Supplementary Information

Identification of effector candidate genes of *Rhizoctonia solani* expressed during infection on *Brachypodium distachyon*

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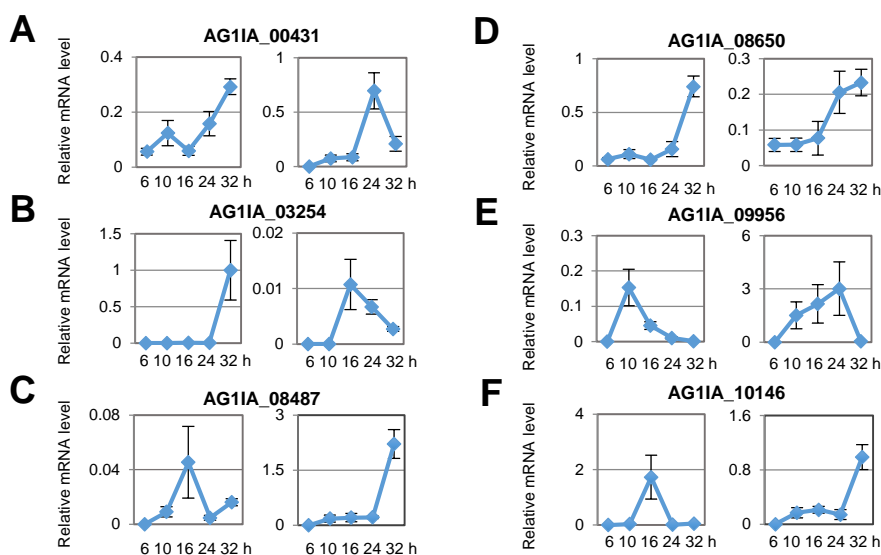
Supplementary Figure S1. Expression profiles of *RsSEPGs* during inoculation of *Rhizoctonia solani* on detached leaves of *Brachypodium distachyon* which showed different expression patterns in two independent experiments. (**A** to **F**) Expressions of *AG1A_00431* (cluster 4 or 6) (**A**), *AG1IA_03254* (cluster 6 or 3) (**B**), *AG1IA_08487* (cluster 3 or 6) (**C**), *AG1IA_08650* (cluster 6 or 5) (**D**), A*G1IA_09956* (cluster 2 or 3) (**E**), and *AG1IA_10146* (cluster 3 or 6) (**F**) of *R. solani*, which were classified into different clusters with their expression patterns, on *B. distachyon* at 6, 10, 16, 24, and 32 hours post inoculation with the improved method. Gene expressions were examined using qRT-PCR using cDNA prepared with total RNAs extracted from *R. solani*-inoculated *B. distachyon* leaves at each time point. Graphs display the relative mRNA level of each gene normalized by *18S rRNA* as internal control. The data are presented as means with standard error of 4 biological replicates. The results of two independent experiments were shown.

Supplementary Table S1. Summary of RNA-seq data of *Rhizoctonia solani* growing on PDA medium, and *R. solani* in detached leaves of *Brachypodium distachyon* Bd21

Sample name	Biological replicate	Mapping results to the *B. distachyon* genome					Mapping results to the *R. solani* genome				
		Number of total reads	Number of mapped reads	Mapped (%)	Properly paired (%)	Number of unmapped reads	Number of total reads	Number of mapped reads	Mapped (%)	Number of properly paired reads	Properly paired (%)
On PDA medium	1						75,820,544	62,663,857	82.65	52,149,370	68.78
	2						71,975,102	59,730,523	82.99	48,662,366	67.61
	3						74,022,804	60,630,695	81.91	50,261,484	67.90
In *B.distachyon* leaves (8 hpi)	1	51,894,736	48,761,392	93.96	85.98	3,133,344	1,812,936	36,066	1.989	30,258	1.669
	2	64,192,412	60,122,908	93.66	85.26	4,069,504	2,480,250	13,589	0.548	11,340	0.457
	3	60,687,308	56,567,307	93.21	83	4,120,001	2,586,980	11,334	0.438	9,098	0.352

Supplementary Table S3. *Rhizoctonia solani AG1IA secretory effector protein* genes (*RsSEPGs*) with their expressions during infection and agar medium

