

Two yeast plasmids that confer nourseothricin-dihydrogen sulfate and hygromycin B resistance in *Neurospora crassa* and *Cryphonectria parasitica*.

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Fungal Genetics Newsletter 54:12-13

Two plasmids that were previously used with yeast, pRS41N and pRS41H, were found to confer clonNAT and hygromycin B resistance, respectively, in the filamentous fungi *Neurospora crassa* and *Cryphonectria parasitica*. These plasmids are suitable for routine cloning and for use in forcing heterokaryons and are available through the FGSC.

Identification of novel resistance markers is important to further our ability to manipulate and characterize genetic elements in filamentous fungi. Hygromycin B (hygB) inhibits protein translation and has been used extensively as a selectable marker in filamentous fungi (Rao *et al.*, 1985). Alternative, affordable selectable markers are desirable for various applications, including for forcing heterokaryons. Previously, nourseothricin-dihydrogen sulfate (clonNAT) has been used as a selectable marker in fungi (Kück and Hoff, 2006). This aminoglycoside binds to ribosomes and results in inhibition and errors in protein synthesis (Cundliffe, 1989). Here we report that two vectors, pRS41N (clonNAT resistance) and pRS41H (hygB resistance), that were previously characterized in *S. cerevisiae* but not in filamentous fungi, are appropriate transformation vectors and suitable for forcing heterokaryons in *Neurospora crassa* and *Cryphonectria parasitica*.

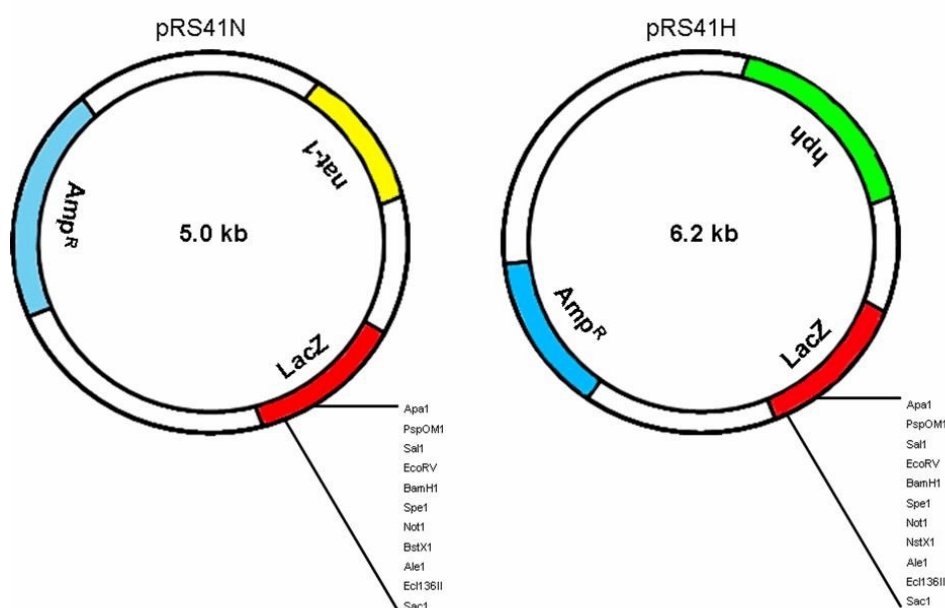


Figure 1. Yeast plasmids pRS41N and pRS41H that confer clonNAT and hygB resistance, respectively, to *N. crassa* and *C. parasitica*. Unique restriction sites in the multiple cloning site are indicated.

Plasmids pRS41N and pRS41H (Figure 1) were derived from pRS416 as described in Taxis and Knop (2006). Resistance genes in both plasmids are driven by the TEF promoter from *Ashbya gossypii*, and *S. cerevisiae* *CYC1* and *ADH1* terminators are used in pRS41H and pRS41N, respectively. This contrasts to previously characterized vectors such as pCB1004 (Carroll *et al.*, 1994) and pD-NAT1 (Kück and Hoff, 2006) that use *Aspergillus nidulans* *trpC* promoter and terminator. Transformations with pRS41N and pRS41H were done with a PEG-mediated transformation protocol (Smith *et al.*, 2000). Selection of *N. crassa* pRS41N transformants was on Vogel's minimal medium containing 200 ug/ml of clonNAT (Werner Bioagents, Jena, Germany) and yielded ~20 colonies / ug plasmid DNA. Similarly, *C. parasitica* transformed with pRS41H, was selected on Potato Dextrose Agar (PDA)

containing 100 ug/ml of clonNAT and ~10 colonies/ug of plasmid were recovered. Stable transformation frequencies of ~12 colonies/ug were achieved with both species using pRS41H by selection for resistance to hygB at 200 ug/ml and 30 ug/ml for *N. crassa* and *C. parasitica*, respectively.

Eleven unique restriction sites are available in the multi-cloning sites of pRS41N and pRS41H to facilitate blue-white selection. We used the *SacI* and *SpeI* restriction sites to clone *un-24^{PA}* into pRS41N and transformed this construct into *N. crassa* strain C9-2, which contains *un-24^{OR}*. The transformants had the characteristic self-incompatible phenotype (Micali and Smith, 2006) indicating that *un-24^{PA}* was expressed as a transgene using this system. We further tested whether pRS41N and pRS41H could be used to force heterokaryons. Strains of both species were transformed separately with pRS41N and pRS41H. Small (~0.3 mm³) agar blocks containing hygB- and clonNAT-resistant hyphae were grown for two days on nonselective medium and then transferred to medium containing both clonNAT and hygB at the concentrations given above. Heterokaryon outgrowths of both species were evident within ~2 days and subsequently grew at near wild-type rates in clonNAT-hygB medium. We conclude that pRS41N and pRS41H are suitable as general transformation vectors and are particularly useful for generating forced heterokaryons in both fungal species.

Acknowledgements

This work was supported by OGS and NSERC CGS to RPS and an NSERC Discovery Grant to MLS. Thank you to Dr. Christof Taxis for the plasmids used in this study. Thanks to Dr. John Vierula for his helpful suggestions with this manuscript.

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