

Table S1. Gene-specific primers used for PCR amplification.

Name	Purpose	Forward primer (5'-3')	Reverse primer (5'-3')
<i>FOX</i>	FOX insertion gene cloning	TTTTACGACTCAATGACAAGA	CGATGGGGGTGTTCTGCTGGT
<i>PtoLAC14</i>	Gene cloning	CGGGATCCATGGAGTACGCTTGCTGGCTCC	CGGGATCCTCAACATTTTGGAAAGGTCGCTT
	Cas9-Target1	GTCACCCTGCTTTGGTCCAGTGCA	AAACTGCACTGGACCAAAGCAGGG
	Cas9-Target2	GTCAGCGTTTAGGCAAGACAACCA	AAACTGGTTGTCTTGCCTAAACGC
	Cas9-Seq	GGCGATATGGAGTACGCTTG	ATTCAGCTAATAACAACAACC
	Q-PCR	TGCCTCTTCCCTGCTTTGGTCC	GCCATTGACGGTAACAATCGGC
<i>Hyg</i>	PCR	ATCGGACGATTGCGTCGCATC	GTGTCACGTTGCAAGACCTG
<i>PtoUBQ</i>	Q-PCR	GTTGATTTTTGCTGGGAAGC	GATCTTGGCCTTCACGTTGT
<i>ProAtLAC4</i>	Promoter cloning	ACTGAGCTCACCATAGTTTTTCATTGGAC	ATACCATGGCTCCCTCTCTATCTTTCTC
<i>AtGAPDH</i>	Q-PCR	GCAACATACGACGAAATCAAGAA	CGACACGAGAACTGTAACCCC

Table S2. Information of related genes.

No.	Genes	Accessions	Sequence identity to PtrLAC14	References
1	<i>PtrLAC2</i>	<i>Potri.001G184300.1</i>	61.24	Bryan <i>et al.</i> , 2016
2	<i>PtrLAC3</i>	<i>Potri.001G206200.1</i>	67.26	Bryan <i>et al.</i> , 2016
3	<i>PtrLAC14</i>	<i>Potri.006G096900.1</i>	100	Lu <i>et al.</i> , 2013
4	<i>AtLac4</i>	<i>AT2G38080.1</i>	75.85	Berthet <i>et al.</i> , 2011
5	<i>AtLac17</i>	<i>AT5G60020.1</i>	55.88	Berthet <i>et al.</i> , 2011
6	<i>SofLAC</i>	<i>SCVPRZ3027A08.g</i>	57.54	Cesarino <i>et al.</i> , 2013
7	<i>BdLAC5</i>	<i>Bradi1g66720.1</i>	56.99	Wang <i>et al.</i> , 2015

Ptr: *P. trichocarpa*, At: *A. thaliana*, Sof: *Saccharum officinarum*,
Bd: *Brachypodium distachyon*.

Table S3. Cell wall composition (% dry matter) of raw material.

Samples	Cellulose	Hemicelluloses	Lignin
WT1	36.73 ± 0.91	22.16 ± 0.08	23.70 ± 0.83
OE-L1	36.62 ± 0.55	22.30 ± 0.03	26.52 ± 0.87
OE-L2	36.95 ± 0.59	22.30 ± 0.31	26.32 ± 0.76
OE-L5	37.07 ± 1.14	22.33 ± 0.41	27.35 ± 0.66
WT2	37.79 ± 0.69	22.30 ± 0.25	22.39 ± 0.24
KO-L1	36.73 ± 0.91	22.33 ± 0.41	20.88 ± 0.23
KO-L3	36.90 ± 0.99	23.30 ± 0.31	20.72 ± 0.11

Table S4. Lignin composition of raw material.

