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Title:

Optimization of Isoelectric Focusing Parameters towards the Development of a Complement to the AMES Test

Abstract:

The AMES test is designed to detect the presence of a mutagenic compound. It has a number of inherent flaws resulting in some compounds reporting falsely as positives or negatives. The RIT Proteomics group is developing a method, utilizing 2D gel electrophoresis, which would act to complement the AMES test. Potential carcinogenicity of various compounds will be determined through analysis of the protein expression of *Pseudomonas pudita* KT2440 grown in the presence of the compound. Clear separation of protein spots on the polyacrylamide gels is necessary to perform accurate analysis using Phoretix software. The goal of this project is to increase reproducibility and resolution of 2D electrophoretic gels through a variation of multiple parameters involving both the rehydration and isoelectric focusing steps. Two common rehydration techniques for IPG strips - active and passive rehydration - are evaluated in replicate sets. Direct comparison of varying times is performed with both processes. A standard isoelectric focusing program and one that has been newly developed and differs in voltage settings and ramping modes will also be examined.