```
                    *         20        *         40        *         60        *         80
Intron 3 of LA¹ : GAGAATAAGCCATCGGACACACCACCAGAAC------------------------------------------------- :  31
Intron 3 of la¹ : GAGAATAAGCCATCGGACACACCACCAGAACTAGGCCTGGGCGTTCGGGGCCCCGTCGGATTCGGTTCGGTTAATTTGGAT :  81
Intron 3 of LA² : GAGAATAAGCCATCGGACACACCACCAGAAC------------------------------------------------- :  31
Intron 3 of la² : GAGAATAAGCCATCGGACACACCACCAGAAC------------------------------------------------- :  31

                    *        100        *        120        *        140        *        160
Intron 3 of LA¹ : -------------------------------------------------------------------------------- :   -
Intron 3 of la¹ : TTTCGGATTTTCGGTATTATGTTCATAAGCCCCATTCGGTTTTTTTCTCTTATTGAATGGGTTTCGGTTCGGTTCGGTTTT :  162
Intron 3 of LA² : -------------------------------------------------------------------------------- :   -
Intron 3 of la² : -------------------------------------------------------------------------------- :   -

                    *        180        *        200        *        220        *        240
Intron 3 of LA¹ : -------------------------------------------------------------------------------- :   -
Intron 3 of la¹ : TTCCGGATTCGGTTCGGATTGGATCGGATTGTAACCAATGTTTGAAATCGTCAAAAAAATATTTTTTTAAATCATGAAAAA :  243
Intron 3 of LA² : -------------------------------------------------------------------------------- :   -
Intron 3 of la² : -------------------------------------------------------------------------------- :   -

                    *        260        *        280        *        300        *        320
Intron 3 of LA¹ : -------------------------------------------------------------------------------- :   -
Intron 3 of la¹ : ATGACAAAAAAAAACTCAAAATATATAAATTATTCACAAAACTAATTAAAAATTAGTTAATTTATCTAAACTAACTGAAAA :  324
Intron 3 of LA² : -------------------------------------------------------------------------------- :   -
Intron 3 of la² : -------------------------------------------------------------------------------- :   -

                    *        340        *        360        *        380        *        400
Intron 3 of LA¹ : -------------------------------------------------------------------------------- :   -
Intron 3 of la¹ : GTATTAAAAATAATAAATAAAATAAATTATAAAATATTTAGAATATTATAAAATATCTGAATTACTTTAATATATATAAAA :  405
Intron 3 of LA² : -------------------------------------------------------------------------------- :   -
Intron 3 of la² : -------------------------------------------------------------------------------- :   -

                    *        420        *        440        *        460        *        480
Intron 3 of LA¹ : -------------------------------------------------------------------------------- :   -
Intron 3 of la¹ : ATATTAGCATATTTAACTAACTTTGGATGTCTTCGGATCCTAGTTTGGATTTCGGTTCGGTTCAAGTTATAACCAAACCCC :  486
Intron 3 of LA² : -------------------------------------------------------------------------------- :   -
Intron 3 of la² : -------------------------------------------------------------------------------- :   -

                    *        500        *        520        *        540        *        560
Intron 3 of LA¹ : -------------------------------------------------------------------------------- :   -
