

Supplementary information for
Identifying transcription factors that reduce wood recalcitrance and improve
enzymatic degradation of xylem cell wall in poplar

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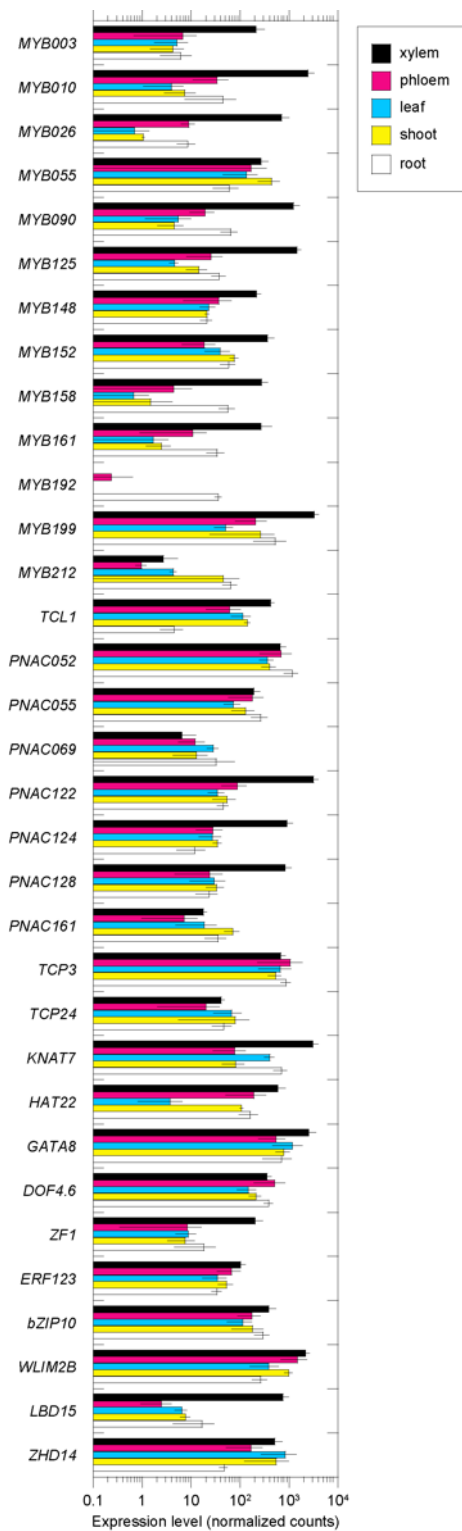


Figure S1. Expression pattern of target TFs in xylem, phloem, leaf, shoot, and root tissues in poplar. RNA sequencing dataset (GSE81077)¹ was retrieved and re-analyzed.



Figure S2. Gene expression patterns of 33 target TFs during xylem formation (gene names in magenta) together with 40 biosynthetic enzymes of cellulose, hemicellulose and lignin (gene names in black), based on the information deposited in the AspWood database (<http://aspwood.popgenie.org/aspwood-v3.0/>)². Classification of stem tissue was referred to Kumar et al (2019)³. CA-RE; cambium and radial expansion zone. PW-SW; Primary to secondary wall transition.

