Mattern. I.E., P.J. Punt and C.A.M.J.J. Van den Hondel

A vector of *Aspergillus* transformation conferring phleomycin resistance.

Recently, transformation of *Aspergillus* species with vector pAN7-1, conferring resistance to hygromycin B was reported (Punt *et al.* 1987 Gene 56:117-124). Here we describe a transformation vector (pAN8-1, Fig. 1) containing the *Streptococcus hindustanus* phleomycin resistance gene (obtained from G. Tiraby, Toulouse, France) flanked by the promoter region of the highly expressed *A. nidulans gpd* gene, and the terminator region of the *A. nidulans trpC* gene.

Transformation of *A. nidulans* and *A. niger* was achieved with this vector at frequencies of 1 to 20 transformants per ug pAN8-1 DNA. These frequencies are similar to those found for transformation with pAN7-1. Transformants could be selected at low concentrations of phleomycin (5-10 ug/ml for *A. niger*, 10-20 ug/ml for *A. nidulans*).

A. oryzae, which cannot be transformed with pAN7-1 because of its innate insensitivity to hygromycin B, is inhibited in its growth at 50-100 ug/ml phleomycin. Phleomycin resistant transformants were obtained by cotransformation of an *A. oryzae pyrG* mutant with pAB4-1 (containing the *A. niger pyrG* gene) and pAN8-1 (Mattern *et al.* 1987, MGG 210:460-461). Experiments are in progress to achieve direct selection of phleomycin resistant transformants of *A. oryzae*.

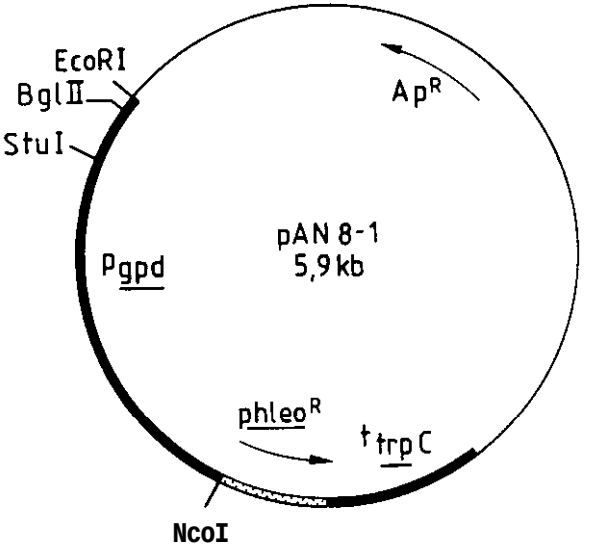

Figure 1. Vector pAN8.1. A 0.4 kb NcoI-StuI fragment from pUT701 (G. Tiraby, unpublished) containing the coding region of the *S. hindustandus* phleomycin resistance gene was ligated into pAN52-3, which was cut with HindIII, treated with T4 polymerase and subsequently cut with NcoI. Vector pAN52-3 is a derivative of pAN52-1 (Punt *et al.* 1987 Gene 56:117-124) in which the unique BamHi site was converted into a Hind III site by site directed mutagenesis.

- - Medical Biological Laboratory TNO, P.O. Box 45, 2280 AA Rijswijk, The Netherlands