Appendix 1.

VSX1 Primers and conditions.

VSX1 PCR conditions

PCR CONDITIONS:

Used FastStart Taq enzyme kit from Roche, with 3.5mM final concentration of $MgCl_2$.

Primer sequences as follows:

Exon1	VSX1_Ex1aF:	5'-TGCTTGCTAAGGAACCATGA-3'
	VSX1_Ex1cR:	5'-CTCAGAGCCTAGGGGACAGG-3'
Exon2	VSX1_Ex2F:	5'-AAATCCAGGAAATAGAGGGGA-3'
	VSX1_Ex2R:	5'-AGATGCAGGTGCCATAAACC-3'
Exon3	VSX1_Ex3F:	5'-CTGTGTGTTTTGGGGTCCTT-3'
	VSX1_Ex3R:	5'-GTGGTATCTTTGGAGCGGAG-3'
Exon4	VSX1_Ex4F:	5'-TCTCCTGCCTCCAACCAG-3'
	VSX1_Ex4R:	5'-CTCCTACAACACCTCGAGCC-3'
Exon5	VSX1_Ex5aF2:	5'-CAATTCTAGTGGGATTTAGAGAACA-3'
	VSX1_Ex5bR:	5'-CCTTTGACAGTGGGACCTGT-3'
Exon6	VSX1_Ex6F:	5'-ATCTCTTTGCATTCTCAGAGGGTGAGA-3'
	VSX1_Ex6R:	5'-TATTCCTGCTGCATGGGTCCATTTGT-3'
Exon7	VSX1_Ex7aF2:	5'- CAGTCCCACCTTTCCCTCTCTCT-3'
	VSX1_Ex7cR2:	5'- CTGCCCTAACACTCAGGGTC-3'

PCR cycle as follows:

	temperature	time
initial denaturation	96°C	5min
35x -denaturation	94°C	30secs
-annealing	*°C	1min
-extension	72°C	1min
final extension	72°C	10min

*56°C for exons 1, 2, 3, 7
58°C for exon 4
61°C for exon 5
60°C for exon 6

5uL of product was visualised on 1.5% agarose gel, and the remaining 20ul cleaned with HighPure PCR purification kit from Roche.

VSX1- Control screen for c.173C>T, p.Pro58Leu

HRM CONDITIONS:

- Used SensiMix HRM kit from Quantace, with 3mM final concentration of $MgCl_2$.
- Primers were designed using NCBI primer-BLAST. Sequences as follows:
  VSX1_Ex1aF2: 5'-TTGCTAAGGAACCATGACCG-3'
  VSX1_Ex1aR2: 5'-ACAGAGGAGGCCGAGTCC-3'
- HRM cycle as follows:

	degC	time
initial denaturation	95	10min
50x -denaturation	95	10secs
-annealing	58	15secs
-extension	72	20secs
HRM step	87-97	N/A

PCR CONDITIONS:

- Used FastStart Taq enzyme from Roche, with 3.5mM final concentration of $MgCl_2$.
- Primer sequences as follows:
  VSX1_Ex1aF: 5'-TGCTTGCTAAGGAACCATGA-3' -
  VSX1_Ex1cR: 5'-CTCAGAGCCTAGGGGACAGG-3'

- PCR cycle as follows:

	degC	time
initial denaturation	96	5min
35x -denaturation	94	30secs
-annealing	56	1min
-extension	72	1min
final extension	72	10min

- 5uL of product was visualised on 1.5% agarose gel, and the remaining 20ul cleaned with HighPure PCR purification kit from Roche.

VSX1- Control screen for c.731A>G, p.His244Arg

HRM CONDITIONS:

- Used High Resolution Melting Master kit from Roche, with 2mM final concentration of $MgCl_2$.
- Primers were designed using NCBI primer-BLAST. Sequences as follows:
  VSX1_Ex4F 5'-TCTCCTGCCTCCAACCAG-3'
  VSX1_Ex4R 5'-CTCCTACAACACCTCGAGCC-3'
- HRM cycle as follows:

	degC	time
initial denaturation	95	10min
50x -denaturation	95	10secs

-annealing	61	15secs
-extension	72	20secs
HRM step	87-97	N/A

PCR CONDITIONS:

- Used AmpliTaq enzyme from Applied Biosystems, with 1.5mM final concentration of $MgCl_2$.
- Primer sequences as above.
- PCR cycle as follows:

	degC	**time**
initial denaturation	96	5min
35x -denaturation	94	30secs
-annealing	56	1min
-extension	72	1min
final extension	72	10min

- 5uL of product was visualised on 1.5% agarose gel, and the remaining 20ul cleaned with HighPure PCR purification kit from Roche.

SEQUENCING CONDITIONS:

- Used BigDye Terminator from Applied Biosystems.
- Sequenced in both directions, using above PCR primers.
- Sequencing products cleaned by EtOH/NaOAc precipitation, then sent to Allan Wilson Centre (Massey University) for capillary separation.
- Sequencing analysis done using CodonCode Aligner version 3.5.6

REFERENCE SEQUENCES:

- Genomic: NCBI Reference Sequence NG_008101.1
- mRNA: NCBI Reference Sequence NM_014588.4