Universitat de Girona
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Reverse genetics in Arabidopsis using multiplex CRISPR-Cas9 system for phellem formation study

Societat Catalana de BIOLOGIA
I JORNADES DE BIOLOGIA DE GIRONA

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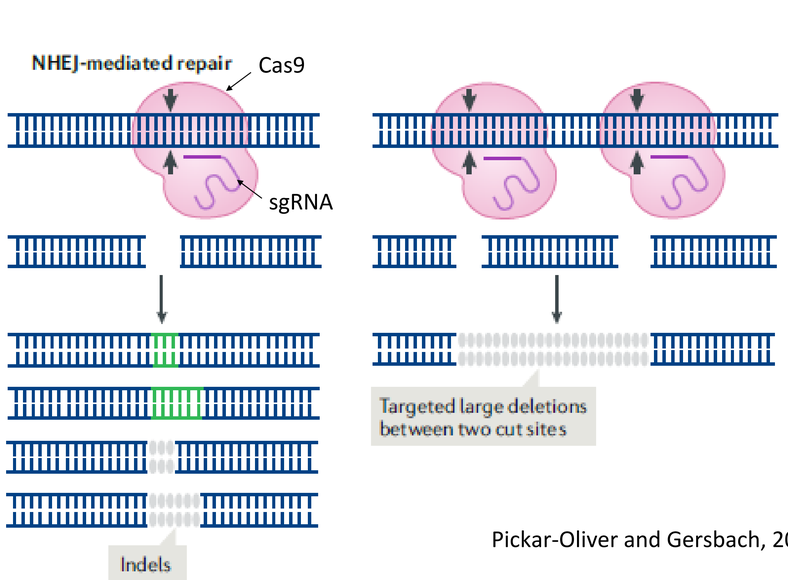
Secondary growth occurs in mature stems and roots of some dicotyledonous plants, like *Arabidopsis thaliana*, and gymnosperms. This growth involves the rupture of the epidermis and, consequently, the lack of its protecting function. In response, the plant forms a new protecting structure: the **periderm**. This is an apoplastic barrier formed by three layers: phellem or cork, phellogen and phelloderm. **Phellem** is the tissue that confers this protection by accumulating lignin and suberin within their cell walls following a spatio-temporal pattern. Transcriptomic studies identified some members of a multigenic family that could guide this modification.

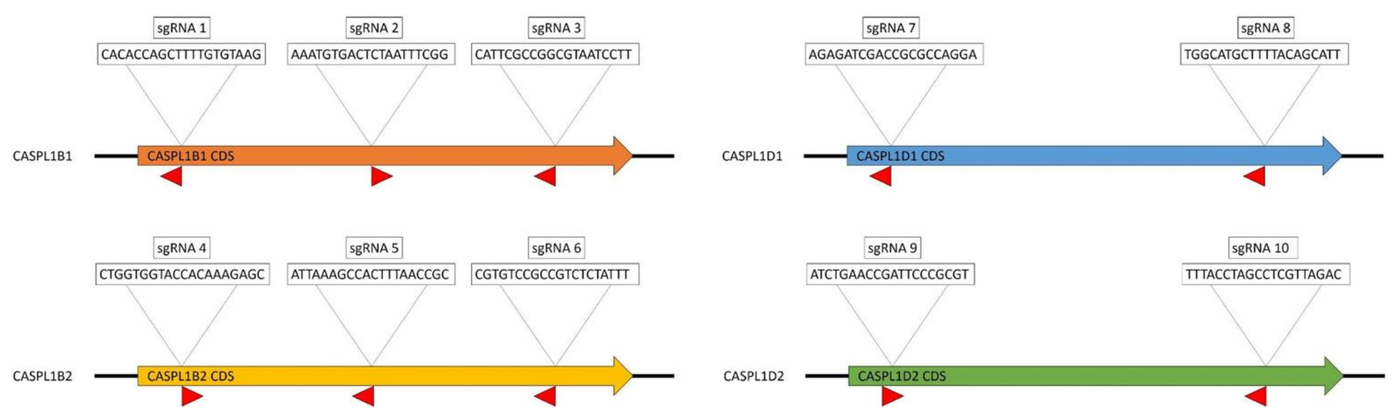
Aim: determine the function of the candidate genes by reverse genetics. We will approach it by generating Arabidopsis quadruple knock-out mutants **using a multiplex CRISPR/Cas9 system** to avoid genetic redundancy and perform phenotypic analysis in the root phellem. Here we present the mutant obtaining procedure.

