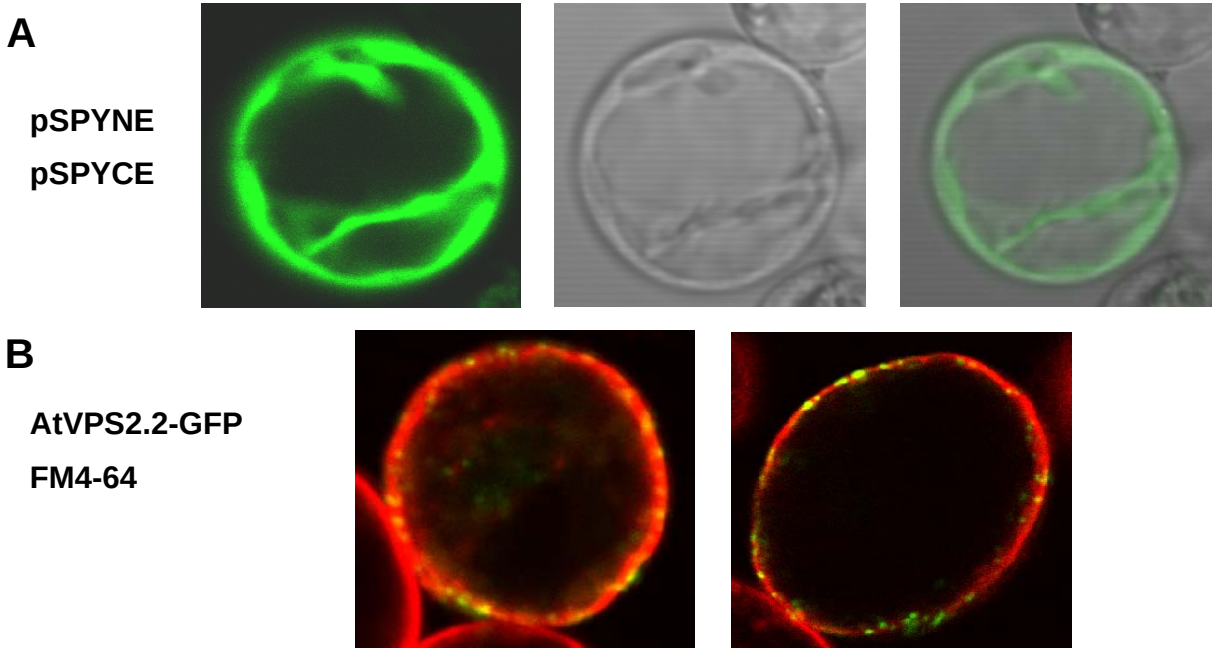
Supporting Information

Material and Methods:

The plasma membrane of transformed protoplasts was visualized with the membrane selective amphiphilic styryl dye FM4-64 by adding a 2000x stock solution (aqueous 16.4 mM SynaptoRed C2, Biotium, Germany) to the sterile protoplast incubation media (Gamborg's B5 basal salts, 0.34 M glucose, 0.34 M mannitol, pH 5.5) 30 min before observation with the confocal laser scanning microscope (CLSM) (TCS-SP2 Leica, Germany). Excitation wavelengths were 488 nm (argon laser) for GFP and FM4-64. Emissions were detected between 500-535 nm for GFP and 610–680 nm for FM4-64. Images were taken with a long distance 63x water-immersion objective. Pictures were prepared for publication with the Leica LAS AF Lite, ImageJ and Paint Shop Pro 5 software.



Supplemental Figure S1. Single scans through transformed protoplasts

A) Protoplast transformed with the empty BiFC vectors pSPYNE and pSPYCE which occasionally and spontaneously interact in the cytoplasm. The left image shows the GFP emission, in the middle is the transmission image of the same protoplast and on the right the overlay between the GFP and transmission image. **B)** Protoplasts transformed with AtVPS2.2-GFP and stained with FM4-64. The orange-yellow speckles are AtVPS2.2-GFP labelled compartments that co-localized with the plasma membrane.

