meric in SDS-PAGE following boiling in loading buffer might in fact be oligomeric, leading to multiple or broadened peaks in size exclusion or ion-exchange chromatography. Therefore, we recommend adding EDTA to eluted IMAC fractions immediately after collection. Excess EDTA and EDTA-metal complexes can then be removed by dialysis or size-exclusion chromatography.

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Expression Vector Containing an N-Terminal Epitope Tag for Dictyostelium discoideum


For studies of gene function, an epitope tagging system can provide several advantages. Epitope tagging involves the fusion of a small, well-characterized epitope (usually in the form of a short peptide) with another protein. This method has the obvious advantage of bypassing the requirement for antibodies specifically against the protein of interest. Furthermore, an epitope-tagged protein can readily be distinguished from the endogenous protein.

The cellular slime mold Dictyostelium discoideum is a eukaryote used as a model system to study the processes of development and many aspects of cell biology. To date, several expression vectors for this organism have been designed for epitope tagging on the C termini of proteins (1,8), but very few N-terminal tags are available (2,9,12). Many proteins, such as ras proteins, do not permit C-terminal tags because posttranslational processings on their C termini are essential for their function. The green fluorescent protein (GFP) has been used as an N-terminal tag in several Dictyostelium studies (9,12). The application of this GFP tag, however, can be limited by its high molecular weight (9). We describe here the successful application of a new N-terminal epitope-tagged expression vector for studying gene functions in Dictyostelium discoideum.

The peptide used for epitope tagging contained the first 12 amino acids of the phage T7 gene 10 (7). Antibodies to this peptide are commercially available (Novagen, Madison, WI, USA). To construct the vector, we started with the Dictyostelium shuttle vector EXP4(+) (4). It can be easily maintained and amplified in E. coli in the presence of the selective drug ampicillin. The EXP4(+) vector contains the G418-selectable marker, which is used for transforming DNA into the Dictyostelium genome (6). It also contains a well-characterized Dictyostelium constitutive promoter, the actin 15 promoter (3), which has a known expression pattern.

To construct an efficient epitope-
tagged expression plasmid for Dictyostelium, two additional factors have been considered. First, the Dictyostelium genome is extremely codon-biased. Dictyostelium prefers AT-rich sequences, because the average GC content of the coding region is only around 30%–40%, and the non-coding 5′ and 3′ regions and introns are less than 20% GC (10). For example, CGC is a rarely used arginine codon (0.8%) than 20% GC (10). For example, CGC (Figure 1). The in-frame multiple cloning site start (ATG) of the T7 gene. This result – inserted a series of 5 As in front of the ATG initiation codon (5). There is a rarely used arginine codon (0.8% usage), while CGT (40%) and AGA (56%) are more preferred. To optimize the expression in Dictyostelium, several codons of the T7 gene have been changed to favor Dictyostelium usage (Figure 1).

The majority of known Dictyostelium coding regions have a series of adenosines, usually 5 to 6, in front of the ATG initiation codon (5). Therefore, in a second modification, we inserted a series of 5 As in front of the start (ATG) of the T7 gene. This resulted in the plasmid called pCFC5 (Figure 1). The in-frame multiple cloning site (MCS) immediately adjacent to the T7 tag allows for the ready insertion of a Dictyostelium gene of interest.

We have successfully used pCFC5 to express a Dictyostelium RasG gene in the wild-type strain KAX-3 (Figure 2). The shift in apparent molecular weight is not unusual for epitope-tagged proteins (11). The low level of overexpression, when compared to the endogenous ras protein, is likely due to the deleterious effects of ras overexpression during selection (unpublished observations), rather than anything inherent to the construct. The small T7 epitope does not interfere with Dictyostelium cellular functions (data not shown) and can be used in Western blots and immunoprecipitation (data not shown) studies using the commercial anti-T7 antibodies.

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