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A SITE-DIRECTED MUTAGENESIS INTERROGATION OF THE GENE ENCODING
THREONINE DEHYDRATASE/DEAMINASE (TD) IN *ARABIDOPSIS THALIANA*

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TD is feedback inhibited by isoleucine, the end product of this biosynthetic pathway. Mutagenized seed stocks yielded variants of TD that were insensitive to feedback inhibition and led to isoleucine overproduction. To maximize the overproduction of isoleucine, several point mutations will be site-directed in *OMRI*, the wild type allele that encodes TD in *Arabidopsis thaliana*. One such point mutation has been introduced in the conserved regulatory region of TD, and the mutant allele has been cloned in the prokaryotic expression vector pTrc99A. This vector was introduced into the *E. coli* strain DH5a. The effects of this mutation on TD's regulatory properties and the overproduction of isoleucine will be assessed. Several additional site-directed point mutations will be introduced in *OMRI*. The point mutations that prove to lead to isoleucine overproduction in *E. coli* will be cloned into a plant expression vector and engineered into wild type *Arabidopsis*. Enzyme assays and HPLC analysis in transgenic plants will identify the specific point mutations that alter TD feedback regulation and lead to overproduction of isoleucine respectively.