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 $MgSO_4$
(A) 0.1M Citric acid 0.8M $(NH_4)_2SO_4$ 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 $(NH_4)_2SO_4$ 1% sucrose pH6.0
(D) 25% PEG 8000 100mM $CaCl_2$ 0.6M KCl 10mM Tris-HCl pH7.5
(E) 0.6M KCl 100mM $CaCl_2$ 10mM Tris-HCl pH7.5.
All solutions are prepared in distilled water and made sterile by autoclaving.

Media you will need.
YGUUV: 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.
Innoculate 10^9 fresh conidia into 50ml YGUUV and incubate at 32^{o}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 $MgSO_4$
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^{o}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^{o}C and pour onto YAS plate prewarmed in 42^{o}C.

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

From the May laboratory