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Abstract

The *qa-2+* gene has been widely used as a selectable marker in Neurospora transformations (Akins and Lambowitz 1985. Mol. Cell. Biol. 5:2272-2278). A plasmid (pMSN1) has been constructed to facilitate the cloning of selected genes into a *qa-2+* vector. The system takes advantage of the blue/white screening possible when cloned fragments are inserted into the *E. coli lacZ* (beta-galactosidase) gene, and also facilitates subsequent sequencing of the cloned genes.

A *qa-2*+-pGEM vector for Neurospora transformations

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The *qa-2*+ gene has been widely used as a selectable marker in Neurospora transformations (Akins and Lambowitz 1985. Mol. Cell. Biol. 5:2272-2278). A plasmid (pMSN1) has been constructed to facilitate the cloning of selected genes into a *qa-2*+ vector. The system takes advantage of the blue/white screening possible when cloned fragments are inserted into the *E. coli lacZ* (beta-galactosidase) gene, and also facilitates subsequent sequencing of the cloned genes.

The *qa-2*+ gene of *N. crassa* was inserted into a non-coding region (the *Nde*I site) of the parental pGEM-3Zf(+) (Promega) to create the pMSN1 vector (Figure 1). After pGEM-3Zf(+) was digested with *Nde*I, the recessed 3' ends were filled in with the Klenow fragment of DNA polymerase I and the blunt-ended molecules were phosphatase-treated (Cobianchi and Wilson 1987. Methods Enzymol. 152:94-110). An approximately 2.5 kb *Bam*HI fragment (containing the 2.1 kb *Bam*HI-*Hin*dIII fragment of the *qa-2*+ gene (Geever et al. 1989. J. Mol. Biol. 207:15-34) plus a 345 bp *Hin*dIII-*Bam*HI fragment from pBR322) was made blunt with Klenow fragment, gel-purified and ligated to the prepared pGEM-3Zf(+) vector. The 5.8 kb pMSN1 plasmid was transferred into NM522 by selecting ampicillin resistant transformants (Miller 1987. Methods Enzymol. 152:145-170).

The pMSN1 vector contains a multiple cloning site within the coding sequences of the *lacZ* gene. When an *E. coli* strain harboring the *lacZ* M15 gene on an F' (such as NM522; Gough and Murray 1983. J. Mol. Biol. 166:1-19) is transformed with pMSN1, those transformants form blue colonies on indicator plates containing IPTG and X-Gal. Strains harboring recombinant vectors with inserts at the multiple cloning site of pMSN1 (which disrupt the *lacZ* gene) form white colonies on indicator plates. The pMSN1 vector contains SP6 and T7 RNA polymerase promoters that flank the multiple cloning site, allowing the in vitro synthesis of RNA from either strand of the cloned inserts. Also, pMSN1 contains the origin of replication of the filamentous bacteriophage f1, which facilitates the production of single-stranded DNA for sequencing.

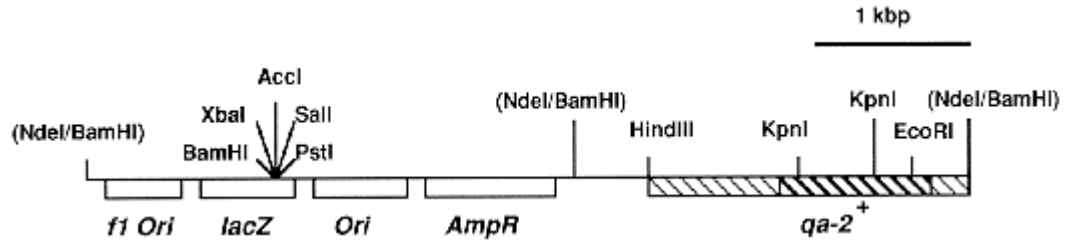

Figure 1. The pMSN1 vector (approximately 5.8 kb). The circular construct has been linearized at one of the destroyed *Nde*I/*Bam*HI sites (indicated in parentheses). The open boxes represent regions within the pGEM-3Zf(+) sequence, while the dark-slashed and light-slashed boxes

indicate coding and non-coding areas, respectively, with the Neurospora *qa-2+* insert. The positions of the beta-galactosidase gene (*lacZ*), the ampicillin resistance gene (*AmpR*), the origin of replication for growth in *E. coli* (Ori), and the filamentous bacteriophage f1 origin of replication (f1 Ori) are shown. The five unique enzymes (*Bam*HI, *Xba*I, *Acc*I, *Sal*I and *Pst*I) cutting within the multiple cloning in the *lacZ* gene are shown. The multiple cloning site also contains sites for *Eco*RI, *Sac*I, *Kpn*I, *Sma*I, *Ava*I, *Hinc*II, *Sph*I and *Hin*dIII, which cut within the Neurospora sequence (not shown). Selected sites within the *qa-2+* insert are indicated. The plasmid is drawn approximately to scale.

The recombinant vectors are introduced into Neurospora by transformation of *qa- 2;aro-9* spheroplasts and selection for prototrophy (growth in the absence of aromatic amino acid supplement; Akins and Lambowitz 1985. Mol. Cell. Biol. 5:2272-2278). We examined the progeny obtained with recombinant pMSN1 constructs (Nelson and Metzenberg 1992. Genetics, in press) and showed that nearly half contained single inserts of the transforming DNA sequences. The transformants can be analyzed for recombinant DNA phenotypes and/or used in RIP-mediated gene disruption experiments (Selker et al. 1989. Fungal Genetics Newsl. 36:76-77).

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