10.1071/FP09271\_AC © CSIRO 2010 Accessory Publication: Functional Plant Biology, 2010, 37(8), 767–784.

## Table S1. Oligonucleotide primers used to obtain full length coding sequence of berry expressed LOXs

The sequences in bold were incorporated in order to facilitate directional cloning of a PCR fragment into pENTR<sup>TM</sup>/TEV/D-TOPO® vector; the predicted translation initiation codon is underscored

Gene	Forward primer	Reverse primer			
VvLOXA	5'- <b>CACCATG</b> TTCAAGACTCAGGTCCA-3'	5'-TCAAATGGAGATACTGTATGGA-3'			
VvLOXC	5'- <b>CACCATG</b> ATTCATTCAATTGTTGGT-3'	5'-TTAGATGGAGACACTGTTGGGAATC-3'			
VvLOXO	5'- <b>CACCATG</b> GCAGTGGTTAAAGAAATCAT-3'	5'-TCATATCGACACACTGTTTGGAATCCC-3'			

## Table S2.Oligonucleotides used to amplify VvLOXA and VvLOXO for cloning into pENTR<sup>TM</sup> TEV/D-TOPO® vectors

Primer name	Sequence
LOXAFL_TOPO	5'-CACCTTCAAGACTCAGGTCCAC-3'
LOXA-TP_TOPO	5'-CACCGTTGGCTACGTCCCTGCCAACAT-3'
LOXATerm	5'-TCAAATGGAGATACTGTATGGAACT-3'
LOXOFL_TOPO	5'-CACCGCAGTGGTTAAAGAAA-3'
LOXO-TP_TOPO	5'-CACCCCTGTTGCGGCTGTGA-3'
LOXOTerm	5'-TCATATCGACACACTGTTTGGAATCCC-3'

## Table S3.Oligonucleotide primers used in real-time qPCR experiments

Gene	Forward primer	Reverse primer
Actin	5'-CTTGCATCCCTCAGCACCTT-3'	5'-TCCTGTGGACAATGGATGGA-3'
GAPDH	5'-ACTGCCTTGCTCCTCTTGCGAAG-3'	5'-CCAGTGCTGCTAGGAATGATGTTGAATG-3'
SAND	5'-TGCCTGTTGATACATCTCCTCGCTCTG-3'	5'-TGGTGGTGAGAACTCCGAAGATACATACTG-3'
EF1	5'-GTGCGTCATAGTTTTCTGCCTTCTTCCTTG-3'	5'-CTCAACCAGTTATCTGCCACCGCCTATC-3'
VvLOXA	5'-GCAAATCAAAGGGACAACGCTGTATGG-3'	5'-TGCTTCCACTGCGGCTTCC-3'
VvLOXC	5'-TGGTGGAAGGAAGTCAGGGAAGAG-3'	5'-TGGGCGGTTTGGGAGGTAGC-3'
VvLOXD	5'-ACCCACCAAATCGTCCCACACTATG-3'	5'-ACCTCTTCGTTGTCTGTCCACTCTG-3'
VvLOXO	5'-TTCCACCCACTCGCCTGATG-3'	5'-GCACCGCACCTGTTTCTTCG-3'





Fourteen out of eighteen identified in the grape genome LOX genes were mapped with respect to the predicted grape chromosomes. The sequences of other four genes, *VvLOXD*, *VvLOXP*, *VvLOXR* and *VvLOXS* could not be linked to any of the existing chromosomes. Nineteen nuclear chromosomes, mitochondrial (Mt) and plastidial (Pltd) sub-genomes are schematically depicted. The arrows indicate 5' to 3' direction for the predicted coding sequences.

