# A Comparison of Hygromycin and Paromomycin Selection Strategies in the Genetic Transformation of Seven *Lolium, Festuca, Poa*, and *Agrostis* Species

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## Presenter Information

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**Introduction** Hygromycin selection for the *hpt* gene, expressed from the CaMV-35S promoter, has been successful in transgenesis of a limited number of grass species. As an alternative to *hpt* selection Altpeter *et al.,* (2000) reported successful transformation using paromomycin selection for the *nptII* gene expressed by the maize ubiquitin promoter. We have tested the utility of a number of selection cassettes using previously sporadically transformable species which nevertheless had very good tissue culture and regeneration protocols.

**Materials and methods** Callus and cell suspension cultures were co-transformed with genes of interest and either the *hpt* gene encoding hygromycin resistance expressed from the CaMV-35S promoter, or the *nptII* gene encoding paromomycin resistance expressed from the maize ubiquitin promoter. Transformations were performed by bombardment in a particle inflow gun at various parameters depending on the tissue and species involved (Dalton, 1999). The results in Table 1 were collated from a large number of experiments and no account was taken of the effect of different genes of interest on regeneration except *L.multiflorum* and *F.arundinacea*, where large numbers allowed comparisons with co-transformations involving similar genes of interest.

**Results** There was genotype-associated variation in resistance to both paromomycin and hygromycin of cell cultures. However, paromomycin did not inhibit embryogenesis as much as hygromycin and many cultures in which regeneration was inhibited were still able to develop embryoids. Increasing the numbers of subcultures to fresh paromomycin-containing media reduced the numbers of escapes. Transformation frequency in terms of the number of bombardments in which plants were regenerated was greater in *L.perenne* when *hpt* was expressed from the CaMV-35S promoter and when *nptII* was expressed from the maize ubiquitin promoter. Selection with the *ubi-nptII* construct improved the recovery of transformants in all species.

**Table 1** Transgenic plant regeneration under hygromycin (*CaMV-hpt*) and paromomycin (*ubi-nptII*) selection

| Species | Tissue | Selection | Successful bombardments | Percentage | No. of plants | No. plants per bombardment |
|---|---|---|---|---|---|---|
| *F.arundinacea* | cell suspension | *CaMV-hpt* | 60/108 | 56 | 112 | 1.0 |
| *F.arundinacea* | cell suspension | *ubi-nptII* | 20/44 | 45 | 63 | 1.4 |
| *L.multiflorum* | cell suspension | *CaMV-hpt* | 21/63 | 33 | 34 | 0.5 |
| *L.multiflorum* | cell suspension | *ubi-nptII* | 18/25 | 72 | 35 | 1.4 |
| *L.temulentum* | cell suspension | *CaMV-hpt* | 18/161 | 11 | 37 | 0.2 |
| *L.temulentum* | cell suspension | *ubi-nptII* | 36/91 | 40 | 58 | 0.6 |
| *L.perenne* | callus | *CaMV-hpt* | 10/67 | 15 | 12 | 0.2 |
| *L.perenne* | callus | *CaMV-nptII* | 0/15 | 0 | 0 | 0 |
| *L.perenne* | callus | *ubi-hpt* | 1/16 | 6 | 1 | 0.1 |
| *L.perenne* | callus | *ubi-nptII* | 7/20 | 35 | 7 | 0.4 |
| *P.pratensis* | callus | *CaMV-hpt* | 5/49 | 10 | 8 | 0.2 |
| *P.pratensis* | callus | *ubi-nptII* | 14/108 | 13 | 31 | 0.3 |
| *F.rubra* | callus | *CaMV-hpt* | 1/7 | 14 | 1 | 0.1 |
| *F.rubra* | callus | *ubi-nptII* | 5/9 | 55 | 6 | 0.7 |
| *A.stolonifera* | callus | *CaMV-hpt* | 5/53 | 9 | 9 | 0.2 |
| *A.stolonifera* | callus | *ubi-nptII* | 6/26 | 23 | 6 | 0.2 |

**Conclusions** Different commonly used constitutive promoters improve transformation frequencies when selecting for different marker genes, suggesting that relative differences in expression are important for different selection regimes. However, over the 7 grass species use of the *ubi-nptII* construct generally doubled the mean number of successful bombardments from 21% to 40% and the number of plants regenerated per bombardment from 0.34 to 0.71.

**References**

Altpeter *et al.* (2000). Generation of large numbers of independently transformed fertile ryegrass (*Lolium perenne* L.) plants of forage and turf-type cultivars *Mol Breeding.6:519-528*

Dalton *et al.* (1999) Co-transformed, diploid *Lolium perenne* (perennial ryegrass), *Lolium multiflorum* (Italian ryegrass) and *Lolium temulentum* (Darnel) plants produced by microprojectile bombardment. *Plant Cell Reports 18:721-726*