
The influence of viticultural treatments on the accumulation of flavonoid compounds in grapes and their contribution to wine quality

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APPENDIX 2A

Buffers, solutions, reagents & media

Solutions, growth medias, buffers and reagents commonly used in this investigation are shown in the tables below. All chemicals were analytical or molecular biology grade and obtained from BD Biosciences (<http://www.bdbiosciences.com>) or Sigma-Aldrich (<http://www.sigmaaldrich.com>). Solutions were prepared with nanopure or deionised water and autoclaved as necessary. The sources of all other supplies used in this investigation are indicated in the relevant methods section.

Table 2A.1 Suppliers of chemicals, enzymes, growth media & other miscellaneous consumables

Manufacturer	Products	Website
BD Biosciences (CloneTech Laboratories)	Universal Genome Walking™ Kit	http://www.bdbiosciences.com
Extrasynthase	HPLC standards (malvidin-3-glucoside, quercetin-3-glucoside)	No website
Integrated Sciences (Stratagene)	PicoMaxx Taq The QuikChangeR II Site-Directed Mutagenesis Kit	http://www.integratedsci.com.au
Invitrogen	Hi-Fi, pfx, pfu and platinum Taq, electroporation cuvettes, Oligonucleotides (primers)	http://www.invitrogen.com
Promega	Dual-luciferase® reporter assay system, Molecular weight markers	http://www.promega.com
Qiagen	QIA gel extract, QIA plasmid prep, Qiagen PCR cloning kit, RNeasy plant RNA Kit, RNase free DNase Set	http://www.qiagen.com
Quantum Scientific (Fermentas)	Restriction endonucleases	http://www.quantum-scientific.com.au
Roche Diagnostics	Restriction endonucleases	http://www.roche-diagnostics.com

Table 2A.2 Downloadable sequence management programs & online databases

Program/ Database	Website
Primer 3 program	http://www-genome.wi.mit.edu/cgi-bin/primer/primer3 www.cgi
Chromas program	http://www.techneysium.com.au
ClustalW Alignment program	http://www.ebi.ac.uk/clustalW/
GeneDoc Display program	http://www.psc.edu/biomed/genedoc
BLAST analysis	http://www.ncbi.nlm.nih.gov/BLAST/
PLACE database	http://www.dna.affrc.go.jp/htdocs/PLACE
TIGR database	http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=grape .
NCBI database	http://www.ncbi.nlm.nih.gov/
Trace database	http://www.ncbi.nlm.nih.gov/Traces/trace.cgi?

Table 2A.3 Solutions (& their composition) commonly used in this research

Solution	Composition
Analytical Methods	
Acidulated methanol	1.0 mL of acidified methanol (1% HCl v/v; 3mL conc HCL + 97mL methanol)
70% aqueous acetone extraction solvent	70% (v/v) aqueous acetone + 0.1% ascorbate. Add ascorbate fresh when ready to extract and store in dark at 4°C
Phloroglucinol buffer	0.25 g ascorbate, 1.25 g phloroglucinol, 215 mL conc. HCl, 25 mL methanol. Store in dark at 4°C. Lasts up to 3 weeks
Sodium acetate	200 mM, pH 7.5
Buffer A (model wine)	0.5 g potassium bitartrate in 800ml distilled water, add 120 mL of 96% ethanol. Adjust pH to 3.3 with hydrochloric acid (HLC) and make volume up 1.0 L with water
Buffer B (washing buffer)	9.86g of sodium chloride(NaCl) in 500 mL distilled water, add 12 mL glacial acetic acid and adjust pH to 4.9 with sodium hydroxide (NaOH) and make volume up to 1.0 L with distilled water
Buffer C (resuspension buffer)	50g of SDS in 800 mL distilled water, add 50 mL triethanolamine and monitor pH, when stabilises adjust pH to 9.4 with HCL and make volume to 1.0 L with distilled water
Ferric Chloride Reagent	2.7g ferric chloride (FeCl ₃) in 800 mL distilled water, add 800mL of concentrated HCl (12.1 N; 33-37% HCl) and make volume up 1.0 L with water
Protein Solution	Mix 25 mL Bovine Serum Albumin with 25 mL Buffer B. Store at 4°C.
Molecular Methods	
DNA loading dye (10×)	78% glycerol, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol, 10 mM EDTA
RNA loading dye (10×)	70% (v/v) deionised formamide, 10% (v/v) formaldehyde, 6% (v/v) agarose-gel loading dye (50% (v/v) glycerol, 1 mM EDTA, 0.4% (w/v) bromophenol blue), 14% (v/v) MOPS/EDTA buffer
RNA extraction buffer 1	5 M sodium perchlorate, 0.3 M Tris-HCl (pH 8.3), 1% (w/v) SDS, 1% (v/v) β-mercaptoethanol, 8.5% (w/v) PVPP, 2% (w/v) PEG 4000
RNA extraction buffer 2	5 M sodium perchlorate, 0.3 M Tris-HCl (pH 8.3), 1% (w/v) SDS, 1% (v/v) β-mercaptoethanol
TBE buffer	90 mM Tris-HCl, 90 mM borate, 2 mM EDTA, (pH 8.3)
TAE buffer	10 mM Tris-HCl (pH 7.6), 1 mM EDTA (pH 7.6)
LB (bacterial growth media)	Liquid = 1% (w/v) bacto-tryptone, 0.5% (w/v) yeast extract, 1% (w/v) NaCl, pH 7.0. Solid = add 1.2% (w/v) bacto-agar.
SOC (bacterial growth media)	Liquid = 2% (w/v) bacto-tryptone, 0.5% (w/v) yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl ₂ , 20mM MgSO ₄ , 20 mM sucrose, pH 7.0. Solid = add 1.2% (w/v) bacto-agar.
Grape Cormier (GC) (plant growth media)	Liquid = 0.3% (w/v) Gamborg's B-5 basal medium, 3% (w/v) sucrose, 0.025% (w/v) casein hydrolysate, 0.93 μM kinetin, 0.54 μM NAA, pH 5.7-5.8. Solid = add 0.8% bacto-agar
Ampicillin	100 μg/mL (in water)
XGal	(40μg/mL) 100 mg 5-bromo-4 chloro-3-indol-β-D-galactoside dissolved in 2 mL N, N'-dimethyl-for-amide.

APPENDIX 2B

HPLC chromatograms

Shiraz grape skin and wine samples were analysed in triplicate. The method of HPLC separation for grape and wine anthocyanins and flavonols was kindly provided by Mark Downey (2002), and is also described in Bogs et al. (2005) and Takos et al. (2006). The absorbance was measured at 520 nm (anthocyanins), 353 nm (flavonols), 320 nm (hydroxycinnamic acids) and 280 nm (total phenolics). Anthocyanin and flavonol peaks were identified by three means: a) comparison of their elution order with the published separations (Cheynier and Rigaud 1986; Wulf and Nagel 1978), b) comparison of the elution time and absorbance spectra with a commercial standards of malvidin-3-glucoside and quercetin-3-glucoside (Extrasynthese, France) c) LC-MS analysis (**Chapter 2, Section 2.2.1.3**). In both grapes and wine, the anthocyanin and flavonol compounds identified were expressed as malvidin-3-glucoside and quercetin-3-glucoside equivalents respectively, based on commercial standards (Extrasynthese, France).

Figure 2B.1 Typical Shiraz skin HPLC chromatogram

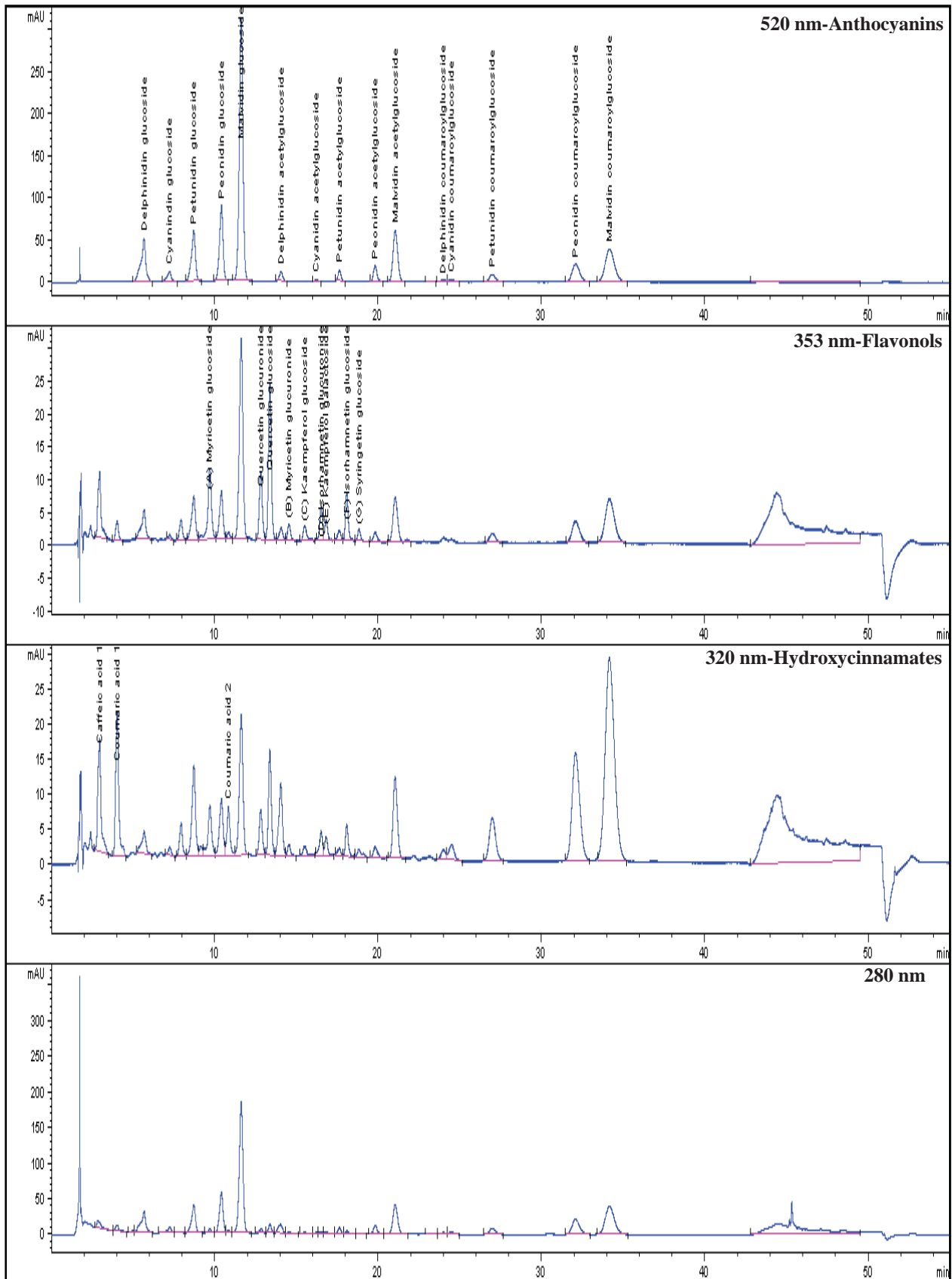
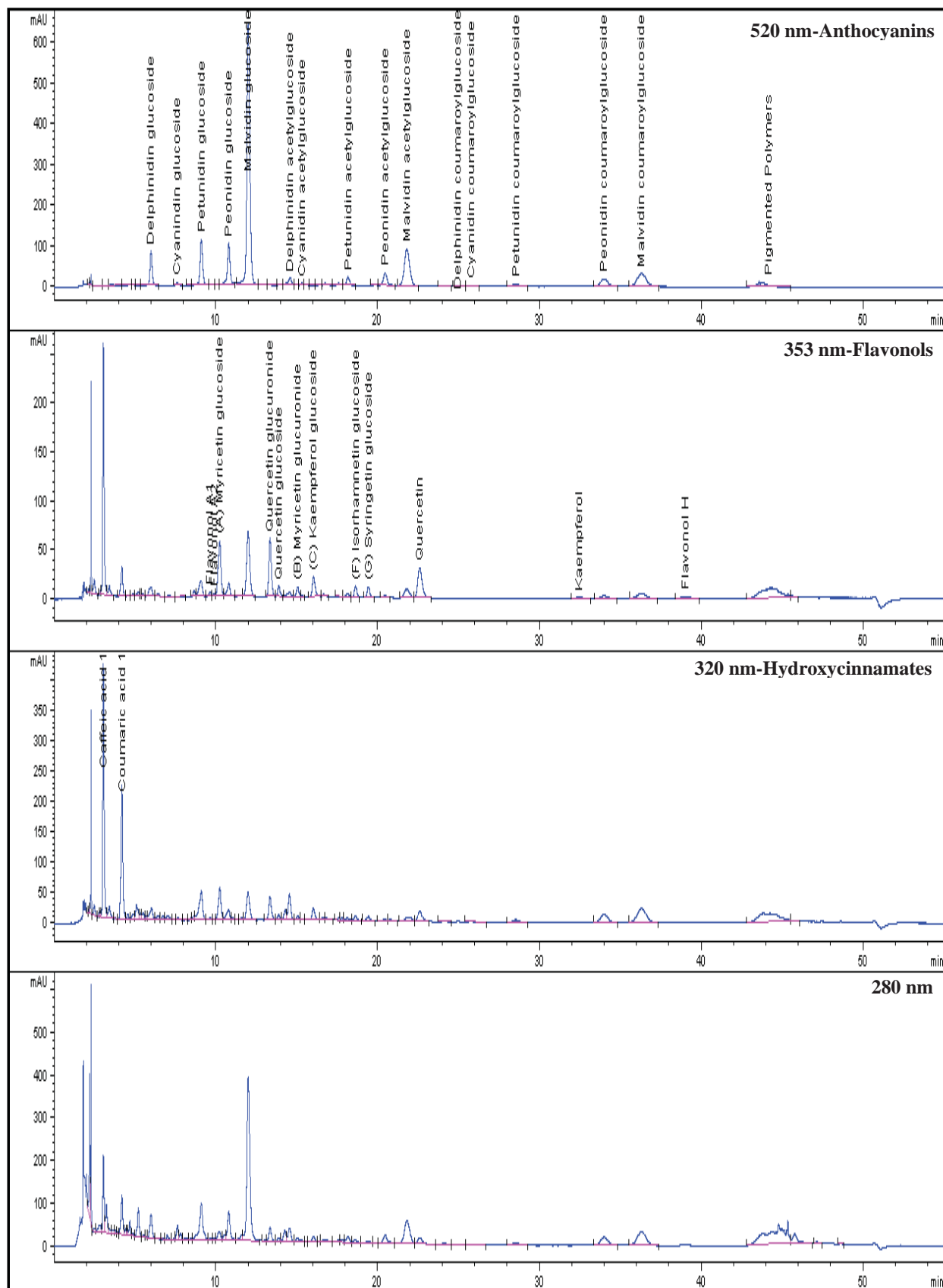


Figure 2B.2 Typical Shiraz wine HPLC chromatogram



APPENDIX 2C

Table of primers

Table 2C.1 RT-PCR primers used for analysis of flavonoid gene expression

Gene	Accession #	Reference	Forward primer	Reverse primer
<i>VvFLS1</i>	AY257978	Downey et al. 2003b	CAGGGCTTGCAGGTTTTAG	GGGTCTTCTCCTTGTTACAG
<i>VvFLS2</i>	AY257979	Downey et al. 2003b	TGAGCTGGCTATAGGTCCTC	TGCATGTACACTGGAAAAGG
<i>VvUFGT</i>	X75968	Downey et al. 2004	TAACACATTGTGGATGGAACAT	ACCTTCAATTCTCACTCCAATCTC
<i>VvLDOX</i>	X75966	Bogs et al. 2005	ACCTTCATCCTCCACAACAT	AGTAGAGCCTCCTGGGTCTT
<i>VvCHI</i>	X75963	Unpublished	GGATGAGAAAATTGAAGCTGAG	TACAGGAACAAGAGGAACCAAC
<i>VvUbiquitin1 (VvUBI1)</i>	TC32075	Downey et al. 2003b	GTGGTATTATTGAGCCATCCTT	AACCTCCAATCCAGTCATCTAC

Table 2C.2 RT-PCR primers used in testing potential *AtMYB12* homolog candidate genes

Assigned TC #	TC #	Forward primer	Reverse primer
TC1	TC46393	ATAATAGCGATACGTGCGGTGT	AATAATCGGGACGCTTCG
TC2	TC41702	TCATAATCACATCAGCAGCAG	GGAAATCAGGTGCTAGTTTTGTG
TC3	TC47032	GAAGTGGGGTTTGGATTATGTA	CAAATAAGGGAAGAGGAGAAAAG
TC4	TC47565	AGAATTCTAGGGTTTGGGTTGT	AATTCAAATTCTTGATCGGAAG
TC5	TC45624	CTAGCAGCCAACACCTATTTCAT	ATTCCACATGCCTTTCTTTGTA
TC6	TC44301	GAGGAGCAACGTCTTGTAATTC	TTCTCTTCTGCTCTCTCTGCT

