

# Activation of Regeneration Candidate Genes

## Background

In 2019, Dr. Hairong Wei selected some regeneration candidate genes based on network analyses. Also, RNA-Seq from the callus experiments (type II vs. I and fast vs. slow) were used as the reference. In total, 31 genes were selected. Among these 31 genes, 9 genes were selected for the validation using various strategies.

## New strategy for validation

Yiping Qi shared a gene expression activation system with CRISPR/dCas9. We would like to try the system to validate all 31 genes and other genes that can be possibly added.

Briefly, guide RNAs are designed for genes to be validated on their promoter regions. These guide RNAs are pooled to make CRISPR constructs, which are transferred to the embryotic callus tissue. A marker gene GFP is included in each construct. The construct pool should cover all genes with guide RNAs designed. The callus cells with an effective activation are anticipated to grow faster and better than other callus cells. We, therefore, are able to observe fluorescent sectors that can be dissected for further culture and analysis.

In the current design, we added the genes that were up-regulated in both type II and fast-growth calli as compared to their respective controls. In total 15 genes were identified and three of them are in the 2019 candidate list. Therefore, 12 new genes are added. In total, 43 genes are in the updated regeneration candidate gene list.

## Plan

In Qi's activation system, the vector pYPQ141D2.0 is used for expressing one guide RNA; the vectors pYPQ13[1-4]-tRNA2.0 are used for expressing multiple guide RNAs. Our plan is to design 1-2 guide RNAs for a gene if possible, then use pYPQ13<sup>1</sup>-tRNA2.0 and pYPQ13<sup>2</sup>-tRNA2.0 to integrate guide RNAs. In final constructs, two genes or one gene can be target by each construct.

A concern was raised that the number of combinations of guide RNAs requires thousands of clones to be made. The experiment will tell if this is possible. As a backup plan, pYPQ141D2.0 will be used to express one guide RNA. However, the design of the guide RNA construct for pYPQ141D2.0 is different from that for pYPQ13[1-4]-tRNA2.0. First, the overhang of guide RNA oligo pairs is different; Second, the 1st base of the guide RNA oligo must be A to be compatible to the rice U3 promoter that is used.

Based on the information collected, we will order all oligos designed for pYPQ13[1-4]-tRNA2.0. Some oligos with A as the 1st base from one of the pair can be used for pYPQ141D2.0. Therefore, only oligos from one pair need to be ordered.