# TRANSIENT ASSAYS FOR B-GLUCURONIDASE

Judelson procedure

Extraction buffer

1.25 ml 1M $NaPO_4$ pH 7.0
0.5 ml 0.5M EDTA
0.25 ml 10% Triton X-100
25 mg N-lauryl sarcosine
15 µl B-mercaptoethanol
34 µl 0.1M PMSF in ethanol
water to 25 ml

Assay buffer

add 4-methylumbelliferyl glucuronide to extraction buffer to 1 mM final (7 mg/ 20 ml).

Treatment of protoplasts with DNA

Typically, treat 2 x $10^7$ protoplasts with DNA. Add 10-fold media, and incubate 16 to 48 hr.

Making extract

Gently pellet protoplasts from the media, and wash once with osmoticum. The tissue can be frozen at -80C if desired, and processed later. (Keep on ice from now on).
Add ca. 200 µl cold extraction buffer, mix, and sonicate 30 sec.
Spin 1 min. in microfuge, and save supernatants (or just work with the supernatants in the original tube).

GUS assay

Add equal amounts of protein (5-20 µg as determined by Bradford assay of 10-20 µl of extract) to each reaction (should be less than 100 µl of extract).
Add extraction buffer to each tube to equalize volumes.
Add 200 ~1 assay buffer to tube, mix, incubate 37 C 1-4 hr or longer; include no extract and $T_o$ controls.
Stop with 1.25 ml of 0.2 M sodium carbonate.
Read relative fluorescence.
Standardize to 10 nM or 100 nM 4-MU in sodium carbonate.