

## Improved selection for inositol-utilization following transformation of *Neurospora crassa*

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# Improved selection for inositol-utilization following transformation of *Neurospora crassa*

## **Abstract**

Plasmids based on the cloned inositol gene of *Neurospora crassa* are potentially very useful as a transformation marker, or for techniques like insertional mutagenesis.

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**Improved selection for inositol-utilization following transformation of *Neurospora crassa***Robert L. Metzenberg<sup>1,2</sup><sup>1</sup>Department of Biological Sciences, Stanford University, Stanford, California 94305-5020; <sup>2</sup>present address, Dept. of Chemistry and Biochemistry, University of California, Los Angeles CA 90095-1569

Plasmids based on the cloned inositol gene of *Neurospora crassa* are potentially very useful as a transformation marker, or for techniques like insertional mutagenesis. Transformation is generally done on spheroplasts stabilized with sorbitol, or by electroporation of conidia, also suspended in sorbitol. For transformation to inositol independence, however, this gives an unacceptable background of non-transformants that grow on the nominally inositol-free medium. It appears that sorbitol always contains a trace of inositol that cannot be completely removed by recrystallization. It seems possible that sorbitol, which differs from inositol only by a pair of hydrogens, spontaneously generates the latter by air oxidation. Substituting  $\alpha$ -methyl glucoside for the sorbitol on an equimolar basis can circumvent this problem.  $\alpha$ -Methyl glucoside works well as an osmoticum, and gives clean backgrounds in transformation of *inl* mutants to inositol independence.