Supplemental Table S1 AtVPS2.2-GFP interacting proteins identified in the three independent the pull down screens.

Proteins are sorted by abundance of identified peptides. Indicated are the AGI and accession numbers, molecular weight, number of unique peptides, number of assigned peptides and sequence coverage.

all 3 AtVPS2.2- GFP, no wild type, no GFP control
2 AtVPS2.2- GFP, no wild type, no GFP control
1 AtVPS2.2- GFP, no wild type, no GFP control

					2007 April			2007 June								2008 January							
					AtVPS2.2-GFP			wild type				AtVPS2.2-GFP				35S:GFP				AtVPS2.2-GFP			
#	Identified Proteins (84)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Nr. of unique peptides	Nr. of assigned peptides	Sequence coverage	Nr. of unique peptides	Nr. of assigned spectra	Sequence coverage	Quant. value	Nr. of unique peptides	Nr. of assigned spectra	Sequence coverage	Quant. value	Nr. of unique peptides	Nr. of assigned spectra	Sequence coverage	Quant . value	Nr. of unique peptides	Nr. of assigned spectra	Sequence coverage	Quant. value
1	glycosyl hydrolase family 1 protein [Ar	gil15219623	60 kDa	WAHR	12	41	25%	16	120	26%	103	12	47	22%	47	9	21	18%	32	10	38	23%	57
2	PYK10 (phosphate starvation-respor	gil15232626 (	60 kDa	WAHR	7	29	15%	9	101	20%	166	8	34	16%	71	5	29	12%	66	6	34	16%	96
3	PBP1 (PYK10-BINDING PROTEIN 1) [A	gil15228198	32 kDa	WAHR	2	2	5,0%	16	72	72%	62	12	41	54%	41	17	56	42%	84	16	62	42%	93
4	jacalin lectin family protein [Arabidop	gil15228218	72 kDa	WAHR	11	29	20%	19	123	27%	106	11	24	18%	24	10	18	14%	27	15	22	24%	33
5	beta-glucosidase (PSR3.2) [Arabido	gil15219605 (-	60 kDa	WAHR	8	15	16%	9	65	20%	139	8	34	16%	74	7	14	14%	48	8	19	18%	74
6	jacalin lectin family protein [Arabidop	gil15225232	49 kDa	WAHR	21	72	46%	12	28	31%	24	9	13	20%	13	8	9	21%	14	4	5	11%	8
7	ADL1 (ARABIDOPSIS DYNAMIN-LIKE	gil30693985	68 kDa	WAHR	18	22	29%	8	10	15%	9	17	27	28%	27	0	0	0	1	20	28	33%	42
8	ADL3 (ARABIDOPSIS DYNAMIN-LIK	gil15218837	100 kDa	WAHR	0	0	0	8	8	9,0%	7	28	59	34%	59	0	0	0	1	12	16	13%	24
9	ZW9 [Arabidopsis thaliana], gil89793	gil18406229	45 kDa	WAHR	10	29	22%	7	25	15%	22	7	18	16%	18	5	12	14%	18	3	7	7,3%	11
10	meprin and TRAF homology domain-	gil18421006	40 kDa	WAHR	4	5	10%	13	35	28%	30	10	25	23%	25	5	6	13%	9	11	17	22%	26
11	dynamin, putative [Arabidopsis thalia	gil21537304	69 kDa	WAHR	11	12	21%	3	3	5,7%	3	16	19	27%	19	0	0	0	1	13	15	20%	23
12	clathrin heavy chain, putative [Arabid	gil42563757	193 kDa	WAHR	0	0	0	20	21	12%	18	18	23	11%	23	0	0	0	1	6	7	4,4%	11
13	elongation factor 1-alpha [Arabidops	gil110741201 (	49 kDa	WAHR	4	6	9,6%	2	16	5,1%	21	4	19	8,9%	25	2	2	5,3%	8	2	3	5,1%	8
14	JR1 (Jacalin lectin family protein) [Ara	gil30684083	48 kDa	WAHR	17	33	28%	11	14	24%	12	3	3	5,1%	3	3	4	10%	6	2	3	7,1%	5
15	GAPC-2; glyceraldehyde-3-phosph	gil15222848	37 kDa	WAHR	0	0	0	7	19	24%	16	9	19	33%	19	0	0	0	1	5	6	15%	9