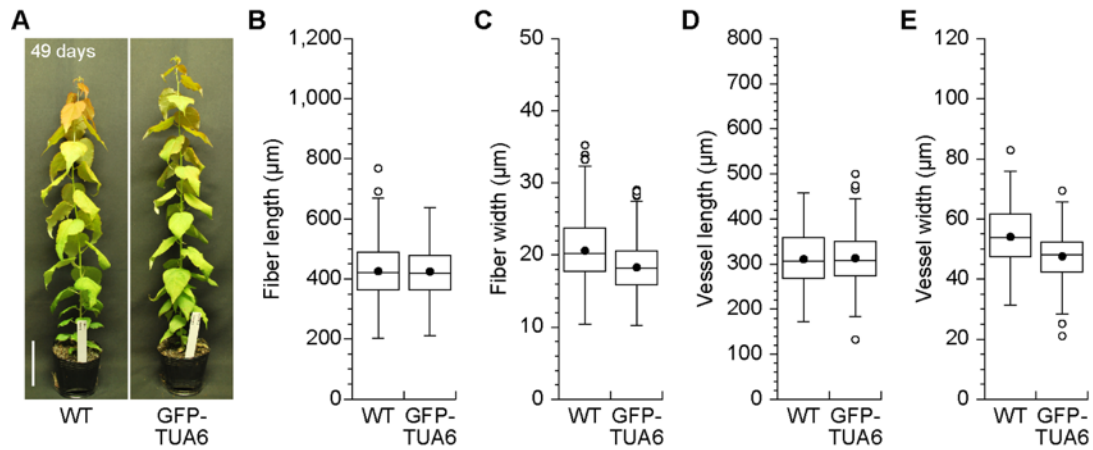


Figure S3. Transgenic hybrid aspen overexpressing GFP-TUA6 and wild-type (WT) plants. Phenotypes of 49-days-old WT and GFP-TUA6 (A). Scale bar = 10 cm. Xylem fiber lengths (B) and widths (C), and vessel element lengths (D) and widths (E). Stem samples were macerated in a solution of 30% hydrogen peroxide and glacial acetic acid (1:1) at 95°C for five hours. Xylem cells were imaged using a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany). Length and width were measured using ImageJ software. Six hundred fiber cells and 195 vessel cells were estimated from three trees per genotype.

Table S1. Oligonucleotides used in this study.

Primer Name	Sequence (5'-3')	Target Gene	Experiment
MYB003.TOPO.for	CACCATGATGAGGAAGCCGGAT	<i>Pt</i> × <i>tMYB003</i>	Cloning of target gene
MYB003.TOPO.rev	TACTACTTGAAATCAAGTAAAGG	<i>Pt</i> × <i>tMYB003</i>	Cloning of target gene
MYB010.TOPO.for	CACCATGATGGGTCACCATTCTTGCTGC	<i>Pt</i> × <i>tMYB010</i>	Cloning of target gene
MYB010.TOPO.rev	TAGAGATGAAGGGAAAGAAGAGA	<i>Pt</i> × <i>tMYB010</i>	Cloning of target gene
MYB026.TOPO.for	CACCATGATGGAAGATTTTAGGGGTAGC	<i>Pt</i> × <i>tMYB026</i>	Cloning of target gene
MYB026.TOPO.rev	AGAGATCCCACACCAAGAAAG	<i>Pt</i> × <i>tMYB026</i>	Cloning of target gene
MYB055.TOPO.for	CACCATGGGAGGCACTCTTGTTGCT	<i>PniMYB055</i>	Cloning of target gene
MYB055.TOPO.rev	AATATATCCATATGATGCTGTAAG	<i>PniMYB055</i>	Cloning of target gene
MYB090.TOPO.for	CACCATGATGTGTACTAGAGGCC	<i>Pt</i> × <i>tMYB090</i>	Cloning of target gene
MYB090.TOPO.rev	AGCTGAGAAAAATCAATGAATG	<i>Pt</i> × <i>tMYB090</i>	Cloning of target gene
