

## **Transcript switches for fine-tuning of transgene expression**

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### **Project Goals: The project is aimed at developing novel tools to achieve stringent and precise control of transgene expression.**

Since its first establishment around 30 years ago, plant transgenic techniques have been widely used for basic biological research as well as for improving agronomic traits in crop plants. To maximize the benefit and minimize the side effect of genetic manipulation of plants, stringent and precise control of transgene expression is desired. In the current report, we will present two two-component systems to support tight transgene expression: a transgene activation switch (TAS) and a transgene repression switch (TRS) were developed. The TAS is based on an alternative splicing mechanism and involved a synthetic splicing cassette and a splicing factor in pair. By employing TAS, effector proteins in effector-triggered immunity were tightly regulated and background hypersensitive response was prevented. The strategy allowed for the first time the generation of healthy transgenic Arabidopsis that initiated hypersensitive response after supply of an artificial inducer. In contrast to TAS that allows transgene to be expressed when the activator protein is present, TRS will eliminate expression of the transgene when the repressor protein is present. Using both GFP and DsRed reporters and different promoter combinations, over two order of magnitude of transgene repression were achieved with TRS as well as simultaneous repression of multiple transgenes. TRS was validated in monocotyledonous and dicotyledonous species using transient and stable expression approaches. To further increase expression flexibility, several TRS systems are in current development. TAS and TRS demonstrate the possibility of fine tuning transgene expression by layering regulatory elements. The tools are likely applicable across species boundaries and may be used as standard elements for plant synthetic biology in the future.

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