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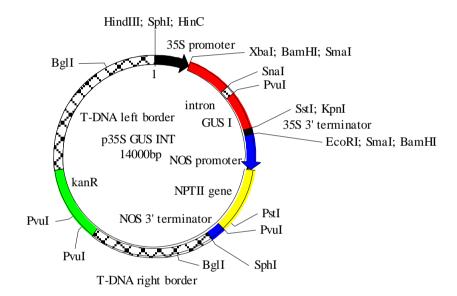
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## Supplementary data

Factors influencing the efficiency of  $Agrobacterium\ tume faciens$ -mediated transformation and regeneration in Brussels sprouts

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Supplementary Fig 1. P35SGUS intron plasmid used for Agrobacterium tumefaciens - mediated transformation of Brussels sprouts.



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7 d old seedlings of cultivar Winter Pick Cut 0.5 x 0.5 cm leaf sections across mid rib Immediate inoculation or 2 d preconditioning at 21±1°C in darkness abaxially on callus induction medium (CIM; MS + 0.1 mg L<sup>-1</sup> 2,4-D + 0.5 mg L<sup>-1</sup> BAP solidified with 7 g L<sup>-1</sup> phytaGel (pH 5.7) 10 min inoculation with overnight culture of A. tumefaciens strain LBA 4404 (OD600 = 0.300) 2 d co-cultivation at 21±1°C in darkness on sterile filter paper saturated with liquid cocultivation medium (CM; MS + 0.1 mg L<sup>-1</sup> 2,4-D + 2.0 mg L<sup>-1</sup> BAP) Eliminate A. tumefaciens with 150 mg L<sup>-1</sup> cefotaxime in MS basal medium (4 - 5 washes of 5 min each) Culture for 7 d abaxially on shoot regeneration medium (SRM; MS + 2 mg L-1 BAP\*1 + 5 mg L<sup>1</sup> AgNO3 + 150 mg L<sup>1</sup> cefotaxime. Test random explants for GUS expression Select putatively transformed shoots after 14 d on shoot regeneration selection medium (SRSM; SRM + 50 mg L<sup>-1</sup> kanamycin<sup>-2</sup>) subculture every 10-14 d. Test random green shoots for GUS expression regularly Transfer shoot to rooting medium (MS + 0.1 mg L<sup>-1</sup> NAA + 150 mg L<sup>-1</sup> cefotaxime + 50 mg L-1 kanamycin) "1 higher hormone levels, 5.0 mg L-1 BAP + 1.0 mg L-1 IBA may increase shoot regeneration per explants \*2Kanamycin kill curve for Brussels sprouts cultivars was previously determined by Gu (1998). Appropriate selection antibiotic and concentration to be determined if different

cultivars and A. tumefaciens strains are used.