Methodology basis: The NHEJ-mediated repair produces targeted indels or targeted large deletions when using one or multiple sgRNAs, respectively

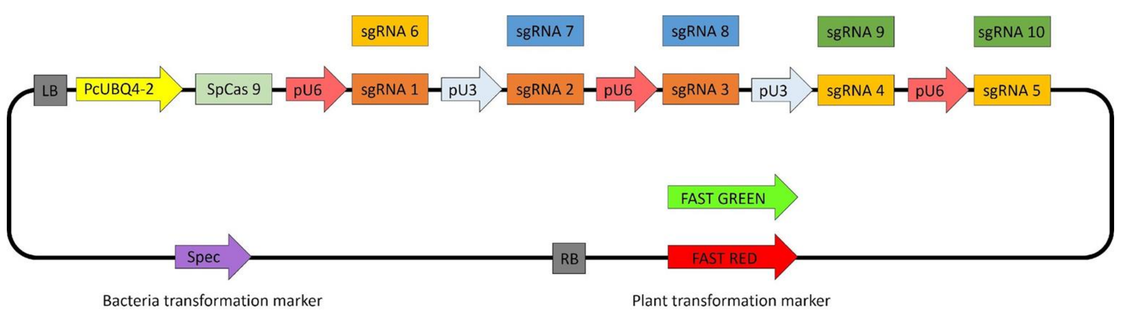


Pickar-Oliver and Gersbach, 2019

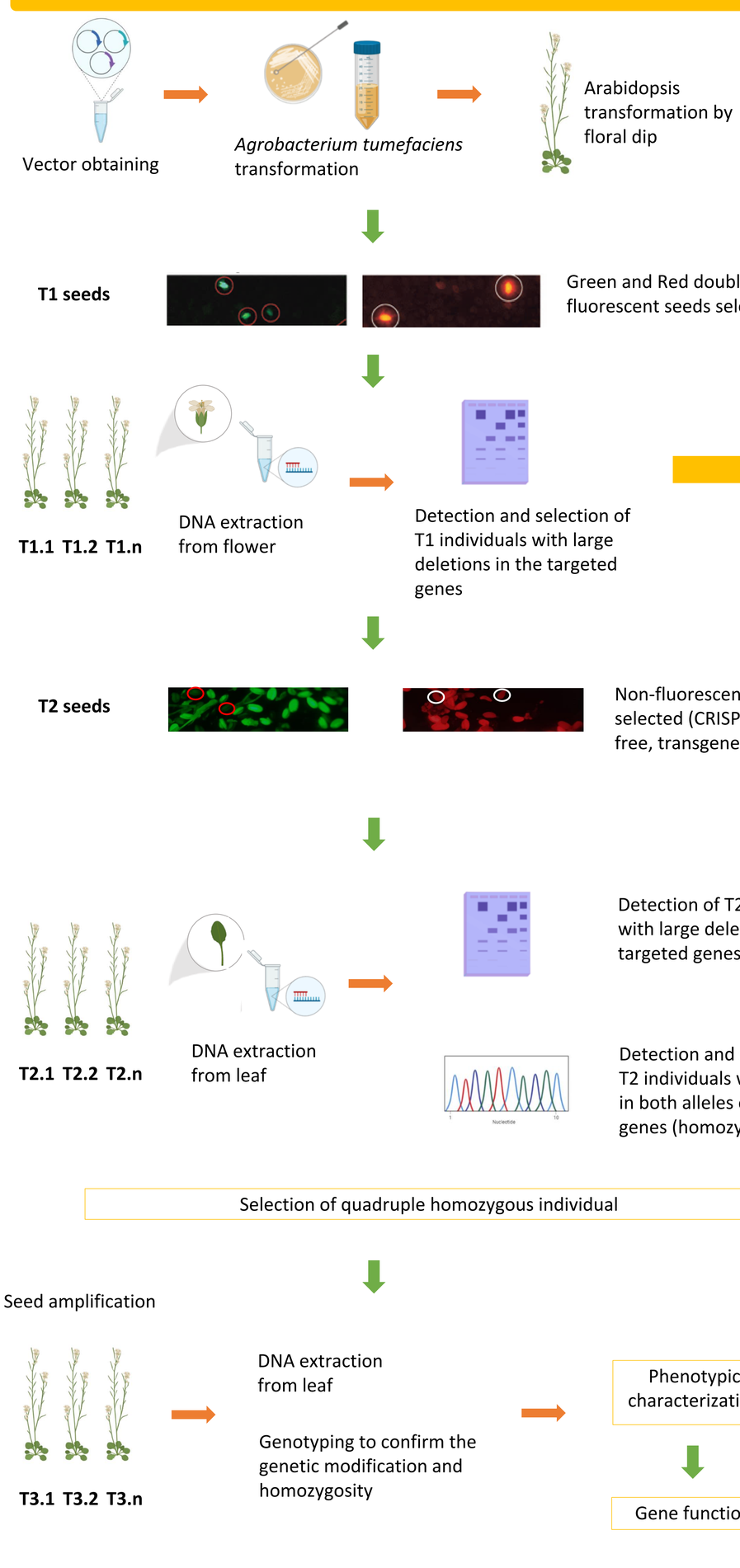
Cloning strategy to produce CRISPR-Cas9 binary vectors to knock-out 4 genes. sgRNA1 to sgRNA5 were cloned in a vector with FastRED as a plant transformation marker and sgRNA6 to sgRNA10 were cloned in a vector with FastGREEN transformation marker



