CRISPR/Cas-9 Knockout of LOX3 in *Oryza sativa*

Hannah Jo
Cornell University

Peng Yan, Mo Yi
CNHRRDC

Purpose:

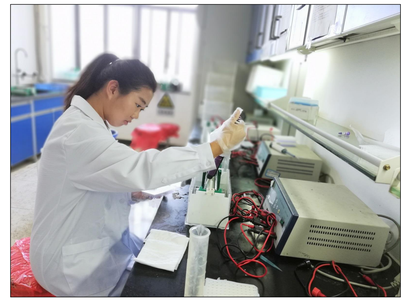
The objective of this experiment was to knockout the Lipoxegenase-3 (LOX3) gene from *Oryza sativa* using the targeted genome editing technology of CRISPR/Cas-9.

Background:

- CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats systems
- Two components: guide RNA (gRNA) and the associated endonuclease, which in this case is Cas-9
- CRISPR/Cas-9 system used to knockout a target gene in rice, *Oryza sativa*, and replace it with an alternative sequence.
- LOX3: gene responsible for catalyzing the peroxidation of polyunsaturated fats in rice seeds; lipid metabolism affects lipid membrane degradation and seed deterioration, thus affecting seed longevity.
- Current storage time and viability of rice seeds is 2 years. Removing LOX3 from the gene of *Oryza sativa*, will maintain the integrity of polyunsaturated fatty acids for a longer period of time.

Method:

1) Locate the desired Cas9 target site in the targeted gene, LOX3, and design the gRNA.
2) Design primers to confirm the mutation of
3) Copy the gRNA into the cloning vector and confirm the successful construction with agarose gel electrophoresis.
4) Clone the newly constructed vector expressing the gRNA into the destination vector carrying the Cas-9 expression.
5) Cultivate E. coli colonies inoculated with the final vector, and identify positive mutants.
6) Sequence the plasmid to verify the positive result.



Results:

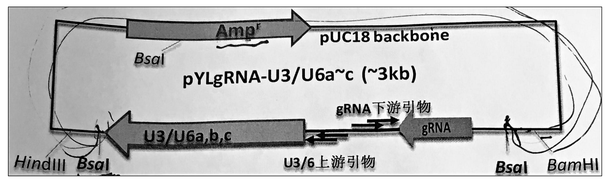


Fig. 1: Cloning Vector

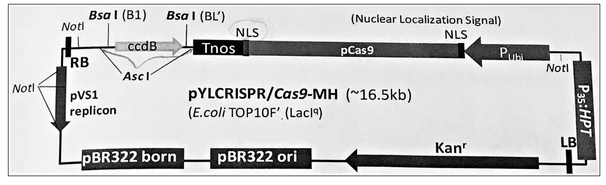


Fig. 3: Destination Vector

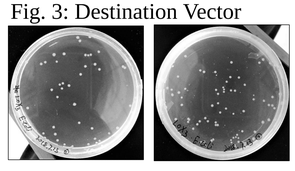


Fig. 4: E. coli Colonies

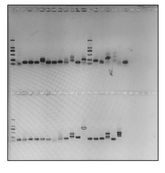


Fig. 5: Confirmation of positive mutant.

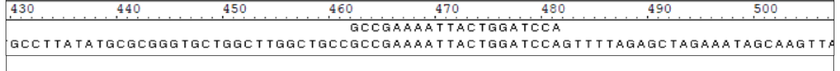


Fig. 6: (Top line) designed gRNA; (Bottom line) LOX3 successfully knocked out

- Site G chosen to be the Cas9 target site in LOX3.
- One out of 36 E. coli single colonies cultivated, resulted in a successful mutation.

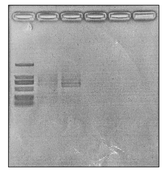


Fig. 2: Confirmation of construction of new vector.

Conclusion & Future Work:

- CRISPR/Cas-9 was successfully used to knockout LOX3 from *Oryza sativa*.
- Transfer successfully mutated DNA into desired line of rice for planting to create a new transgenic line of rice; observe polyunsaturated fat levels of transgenic seeds over time.

Personal Experience:

My eight weeks in Changsha, China were eye-opening, awe-inspiring, and life-changing. The professional experience and life experience I gained from this internship is truly incredible. I am so grateful to have worked at an international research institute, collaborating with scientists to develop improved varieties of rice and fight against world hunger

Acknowledgements:

This research was made possible by the World Food Prize and the China National Hybrid Rice Research & Development Center.