Samples	Lignin monolignols ($\mu\text{mol/g biomass}$)			Lignin monolignols (% total)		
	G	S	H	G	S	H
WT1	143.42 \pm 3	203.45 \pm 8	1.44 \pm 0.1	41.18	58.41	0.41
OE-L1	160.05 \pm 4	195.28 \pm 5	1.64 \pm 0.1	44.84	54.71	0.46
OE-L2	165.01 \pm 5	203.48 \pm 8	2.46 \pm 0.2	44.48	54.86	0.66
OE-L5	165.27 \pm 7	198.55 \pm 6	1.81 \pm 0.1	45.20	54.30	0.50
WT2	172.24 \pm 6	206.20 \pm 4	2.40 \pm 0.2	45.23	54.14	0.63
KO-L1	154.64 \pm 5	207.97 \pm 5	2.41 \pm 0.2	42.36	56.97	0.66
KO-L3	155.20 \pm 5	210.82 \pm 6	2.71 \pm 0.3	42.09	57.17	0.74

PtoLAC14	.MEYACWLRFMFLAVCIFFPAIVQC.RVREYKFNVMKNTTRLCSSRFIVT..VNGRFPGPTLYAREDDIVLVKVVNVKYNVSIHWHGIRQLRGTGWADGPAYITQCPIC.	105
PtrLAC2	.MENYRARAIIILLVIFIFPAIVEC.EVRLYDFRVVLIINTTKLCSTKSIIVT..INGKFPGPPTIYAREGDVNVNIKLTNEVQYVNIHWHGVRQLRGTGWSGDPAYITQCPIC.	105
PtrLAC3	.MEYYQAR.TMLLVIFIFPAIVEC.KVRLYDFRVVLIINTTKLCSSKSIPTPTINGKFPGPPTIYAREGDVNVNIRLTNEVQYVNVVHWHGVSSCFGTGWADGPAYITQCPICF.	107
AtLAC4	MGSHMVVFTFLVSFFSVFPAESES.MVREYKFNVMKNTTRLCSSKFTVT..VNGRFPGPTIYAREDDILIKVVNVKYNVSIHWHGVRQVIRGTGWADGPAYITQCPIC.	106
AtLAC17	.MALQLLAVSFCVLLLPQCAF.G.ITREYTLTKMNVTRLCSTKSLVS..VNGQFPGPKLIAREGDVILKVVNVQVFNISLHWHGIRQLRGTGWSGADGPAYITQCPIC.	104
BdLAC5	MGAKCLSLVFLGTSLLPQLLAAMTRMYTENVTKKVTRLCNTRAIPT..VNGKFPGPKIVTREGDRVVVKKVNVNKHVNIHWHGVRQLRGTGWSGDPAYITQCPIC.	107
SofLAC	MEAPCLALLLFGTLLVLPQSSHG.ATRYTTFNVTLKVTRLCTTRAIPT..VNGKFPGPKIVTREGDRVVVKKVNVNKHVNDNITIHWHGVRQLRGTGWSGDPAYITQCPIC.	106
Signal peptid		
PtoLAC14	.PGQSYVYNFTLPGQRTLWHAHILWLRATVHGAMVVLPRKGPYPFF.KPEKEVIVVIA..EWWKSDTEAVINEAKSGIAPNVSDAHTINGHPGAVSTCSSCGCFTL	211
PtrLAC2	.PGQSYLYNFTLPGQRTLWHAHISWLRATVHGAIIVLPKRGVYPFF.KPEKEKIIILG..EWWKADVEAVVNOACTGTLPNISDAHTVNGCTGAVFGCPSGCFTL	210
PtrLAC3	TGGQSYLYNFTLPGQRTLWHAHISWLRATVHGAIIVFPKKGVPYPFF.KPEKEKIIILSFTTEWWKADVEAVVNOACTGTLPNISDAHTVNGCTGAVFGCSTSGCFTL	215