Table 2C.3 RT-PCR primers used in testing potential microarray candidate genes

Assigned TC #	TC #	Unigene code	Forward primer	Reverse primer
TC7	TC39225	Vvi.10003	CAAACCCCTAGTGAAAGCACA	TAAAGGCACGCAAAGTACAAG
TC8	TC38623	Vvi.6975	CCCTCCACATTATCATTCTT	ACTGCATCAAAGTCTGAAAGGT
TC9	TC40540	Vvi.6616	AATCTCCTCCTTCTTCACTGC	AAGTTTCTGTACCCACCAAA
TC10	TC43962	Vvi.5444	TCGTCTGATGATATGAAGATGG	GTTGATTAGATGTGGGATTTGG
TC11	TC45686	Vvi.1267	GACGCTCAAACAAAAAGTAAAA	CTCCACACCTAAAAGATAGAGCA
TC12	TC49275	Vvi.3440	TATTGAAGAACCACAGCAAG	GTTCTACTTTTGGTGCCATTTC
TC13	TC46999	Vvi.7854	ATAAGCAGTTGGGTTCCAATA	TCTTTAAGTGGGTTCAACAGC

Table 2C.4 General primers used in PCR cloning

Role	Section	Primer name	Forward primer	Primer name	Reverse primer
Genomic Walking	5.2.1	<i>FLS1</i> pout	GCTTTCTTGCCCTCCACTTCTT TTTG	<i>FLS1</i> pnest	GTGAGCTTCTCCTCATCTGGG TCACT
Cloning into pLUC	5.2.1	LUCBAM	ATAGGATCCTCATTGTATCTTG TTGGCATATTT	LUCXHO	ATACTCGAGACTGCTTCCCTC TCTCTTACT
<i>VvFLS1</i> Intron isolation	5.3.1	<i>FLS1</i> INT	CCTCTCCATAACATCTG	<i>FLS1</i> INT	CCACTTTCATACTCCTC

APPENDIX 3A

South Australian grape growing regions:

The Riverland & McLaren Vale

Table 3A.1 Riverland and McLaren Vale regional characteristics

	Riverland (warm)	McLaren Vale (cool)
Climate details ^{a,b}		
Altitude (m)	65.5	50-200m
Longitude (°E)	140.6	138.33
Latitude (°S)	-34.28	35.14
Mean January temperature (°C)	23.2	23.4 ^c
Heat degrees days (cut off 19°C)(Oct-Apr) ^d	1767	1767
Sunshine hours (hrs)(Oct-Apr)	2002 ^e	1765
Relative humidity 3PM (%)(Oct-Apr)	30	46
Annual rainfall (mm)	261	513
Growing season rainfall (mm)(Oct-Apr)	130	168
General description	Inland region, with high summer temperatures	Warm summers, with cool afternoon sea breezes
Vintage details ^f		
Current area (Ha)	19,551	6437
Total crushed (T)	369,173	43,428
Estimated value of total crush (\$ million)	213	70
Top 3 varieties	Shiraz, Cabernet Sauvignon, Chardonnay	Shiraz, Cabernet Sauvignon, Chardonnay
Vineyard details ^g		
Soil type	The river valley terraces are sandy and the mallee highlands are sandy/ clay. Salt accumulation is widespread	Variable sand/ clay soils
Management practices	Vineyards are drip irrigated from River Murray (~5-7ML/Ha/season). Vines are trellised and most are mechanically pruned and harvested. Average yields range from 10-25 T/Ha	Vineyards are drip irrigated from mains supply (~0.8-1.5ML/Ha/season). Most vines are trained and either hand or mechanically pruned/ harvested. Average yields range from 5-15 T/Ha
Winery Details		
Region winery	Berri Estates Winery	Tintara Winery
Management practices	Fermented in large volumes, in close stainless steel vessels. Most wines are available to consumers with 12 months of harvest	Fermented in small volumes in open and closed stainless steel and cement vessels. Most wines aged in oak barrels and can it can take up to 36 months before wine makes it to consumers.
Wine product	Bulk to semi-premium (\$5/L-\$20/bottle)	Semi-premium to premium (\$5/bottle-\$55/bottle)
NOTES		
^a -Climate data for the Riverland is derived from Berri Station # 024002 (est. 1915), unless noted otherwise (Australian BOM Nov 2005)		
^b - McLaren Vale climate details derived from Noarlunga Weather Station (est. 2000), which is ~8 Km North of McLaren Vale), unless noted otherwise (AWBC Nov 2005)		
^c - The mean January temperature suggested by HWC (Sas and Lim 2003) is closer to 21.4°C (as detailed in Section 3.1.1.1.2)		
^d - Calculation of heat degree days as developed by Gladstones 1992		
^e - Sunshine hours for the Riverland calculated from Gladstones 1992		
^f -Vintage data obtained from Phylloxera and Grape Industry Board of South Australia 2003		
^g -Vineyard descriptions as described by Dry et al. 2004b		

NOTE: This figure is included on page 244 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3A.1 Map of South Australia grape growing regions Riverland & McLaren Vale. Shiraz berry samples were received from two SA viticultural regions; Riverland (Berri Estates Winery 34° 26' South, 140° 61' East) & McLaren Vale (Tintara Winery 35° 21' South, 138° 52' East). Fruit was collected from McLaren Vale, whilst couriered overnight from the Riverland. Map downloaded from Google Earth (Nov 2005).

APPENDIX 3B

Supplementary results for Chapter 3 (Flavonoid composition of Shiraz Grapes)

McLaren Vale & Riverland sample details

and

Data sets (On CD)

FILES ON CD: *(Provided as Microsoft Excel documents)*

<u>Appendix 3B MV03</u>	McLaren Vale, 2003 data set
<u>Appendix 3B MV04</u>	McLaren Vale, 2004 data set
<u>Appendix 3B RL03</u>	Riverland, 2003 data set
<u>Appendix 3B RL04</u>	Riverland, 2004 data set

KEY

For data sets in Appendix 3B (On CD)

MV03	McLaren Vale, 2003
MV04	McLaren Vale, 2004
RL03	Riverland, 2003
RL04	Riverland, 2004
CSIRO	Commonwealth Science and Research Organisation
HWC	Hardy Wine Company
GQS	Grape Quality Stream
H	High Quality
L	Low Quality
SPEC	UV-VIS spectrophotometric analysis
PGA	Phloroglucinol analysis
PPA	Protein precipitation assay
BM	Benchmarking
MA	Maselli
T	Totals
D	Delphinidin
C	Cyanidin
PT	Petunidin
PO	Peonidin
M	Malvidin
G	Glucoside
AG	Acetyl-glucoside
CG	Coumaroyl-glucosides
pp	Pigmented polymers
Q	Quercetin
M	Myricetin
K	Kaempferol
S	Syringetin
GR	Glucuronide
GS	Glucoside
EGC	Epigallocatechin
C	Catechin
E	Epicatechin
ECG	Epicatechin gallate
A	Adducts
SK	Skin
SD	Seed

3B.1. McLaren Vale

3B.1.1 MV03

3B.1.1.1 CSIRO data

Grape samples

GQS	No. of samples	Sample name
1H	6	MLE 1, 2, 3, 4, 5, 6
2	6	MLT 10, 13, 15, 16, 17, 18
3L	13	MLO 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 32
	25	

Parameters - See Section 3B.3
 -Berry factors
 -Grape flavonoid (anthocyanin, flavonol, tannin) content and composition

Wine samples

GQS	No. of samples	Sample details
1H	6	
2	6	
3L	10	No MLO32, (MLO20&21), (MLO29&31)
	22	

Parameters - See Section 3B.3
 -Wine anthocyanin and flavonol content and composition
 -(&) Indicates grape samples which were blended during winemaking (i.e. same measurements)

3B.1.1.2 HWC data

Vineyard assessment (also referred to as Benchmarking factors (BM))

GQS	No. of samples	Sample name/details
1H	5	No MLT2
2	6	
3L	9	No MLO32, MLO28, (MLO20&21), (MLO29&31)
	20	

Parameters - See Table 3B.1
 -(&) Indicates grape samples which measurements were the same

Weighbridge factors

GQS	No. of samples	Sample name/details
1H	5	No MLT2
2	6	
3L	9	No MLO32, MLO28, (MLO20&21), (MLO29&31)
	20	

Parameters
 -Bunch weight (g), berry weight (g)
 -Maselli analysis (MA): baume, pH, TA
 -Anthocyanin (NIR colour (mg/g berry)) No MLO32, MLO24, MLE2 (MLO20&21) (MLO29&31)
 -(&) Indicates grape samples which measurements were the same

Wine factors

GQS	No. of samples	Sample name/details
1H	6	
2	6	
3L	10	No MLO32, (MLO20&21), (MLO29&31)
	22	

Parameters
 -Wine colour density, hue (**Note** There are many missing data points)
 -Wine grade: 1(High quality) → 6(Low quality)
 -(&) Indicates grape samples which were blended during winemaking (i.e. same measurements)

Pricing factors

GQS	No. of samples	Sample name/details
1H	6	2X MLE5, 2X MLE3, 3X MLE6
2	6	2X MLT18, 2X MLT13, 2X MLT17, 2X MLT16
3L	10	No MLO32, (MLO20&21), (MLO29&31), 2X MLO26, 2X MLO28
	22	
Parameters		
-\$/Tonne, \$/bottle -(X) Indicates more than one data point for each sample. The weighted average was calculated -(&) Indicates grape samples which were blended during winemaking (i.e. same measurements)		

3B.1.2 MV04

3B.1.2.1 CSIRO data

Grape samples

GQS	No. of samples	Sample name/details
1	7	M 4, 5, 14, 15, 20, 22, 23
2	7	M 1, 3, 6, 8, 12, 13, 16
3	10	M 2, 7, 9, 10, 11, 17, 18, 19, 21, 24
	24	
Parameters - See Section 3B.3		
-Berry factors -Grape flavonoid (anthocyanin, flavonol) content and composition -Grape tannin content		

Wine samples

GQS	No. of samples	Sample name/details
1	6	(M14&M15)
2	6	(M6&M8)
3	8	(M10&M11), (M18&M19)
	20	
Parameters - See Section 3B.3		
-Wine anthocyanin and flavonol content and composition -(&) Indicates grape samples which were blended during winemaking (i.e. same measurements)		

3B.1.2.2 HWC data

Vineyard assessment (also referred to as Benchmarking factors (BM))

GQS	No. of samples	Sample name/details
1	5	No M4, M15
2	7	
3	9	No M9
	21	
Parameters - See Table 3B.1		

Weighbridge factors

GQS	No. of samples	Sample name/details
1	7	
2	7	
3	8	No M9, M24
	20	
Parameters		
-Bunch weight (g), (No berry weight) -Maselli analysis (MA): baume, pH, TA -Anthocyanin (NIR colour (mg/g berry) and Col2Bx)		

Pre-ferment factors

GQS	No. of samples	Sample name/details
1	5	
2	5	
3	5	
	15	
Parameters		
-TSO ₂ , TA, pH, Be, NH ₄ , FAN, YAN		
-Note There are many missing data points		

Wine factors

GQS	No. of samples	Sample name/details
1	6	(M14&M15)
2	6	(M6&M8)
3	8	(M10&M11) (M18&M19)
	20	
Parameters		
-Wine grade: 1(High quality) → 7(Low quality) No M3, M21		
-Somers measurements:		
-colour density, hue, chemical age 1, chemical age 2, degree of ionisation, total anthocyanins (mg/L), total phenolics (mg/L), A420, pressed baume		
-(&) Indicates grape samples which were blended during winemaking (i.e. same measurements)		

3B.2. Riverland

3B.2.1 RL03

3B.2.1.1 CSIRO data

GQS	No. of samples	Sample name/details
1H	13	RL 1, 13, 15, 17, 2, 23, 25, 27, 3, 37, 38, 41, 7
2	17	RL 16, 19, 20, 21, 22, 24, 26, 28, 30, 31,32, 33, 35, 45, 46, 49, 50
3	9	RL 29, 34, 36, 4, 48, 5, 54, 55, 6
4	8	RL 10, 14, 39, 40, 42, 43, 44, 8
5L	4	RL 18, 51, 53, 9
Unclassified	4	RL 11, 12, 47, 52
	55	
Parameters - See Section 3B.3		
-Berry factors		
-Grape flavonoid (anthocyanin, flavonol) content and composition		

3B.2.1.2 HWC data

GQS	No. of samples	Sample name/details
1H	13	
2	17	
3	9	
4	8	
5L	4	
Unclassified	0	No RL11, RL12, RL47, RL52
	51	
Parameters		
-Vineyard assessment (benchmarking) - See Table 3B.1		
-Berry weight (g), baume		
-Anthocyanins (NIR colour (mg/g berry))		
-Enduse/ Wine grade: 1(High quality) → 5(Low quality) No RL11, RL12, RL52		

3B.2.2 RL04

3B.2.2.1 CSIRO data

GQS	No. of samples	Sample name/details
1H	13	R 10, 13, 17, 18, 28, 29, 3, 30, 32, 4, 6, 8, 9
2	10	R 1, 11, 12, 14, 15, 16, 35, 43, 5, 7
3	11	R 2, 26, 27, 33, 34, 40, 45, 49, 52, 53, 54
4	10	R 20, 22, 23, 31, 36, 37, 38, 39, 41, 55
5L	11	R 19, 21, 24, 25, 42, 44, 46, 47, 48, 50, 51
	55	
Parameters - See Section 3B.3		
-Berry factors		
-Grape flavonoid (anthocyanin, flavonol) content and composition		
-Grape tannin content		

3B.2.2.2 HWC data

GQS	No. of samples	Sample name/details
1H	13	
2	10	
3	11	
4	10	
5L	11	
	55	
Parameters		
-Vineyard assessment (benchmarking) - See Table 3B.1		
-Berry weight (g), baume		
-Anthocyanins (NIR colour (mg/g berry))		
-Enduse/ Wine grade: 1(High quality) → 4(Low quality) No R55, R31		

Table 3B.1 Vineyard assessment measurements recorded by Hardy Wine Company (HWC). Data is also known as benchmarking factors (BM). * indicates data was provided; blank cells indicate data was not provided.

NOTE: This table is included on page 251 of the print copy of the thesis held in the University of Adelaide Library.

3B.3. CSIRO measurements

3B.3.1 Berry factors

-Sampled all data sets: MV03, MV04, RL03, RL04

-100 berries were randomly selected for processing

-Measurements are an average of 100 berries

-Data: Berry weight (g), skin weight (g), seed weight (g), number of seeds, °Brix

-Calculated data: Skin weight per berry weight (also expressed also a proportion (%)), seed weight per berry weight (also expressed also a proportion (%))

3B.3.2 Grape & wine flavonoid content & composition

3B.3.2.1 HPLC analysis of anthocyanins & flavonols

3B.3.2.1.1 Grape

-Sampled all data sets: MV03, MV04, RL03, RL04

-Samples consisted of 100 berry skins, pooled together and finely ground to a powder

-Samples were analysed in triplicate and are expressed mg/g berry

-See **Appendix 2B, Figure 2B.1** for typical Shiraz grape HPLC chromatogram

- **Anthocyanins (A520)**

-Data: 15 anthocyanin compounds detected:

Delphinidin glucoside, cyanidin glucoside, petunidin glucoside, peonidin glycoside, malvidin glucoside, delphinidin acetyl-glycoside, cyanidin acetyl-glycoside, petunidin acetyl-glycoside, peonidin acetyl-glycoside, malvidin acetyl-glycoside, delphinidin coumaroyl-glycoside, cyanidin coumaroyl-glycoside, petunidin coumaroyl-glycoside, peonidin coumaroyl-glycoside, malvidin coumaroyl-glycoside

-Calculated data: Totals (T=sum of each compound type)

TAnthocyanins, TGlucosides, TAcetyl-glycosides, TCoumaroyl-glycosides, TDelphinidins,

TCyanidins, TPetunidins, TPeonidins, TMalvidins

- **Flavonols (A353)**

-**Data:** 9 flavonol compounds detected:

Quercetin glucoside, quercetin glucuronide, flavonol A (myricetin glucoside), flavonol B (myricetin glucuronide), flavonol C (kaempferol glucoside), flavonol D (isorhamnetin glucuronide), flavonol E (kaempferol galactoside), flavonol F (isorhamnetin glucoside) flavonol G (syringetin glucoside)

-**Calculated:** Totals (T=sum of each compound type)

TFlavonols, TQuercetin-glycosides, TMyricetin-glycosides, TKaempferol-glycosides, TIsorhamnetin-glycosides

- **Hydroxycinnamaic acids (A320)**

-**Data:** Caffeic acid 1, Coumaric acid 1, Coumaric acid 2

-**Calculated data:** Totals (T=sum of each compound type) TCoumaric acids

3B.3.2.2.2 Wine

-Sampled data sets: MV03, MV04

-Raw wine samples were analysed in triplicate and are expressed g/L

-Abbreviated forms of the compounds listed (See **Key**)

-See **Appendix 2B, Figure 2B.2** for typical Shiraz wine HPLC chromatogram.

