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Degenerate PCR Cloning of Isopropylmalate Synthase, The Regulatory Enzyme of the Leucine Biosynthetic Pathaway, From Arabidopsis Thaliana

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DEGENERATE PCR CLONING OF ISOPROPYLMALATE SYNTHASE, THE REGULATORY ENZYME OF THE LEUCINE BIOSYNTHETIC PATHWAY, FROM *ARABIDOPSIS THALIANA*

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The biosynthesis of the essential amino acid leucine involves the participation of isopropylmalate synthase (IPMS), isopropylmalate isomerase, isopropylmalate dehydrogenase, and a transaminase. These enzymes are well characterized in yeast and bacteria. Functional cloning attempts in *Arabidopsis* have elucidated the sequences of isopropylmalate isomerase and isopropylmalate dehydrogenase, but not isporopylmalate synthase. Isopropylmalate synthase is the site of negative feedback inhibition by the end product leucine. Being the regulatory enzyme in leucine biosynthesis, IPMS is highly conserved among organisms of different taxa. Utilizing amino acid alignments of bacteria, yeast, and two plant species, conserved regions of IPMS were identified and degenerate PCR primers were constructed. PCR yielded a 1.3 kb fragment, which will be purified, cloned, and sequenced. Upon confirmation of being a fragment of IPMS, the 1.3 kb fragment will be used as a probe to screen cDNA and genomic DNA libraries of *Arabidopsis*. Complementation tests using a leucine auxotrophic *E. coli* strain will be used to confirm the functionality of *Arabidopsis* IPMS. Molecular and biochemical characterization will be used to identify the regulatory regions of IPMS of *Arabidopsis thaliana.*