AtLAC4	.PGQVYTYNFTLPGQRTLWHAHILWLRATVYGALVILPKRGVYPFF.KPENEKIVLVG..EWWKSDTEAVINEAKSGIAPNVSDSHMINGHPGPVRNCPSCG.YKL	211
AtLAC17	.TGQSYVYNFTLPGQRTLWHAHISWLRSTVYGLIILPKRGVYPFF.KPEKEVEMIFG..EWFNADTEAIRQACTGGGPNVSDAHTINGHPGPLYNCSAKTDFRL	210
BdLAC5	.TGQSYVYNFTLPGQRTLWHAHVSWMRATVYGPVILPKLGVYPFF.KPEKDVIMFG..EWFNVDEAIIAQACTGGGPNVSDAHTINGHPGPLYNCSSTDFKL	213
SofLAC	.TGQSYVYNFTLPGQRTLWHAHVSWMRATVYGPVILPKLGVYPFF.KPEKEVEIIFG..EWFNADTEAIIAQACTGAPNISDAHTINGHPGPLYNCSSTDFKL	213
PtoLAC14	FVKSGETVYLRRLIN..AALNEELFFKIAGHKLVVEVDATVYKPEFTDVTLLIAPGOTTNVLVTTKKN...TGKYLVA..ASPFMFS.FLAVDNNVTAIFLRYSGALA..	310
PtrLAC2	HVESGKTYLLRLIN..AALNDELFFKIAGHNITVVEVDAAVYKPESTDTFIFIPGOTTNALLTAKS...VGKYLMA..VSPFMDT.VVAVDNNVTAIFLRYMKGTTA..	309
PtrLAC3	HVESGKTYLLRIIFTAALNDELFFKIAGHNITVVEVDATVYKPESTDTFIFIPGOTTNALLTAKS...IGKYLIIFTVSPFMDT.VVAVDNNVTAIFLRYNEPLH..	318
AtLAC4	SVENGYTYLLRLVN..AALNEELFFKVAGHIEFTVVEVDAAVYKPEFTDVTLLIAPGOTTNVLLTAKS...AGKYLVT..ASPFMFA.FLAVDNNVTAIFLRYMKGTTA..	310
AtLAC17	FVKKGKTYLLRLIN..AALNDELFFSIANHTVTVVEADAIYVYKPEFTETILLIAPGOTTNVLLKTKSS..YPSASFFMT..ARPYVIG.CCTFDNSTVAGILEVEPPKQTK	313
BdLAC5	KVQEGKWYLLRLIN..AALNDELFFSIANHTLITVDVDASVYKPEFTDVTLLIAPGOTTNVLLHAKPEDEGCQPAHMLL..AREYATSRGCTYDNTVVAVLEYSPPGQ..	317
SofLAC	KVLEKGWYLLRLIN..AALNDELFFSIANHTLITVDVDAAVYKPEFTDVTLLIAPGOTTNVLLRAEPDAGCPAAHMLL..AREYGTGCECTFDNTVVAVLEYPAPGH..	317
PtoLAC14NSPTILTTFEPFNATAVANCFTNSLRSLNSRFF..PAKVPINVDHNLFFTVSLGVNPC...PSCKAGN..GSRVVASINNVTFVMPPTALLQAHFL.NIS	402
PtrLAC2FSPEVLTITPAINATPVITSTFMDLRLSLNSKRK..PANVPITVDHSLMFTIGVGDIPC...ATCVNG...SKAVCAINNIFFMPTTALLQAHYY.SIS	399
PtrLAC3SPHISLTTTPAINATPATSTFMDKLRSLNSKRETYPANVPITVDHILMFTIGVGDIPC...ATCTNG...SKAVADINNVSEFMPPTALLQAHYY.NIS	410
