

Supplementary Materials:

**Oryza sativa Japonica Group DNA, chromosome 6, cultivar: Nipponbare, complete sequence**

Sequence ID: [AP014962.1](#) Length: 31248787 Number of Matches: 2

Range 1: 21954299 to 21954934 [GenBank](#) [Graphics](#) [▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1175 bits(636)	0.0	636/636(100%)	0/636(0%)	Plus/Plus

Features: [os06g0567900](#)  
[os06g0567950](#)

Query	1	GGGCGCTACAGGTGGTCCATCAACAACGTGTCCCTGACGCTGCGGGGACGCGGTACCTG	60
Sbjct	21954299	GGGCGCTACAGGTGGTCCATCAACAACGTGTCCCTGACGCTGCGGGGACGCGGTACCTG	21954358
Query	61	GGGCGCTTCCACCACGGGCTCCAGGACAGCGGTTTCGACGCGTCCGGGAGCGCGCGGCG	120
Sbjct	21954359	GGGCGCTTCCACCACGGGCTCCAGGACAGCGGTTTCGACGCGTCCGGGAGCGCGCGGCG	21954418
Query	121	GCGTTCCCGGAGGACTACGACGTGATGAGGCCGCGGGGAAACAACGCGACGACGCGGAGC	180
Sbjct	21954419	GCGTTCCCGGAGGACTACGACGTGATGAGGCCGCGGGGAAACAACGCGACGACGCGGAGC	21954478
Query	181	GACAGGGTGTTCGGCTGCGACACGGCGGGGTGGTGGACGTGGTCCAGAACGCCAAC	240
Sbjct	21954479	GACAGGGTGTTCGGCTGCGACACGGCGGGGTGGTGGACGTGGTCCAGAACGCCAAC	21954538
Query	241	ATGCTGAGGGAGGAGGTGAGCGAGACGCCACCCGTGGCACTCCACGGCCACGACTTCTGG	300
Sbjct	21954539	ATGCTGAGGGAGGAGGTGAGCGAGACGCCACCCGTGGCACTCCACGGCCACGACTTCTGG	21954598
Query	301	GTGCTCGGCTACGGGACGGCGGTACGACCCGGCGGCAACGGCGCGGGCTCAACGCC	360
Sbjct	21954599	GTGCTCGGCTACGGGACGGCGGTACGACCCGGCGGCAACGGCGCGGGCTCAACGCC	21954658
Query	361	GCCGACCCGCGCTGCGGAAACACGGCGGTGGTCTTCCCGCACGGGTGGACGGGCTTCGG	420
Sbjct	21954659	GCCGACCCGCGCTGCGGAAACACGGCGGTGGTCTTCCCGCACGGGTGGACGGGCTTCGG	21954718
Query	421	TTGCTCGCCAAACAACCGGGCGGTGGCGTTCCACTGCCACATCGAGCCGACCTCCAC	480
Sbjct	21954719	TTGCTCGCCAAACAACCGGGCGGTGGCGTTCCACTGCCACATCGAGCCGACCTCCAC	21954778
Query	481	ATGGGCATGGGGTGTCTTTCGTCGAGGGGGAGGACAGGATGCACGAGCTCGACGTGCC	540
Sbjct	21954779	ATGGGCATGGGGTGTCTTTCGTCGAGGGGGAGGACAGGATGCACGAGCTCGACGTGCC	21954838
Query	541	AAGGACGCCATGGCGTGGGCTCGTCCAGGACGGCGCCACGCGCTCACCCCGCA	600
Sbjct	21954839	AAGGACGCCATGGCGTGGGCTCGTCCAGGACGGCGCCACGCGCTCACCCCGCA	21954898
Query	601	ACGCCGCTGCCCTCCGTCGCGCGCGCGCGCCATGA	636
Sbjct	21954899	ACGCCGCTGCCCTCCGTCGCGCGCGCGCGCCATGA	21954934

**Figure S1.** Sequence alignment of the *REL1* interacting protein 5 (*RIP5*) obtained from the Y2H screening experiment with the genome of Nipponbare.