Intron 3 of la¹ : AAAGTATTAAGATCACAAGTCCCGTTCGGATATTTTTGTATAACGGTTTGGATCCGGTTTCGGTTTTTTCGGTTCGGGTTT :  567
Intron 3 of LA² : -------------------------------------------------------------------------------- :   -
Intron 3 of la² : -------------------------------------------------------------------------------- :   -

                    *        580        *        600        *        620        *        640
Intron 3 of LA¹ : ----------------------------------------------------CATGGCCATGTTGAAAACGACGAGTCTTGGGTT :  64
Intron 3 of la¹ : AGGTAAGTAATTCGGGTTGGGGTAGAAACGCCCAGGCCTAACCAGAACCATGGCCATGTTGAAAACGACGAGTCTTGTTGTT :  648
Intron 3 of LA² : ----------------------------------------------------CATGGCCATGTTGAAAACGACGAGTCTTGGGTT :  64
Intron 3 of la² : ----------------------------------------------------CATGGCCATGTTGAAAACGACGAGTCTTGGGTT :  64

                    *        660        *        680        *        700        *        720
Intron 3 of LA¹ : CCGGTAATCTTTCC--CTCTCATAGT-------------ATTTTTTTTTCCTTTTTTGAAATTATT-TCCTTTTAATTTT :  128
Intron 3 of la¹ : CCGGTAATCTTTCC--CTCTCATAGT-------------ATTTTTTTTTTCTTTTTTGAAATTATT-TCCTTTTAATTTT :  712
Intron 3 of LA² : CCGGTAATCTTTCATACTACTCTCATAGTTTCTCTTGACTTTTTATTTATTTCTTTGTTTTTCGGAACTATTCTTATGTCTATGTT :  145
Intron 3 of la² : CCGGTAATCTTTCATACTACTCTCATAGTTTCTCTTGTCTTTTATTTATTTGTTTGTTTTTCGGAACTATTCTTATGTCTATGTT :  145

                    *        740        *        760        *        780        *        800        *
Intron 3 of LA¹ : CTTAGGATTCTATGTATTTATTTTA---ATTAATA------------------------------TTTTTCCAG--------- :  169
Intron 3 of la¹ : CTTAGGATTCTATGTATTTATTTTA---ATTAATA------------------------------TTTTTCCAG--------- :  753
Intron 3 of LA² : CTTAGGATTCTATATCTTTATTTTATTAGTTTATG------------------------------TTTTCAG---------- :  187
Intron 3 of la² : CTTAGGATTCGACGTGTTTATTTTATTTTATGATTTTCTTAGTGGTTTATAGTAGTAACTCTTTTTCAGAAAATGAAA :  226

                      820        *        840        *        860        *        880        *
Intron 3 of LA¹ : ---------TTCGAGGCTAGGACGACCATTT------GTCAGA-ACTGT----CGTTTAGCTGTAGTAA-AAAATGATTTA :  229
Intron 3 of la¹ : ---------TTCGAGGCTTGGACGACCACTT------GTCAGA-ACTGT----CGTTTAGCTGTAGTAA-AAAATGATTTT :  813
Intron 3 of LA² : ---------TTTGAGGCCAGACCGACCATTT------GTTAG--GTTGT----TTTTTCAGCTATAGTAAAAAAAACAATTTG :  248
Intron 3 of la² : AATCAATACTTTTTATACTACTCCCTCCGTTTCAAAATGTTACATGTTTAGGTTTTTCACACATATTAATAAAACATATTA :  307
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                    900        *       920        *       940        *       960        *
Intron 3 of LA¹ : -----------AAGTG------------------------------------------------------------------ :  234
Intron 3 of la¹ : -----------AAGTG------------------------------------------------------------------ :  818
Intron 3 of LA² : ----------CAAGTGTA----------------------ATAGTTC---ACCAGT------------------GAT :  272
Intron 3 of la² : AATCTTAACCATTAAATGTATAATTTTTTGTGATTAACTATTTCCCATAACTTTTAACCAATCAAAATTCAATAAGTACAAT :  388

                    980        *      1000        *      1020        *      1040        *
Intron 3 of LA¹ : -----------------GTTTATAGT-----------ACT------------------------------------------ :  246