Design of the sgRNA to produce targeted mutation in the 4 CASPL candidate genes

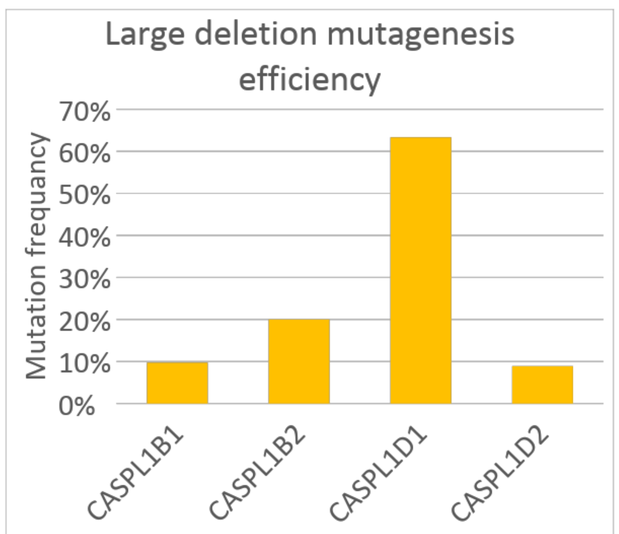


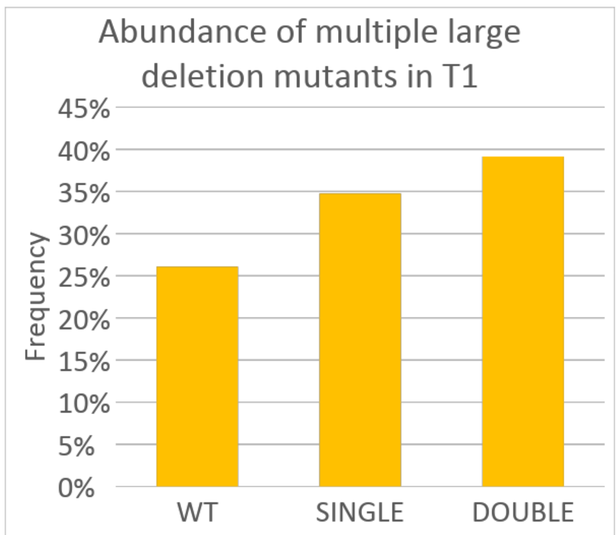
WORKFLOW



RESULTS

T1 line	sgRNA targeting sites					T1 line	sgRNA targeting sites				
	1B1	1B2	1D1	1D2	Sum		1B1	1B2	1D1	1D2	Sum
1					0	19					2
2					2	20					2
4					1	21					1
6					1	23					1
7					0	24					1
8					1	30					2
10					2	31					2
11					1	35					0
12					2	39					2
13					2	41					1
15					0	42					0
18					0						





CONCLUSIONS

- The two vectors are functional and fluorescent marker selection is feasible
- At least two sgRNA per gene are functional, detected by gene deletion
- CASPL1D1 has the highest mutagenesis efficiency by gene deletion analysis.

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