		690	700	710	720	730	740	750
	LOX1 Gm 3	SHATINTHAVVERI	гттал мвнт з	VVHBTYKTTF	E HYBDD - MI	NTNGTABLSTV	MDG	
	LOV1 Be 2	SHADNDHAVVEDI	EVITATINE HTS	CTHERTYKITT		NTNST.ADT.ST.V	MDG	
Г	LOX1_LC_1	SHWENTHAVVEP	TIATNERLS	VVHELHKLLI		NINALARNVLV	WAE	
ե	LOX1_Gm_4	. SHWLSLHAIVER	E V LAHNRQLS	VVHBLYKLLE	EEXRDU-MI	NLNSLARKALV	<b>A</b> D	GTTDKTEPM
- C	LOX1_Gm_5	SHWENTHAVVEPI	FVIATNRHLS	VVHPIYKLLE	FPHYRDT-MI	NINSLARKSLV	MAD	GIIBKTFLW
E	LOX1_Gm_6	SHWLNTHAVMEP	FAIATNRHLS	A THEIAKTT?	( P H Y K D 🛛 – I I	NINGLARQSLI	NAG	GIIEQTFLP
de la	LOX1 Ps 2	ISHWINTHAVIEPI	IVIATNROLS	VVHPINKLLA	PHYR DIMMI	NINALARDSLI	NAN	CLIDRSFLP
10-	LOX1_Ah_1	[SHWINTHAVIER]	IVIATNROLS	VINCTIANS	RHYRDR - MI	NINALARONLI	NSD	GIIDRTFLP
17.	LOX1_Gm_1	SHATINTHA AMERI	V T A M H B H T S	VTHETYKTT	PHYBNN-MI	NTNALABOSILT	M A N	
192	LOX1_Gm_2	ISHMENTHAVIED	TTATNEHTS	ATHPTYKTT	PHYBDM - MI	NTNALABOSILT	MAD	
_		SHWIGTHAVIED	T T A S N P O T S			N T N A T. A D H V T. T	N S D	
l nE 👘								
							N A G	
142							NGG	
111147	LOX1_St_1	SEWDNTEAVIEF	VIATNRQLS	VTHETHKTTI		NINASARQIIIV	WAG	GVLDSTVEQ
114 5	LOX1_St_3	SHWENTHAVIEE	Y LATNRQLS	A THETHKTT J	E E F R D H - M	INASARQILL	NAG	GATBRIALG
	LOX1_At_1	SHWMQIHASIEB	E V LAHNRQLS	A T E B A E K T T E	EBEFRDU – MI	N LNALAR QILL	<u>N</u> GG	CIEDIAAEB
114-	LOX1_Pd_1	SHWLNTHAVCEP	VVIATNRQLS	VVHPIYKLLH	I P H F R D T - M I	NINAFARQIVI	NAG	CILDTTVFP
ዛር	WLOXB	SHWLNTHAAIEPI	FVIATNRQLS	VLHPIHKILH	I <b>PH</b> F <b>R</b> D <b>T</b> MN ·	- INALARQILI	NAG	GVVESTVFP
1.2	WLOXC	SHWINTHAAIEPI	FVIATNRQLS	VLHPIHKLLH	I P H F R D T M N ·	- INALARQILI	NAG	C V V D S T V F P
le l	LOX1 Hv 1	SHWLNTHAVMEPI	FVISTNRHLS	VTHEVHKLLS	EHYRDT-M	TINALARQTLI	NAG	GIFDMTVFP
IL .	LOX1_Os_1	SHWENTHAVMERI	IVIATNROLS	VAHEVHKITTI	RHYRDR - M	ТТАНАВОТІТ	NGG	GIFDMTVFP
lle -	LOX2_7m_6	THWHNTHASMEP	TVTAANBOTS	VIHPTHRITH	PHFRKM-TI	HTNAVABOTTV	GSGDORKDG	SVFRGTDDVTYFP
41 -	Pt LOX	SHWIHTHAVVEPI	TTATRBOTS	VMHPTNWTTP	PHFKDMMH	TNADABSTLT	NS	
lin_	WEOXD	SHMUNTHAVVEDI	TTARBBOTS	VMHEVHENTI	PHFKDMMH	- TNALSBSITT	NSG	