- **Anthocyanins (A520)**

-**Data:** 16 anthocyanin compounds detected:

Delphinidin glucoside, cyanidin glucoside, petunidin glucoside, peonidin glycoside, malvidin glucoside, delphinidin acetyl-glycoside, cyanidin acetyl-glycoside, petunidin acetyl-glycoside, peonidin acetyl-glycoside, malvidin acetyl-glycoside, delphinidin coumaroyl-glycoside, cyanidin coumaroyl-glycoside, petunidin coumaroyl-glycoside, peonidin coumaroyl-glycoside, malvidin coumaroyl-glycoside

-Additional peak= Pigmented polymers (pp). Incorporates large integrated peak at the end of the HPLC chromatogram

-**Calculated data:** Totals (T=sum of each compound type)

TAnthocyanins (TAnthocyanins + pp), TGlucosides, TAcetyl-glycosides, TCoumaroyl-glycosides, TDelphinidins, TCyanidins, TPetunidins, TPeonidins, TMalvidins

- **Flavonols (A353)**

-Data: 12 flavonol compounds detected:

Quercetin glucoside and quercetin glucuronide, flavonol A (myricetin glucoside), flavonol B (myricetin glucuronide), flavonol C (kaempferol glucoside), flavonol F (isorhamnetin glucoside) flavonol G (syringetin glucoside)

-No flavonol D (isorhamnetin glucuronide), flavonol E (kaempferol galactoside)

-Additional peaks (unknown)= flavonols A1, flavonols A2, flavonol H (not detected in 2004)

-Additional peaks (flavonol aglycones)= quercetin, kaempferol (not detected in 2004)

-Calculated: Totals (T=sum of each compound type)

TFlavonols, TQuercetin-glycosides, TMalvidin-glycosides

- **Hydroxycinnamaic acids (A320)**

-Data: Caffeic acid1 and Coumaric acid1

3B.3.2.2 UV-VIS Spectrophotometric analysis

3B.3.2.2.1 Grape

-Method based on Iland et al. (2000)

-Sampled all grape data sets: MV03, MV04, RL03, RL04

-Sampled wine data sets: MV03, MV04

-Grape and wine extracts (from HPLC analysis) were diluted in acidulated methanol

-Samples were analysed in triplicate; grape data expressed mg/g berry, wine data expressed g/L

-Data: (SPEC)-Total anthocyanins (A520), Total phenolics (A280)

3B.3.2.2.2 Wine

-Method as described by Somers and Evans (1977)

-Sampled wine data set: MV04

-Wine samples were analysed in triplicate and are expressed mg/L.

-Data: Colour density, hue, degree of red pigment colouration (%), total red pigments, total phenolics, total anthocyanins (mg/L), chemical age I, chemical age II, degree of ionisation of anthocyanins (%)

3B.3.2.3 Analysis of skin & seed tannins

3B.3.2.3.1 PGA

- Method based on that by Downey et al. (2003a) and Kennedy and Jones (2001)
- Sampled grape data set: MV03 (skin and seed)
- Samples consisted of 100 berry skins (seeds from 100 berries), pooled together and finely ground to a powder
- Only acid hydrolysis component of method performed
- Samples were analysed in triplicate and are expressed mg/g berry

- Skin

-Data: 7 tannin compounds detected:

Epigallocatechin-adduct, catechin-adduct, epicatechin-adduct, catechin, epicatechin gallate-adduct, epicatechin, epicatechin gallate

-Calculated data: Totals (T=sum of each compound type)

TSkin tannins, TSkin adducts, TSkin free monomers

- Seed

-Data: 6 tannin compounds detected:

Catechin-adduct, epicatechin-adduct, catechin, epicatechin gallate-adduct, epicatechin, epicatechin gallate

-Calculated data: Totals (T=sum of each compound type)

TSeed tannins, TSeed adducts, TSeed free monomers

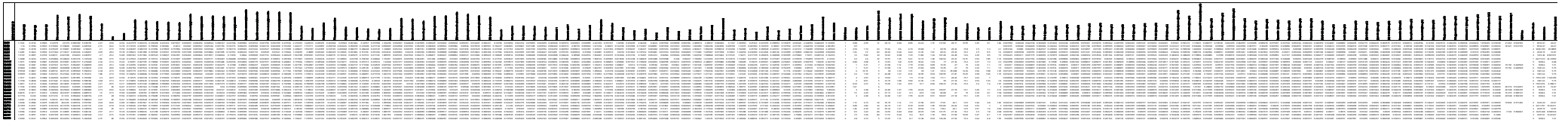
3B.3.2.3.2 PPA

- Method based on that by Downey and Adams (2005) and Harbertson et al. (2002)
- Sampled grape data set: MV04, RL04 (skin and seed)
- Samples consisted of: 100 berry skins (or seeds from 100 berries), pooled together and finely ground to a powder
- Samples were analysed in triplicate and are expressed mg/ g berry

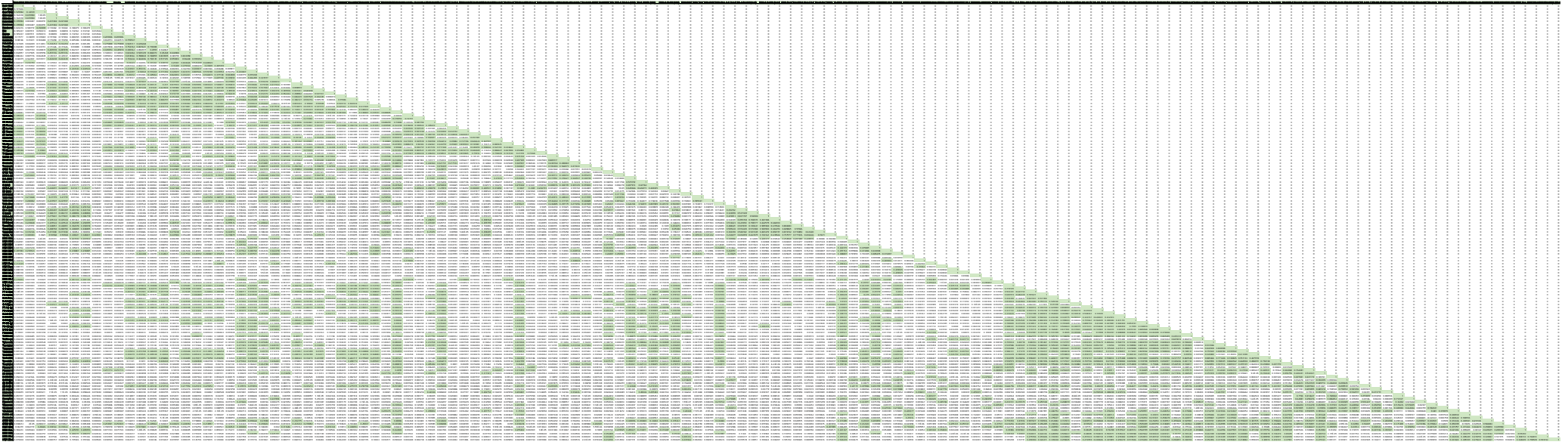
-Data: Total skin tannins, Total seed tannins

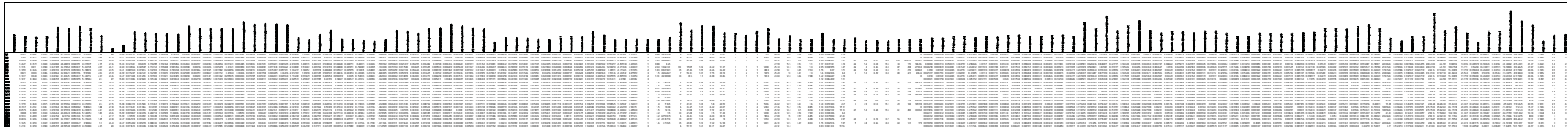
-Calculated data: Totals (T=sum of each compound type).

Total tannins (skin + seed)

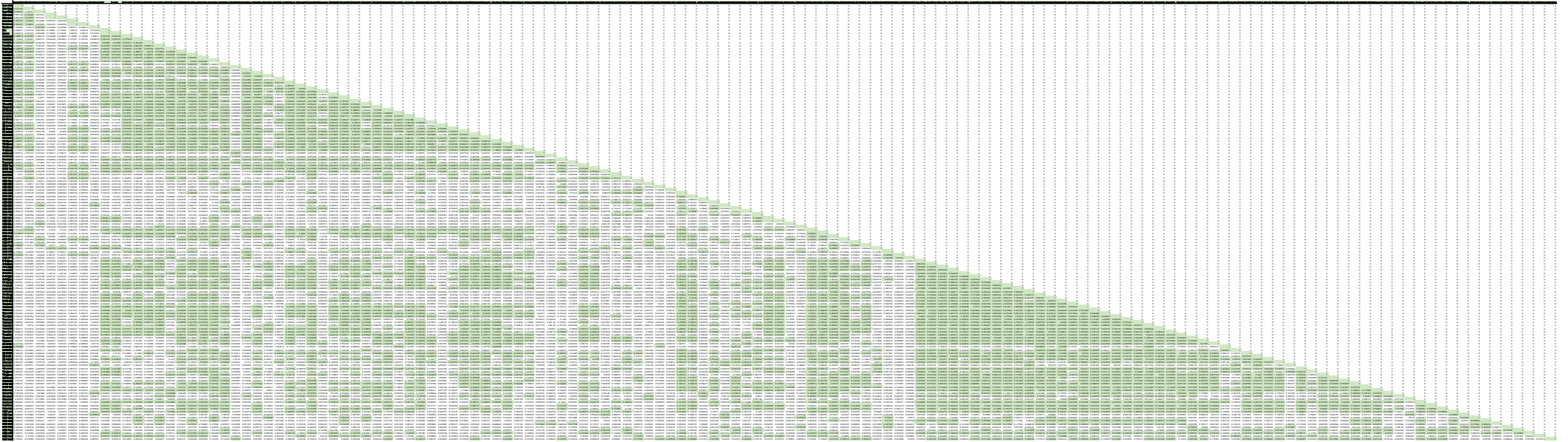


This table displays R values for MVI5, organized in a triangular format. The values are arranged in rows, with the number of columns decreasing from left to right. The values are small, multi-digit numbers, likely representing specific data points or calculations related to MVI5. The table is bounded by a thick black line at the top and bottom, and a vertical line on the left side.





The image displays a large, dense table of R values for MV04, organized in a triangular grid. The table is mostly illegible due to the small font size and high density of text. The data is organized in a triangular grid, with the number of columns decreasing from left to right. The table is bounded by a thick black line at the top and bottom, and a vertical line on the left side. The text within the table is too small to read, but it appears to be a list of numerical values arranged in rows and columns.



APPENDIX 3B RL03 - Mean Values

Table with 100 columns (RL01-RL100) and 100 rows (R1-R100). Each cell contains a numerical value representing mean values for various parameters.

APPENDIX 3B RL03 - R Values

Key	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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Key	1301	1302	1303	1304	1305	1306	1307	1308	1309	1310	1311	1312	1313	1314	1315	1316</																																																																																				

APPENDIX 4A

Maps of experimental plots

(Light induction of flavonol biosynthesis)

NOTE: This figure is included on page 256 of the print copy of the thesis held in the University of Adelaide Library.

Figure 4A.1 Map of Slate Creek Vineyard, Willunga. Light induction experiments were conducted in a commercial vineyard, Harvey's Slate Creek, in Willunga, South Australia (34° 46' South, 138° 32' East). Varieties sampled included Shiraz & Chardonnay vines grown on modified Scott-Henry trellises. The experimental plot included a single block each for 180 Shiraz vines (6 rows & ten panels) & 72 Chardonnay vines (four rows & six panels). Boxes were applied to both sides of the row & a two-panel & two-row buffer was employed to avoid end effects. Map downloaded from Google Earth (Nov 2005).

NOTE: This figure is included on page 257 of the print copy of the thesis held in the University of Adelaide Library.

Figure 4A.2 Map of Coombe Vineyard, Adelaide University Waite Campus. Diurnal experiments were conducted at the Coombe Vineyard on the Waite Campus of Adelaide University (Adelaide, South Australia, latitude 34° 56' South, longitude 138° 36' East). The experiment plot included two N-S facing rows of 180 Shiraz (clone BVRC12S) vines, & a two-panel & five-row buffer was employed to avoid end effects. Map downloaded from Google Earth (Nov 2005).

APPENDIX 4B

Supplementary results for Chapter 4 (Light induction of flavonol biosynthesis)

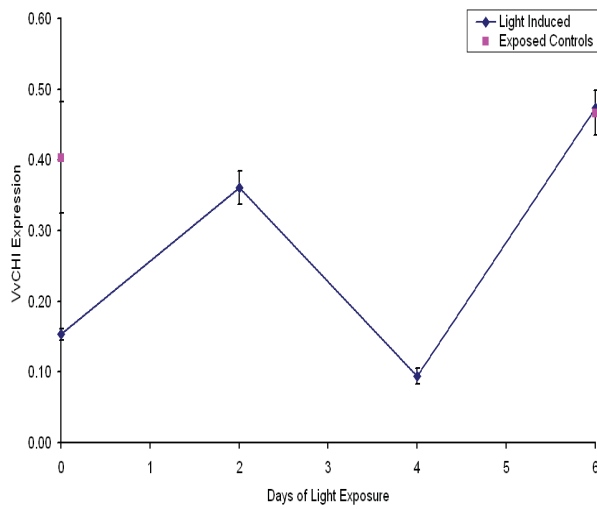
The influence of bunch light exposure on expression of *VvLDOX* & *VvCHI* in Shiraz & Chardonnay berries during development

The influence of bunch light exposure on expression of *VvLDOX* and *VvCHI* in Shiraz and Chardonnay berries during development are in graphs below. Detailed descriptions of samples and key sampling dates are discussed in **Chapter 4**. Boxes were applied to vines at budburst in the 2003-2004 season. At each stage in development, boxes were removed from vines and berries/inflorescences were randomly sampled from shaded (now Light Induced (LI)) along with nearby control berries (Exposed Controls (EC)). Light induced samples were taken every 2nd day for 6 days, when another exposed control sample was taken. At pre-veraison and post-veraison time-points, an additional shaded control (SC) sample was also taken on day 6. For each sample, 100 berries (5-10 inflorescences) were pooled together. Error bars represent the standard error (\pm SEM) for three analytical replicates. Expression of *VvLDOX* and *VvCHI* is relative to *VvUBI1* expression determined by RT-PCR.

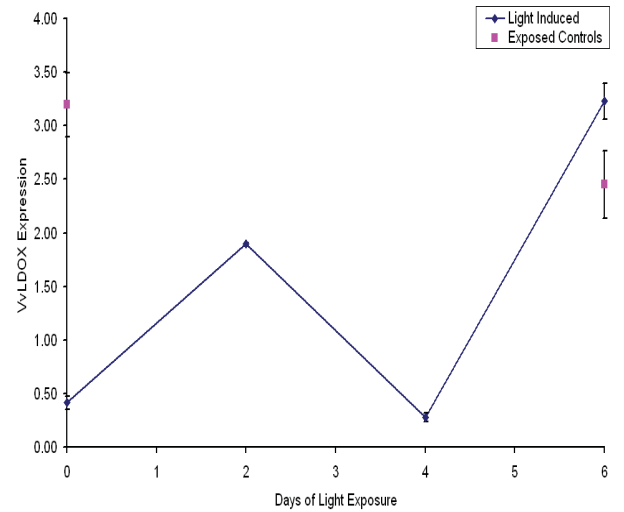
Figure 4B.1 RT-PCR expression of *VvCHI* & *VvLDOX* genes in Shiraz berries during development

