

Electronic supplementary materials

Title: Targeted CRISPR/Cas9-based knock-out of the rice orthologs *TILLER ANGLE*

CONTROL 1 (TAC1) in poplar induced erect leaf habit and shoot growth

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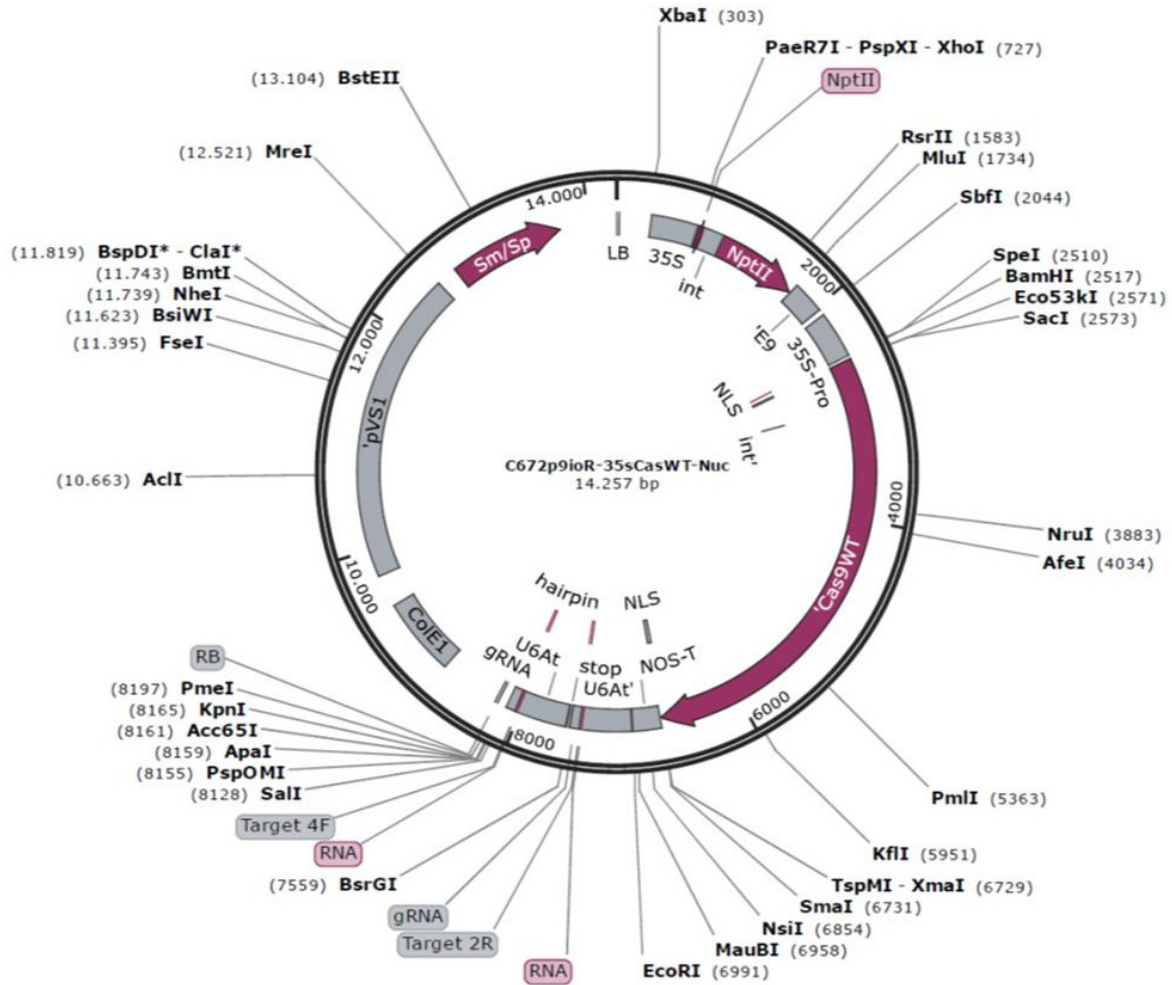


Figure S1: C672p9ioR-35sCasWT-Nuc plasmid (14.257 bp) (sequence is available by DNA Cloning Service, Hamburg, Germany, upon request) for knockout of the homologous rice ortholog *TILLER ANGLE CONTROL 1* (*TAC1*) in poplar, namely Potri.014G102600 ("TAC-14"), and its paralog Potri.002G175300 ("TAC-2") in the *P. × canescens* clone INRA 717-1B4.

