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Isolation, Amplification, and Expression of BCHC

Eileen Forde Illinois Wesleyan University

David Bollivar, Faculty Advisor *Illinois Wesleyan University*

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THE JOHN WESLEY POWELL STUDENT RESEARCH CONFERENCE - APRIL 2003

Poster Presentation P15

ISOLATION, AMPLIFICATION, AND EXPRESSION OF BCHC

<u>Eileen Forde</u> and David Bollivar* Department of Biology, Illinois Wesleyan University

The bchC gene of Chloroflexus aurantiacus has been identified by the use of computer based sequence homology searches. To test for function of the proposed gene, it was necessary to create a system so that the polypeptide encoded by the gene could be expressed and identified. Oligonucleotide primers were designed to amplify the bchC region so that this gene could be cloned. The amplified gene was cloned and sequenced, and ultimately placed in an expression vector that resides in the bacterium E. coli. The protein was then expressed using an arabinose induction system. SDS-PAGE and Western analysis have been used to confirm that the polypeptide is expressed properly. Enzymatic assays were then performed to test for function of the bchC gene product. The demonstration of the enzyme activity, 2-hydroxyethyl bacteriochlorophyllide oxidase, was successful allowing the designation of this gene as sufficient for the enzymatic activity.