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Abstract

Resistance to hygromycin B is an important dominant selectable marker in fungal transformation. Our goal was to improve vectors for hygromycin selection by making the gene more compact, by eliminating sites for commonly used restriction enzymes, and by subcloning the modified gene into convenient vectors. These improvements were made by modifying pCSN43 (Staben et al. 1989 Fungal Genetics Newsl. 36:79-81) through three rounds of megaprimer mutagenesis (Aiyar and Leis, 1993 Biotechniques 14:366-368), a technique based on polymerase chain reaction amplification. Plasmid pCSN43 has a 2.4 kb SalI fragment containing the bacterial hph gene (Gritz and Davies, 1983 Gene 25:179-188), encoding hygromycin B phosphotransferase, under control of the Aspergillus nidulans trpC promoter and terminator (Mullaney et al. 1985 MGG 199:37-45)

Improved Vectors for Selecting Resistance to Hygromycin

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Four restriction enzyme sites in the hph gene were eliminated through single base pair changes. The changed base pair is shown by lower case letters: EcoRI (GAgTTC); PstI (CTGgAG); SstII (CCGCGc); BamHI (GGtTCC). These changes maintained the hph amino acid sequence. While eliminating a fifth site, the ClaI site, the sequence near the translation initiation ATG, the Kozak sequence, was changed from ATCGATATG to ATCcAaATG. The latter matches the consensus Kozak sequence for Neurospora crassa (Bruchez and Eberle, 1993 Fungal Genetics Newsl. 40:85-88, 1993). The primers used for the final amplification introduced restriction sites at both ends of the gene and eliminated the trpC terminator which is unnecessary for function.

The product from the final mutagenesis (Fig. 1b) was a 1.4 kb fragment with EcoRI, SalI and HpaI fragments at both ends. The HpaI sites were introduced to provide a convenient blunt end fragment for subcloning without the need for Klenow fill-in. The final product lacks the original BamHI, ClaI, EcoRI, PstI, and SstII sites as well as the trpC terminator.

The engineered hph gene was subcloned as follows. Plasmid pCB1003 was produced by ligating the final mutatgenesis product into the EcoRI site of pUC19. Plasmid pCB1004 was produced by ligating the SalI fragment of pCB1003 into the BclI site of pBC SK+. This was performed by half site fill-in, thereby destroying both restriction sites. Plasmid pCB1004 has chloramphenicol resistance, has a functional lacZ gene allowing blue/white screening, and has unique sites for all the restriction enzymes that cut in the "SK" polylinker. These plasmids transform Magnaporthe grisea strains 4091-5-8 and CP987 with the same efficiency as pCSN43 and confer the same level of resistance to hygromycin B.

Fig 1a

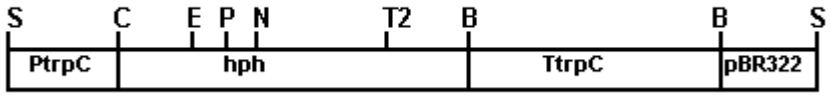
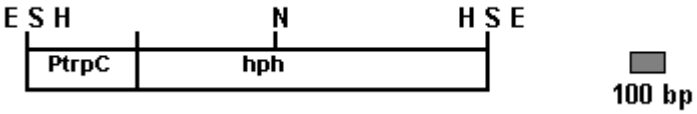


Fig 1b



B=BamH1 C=ClaI E=EcoRI N=NdeI P=PstI S=SalI T2=SstII H=HpaI

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