GeneID [a]	Correction of gene model	Loss of signal peptide after annotation calibration	Length (aa)	Cluster [b]	Expression timing in Zheng et al (2013) [c]	Detection of transcripts in Xia et al (2017)	Annotation [d]	Expression levels on PDA medium (FPKM) [e]			
								Rep1	Rep2	Rep3	AVG
AG1IA_00153	yes	yes	183>	n.a.	24 hpi		hypothetical protein	12.11	23.17	22.26	19.18
AG1IA_00157	yes	no	168	2	24 hpi		polysaccharide deacetylase domain-containing protein	4.51	7.52	9.41	7.15
AG1IA_00172 [f]	n.a.	n.a.	-	n.a.	n.a.		Cysteine proteinase	12.46	18.47	22.04	17.66
AG1IA_00273	yes	no	146	5	10, 18 hpi	yes	hypothetical protein	92.34	48.50	32.69	57.84
AG1IA_00431	no	no	232	4 or 6	n.a.		GILT domain-containing protein	215.43	168.17	173.44	185.68
AG1IA_00478	yes	no	90	2	n.a.		hypothetical protein	20.93	25.13	26.58	24.21
AG1IA_00529	yes	yes	160	n.a.	n.a.		hypothetical protein	8.07	6.39	7.29	7.25
AG1IA_00669 [h]	yes	no	294>	4	10 hpi		glycosyl hydrolase family 61 domain-containing protein	91.92	140.04	126.43	119.47
AG1IA_00684	yes	no	67	6	10, 18 hpi		hypothetical protein	0.27	0.71	0.69	0.55
AG1IA_00763	n.a.	n.a.	68	n.d.	24 hpi		hypothetical protein	1.30	0.00	0.00	0.43
AG1IA_00879	n.a.	n.a.	156	n.d.	10 hpi		hypothetical protein	6.56	8.45	7.23	7.41
AG1IA_00951	yes	no	134	6	n.a.		deuterolysin metalloprotease (M35) family domain-containing protein	3107.04	2100.49	1561.70	2256.41
AG1IA_01042	yes	no	170	2	n.a.	yes	hypothetical protein	13.98	8.07	9.42	10.49
AG1IA_01512	yes	no	78	4	n.a.	yes	hypothetical protein	19.66	17.26	16.02	17.65
AG1IA_01599	yes	yes	391	n.a.	n.a.		lipoic acid synthase	86.49	86.45	91.46	88.13
AG1IA_01603	yes	no	85	2	n.a.		DUF1524 domain containing-protein	10.51	14.77	14.81	13.36
AG1IA_01604	yes	no	211	2	n.a.	yes	DUF1524 domain containing-protein	15.46	18.00	14.69	16.05
AG1IA_01605	no	no	56	2	n.a.		hypothetical protein	148.22	164.07	160.04	157.44
AG1IA_01933	n,a,	n.a.	88	4	n.a.		hypothetical protein	0.91	2.65	2.15	1.90
AG1IA_02138	yes	yes	290>	n.a.	n.a.		hypothetical protein	58.78	74.98	67.94	67.23
AG1IA_02490 [f]	n.a.	n.a.	-	n.a.	n.a.		hypothetical protein	0.59	0.00	0.00	0.20
AG1IA_02878/79/80 [g]	yes	yes	759	n.a.	24 hpi		DNA binding domain-containing protein	35.98	47.58	45.14	42.90
AG1IA_02940	yes	no	209	6	n.a.		DUF3455 domain containing-protein	430.26	251.35	207.44	296.35
AG1IA_03047	n.a.	n.a.	68	5	n.a.		hypothetical protein	1.30	0.73	0.00	0.67
AG1IA_03254	no	no	111	3 or 6	10 hpi		hypothetical protein	9.03	12.85	14.38	12.09
AG1IA_04006	no	no	54	2	n.a.		hypothetical protein	8.63	21.74	13.00	14.46
AG1IA_04108	yes	yes	52	n.a.	24 hpi		hypothetical protein	2.12	4.91	6.18	4.40
AG1IA_04191	yes	yes	98	n.a.	10 hpi		hypothetical protein	0.62	1.00	0.16	0.59
AG1IA_04237	yes	no	138	5	24 hpi		hypothetical protein	0.49	0.52	0.12	0.38

