Regulated expression of foreign genes fused to *lac*: control by glucose levels in growth medium

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Submitted January 10, 1990

Expression of cloned genes from the *E. coli lac* promoter has been used by many investigators to produce a variety of foreign or mutant gene products. The commonly used expression plasmids are the pUC series of plasmids, or derivatives thereof (1). The expression of genes cloned downstream of the *lac* promoter can be conveniently induced by addition of β-isopropylthiogalactoside (IPTG), however, in the absence of inducer there is a basal level of expression from this promoter (2). It is this 'leakiness' which has limited the utility of this cloning strategy when the cloned gene product may be lethal to the cell. This is unfortunate because many of the initial cloning steps are carried out in this plasmid type and procedures for rapid sequencing of these plasmids are available.

We have been studying the expression of the *E. coli* gene for translation initiation factor 3 (IF3) (3). In the course of these experiments we developed a method for circumventing the inherent 'leakiness' of *lac* by growing the transformed cells harboring the recombinant pUC plasmid in the presence of small amounts of glucose. The plasmid pDD1 contains the gene for IF3 under the control of *lac* in pUC19. Overnight cultures of JM107-transformed cells were inoculated into 250 mL of L broth in the presence or absence of glucose, grown at 37°C to OD_{600} = 0.9 and subsequently induced for three hours with 0.4 mM IPTG. Cells were harvested after induction and analyzed for IF3 production by immunoblotting. When the initial growth is carried out in the presence of 0.4% glucose induction by IPTG is inhibited (lane 2). In contrast, cultures grown in lower concentrations of glucose (0.05%−0.1%) can yield maximal overproduction of the cloned gene product after IPTG induction (lanes 5 and 9). The key observation is that in the absence of IPTG the levels of IF3 are identical to the amounts observed in the pUC19-transformed control (lanes 4, 6, 8 and 10). These results can be explained in the following manner. The presence of glucose in the growth medium represses expression from *lac* (4) during log phase growth but the glucose is consumed by the time the cultures achieve an OD_{600} of 0.9. At this point the addition of IPTG will result in induction of expression from the *lac* promoter.

Thus, it is possible to repress transcription from the *lac* promoter to virtually 100% by the initial addition of low concentrations of glucose, thereby eliminating any deleterious background expression. We believe that with the modified growth conditions defined here pUC-derived plasmids may be employed in studies requiring large-scale expression of potentially lethal gene products with relative ease.

ACKNOWLEDGEMENT

This work was supported by NIH grant GM29265.

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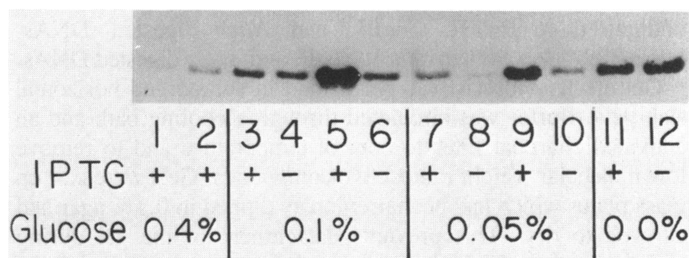

Figure. Immunoblot analysis of IF3 synthesized in JM107-transformed strains. Lanes 1, 3, 4, 7 and 8: pUC19; lanes 2, 5, 6 and 9–12: pDD1.

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