Alignment of OsAAO2.seq (upper line) and RIP5\_partial\_region.seq (lower line)

Identity = 100 % (636 / 636). Gap = 66.56 % (1266 / 1902)

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OsAAO2.txt      ATGGCCGCCCCCTGACGCTCTCTCTGTCGCCCCCCCCCCCCATCGCCGCGGCTCTCTCCCGCCATGCGCCGCGCCGCGCCGACGCTGGAGCTGGACCTGGACCTAGCTACTCTGGCCGCGCACTGCCACGCGCGT
RIP5_partial_region.txt

OsAAO2.txt      ATGATCGGGATAAACCGCAGGTTCCCGGGCCCAACATCACCCGCGCCCGCCGCGCAGTGCAGCTCAGCATGAACAACAGATGCACACGAGGCGCTGCTATCCACTGGCAGCGCATCAGACAGTTCGCCACGCGCTGGCGGAC
RIP5_partial_region.txt

OsAAO2.txt      GGGACGGATCGATATCCCACTGCGCAGTGAACCGCGCCGACAGCTGTCTACAGTTCTGTCGCGCAAGACGGGCACTACTTCTACCCAGCGCACTTCGGGATCGAGCGCGCCCGCGGCTGTACGGTTCCTCTATGCTCTCGAC
RIP5_partial_region.txt

OsAAO2.txt      TCCCGGAGCAGCCGAGCGGTTCCCGCAGCAGTACGACAGCGCGGCACTCCCATGATGCTCTCAGCGACTGCTGGCACCAGAACGCTCTACGCCAGCGCCCGGACTCGACGGCAAGCAGGCACTTCAGTGGATCGCGGAG
RIP5_partial_region.txt

OsAAO2.txt      CCCAGACGATCTTCAATCGGACAGCAGTTCGAGTGCACGCTGGCCGACGAGGAAGGTTTGAGAACTCTCAACGAGAACCTGGAGCCTGCTCGACGACCAAGATCTGCCAGCCAGGAAAGTCCCTGAGGAG
RIP5_partial_region.txt

OsAAO2.txt      ACGCGTCCCGCGCTGCTCCCGCAGCAGCAGTCCCGCTCTGCTGTTCAATGTGACGACCGGCAAGCTTACCGCTTAGATCGCCAGCACCTCCCTTTCTCTCTCAAGCTCAAGATTAAAGCCGACAGATGACGCTGCTG
RIP5_partial_region.txt

OsAAO2.txt      CAGCCGACGCGAACCCAGTGGACCGCTCTGCTCGACGACATGACATCTACTCCCGGAGAGCTACTCCCTCTCTCAAGCGCGACAGAAAGCCGCGGAGCTACTGGATCTCCCTCGCGCTCAGGGCGCCGACCCCAAGCGGTG
RIP5_partial_region.txt

OsAAO2.txt      CCGCGCTCCCATCTCAGCTACGGCAAGCGCAACCGCCCGCCGCGCTCCAGTGCAGCCGCGGAGCCCGCTGACCGCGGCTGGAGCAGCAGCCAGCCAGCGCTTCACTACAGCATCAGCGCGCGAAGGACAC
RIP5_partial_region.txt

OsAAO2.txt      AAGCCGCGCCCGCCCGCCGCGCAGCAGTCTCTCTCAACAGCGAGACCTGATGAGCGCGCTACAGTGTCTCCATCAACAGCTGTCCCTGACCTCGCGCGGACCGCTACTGGCGCTTCAGCAGCGGCTCCAG
RIP5_partial_region.txt

