Cas9 Expression In *Pichia pastoris* GS115

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Introduction: The CRISPR-Cas9 system has revolutionized the field of genetic engineering. Originated from bacteria's immune system, it was adapted for genome editing in different organisms, including yeasts, allowing in a simple manner, the deletion and insertion of genes by a sole enzyme, Cas9, which performs a double strand break in the targeted DNA, guided by a specifically designed small guide chimeric RNA. *Pichia pastoris* is an important resource for biotechnological and pharmaceutical approaches including heterologous protein production. Facilitating the genome edition in this organism could certainly be beneficial to the research and development of bioproducts. **Objectives:** Due to the large convenience and effectiveness in the usage of CRISPR genome-editing tool, this project aims to express Cas9 in *P. pastoris* to evaluate and further study Cas9 expression in this organism. **Material and Methods:** The Cas9 gene was amplified from the pX458 vector (Addgene) by PCR using specific primers and subsequently subcloned into the pGEM-Teasy vector (Promega). Digest reactions using restriction enzymes were conducted and the Cas9 gene was then cloned into the pPICHOLI-1 expression vector (MoBiTech®). pPICHOLI-1/Cas9 plasmid was used to transform *P. pastoris* GS115 strain and Cas9 expression was induced with methanol using the AOX1 promoter. Western blot with a monoclonal anti-Cas9 antibody was performed to confirm its expression. **Results and Discussion:** A Cas9 band with the expected size of approximately 5Kb was detected by PCR. The pGEM-Teasy/Cas9 and pPICHOLI-1/Cas9 constructions were both confirmed by restriction enzyme digestion and sequencing. Cas9 expression in *P. pastoris* GS115 was detected only in positive clones in the western blot, confirming its expression, and a high expressing clone was selected. Purification steps will follow. **Conclusions:** Cas9 was successfully expressed in *P. pastoris*, thus facilitating future studies regarding genome editing and protein production in referred organism.

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