41 _	LOV2 At 4	NHMIRTHASMEP	V T T A T N P O T S			ETNARABKSTT	NGG	
- Ll rL		'NHWIDDTHACMED					NGG	
	WLOVE			CMHPTFKTTK				
40.6	VILOAR			CMHDTRMTTM				
1147	VVLOXS			A MH D T R R T T R			NAG	
11 17	VVLOXO						NAD	
11 1	LOX2_St_2	NHWERTHASLEPI	E L L A A H R U L S	AMHELYKLLI		EINGLARUSLI	NAD	
ՍՆՐ	LOX2_At_2	NHWERLHACLER	FILAAHRQLS	AMERIFKLLI	BEMRYN-LI	BINALARQSLI	SAD	GVIDGGETA
1 5	LOX2_At_3	NHWERLEACLEE	FILAAHRQLS	AMHELFKLLI	BEMRYN-LI	BINAVARQULI	SAD	GVIDSCETA
In the second se	LOX2_Zm_10	SHWIRTHCCVEP	YIIAANRQLS	R LHPVYR LLH	$\mathbf{I} \mathbf{P} \mathbf{H} \mathbf{F} \mathbf{R} \mathbf{Y} \mathbf{T} - \mathbf{M}$	EINALAREALI	N A D	GIIDESFWP
	LOX2_Os_1	THWERTHCAVEP	YIIAANRQLS	EMHPIYQLLF	$\mathbf{P}\mathbf{H}\mathbf{F}\mathbf{R}\mathbf{Y}\mathbf{T}-\mathbf{M}\mathbf{I}$	RINARARSALI	SAG	GIIDRSFSP
llr –	LOX2 Hv 1	SHWLRTHACTEP	YIIAANRQLS	QMHEVYRLLH	I <b>PHFRFT-</b> MI	EINAQARAMLI	NAG	GIIEGSFVP
ւր	LOX2Os 2	SHWLRTHCCVEP	YVIAANRRLS	QMHEIYRLLH	I <b>PHFRFT-</b> MI	EINAQARGMLI	NAN	GIIESAFAP
le le	LOX2 At 1	SHWLRTHACTEP	YIIAANRQLS	AMHPIYRLLH	IPHFRYT-MI	EINARARQSLV	NGG	GIIDTCFWP
լը	LOX2 Bn 2	SHWIRTHCCIEP	YIIAANRQLS	AMHPIYRLLH	IPHFRYT-MI	EINARARQVLI	NEG	GIIDSRFWP
ll e	LOX2 St 1	SHWIRTHCCTEP	YIIASNRQLS	AMHPIYRLLH	IPHFRYT-MI	EINALAREALI	NAN	GVIESSFFP
- Dde	WLOXJ	'VHMMRRHHCCTPP	YIIAANROLS	AMTERYR	IPHLRYTME	- INALARESLI	NAG	GIIDSCFSP
112	WLOXK	'- HWIRTHCCTPP	YITAANROLS	AMHETYRTTH	I RH FRYMME	TNALAOESLI	NAG	CIIDSCFSP
76	WL OXA	SHWWRRTHCVTFF	Y T T A T N R O L S	AMHETYRTT	I PH FRYTME	TNALAREALT	NAD	GIIDSAFTP
	WLOXE	SHWIRRTHCVTPP	Y T T A UN B O L S	VMHETYBTTH	PHFBY MH	TNABABESTT	MAE	GTTDSSFSP
ղե	WL OXE	SHWIRTHCVTEP	Y T T A T N R O L S	VMHENTYRTTH	RHFRYTMH.	TNALAREST	NAD	GIIBTSESP
- Ч <sub>-</sub>	WI OXG	SHWIBTHCATED	Y T T A T N B O T S	VMHPTYRTTN	PHCBYTMK	TNALABOVIT	SAD	
Ч.	WLOXH	SHWIRTHCATER	VVTATINGTS	VMHPTYKTTH		- TNALABEVI.T	MAD	
- 4	WLOXI	SHWIRTHCATER	TATNBOLS	AMHETYKTTH			MAD	
								•
						-		•