AG1IA_04267/8 [g]	yes	yes	478	n.a.	n.a.		Viral tegment protein/pectinesterase inhibitor	53.65	55.73	54.81	54.73
AG1IA_04298	yes	no	218	4	n.a.		emp24/gp25L/p24 family/GOLD domain-containing protein	66.30	64.66	71.39	67.45
AG1IA_04737	n.a.	n.a.	140	n.d.	n.a.		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_04814	n.a.	n.a.	145	n.d.	10 hpi		hypothetical protein	0.22	0.36	0.23	0.27
AG1IA_04819	no	no	151	5	n.a.		fungal cellulose binding domain-containing protein	0.00	0.00	0.00	0.00
AG1IA_04889 [f]	n.a.	n.a.	-	n.a.	n.a.		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_05179	yes	yes	60	n.a.	n.a.	yes	hypothetical protein	0.13	0.29	0.00	0.14
AG1IA_05291	no	no	230	3	24 hpi	yes	hypothetical protein	35.87	41.33	33.47	36.89
AG1IA_05500	yes	no	227>	2	n.a.		hypothetical protein	780.36	928.89	845.63	851.63
AG1IA_05601 [f]	n.a.	n.a.	-	n.a.	24 hpi		histone acetyl transferase	15.28	11.79	19.81	15.63
AG1IA_05783	no	no	90	5	n.a.		hypothetical protein	27.82	56.66	58.08	47.52
AG1IA_05938	yes	no	189	1	n.a.		hypothetical protein	28.44	78.85	63.30	56.86
AG1IA_06169	n.a.	n.a.	184	n.d.	24 hpi		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_06325	no	no	142	2	n.a.	yes	FKBP-type peptidyl-prolyl cis-trans isomerase domain-containing protein	149.73	212.66	194.36	185.58
AG1IA_06613 [h]	yes	no	218	2	n.a.	yes	DUF3129 domain containing-protein	183.12	312.28	273.76	256.39
AG1IA_06710 [h]	yes	no	38	6	n.a.		hypothetical protein	0.00	0.63	0.40	0.34
AG1IA_06739	yes	no	221	4	18, 24 hpi		hypothetical protein	9.26	7.92	9.28	8.82
AG1IA_06741	yes	yes	59	n.a.	18, 24 hpi		hypothetical protein	1.26	0.00	1.68	0.98
AG1IA_07047	yes	no	154	6	24 hpi		hypothetical protein	31.69	30.05	25.03	28.92
AG1IA_07075	yes	no	117	5	n.a.	yes	CFEM domain-containing protein	1.57	4.68	3.22	3.15
AG1IA_07122	n.a.	n.a.	133	5	n.a.		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_07262	n.a.	n.a.	257	n.d.	n.a.	yes	hypothetical protein	0.25	0.10	0.20	0.18
AG1IA_07267 [h]	yes	no	159	1	n.a.	yes	hypothetical protein	5.80	6.46	8.31	6.86
AG1IA_07490	yes	yes	129	n.a.	n.a.		Yippee domain-containing protein	10.59	10.34	12.10	11.01
AG1IA_07958 [h]	yes	no	306	4	18, 24 hpi		igA peptidase M64 domain-containing protein	13.85	10.61	12.07	12.18
AG1IA_07966	yes	no	142	1	10 hpi		hypothetical protein	8.19	9.09	9.30	8.86
AG1IA_08025	no	no	238	2	n.a.		DUF3421 domain containing-protein	38.43	44.08	37.76	40.09
AG1IA_08061	n.a.	n.a.	184	5	10, 18 hpi		hypothetical protein	0.23	0.57	0.08	0.30
AG1IA_08487 [h]	yes	no	144	3 or 6	24 hpi		Ribonuclease domain-containing protein	384.71	416.22	430.21	410.38
AG1IA_08488	yes	no	149	2	24 hpi	yes	Ribonuclease domain-containing protein	2.28	3.45	0.98	2.24
AG1IA_08577	n.a.	n.a.	49	n.d.	n.a.		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_08615	yes	no	225	1	10 hpi	yes	hypothetical protein	24.78	25.15	25.99	25.31
AG1IA_08650	yes	no	213>	5 or 6	24 hpi		hypothetical protein	23.90	58.04	48.62	43.52
AG1IA_08777	yes	no	160	6	24 hpi		plastocyanin-like domain-containing protein	0.48	0.72	0.77	0.65
AG1IA_08781 [f]	n.a.	n.a.	-	n.a.	18, 24 hpi		Cuticle-degrading protease	61.13	42.02	19.48	40.87