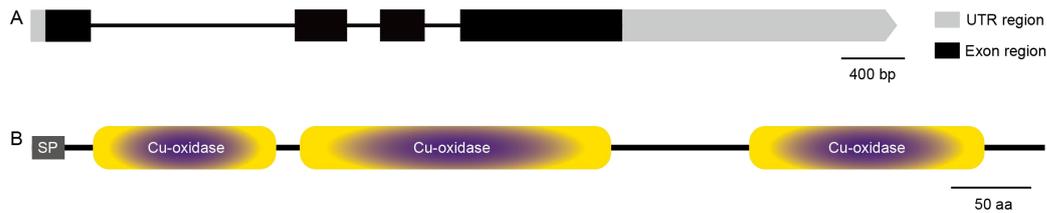
OsAAO2.txt      GACACCGCTTCAGCGCTCCCGCAGCCCGCCCGCCGCTCCCGGAGCACTACGACGTGATGAGCCCGCGGCAACACCGCCAGCAGCCGAGCGAGAGGTTCCCGGTCGCAACCGCGGCTGCTGAGCTGCTCTCAGAAC
RIP5_partial_region.txt

OsAAO2.txt      CCAGATCTCTGGGAGAGCTGACGCGACGACCGCTGCGACCTGACCGCGGAGGTTCTGGCTCTGCTGCTGCGCTAGCGCGCGGCTAGCAGCGCGCGCGCTAAGCGCGCGCGCGCGCGCGCTCCGAGAGG
RIP5_partial_region.txt

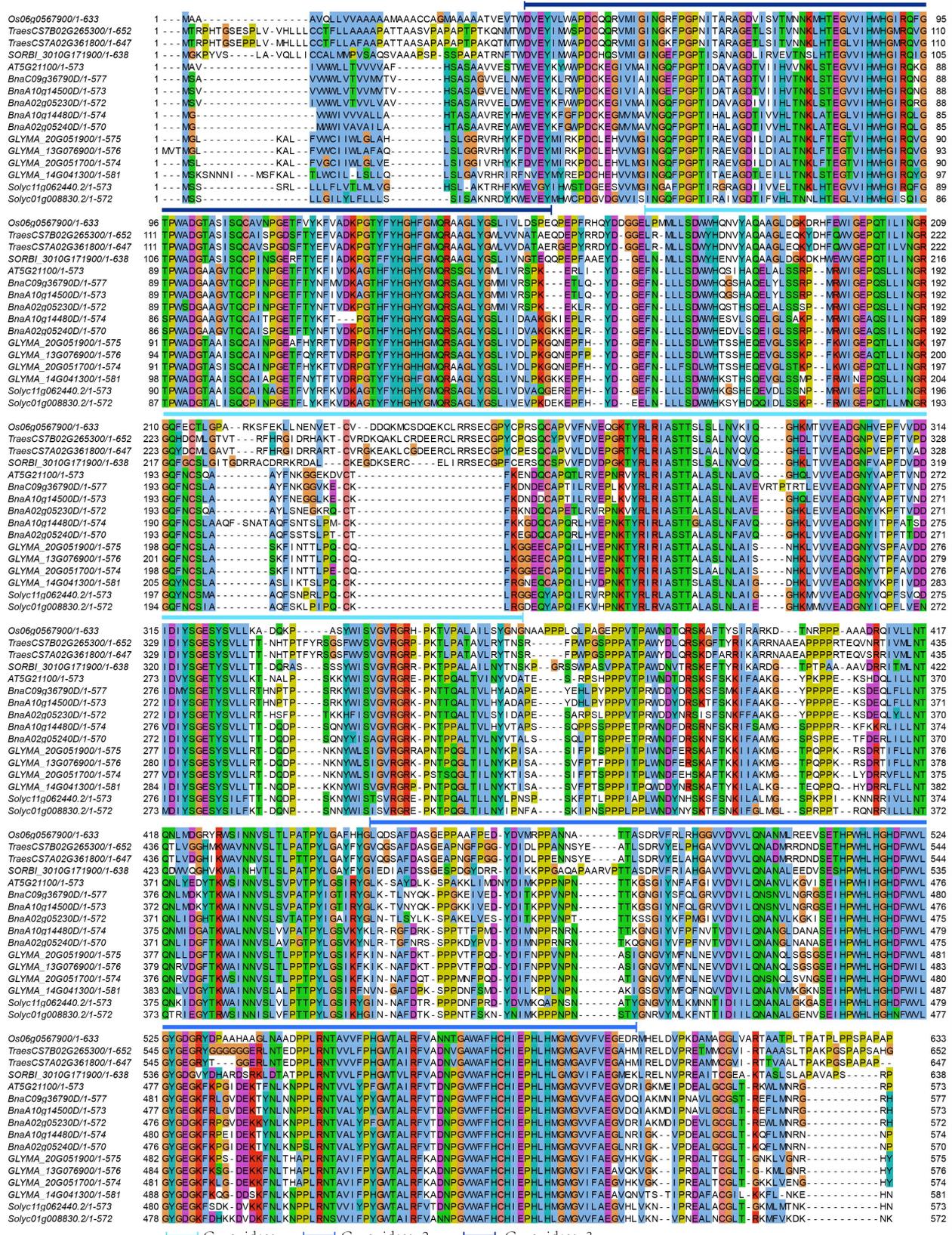
OsAAO2.txt      CGCGTCTCTCCCGACGGCTCGACCGCCCTTCGGTTCGTCGCGCAACACCGCCCGCTCCGCTTCACTCCACATCGAGCGCCACTCCACATGGGATCGCGCTGCTCTCTGTCAGCGGGAGCAGCATGCACGACTCCAG
RIP5_partial_region.txt

