S. cerevisiae Δpho84 complementation: metal toxicity

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Abstract

Testing for metal toxicity as a result of addition of a putative phosphate transporter to a *S. cerevisiae Δpho84* strain

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Protocol

Step 1.

Grow 10 ml *S. cerevisiae* strains BY5676 p416 GPD, BY2959 p416 GPD and BY2959 pXXX [p416 GPD + gene of interest] in Sc-ura medium for 16 hours at 30°C, 200 rpm shaking. Grow each strain in triplicate (3 independently obtained transformants).

Step 2.

Centrifuge cells at 1,810 x *g* for 3 minutes, wash twice and re-suspend in water.

Step 3.

Dilute cells to an OD_{600} of 1.0 and prepare a dilution series (down to 10^{-4}).

Step 4.

Spot 10 µl of each dilution onto Sc-ura plates containing each metal to be assayed. Use a Sc-ura plate with no added metal as a positive control to ensure that all strains grow equally well

Metals to try (try concentrations either side of these suggested starting points):

Metal	Suggested starting concentration
Potassium chromate	300 µM
Cobalt chloride	1 mM
Zinc chloride	10 mM
Sodium arsenate	3 mM
Manganese chloride	10 mM
Copper chloride	1 mM

Step 5.

Incubate plates for 72 hours at 30°C before imaging on a G:BOX imaging system (Syngene) to identify compounds on which toxicity was restored by the addition of pXXX.

Use these results to optimise compound concentrations for a second run.