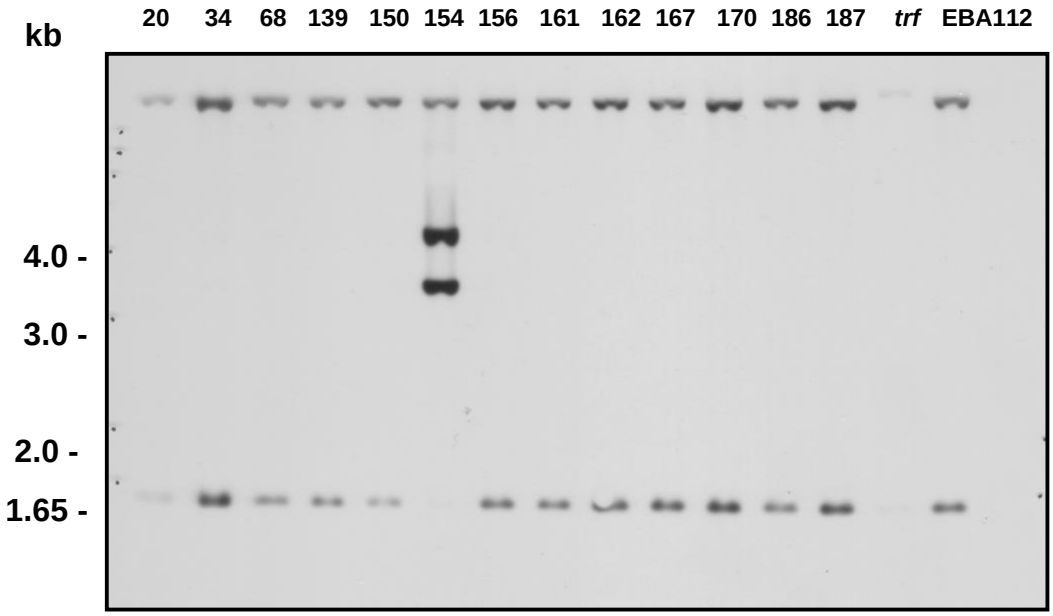

kb
20
34
68
139
150
154
156
161
162
167
170
186
187
trf
EBA112
4.0 -
3.0 -
2.0 -
1.65 -