Only part of the alignment is shown containing important functional motifs and amino acid residues. The open triangles indicate amino acid residues involved in binding of catalytic atom of iron; black diamond and black triangle indicate amino acid residues involved in determining LOXs regiospecificity and stereospecificity respectively.



Fig. S2. (continued) Amino acid sequence alignment of the predicted Pinot noir LOXs and characterised plant LOXs

Only part of the alignment is shown containing important functional motifs and amino acid residues. The open triangles indicate amino acid residues involved in binding of catalytic atom of iron.

		990	1,000	1,010	1,020	1,030	1,040	1,050	1,058
<b>F</b>	LOX1 Gm 3	ALEAFKR	FGNKLAQIENKI	SERNNDEKLR-	- N R C Ġ P V	OMPYTLLL-		<u>e la fr<b>gip</b></u> n	SISI
F	LOX1 Ps 3	ALEAFKK	FGNKLAEIDKKI	TORNNDEKLR-	- N R H G P V	EMPYTLLYP-	SKE	ELTFRGIPN	SISI
le l	LOX1 Lc 1	PKEAFKR	FGKKLAEIDEKI	TQRNNDESLR-	- N R Y G P V	KMPYTLLYP-	SEE	ELTCRGIPN	SISI
lde -	LOX1 Gm 4	PLEAFKR	FGKKLEEIDKKI	JIEKNKDETLR-	- NRYGPA	KMPYTLLYP-	SSEE	ELTFRGIPN	SISI
լլե	LOX1_Gm_5	PLEAFKR	FGKNLEEIDKKI	JIEKNNDETLR-	- NRYGPA	KMPYTLLYP-	SSEE	ELTFRGIPN	SISI
ll e	LOX1_Gm_6	ALEAFKK	FGNKLAEIDGKI	TQRNNDPSLK-	SRHGPV	QLPYTLLHR-	SEE	EMSFKGIPN	SISI
llde	LOX1 Ps 2	ALQAFQK	FGNKLAEIDAKI	TNKNNDPSLY-	- HRVGPV	QLPYTLLHP-	SKE	ELTFRGIPN	SISI
414-	LOX1_Ah_1	ALEAFQR	FGNKLSEIDEKI	JTEKNKDGRLS-	-NRIGPV	ELPYTLLHP-	TSNE	ELTFRGVPN	SISI
11 6-	LOX1_Gm_1	ALQAFQK	FGNKLKEIEEKI	JVRRNNDPSLQG	SNRL <b>G</b> PV	QLPYTLLYP-	SSEE	ELTFRGIPN	SISI
11 2	LOX1_Gm_2	ALQAFQK	FGNKLKETDEKI	ARKNNDQSLS-	-NRLGPV	QLPYTLLHP-	NSEE	<u>e l T</u> C R <b>G I P N</b>	SISI
ll ar	LOX1_At_2	PLEAFKR	FGKELELIDNNI	IRRNNDKRFK -	-NRTGPV	NIPYTLLYPN	TTDYTREG	G I I G K G I P N	SVSI
11 []	WLOXL -	PLAAFER	FGSRLRGIDTRI	NQMNQ DRRWNN	IRF-GPV	EMQYTLLYEN	TSDYSRQG	🖥 L A G K <b>G I P N</b>	SVSI
1J r	LOX1_Nt_1	PLSAFAR	FGKKLSDIDDQI	MQMNVDEKWK -	-NRSGPV	KVPYTLLFP-	TSEG	<u>e l t g k g t p n</u>	SVSI
1016-	LOX1_St_1	PLAAFDR	FGKKLTDIEKQI	IQRNGDNILT-	- N R S G P V	NAPYTLLFP-	TSEG	<b>g</b> l <b>h</b> g k <b>g i p n</b>	SVSI
114 5	LOX1_St_3	PLAAFDK	FGKKLTDIDKQI	IQRNGDNILT-	-NRSGPV	NAPYTLLFP-	TSEG	<u>e</u> l n g k <b>g i p n</b>	SVSI
II In	LOX1_At_1	ALEAFEK	FGEKVKEIDKNI	DERMDDETLK-	- NR TGLV	KMPYTLLFP-	SSEG	ë v n g r <b>g i p n</b>	SVSI
114-	LOX1_Pd_1	PLKAFDK	FGRKLAKIDDRI	TSMNNDEKLK-	- NR V <b>G</b> P V	KMPYTLLFP-	TSGG	<u>e</u> l n g r <b>g i p n</b>	SVSI
ዛሬ	WLOXB	PLKAFEK	FGRKLADIBERI	IDRNGNERFKN	IRV-GPV	KIPYTLLYPT	S E G	ELEGKGIPN	SVSI
1.5	WLOXC	PLKAFEK	FGRKLADIDEM	IDRMGNERFKI	IRV- <b>G</b> PV	KIPYTLLYPT	S E G	ELEGKGIPN	SVSI
L.C.	LOX1_Hv_1	ALEVEKR	SDRLVELDSKV	VGMMHDPELK-	NRNGPA	KFEYMLLYEN	TSDHKGAAA	ELMAKGIEN	SISI
115	LOX1_Os_1	ALEAFKR	GARLTELDSRV	VAMNKDPHRK-	NRVGPT	NFEYTLLYEN	TSDLKGDAA	ELSARGIEN	SISI
Иг	LOX2_Zm_6	AAEALAE	RARLEEVAGN	DRRMADPALK-	NRTCQV	EVENTLLKE-	TAQP	<u>e l v lr g i pn</u>	SITV
ll-F	Pt_LOX	VQQKBKK	ENEHLQELDKK	IQRMKDPKFK-	NRSGPA	KIEYELLYED	TSNVGPRW	<u>e i mrkgipn</u>	$S \vee S \perp$
015	WLOXD	VRQREEK	ERENLQKVDRK.	LVRMRDPKLKI	VRK-GPA	KIEYKLLYED	TSNIGIGR	<u>e i ng kg i pn</u>	$S \perp S \perp$
	LOX2_At_4	VVKYENK	ESEELVKLDKT	NERMKDKKLK-	NRTGAG	WEEXETTEN	'SPH	evngrg len	$S \perp S \perp$