MYB125.TOPO.for	CACCATGATGGGAAGGCAACCTTGCTGTG	<i>Pt</i> × <i>tMYB125</i>	Cloning of target gene
MYB125.TOPO.rev	GCGATTGAGTCCATCTCTA	<i>Pt</i> × <i>tMYB125</i>	Cloning of target gene
MYB148.TOPO.for	CACCATGGGACGACATTCTTGTTGTTG	<i>Pt</i> × <i>tMYB148</i>	Cloning of target gene
MYB148.TOPO.rev	TATCTGGTGAAGCCATCGA	<i>Pt</i> × <i>tMYB148</i>	Cloning of target gene
MYB152.TOPO.for	CACCATGGGAAGGCAACCATGT	<i>PniMYB152</i>	Cloning of target gene
MYB152.TOPO.rev	CAAGAGCCCATTGTCCAAG	<i>PniMYB152</i>	Cloning of target gene
MYB158.TOPO.for	CACCATGATGGAGGAATCTTTGGCTGGT	<i>Pt</i> × <i>tMYB158</i>	Cloning of target gene
MYB158.TOPO.rev	AGAAGATATACCCACACCAAG	<i>Pt</i> × <i>tMYB158</i>	Cloning of target gene
MYB161.TOPO.for	CACCATGATGTCTAGCAGAGGCCA	<i>Pt</i> × <i>tMYB161</i>	Cloning of target gene
MYB161.TOPO.rev	CAATCCACTAACTTGGTTTCCAGC	<i>Pt</i> × <i>tMYB161</i>	Cloning of target gene
MYB192.TOPO.for	CACCATGATGGGAAAAGGGAGAGCAC	<i>Pt</i> × <i>tMYB192</i>	Cloning of target gene
MYB192.TOPO.rev	TAAAGAAGGCCACAAATGAA	<i>Pt</i> × <i>tMYB192</i>	Cloning of target gene
MYB199.TOPO.for	CACCATGATGGGTAGACAACCTTGTTG	<i>Pt</i> × <i>tMYB199</i>	Cloning of target gene
MYB199.TOPO.rev	ATGCTTGCCACCCATGTCTA	<i>Pt</i> × <i>tMYB199</i>	Cloning of target gene
MYB212.TOPO.for	CACCATGATGGATGTTAAAGGCAACAGCAAC	<i>Pt</i> × <i>tMYB212</i>	Cloning of target gene
MYB212.TOPO.rev	CAAGTTGTTTAGCTGCTGCTGAAGAAC	<i>Pt</i> × <i>tMYB212</i>	Cloning of target gene
TCL1.TOPO.for	CACCATGGATAGACGTCGAAGAAGCG	<i>Pt</i> × <i>tTCL1</i>	Cloning of target gene
TCL1.TOPO.rev	AGAGGTATTCGGAATTACATCTCT	<i>Pt</i> × <i>tTCL1</i>	Cloning of target gene
PNAC052.TOPO.for	CACCATGCTCTTGCAGCAATCCAGGTG	<i>Pt</i> × <i>tPNAC052</i>	Cloning of target gene

