

A series of vectors for fungal transformation

Jim Sweigard, Forrest Chumley, Anne Carroll, Lennie Farrall, and Barabara Valent - Central Research and Development, DuPont, P. O. Box 80402, Wilmington, DE 19880-0402 USA

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* *ILV1* 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* *ILV1* 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 *SaII* 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 *SaII* fragments into the polylinker of pUC, pBluescriptII, and pBC vectors. They were also cloned as *SaII* fragments into the *XhoI* site of a modified polylinker in pBluescript II and pBC (pCB1519 and pCB1520, respectively) where the *XhoI* site is flanked on both sides by *SmaI* 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 sulfonyleurea resistance

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

^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*II site (half-site filled in)

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

Vector Polylinker	Polylinker Restriction Enzymes ^B
SK or KS in pBC HindIII	SstI ^ SstII* NotI XbaI SpeI BamHI SmaI PstI EcoRI EcoRV
or pBluescript	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 sulfonyleurea (^) resistance.

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