A series of vectors for fungal transformation

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We report a new fungal selectable marker that confers resistance to chlorimuron ethyl, a sulfonylurea herbicide. This gene as well as genes that confer resistance to hygromycin and bialaphos have been engineered to be compact and to eliminate sites for most common restriction enzymes. These three selectable markers have been used to construct a series of vectors for fungal transformation.

We have modified three dominant selectable markers for fungal transformation. First, we cloned a sulfonylurea resistant allele of the *Magnaporthe grisea ILV*1 gene using the *Saccharomyces cerevisiae ILV2* gene as a heterologous probe. These genes encode acetolactate synthase, an enzyme involved in isoleucine and valine synthesis. Sulfonylureas (specifically chlorimuron ethyl, the active ingredient of the herbicide Classic[reg].) inhibit acetolactate synthase. The sulfonylurea resistant allele of *M. grisea ILV*1 has been subcloned as a 2.8 kb fragment and modified by the elimination of eight restriction enzyme sites (GenBank AF013601). Second, a chimeric gene for bialaphos resistance (Pall and Brunelli 1993 Fungal Genet. Newsl. **40**:59-63) has been further modified by eliminating five restriction enzyme sites and by removing the transcription terminator (GenBank AF013602). Third, a chimeric gene conferring hygromycin resistance (GenBank) has been reported previously (Carroll *et al*. 1994 Fungal Genet. Newsl. 41:22) and is included here for completeness. All three selectable markers have had *Sal*I sites introduced at both ends.

The three selectable markers were cloned into various plasmids both within and outside the polylinker (Table 1). First, the genes were cloned as *Sal*I fragments into the polylinker of pUC, pBluescriptII, and pBC vectors. They were also cloned as *Sal*I fragments into the *Xho*I site of a modified polylinker in pBluescript II and pBC (pCB1519 and pCB1520, respectively) where the *Xho*I site is flanked on both sides by *Sma*I sites. Second, the selectable markers were cloned into common cloning vectors outside the polylinker, thus leaving the *lacZ* gene intact. Most of the restriction enzyme sites in these polylinkers are unique (Table 2). We find that these vectors allow flexible and facile cloning options due to the presence of three fungal selectable markers available as many different restriction fragments including blunt fragments, a range of polylinker choices with blue/white screening, and a choice of bacterial selection for ampicillin or chloramphenicol resistance. All of these plasmids have been deposited in the FGSC.

We have successfully used these vectors to transform *M. grisea* as described previously for hygromycin selection (Sweigard *et al.* 1995 Plant Cell **7**:1221-1233). Defined complex medium [yeast nitrogen base without amino acids (Difco) 1.7 (g/l); asparagine, 2; NH4NO3, 1; glucose, 10; pH to 6.0 with Na2HPO4) was used to select for bialaphos and sulfonylurea resistance. Bialaphos (25 ug/ml) and chlorimuron ethyl (100 ug/ml) were dissolved in water and dimethylformamide, respectively, and added to media after autoclaving. Chlorimuron ethyl can be purchased from Chem Service, P. O. Box 3108, West Chester, PA 19381-3108, 610-692-

3026. We have not tested whether the formulated herbicide Classic[reg]. can be used for selection instead of the technical material.

Table 1. Plasmids with bialaphos, hygromycin and sulfonylurea resistance

Base plasmid [E] /cloning site for fungal selectable marker	Baialaphos resistance	Hygromycin resistance	Sulfonylurea resistance
Selectable marker in polylinker			
pUC19& or pUC118*/SalI	pCB1517[&]	pCB1003 [& A]	pCB 1528*
pBluescript II SK-/ SalI	pCB1635	pCB1636	pCB1637(NOT AVAILABLE)
pBC KS-/ SalI	pCB1546	pCB1490	pCB1551
pCB1519/XhoI	pCB1524[B]	[C]	not made
pCB1520/XhoI	pCB1525[B]	[D]	pCB1550[B]
Selectable marker outside lacZ gene			
pBluescript II SK-& or KS+*/SspIF	pCB1530[&]	pCB1179*	pCB1532[&]
pBC KS-& or pBC SK+/BclIG	pCB1531	pCB1004[A]*	pCB1533[&]
pLitmus 28/HpaIF	pCB1534	pCB1535	pCB1536
pLitmus 39/HpaIF	pCB1537	pCB1538	pCB1539

[A]Reported previously (Carroll et al. 1994 Fungal Genet. Newsl. **41**:22)
[B] Digestion of these plasmids with *Sma*I yields the respective selectable marker as a blunt restriction fragment
[C, D] The hygromycin resistant equivalents of these plasmids were not constructed because a blunt fragment can be obtained by *Hpa*I digestion of pCB1003 and pCB1004, respectively.
[E] pBluescript II and pBC, Stratagene; pLitmus, New England Biolabs; pCB#, this paper. All the base plasmids are ampicillin resistant except pBC KS- and pCB1520 which are chloramphenicol resistant.
[F] Selectable marker obtained as *Sma*I fragment from pCB1525 or pCB1550 or as *Hpa*I fragment from pCB1004.
[G] Selectable marker cloned as *Sal*I fragment (half-site filled in) into the *Bcl*I site (half-site filled in)

Table 2. Restriction enzyme sites in the polylinkers of plasmids with intact *lac*Z geneA

Vector Polylinker	Polylinker Restriction Enzymes[B]
SK or KS in pBC or pBluescript	SstI ^ SstII* NotI XbaI SpeI BamHI SmaI PstI EcoRI EcoRV HindIII ClaI SalI XhoI ApaI KpnI

Litmus 28	SnaBI SpeI BglII NsiI BssHII^ BsiWI XhoI EcoRI PstI EcoRV BamHI HindIII NcoI^# AatII^# AgeI^ XbaI AvrII SstI^ KpnI StuI^ AflII*#
Litmus 39	SnaBI SpeI SalI SphI*^ I BsrGI BspEI# MluI^ EagI*# NheI^ EcoRI BamHI EcoRV PstI HindIII KasI^ NgoMI*^ MfeI^ ApaI StuI^ AflII*#

[A] These are the plasmids in the second part of Table 1
[B] All restriction enzyme sites in the vector polylinker are unique for the entire plasmid including the fungal selectable marker except for those sites indicated where a restriction site is also present in the fungal selectable marker for bialaphos (*), hygromycin (#), or sulfonylurea (^) resistance.

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