PNAC052.TOPO.rev	GAAGAAGAAGTAAAATGGAACGAAGC	<i>Pt</i> × <i>t</i> PNAC052	Cloning of target gene
PNAC055.TOPO.for	CACCATGGAAGAGAGCAACACAAACCCCAAAAC	<i>Pt</i> × <i>t</i> PNAC055	Cloning of target gene
PNAC055.TOPO.rev	ATCCACAAGATCATCCAGCTCTATAAAATC	<i>Pt</i> × <i>t</i> PNAC055	Cloning of target gene
PNAC069.TOPO.for	CACCATGGATGAAATGCCATTACAGCTTGATC	<i>Pt</i> × <i>t</i> PNAC069	Cloning of target gene
PNAC069.TOPO.rev	GAAATTCAAAATGTCTGCATAATGGGCAA	<i>Pt</i> × <i>t</i> PNAC069	Cloning of target gene
PNAC122.TOPO.for	CACCATGACTTGGTGAATGACTG	<i>Pni</i> PNAC122	Cloning of target gene
PNAC122.TOPO.rev	AGGGATACGAGAAGATCC	<i>Pni</i> PNAC122	Cloning of target gene
PNAC124.TOPO.for	CACCATGATGACATGGTGAATAATAATTC	<i>Pt</i> × <i>t</i> PNAC124	Cloning of target gene
PNAC124.TOPO.rev	TTTTCTCTGAAGCTTTCCCTTG	<i>Pt</i> × <i>t</i> PNAC124	Cloning of target gene
PNAC128.TOPO.for	CACCATGAGTACTAAGTGAATATGGC	<i>Pt</i> × <i>t</i> PNAC128	Cloning of target gene
PNAC128.TOPO.rev	CCCATGATGATCCTGGTT	<i>Pt</i> × <i>t</i> PNAC128	Cloning of target gene
PNAC161.TOPO.for	CACCATGGCTGCCAATCTTCCCTCT	<i>Pt</i> × <i>t</i> PNAC161	Cloning of target gene
PNAC161.TOPO.rev	ATTTGGCAAATTTATTCATCAAG	<i>Pt</i> × <i>t</i> PNAC161	Cloning of target gene
TCP3.TOPO.for	CACCATGGATCCCAAGGGCTCTAACTCAA	<i>Pt</i> × <i>t</i> TCP3	Cloning of target gene
TCP3.TOPO.rev	CTGCCCTGATCCTTGTGAATCATCT	<i>Pt</i> × <i>t</i> TCP3	Cloning of target gene
TCP24.TOPO.for	CACCATGAAGAACAACGGAGAAATTATTCAGG	<i>Pt</i> × <i>t</i> TCP24	Cloning of target gene
TCP24.TOPO.rev	ATTCTGAGAATTTGGAGAAGAGTGTGGC	<i>Pt</i> × <i>t</i> TCP24	Cloning of target gene
KNAT7.TOPO.for	CACCATGCAAGAACCAAACCTGGGC	<i>Pni</i> KNAT7	Cloning of target gene
KNAT7.TOPO.rev	CCTTTTGCCTTGGACTCA	<i>Pni</i> KNAT7	Cloning of target gene
HAT22.TOPO.for	CACCATGATGGGTTTTGGAACACTACTGATGATC	<i>Pt</i> × <i>t</i> HAT22	Cloning of target gene
HAT22.TOPO.rev	GCAAGCTGCGGATGAGTGGGTATC	<i>Pt</i> × <i>t</i> HAT22	Cloning of target gene
GATA8.TOPO.for	CACCATGATTGGACAGACAAATACTACTAGTAAT	<i>Pni</i> GATA8	Cloning of target gene
GATA8.TOPO.rev	TATGTAATCCATTGCAGGGTTGCTC	<i>Pni</i> GATA8	Cloning of target gene
DOF4.6.TOPO.for	CACCATGGATACTGCTCAGTGGCCACAG	<i>Pt</i> × <i>t</i> DOF4.6	Cloning of target gene
DOF4.6.TOPO.rev	CCATGATCCTCCACCTAACATT	<i>Pt</i> × <i>t</i> DOF4.6	Cloning of target gene
ZF1.TOPO.for	CACCATGAAGAAGAATCAAGAAAAGGTGCA	<i>Pni</i> ZF1	Cloning of target gene
ZF1.TOPO.rev	GTTGGCTATAAGACCCACCAGTG	<i>Pni</i> ZF1	Cloning of target gene
ERF123.TOPO.for	CACCATGGATCCCTGTCTCAGTCTCTGTGTC	<i>Pt</i> × <i>t</i> ERF123	Cloning of target gene
ERF123.TOPO.rev	GAGGGAGTCTATCCATTCTATGTCCAG	<i>Pt</i> × <i>t</i> ERF123	Cloning of target gene
bZIP10.TOPO.for	CACCATGAATAGTGTCTTCTCAGTGGACGA	<i>Pt</i> × <i>tb</i> ZIP10	Cloning of target gene
bZIP10.TOPO.rev	TTTCTTTTGGCATCCCTCGCAACC	<i>Pt</i> × <i>tb</i> ZIP10	Cloning of target gene
WLIM2B.TOPO.for	CACCATGTCGTTTACTGGTACCCAACAGAA	<i>Pt</i> × <i>t</i> WLIM2B	Cloning of target gene