FLOWERING - SHIRAZ

VvCHI

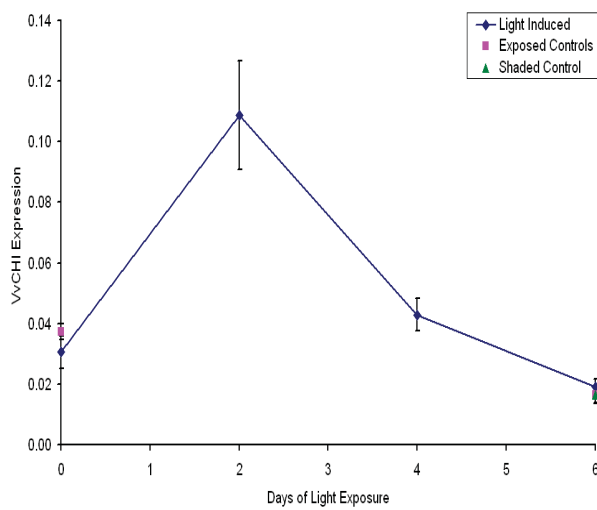


VvLDOX

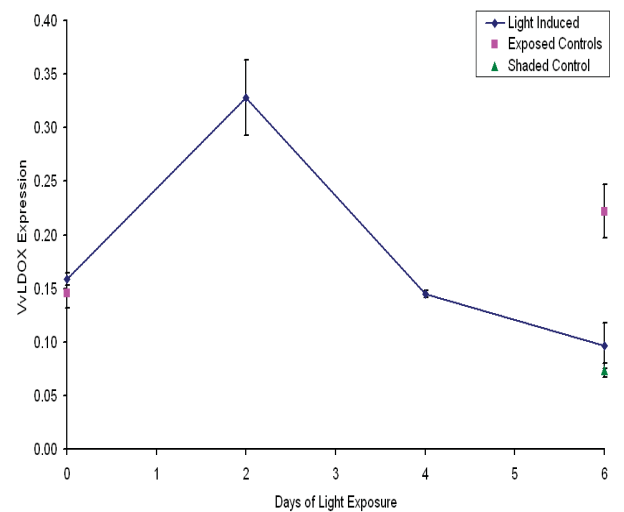


PRE-VERAISON - SHIRAZ

VvCHI

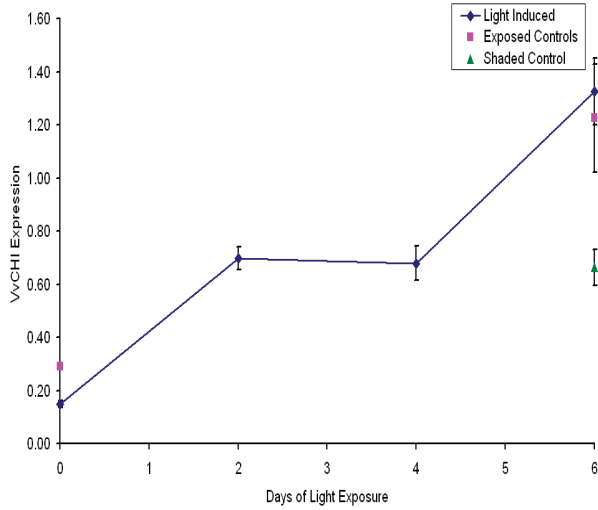


VvLDOX

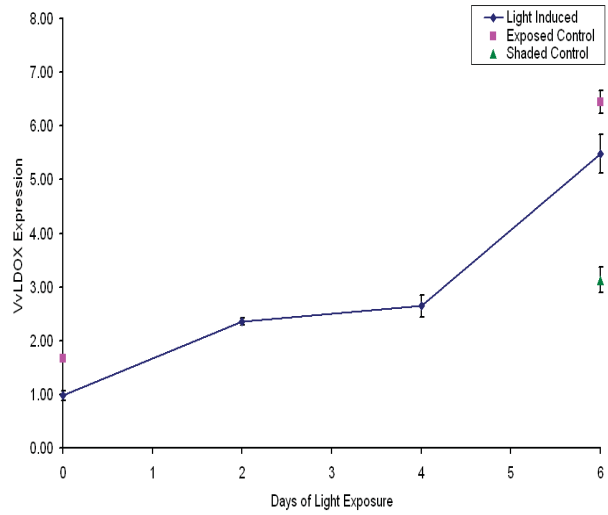


POST-VERAISON - SHIRAZ

VvCHI

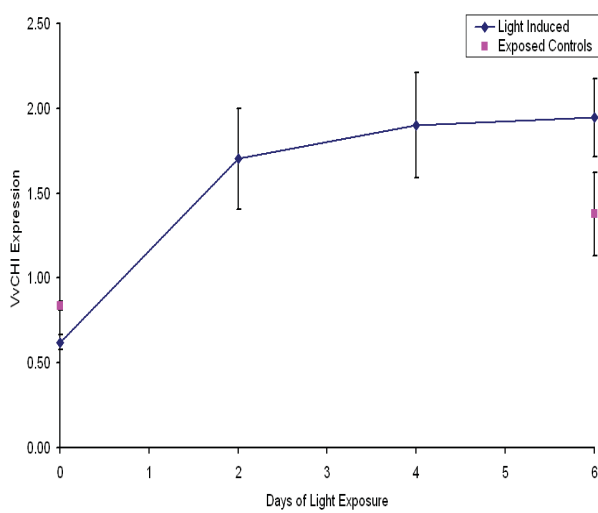


VvLDOX



HARVEST - SHIRAZ

VvCHI



VvLDOX

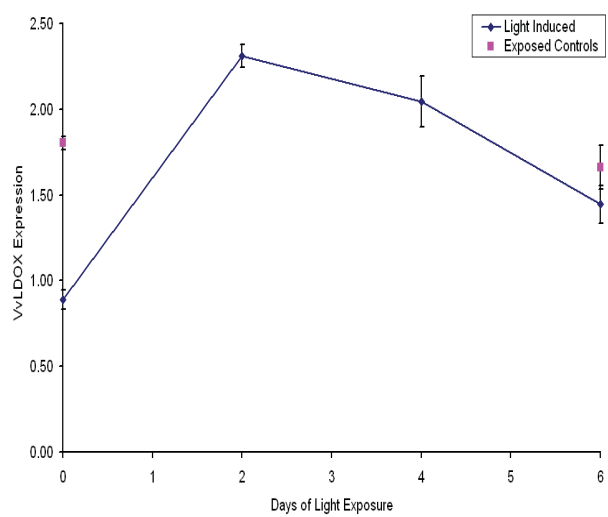
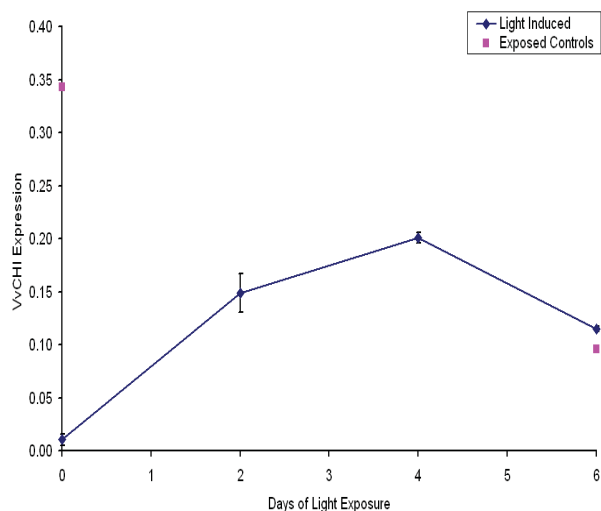


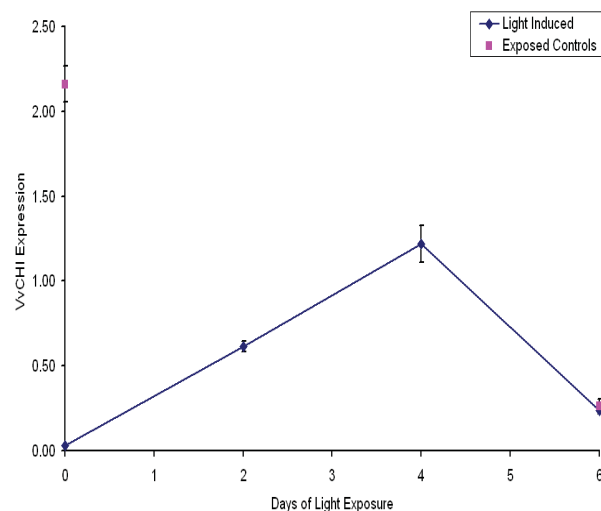
Figure 4B.2 RT-PCR expression of *VvCHI* & *VvLDOX* genes in Chardonnay berries during development

FLOWERING - CHARDONNAY

VvCHI

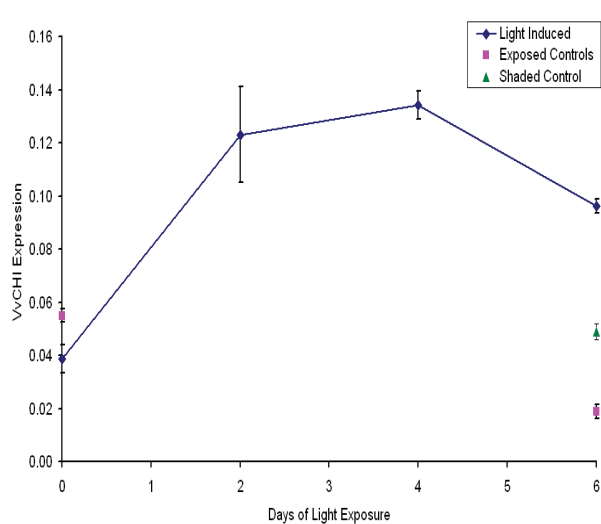


VvLDOX

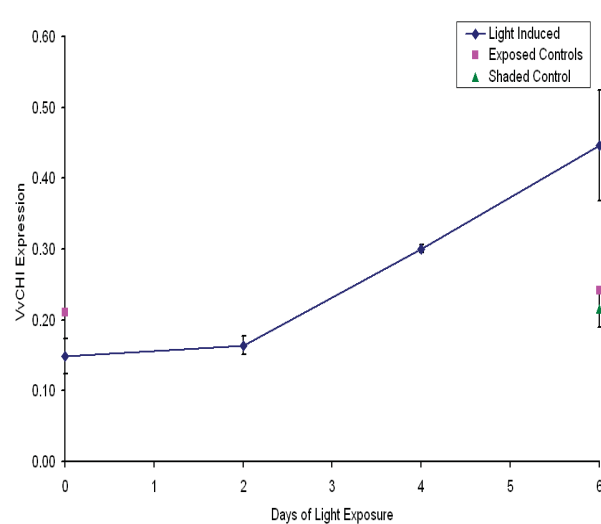


POST-VERAISON – CHARDONNAY

VvCHI

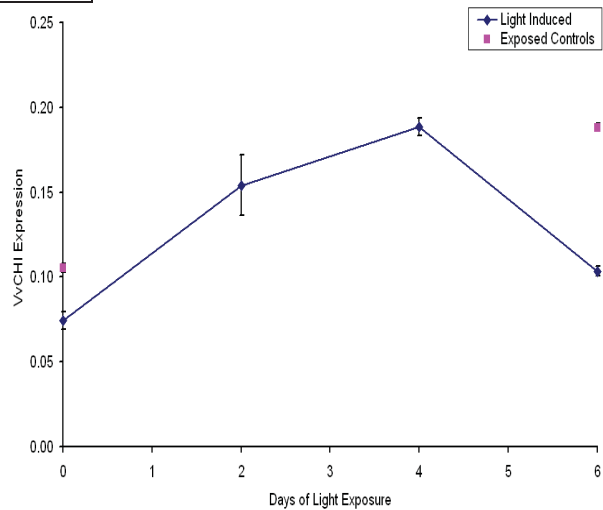


VvLDOX

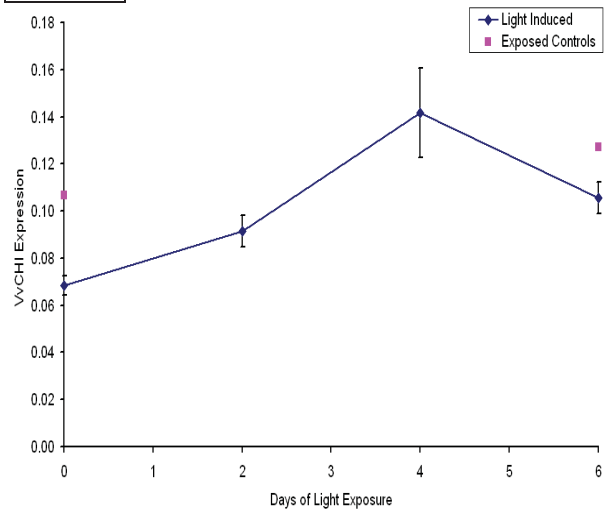


HARVEST - CHARDONNAY

VvCHI



VvLDOX



APPENDIX 5A

Supplementary results for Chapter 5 (Molecular mechanisms of flavonol biosynthesis)

Microarray data (On CD)

FILE ON CD: Appendix 5A Microarray data (Provided as a Microsoft Excel document)

Total RNA was extracted from light-exposed and dark-control Chardonnay cells (as described in **Chapter 5**). Microarray analysis involved hybridisations using the Affy-1-cycle labelling technique. The *V. vinifera* cDNA microarray slide contained 16,602 unique sequences, (part of the NCBI grapevine database <http://www.ncbi.nlm.nih.gov/>). The microarray procedures and *V. vinifera* slide information are available on the Affymetrix website (<http://www.affymetrix.com>).

The raw data was analysed in Avadis (V4) (Strand Life Sciences, USA). The probe level was corrected using a normalisation method (GC-RMA), which accounts for technical bias in a sample. The data was then log transformed (base 2) which compresses the data, allowing large differences to stand out. Differential expression analysis (DEA) was performed on each gene, resulting in the following headings:

-Corrected p value. An unpaired t-test was conducted on the technical replicates; the correction type, Benjamini-Hochberg. The corrected p value indicates which genes significantly changed in response to light, where $p < 0.001$ is highly significant and ns is not significant.

-Rank. These values are closely associated with the corrected p value, ranging from 0-16,601.

-Absolute value. These values are used to indicate the difference in gene expression between the light and dark samples. It is also represented as L-D.

-Regulation. Indicates whether genes are up/down regulated in response to light. This is quantified as the ratio of L/D (additional calculation-no correction), where values > 1.0 indicated up-regulation in response to light.

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Year	Country	Value
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1978	Spain	6.5
1979	Spain	7.0
1980	Spain	7.5
1981	Spain	8.0
1982	Spain	8.5
1983	Spain	9.0
1984	Spain	9.5
1985	Spain	10.0
1986	Spain	10.5
1987	Spain	11.0
1988	Spain	11.5
1989	Spain	12.0
1990	Spain	12.5
1991	Spain	13.0
1992	Spain	13.5
1993	Spain	14.0
1994	Spain	14.5
1995	Spain	15.0
1996	Spain	15.5
1997	Spain	16.0
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1999	Spain	17.0
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2002	Spain	18.5
2003	Spain	19.0
2004	Spain	19.5
2005	Spain	20.0
2006	Spain	20.5
2007	Spain	21.0
2008	Spain	21.5
2009	Spain	22.0
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2011	Spain	23.0
2012	Spain	23.5
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2018	Spain	26.5
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APPENDIX 5B

Supplementary results for Chapter 5 (Molecular mechanisms of flavonol biosynthesis)

Testing potential transcription factor candidate genes by RT-PCR expression analysis

To further investigate the expression pattern of candidate genes TC10 and TC12 (and compare it to *VvFLS1* expression) cDNA from the Shiraz and Chardonnay light induction series and the Shiraz hourly induction series as described in **Chapter 4** was used in RT-PCR expression analysis (**Figure 5B.1 & 5B.2**). Both candidates gene expression was lower in the shaded fruit compared to the exposed fruit and expression was increased in light induced samples. However, there was a difference in the magnitude of expression different between the candidates, where TC12 expression was greater. These results suggest these two putative transcription regulators (TC candidate 10 and 12) may be involved in the regulation of *VvFLS1* expression (based on expression patterns) and subsequently warrants further investigation.

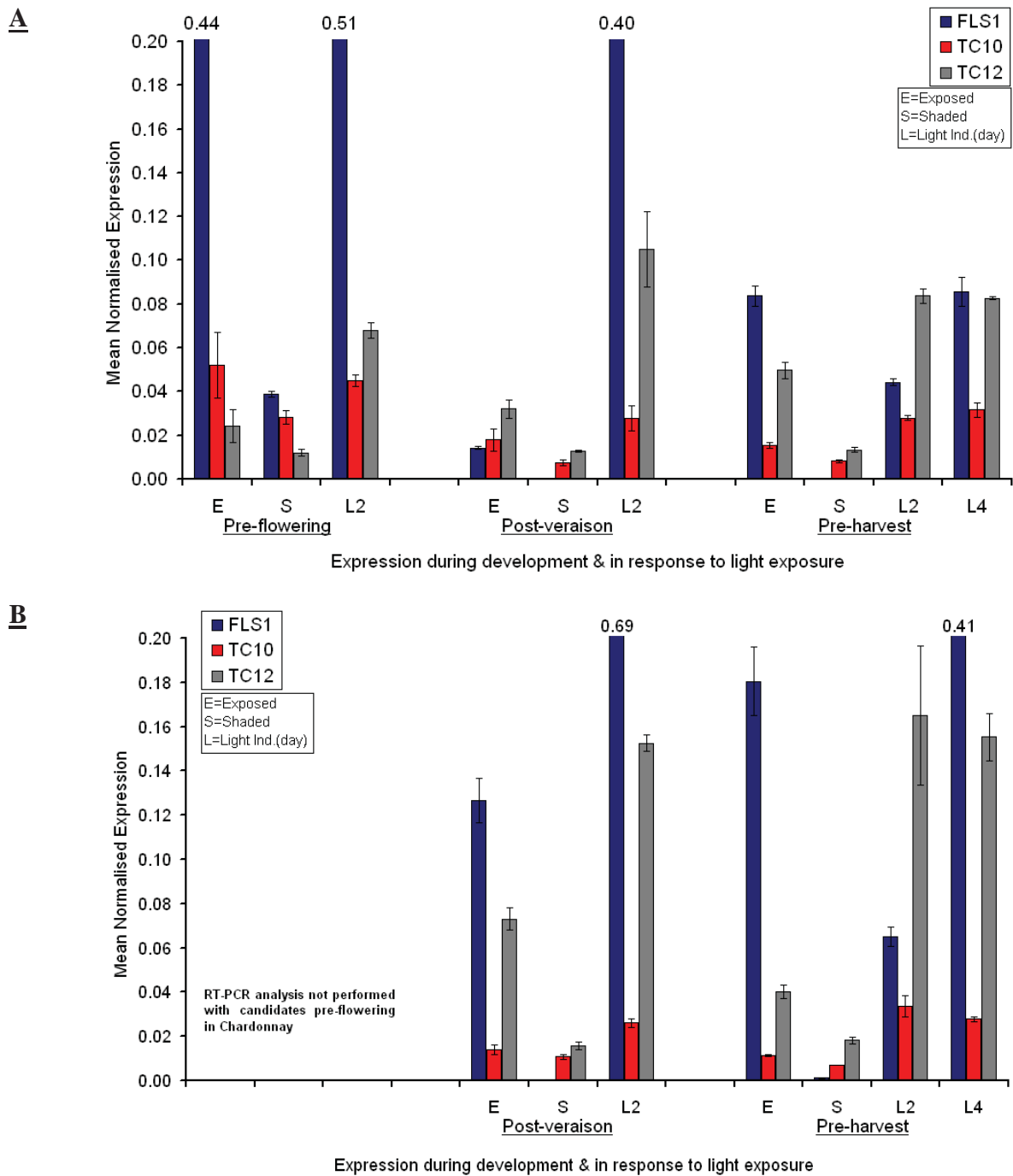


Figure 5B.1 RT-PCR expression pattern for candidate genes (TC10 & TC12) in Shiraz & Chardonnay fruit.