Inside of the ring: LB: left border; 35S: cauliflower mosaic virus 35S promoter; Int: StLS1 (*Solanum tuberosum* rbcL-large subunit 1)-intron; NptII: coding sequence of kanamycin resistance gene; 'E9: terminator Rbcs-E9; 35S-Pro: cauliflower mosaic virus 35S promoter region; 'Cas9WT: Cas9 coding sequence of Cas9 wildtype gene (*Streptococcus pyogenes*); NLS: Nuclear localization sequence, for Cas9 sequence including NLS [55]; NOS-T: nopaline synthase terminator; U6At/U6At': coding U6 promoter (*Arabidopsis thaliana*) followed by gRNA sequence; RB: right border of T-DNA; ColE1: origin of replication; 'pVS1: origin of replication in *A. tumefaciens*; Sm/Sp: coding sequence of streptomycin/spectinomycin resistance gene.

Outside of the ring: Positions of gRNAs (RNA), and Targets 4F and 2R as well as of the restriction site (bp) of various restriction enzymes are given.

Table S1: Putative *PpeTAC1* (EMBL acc.no. KF218366; [43]) homologous genes in *P. trichocarpa*. To find homologous sequences in *P. × canescens* (*P. tremula* × *P. alba*, clone INRA 717-1B4 or P1), and *P. tremula* v1.1 (PopGenIE), transcript sequences of the two *P. trichocarpa* genes were blasted against <https://urgi.versailles.inra.fr/blast/blastresult.php?pathJob=2&jobid=160431359012&opt=none> and <http://popgenie.org/blast>. *Transcript sequence of Potri.014G102600 was blasted; **Transcript sequence of Potri.002G175300 was blasted.

<u>Species</u>	<u>Gene/sequence ID</u>	<u>Score</u>	<u>E-value</u>
<i>P. trichocarpa</i>	Potri.014G102600	183.4	8.7 e ⁻⁴⁴
	Potri.002G175300	172.6	1.6 e ⁻⁴⁰
<i>P. × canescens</i>	scaffold_37509*	1052	0.0
	scaffold_1688*	1052	0.0
	scaffold_72118*	1047	0.0
	scaffold_170684*	944	0.0
	scaffold_333464*	773	0.0
	scaffold_74020*	639	0.0
	scaffold_47155*	639	0.0
	scaffold_333464**	1106	0.0
	scaffold_47155**	933	0.0
	scaffold_74020**	928	0.0
	scaffold_72118**	764	0.0
	scaffold_37509**	753	0.0
	scaffold_1688**	753	0.0
	scaffold_170684**	679	0.0
<i>P. tremula</i>	Potra003475*	506.7	3.98 e ⁻¹⁶
	Potra001079*	237.9	7.28 e ⁻¹⁴
	Potra001079**	465.7	1.52 e ⁻¹⁸
	Potra003475**	229.5	6.78 e ⁻¹⁴

Table S2: Primers used in this study to amplify the *Cas9*, *NPT-II*, “TAC-14”, and “TAC-2” genes in PCR-reactions and for Sanger-sequencing.

<u>Gene</u>	<u>Primer name</u>	<u>Sequence (5'... 3')</u>	<u>Annealing temperature</u>	<u>Expected amplicon size</u>
<i>Cas9</i>	f-Cas9	GCT CCA GAC AAG AAG TAC AGC	58 °C	~930 bp
	r-Cas9	TGT TCA CGC GAA GGA TGT CG		
<i>NPT-II</i>	f-nptII	TTG AAC AAG ATG GAT TGC ACG	60 °C	~960 bp
	r-nptII	AAG AAG GCG ATA GAA GGC GA		
“TAC-14”	f-Potri14	ATG GGC TAG CTA GAA ATG TG	56 °C	~500 bp
	r-Potri14	CCT GAT TTG GTC TCA ATT TCA		
“TAC-2”	f-Potri2	AGA TGG GCT CGC TGG AAA TGT G	63 °C	~550 bp
	r-Potri2	CCT GGT TCA GTC TCA ACT TCA		