WLIM2B.TOPO.rev	AGCCTCTGGCACAGAAGCCGCTG	<i>Pt</i> × <i>t</i> WLIM2B	Cloning of target gene
LBD15.TOPO.for	CACCATGTCCAGAGACAGGGAGAGA	<i>Pt</i> × <i>t</i> LBD15	Cloning of target gene
LBD15.TOPO.rev	ATCAAAATATGGGACATTATTATC	<i>Pt</i> × <i>t</i> LBD15	Cloning of target gene
ZHD14.TOPO.for	CACCATGGACATAACCCAGCAACAGCAAC	<i>Pt</i> × <i>t</i> ZHD14	Cloning of target gene
ZHD14.TOPO.rev	AGAAGATGAAGAAGAACCATTAGTCC	<i>Pt</i> × <i>t</i> ZHD14	Cloning of target gene
NLS-TagRFP.TOPO.for	CACCATGCCTAAGAAGAAGCGTAAGGTTGGAGGAGTGTCTAAGGGCGAAG	<i>TagRFP</i>	Cloning of TagRFP
TagRFP.TOPO.rev	ATTAAGTTTGTGCCCCAGTTTG	<i>TagRFP</i>	Cloning of TagRFP
PtUBQ.for	GGTTGATTTTGTCTGGGAAGC	<i>Pt</i> × <i>t</i> UBQ	Real-time PCR
PtUBQ.rev	GATCTTGGCCTTCACGTTGT	<i>Pt</i> × <i>t</i> UBQ	Real-time PCR
TagRFP.for	CAGGACGGCTGCCTCATCT	<i>TagRFP</i>	Real-time PCR
TagRFP.rev	GCCGTTGGATGGGAAGTTC	<i>TagRFP</i>	Real-time PCR
Ptt18S.RT.for	TCAACTTTCGATGGTAGGATAGTG	<i>Pt</i> × <i>t</i> 18S	Real-time PCR
Ptt18S.RT.rev	CCGTGTCAGGATTGGGTAATTT	<i>Pt</i> × <i>t</i> 18S	Real-time PCR
PAL1.RT.for	CGTGAAGATGTCTTTGCTTACGCC	<i>Pt</i> × <i>t</i> PAL1	Real-time PCR
PAL1.RT.rev	GCGTTCTTCTCATTCTCTCCATTTG	<i>Pt</i> × <i>t</i> PAL1	Real-time PCR
PAL2.RT.for	GCTGTGGGACCCAATGGAGAAA	<i>Pt</i> × <i>t</i> PAL2	Real-time PCR
PAL2.RT.rev	GAACCAACAGCAGTACCATTTACA	<i>Pt</i> × <i>t</i> PAL2	Real-time PCR
PAL3.RT.for	CGTGAATATGTCTTTGCCTATGTGG	<i>Pt</i> × <i>t</i> PAL3	Real-time PCR
PAL3.RT.rev	TCCATTTTCCAACGCATGGTCAAC	<i>Pt</i> × <i>t</i> PAL3	Real-time PCR
PAL4.RT.for	GCAATTGGACCCAATGGAGAGC	<i>Pt</i> × <i>t</i> PAL4	Real-time PCR
PAL4.RT.rev	CAGAACCCACTGCAGTACTATTACT	<i>Pt</i> × <i>t</i> PAL4	Real-time PCR
C4H2.RT.for	AAACCCTGAGGCTGCAACCAA	<i>Pt</i> × <i>t</i> C4H1	Real-time PCR
C4H2.RT.rev	CTCCTCTCACCATTCAAAGCCTTG	<i>Pt</i> × <i>t</i> C4H1	Real-time PCR
C4H1.RT.for	AAACCCCGAGGCTGCAACTCAT	<i>Pt</i> × <i>t</i> C4H2	Real-time PCR
C4H1.RT.rev	GCTCCTCTCACCATTCAAAGCCTTA	<i>Pt</i> × <i>t</i> C4H2	Real-time PCR
4CL3.RT.for	CACACAGAGGTGCCATGATC	<i>Pt</i> × <i>t</i> 4CL3	Real-time PCR
4CL3.RT.rev	GCCTGTGTTATCAGAAGCTTTGCTC	<i>Pt</i> × <i>t</i> 4CL3	Real-time PCR
4CL5.RT.for	CACACAGGGGTGCCATTACT	<i>Pt</i> × <i>t</i> 4CL5	Real-time PCR
4CL5.RT.rev	GCCTGTGTTATCAAAAAGCTTTGCTC	<i>Pt</i> × <i>t</i> 4CL5	Real-time PCR
CSE1.RT.for	CCATGAGAGAAATCGCCAGAGCC	<i>Pt</i> × <i>t</i> CSE1	Real-time PCR
CSE1.RT.rev	ACGTCACCCCATCGGCGGTC	<i>Pt</i> × <i>t</i> CSE1	Real-time PCR
CSE2.RT.for	CCATGAGGGAAATTGCTAGGATG	<i>Pt</i> × <i>t</i> CSE2	Real-time PCR

