KCl Transformation for Aspergillus

Solutions:
A. 1.1M KCl, 0.1M citric acid pH5.8 with KOH
B. 1% yeast extract, 2% sucrose, 40mM glucose, 2X vitamin mix
C. 0.6M KCl, 0.05 M citric acid, 1% sucrose pH5.8 with KOH
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 and top agar for plating transfomants is made with 0.6MKCl to stabilize the transformant protoplasts from osmotic lysis. The standard media formulations are used except that the nutritional supplement you are selecting for is not added. For example, you can use YAG with 0.6M KCl without uridine and uracil for selection of pyrG89 mutant strain transformations.

Making Protoplasts:
Inoculate 10^9 fresh conidia into 50ml media 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
100µl glucuronidase

Filter sterilize the lytic mix.

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 15 minutes.

Add 1ml solution D and incubate at room temp. for 15 minutes.

Aliquot 100-200µl of mix into 3ml 0.6M KCl top agar media at 45^{o}C and pour onto 0.6M KCl media 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 for some strains.

Freezing protoplasts:
Extra protoplasts can be stored frozen at $^-$80^{o}C after the addition of solution D and DMSO, in the following manner. To 1ml of protoplasts add 0.5ml of solution D and 15µl of DMSO, mix thoroughly and freeze in 100µl portions.

To transform frozen protoplasts.

Thaw the protoplasts on ice, add transforming DNA, incubate 15 minutes on ice.

Add 1ml of solution D and continue as above.