A Expression of *VvFLS1* & candidate genes (TC10 (TC43962) & TC12 (TC49275)) during development in Shiraz skins, pre-flowering (12/11/03), post-veraison (2/2/04) & pre-harvest (23/2/04). At each time-point, on day 0, shaded fruit (S) & exposed fruit (E) was sampled. The boxes were removed & light induced fruit (L) was sampled on either day 2 (L2) or day 4 (L4) (as described in **Chapter 4**). Data is expressed relative to the expression of *VvUBI1Q1*. Error bars represent the standard error (\pm SEM) for three analytical replicates.

B Expression of *VvFLS1* & candidate genes (TC10 (TC43962) & TC12 (TC49275)) during development in Chardonnay skins, post-veraison (2/2/04) & pre-harvest (23/2/04). At each time-point, on day 0, shaded fruit (S) & exposed fruit (E) was sampled. The boxes were removed & light induced fruit (L) was sampled on either day 2 (L2) or day 4 (L4) (as described in **Chapter 4**). Data is expressed relative to the expression of *VvUBI1Q1*. Error bars represent the standard error (\pm SEM) for three analytical replicates.

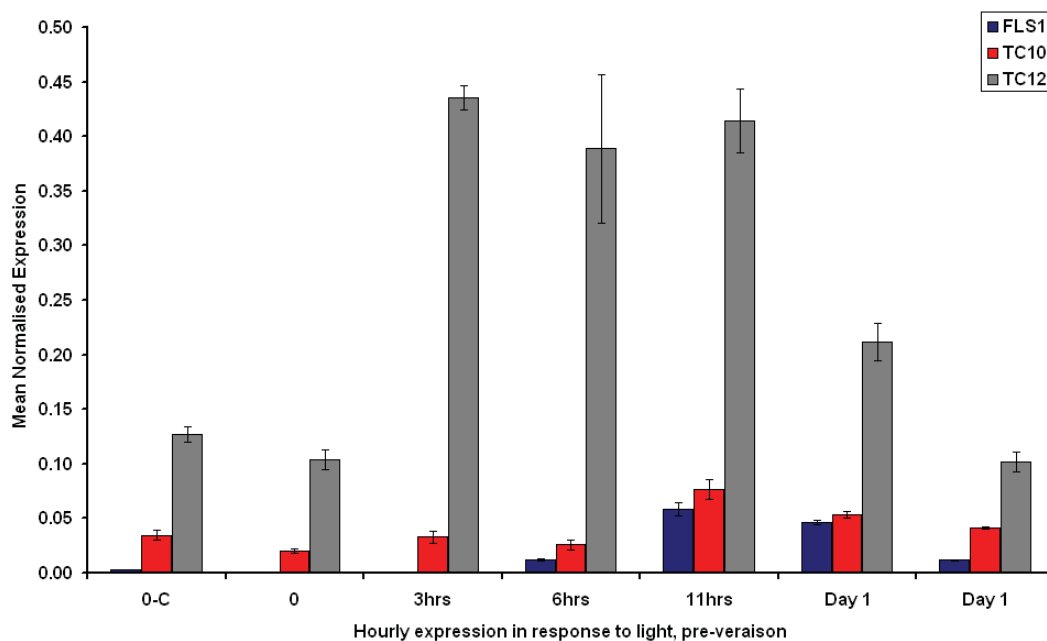


Figure 5B.2 RT-PCR expression pattern for candidate genes (TC10 & TC12) in Shiraz (hourly induction). Expression of *VvFLS1* & candidate genes (TC10 (TC43962, TC12 (TC49275) in Shiraz skins, pre-veraison (9/1/05). Boxes were removed on Day 0 & berry skins sampled (along with exposed controls (0-C). Berries continued to be sampled 3, 6 11 hrs & on day 1 & 2 (as described in **Chapter 4**). Data is expressed relative to the expression of *VvUBI1*. Error bars represent the standard error (\pm SEM) for three analytical replicates.

APPENDIX 5C






Supplementary results for Chapter 5 (Molecular mechanisms of flavonol biosynthesis)

Nucleotide sequence alignment of grapevine *VvFLS1* genomic sequences (On CD)

FILE ON CD: Appendix 5C FLS nucleotide alignment (Provided as Microsoft Word document)

Sequences aligned included: *VvFLS1* promoter1 (Coding sequence AY257978, genomic sequence (this investigation), *VvFLS4cs* (AB092591), *VvFLS1pn* (unpublished), VvTC46143. Full-length genomic sequences were aligned using ClustralW (<http://www.ebi.ac.uk/clustralW/>) (Thompson et al. 1994) and displayed by GenDoc (<http://www.psc.edu/biomed/genedoc>). Dashes indicate introduced gaps. Shading represents the degree of sequence identity at each nucleotide position: nucleotides conserved between sequences are shaded in black and dark grey shading indicates conserved nucleotides in two out of three of the sequences.

KEY:

-  2Kb and 1.5Kb mark in the upstream genomic sequence (from the start codon (ATG))
-  Start codon (ATG)
-  Start and end of intron sequence
-  Primers used to isolate the *VvFLS1* promoter sequence(s)
-  Key DNA binding motifs/elements






APPENDIX 5C

Supplementary results for Chapter 5 (Molecular mechanisms of flavonol biosynthesis)

Nucleotide sequence alignment of grapevine *VvFLS1* genomic sequences

Sequences aligned included: *VvFLS1* promoter1 (Coding sequence AY257978, genomic sequence (this investigation), *VvFLS4cs* (AB092591), *VvFLS1pn* (unpublished), VvTC46143. Full-length genomic sequences were aligned using ClustralW (<http://www.ebi.ac.uk/clustralW/>) (Thompson et al. 1994) and displayed by GenDoc (<http://www.psc.edu/biomed/genedoc>). Dashes indicate introduced gaps. Shading represents the degree of sequence identity at each nucleotide position: nucleotides conserved between sequences are shaded in black and dark grey shading indicates conserved nucleotides in two out of three of the sequences.

KEY:

-  2 Kb and 1.5 Kb mark in the upstream genomic sequence (from the start codon (ATG))
-  Start codon (ATG)
-  Start and end of intron sequence
-  Primers used to isolate the *VvFLS1* promoter sequence(s)
-  Key DNA binding motifs/elements

Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*           20           *           40           *           60
VvFLS4cs : ----- : -
VvFLS1pn : ATTGTTATAATTATGCTTGAATATTTGCACCTTCGTGTTTCAACTTTCAACACTACCATT : 60
VvFLS1   : ----- : -

*           80           *           100          *           120
VvFLS4cs : ----- : -
VvFLS1pn : TCAAATTATTGAAAGGAGCGGAAACAACACTATGAGATGAGGGTGAAGTATAAATTTGTAT : 120
VvFLS1   : ----- : -

*           140          *           160          *           180
VvFLS4cs : ----- : -
VvFLS1pn : ATAAGAAATAAAATATTTGTTAAGCTTTGTATTTTCTCGACATATTCAGCTACTATCAA : 180
VvFLS1   : ----- : -

*           200          *           220          *           240
VvFLS4cs : ----- : -
VvFLS1pn : GTTTTGACATGATTTTTTATCGAACTCCATTGTTATTTTGTCTTGCTCATGGCATATCGC : 240
VvFLS1   : ----- : -

*           260          *           280          *           300
VvFLS4cs : ----- : -
VvFLS1pn : TCTGGTACTCATATTTGAAAGATGTTGCCATATAATGTTTTATTATCGTATCATGTATGT : 300
VvFLS1   : ----- : -

*           320          *           340          *           360
VvFLS4cs : ----- : -
VvFLS1pn : CAAGGTGTTAACAAGCCACCCCTGTCAAAATGAAGATTGATGAAATAATAGAAAAGTTCA : 360
VvFLS1   : ----- : -

*           380          *           400          *           420
VvFLS4cs : ----- : -
VvFLS1pn : ACAAACAGTGGGTTTTAACTCACTAATTTGAAAAAATTTAATAATAGCATATATTTTAA : 420
VvFLS1   : ----- : -

*           440          *           460          *           480
VvFLS4cs : ----- : -
VvFLS1pn : AATATTTATAAATGGATAATCATAAAAATGATTTATAAAAATTGGTTTTTTTTTTAAWAT : 480
VvFLS1   : ----- : -

*           500          *           520          *           540
VvFLS4cs : ----- : -
VvFLS1pn : TTTTTCAAATTAGTGAGTCTTAACTAGGTATTAGAATTATAAAAAATGACAATAGTCTAG : 540
VvFLS1   : ----- : -

*           560          *           580          *           600
VvFLS4cs : ----- : -
VvFLS1pn : CAATTATCCAATCACACTATATTGAAAAGATATTAATAAAAAAATTAATCACTATAATTG : 600
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*           620           *           640           *           660
VvFLS4cs : ----- : -
VvFLS1pn : TAAGCTGGTATATGCACCTTTTGACTCAAACGTAAGGTTATATTTTGATATTGAAAAGAC : 660
VvFLS1   : ----- : -

*           680           *           700           *           720
VvFLS4cs : ----- : -
VvFLS1pn : AGTACCTCAATTAGAATAGGGAGAATTGTGTTTTGGGCCCAAAAATTAACATTTAGTCAT : 720
VvFLS1   : ----- : -

*           740           *           760           *           780
VvFLS4cs : ----- : -
VvFLS1pn : CCAACCATCCATATTTAAGCTCAGGACCCAAACAAAGTATGAAAATTAAGATGAAGGATA : 780
VvFLS1   : ----- : -

*           800           *           820           *           840
VvFLS4cs : ----- : -
VvFLS1pn : TTGTCTTCCATTAATTCCTAAAATACCCTTATCCCTTATATGCCTACAAGTGAGCGTGAA : 840
VvFLS1   : ----- : -

*           860           *           880           *           900
VvFLS4cs : ----- : -
VvFLS1pn : TTTTAATTTTTTTTTGTATTTTTTTTTTCTTTTCTATTTTCTATTAATATTACTCATTTTC : 900
VvFLS1   : ----- : -

*           920           *           940           *           960
VvFLS4cs : ----- : -
VvFLS1pn : TAACTTTTTTTTTTCTTTTTATTCCAATATATATTTCTATTTTATGTCAAATAAAAAACAA : 960
VvFLS1   : ----- : -

*           980           *           1000          *           1020
VvFLS4cs : ----- : -
VvFLS1pn : TAATATTTTTTTTATTTTTTCATTTTTAAAGATATTGAATCTTTTTGCTCTTATTTAAAAGCA : 1020
VvFLS1   : ----- : -

*           1040          *           1060          *           1080
VvFLS4cs : ----- : -
VvFLS1pn : ATGATAAAAATTTTGGGATTTTTTCATCTAAATATTTTTTATCTTTTTGTATTTACCTTTT : 1080
VvFLS1   : ----- : -

*           1100          *           1120          *           1140
VvFLS4cs : ----- : -
VvFLS1pn : AGTTTAATTTTCATTTTTTATTTTAAATTTATACTACCCTTTCATCTACATTTTTTCTCTT : 1140
VvFLS1   : ----- : -

*           1160          *           1180          *           1200
VvFLS4cs : ----- : -
VvFLS1pn : ATTTTTAATTATTTTTGATATTTTCTACATTAACCATATTTTAAAAAATTTTAAAATTAC : 1200
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

```

*      1220      *      1240      *      1260
VvFLS4cs : ----- : -
VvFLS1pn : CTATCACTTCACTTTATTATATATATATATATATATATATATATCTTTTTGGATTTTTTAATTTTTTA : 1260
VvFLS1   : ----- : -

*      1280      *      1300      *      1320
VvFLS4cs : ----- : -
VvFLS1pn : ATGTTTTTATTTTTAAAATTTTTAAAAAGATAAATTTGTTGAAAAAAAAAAAACTAAGATAT : 1320
VvFLS1   : ----- : -

*      1340      *      1360      *      1380
VvFLS4cs : ----- : -
VvFLS1pn : GAATTTCTAATATTATATTAATAATTTTTCTTATCTAGATAGACTAATGCAAAGTATTTT : 1380
VvFLS1   : ----- : -

*      1400      *      1420      *      1440
VvFLS4cs : ----- : -
VvFLS1pn : GACCCATAACAAGAAAATTGTCAATACATTTTTTTAGTTAAACTTTAAAAATTAAGTTTC : 1440
VvFLS1   : ----- : -

*      1460      *      1480      *      1500
VvFLS4cs : ----- : -
VvFLS1pn : AAATAAAATATATTATATAAAAATTTTATCTAGAAATTATTGAAAGTGGTATTTAATTTTTT : 1500
VvFLS1   : ----- : -

*      1520      *      1540      *      1560
VvFLS4cs : ----- : -
VvFLS1pn : TTATTTATTGACTTTGAAACTTATCTAATTTTTTACTTGAAAGGTAATAGAACATGTTTA : 1560
VvFLS1   : ----- : -

*      1580      *      1600      *      1620
VvFLS4cs : ----- : -
VvFLS1pn : CAAAAAATAAAATAAATAGTTTCTCAGTTTTTAAATAAATGAGTATAAATATATTAAGA : 1620
VvFLS1   : ----- : -

*      1640      *      1660      *      1680
VvFLS4cs : ----- : -
VvFLS1pn : GAATTAAGATAATCTTAGTAAAAATTTTGATAAATATGATTATAATTTTAAATATAGAT : 1680
VvFLS1   : ----- : -

*      1700      *      1720      *      1740
VvFLS4cs : ----- : -
VvFLS1pn : TCATTTGGATAAAAATTTACAAAAAATAATATAGTGAAAAGAAATAACATTGATAAAA : 1740
VvFLS1   : ----- : -

*      1760      *      1780      *      1800
VvFLS4cs : ----- : -
VvFLS1pn : CTACAAAAACAAAATATGCAATTACAAATTTTTTGATGATGAAATTTAAAAATGAAATTCG : 1800
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

```

*      1820      *      1840      *      1860
VvFLS4cs : ----- : -
VvFLS1pn : AAACAATTCTTGAGTGAGAGAATTTGAGTGGGGGAAAAATCTTTGAGTGGAAAAAATAG : 1860
VvFLS1   : ----- : -

*      1880      *      1900      *      1920
VvFLS4cs : ----- : -
VvFLS1pn : GAAAATTTTTCAAATCACTTGAAATCCTTAACAAAAAGTAGTCTTGTACACCCTTGTAT : 1920
VvFLS1   : ----- : -

*      1940      *      1960      *      1980
VvFLS4cs : ----- : -
VvFLS1pn : AATTAGTAAATTTGTCTTGTATAGTTAGTGTAAATACCTTGTATAAATAACTCAAATAACTC : 1980
VvFLS1   : ----- : -

*      2000      *      2020      *      2040
VvFLS4cs : ----- : -
VvFLS1pn : AAAATTTTATTTCTTATGTGTTAATTTTTGTAGGGAATGTCTTAATGAAATAGTTTGTA : 2040
VvFLS1   : ----- : -

*      2060      *      2080      *      2100
VvFLS4cs : ----- : -
VvFLS1pn : AAAAAAGAAAAAAACTATCAATAATCGTCTTAATATCAAATTCAAGTTGTTTAAAAA : 2100
VvFLS1   : ----- : -

*      2120      *      2140      *      2160
VvFLS4cs : ----- : -
VvFLS1pn : TCGTACAAAACCTATTAGGAGCCTTGTACACCCTTGTACACTTAACACATTTCCCTTGTA : 2160
VvFLS1   : ----- : -

*      2180      *      2200      *      2220
VvFLS4cs : ----- : -
VvFLS1pn : CAATTAGTATAAATACCTTTGTACAATGACATAAATTTTCATATTTGTCATAACTTAAACGT : 2220
VvFLS1   : ----- : -

*      2240      *      2260      *      2280
VvFLS4cs : ----- : -
VvFLS1pn : ATTTTCAAAAAGAGGCAATATAGAAAAATAAAAAATAAAAACTAGAAAAATATTTTAAACC : 2280
VvFLS1   : ----- : -

*      2300      *      2320      *      2340
VvFLS4cs : ----- : -
VvFLS1pn : AAACCTCAAATATAATCTATRTAATTTTTAGTTACTTGTTTTCATCTAAAACAAGTAAATA : 2340
VvFLS1   : ----- : -

