Applications of CRIPSR/Cas9 breeding using flowering stimulation and precision lighting

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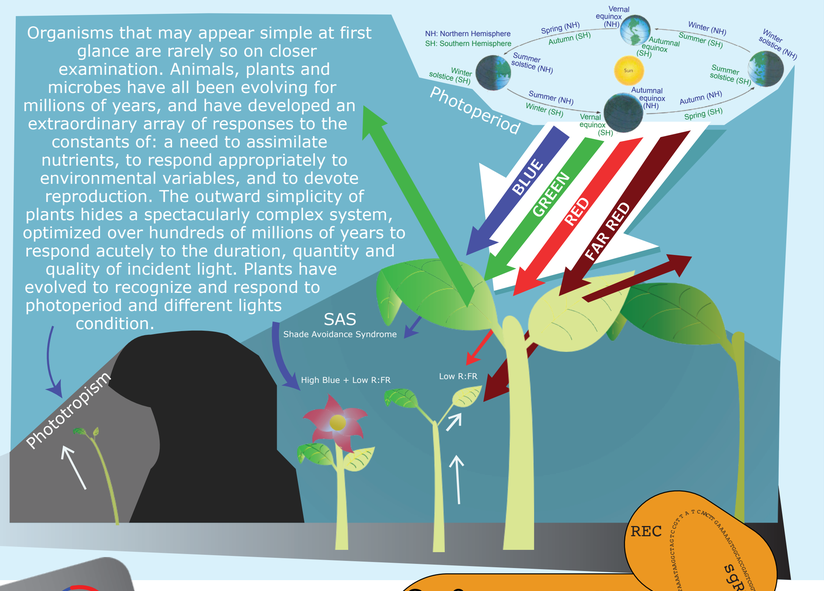
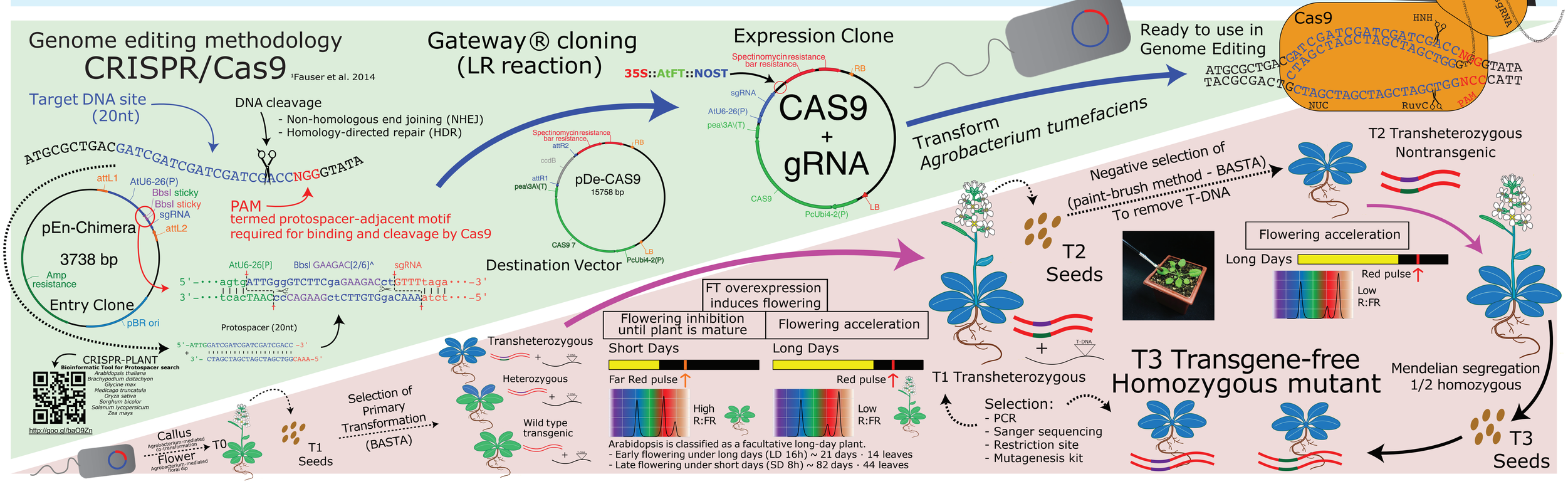
CRISPR/Cas9 has been shown to be an excellent tool for genome editing. In plants, it is starting to be used in breeding to create improved varieties that are genetically modified but transgene-free. This possibility is particularly interesting in the forestry industry. Because they represent a renewable resource, the demand for wood products is expected to continue to increase into the future. Breeding is one way of increasing the productivity of trees and forests, but genetic improvement of long-lived species like trees is problematic because the long generation times make incorporation of new characteristics prohibitively long-term. In most countries, tight regulations opposing the use of transgenics use in the field make direct integration of novel genetic material undesirable. CRISPR/Cas9 has several major advantages over previous transgene-based approaches and can work alongside conventional breeding programs by directly improving known yield-related loci or genes. In this work, we target reporter genes in Arabidopsis thaliana by using a modified CRISPR/Cas9 system and have added a strong ubiquitous CaMV 35S promoter, driving the FLOWERING LOCUS T (FT) gene. Ectopic expression of FT accelerates sexual development. To regulate the acceleration of flowering time to get viable flowers, we use precision lighting with different ratios of Blue, Red and Far Red light. The CRISPR/Cas9 mutated plants flower earlier than conventional plants as a result of the ectopic FT expression. This results in the rapid recovery of the second generation (F2) in Arabidopsis. We will use this technology to accelerate breeding in arboreal species such as Eucalyptus.

