

The effect of repeated freeze-thaw cycles on cryopreserved *Neurospora crassa* samples.

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Abstract

To better characterize handling parameters for the arrayed mutants prepared for the *Neurospora* functional genomics program, we have put 7 day old conidia from strain FGSC 2489 through a series of cycles of freezing at -80 C in 25% glycerol and 3.5% reconstituted non-fat dry milk.

Brief Notes**A simple way to make a dilution series**

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Fungal Genetics Newsletter 53:37

Often one needs to determine a suitable concentration of a previously untested nutrient or inhibitor to use in subsequent experiments. A series with relative concentrations of 100, 30, 10, 3, 1... gives a good estimate. However, making such a series by diluting from one tube to its neighbor requires different amounts of diluent for different tubes, as well as resetting the pipetting device at each transfer -- a tedious and mistake-prone process. Alternatively, one can make a series of three-fold dilutions. This is easy, but gives daunting relative numerical values for concentration: 100, 33.3, 11.1, 3.70, 1.23,.... I find it useful to make successive dilutions of 3.16-fold (the square root of 10) so that relative concentrations are 100, 31.6, 10, 3.16, 1,.... I prepare 3.16 ml. of the most concentrated solution, set up successive tubes with 2.16 ml. of diluent, and pipet 1.0 ml. from one tube to the next. I discard 1 ml. from the penultimate tube, saving the final tube for the zero-concentration control.

The effect of repeated freeze-thaw cycles on cryopreserved *Neurospora crassa* samples.

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To better characterize handling parameters for the arrayed mutants prepared for the *Neurospora* functional genomics program, we have put 7 day old conidia from strain FGSC 2489 through a series of cycles of freezing at -80 C in 25% glycerol and 3.5% reconstituted non-fat dry milk. This is prepared by mixing autoclaved 50% glycerol with 7% milk that had been sterilized in a pressure cooker for 20 minutes. Samples were frozen in 2 ml screw-cap vials without control of freezing rate. Thawing was carried out on the bench. We diluted spores in sterile water and plated them on agar-solidified medium containing 1X Vogels salts and 1X FGS. Plates were incubated at 30 C until countable colonies were visible. Two replicates beginning with independent conidiating cultures were carried out and the results are presented in Table 1.

Table 1

Freeze- thaw cycles	Viability of conidia ¹				
	1	2	3	4	5
First Replicate	ND ²	2.7 X 10 ⁻¹	2.0 X 10 ⁻¹	1.1 X 10 ⁻¹	7.0 X 10 ⁻²
Second Replicate	8.3 X 10 ⁻¹	2.3 X 10 ⁻¹	3.8 X 10 ⁻¹	1.8 X 10 ⁻¹	5.0 X 10 ⁻²

¹ Viability is calculated as the ratio of the viable conidia to the plated conidia.

² Not Determined

Freeze thaw cycles do decrease the recovery of *Neurospora crassa* conidia and the decrease is linked to the number of freeze-thaw cycles. For practical uses, the ability to recover a strain following cryopreservation is robust when one begins with a significant number of conidia. In the first replicate, we started with 5 X 10⁶ conidia per ml and in the second we started with 6.5 X 10⁶ conidia per ml.

Because *Neurospora* conidia are hydrophobic they have a tendency to form clumps. This may account for some of the variability in numbers (for example the apparent increase in viability between cycles 2 and 3 in the second replicate). This is not a practical problem when one begins with great numbers of conidia and the goal is to maintain viability.

The ability to recover mutant strains may be reduced depending on the specific characteristics of the strain. Nevertheless, the ability to thaw and re-freeze strains arrayed as glycerol stocks demonstrates the usefulness of this resource.