AtLAC4SSPTILTTFEPFNATSIANNFTNSLRSLNSKKY..PALVPITIDHLLFFTVGLGLNAC...PTCKAGN..GSRVVASINNVTFMMPKTALLPAHYF.NTS	402
AtLAC17	GAHSRTSIKNIQLEKPIIPALINDTNEATKESNKLRLSLNSKRE..PANVPINVDKRFVFTVGLGTPNCHKNCCCGPTNTMFAASISNIFMPTKALLQSHYSGSH	421
BdLAC5IKSRPTLFRPTLPVNDTSEAFANYSAKHSRLASSEY..PANVPRRIDRFFFAVGLGTPC...PTHQCNGPTNITKFSASMNNVSEFMPPTALLKAHYDNTA	416
SofLACIKSLPFRPSLPAINDTAFAANYSARLSRLATPDY..PANVPRGVDRSFFFAVGLGTPC...PANCQCCP.NGSMFTASMNNVSEFMPPTALLQAHYN.NIA	414
PtoLAC14	G..VFTTDFPAKPPHFNYTGT.PEINLCKSGTKVYRLSYNSTVQLVMQDTGIIISPENHEI..HLHGFNFVAVGRCVGNMYPKIDTKKFNLDVPERNTIGVPSGGWVA	507
PtrLAC2	G..VFTTDFPAPPNSEFNYTGNTAINLCTINGTRTFRIANSTVQLVLOGTIIIPESHEF..HLHGFNFVAVGRCVGNMYPKIDTKKFNLDVPERNTISVPTAGWAA	505
PtrLAC3	GFTVFTTDFPAKPPISFNYTGNTAMNLTNGTRAMRLAENSAVQVLOGTIIIPESHEFFTHLHGFNFVAVGRCVGNMYPKIDTKKFNLDVPERNTISVPTAGWIA	520
AtLAC4	G..VFTTDFPKNPPHFNYSGG.SVINMATETGTRVYRLRYNATVQLVQDTGVIPENHEV..HLHGFNFVAVGRCVGNMYPKIDTKKFNLDVPERNTIGVPSGGWVV	507
AtLAC17	G..VWSPKFPWSEPIVEFNYTGT.PENNTNVSNGTINLMLVLENTSYVELVQDTSILGAESEHL..HLHGFNFVAVGRCVGNMYPKIDTKKFNLDVPERNTIGVPSGGWAA	526
BdLAC5	G..VYTDAPFPAFTQHFNYTGT.PENNTNVSNGTKVAVLEYNASVEVVLQDTSILGAESEHL..HLHGFDFVAVGRCVGNMYPKIDTKKFNLDVPERNTIGVPSGGWVA	521
SofLAC	G..VMTTDFPVPLEFNYTGT.PENNTNVSNGTKVVVLQYNTSYVEVVLQDTSILGAESEHL..HLHGFDFVAVGRCVGNMYPKIDTKKFNLDVPERNTIGVPSGGWVA	519
PtoLAC14	IRFRADNPGVWFM..HCHLEVHTTWGLKMAFLVINGRGKRESLLPPPSDLPK	557
PtrLAC2	IRFRADNPGVWEL..HCHLEVHTTWGLKMFVVVINGRGDESLLPPPSDLPN	555
PtrLAC3	IRFRADNPGVWFLFTHCHLEVHTTWGLKMAFVVVINGRGPNESILPPPSDLPT	572
AtLAC4	IRFRADNPGVWFM..HCHLEVHTTWGLKMAFLVINGRGPNQSILPPPKDLPK	557
AtLAC17	IRFRADNPGVWFM..HCHLEVHTSWGLRMAFLVLDGCKPKDQKLLPPPADLPK	576
BdLAC5	IRFRADNPGVWFM..HCHLEVHTSWGLKMAVVDGELPKDQKLMPPPSDLPK	571
SofLAC	IRFRADNPGVWFM..HCHLEVHTSWGLKMAVVDGELPKDQKLMPPPADLPM	569

