Dear Aspergillus community:

With the shutting down of Interspex, finding usable protoplasting enzymes has become even more problematic. We have recently found that the commercially available winemaking enzyme Vinoflow FCE (made by Novo) is an excellent protoplasting enzyme. It has pectinase and beta 1,3-1,6 glucanase activity. Novo makes a number of enzymes with similar names so be careful to obtain the correct enzyme. Vinoflow FCE does not work well with germinating spores, but efficiently protoplasts hyphae. It is inexpensive ($31.56/100 g in the U.S.).

In the U. S. it can be purchased from Gusmer Enterprises, which has bought out the former distributer, The Wine Lab. The URL is:

http://www.thewinelab.com/Catalog_i3459923.html?catId=136139

We use it as follows although I suspect other procedures will work. This procedure works very reliably in our hands and we typically get lots of protoplasts that yield hundreds of transformants per microgram of transforming DNA (fusion PCR products or non-replicating plasmids), and thousands of transformants per experiment. The procedure should scale up or down, but since we are only spending about $0.40 per experiment, and are getting lots of protoplasts, we have not experimented with different scales.

Note that this procedure produces protoplasts, not sphaeroplasts. They are extremely sensitive to detergents or osmotic shock (i.e. if, during the transformation, if you treat them with PEG that is not osmotically balanced with KCl or some other osmotic balancer, they will lyse). However, because of their small size they are surprisingly resistant to physical shear. We vortex them during our transformation procedure to break up clumps and do not get significant lysis. However, after $CaCl_2$ addition they are less resistant to shear.

Procedure:

1. Inoculate 1 X 10^8 spores into 20 ml of complete medium and incubate with shaking for 13-14 hours at 30ºC.

2. Harvest the hyphae by centrifugation or filtration (through sterile miracloth) and resuspend in 8 ml of medium.

3. Add 8 ml of 2X protoplasting solution that has been filter sterilized. The 2X protoplasting solution consists of 1.1 M KCl, 0.1 M citric acid (the KCl, citric acid solution is made first and the pH is then adjusted to 5.8 with freshly made 1.1 M KOH. The addition of KOH increases the volume but roughly maintains the osmolarity) and 128 mg/ml Vinoflow FCE. The final concentration of Vinoflow FCE is, thus, 64 mg/ml. We normally use low protein binding filters to filter the 2X protoplasting solution and run

a little medium through the filter to remove any residual detergent that might be present before filtering the 2X protoplasting mixture.

4. Monitor the protoplasting microscopically. Protoplasts begin to form in the first hour and we usually stop the protoplasting after a couple of hours.

5. There will be some residual undigested hyphal material that will need to be removed. We normally do this by layering the protoplasting mixture onto a sucrose cushion (1.2 M) and centrifuging at 1800 X g for10 minutes at 4ºC). Hyphal remnants go through and protoplasts are collected at the interphase. The protoplasts are mixed with 0.6 M KCl and spun down before going through our regular washing and transformation procedures.

6. We normally divide the protoplasts into aliquots before transforming. We often transform with 10 or more fusion PCR fragments plus positive and negative controls. The procedure above produces enough protoplasts to divide into at least 10-15 useful aliquots.

I hope this is helpful.

Berl Oakley