AG1IA_08891	yes	yes	147	n.a.	n.a.	yes	hypothetical protein	59.57	47.90	41.50	49.65
AG1IA_08892	yes	yes	169	n.a.	n.a.	yes	hypothetical protein	63.82	47.94	45.15	52.30
AG1IA_08957	yes	yes	32	n.a.	n.a.		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_09055	yes	no	265	5	n.a.	yes	thaumatin-like protein	2.67	2.82	2.26	2.58
AG1IA_09060	yes	no	253	4	n.a.	yes	thaumatin-like protein	5.50	6.78	5.84	6.04
AG1IA_09137 [f]	n.a.	n.a.	-	n.a.	n.a.		hypothetical protein	5.06	2.22	3.63	3.64
AG1IA_09202	yes	no	184	2	n.a.	yes	hypothetical protein	106.32	205.36	185.35	165.68
AG1IA_09203	yes	no	181	2	n.a.	yes	hypothetical protein	38.83	52.21	43.66	44.90
AG1IA_09207	yes	no	182	1	24 hpi	yes	hypothetical protein	316.59	322.34	235.00	291.31
AG1IA_09229	yes	no	99	4	n.a.		hypothetical protein	2.50	1.32	1.04	1.62
AG1IA_09275	yes	yes	158	n.a.	n.a.		hypothetical protein	521.10	414.59	355.22	430.30
AG1IA_09643	n.a.	n.a.	66	n.d.	n.a.		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_09728	yes	no	67	5	n.a.		hypothetical protein	1.24	1.54	0.74	1.18
AG1IA_09836	yes	yes	147	n.a.	18 hpi		hypothetical protein	47.64	41.95	36.13	41.91
AG1IA_09837	n.a.	n.a.	131	n.d.	10 hpi		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_09956	no	no	84	3 or 4	n.a.	yes	hypothetical protein	173.67	148.51	123.75	148.64
AG1IA_10146	yes	no	82	4 or 6	n.a.		hypothetical protein	38.00	98.71	86.25	74.32
AG1IA_10180	yes	yes	146	n.a.	n.a.		hypothetical protein	2.23	2.63	3.06	2.64
AG1IA_10284	yes	no	80>	1	n.a.		hypothetical protein	0.00	0.00	0.00	0.00
AG1IA_10296 [f]	n.a.	n.a.	-	n.a.	10 hpi		hypothetical protein	24.79	20.71	24.11	23.20
AG1IA_10375	yes	yes	168>	n.a.	n.a.		valine-tRNA ligase	72.02	72.31	64.21	69.52
AG1IA_10403	yes	yes	152>	n.a.	n.a.		hypothetical protein	9.10	8.83	12.65	10.19
AG1IA_10405	yes	yes	65>	n.a.	18, 24 hpi	yes	hypothetical protein	0.40	0.45	0.81	0.55
AG1IA_10478	no	no	93	5	n.a.		hypothetical protein	12.03	14.08	17.09	14.40

[a] Finally selected *RsSEPGs* after verification of gene model were shown in bold letter.

[b] Cluster numbers categorized with their expression profiles during infection on *Brachypodium distachyon* in this study as shown in Fig. 2.

[c] Expression timing (hours) after inoculation on rice plants determined by Zheng et al. (2013).

[d] Annotations were obtained from top hit of blastP search.

[e] FPKM (fragments per kilobase of exon per million reads mapped) of 3 independent RNA-seq analysis using total RNA prepared from growing hyphae of *R. solani*.

[f] Transcripts were detected in reverse strand of these genes in RNA-seq results.

[g] A series of transcript was detected across multiple annotated genes.