Fig. S1 Multiple alignment of *PtoLAC14* with other plant laccase proteins known to be involved in lignification. Ptr: *P. trichocarpa*, At: *A. thaliana*, Sof: *Saccharum officinarum*, Bd: *Brachypodium distachyon*.

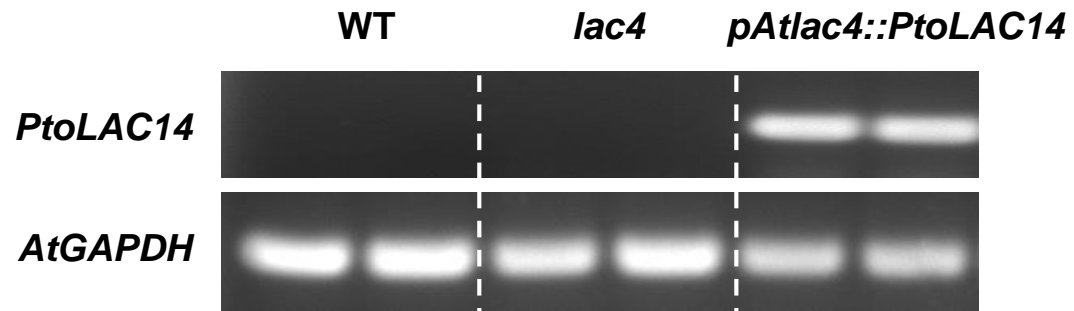


Fig. S2 Expression of *PtoLAC14*.

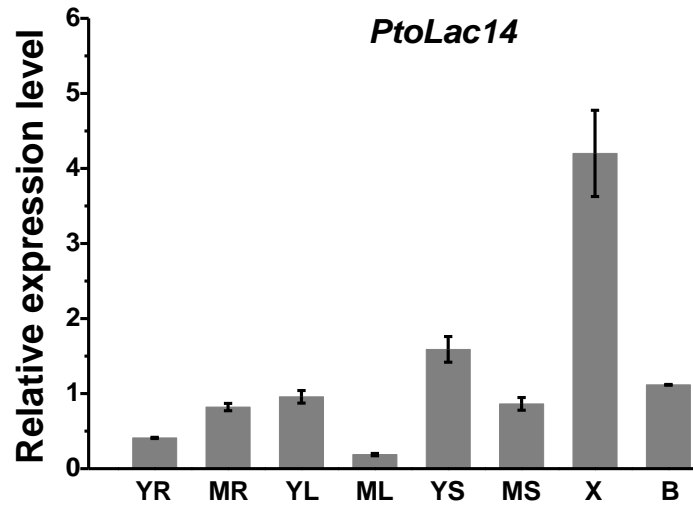


Fig. S3 The expression profiling of *PtoLAC14* by Q-PCR analysis.
YR, young root; MR, mature root; YL, young leaf; YL, ML, mature leaf;
YS, young stem; MS, mature stem; X, xylem; B, bark.