Intron 3 of la¹ : -----------------GTTTATAGT-----------ACT------------------------------------------ :  830
Intron 3 of LA² : TGATTATCTTAGTGGTTTACAGT--------AGTAACT-----------CTTTCAAAAAATGAAAAAT----------CAA :  324
Intron 3 of la² : TAATTTTCTTGA-AGTTTACAATTTATCATTATTAACTACTAAAAATTGCATTGAAAAGTAAAAAATGTAACTTTGACAA :  468

                   1060        *      1080        *      1100        *      1120        *
Intron 3 of LA¹ : -------------------------------------------GTAGTTAACTTTAACAACGGACCACTTATATTCGACGT :  284
Intron 3 of la¹ : -------------------------------------------GTAGTTAACTTTAACAACGGACCACTTATATTCGACGT :  868
Intron 3 of LA² : TACTTTT---------AT------------------ACTAGTACCTAACTTTAACCATGGACCACTTATATTCGAT-C :  374
Intron 3 of la² : AAATTTTTGCTAGAAAATGGATTATTTAGAAACGGAGGGAGTAGTACCTAACTTTAACCATGGACCACTTATATTCGAT-C :  548

                   1140        *      1160        *      1180        *      1200        *
Intron 3 of LA¹ : CATTGGCATAAAATGATTCTCCTCGATATTCGTTTACTTTTCTTACTATTTTTCT-----GTTTTGGAGCTTTCAT---- :  355
Intron 3 of la¹ : CATTGGCATAAAATGATTCTCCTCGATATTCGTTTACTTTTCTTAGTATTTTTCT-----GTTTTGGAGCTTTCAT---- :  939
Intron 3 of LA² : CATTAGCATAAAATGATTCTCCTCGAAATCCGTTTACTTTTCTTCATTATTTTTTCAAACAGTTTTGGCGTTTTGTTGCA :  455
Intron 3 of la² : CATTAGCATAAAATGATTCTCCTCGAAATCCGTTTACTTTTCTTCATTATTTTTATCAAACAGTTTTGGCGTTTTGTCTGCA :  629

                   1220        *      1240        *      1260        *      1280        *
Intron 3 of LA¹ : ------AGAACTAATAA--AAAGAAAAAACTTATATAA-------CACACCACATGCAGTTAATTAATTTCAACATATAATAT :  422
Intron 3 of la¹ : ------AGAACTAATAA--AAAGAAAAAACTTATATAACATTAAACACACCACATGCAGTTAGTTAATTCCAACATATAATAT : 1013
Intron 3 of LA² : ATCTTGAAAACTATTATGAAATTGAAAACTTATA---------CACATCACATGCAATCAATTAATACGAATATATAACCA :  527
Intron 3 of la² : ATCTTGAAAACTATTAT-AAATTGAAAACTTATA---------CACATCACATGCAATCAATTAATACGAATATATAACCA :  700

                   1300        *      1320        *      1340        *      1360        *      1380
Intron 3 of LA¹ : TAACTATAA-TTTTAAAT-ATTACTCATTCC-------GTTTCGCAAAGAGTGTCGTTTTAGA------------------ :  476
Intron 3 of la¹ : TAACTATAA-TGTTAAAT--------------------------------------------------------------- : 1030
Intron 3 of LA² : TAAGGACAAATTTTCAATGAATATTTAATA--------------------------------------------------- :  557
Intron 3 of la² : GAAGGACAAATTTTCAATGAATATTTAATACTCCCTCTGTTTTATAATAAGTGTCATTTTAGATTTATGCACACGGATTAA :  781

                               *      1400        *      1420        *      1440        *      1460
Intron 3 of LA¹ : -GAAATTTTTCATTATA---------------------------------------------------------------- :  492
Intron 3 of la¹ : -------------------------------------------------------------------------------- :    -
Intron 3 of LA² : -------------------------------------------------------------------------------- :    -
Intron 3 of la² : GAAAACATTTAATTTTACATATTTTCTATATAAAAACATCATTTCCCATACACCTAACTATATTTCAACCATAGAAAATAA :  862

                               *      1480        *      1500        *      1520        *      1540
Intron 3 of LA¹ : -------------------------------------------------------------------------------- :    -
Intron 3 of la¹ : -------------------------------------------------------------------------------- :    -