CSE2.RT.rev	CATGCCACCCCATCAGCCGTG	<i>Pt</i> × <i>iCSE2</i>	Real-time PCR
HCT1.RT.for	TTTTATAGGCCACAGGTGCCT	<i>Pt</i> × <i>HCT1</i>	Real-time PCR
HCT1.RT.rev	CCTGCCATAGGGTAGAATGGT	<i>Pt</i> × <i>HCT1</i>	Real-time PCR
HCT6.RT.for	GGCTGAGACCACCTCAGTTATAGA	<i>Pt</i> × <i>HCT6</i>	Real-time PCR
HCT6.RT.rev	CGCCAGAGTAATCCACAGTAGGT	<i>Pt</i> × <i>HCT6</i>	Real-time PCR
C3'H3.RT.for	CTCAGGCCCTTCCTTACTTACAATG	<i>Pt</i> × <i>C3'H3</i>	Real-time PCR
C3'H3.RT.rev	GTAGCCACCAACTTTCACATTGGC	<i>Pt</i> × <i>C3'H3</i>	Real-time PCR
CCoAOMT1.RT.for	AGCCAGGCAGGAAGGCACCAG	<i>Pt</i> × <i>CCoAOMT1</i>	Real-time PCR
CCoAOMT1.RT.rev	CATGCATTCAGGCTCTCTTGGG	<i>Pt</i> × <i>CCoAOMT1</i>	Real-time PCR
CCoAOMT2.RT.for	ACTCAGGCCGGAAGGCATCAA	<i>Pt</i> × <i>CCoAOMT2</i>	Real-time PCR
CCoAOMT2.RT.rev	ATGCATTCAGGCTCTCTTGGG	<i>Pt</i> × <i>CCoAOMT2</i>	Real-time PCR
CCoAOMT3.RT.for	AACTAGTGCATATCCTGGGGAGC	<i>Pt</i> × <i>CCoAOMT3</i>	Real-time PCR
CCoAOMT3.RT.rev	CCTTCATCAACTGGCACAGACATC	<i>Pt</i> × <i>CCoAOMT3</i>	Real-time PCR
CCR7.RT.for	AAACCAGAAGCTAAGGGATCTGGG	<i>Pt</i> × <i>CCR2</i>	Real-time PCR
CCR7.RT.rev	TTCACAGACTCTTCTGCAGCTTG	<i>Pt</i> × <i>CCR2</i>	Real-time PCR
F5H1.RT.for	ACTACTCCGAAGAGGCTGAAACC	<i>Pt</i> × <i>F5H1</i>	Real-time PCR
F5H1.RT.rev	CTCGTTTACTTTTGTCTCTTCACTG	<i>Pt</i> × <i>F5H1</i>	Real-time PCR
F5H2.RT.for	AAGCCAATATAGGCAAGCCTGTGAATC	<i>Pt</i> × <i>F5H2</i>	Real-time PCR
F5H2.RT.rev	ATTTTtagccccgaaagctgctctg	<i>Pt</i> × <i>F5H2</i>	Real-time PCR
COMT1.RT.for	GGGAATGTCTGACCACTTACCATTA	<i>Pt</i> × <i>COMT1</i>	Real-time PCR
COMT1.RT.rev	AGCTCCAGTCCCGCCACCAACAT	<i>Pt</i> × <i>COMT1</i>	Real-time PCR
COMT2.RT.for	AGCACAATCGTCTCCAAGTACCCT	<i>Pt</i> × <i>COMT2</i>	Real-time PCR
COMT2.RT.rev	AACATTCTCCACACCAGGGAAAGC	<i>Pt</i> × <i>COMT2</i>	Real-time PCR
CAD1.RT.for	TGTGGTGAGAAATTCCTGATGGG	<i>Pt</i> × <i>CAD1</i>	Real-time PCR
CAD1.RT.rev	CTCTTAGCCCACTCTGTTTCAG	<i>Pt</i> × <i>CAD1</i>	Real-time PCR
CESA1-B.RT.for	GGATGGAGATGAGGATGAAGATGAT	<i>Pt</i> × <i>CESA1-B</i>	Real-time PCR
CESA1-B.RT.rev	ACCCTGCCACTGACGCCTTGCC	<i>Pt</i> × <i>CESA1-B</i>	Real-time PCR
CESA3-A.RT.for	GGTGGATCCCGGAAGAAGAGTTTCAG	<i>Pt</i> × <i>CESA3-A</i>	Real-time PCR
CESA3-A.RT.rev	AAGTAGGGTCAACATGCTTGCTCG	<i>Pt</i> × <i>CESA3-A</i>	Real-time PCR
CESA6-A.RT.for	GCACTTATTGTACCACCACACATG	<i>Pt</i> × <i>CESA6-A</i>	Real-time PCR
CESA6-A.RT.rev	TTGGAACCATTGGTCTTGGTTGAG	<i>Pt</i> × <i>CESA6-A</i>	Real-time PCR
CESA6-B.RT.for	GCACTTATTGTTCCACCGAGTC	<i>Pt</i> × <i>CESA6-B</i>	Real-time PCR

CEA6-B.RT.rev	TTGGAACCATTTGGTCTTGGTTGAG	<i>Pt</i> × <i>i</i> CEA6-B	Real-time PCR
CEA4.RT.for	GGTGCATCCATGCTCCTTTT	<i>Pt</i> × <i>i</i> CEA4	Real-time PCR
CEA4.RT.rev	GAACCCACCTTCTAGCAAACCTCA	<i>Pt</i> × <i>i</i> CEA4	Real-time PCR
CEA7-B.RT.for	GATACATGTGCATCCTGCTTCTAA	<i>Pt</i> × <i>i</i> CEA7-B	Real-time PCR
CEA7-B.RT.rev	CATCTCCATCTTAGTCAGTTTATAC	<i>Pt</i> × <i>i</i> CEA7-B	Real-time PCR
CEA8-B.RT.for	TTGCTGAGCTACCTCCAATAAG	<i>Pt</i> × <i>i</i> CEA8-B	Real-time PCR
CEA8-B.RT.rev	AGGGAAACTACAACGAGGATCA	<i>Pt</i> × <i>i</i> CEA8-B	Real-time PCR

References

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