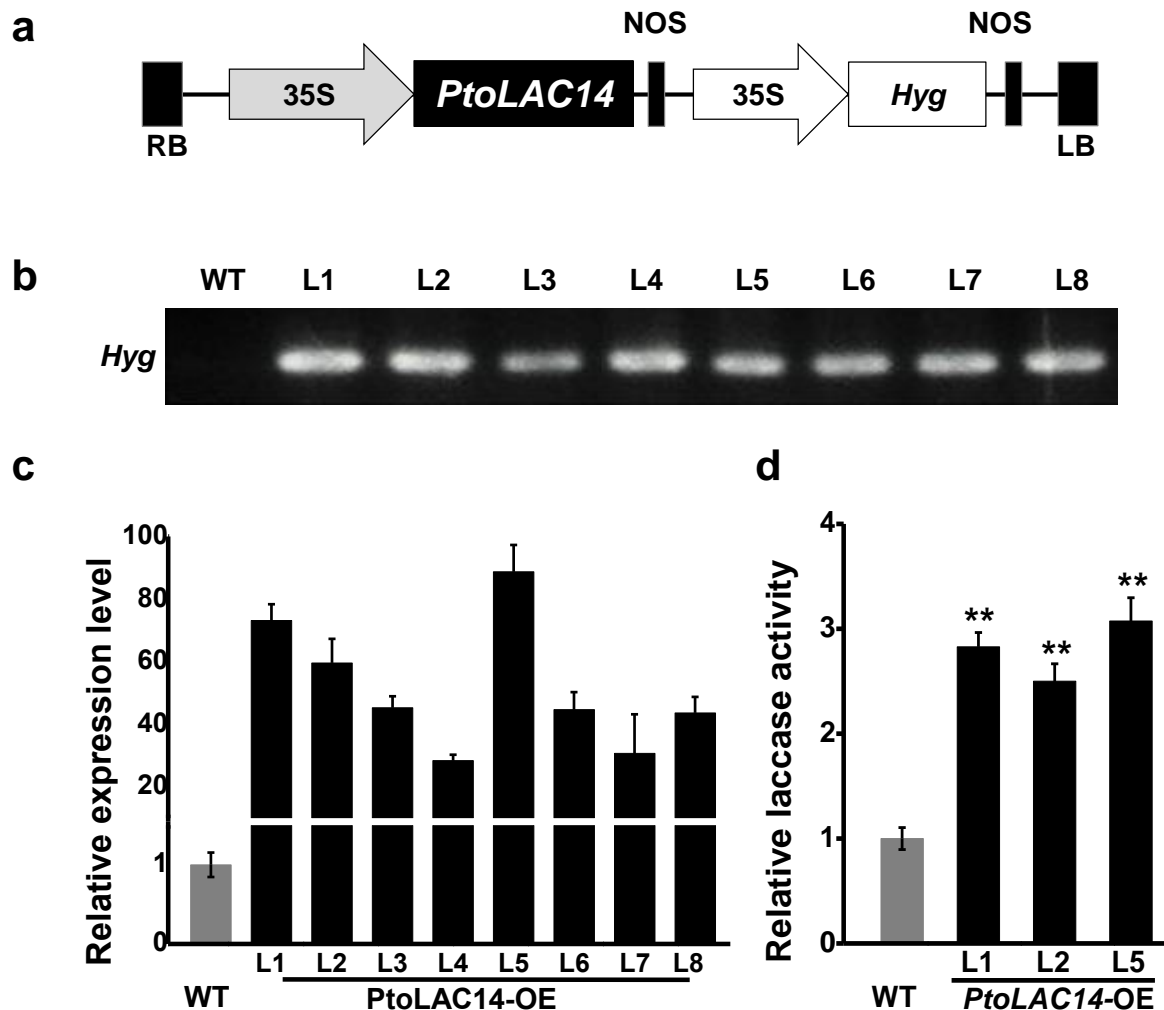
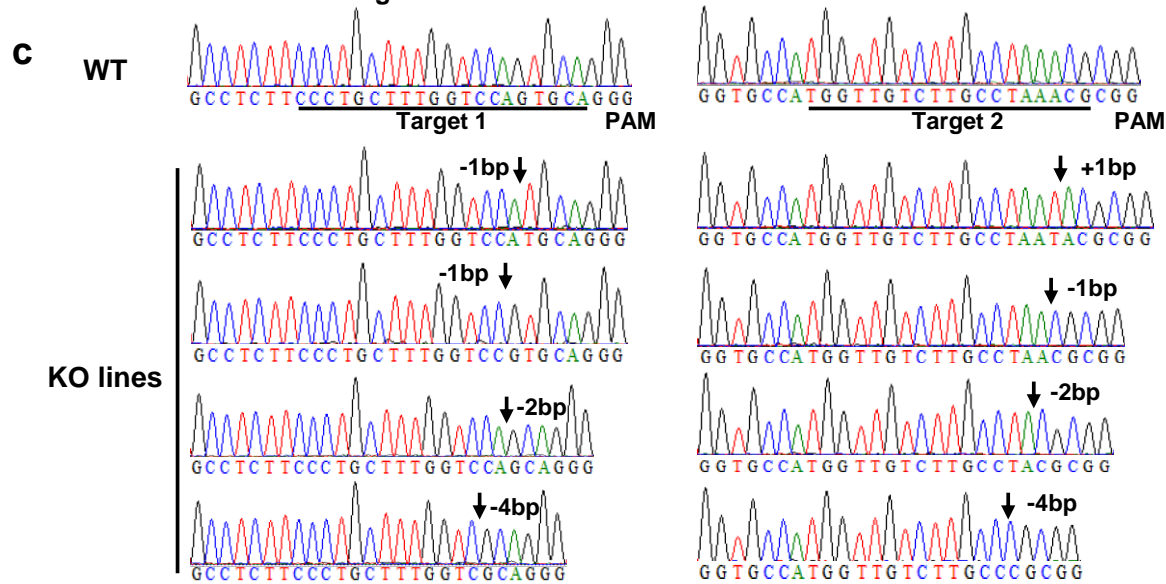
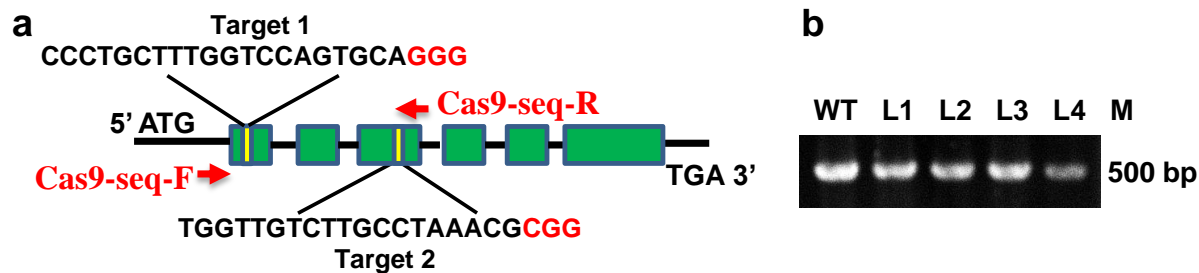