*      2360      *      2380      *      2400
VvFLS4cs : ----- : -
VvFLS1pn : TACCTTTCRACCCTTTTATATATATAAAAAAATCAATCTCTACTATGTATTGATATAATC : 2400
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

```

*           2420           *           2440           *           2460
VvFLS4cs : ----- : -
VvFLS1pn : CACTTCTAAAATTAGGTCATATGAAAACATTTTAATTGAGTACTTAAAATAAAAACTCTC : 2460
VvFLS1   : ----- : -

*           2480           *           2500           *           2520
VvFLS4cs : ----- : -
VvFLS1pn : CATCCATTCTYATTTTAATYAGTACTTAAAATAAAAACTCTCAATCCATCCCCYTTCTT : 2520
VvFLS1   : ----- : -

*           2540           *           2560           *           2580
VvFLS4cs : ----- : -
VvFLS1pn : TATTTTTTCYTTTTCTTATTTTAATAAATTTTCAATTMATTCAAATCATTAAAAAAATCC : 2580
VvFLS1   : ----- : -

*           2600           *           2620           *           2640
VvFLS4cs : ----- : -
VvFLS1pn : TTAATAAACAAATTTGTAGCATTTTCATATAACTTATTACTAAAAGTCATGTTCAAAAAGT : 2640
VvFLS1   : ----- : -

*           2660           *           2680           *           2700
VvFLS4cs : ----- : -
VvFLS1pn : TGTTAAAWTARGGAAGGAAGAAGATTAAAAAAAAAAAAAATTGAACCTTTTATTTTATAAT : 2700
VvFLS1   : ----- : -

*           2720           *           2740           *           2760
VvFLS4cs : ----- : -
VvFLS1pn : AGTAAAAAAAAAACTTTAAAATACTTTTTACTATAAAAAATAAAATAAAATAGGGAATATAT : 2760
VvFLS1   : ----- : -

*           2780           *           2800           *           2820
VvFLS4cs : ----- : -
VvFLS1pn : TTATTTTAAATGGTATTTTAATCATTAAATTTATTTACTTCTAAAATGTAAACGATAAAAA : 2820
VvFLS1   : ----- : -

*           2840           *           2860           *           2880
VvFLS4cs : ----- : -
VvFLS1pn : TAAATATAAAAGATAATTTTTATTTATTTAAATTAATATTTTTTTTAAAAAGTATTTCTAA : 2880
VvFLS1   : ----- : -

*           2900           *           2920           *           2940
VvFLS4cs : ----- : -
VvFLS1pn : AATAGTCTTTGAATCATTAATATAATTTTTGTWTATTTATAATTTAAAAWTAAAAAATAA : 2940
VvFLS1   : ----- : -

*           2960           *           2980           *           3000
VvFLS4cs : ----- : -
VvFLS1pn : TGTAATTTTTCAATTATTTATAAAAAGATTTTAAAAAAWTGAAAAATCCAAAATGTTAA : 3000
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

```

*      3020      *      3040      *      3060
VvFLS4cs : ----- : -
VvFLS1pn : ATAAGRAAGAAGAGTGGCAAGTAGTAGAATAAAGACAAAAATGAGTCAAGTGGGTATTTT : 3060
VvFLS1   : ----- : -

*      3080      *      3100      *      3120
VvFLS4cs : ----- : -
VvFLS1pn : TGTCCATTACTATCTCATATCCTTGRATTTAAATATGGGTGGTTGGATGACCAAATGTTA : 3120
VvFLS1   : ----- : -
                2Kb from start codon      MYBPZM      EBOXBNNAPA

*      3140      *      3160      *      3180
VvFLS4cs : ----- : -
VvFLS1pn : ATTTTTGGGCCAGCTGAGCCCAAAACACAATTCCTCCATTAGAATATGGTTATGTTGTAG : 3180
VvFLS1   : ----- : -
                EBOXBNNAPA

*      3200      *      3220      *      3240
VvFLS4cs : ----- : -
VvFLS1pn : GTGTCTTATGAATGCTACGACAGGTACAAGAAGCTGGTATGGCTTTTTCTATTAGAAAGTT : 3240
VvFLS1   : ----- : -

*      3260      *      3280      *      3300
VvFLS4cs : ----- : -
VvFLS1pn : AAAATACAACCAAAATTACAATGATACATTTTACTAAATATTAGATGATTGATCAAGATG : 3300
VvFLS1   : ----- : -

*      3320      *      3340      *      3360
VvFLS4cs : ----- : -
VvFLS1pn : ATCATAAATGAATAGTTAGGCTTGGTGGAGCAGCAGGATCACCATGGTTGCAAGAGAAGC : 3360
VvFLS1   : ----- : -

*      3380      *      3400      *      3420
VvFLS4cs : ----- : -
VvFLS1pn : TAAGTGGTTAAGGAATTTATTAATGGAAATATTGTTTGGCCTTAGCCAATGCCTTTAAT : 3420
VvFLS1   : ----- : -

*      3440      *      3460      *      3480
VvFLS4cs : ----- : -
VvFLS1pn : ATCTATATGTTGTGACAGGTTGCTTGTGAAGGGCATATAGCTAAATTTACAATGTATGG : 3480
VvFLS1   : ----- : -

*      3500      *      3520      *      3540
VvFLS4cs : ----- : -
VvFLS1pn : TCATTACAATTGACTTTGTAATAAATCAAGACACAGGCTTAAGACATAGCTATGTGAGACAA : 3540
VvFLS1   : ----- : -
                EBOXBNNAPA

*      3560      *      3580      *      3600
VvFLS4cs : ----- : -
VvFLS1pn : TTAICTACCAATGGAGTAATTAACAATTGATTTGTTAAATCATGCCCAAATTTGGCACAC : 3600
VvFLS1   : ----- : -
                EBOXBNNAPA      1.5Kb from start codon

