Resource Summary Report

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gRNA design and cloning with BbsI into Loop plasmid L1_lacZgRNA-Ck2/3

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Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.94ah8se

Authors: Eftychis Frangedakis, Marta Tomaselli, Susana Sauret-Gueto

Group: OpenPlant Project

Summary: This protocol explains how to design and clone the guide RNA target sequence into a L1 plasmid ready to accept the gRNA by cloning with BbsI. L1 plasmids are L1_lacZgRNA-Ck2 and L1_lacZgRNA-Ck3.If one gRNA target sequence is cloned into the Ck2 plasmid and another one into Ck3 one, the two L1_gRNA transcription units can be combined with an antibiotic resistance transcription unit and a MpEF1??:Cas9 transcription unit via L2 SapI Loop assembly. This allows for dual gRNA editing.

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