[h] Prediction as an effector protein with EffectorP 1.0 was lost in the calibrated cDNA sequences of these 6 genes.

n.d., not detected

n.a., not applicable

Supplementary Table S4. Primers used in this study

Gene	Primer sequence	Gene	Primer sequence
AG1IA_00157	CCCATGCTCACTTGGCTTC CCCTTACTTCGTCATCATACTCACC	AG1IA_07122	TCTCTAGTCAGCCGCCCATC GATTTGTTCTCTGGTTTCGAGGTC
AG1IA_00273	CCGCAATGCAAGTCAAAGG GCAAAGGGACGAAGGTGTG	AG1IA_07262	TTGATCTTGTGCTCTGCCATC CTTTCCTCCGCCATTACACC
AG1IA_00431	GGGATCGACTGGGATAAAAGTG ATCGCACCTTGCCGTTG	AG1IA_07267	GTCGACTGGCTCACACCTTC ACGCACTAAACAGCGCAAAC
AG1IA_00478	GGGTTGACGAGTTTGCTTCC TAATAGCGTGGTCCGTTCTTCC	AG1IA_07958	GCGATCAATATCAACGACCAAG TGAGAGGGAGTATGACGGAGTG
AG1IA_00669	TGTTCTCTTCGCTTGTATCGTTTG ACGACGGATTGGAGACTTGG	AG1IA_07966	GATCCATGCTATGCCAGTACCTC GTCGCCATCCACAACCATC
AG1IA_00684	GGCAAGTTCCAAGGGAGTG AAATCCAACGACCGGAGAAG	AG1IA_08025	GACTCTTGCAACCACAGTCTCC TTCCTCTGCCTTTCGTATCC
AG1IA_00763	TGGGAGAAAGGGACAGCAAG ACAGTGATTTGCCTCGTTCG	AG1IA_08061	GGTCGCAGTTTCATCATCTCC TTGCGGCGAATCCAAAG
AG1IA_00879	TGACTGAGATGGAGGAAATCACAC TTCCTGGTAGCCTGTAATCGTAATC	AG1IA_08487	CACGTTTTCAATAACAGGGAAGG TAAACCGACGAGCGGAAAAG
AG1IA_00951	GGCCGATGTCGAGAACTTG TACACCGCGAGGGTCTTTG	AG1IA_08488	CTTTGTTCGAGTTCCCTATTTTCC AGCCCCTTATACTGCCGATG
AG1IA_01042	CATCTGGGATTGACGCTTATGTC TTCCTGCTGCCGATTTCC	AG1IA_08577	CTTGCTTGTGCCTAATACGACTG TTTGACGGGGTGGCTTG
AG1IA_01512	GGCCTCAGGAAGACACAAGG TGGAAGGGATAGGTGGGAAAC	AG1IA_08615	CCCTCTTGTAATCAACGTCTTGG CTACTGCCTCCTTGTTCATCTGG
AG1IA_01603	CTAAATTGGTTGTTGCTG CGCTCTCGTGTATCGCATTTAC	AG1IA_08650	TTTACGACCAACCCGCCTAC CCTGGTTAAGTCCAGCCAACTC
AG1IA_01604	CGAAGGCAGATGCGATACAC TCATAGTCCGAAGTCCAAAGTCC	AG1IA_08777	CCAAGGAATCAGGAGGAAAGTG TCCAAAAGGGTCCGTGTAATG
AG1IA_01605	CGCTTCGCTTATTTGATTGCTC GGGTCGGTTGTCGCTTTAC	AG1IA_09055	TGCGAATGTAGTCGCTCACC TAAACCCGCCAACCAAACTC
AG1IA_01933	AGGCACCGGCTAATTGTTG CCAGACGATCCCAAATCAACTC	AG1IA_09060	CGCCTGTCCATTCACCATC CCAAATACGACCAGCCTTCC
AG1IA_02940	AACACATCGCTCTTGGATATGG TTCTGGGTCGGTTTTGGTG	AG1IA_09202	AGGCTTTCTGCTCGCCTTC GTTGTCCACTCCCTCCACTACC
