

## Aspergillus Transformation for pyrG89 Strains

Things you will need for making protoplasts.

Proteins:

BSA, Novozyme 234 (Novo Industri), Driselase (Sigma), Glucuronidase (Sigma No. G-0762)

Solutions:

1M MgSO<sub>4</sub>

(A) 0.1M Citric acid 0.8M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> pH5.8 with KOH

(B) 1% yeast extract 2% sucrose 40mM glucose 2X vitamin mix and other media supplements as required for your strain

(C) 0.05M citric acid 0.4M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1% sucrose pH6.0

(D) 25% PEG 8000 100mM CaCl<sub>2</sub> 0.6M KCl 10mM Tris-HCl pH7.5

(E) 0.6M KCl 100mM CaCl<sub>2</sub> 10mM Tris-HCl pH7.5.

All solutions are prepared in distilled water and made sterile by autoclaving.

Media you will need.

YGUV: 0.5% yeast extract, 20mM glucose, 5mM uridine, 10mM uracil, vitamin mix and other supplements as required for your strain. Sterilized by autoclaving.

YAS: 0.5% yeast extract, 20mM glucose, 1M sucrose for top agar and 0.2 M in plates, 1% agar for top and 1.5% agar for plates, vitamin mix and other supplements as required by your strain.

Making Protoplasts.

Inoculate 10<sup>9</sup> fresh conidia into 50ml YGUV and incubate at 32°C for 5.5-6 hours.

Harvest by centrifugation in the clinical centrifuge for 5 minutes on setting 7.

Resuspend in 40ml of lytic mix.

Lytic Mix

20ml solution A

20ml Solution B

0.4ml 1M MgSO<sub>4</sub>

200mg BSA

100-200mg Novozyme 234

100mg Driselase (optional)

100µl glucuronidase

Allow to dissolve, spin 5 minutes to sediment insoluble material and filter sterilize.

Incubate at 32°C with shaking for 2 or more hours. The time depends on how well the protoplasting is going.

Harvest by centrifugation for 5 minutes on setting 7 and wash 2X in solution C.

Resuspend pellet in 1ml solution E.

The transformation.

Add 100µl protoplasts to a microfuge tube with 2-4µg of plasmid DNA in not more than 15µl of TE.

Add 50µl of solution D and incubate on ice 20 minutes.

Add 1ml solution D and incubate at room temp. for 30 minutes.

Aliquot 100-200µl of mix into 3ml YAS TOP at 45°C and pour onto YAS plate prewarmed in 42°C.

Incubate plates at 32°C for 3-4 days. If you are not working with a temperature sensitive mutant you may incubate them at 37°C.

Protoplasts can be stored on ice and used the next day for transformation. It has been noted that the transformation efficiency is increased by this overnight storage.