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

```

*           3620           *           3640           *           3660
VvFLS4cs : ----- : -
VvFLS1pn : CCCTAGATGAAAAGCCTTGCTAGGGATTTAGTATGGAAAATATAAAGAAGAATGAGACTA : 3660
VvFLS1   : ----- : -

*           3680           *           3700           *           3720
VvFLS4cs : ----- : -
VvFLS1pn : AAGCCAACATCTATTATATCACCGATAATGGAACCTTAACCTAATGGCTTGAAATATCACA : 3720
VvFLS1   : ----- : -
                [REBOXIPCCHS1]

*           3740           *           3760           *           3780
VvFLS4cs : ----- : -
VvFLS1pn : TCCTTAGGTTCAAGGAGTAAAAGTATAACATATGTTGTGATTGCAAGCGCTATAATATTA : 3780
VvFLS1   : ----- : -
                [REBOXIPCCHS1]                [EBOXBNNAPA]

*           3800           *           3820           *           3840
VvFLS4cs : ----- : -
VvFLS1pn : ATGACATGTMTCAAAAGTAAAAGGGCTYGATACCTGTAATGTGAAGGTTAATAGTTTTAC : 3840
VvFLS1   : ----- : -

*           3860           *           3880           *           3900
VvFLS4cs : ----- : -
VvFLS1pn : AACCTATTTATGTTGGAATATTGTGATAACTAGAGGGTCGACATTCTTGATAAGCCTACC : 3900
VvFLS1   : ----- : -
                [IBOXCORE] [MYBPZM]

*           3920           *           3940           *           3960
VvFLS4cs : ----- : -
VvFLS1pn : CATGTGAGTGTAGGGAATTAGAAAGATGTCTTTCTAAAGATATATAGGACCTTACATAAA : 3960
VvFLS1   : ----- : -
                [EBOXBNNAPA]

*           3980           *           4000           *           4020
VvFLS4cs : ----- : -
VvFLS1pn : TGGGTATGTAATAACGTGATGCGACCCAAATTGAACGAGTGTGATGTCCAAAAAATGCAA : 4020
VvFLS1   : ----- : -
                [ACE SITE]

*           4040           *           4060           *           4080
VvFLS4cs : ----- : -
VvFLS1pn : CCTTAGATTAAAATGTGCTTTGAAATCTTTAAAAGAGGGCCGAGGGGTGACCATTGCATT : 4080
VvFLS1   : ----- : -

*           4100           *           4120           *           4140
VvFLS4cs : ----- : -
VvFLS1pn : CATTCAATCAACCATCAAATTCCTCGATTCTTTCCATCATTTCACAACCTTTTAGCATTAT : 4140
VvFLS1   : ----- : -

*           4160           *           4180           *           4200
VvFLS4cs : ----- : -
VvFLS1pn : ATTTTCTTCATGAATCAAATTTTTATATACTTTTATTGTAAAAAACATTACAAATATT : 4200
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*           4220           *           4240           *           4260
VvFLS4cs : ----- : -
VvFLS1pn : TTTGACAGGCGAGTTATATTTTGAGGATATTTGGGATCTGCTTTCATCAATGTACAATGG : 4260
VvFLS1   : ----- : -
                               MYBCORE

*           4280           *           4300           *           4320
VvFLS4cs : ---CCTACAAACATAGCTTCATTGTATCTTGGGGGCATATTTGTTATACTCGTGCATTAC : 57
VvFLS1pn : AGGCCTACAAACATAGCTTCATTGTATCTTGGGGGCATATTTGTTATACTCGTGCATTAC : 4320
VvFLS1   : ---CCTACAAACATAGCTTCATTGTATCTTGGGGGCATATTTGTTATACTCGTGCATTAC : 57
                               CCTACAAACATAGCTTCATTGTATCTTGGGGGCATATTTGTTATACTCGTGCATTAC
                               LucBAM-F

*           4340           *           4360           *           4380
VvFLS4cs : AAAGTTGGTGCCCAAAGTGACTCTCTACTACACCTTGAAGCATTCTAATTCCAATTTTAT : 117
VvFLS1pn : AAAGTTGGTGCCCAAAGTGACTCTCTACTACACCTTGAAGCATTCTAATTCCAATTTTAT : 4380
VvFLS1   : AAAGTTGGTGCCCAAAGTGACTCTCTACTACACCTTGAAGCATTCTAATTCCAATTTTAT : 117
                               AAAGTTGGTGCCCAAAGTGACTCTCTACTACACCTTGAAGCATTCTAATTCCAATTTTAT

*           4400           *           4420           *           4440
VvFLS4cs : TATTTACATTATCTCTTTTCGCACATCATCAATTTCAATATCATGGTACCCACAGTTTTTC : 177
VvFLS1pn : TATTTACATTATCTCTTTTCGCACATCATCAATTTCAATATCATGGTACCCACAGTTTTTC : 4440
VvFLS1   : TATTTACATTATCTCTTTTCGCACATCATCAATTTCAATATACCATACCCACAGTTTTTC : 176
                               TATTTACATTATCTCTTTTCGCACATCATCAATTTCAATATcAtggTACCCACAGTTTTTC
                               BOXCORE

*           4460           *           4480           *           4500
VvFLS4cs : TCTAGTGGATGATTGCGAAGTTAGAAGTATGAGAAACGACAAAAGTTAGAGAGCGACTTTTT : 237
VvFLS1pn : TCTAGTGGATGATTGCGAAGTTAGAAGTATGAGAAACGACAAAAGTTAGAGAGCGACTTTTT : 4500
VvFLS1   : TCTAGTGGATGATTGCGAAGTTAGAAGTATGAGAAACGACAAAAGTTAGAGAGCGACTTTTT : 236
                               TCTAGTGGATGATTGCGAAGTTAGAAGTATGAGAAACGACAAAAGTTAGAGAGCGACTTTTT

*           4520           *           4540           *           4560
VvFLS4cs : AAGGACTATTATCAAAAAAGAGAGTTGCCAGCAAGTTGTCATTTTCCCATCTTTATAGCT : 297
VvFLS1pn : AAGGACTATTATCAAAAAAGAGAGTTGCCAGCAAGTTGTCATTTTCCCATCTTTATAGCT : 4560
VvFLS1   : AAGGACTATTATCAAAAAAGAGAGTTGCCAGCAAGTTGTCATTTTCCCATCTTTATAGCT : 296
                               AAGGACTATTATCAAAAAAGAGAGTTGCCAGCAAGTTGTCATTTTCCCATCTTTATAGCT
                               BOXCORE

*           4580           *           4600           *           4620
VvFLS4cs : AGTTAGTTTCTGTTGTTCAAACAGATCTCACTTTTCTCCATCACTCCATCTCCATTTTGA : 357
VvFLS1pn : AGTTAGTTTCTGTTGTTCAAATAGATCTCACTTTTCTCCATCACTCCATCTCCATTTTGA : 4620
VvFLS1   : AGTTAGTTTCTGTTGTTCAAACAGATCTCACTTTTCTCCATCACTCCATCTCCATTTTGA : 356
                               AGTTAGTTTCTGTTGTTCAAACAGATCTCACTTTTCTCCATCACTCCATCTCCATTTTGA
                               MYBCORE

*           4640           *           4660           *           4680
VvFLS4cs : ATATTTATTTCAAAGTTACCCAAAATGTGTATACTTTAATAGTCACAACCACCTCTTTA : 417
VvFLS1pn : ATATTTATTTCAAAGTTACCCAAAATGTGTATACTTTAATAGTCACAACCACCTCTKTA : 4680
VvFLS1   : ATATTTATTTCAAAGTTACCCAAAATGTGTATACTTTAATAGTCACAACCACCTCTTTA : 416
                               ATATTTATTTCAAAGTTACCCAAAATGTGTaTACTTTAATAGTCACAACCACCTCTtTA

*           4700           *           4720           *           4740
VvFLS4cs : AACCAAACCTAACTCAATTATGCGATTTCTACAGCAGATTAAGAATTGTCGAATTGGGA : 477
VvFLS1pn : AACCAAACCTAACTCAATTATGCGATTTCTACAGCAGATTAAGAATTGTCGAATTGGGA : 4740
VvFLS1   : AACCAAACCTAACTCAATTATGCGATTTCTACAGCAGATAAAGAATTGTCGAATTGGGA : 476
                               MYBPLANT [REBOXIPCCHS] CAATTATGCGATTTCTACAGCAAAAGAATTGTCGAATTGGGA
                               BOXCORE

*           4760           *           4780           *           4800
VvFLS4cs : AATCTGTATGGCGTGGCTGTTGATGGTCCGGCTGTACCCGTCCATTTCAAGAGTGATGAAG : 537
VvFLS1pn : AATCTGTATGGCGTGGCTGTTGATGGTCCGGCTGTACCCGTCCATTTCAAGAGTGATGAAG : 4800
VvFLS1   : AATCTGTATGGCGTGGCTGTTGATGGTCCGGCTGTACCCGTCCATTTCAAGAGTGATGAAG : 536
                               AATCTGTATGGCGTGGCTGTTGATGGTCCGGCTGTACCCGTCCATTTCAAGAGTGATGAAG
                               MYBCORE PALBOXAPC

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*           4820           *           4840           *           4860
VvFLS4cs : AGATGCATTAATAAGATCGCACCAATAGGAAAACTACCCTCGTGGGCAGGGTTGGTAAGA : 597
VvFLS1pn : AGATGCATTAATAAGATCGCACCAATAGGAAAACTACCCTCGTGGGCAGGGTTGGTAAGA : 4860
VvFLS1   : AGATGCATTAATAAGATCGCACCAATAGGAAAACTACCCTCGTGGGCAGGGTTGGTAAGA : 596
          AGATGCATTAATAAGATCGCACCAATAGGAMREATCHSCCCTCGTGGGCAGPALBOXLPCMYBPZM
          BOXICOREDCPAL

*           4880           *           4900           *           4920
VvFLS4cs : GCCATTATGCGAACATCCTCGTCACACGTCACCCTTTGGTTACCGCCTTTGAATTACTTG : 657
VvFLS1pn : GCCATTATGCGAACATCCTCGTCACACGTCACCCTTTGGTTACCGCCTTTGAATTACTTG : 4920
VvFLS1   : GCCATTATGCGAACATCCTCGTCACACGTCACCCTTTGGTTACCGCCTTTGAATTACTTG : 656
          GCCATTATGCGAACATCCTCGTCACACCTCACCCTTTGGTTACCGCCTTTGAATTACTTG
          ACEATCHS

*           4940           *           4960           *           4980
VvFLS4cs : GCTTCCCCTTGTACGCGTGCTTTCTTTCTCGCCCTTCCATGATGACTGCTCGTCTGTGA : 717
VvFLS1pn : GCTTCCCCTTGTACGCGTGCTTTCTTTCTCGCCCTTCCATGATGACTGCTCGTCTGTGA : 4980
VvFLS1   : GCTTCCCCTTGTACGCGTGCTTTCTTTCTCGCCCTTCCATGATGACTGCTCGTCTGTGA : 716
          GCTTCCCCTTGTACGCGTGCTTTCTTTCTCGCCCTTCCATGATGACTGCTCGTCTGTGA

*           5000           *           5020           *           5040
VvFLS4cs : TTCATTAATGACAGGCTCTATAAAAGTTGGATCAGACTTAGAAGCCCAAACCCAAGACAA : 777
VvFLS1pn : TTCATTAATRACAGGCTCTATAAAAGTTGGATCAGACTTAGAAGCCCAAACCCAAGACAA : 5040
VvFLS1   : TTCATTAATGACAGGCTCTATAAAAGTTGGATCAGACTTAGAAGCCCAAACCCAAGACAA : 776
          TTCATTAATgACAGGCTCTATAAAAGTTGGATCAGACTTAGAAGCCCAAACCCAAGACAA

*           5060           *           5080           *           5100
VvFLS4cs : CACAGAAGAAAGAGTAAGAGAGAGAGGGAAGCAGTATCGAATTAAGACAGTCCAAGCCA : 837
VvFLS1pn : CACAGAAGAAAGAGTAAGAGAGAGAGGGAAGCAGTATCGAATTAAGACAGTCCAAGCCA : 5100
VvFLS1   : CACAGAAGAAAGAGTAAGAGAGAGAGGGAAGCAGTATCGAATTAAGACAGTCCAAGCCA : 836
          CACAGAAGAAAGAGTAAGAGAGLucXHO-RGAAGCAGTStart CodonTAAAGACAGTCCAAGCCA

*           5120           *           5140           *           5160
VvFLS4cs : TTGCCTTTTTCGTCCATGTCCGCGGGCATCATCCCTTCCGAGTTCATAAGGTCTGAGAAAG : 897
VvFLS1pn : TTGCCTTTTTCGTCCATGTCCGCGGGCATCATCCCTTCCGAGTTCATAAGGTCTGAGAAAG : 5160
VvFLS1   : TTGCCTTTTTCGTCCATGTCCGCGGGCATCATCCCTTCCGAGTTCATAAGGTCTGAGAAAG : 896
          TTGCCTTTTTCGTCCATGTCCGCGGGCATCATCCCTTCCGAGTTCATAAGGTCTGAGAAAg

*           5180           *           5200           *           5220
VvFLS4cs : AGCAACCTGCTATCACAACCTTTCATGGGTATATCCCGCAAGTCCCCACCATTGATCTCA : 957
VvFLS1pn : AGCAACCTGCTATCACAACCTTTCATGGGTATATCCCGCAAGTCCCCACCATTGATCTCA : 5220
VvFLS1   : AACAACCTGGCTATCACAACCTTTCATGGGTATATCCCGCAAGTCCCCACCATTGATCTCA : 956
          AgCAaCCTGCTATCACAACCTTTCATGGGTATATCCCGCAAGTCCCCACCATTGATCTCA

*           5240           *           5260           *           5280
VvFLS4cs : GTGACCCAGATGAGGAGAAGCTCACGCGTTTGATTGTCGAGGCCAGCAGCGAGTGGGGGA : 1017
VvFLS1pn : GTGACCCAGATGAGGAGAAGCTCACGCGTTTGATTGTCGAGGCCAGCAGCGAGTGGGGGA : 5280
VvFLS1   : GTGACCCAGATGAGGAGAAGCTCACGCGTTTGATTGTCGAGGCCAGCAGCGAGTGGGGGA : 1016
          GTGACCCAGATGAGGAGAAGCTCACGCGTTTGATTGTCGAGGCCAGCAGCGAGTGGGGGA

*           5300           *           5320           *           5340
VvFLS4cs : TGTTCCAGATTGTGAACCATGGCATTCCCAGTGACGTTATCAGCAACTTGCAGAAAGTTG : 1077
VvFLS1pn : TGTTCCAGATTGTGAACCATGGCATTCCCAGTGACGTTATCAGCAACTTGCAGAAAGTTG : 5340
VvFLS1   : TGTTCCAGATTGTGAACCATGGCATTCCCAGTGACGTTATCAGCAACTTGCAGAAAGTTG : 1076
          TGTTCCAGATTGTGAACCATGGCATTCCCAGTGACGTTATCAGCAACTTGCAGAAAGTTG

*           5360           *           5380           *           5400
VvFLS4cs : GCAAGGAGTTCTTTGAGCTCCCACAAGAGGAGAAGGAACTTTATGCTAAGTCTCCTGACT : 1137
VvFLS1pn : GCAAGGAGTTCTTTGAGCTCCCACAAGAGGAGAAGGAACTTTATGCTAAGTCTCCTGACT : 5400
VvFLS1   : GCAAGGAGTTCTTTGAGCTCCCACAAGAGGAGAAGGAACTTTATGCTAAGTCTCCTGACT : 1136
          GCAAGGAGTTCTTTGAGCTCCCACAAGAGGAGAAGGAACTTTATGCTAAGTCTCCTGACT

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*           5420           *           5440           *           5460
VvFLS4cs : CCAAGTCTATTCAAGGCTATGGAAGCAAGCTTCAAAAAGAAGTGGAGGGCAAGAAAGCTT : 1197
VvFLS1pn : CCAAGTCTATTCAAGGCTATGGAAGCAAGCTTCAAAAAGAAGTGGAGGGCAAGAAAGCTT : 5460
VvFLS1   : CCAAGTCTATTCAAGGCTATGGAAGCAAGCTTCAAAAAGAAGTGGAGGGCAAGAAAGCTT : 1196
          CCAAGTCTATTCAAGGCTATGGAAGCAAGCTTCAAAAAGAAGTGGAGGGCAAGAAAGCTT

*           5480           *           5500           *           5520
VvFLS4cs : GGGTCGATCACCTCTTCCATAACATCTGGCCTCCTCCTGCCATTGACTATCAGTTCTGGC : 1257
VvFLS1pn : GGGTCGATCACCTCTTCCATAACATCTGGCCTCCTCCTGCCATTGACTATCAGTTCTGGC : 5520
VvFLS1   : GGGTCGATCACCTCTTCCATAACATCTGGCCTCCTCCTGCCATTGACTATCAGTTCTGGC : 1256
          GGGTCGATCACCTCTTCCATAACATCTGGCCTCCTCCTGCCATTGACTATCAGTTCTGGC

*           5540           *           5560           *           5580
VvFLS4cs : CTAAAAAACCACCTACTTACAGGTTTGAACATTCGTTAACGTAGCTATTTTTTTCCTTT : 1317
VvFLS1pn : CTAAAAAACCACCTACTTACAGGTTTGAACATTCGTTAACGTAGCTATTTTTTTCCTTT : 5580
VvFLS1   : CTAAAGAAACCACCTACTTACAGGTTTGAACATTCGTTAACGTAGCTATTTTTTTCCTTT : 1316
          CTAAaAAACCACCTACTTACAGGTTTGAACATTCGTTAACGTAGCTATTTTTTTCCTTT
          Start of Intron

*           5600           *           5620           *           5640
VvFLS4cs : TTTTTGAAAAAAAAAAAAAAAAA--GCTGATTAACACACGTCTCACACTCACAGGGTTAAG : 1376
VvFLS1pn : TTTTTGAAAAAAAAAAAAAAAAAAGCTGATTAACACACGTCTCACACTCACAGGGTTAAG : 5640
VvFLS1   : TTTTTGAAAAAAAAAAAAAAAAA--GCTGATTAACACACGTCTCACACTCACAGGGTTAAG : 1374
          TTTTTGAAAAAAAAAAAAAAAAAa GCTGATTAACACACGTCTCACACTCACAGGGTTAAG

*           5660           *           5680           *           5700
VvFLS4cs : TAATATTAATGTACAAGTCTTAACGTCTTCAATATAACGTGAGAGTAGGCATGATATATA : 1436
VvFLS1pn : TAATATTAATGTACAAGTCTTAACGTCTTCAATATAAYGTGAGAGTAGGCATGATATATA : 5700
VvFLS1   : TAATATTAATGTACAAGTCTTAACGTCTTCAATATAACGTGAGAGTAGGCATGATATATA : 1433
          TAATATTAATGTACAAGTCTTAACGTCTTCAATATAAcGTGAGAGTAGGCATGATATATA

*           5720           *           5740           *           5760
VvFLS4cs : TAGAACATTTGGAGATCAGCTTTTTAAATCCTGTTGGATATGCGTAGGAGACCCAAAAA : 1496
VvFLS1pn : TAGAACATTTGGAGATCAGCTTTTTAAATCCTGTTGGATATGCGTAGGAGACCCAAAAA : 5760
VvFLS1   : TAGAACATTTGGAGATCAGCTTTTTAAATCCTGTTGGATATGCGTAGGAGACCCAAAAA : 1493
          TAGAACATTTGGAGATCAGCTTTTTAAATCCTGTTGGATATGCGTAGGAGACCCAAAAA

*           5780           *           5800           *           5820
VvFLS4cs : GGATCTCAAAATGTAATAATTAATAAAAAATTACACTGAAATATAAATATGAGATAGATG : 1556
VvFLS1pn : GGATCTCAAAATGTAATAATTAATAAAAAATTACACTGAAATATAAATATGAGATAGATG : 5820
VvFLS1   : GGATCTCAAAATGTAATAATTAATAAAAAATTACACTGAAATATAAATATGAGACAGATG : 1553
          GGATCTCAAAATGTAATAATTAATAAAAAATTACACTGAAATATAAATATGAGATAGATG

*           5840           *           5860           *           5880
VvFLS4cs : GTGTTTGATAAAATTTAATAATTAATTTAAAGTTAAATTATATTTAATATGTTTGATTTA : 1616
VvFLS1pn : GTGTTTGATAAAATTTAATAATTAATTTAAAGTTAAATTATATTTAATATGTTTGATTTA : 5880
VvFLS1   : GTGTTTGATAAAATTTAATAATTAATTTAAAGTTAAATTATATTTAATATGTTTGATTTA : 1613
          GTGTTTGATAAAATTTAATAATTAATTTAAAGTTAAATTATATTTAATATGTTTGATTTA

*           5900           *           5920           *           5940
VvFLS4cs : ATATTTATTATTTAATTTTTTAAAGTAAAATTAATTTAAAATTGATTTTTAAATCATTCAA : 1676
VvFLS1pn : ATATTTATTATTTAATTTTTTAAAGTAAAATTAATTTAAAATTGATTTTTAAATCATTCAA : 5940
VvFLS1   : ATATTTATTATTTAATTTTTTAAAGTAAAATTAATTTAAAATTGATTTTTAAATCATTCAA : 1673
          ATATTTATTATTTAATTTTTTAAAGTAAAATTAATTTAAAATTGATTTTTAAATCATTCAA

*           5960           *           5980           *           6000
VvFLS4cs : TTGATATATTTATTTAATTGATTTAACTAGTATTTATATTTTTTAATTTTTAAAAAATA- : 1735
VvFLS1pn : TTGATATATTTATTTAATTGATTTAACTAGTATTTATATTTTTTAATTTTTAAAAAATA : 6000
VvFLS1   : TTGATATATTTATTTAATTGATTTAACTAGTATTTATATTTTTTAATTTTTAAAAAATA : 1733
          TTGATATATTTATTTAATTGATTTAACTAGTATTTATATTTTTTAATTTTTAAAAAATA

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*           6020           *           6040           *           6060
VvFLS4cs : ATTTATTTTATAAAACATCATATTTAAAGTATTATATAAATATATAAAAATAATTATAAAATATT : 1795
VvFLS1pn : ATTTATTTTATAAAACATCATATTTTAAAGTATTATATAAATATATAAAAATAATTATAAAATRTT : 6060
VvFLS1   : ATTTATTTTATAAAACATCATATTTGAAGTATTATATAAATATATAAAAATAATTATAAAATGTT : 1793
          ATTTATTTTATAAAACATCATATTT  AAGTATTATATAAATATATAAAAATAATTATAAAAT  TT

*           6080           *           6100           *           6120
VvFLS4cs : TATCATAAAAAAATAAGTCATGAATATAAAATTTATAATTTAAAATATACCTTTCACTAATA : 1855
VvFLS1pn : TATCATAAAAAAATAAGTCATGAATATAAAATTTATAATTTAAAATATATTTTTYACTRATA : 6120
VvFLS1   : AATCATAAAAAAATAAGTCATGAATATAAAATTTATAATTTAAAATATACCTTTCACTGATA : 1853
          ATCATAAAAAAATAAGTCATGAATATAAAATTTATAATTTAAAATATACCTTTCACT  ATA

*           6140           *           6160           *           6180
VvFLS4cs : TAAATTACCATATAATAAAAGTGATTTAAGATTAAAAATAATTTAAT-----A : 1901
VvFLS1pn : TAAATTACCGTRATAAAAGTGATTTAAGATTAAAAATAATTTAATTTTTAATTTAATA : 6180
VvFLS1   : TAAATTACTGTGATAAAAGTGATTTAAGATTAAAAATAATTTAATTTTTAATTTAGTA : 1913
          TAAATTACcgT  ATAAAAGTGATTTAAGATTAAAAATAATTTAATtattttaattta  tA

*           6200           *           6220           *           6240
VvFLS4cs : CTTAAAATTAGAAATAATTTTAAATTATAATAATTTATTAAATAATATTAATTAATTTA : 1961
VvFLS1pn : CTTAAAATTAAAAATAATTTTAAATTATAATAATTTATTAAATAATATTAATTAATTTA : 6240
VvFLS1   : CTTAAAATTAAAAATAATTTTAAATTATAATAATTTATTAAATAATATTAATTAATTTA : 1973
          CTTAAAATTAAaAAATAATTTTAAATTATAATAATTTATTAAATAATATTAATTAATTTA

*           6260           *           6280           *           6300
VvFLS4cs : TCAAACACTCTCTATCACTTTATAAAATGATGAGAAGATTTTCTGAACAAAATCTCTTTG : 2021
VvFLS1pn : TCAAACAC-CTCTCTCACTTTATAAAATGATGAGAAGATTTTCTGAACAAAATCTCTTTG : 6299
VvFLS1   : TCAAACACTCTCTGTCACTTTATAAAATGATGAGAAGATTTTCTGAACAAAATCTCTTTG : 2033
          TCAAACACTCTCT  TCACTTTATAAAATGATGAGAAGATTTTCTGAACAAAATCTCTTTG

*           6320           *           6340           *           6360
VvFLS4cs : TTTTTGTTTTTTCAATTTAAAAGGCATTATTATTATTTGTCCTTGTGATTTTTTTTCCTT : 2081
VvFLS1pn : TATTT-TTTTTTAAATTTAAAAGGCATTATTATTATTTGTCCTTGTGATTTTTTTTCCTT : 6358
VvFLS1   : TATTT-TTTTTTAAATTTAAAAGGCATTATTATTATTTGTCCTTGTGATTTTTTTTCCTT : 2091
          TaTTT  tTTTTTTtAAATTTAAAAGGCATTATTATTATTTGTCCTTGTGATTTTTTTtTCCTT

*           6380           *           6400           *           6420
VvFLS4cs : TCTATTTATTTATGGTTAAGTGTTCAAAGTGGTATTATTGTCAAAGAAATAATAAAGGTA : 2141
VvFLS1pn : TCTATTTATTTATGGTTAAGTGTTCAAAGTGGTATTATTGTCAAAGAAATAATAAAGGTA : 6418
VvFLS1   : TCTATTTATTTATGGTTAAGTGTTCAAAGTGGTATTATTGTCAAAGAAATAATAAAGGTA : 2151
          TCTATTTATTTATGGTTAAGTGTTCAAAGTGGTATTATTGTCAAAGAAATAATAAAGGTA

*           6440           *           6460           *           6480
VvFLS4cs : GCATATTTTCATTGGACCGTACACGTTTATGATATTTCAATGGTTCATATGTTGGACACA : 2201
VvFLS1pn : GCATATTTTCATTGGACCGTACACGTTTATGATATTTCAATGGTTCATATGTTGGACACA : 6478
VvFLS1   : GCATATTTTCATTGGACCGTACACGTTTATGCTATTTCAATGGTTCATATGTTGGACACA : 2211
          GCATATTTTCATTGGACCGTACACGTTTATGaTATTTCAATGGTTCATATGTTGGACACA

*           6500           *           6520           *           6540
VvFLS4cs : TTGCATTTTTAGTTATCGTACACTTGTCTAATTTAAGAATGACCTTCATATTTCCCGATC : 2261
VvFLS1pn : TTGCATTTTTAGTTATCGTACACTTGTCTAATTTAAGAATGACNTTCATATTTCCCGATC : 6538
VvFLS1   : TTGCATTTTTAGTTATCGTACACTTGTCTAATTTAAGAATGACCTTCATATTTCCCGATC : 2271
          TTGCATTTTTAGTTATCGTACACTTGTCTAATTTAAGAATGACcTTCATATTTCCCGATC

*           6560           *           6580           *           6600
VvFLS4cs : GAAAAGGAAAAATTATT--TTTTTGTAAATAAAAATACCTTACAATAAATTAATTT--GGTT : 2318
VvFLS1pn : GAAA-GGAAAAATTATT--TTTTT-GTAATAAAAATACCTTACAATAAATTAATTT--GGTT : 6593
VvFLS1   : GAAAAGGAAAAATTATTATTTTTTAAATAAAAATACCTTTACAATAAATTAATTTGGTT : 2331
          GAAAaGGAAAAATTATT  TTTTTtgTAATAAAAATACCTTaCAATAAATTAATTT  GGTT

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*           6680           *           6700           *           6720
VvFLS4cs : GTATAAATGAACAAATAGGGT GAGTAGATCCATACATGTCTTTATAAACATTGTTGTAAT : 2436
VvFLS1pn : GTATAAATGAACAAATAGGGT GAGTAGATCCATACATGTCTTTATAAACATTGTTGTAAT : 6713
VvFLS1   : GCATAAATGAACAAATAGAGT GAGTAGATCCAAACATGTCTTTATAAACATTGTTGTAAT : 2449
          GtATAAATGAACAAATAGgGT GAGTAGATCCatACATGTCTTTATAAACATTGTTGTAAT

*           6740           *           6760           *           6780
VvFLS4cs : TGCAGAGCTGCGAACGAGGAGTATGCAAAGTGGCTGCAAGGGGTGGCAGACAAGCTGTTT : 2496
VvFLS1pn : TGCAGAGCTGCGAACGAGGAGTATGCAAAGTGGCTGCAAGGGGTGGCAGACAAGCTGTTT : 6773
VvFLS1   : TGCAGAGCTGCGAACGAGGAGTATGCAAAGTGGCTGCAAGGGGTGGCAGACAAGCTGTTT : 2509
          TGCAGAGCTGCGAACGAGGAGTATGCAAAGTGGCTGCAAGGGGTGGCAGACAAGCTGTTT
          End of Intron

*           6800           *           6820           *           6840
VvFLS4cs : GGTTCGTCTGTCACTGGGGTTAGGTCTGGGAGAGGGTACGTTGAAGGAAAGCGTCGGTGGG : 2556
VvFLS1pn : GGTTCGTCTGTCACTGGGGTTAGGTCTGGGAGAGGGTACGTTGAAGGAAAGCGTCGGTGGG : 6833
VvFLS1   : GGTTCGTCTGTCACTGGGGTTAGGTCTGGGAGAGGGTACGTTGAAGGAAAGCGTCGGTGGG : 2569
          GGTTCGTCTGTCACTGGGGTTAGGTCTGGGAGAGGGTACGTTGAAGGAAAGCGTCGGTGGG

*           6860           *           6880           *           6900
VvFLS4cs : GATGAATTGTTGTATCTTCTGAAAATAAATTATTACCCACCATGTCCTCGTCCAGACCTG : 2616
VvFLS1pn : GATGAATTGTTGTATCTTCTGAAAATAAATTATTACCCACCATGTCCTCGTCCAGACCTG : 6893
VvFLS1   : GATGAATTGTTGTATCTTCTGAAAATAAATTATTACCCACCATGTCCTCGTCCAGACCTG : 2629
          GATGAATTGTTGTATCTTCTGAAAATAAATTATTACCCACCATGTCCTCGTCCAGACCTG

*           6920           *           6940           *           6960
VvFLS4cs : GCTCTTGGGGTGGTTGCCCATACTGATATGTCCGCCATTACCATTCTTATCCCAAATGAA : 2676
VvFLS1pn : GCTCTTGGGGTGGTTGCCCATACTGATATGTCCGCCATTACCATTCTTATCCCAAATGAA : 6953
VvFLS1   : GCTCTTGGGGTGGTTGCCCATACTGATATGTCCGCCATTACCATTCTTATCCCAAATGAA : 2689
          GCTCTTGGGGTGGTTGCCCATACTGATATGTCCGCCATTACCATTCTTATCCCAAATGAA

*           6980           *           7000           *           7020
VvFLS4cs : GTTCAGGGCTTGCAGGTTTTTAGAGACGATCATTGGTTTGATGTCAAGTATATCTCCAAC : 2736
VvFLS1pn : GTTCAGGGCTTGCAGGTTTTTAGAGACGATCATTGGTTTGATGTCAAGTATATCTCCAAC : 7013
VvFLS1   : GTTCAGGGCTTGCAGGTTTTTAGAGACGATCATTGGTTTGATGTCAAGTATATCTCCAAC : 2749
          GTTCAGGGCTTGCAGGTTTTTAGAGACGATCATTGGTTTGATGTCAAGTATATCTCCAAC

*           7040           *           7060           *           7080
VvFLS4cs : GCTCTTGTCAATTCATGTCCGTGACCAGTTGGAGGTACAAAATTGAAAGACTCGATTTCAAT : 2796
VvFLS1pn : GCTCTTGTCAATTCATGTCCGTGACCAGTTGGAGGTACAAAATTGAAAGACTCGATTTCAAT : 7073
VvFLS1   : GCTCTTGTCAATTCATGTCCGTGACCAGTTGGAGGT----- : 2784
          GCTCTTGTCAATTCATGTCCGTGACCAGTTGGAGG Start of Intron gaagactcgatttcaat

*           7100           *           7120           *           7140
VvFLS4cs : TTATCGTCTCGGCTTAATTAGATTTGCAAAAAGCAACTGTGATTTAAAACGCACGTTGAA : 2856
VvFLS1pn : TTATCGTCTCGGCTTAATTAGATTTGCAAAAAGCAACTGTGATTTAAAACGCACGTTGAA : 7133
VvFLS1   : ----- : -
          ttatcgtctcggcttaaattagatttgcaaaaagcaactgtgatttaaaacgcacgttgaa

*           7160           *           7180           *           7200
VvFLS4cs : AAAAAATCTACAATCAATTTTATTATTATTTTCTATGTTGTAGGAAGGCACACTTTTCCCA : 2916
VvFLS1pn : AAAAAATCTACAATCAATTTTATTATTATTTTCTCTGTTGTAGGAAGGCACACTTTTCCCA : 7193
VvFLS1   : ----- : -
          aaaaaatctacaatcaatTTTATTATTATTTTCT tgtgtaggaaggcacactTTTCCCA

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*       7220           *       7240           *       7260
VvFLS4cs : CGTTATTAATAAAGGTTACGCGCTGAGGGGCGGTGCCATAATAACGGGCATCTCCTTTC : 2976
VvFLS1pn : CGTTATTAATAAAGGTTACGCGCTGAGGGGCGGTGCCATAATAACGGGCATCTCCTTTC : 7253
VvFLS1   : ----- : -
          cgttattaataaaggttcacgcgctgaggggcggtgccataataacgggcatctcctttc

*       7280           *       7300           *       7320
VvFLS4cs : TGGGCTTCTGGGTTTCGTAGGGGGTCATAACTCCTAGCTAGCCATGATTAGCCGTAATCAT : 3036
VvFLS1pn : TGGGCTTCTGGGTTTCGTAGGGGGTCATAACTCCTAGCTAGCCATGATTAGCCGTAATCAT : 7313
VvFLS1   : ----- : -
          tgggcttctgggttcgtagggggtcataaactcctagctagccatgattagccgtaatcat

*       7340           *       7360           *       7380
VvFLS4cs : ATTCTATACTAATACTATGATGCATGAACCCAGATCTTGAGCAACGGAAAATACAAGGCG : 3096
VvFLS1pn : ATTCTATACTAATACTATGATGCATGAACCCAGATCTTGAGCAACGGAAAATACAAGGCG : 7373
VvFLS1   : ----- : -
          attctataactaataactatgatgcatgaaccagatcttgagcaacggaaaatacaaggcg

*       7400           *       7420           *       7440
VvFLS4cs : GTGCTTCACAGGACGACCGTGAACAAGGAGAAGACGAGGATGTCATGGCCGGTGTCTCTTG : 3156
VvFLS1pn : GTGCTTCACAGGACGACCGTGAACAAGGAGAAGACGAGGATGTCATGGCCGGTGTCTCTTG : 7433
VvTC46143 : -----TCTCTTG : 2789
          gtgcttcacaggacgacCGTgaacaaggagaagacgaggatgtcatgcccgatTCTCTTG
                                     End of Intron

*       7460           *       7480           *       7500
VvFLS4cs : GAGCCACCGTCTGATCAGGTGGTAGGGCCTCTTCCTCAGCTTGTCAACGAAGAGAATCCT : 3216
VvFLS1pn : GAGCCACCGTCTGATCAGGTGGTAGGGCCTCTTCCTCAGCTTGTCAACGAAGAGAATCCT : 7493
VvTC46143 : GAGCCACCGTCTGATCAGGTGGTAGGGCCTCTTCCTCAGCTTGTCAACGAAGAGAATCCT : 2849
          GAGCCACCGTCTGATCAGGTGGTAGGGCCTCTTCCTCAGCTTGTCAACGAAGAGAATCCT

*       7520           *       7540           *       7560
VvFLS4cs : GCTAGGTACAAGACCAAGAAATACAGTGATTATGTGTACTGTAAGCTGAACAAGATTCCA : 3276
VvFLS1pn : GCTAGGTACAAGACCAAGAAATACAGTGATTATGTGTACTGTAAGCTGAACAAGATTCCA : 7553
VvTC46143 : GCTAGGTACAAGACCAAGAAATACAGTGATTATGTGTACTGTAAGCTGAACAAGATTCCA : 2909
          GCTAGGTACAAGACCAAGAAATACAGTGATTATGTGTACTGTAAGCTGAACAAGATTCCA

*       7580           *       7600           *       7620
VvFLS4cs : CAGTAGGTGAAGCCAGTGAGTGTTGAATGGATAAATTGTATTGCTTTGCCACTGATATTGT : 3336
VvFLS1pn : CAGTAGGTGAAGCCAGTGAGTGTTGAATGGATAAATTGTATTGCTTTGCCACTGATATTGT : 7613
VvTC46143 : CAGTAGGTGAAGCCAGTGAGTGTTGAATGGATAAATTGTATTGCTTTGCCACTGATATTGT : 2969
          CAGTAGGTGAAGCCAGTGAGTGTTGAATGGATAAATTGTATTGCTTTGCCACTGATATTGT

*       7640           *       7660           *       7680
VvFLS4cs : TCAACACAGTAGAACAATTGCTTTATTTGTTGCTGCGTGAGAACTATGCTGCTTGGTGC : 3396
VvFLS1pn : TCAACACAGTAGAACAATTGCTTTATTTGTTGCTGCGTGAGAACTATGCTGCTTGGTGC : 7673
VvTC46143 : TCAACACAGTAGAACAATTGCTTTATTTGTTGCTGCGTGAGAACTATGCTGCTTGGTGC : 3029
          TCAACACAGTAGAACAATTGCTTTATTTGTTGCTGCGTGAGAAgCTATGCTGCTTGGTGC

*       7700           *       7720           *       7740
VvFLS4cs : GTGAGAATTAAGAAGATGCTTTGTGATCTGCTTTTTAGTTAGTTTTGGTGGTTGTATGTG : 3456
VvFLS1pn : GTGAGAATTAAGAAGATGCTTTGTGATCTGCTTTTTAGTTAGTTTTGGTGGTTGTATGTG : 7733
VvTC46143 : GTGAGAATTAAGAAGATGCTTTGTGATCTGCTTTTTAGTTAGTTTTGGTGGTTGTATGTG : 3089
          GTGAGAATTAAGAAGATGCTTTGTGATCTGCTTTTTAGTTAGTTTTGGTGGTTGTATGTG

*       7760           *       7780           *       7800
VvFLS4cs : TCTTCAAGTACCCGACCCTATGCGATTACCAGATTTGCTCTTACTTCAATAAAAACCTTTT : 3516
VvFLS1pn : TCTTCAAGTACCCGACCCTATGCGATTACCAGATTTGCTCTTACTTCAATAAAAACCTTTT : 7793
VvTC46143 : TCTTCAAGTACCCGACCCTATGCGATTACCAGATTTGCTCTTACTTCAATAAAAACCTTTT : 3145
          TCTTCAAGTACCCGACCCTATGCGATTACCAGATTTGCTCTTACTTCAATAAAAACctttt

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

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*      7820      *      7840      *      7860
VvFLS4cs : ATCTGTTTGATTATCTTTACACGGCCTCATTATTAAGAGTATAATATATATTTTTAAATC : 3576
VvFLS1pn : ATCTGTTTGATTATCTTTACACGGCCTCATTATTAAGAGTATAATATATATTTTTAAATC : 7853
VvFLS1   : ----- : -
          atctgtttgattatctttacacggcctcattattaagagtataaatatattttaaatc

*      7880      *      7900      *      7920
VvFLS4cs : AAAATTTATATTTATTTATTTTTTTTAAAAAAAATGGTAATTCGATATAATATCATAAATT : 3636
VvFLS1pn : AAAATTTATATTTATTTATTTTTTTTAAAAAAAATGGTAATTCGATATAATATCATAAATT : 7913
VvFLS1   : ----- : -
          aaaatTTatatttatttattttt  aaaaaaaatggttaattcgatataaatatcataaatt

*      7940      *      7960      *      7980
VvFLS4cs : CTTGACTTAGTGGAAATGGCCAAATGAAGTCGTTGAAGCAATAAATTCATCATTTAGGAT : 3696
VvFLS1pn : CTTGACTTAGTGGAAATGGCCAAATGAAGTCGTTGAAGCAATAAATTCATCATTTAGGAT : 7973
VvFLS1   : ----- : -
          cttgacttagtggaaatggccaaatgaagtcgttgaagcaataaattcatcatttaggat

*      8000      *      8020      *      8040
VvFLS4cs : CAAGAAATGGGCCTGGCGTGGACGTGGAGAGAT----- : 3729
VvFLS1pn : CAAGAAATGGGCCTGGCGTGGACGTGGAGAGATATYAAACACGCATTTTGCATGGTGTGA : 8033
VvFLS1   : ----- : -
          caagaaatgggcctggcgtggacgtggagagat

*      8060      *      8080      *      8100
VvFLS4cs : ----- : -
VvFLS1pn : ATACATTTGATTTTGCCTATGAATTGATGGAAGGAAATCATTTTCTCATATTTAATTAA : 8093
VvFLS1   : ----- : -

*      8120      *      8140      *      8160
VvFLS4cs : ----- : -
VvFLS1pn : AATATAAAAATATTTAAAAATATTTTAAATTATGTAATTTTATATCAAAAATTTAATA : 8153
VvFLS1   : ----- : -

*      8180      *      8200      *      8220
VvFLS4cs : ----- : -
VvFLS1pn : AATAAAACAAGTTTTAAATAATATAAATATAAAAATATTAATTTTAAAATTATTTTTTAT : 8213
VvFLS1   : ----- : -

*      8240      *      8260      *      8280
VvFLS4cs : ----- : -
VvFLS1pn : TTCTTTCCATTCTCTTTTCCTTCAATTTTTTATATCATAACATAATCTTACCTAATTTATA : 8273
VvFLS1   : ----- : -

*      8300      *      8320      *      8340
VvFLS4cs : ----- : -
VvFLS1pn : CTAAGTGAAGTGTATTTTTCCACGTCACCCCTTCTGAAAAAGAATAATTATTACAAATAT : 8333
VvFLS1   : ----- : -

*      8360      *      8380      *      8400
VvFLS4cs : ----- : -
VvFLS1pn : AATCATTTTTTAAAACTAATTAAAAATATTATAGATCTTAAATTTTAAAATACAATGATAA : 8393
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