1115	WLOXP	VLDMFKK	ESAKLEELDEL.	KGRNKNIHLKN	VRN-GAG	TPEXETTES	8GP		SISI
484	WLOXR	TTRAFYR		EKRMADTSRRF	VRC-GAG	TSPIKLTISS	8GP		SITTV
114-	WLOXS	TTRAFYR		EKRMADISKRI	VRC-GAG	T S F Y K L L T P S	SGP		SLTV
	WLOXO	TURNEVE		EKKWAEFSKKI	NRC-GAG	V LEYELLAES	8		SVSI
11.11		TURAFIA		EVENANTALA-	NRCGAG	V LE L L L A E S V T D V D T T V D S	8		BVBL
- 4 40		TVDAFIG		ERKNADEDRE -	- NRCGAG	VIELEUVES			I N D L N N S T
	LOX2_AL_3	TAPER	ECCDMKEINCE.	DREMEDESEE		TUBYETEKEE			STST
	LOX2_20s_1	VOANDG	RAARTKETEGV	DGBMKDBKTK-	- NRCCAG	TIBYOTMKBE	SDS		S D S T
11-		TNOAFEE	FKESTRKTVEOU	DEWNNDPDBK-	- N B H C A G	MVBVVTTBBS		MVMENCTEN	STST
IIL	LOX2_0s_2	VRAAFGR	ENERMMS TAEM	DCRMKDPERK-	NROCRG	V V P V V T T K P S	VGDPKDMT	SVMENCTEN	STST
1.	LOX2_At 1	TNAAFER	FKGKTOYLNGV	DERNVNTTTK-	NRAGAG	VVKYETTKPT	SEH	SVNGMGV PY	STST
I.L	LOX2 Bn 2	TYAAYEB	FKGKTOYLDGV	DERNVNVSTK-	NBTGAG	VVKYETTKET	SEP	ev n g m g v p y	SVST
ll e	LOX2 St 1	TNAAFEV	<b>F</b> SGK <b>T</b> KEL <b>D</b> GT	DARMNDSKTS-	NBNGAG	VMPYETTKPY	SEP	EV DGKGV PY	STST
9de	WIOXI	TKAAFER	FNGRTKELDGT	DGBMTNLNIK	JRT-GAG	VVPYETTKPF	SKP	EVINGMEV PN	STST
114	WLOXK	IKAAFER	FNGRIKELNGI	DGRNTNLNLKN	IRT-GAG	VVPYELLKPE	SKP	GVRGMGVPN	SISI
16	WLOXA	IKAAFER	FSGRIKEINGI	DARNEDKNLKN	IRH-GAG	VVPYELLKPE	'S G A	SV MGK GV PY	SISI
U	WLOXE	IKEAFNR	<b>F</b> SAK <b>I</b> EEL <b>D</b> RT	DDRNKDNSLKN	IRN-CAG	VVPYELL		KGVRY	SISI
լե	WLOXE	IKEAFNR	FSAKLEELBRTI	DDRNKDNSLKN	IRN- <b>G</b> AG	VVPYELL		KGVPY	SISI
1-	WLOXG	IKKAFER	FAERLKELDKII	NARNKDNSLKN	IRG-CAG	VVPYDVIKPF	SKQ	E V II G E G V P Y	SISI
	WLOXH	IKEAFER	FSARLKELDVI	DARNADNSLKN	IRG – 🔁 AG	VVPYELLKPF	S E A	E V II G K G V P Y	SISI
	- WLOXI	IKEAFER	FSAKIKELGVI	DSRNADDSLKI	IRG – 🖬 AG	VVPYELLKPE	S E A	🛢 V 🏽 G K Ġ V 🖻 Y	SISI
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									_

**Fig. S2. (continued)** Amino acid sequence alignment of the predicted Pinot noir LOXs and characterised plant LOXs Only part of the alignment is shown containing important functional motifs and amino acid residues. The open triangle indicates amino acid residue involved in binding of catalytic atom of iron.



Fig. S3.Phylogenetic tree reconstructed based on the alignment of only the conservedLOX domains of grape and characterised LOX proteins

Conserved LOX domains of grape LOXs and characterised plant LOXs were aligned, and a tree was reconstructed to validate the tree obtained from a full-length LOX alignment (Fig. 2.). The evolutionary relationship was inferred using the Neighbour-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl, Pauling *et al.* 1965) and are in the units of the number of amino acid substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley *et al.* 2007).. Tables 1 and 2 contain gene IDs and accession names for the LOXs used in the alignment.



**Recombinant LOXO-TP Enzyme Kinetics Data** 



**Fig. S4.**The basic kinetic characteristics  $K_m$  and  $V_{max}$  for recombinant LOXA-TP (a) and LOXO-TP (b) were determined by fitting the kinetic rate data with non-liniar fit model described by Michaelis-Menten equation. Inserts on the right are Lineweaver-Burk plots.