Fig. S4 Generation of PtoLAC14-OE transgenic poplars. (a) Diagram of the PtoLAC14-OE vector. (b) The Hyg levels in the PtoLAC14-OE lines. (c) The expression levels of PtoLAC14 in the PtoLAC14-OE lines. The poplar ubiquitin gene was used as an internal control. (d) Quantification of laccase activity with ABTS as the substrate. Data represent mean \pm SD of three biological replicates. Statistical analyses were performed using Student's *t* test as ** $P < 0.01$.



d

Target gene	No. of clones examined	No. of clones with mutations	Mutation rate (%)
PtoLAC14	50	42	84

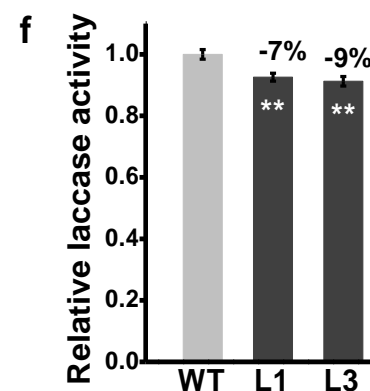
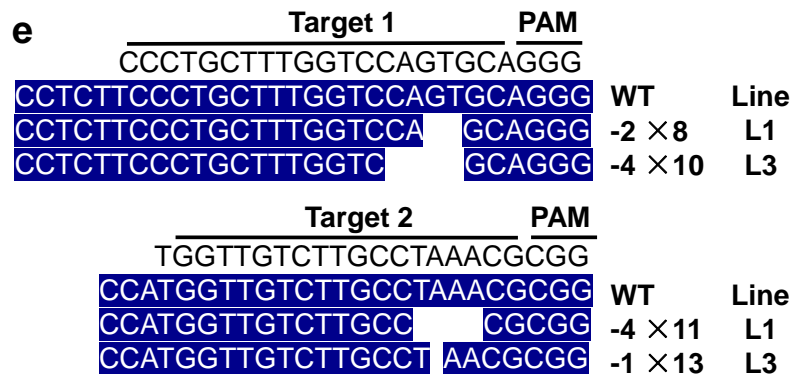


Fig. S5
Collection of
PtoLAC14-
KO
transgenic
poplars.

