

A cassette containing the *bar* gene of *Streptomyces hygroscopicus*: a selectable marker for plant transformation

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The phosphinothricin acetyl transferase gene (*bar*) of *Streptomyces hygroscopicus* ATCC 21705 is a useful marker for the selection of transgenic plants (1). In order to develop a generally useful and available cassette, the *bar* gene was isolated from a *Bam*HI library of genomic DNA of *S. hygroscopicus* cloned in *Bam*HI-cut pUC18 by probing with a synthetic oligonucleotide (corresponding to the complement of the underscored sequence below) derived from the published sequence (2). Site-directed and linker mutagenesis was then used to construct a pUC18 derivative that contained the *bar* coding region preceded by a multiple cloning site. The sequence immediately preceding the translational start codon was changed (indicated by the dotted nucleotides) to conform more closely to that required for optimal translational initiation in eukaryotes

(3). The sequence that follows has been inserted in pUC18 to give pIJ4104. The *Sma*I fragment containing the *bar* coding region has been shown to confer bialaphos resistance in *Streptomyces lividans*. The *bar* nucleotide sequence can be found in the EMBL sequence database as entries SHBRPA, and X17220.

REFERENCES

1. De Block, M., Botterman, J., Vandewiele, M., Dockx, J., Thoen, C., Gossele, V., Rao Movva, N., Thompson, C., Van Montagu, M. and Leemans, J. (1987) *EMBO J.* 6, 2513–2518.
2. Thompson, C.J., Rao Movva, N., Tizard, R., Cramer, R., Davies, J.E., Lauwereys, M. and Botterman, J. (1987) *EMBO J.* 6, 2519–2523.
3. Kozak, M. (1986) *Cell* 44, 283–292.

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EcoRI SstI KpnI SmaI . . . .
GAATTCGAGCTCGGTACCCGGGGATCTACCATGAGCCCAGAACGACGCCCGGCGACATCCGCCGTGCCACCGAGGGCGGA 80
      FM S P E R R P A D I R R A T E A D

CATGCCGGGGTCTGCACCATCGTCAACCACTACATCGAGACAAGCACGGTCAACTTCCGTACCGAGCCGACAGAACCGC 160
      M P A V C T I V N H Y I E T S T V N F R T E P Q E P

AGGAGTGGACGGACGACCTCGTCCGTCTGCGGGAGCGCTATCCCTGGCTCGTCCCGAGGTGGACGGCGAGGTGCGCCGGC 240
      Q E W T D D L V R L R E R Y P W L V A E V D G E V A G

Sali
ATCGCCTACGGGGGCCCTGGAAGGCACGCAACGCCTACGACTGGACGGCCGAGTCGACCGTGTAGCTCTCCCCCGCCA 320
      I A Y A G P W K A R N A Y D W T A E S T V Y V S P R H

CCAGGGACGGGACTGGGCTCCACGGCTCTACACCCACCTGCTGAAGTCCCTGGAGGCACAGGGCTTCAAGAGCGTGGTCCG 400
      Q R T G L G S T L Y T H L L K S L E A Q G F K S V V

SphI SstII SphI
CTGTCAATGGGCTGCCCAACGACCCGAGCGTGGCATGCACGAGGGGCTCGGATATGCCCCCGGGCATGCTGGGGGG 480
      A V I G L P N D P S V R M H E A L G Y A P R G M L R A

KpnI
GCCGGCTTCAAGCACGGGAACCTGGCATGACGTGGGTTTCTGGCAGCTGGACTTCAGCCTGCCGGTACCGCCCGTCCGGT 560
      A G F K H G N W H D V G F W Q L D F S L P V P P R P V

BglII SmaI BamHI PstI SphI HindIII
CCTGCCCGTACCGGATCTGATGACCCGGGGATCCCTGCAGGCATGCAAGCTT 615
      L P V T E I * *
    
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