

**Additional file 3 - Table of PCR analysis results of selected NIP candidates**

"nt" means NIP distance in nucleotides.

nt	FBgn	Plesiomorphic intron	Supposed apo-morphic intron	NIP confirmed	Evidence	Used primers
1	36324	155-1: Dme, Dsi, Dya, Dan, Dmo, Dgr	155-0: Dse	no	Genomic PCR (Dse)	Dse: 5'-CACCGATTCAAAAAGCCATTC-3', 5'- TTTCACCAGAGTTGCGAGTG-3'
3	15572	5-2: Dme, Dsi, Dya, Der, Dan, Dwi, Dmo, Dvi	4-2: Dpe, Dps	yes	RT-PCR (Dpe, Dps)	Dps, Dpe: 5'-TTTCACCAGAGTTGCGAGTG-3', 5'- CCATGCCAGGGCTTCAG -3'
4	27055	314-0: Dme, Dse, Dya, Der, Dan, Dps, Dpe, Dwi	315-1: Dsi	no	RT-PCR (Dsi, Dme, Dse, Dya, Der, Dps, Dpe)	Dps, Dpe: 5'-AGCGTTTATAACAAAAACACAC-3', 5'- TGTTTCAGGATGTAGCGTTCTG-3'; Dya, Der: 5'-CCGTTTTATGAAGCCGATG-3', 5'-CCATGTTGAGAATGTAACGCTC-3'; Dme, Dsi, Dse: 5'-CCAAAAACACACAGGTCATTG-3', 5'-CCATGTTGAGAATGTAACGCTC-3'
4	38858	116-0: Dme, Dsi, Dse, Dya, Der, Dan, Dpe, Dps, Tca	117-1: Dwi	no	RT-PCR (Dwi)	Dwi: 5'-CCGACCACATCGTATCTGC-3', 5'-TTCTGTTTCACCTCTCCGTTG-3'
5	46689	15-0: Dme, Dsi, Dse, Dya, Dpe, Dps	13-1: Der	no	RT-PCR (Dya, Der)	Dya, Der: 5'-CGCACGTACTGCTAAAAATGG-3', 5'- GCTCCACGGTGTACTCCCAC -3'
5			16-2: Dan	yes	RT-PCR (Dan)	Dan: 5'-CGCAAGATGACCGAAATAGTG-3', 5'-TTTTTGTGCTTCAGTCGTGC-3'
6	82831	750-0: Dme, Dse, Dya, Dan	752-0: Der	no	RT-PCR (Dsi, Dya, Der)	Dme, Dsi, Dse: 5'-GCTGATTTTCTCCAGCACG-3', 5'- TCAATGGGAGGCAGGTATATG-3'; Dya, Der: 5- GCTGTGGCAGATCCGAATG-3', 5'- TGTGCTCGTCGCTTGGC-3'