16	jacalin lectin family protein [Arabidop	gil15225068	50 kDa	WAHR	15	35	22%	7	8	16%	7	6	6	13%	6	2	2	7,6%	3	2	3	6,1%	5
17	jacalin lectin family protein [Arabidop	gil15239241	49 kDa	WAHR	19	39	41%	8	8	15%	7	5	6	10%	6	0	0	0	1	0	0	0	1
18	myrosinase-associated protein, puta	gil15221019 (+	43 kDa	WAHR	9	16	27%	6	24	16%	21	0	0	0	1	2	3	5,9%	5	0	0	0	1
19	AAC1 (ADP/ATP CARRIER 1); ATP:AD	gil15231937	41 kDa	WAHR	5	10	14%	8	14	18%	12	3	3	7,9%	3	0	0	0	1	0	0	0	1
20	putative phragmoplastin [Arabidopsis	gil110737889	99 kDa	WAHR	0	0	0	5	5	7,4%	9	13	26	16%	59	0	0	0	1	5	7	6,8%	23
21	VPS2.2 [Arabidopsis thaliana], gil22	gil15241525	24 kDa	WAHR	4	5	19%	0	0	0	1	7	16	31%	16	0	0	0	1	3	6	20%	9
22	ACT12 (ACTIN-12); structural constitu	gil15231447 (-	42 kDa	WAHR	2	3	5,3%	2	2	5,6%	2	3	3	8,8%	3	3	3	11%	5	4	12	13%	18
23	T4D12.15 [Arabidopsis thaliana]	gil8778810	101 kDa	WAHR	0	0	0	2	2	2,0%	2	0	0	0	1	0	0	0	1	0	0	0	1
24	serine/threonine protein kinase, puta	gil15226492 (	40 kDa	WAHR	0	0	0	5	25	22%	22	5	18	22%	18	0	0	0	1	0	0	0	1
25	porin, putative [Arabidopsis thaliana]	gil15232074	29 kDa	WAHR	11	14	29%	5	7	17%	6	2	3	9,4%	3	0	0	0	1	0	0	0	1
26	jacalin lectin family protein [Arabidop	gil15228216 (-	32 kDa	WAHR	5	6	23%	7	13	23%	11	2	2	11%	2	0	0	0	1	3	4	11%	6
27	VPS46.2 [Arabidopsis thaliana], gil15	gil18410249	23 kDa	WAHR	7	14	30%	0	0	0	1	6	11	26%	11	0	0	0	1	3	3	13%	5
28	meprin and TRAF homology domain-	gil18421009	39 kDa	WAHR	7	10	19%	5	6	14%	9	2	2	8,0%	5	0	0	0	1	0	0	0	1
29	HSC70-1 (heat shock cognate 70 kD	gil15241849 (-	71 kDa	WAHR	0	0	0	0	0	0	1	6	9	11%	10	3	3	5,8%	6	7	10	12%	17
30	porin, putative [Arabidopsis thaliana]	gil15240765	30 kDa	WAHR	9	16	22%	7	9	17%	8	0	0	0	1	0	0	0	1	0	0	0	1
31	PLDALPHA1 (PHOSPHOLIPASE D A	gil15232671	92 kDa	WAHR	0	0	0	2	3	2,7%	3	3	4	3,8%	4	3	3	5,7%	5	7	10	12%	15
32	Belongs to the PF00657 Lipase/Acy	gil4587542	48 kDa	WAHR	4	5	13%	5	11	15%	9	0	0	0	1	0	0	0	1	0	0	0	1
33	SH3 domain-containing protein 1 (SH	gil15221714	49 kDa	WAHR	7	9	19%	2	3	5,7%	3	0	0	0	1	0	0	0	1	2	4	4,6%	6
34	STA1 (STARIK 1); ATPase, coupled to	gil15237155 (-	80 kDa	WAHR	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	2	5	1,5%	8
35	jacalin lectin family protein [Arabidop	gil15228199	32 kDa	WAHR	0	0	0	3	6	12%	39	3	3	9,8%	27	2	2	3,0%	39	3	3	6,4%	53
36	DEAD box RNA helicase, putative [Ar	gil15227951 (-	68 kDa	WAHR	2	2	3,3%	0	0	0	1	4	7	7,3%	7	0	0	0	1	0	0	0	1
37	SNF7.1 [Arabidopsis thaliana], gil145	gil15233464 (	24 kDa		0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	3	4	16%	6
38	putative polyubiquitin [Arabidopsis th	gil28436485 (	28 kDa	WAHR	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	3	8	14%	12
39	porin, putative / voltage-dependent a	gil15242210	29 kDa	WAHR	5	7	18%	3	4	12%	5	2	2	9,1%	2	0	0	0	1	0	0	0	1
40	ATGRP8/GR-RBP8 (COLD, CIRCADI	gil15235002	17 kDa	WAHR	0	0	0	2	2	13%	2	3	3	20%	3	2	2	21%	3	2	3	13%	5

