From E. Kafer, Sept.6, 2000

Numbers and Genotypes of the two enclosed strains of *Aspergillus nidulans*:

**FGSC A1061 = EK 4078** *pyrG89 pabaA; wA3; bimD6 pyroA4; chaA1*
**FGSC A1062 = EK 4247** *pyrG89; bimD6; riboB2 chaA1*

Use of strains (used in bimD publication, close to being submitted; comparison with homolog SPO76 from Sordaria macrospora (D. van Heemst et al., 1999, Cell 98:261-271)

These pyrG89;bimD6 strains were most sucessfully used as recipients for homologous and heterologous transformation of bimD6, using primary selection for $Pyr^+$, i.e., growth on media lacking Uridine/Uracil; double selection at 42° also was sucessful (but less informative).

For protoplasting, recipient pyrG89;bimD6 strains were grown at 30 - 32° (overnight) in liquid YG medium (0.5% yeast extract, 2% glucose, Hutner's trace elements and vitamin supplements as needed (May *et al.*, 1985, J. Cell.Biol. 101: 712-19) to which 10 mM uracil and 5 mM uridine (UU) were added (Uracil is disolved in the medium while liquid on a stirring hotplate).
We used the convenient and simple method of mycelial protoplasting by Debets and Bos (1986;FGNL 33: 24; slightly modified: lytic medium SCC: 0.8 M NaCl + 0.2 M $CaCl_2$ , with 5 mg/ml Novozyme; osmotic medium, STC; 1.2 M sorbitol, 10 mM Tris (pH8) + 50mM $CaCl_2$, with 1-2 washes, centrifuging at 2 k for 10 min.

For transformation we used PEG (8000) at 30% with 50 mM $CaCl_2$, and basically followed Osmani et al., 1988 (J. Cell.Biol. 104: 1495-1504). Putative $pyr^+$ transformants were selected on solid YG medium (2% agar in bottom layers, 1% in overlays) with1 M sucrose replacing glucose and for survival assays adding UU (as above).

**NOTE:** 1) Because bimD6 is MMS-sensitive, amino acid requirements are avoided in recipient strains, since all AA mutants are MMS-sensitive in Aspergillus.

2) pyrG89 is cold sensitive so that 25° , the normal permissive temperature for bimD6, is not recommended. We always used 30° as our standard (because we have a 30° incubating room) but 32° may actually produce somewhat faster growth (used as standard by Ron Morris, Greg May,. Steve Osmani, et al.).

3) At 30-32°, suppressor mutations of the pyrG89 cold sensitivity turn up as better growing sectors or colonies (these "su-cs" mutations segregate in crosses; number of genes involved not known).

4) The only adenine mutants tried out with pyrG89;bimD6, were adE8 or adE20, transformants were obtained OK, but barely grew on standard media, while growing and conidiating nicely on transformation medium (1 M sucrose preferred).

Growth of such triple mutant strains, which grew very poorly on standard media (even with high UU and ADE supplementing), was much improved when "su-cs" mutations were present. Unfortunately the latter also caused leakiness for the UU requirement; frequent apparent transformants grew up on selective media, which on purification were not stably transformed.