AG1IA_03047	GACCATTTCCAAGACGACCAG GGGAATCGGGATAATGCAAG	AG1IA_09203	GCCTTTGCTTCCCCTCTTG GTAAGATTGCATTTGCCCTTCC
AG1IA_03254	GAAAGGCACGGATAGGAAAGG GCCGAGTACGGTAACAAAAGG	AG1IA_09207	GTGGAATGCGCGTGTACTG CGTATTTTCCGCCCTTCTTG
AG1IA_04006	TTCGTCCGGCTTGTTCG ACACGGGGTGAAGAGACAGG	AG1IA_09229	GGCTCCACCCAGAGCATAAATAC CGCATTACCTCCTCGCCTAC
AG1IA_04237	GCCCCAGTATCTCTGTTTTGTTG CACAATGACCTCCAAAATTCTTCC	AG1IA_09643	TTTTGTGATTCTGGCCTGTTGTAG CAGACCGCACAGGTCCTAAC
AG1IA_04298	GTCGTAGACAGATCAGAACACCAAC CGAAATAACACTCCCCACCAAG	AG1IA_09728	TGGCCGGACCAACAATAAG CGAGAAAGCGTCATAGAAACAATC
AG1IA_04737	AGCGTGGTGAAGCGAATG CAGACGGATGGCTGTAACGTAG	AG1IA_09837	AAGGTGGTGGGCAGTAACG GGATGAACAACACCCTGATCC
AG1IA_04814	CATAGGAAACGCTCGGCAAC TGGCACTGATGAGACGGTAGAG	AG1IA_09956	TAGCAGAGATTGCTGCACAAGAG ACGGCAACACTCCTTGTATCTTC
AG1IA_04819	CCGTCAGTTTCGTGTAATCACTATG AGATGTGATTGTGGTGGTTGATG	AG1IA_10146	TTTCGTCGCTGCCCAAG CCTGGAAGAGGTGAGCCAAG
AG1IA_05291	GCGCGGCTGTTTTCTTG GACACGAGAGTTGGCGAATG	AG1IA_10284	ATCCGCCACCTCTGTTAGC CAGGTGCTGGCGATACAAC
AG1IA_05500	GGACTGCTGCATTAAGGTTGG GCACGATGGCTGGTTGTAAG	AG1IA_10478	CGACTCCCTTGCCTATTTCTTG CAAGTTTCTCCACTCCCTACGAAC
AG1IA_05783	CCGAGCCGAGAAGATCAAG AGTTATTGGTCGAAGTCAGGGAAG	18S rRNA	AATTCCAGCTCCAATAGCGT TACATACCGTGAGGCAGACC
AG1IA_05938	CAAGAGCAAACGACCGATG GCCAAGGTCCAACAGCAAG		
AG1IA_06169	GACAATCAGGACGACAACGAG ATTGCAGACTTTAGCGAACCAAG	AG1IA_05310	CAACAAGAAAAGTTCACCGAGTATG CGACTTTCGGTTCGTTTGTG
AG1IA_06325	GTGTGTTGGTGAAAAGCGAAAG GGTCCTTGATGCCCAATAGTTC	AG1IA_06618	GCGGGGTTAGTTGTGGTTG GGCTTCATAGCAGGGGAAAAG
AG1IA_06613	TTGTTGTCGTTGCTGCTCTTG CTGCCTTTCCGTTGATACCC	AG1IA_06890	ACCAGGACCACGACAAGGAC TGCTATCCGAGTGCCCAAC
AG1IA_06710	GATACGGCTCGAAGCAACTAAG GGTTTTACTCGCCACTGCTTG	AG1IA_07787	TCCCTTTTCGTTCAGTGTAGTTAGG TTATTGGGCAGGATCGTTTTG
AG1IA_06739	CGCCTCTGGTGGTTCTATCTACTC GAAATCCGAAATGATTGCCTTG	AG1IA_07795	GGATCAACTGGGCAACTCAAC GCAGCTACTACAACATGCACACC
AG1IA_07047	GCGTTGCTATCTGCGTTCC GTAGTCTTCCCGTCCCATCC	AG1IA_08771	AGACCAGTACGCCTTCACCAC CCCGATAAGCAACCAGAACC
AG1IA_07075	CCGATTGCCGTTGGAAG AGTAGAATACACCTGGGCGAAAAC	AG1IA_09161	GCCCATACGCTCTTCCAAAC CACCCAACAGCGACTTCATC