



Plating Method *e.coli* inoculation

Alt method for trouble starters



MSUM Biochemistry &
Biotechnology

Introduction *For working with toxic proteins that are leaky expressers and toxic*

Protocol

1. A single colony of plasmid-containing cells is suspended in 200 ml water and vigorously shaken.
2. The suspension is plated on an LB plate containing the appropriate antibiotic and incubated overnight at 37°C.
3. All colonies (typically 1000-10,000) are scraped-off and suspended in 400 ml LB medium. This suspension usually has an OD600 of approx. 0.4.
4. The culture can be grown to the desired OD and induced to start expression.

Reference: Suter-Crazzolara, C. & Unsicker, K. (1995) Improved expression of toxic proteins in *E. coli*. *BioTechniques* 19, 202-204.