Intron 3 of LA² : -------------------------------------------------------------------------------- :    -
Intron 3 of la² : AATTGGAAAATAAAATTCTTAAATTTTGTATTGAAAAACGAAACGACACTTATTTCGTAACAAAAAATTTCCTCTAAAAC :  943

                               *      1560        *      1580        *      1600        *      1620
Intron 3 of LA¹ : ----------------------------------AAATAAATGTCGTTTTATGATTTCAATGC---------------------- :  521
Intron 3 of la¹ : ----------------------------------ATTAA---------------------------------------------- : 1035
Intron 3 of LA² : --------------------------------GTAAGTAA-------------------------------------------- :  565
Intron 3 of la² : GACACTCTTTGTGAAACGGAGGGAGTAGTAAGTAAGTACTATTCTCTCCGTTTTATATTATTTGTCGTTGTAGAAAAAAAA : 1024

                               *      1640        *      1660        *      1680        *      1700
Intron 3 of LA¹ : ATAATTTATTGATTTTA------------TTTTTAGTTTAATTTTCTATTGGTTGAAATATGATTAGGTATATGGGTAATG :  590
Intron 3 of la¹ : -------------------------------------------------------------------------------- :    -
Intron 3 of LA² : -------------------------------------------------------------------------------- :    -
Intron 3 of la² : ATTGTTTATCGTTTTCAATGCAAAATAATTTTTCACTTTATTTTTTTATTGATTGAAATATGATTAAATGTATAGATAATT : 1105

                               *      1720        *      1740        *      1760        *      1780
Intron 3 of LA¹ : ATGTTTTTCTATAGAAAATATGCAAAATTAAATGTTTTCTTAATCTGTGTGCAAAAAGCTAAAACGACATTTGTTATGAAA :  671
Intron 3 of la¹ : -------------------------------------------------------------------------------- :    -
Intron 3 of LA² : -------------------------------------------------------------------------------- :    -
Intron 3 of la² : ATGTTTTTATTTAGAAAATATATAAAATTATATATTTTCTTAATATGTATGCATAAATCTATAATGACGAGTAATATAAAA : 1186
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                    *        1800         *        1820         *        1840         *        1860
Intron 3 of LA¹ : CAGAGAGAGT---AATTAACATTTTAATCTTAATT-TTGCATTTCAGTTGCCAGAAAAATTATACAAGAAATTGTCCCACA :  748
Intron 3 of la¹ : ---------------TTAACATTTTAATCTTAATT-TTGCATTTCAGTTGCCAGAAAAATTATACAAGAAATTGTCCCACA : 1100
Intron 3 of LA² : ---------------TAGTCATATTAATTTAAATTTATGTATTTCAGTTACCAGAAAAGTTGTACAAGAACTTGCCCCATA :  631
Intron 3 of la² : TGGAGAGAGTGGTAATAGTCATATTAATTTAAATT-ATGTATTTCAGTTACCAGAAAAGTTGTACAAGAACTTGCCCCATA : 1266


                    *        1880
Intron 3 of LA¹ : GTACACGGATGCTCAGATACACCGT :  773
Intron 3 of la¹ : GTACACGGATGCTCAGATACACCGT : 1125
Intron 3 of LA² : GTACTCGGATGCTCAGATACACCGT :  656
Intron 3 of la² : GTACTCGGATGCTCAGATACACCGT : 1291
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Supplementary Figure S4. Nucleotide sequences alignment of intron 3 of *SalFAD3.LA1* and *SalFAD3.LA2* genes in yellow mustard. The sequence of exon 3 was underlined in red while that of exon 4 was underlined in green. The nucleotide sequence of inserted fragment in exon 3 of la[1] was from position 31 to 615. The new intron splicing site GT in the inserted fragment in exon 3 of *la[1]* was underlined in black. The nucleotides in blue rectangle indicated the inserted fragment that remained in the CDS of exon 3 of *la[1]*. The nucleotides in black rectangle indicated the 5 bp direct repeat (5'-AGAAC-3').