

The use of Ignite (Basta;glufosinate;phosphinothricin) to select transformants of bar-containing plasmids in *Neurospora crassa*

Martin L. Pall - Department of Genetics and Cell Biology and Department of Biochemistry/Biophysics, Washington State University, Pullman, WA 99164-4234 The bacterial Basta-resistance (bar) gene has been previously adapted for use as a fungal selectable marker by Avalos et al. (1989 *Curr. Genet.* 16:369-372) and by Straubinger et al. (1992 *Fungal Genet. Newsl.* 39:82-83). Both bialaphos and Basta (Ignite) have been developed for use as herbicides and have only been available as free samples from the chemical companies that have been involved in this development. In *Neurospora crassa*, transformants for the bar gene have been isolated by selecting for resistance to bialaphos, a peptide derivative of phosphinothricin, the active ingredient in Ignite/Basta. However, it has been difficult for many investigators to obtain samples of bialaphos for such studies, limiting the use of this selectable marker. Methods are described here for use of Ignite (Basta; phosphinothricin; glufosinate) to select for bar transformants in *Neurospora*.

Initial studies showed that Ignite was relatively nontoxic to *Neurospora* in standard Vogel's medium N but was considerably more toxic in nitrogen-free medium N supplemented with either nitrate or proline as sole nitrogen source. Ignite at a concentration of 200 micrograms/ml in nitrogen-free medium N containing 0.5% proline and 2% sucrose completely blocked growth where it only partially blocked growth in similar medium containing nitrate and had relatively little effect on growth on normal medium N, which contains ammonium nitrate as nitrogen source. The Ignite is known to act as an inhibitor of glutamine synthetase. It is not clear whether the greater inhibition in proline medium is due to improved transport of Ignite by the proline-grown cultures or whether they are otherwise more sensitive to its action.

Selection for bar gene transformants can be performed as follows: Spheroplasts of germinated conidia of *Neurospora* were prepared and transformed as described previously by Vollmer and Yanofsky (1986 *Proc. Natl. Acad. Sci, USA* 83:4869-4873) with the following changes: The transformed spheroplasts were plated using bottom agar containing nitrogen-free medium N plus 0.5% proline in place of medium N. It also contained 200 to 300 micrograms/ml of Ignite and the usual concentrations of sorbose and other sugars. Similarly, the top agar used contained nitrogen-free medium N plus 0.5% proline and 0.8% agar in place of the usual medium N top agar. Under these conditions, numerous resistant colonies were obtained when using either pJA4 DNA (see Avalos et al. op. cit.) or pBARGPE1, pBARGEM7-2 or related plasmid DNAs, constructed in this laboratory. It should be noted that it is not necessary to germinate the conidia in proline-containing medium in order to obtain efficient transformation.

In a number of experiments, a few colonies were seen on Ignite selection even when no transforming DNA was used. This appears to be due to a significant level of spontaneous mutation to resistance. It is useful, consequently, to use conidia derived from recently cloned cultures in order to minimize this background and to run no-DNA controls with each transformation experiment in order to determine if such background is a problem in a specific experiment.

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