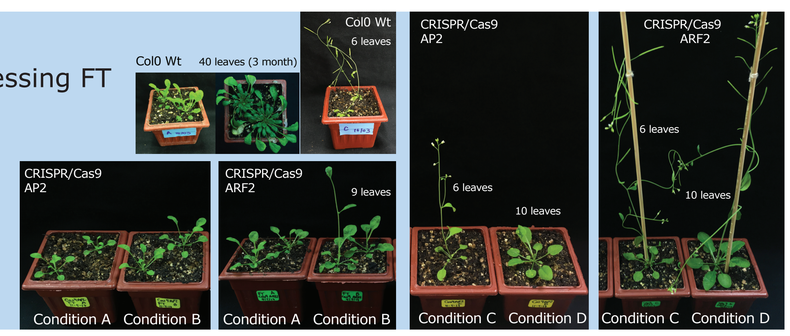




Precision light

Objective 1: Find a "light recipe" that could repress flowering in *Arabidopsis thaliana* used with CRIPSR/Cas9 system overexpressing FT
Objective 2: Find a "light recipe" that could accelerate flowering in *Arabidopsis thaliana* used with CRIPSR/Cas9 system
Under SAS condition, high intensity of Blue light can induce FT expression in the shoot apex

We are currently testing several light conditions, and here we present 4 light recipes:
A) 13uE of blue, 25uE of Red and 13uE of Far Red (High Red:Far Red), Short Day (8h)
B) 13uE of blue, 25uE of Red and 47uE of Far Red (Low Red:Far Red), Short Day (8h) + 15 min FAR RED pulse at 3am
C) 13uE of blue, 25uE of Red and 47uE of Far Red (Low Red:Far Red), Long Day (16h)
D) 13uE of blue, 25uE of Red and 13uE of Far Red (High Red:Far Red), Long Day (16h) + 15 min RED pulse at 3am



The overarching goal of this research is to develop methods to accelerate the production of homozygous, transgene-free, plants with mutations in genes of interest. To this end, we initially expressed the FT gene alongside CRISPR/Cas9 in Arabidopsis thaliana. Ectopic expression of FT accelerates flowering in species as diverse as Arabidopsis and Poplar. Blue light has also been shown to promote flowering in long-day plants and inhibit it in short day plants. We are using the FT CRISPR/Cas9 plants to investigate whether blue or red (LED) wavelengths can suppress flowering even in the presence of ectopic FT. We hope to be able to find a "light recipe" for the suppression of flowering, even in the presence of the flowering inducing FT. If a "light recipes" can be found where precision light can regulate the acceleration or suppression of flowering in the transgenic Arabidopsis plants, it is hoped that it can be found for other plants. We will modify the reporter genes ABH1, SAC9 (bleached cotyledons), PDS3, that results in albino plants and AP2, ARF2 that results in an increased seed size, to test the efficacy of the CRISPR/Cas9 construct. A list of candidate molecular regulators of vascular cambium structure and function has been studied in a previous review paper ([2]Matte et al. 2010) and will be used in subsequent experiments. Genes in lignin biosynthetic pathways, cellulose synthesis or wood formation are also possible targets because of their importance in the forestry industry.

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[1]Fauser, F., Schiml, S. and Puchta, H. (2014), Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*. Plant J, 79: 348–359. doi:10.1111/tpj.12554
[2]Matte Risopatron J.P. (2010), The vascular cambium: molecular control of cellular structure. Protoplasma 247:145–161. doi: 10.1016/j.tplants.2011.11.004

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