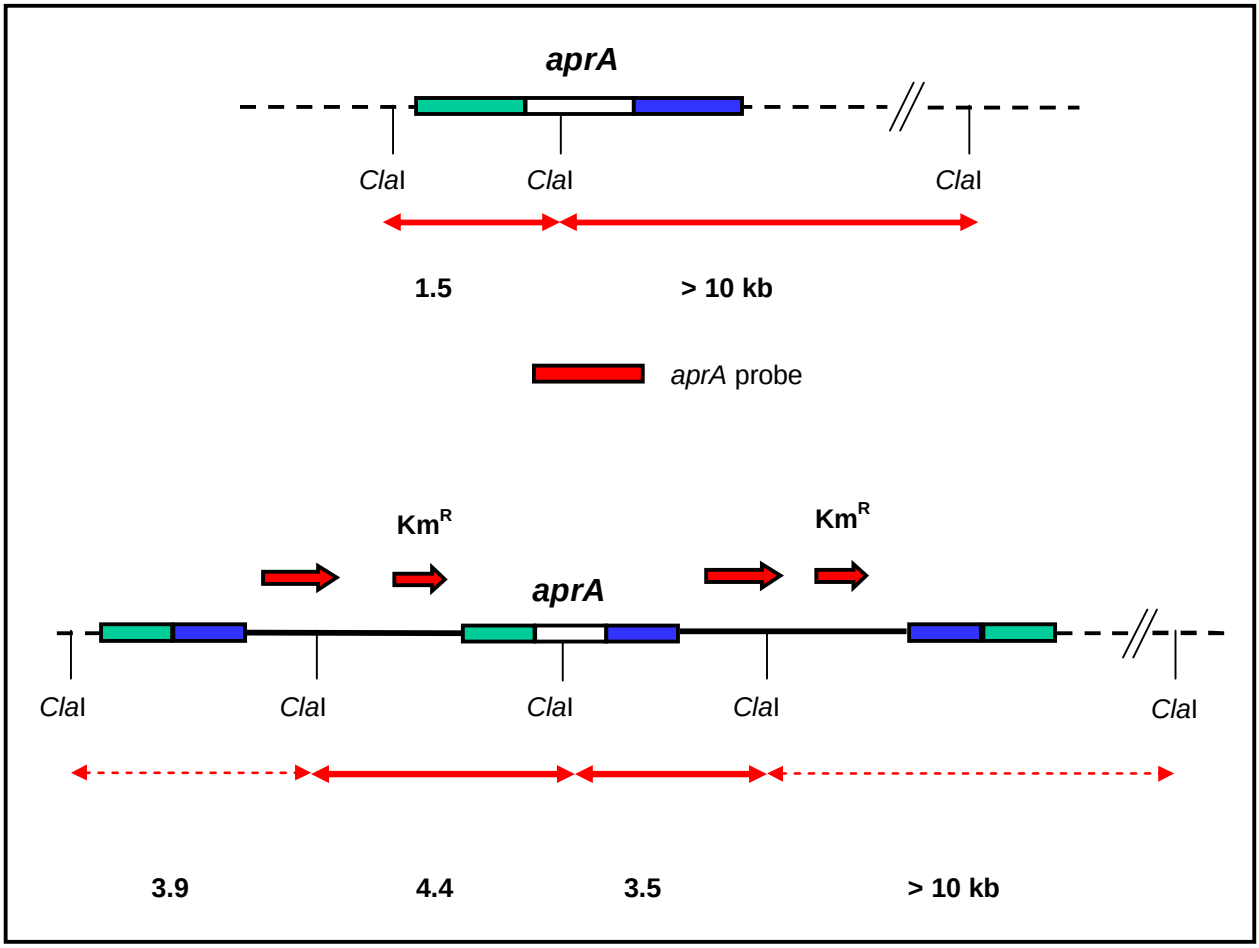
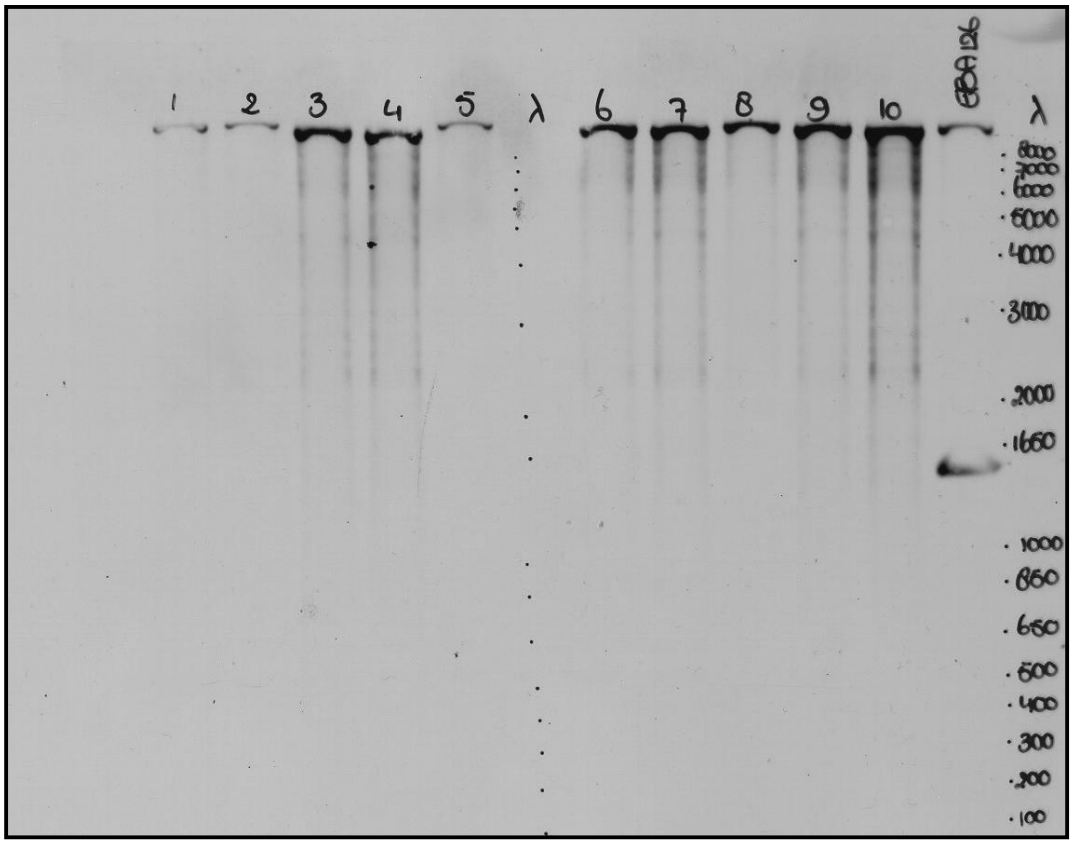