OsAAO2.txt      CTCCCAAGGACCCCATGGCTCCCGCTCTCTCCGACGACCGCCCGCCGCTACCCCGGCAACGCGGCTCCGCTCGCTCCCGCGCCCGCGCCGATG
RIP5_partial_region.txt
  
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**Figure S2.** Sequence alignment of *OsAAO2* and *RIP5*\_partial\_region obtained from the Y2H screening experiment.



**Figure S3.** Gene and protein structure of *RIP5*. (A) Gene structure of *RIP5*. Exon region and UTR region are represented by black boxes and grey boxes. Scale bar, 400 bp. (B) Protein structure of *RIP5*. SP is signal peptide. Scale bar, 50 aa.



**Figure S4.** Multiple sequence alignment of the homologous genes of *RIP5* in arabidopsis, soybean, sorghum, maize, wheat, rape and tomato.

**Table S1.** List of primers used in this study.

Application	Primer	Sequence (5'-3')
plasmid construction	AD-RIP5-F	GCCATGGAGGCCAGTGAATTCATGGCGG CCGCCGTGCAG
plasmid construction	AD-RIP5-R	CAGCTCGAGCTCGATGGATCCTCATGGCG CCGGCGCCGG
plasmid construction	RIP5-pSPYCE-F	TATCCAGATCCAGTGGGATCCATGGCGG CCGCCGTGCAG
plasmid construction	RIP5-pSPYCE-R	CGCACTAGTAAGCTTGGTACCTGGCGCCG GCGCCGGCGA
plasmid construction	RIP5-pRTVnGFP-F	GAGGACTTGAATGAAGGATCCATGGCGG CCGCCGTGCAG
plasmid construction	RIP5-pRTVnGFP-R	CGCACTAGTAAGCTTGGTACCTCATGGCG CCGGCGCCGG
plasmid construction	RIP5-pC-F	AACGATACTCGAGGGGATCCATGGCGG CCGCCGTGCAG
plasmid construction	RIP5-pC-R	GGGAAATTCGCTAGTGGATCCTCATGGCG CCGGCGCCGG
Genotype analysis	V-Cas9-F	AGCCCTGCCTTCATACGCTA
Genotype analysis	V-Cas9-R	CTGCTGGGACTCCGTGGATA
Genotype analysis	P1	GTGATGATCGGGATAAACGGC
Genotype analysis	P2	GTTTCATGGTGACGCTGATCACG
Genotype analysis	P3	CGTGACACACACCACTCACT
Genotype analysis	P4	TGTGTCCCATCCACCCAAAA
Genotype analysis	RIP5OE-F	AGGACCTCGACCTCAACACAAC
Genotype analysis	RIP5OE-R	ACGACGAGCAGCTGCACG
Genotype analysis	rel1-39208	ATTAATCGGCAATAGGTCTC
Genotype analysis	TDNA7777	TCGTGAAAAAGAAGACGTTG
Genotype analysis	rel1-72833	CGAGATAATGCAGGAGTACAA
qRT-PCR	actin-F	TCCAGCAGATGTGGATTGCCAAGG
qRT-PCR	actin-R	TCTGGTACCCTCATCAGGCATCTG
qRT-PCR	qRIP5-F	CCCATTCCAGTTAAATGCCATT
qRT-PCR	qRIP5-R	AAACCACTCTTCATCAGTGAGT
qRT-PCR	OsMYB2-F	GGGCTGAAACGCACAGGCAAGA
qRT-PCR	OsMYB2-R	CTGCTTGGCGTGCTTCTGC
qRT-PCR	POX22.3-F	CAGCTGCTCCAAGGTGAATC
qRT-PCR	POX22.3-R	AGATTGCTTCCAGCAACGAA
qRT-PCR	OsbHLH148-F	AGCTACGCCGACCTCTACG