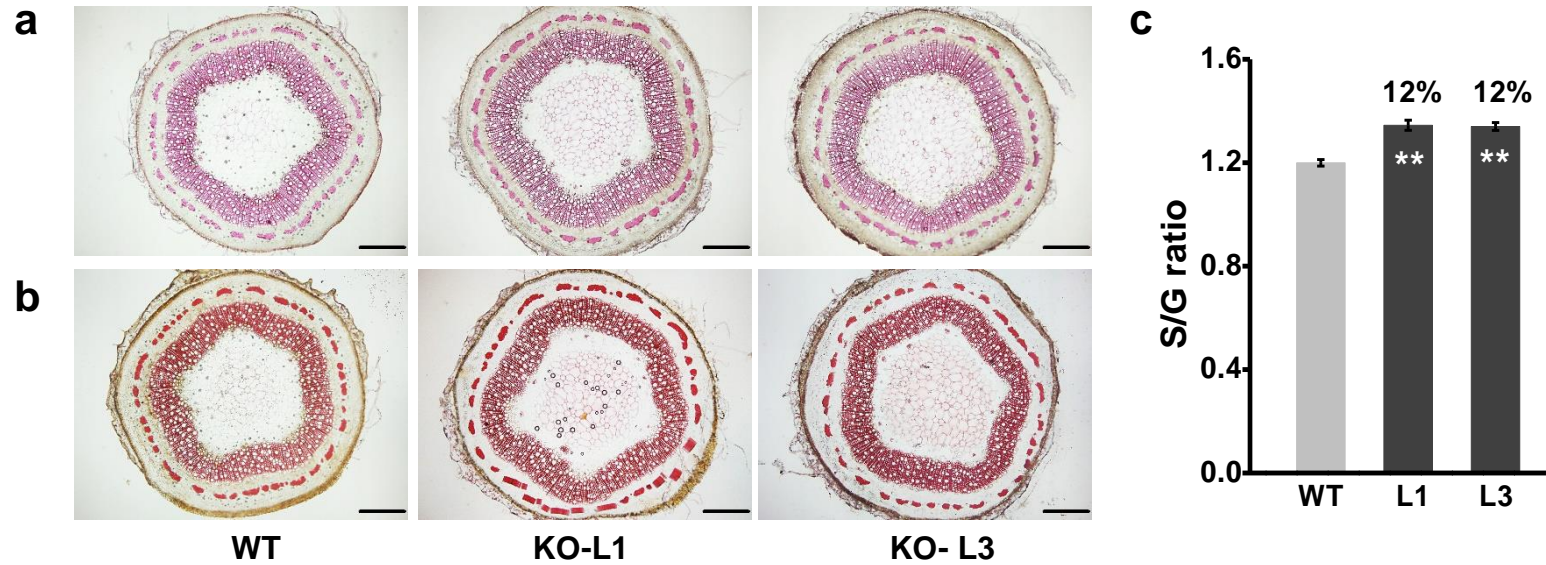


Fig. S6 Lignin and monomer lignin in *PtoLAC14*-KO transgenic poplar lines. (a) Phloroglucinol-HCl staining of stem cross sections from WT and *PtoLAC14*-KO lines. Scale bars as 100 μ m. (b) Histochemical stained with Mäule reagent for lignin monomer composition of stem cross sections from WT and *PtoLAC14*-KO lines. Scale bars as 100 μ m. (c) The monomer lignin S/G ratio in WT and *PtoLAC14*-KO lines. All data as means \pm SD (n = 3). Student's *t*-test was performed between the transgenic line and WT as ***P* < 0.01.