```

*      8420      *      8440      *      8460
VvFLS4cs : ----- : -
VvFLS1pn : TTTATATATATATATATGTATGTATTTTTATTTATTTATAAAATAAATCTTATTTTATAATTA : 8453
VvFLS1   : ----- : -

*      8480      *      8500      *      8520
VvFLS4cs : ----- : -
VvFLS1pn : TTTTAAGATTGGTTGGGWTTACCAAACAGTTAGGCCTTWGGAATTCTATGACTCTTCCAG : 8513
VvFLS1   : ----- : -

*      8540      *      8560      *      8580
VvFLS4cs : ----- : -
VvFLS1pn : CGGTCCTCTTCATCATTGTATTTGTACTCTTCTCTATCTACTTGTACTCCGATTTTCCA : 8573
VvFLS1   : ----- : -

*      8600      *      8620      *      8640
VvFLS4cs : ----- : -
VvFLS1pn : AAAGGTTTTCTTTTTTAAATATAAAAATTAAATTAATTAATAYTCATCCATTATCCTCAACC : 8633
VvFLS1   : ----- : -

*      8660      *      8680      *      8700
VvFLS4cs : ----- : -
VvFLS1pn : CCATAAATATTAGATTAWAAAAATATAAARAAATTAATATAATTCAAATTAATAAAAA : 8693
VvFLS1   : ----- : -

*      8720      *      8740      *      8760
VvFLS4cs : ----- : -
VvFLS1pn : TTTATATATATTTAAATTATTTTCATCTTTATACTAAATAATTAAATAAATATTGATAAT : 8753
VvFLS1   : ----- : -

*      8780      *      8800      *      8820
VvFLS4cs : ----- : -
VvFLS1pn : AACATTATTAAATATTTTTAAATAACTTACTATAAGTCTTTATYCATAAAATATTATAA : 8813
VvFLS1   : ----- : -

*      8840      *      8860      *      8880
VvFLS4cs : ----- : -
VvFLS1pn : TTGGTTTTTCAAATATTTAGAAAGTACTTCTYAAATTTYATCAAAACTTAATTTTCTTTA : 8873
VvFLS1   : ----- : -

*      8900      *      8920      *      8940
VvFLS4cs : ----- : -
VvFLS1pn : AAAAAATATTATCAAATGAACCTTAGTTAAAAAAATTAATAAATAATATATAAAAAATAAWT : 8933
VvFLS1   : ----- : -

*      8960      *      8980      *      9000
VvFLS4cs : ----- : -
VvFLS1pn : AAATTATTTTAAATATATTTTTAYTTTTTCCCTTTTTATTTTTTCTATTTTTTCCCTCCT : 8993
VvFLS1   : ----- : -

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Appendix 5C-Supplementary results for Chapter 5 (Molecular mechanism of flavonol biosynthesis)

```
          *      9020          *      9040          *      9060
VvFLS4cs : ----- : -
VvFLS1pn : ATGCCCKTCATSTTTTTTYTTTTYYTCTYTGCCCTTACTCCRTACCTTTCATTTACATTTTC : 9053
VvFLS1   : ----- : -
```

```
          *      9080          *      9100          *      9120
VvFLS4cs : ----- : -
VvFLS1pn : CATTTCATATAACAAAATGTA CT TATTTCTTTCTTAAATGCTATTTAATATTTATAATT : 9113
VvFLS1   : ----- : -
```

```
          *      9140          *      9160          *      9180
VvFLS4cs : ----- : -
VvFLS1pn : ATTGGAAGTTAACGTRTCATCCTTCTATTAGAGCGAGCAATAACTTTTTATAATAATAAT : 9173
VvFLS1   : ----- : -
```

```
          *      9200          *      9220          *      9240
VvFLS4cs : ----- : -
VvFLS1pn : GAACTCATTTTGTAWAGGTTTGATTATAWAWGGTGTATTTTCTAGAACACTGTTATTM : 9233
VvFLS1   : ----- : -
```

```
          *      9260          *      9280          *
VvFLS4cs : ----- : -
VvFLS1pn : AGAACAYGATAATTAATTCSTTTTTCTAAAGAATGGTGTWTARTTCMAWA : 9283
VvFLS1   : ----- : -
```