Application	Primer	Sequence (5'-3')
qRT-PCR	OsbHLH148-R	GGAAGCTATTATTTGTATGATTGTA
qRT-PCR	OsDT11-F	TGTGTGACGCCGTGCAAT
qRT-PCR	OsDT11-R	CCAGGGCCGGAAGCTAGA
qRT-PCR	OsWRKY76-F	TTCCGAATGCTTTTCTGCTG
qRT-PCR	OsWRKY76-R	ATCGTGAGGCCCGATAGAAG
qRT-PCR	OsLEA3-F	CGGCAGCGTCCTCCAAC
qRT-PCR	OsLEA3-R	CGGTCATCCCCAGCGTG

**Table S2.** Physical and chemical parameters of genes in the *OsAAOs* family

Uniport_entry name	Gene ID	Name	Protein length (aa)	Molecular weight / kDa	Protein isoelectric point (pI)	Instability index	GRAVY	Subcellular localization
A0A0P0WY31_Q5 Z645_ORYSJ	Os01g0816700	OsAAO1	553	61.66	8.83	32.30 (stable)	-0.203	Extracellular
A0A0P0WYB7_Q5 Z5T3_ORYSJ	Os06g0567200	OsAAO2	581	62.98	7.34	35.05 (stable)	-0.075	Chloroplast
Q7XIF0_ORYSJ	Os06g0567900	OsAAO3	633	69.12	5.90	43.86 (unstable)	-0.231	Extracellular
A0A0P0XMD7_B8 BEW5_ORYSJ	Os07g0119400	OsAAO4	545	59.07	7.17	41.87 (unstable)	-0.150	Chloroplast
A0A8J8XW93_Q0J 0J1_ORYSJ	Os09g0365900	OsAAO5	577	62.87	7.38	35.04 (stable)	-0.156	Chloroplast
A0A0P0V9P6_Q94 2J6_ORYSJ	Os09g0507300	OsAAO6	574	62.05	6.98	37.50 (stable)	-0.117	Cell wall

**Table S3.** List of sample sequencing data quality summary in this study.

Sample	Raw_reads	Clean_reads	Clean_bases	Error_rate	Q20	Q30	GC_pct
rip5-ko_PEG_1	39322474	38392556	5.76 G	0.02	98.22	94.82	51.98
rip5-ko_PEG_2	48078672	46927924	7.04 G	0.02	98.22	94.85	52.96
rip5-ko_PEG_3	40686302	39714814	5.96 G	0.02	98.25	94.86	52.61
ZH11_PEG_1	49920428	49295096	7.39 G	0.03	97.6	93.29	51.7
ZH11_PEG_2	45815086	45267186	6.79 G	0.03	97.69	93.5	52.48
ZH11_PEG_3	47386120	46730102	7.01 G	0.03	97.68	93.44	52.65