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Transformation of *Diplonema papillatum* using V5+Neo^R construct

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ABSTRACT

p57-V5+Neo^R plasmid contains V5-tagged aminoglycoside 3'-phosphotransferase gene (APT; conferring resistance to neomycin) flanked by partial regulatory sequences derived from the hexokinase gene of the kinetoplastid *Blastocrithidia* (strain p57).

Plasmid GenBank accession number = MN047315

MATERIALS TEXT

p57-V5+NeoR plasmid.jpg

1 p57-V5+Neo^R plasmid contains V5-tagged aminoglycoside 3'-phosphotransferase gene (APT; conferring resistance to neomycin) flanked by partial regulatory sequences derived from the hexokinase gene of the kinetoplastid *Blastocrithidia* (strain p57).

2 This 1779 bp long sequence is surrounded by *SwaI* restriction sides and was cloned in the pBluescript II SK(+) backbone.

3 Before transformation, the p57-V5+ Neo^R plasmid was cut using *SwaI*, run on the gel and a fragment of 1779 bp containing p57-V5+ Neo^R construct was gel purified

4 3 ug of purified construct was electroporated into *D. papillatum* cells using an established protocol (Kaur *et al,*. 2018). About 18 h after electroporation, a scale of 45 – 83 ug/ml G418 (Geneticin) was added to the media and after about 2 weeks G418-resistant clones were recovered.