A neomycin resistance gene cassette selectable in a single copy state in the *Bacillus subtilis* chromosome

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A neomycin resistance (Nm\(^R\)) gene cassette was constructed as shown in Fig. 1 by the fusion of the promoter region of the repU gene and the gene coding for neomycin resistance both derived from pUB110 (1).

The key feature of the 1.3 kb cassette is that even when integrated into the *B. subtilis* chromosome in a single copy, neomycin resistant transformants can be selected directly because of the addition of the functional repU promoter. Therefore, the use of the neomycin resistance marker together with the widely used chloramphenicol resistance (Cm\(^R\)) gene should facilitate genetic manipulations of *B. subtilis*. For practical use, neomycin (10-20 \(\mu\)g/ml) should be used for selection since selection by kanamycin was not so effective.

![Diagram](image)

Fig. 1. The vector part of pBEST501 and 502 is pGEM4 (Promega Biotec). The repU promoter region (\(\text{repU}\)), nt 4382-4033) was obtained by digestion of pUB110 with TaqI and NcoI. The neomycin resistance gene part (\(\text{NmR}\)), nt 3068-2129) was also generated by exonucleaseIII-mediated deletion. Both plasmids can be selected in *E. coli* by ampicillin (100 \(\mu\)g/ml) or kanamycin (25 \(\mu\)g/ml). (\(\text{APW}\)) and (\(\text{AP}\)) represent the SP6 and T7 promoter, respectively. Nucleotide sequence of multi-restriction enzyme sites is also shown.

REFERENCES
(1) McKenzie et al., Plasmid 15, 93-103 (1986)