 1974. **41**(1): p. 197-202.

Step 6.

Resuspend cells in LPi medium and dilute to an OD_{600} of 0.1.

Step 7.

Inoculate 100 µl into a 96 well plate.

Step 8.

Grow cells at 30°C in a Fluostar Omega Lite microplate reader (BMG Labtech Ltd.). Assess OD_{595} at 10-minute intervals, using continuous double-orbital shaking (200 rpm) between reads.