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Cloning of STH3 gene in pCAMBIA 1303 vector

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STH3 is an *Arabidopsis* B-box protein involved in light dependent development. It has two N terminal B-boxes that interact with bZIP transcription factor HY5, which is a positive regulator of the light signaling pathway of plants. The HY5 is involved in the regulation of transcriptional activity of the promoters containing light responsive elements. Both HY5 and STH3 interact with COP1. STH3 is identified as a positive regulator in photomorphogenesis acting in concert with STH2 and HY5, while also being a target of COP1-mediated ubiquitination. Therefore, we intended to study the role of STH3 in plant systems, by unraveling the nature of interactions of STH3 with light inducible gene promoters in plants. Furthermore to study the effect of STH3 in plant systems by overexpressing the STH3 is expected via carrying out *Agrobacterium* mediated transformation of STH3 gene into rice. Our first attempt of this study was to clone the STH3 gene in pCAMBIA binary vector. We isolated the STH3 gene which has already been cloned into pRTL2 vector, and cloned it again into the plant binary vector pCAMBIA. This was achieved by digesting the available STH3-pRTL2 construct by the enzymes EcoR I and Xba I. Then STH3 gene was cloned into an EcoR I and Xba I double digested pCAMBIA binary vector. Subsequently, the obtained STH3-pCAMBIA recombinant clone was confirmed by two confirmation digestions. Two confirmation digestions were carried out as a EcoR I / Xba I double digestion and a Pst I single digestion. The recombinant STH3-pCAMBIA construct was of ~ 13kb in size. The double digestion and the single digestion resulted in the correct sizes of the fragments ~12kb, ~1kb and ~12kb and ~500bp respectively. These confirmation digestions provided strong evidence for the successful ligation of the plant binary vector pCAMBIA 1303 and the STH3 gene. The recombinant plasmid is ready and will be used to transform into local rice varieties via *Agrobacterium* mediated transformation.