Figure 3A. (*previous page*) Southern hybridisation of EBA126 derived clones for the detection of integration of the pGBBdapr1 vector. Total DNA was isolated from these clones, as well as from the original transformant ("*trf*") and the parental strain EBA112 and digested with *Cla*I. As a probe, a fragment encompassing the *aprA* coding sequence was used. In strains that do not contain an integrated copy of pGBBdapr1 two hybridising fragments (indicated by the heavy arrows in the lower panel) of 1.5, and > 10 kb[1], respectively, were obtained as is the case for the wild-type strains. Among the colonies tested here, clone #154 was shown to contain integration of the vector at the appropriate site, yielding fragments of 4.4 and 3.5 kb, respectively, and this strain was selected for further use. The lower panel shows the integration pattern as deduced from the hybridisation profile of this integrant versus that of the wild-type strains. The dotted arrows represent *Cla*I generated fragments that do not hybridise against the *aprA* probe. The presence of multiple copies of the vector in strain is in agreement with the intensity of the hybridising bands – the wild-type fragments observed in this strain can be explained by the fact that, since integration/excision is a dynamic process, the clone was possibly not pure.

[1] Since the position of the *Cla*I site downstream of the *aprA* region is not known, the expected size of this fragment can only be estimated



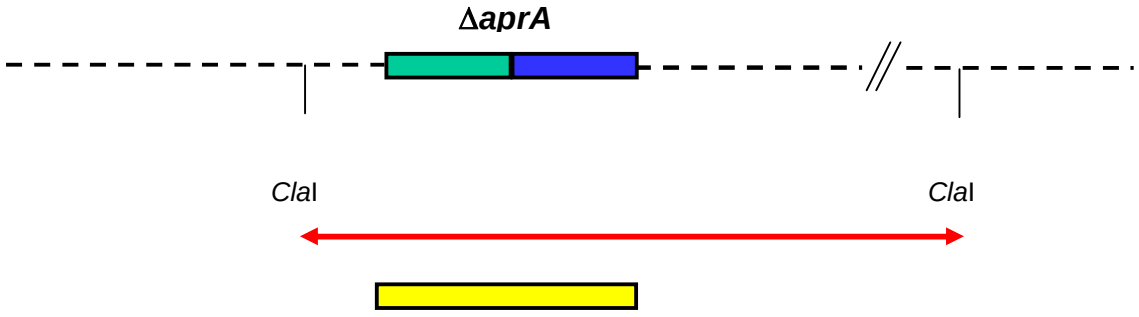


Figure 3B. Southern hybridisation, using a probe specific for the *aprA* flanking sequences, of cured derivatives of strain #154, obtained after several generations of non-selective growth at a temperature permissive for replication of the pE194 origin. All strains tested here show only a single hybridising fragment corresponding to deletion of the *aprA* coding region (see lower panel), as opposed to strain EBA126, which produced the fragments expected for the presence of an intact copy of the *aprA* gene (see also Fig. 3A, lower panel). λ, molecular weight marker (bp).

