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Abstract

The quinic acid inducible qa-2 promoter of *Neurospora crassa* has been used to express cloned genes by a number of different groups. However, most of the commonly available sources of this promoter require extensive sub-cloning and modification before they can be used as effective expression vectors. We report the construction of two plasmids that allow direct cloning and subsequent expression in *N. crassa*. Each plasmid also has a number of useful features to aid in detecting and modifying insert sequences.

Vectors for expression and modification of cDNA sequences in Neurospora crassa.

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The quinic acid inducible qa-2 promoter of Neurospora crassa has been used to express cloned genes by a number of different groups. However, most of the commonly available sources of this promoter require extensive sub-cloning and modification before they can be used as effective expression vectors. We report the construction of two plasmids that allow direct cloning and subsequent expression in N. crassa. Each plasmid also has a number of useful features to aid in detecting and modifying insert sequences.

The first of these plasmids, pMYX2 is a derivative of pGEMQa-2P, originally constructed by S. Kang and obtained from J. Kinsey (University of Kansas Medical Center). The qa-2 promoter region including the QA-1F binding sites and transcriptional start point were cloned as a 1 kilobase (kb) SacI-KpnI fragment into the corresponding restriction sites of the Promega plasmid pGEM3Zf(+). We inserted the trpC terminator and poly-A signal sequences of Aspergillus nidulans (Cullen et al. 1987. Gene 57:21-26) from plasmid pMP6 (M. Plamann, Texas A&M University) as a 0.7 kb BamHI fragment into the unique BamHI of pGEMQa-2P to form an intermediate plasmid, pMYX1. In order to provide a selectable marker for use in N. crassa the benomyl resistant -tubulin gene of pSV50 (Orbach et al. 1986. Mol. Cell. Biol. 6:2452-2461) was inserted into the unique SalI site of pMYX1 as a 2.6 kb SalI fragment to form pMYX2 (Figure 1a). The second of the plasmids reported here, pMYX10 is a derivative of pWF1, originally constructed by Fecke et al. (1993. Fungal Genetics Newsl. 40:34-35). These workers placed both the qa-2 promoter, including all the sequences included in pMYX2, and the qa-4 terminator and poly-A signals into the Pharmacia plasmid pT7T3 to form pWF1 (Fecke et al. 1993. Fungal Genetics Newsl. 40:34- 35). We inserted the hygromycin resistance cassette originally constructed by Staben et al. (1989. Fungal Genetics Newsl. 36:79-81) and modified to include a N. crassa cpc-1 promoter by M. Plamann from plasmid pMP6 as a 3.1 kb HindIII fragment into the unique HindIII site of pWF1 to form pMYX10 (Figure 1b). Insert orientations in both pMYX2 and pMYX10 were determined by restriction mapping.

Both pMYX2 and pMYX10 can be used as expression vectors by cloning the sequences of interest into the unique SmaI site immediately downstream of the qa-2 transcriptional start point in each plasmid. The insert should carry the appropriate translational control sequences necessary for successful expression in N. crassa but does not require the presence of any transcriptional signals. Therefore, these vectors are quite suitable for expression of cDNA encoded gene products. Integration of the recombinant plasmids into the N. crassa genome can be monitored by selecting transformants for the tightly linked resistance markers. The presence of the promoter and insert regions of the plasmids can be verified by either genomic Southern or PCR analysis. Both plasmids include unique bacteriophage T7 promoters immediately upstream of the qa-2 promoter region. Use of a primer specific to the T7 promoter and a single primer homologous to sequences within the insert allows the presence of at least a single, intact recombinant within the genome to be detected by PCR amplification of the qa-2 promoter/insert

junction. In addition, both plasmids contain f1 filamentous phage origins, (+) orientation in pMYX2, (-) orientation in pMYX10, allowing site-directed mutagenesis of recombinants without requiring subcloning from the expression vector.

These vectors have been deposited in the FGSC. This work was supported by an SBIR Phase I award from NIAID to Mycotox, Inc.
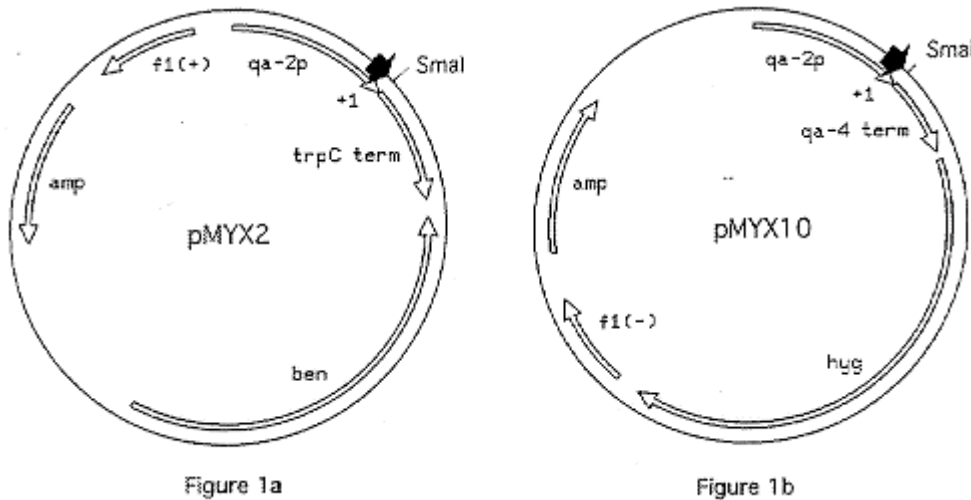

Figure 1: Map of pMYX2 (1a) and pMYX10 (1b). The qa-2 promoter region of each plasmid is indicated as qa-2p. The transcriptional start site is denoted as the large, filled arrow marked +1 on each map. The location of the unique SmaI cloning site is also indicated. The terminator regions are denoted as trpC term for pMYX2 and qa-4 term for pMYX10, indicating the Aspergillus nidulans trpC terminator and the N. crassa qa-4 terminator, respectively. The markers used for selection of transformants are indicated as ben (benomyl resistance) for pMYX2 and hyg (hygromycin resistance) for pMYX10. The f1 origins of replication and the ampicillin resistance genes of each plasmid are denoted as f1(+ or -) and as amp. The complete predicted sequence of pMYX2 is available.