

Department of Biological Sciences  
Seminar Announcement



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Temperature sensitive (Ts) mutants are a powerful tool to study gene function *in vivo*. Ts mutants are typically generated by random mutagenesis followed by laborious screening procedures. Using the *E. coli* cytotoxin CcdB as a model system, we outline simple procedures for generating Ts mutants through site directed mutagenesis. Putative buried, hydrophobic residues are selected through analysis of the protein sequence. Residue burial is confirmed by ensuring that substitution of the residue by Asp leads to protein inactivation. At such sites, a Ts phenotype can typically be generated either by a) substitution of two predicted, buried residues with the eighteen remaining amino acids b) introduction of Lys, Ser, Ala and Trp at 3-4 predicted buried sites or c) expression in an inducible system that permits dose dependent induction. When expressed at an appropriate level, virtually any active mutant (including the wild type protein) will show a Ts phenotype. The methodology was used to generate twenty three tight Ts mutants of CcdB at four predicted buried sites. The same strategy was also used to obtain several Ts mutants of the yeast transcriptional activator, Gal4

### Approaches to the design of temperature sensitive mutants of a globular proteins from amino acid sequence.

**Date:** 22 Jan 2003  
**Time:** 4 - 5pm  
**Venue:** LT32  
**Host:** Prof K Easwaran

**All are welcome**