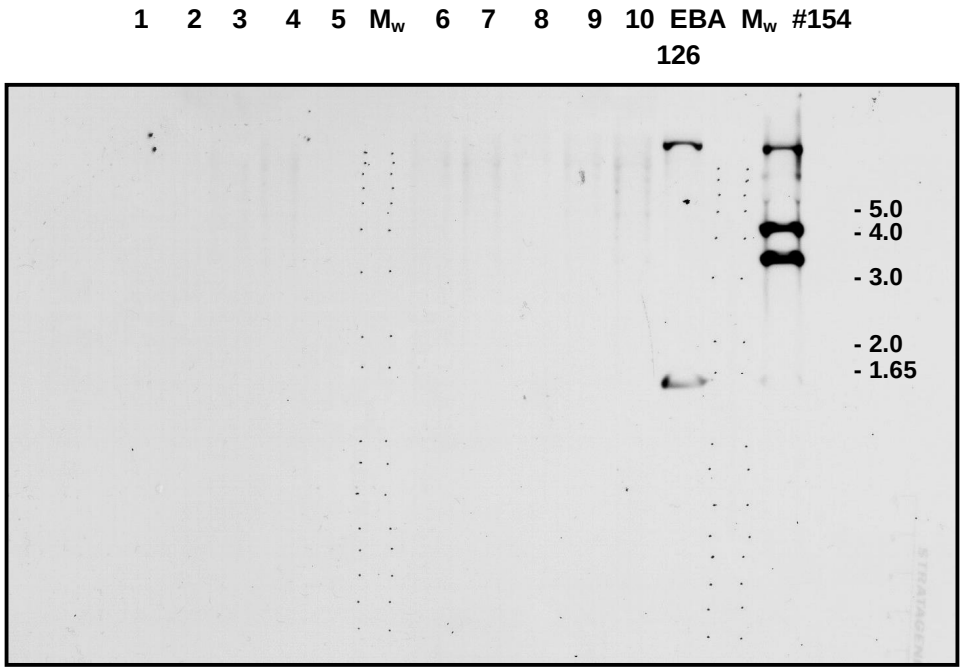


Figure 3C. Southern hybridisation analysis, using the *aprA* specific probe, of the cured strains obtained after several generations of non-selective growth. As controls, strains EBA126 and the original integrant (#154) were tested. In the latter two, *aprA* specific sequences were detected, producing the same patterns as observed previously (see Fig 3A), whereas *aprA* hybridising fragments are completely absent in the Km^S descendants of the original integrant. From the data shown in Figures 3B and 3C it was concluded that the *aprA* gene was successfully deleted from the chromosome of EBA126, and clone number 8 was selected for further use. Mw, molecular weight marker (bp).

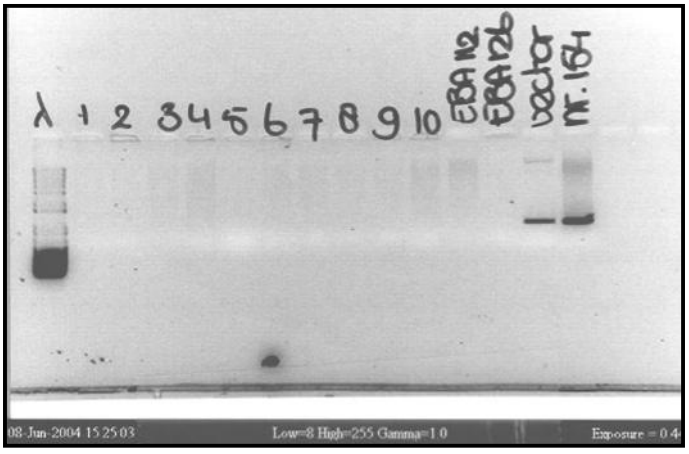


Figure 3D. PCR analysis verifying the absence of the Neo/Kan^R marker of plasmid pGBBdapr1 in the Km^S strains shown in Fig.3C. As negative controls EBA112 and EBA126 chromosomal DNA was used as template for the PCR reaction; the positive controls were pGBBdapr1 (vector DNA) and the integrant strain (#154). λ, molecular weight marker.