# Site-specific mutagenesis studies of PyrD and PyrR genes involved in riboflavin biosynthesis

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Riboflavin (vitamin B2) is the precursor of the flavin cofactors, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). Plants and many microorganisms, synthesized riboflavin but animals must obtain it from dietary sources (Power, 2003). The deaminase and reductase steps in riboflavin biosynthesis are catalyzed by the bifunctional enzyme RibD in *Escherichia coli*. Plants have two homologs of RibD, PyrD (At4g20960) and PyrR (At3g47390). The plant PyrD protein is known to be a degenerate deaminase-reductase in which the reductase domain has lost critical substrate binding residues and hence activity. The plant three-domain PyrR protein has lost the zinc-binding residues and is recently shown lacks deaminase activity. Although the missing zinc finger binding residues of reductase domain in PyrD, and changes in critical substrate binding residues in the reductase domain of PyrR are shown using sequence alignments but have not studied experimentally.

In this project, we used gene synthesis approach to change the critical substrate-binding residues of the deaminase domain of the PyrR gene and the addition of the missing Zinc finger binding residues in the PyrD gene. The mutated genes were then cloned in plasmid pETDuet1. We have tested the mutated PyrD and PyrR genes' functionality in a riboflavin auxotrophic *E. coli* RibD deletant mutant (ΔribD::Kan) strain. The mutated PyrR gene was able to fully complement the riboflavin auxotrophy of *E. coli ΔribD:*:Kan but the mutated PyrD gene failed to complement. We are currently using sequence alignments tools to find any other conserved residues that might be important for the reductase domain function.