41	VPS2.1 [Arabidopsis thaliana], gi|752	gi|18396352 (	25 kDa	WAHR	2	3	7,1%	0	0	0	1	0	0	0	1	0	0	0	1	2	2	7,1%	3
42	GOS12 (GOLGI SNARE 12); SNARE b	gi|15225431	26 kDa	WAHR	0	0	0	3	4	21%	3	2	5	14%	5	0	0	0	1	0	0	0	1
43	MEE5 (maternal effect embryo arrest	gi|15221423	110 kDa	WAHR	0	0	0	0	0	0	1	5	5	5,9%	5	0	0	0	1	0	0	0	1
44	40S ribosomal protein S14 (RPS14A) [	gi|15227588	16 kDa	WAHR	0	0	0	2	2	16%	2	3	5	23%	5	0	0	0	1	0	0	0	1
45	FIB1 (FIBRILLARIN 1) [Arabidopsis tha	gi|30696189 (	33 kDa	WAHR	0	0	0	0	0	0	1	3	3	11%	3	0	0	0	1	0	0	0	1
46	60S ribosomal protein L10 (RPL10A) /	gi|30683726	25 kDa	WAHR	0	0	0	0	0	0	1	5	7	24%	7	0	0	0	1	0	0	0	1
47	HIS4 (Histone H4) [Arabidopsis thalia	gi|15226944	11 kDa		0	0	0	0	0	0	1	2	2	21%	2	4	5	38%	8	0	0	0	1
48	ADL4/ADLP2/DRP1E/EDR3 (DYNAM	gi|18411520 (+	70 kDa	WAHR	0	0	0	0	0	0	1	4	4	6,4%	7	0	0	0	1	2	2	3,5%	6
49	meprin and TRAF homology domain-	gi|15231117	43 kDa	WAHR	3	5	9,3%	3	5	9,3%	4	0	0	0	1	0	0	0	1	0	0	0	1
50	ATNUC-L1/PARL1 (PARALLEL 1); nu	gi|15222009	59 kDa	WAHR	0	0	0	2	2	4,1%	2	3	3	6,5%	3	0	0	0	1	0	0	0	1
51	EMB1507 (EMBRYO DEFECTIVE 1507	gi|15218086	247 kDa	WAHR	0	0	0	0	0	0	1	4	4	2,3%	4	0	0	0	1	0	0	0	1
52	luminal binding protein (BiP) [Arabido	gi|1303695 (+	73 kDa	WAHR	0	0	0	4	4	6,6%	3	3	3	4,3%	3	0	0	0	1	0	0	0	1
53	RPS6 (RIBOSOMAL PROTEIN S6); st	gi|15236042	28 kDa		0	0	0	0	0	0	1	4	4	20%	4	0	0	0	1	2	3	11%	5
54	GLUC (Beta-glucosidase homolog);	gi|15228597	60 kDa	WAHR	4	4	9,4%	0	0	0	1	0	0	0	1	2	2	4,5%	3	0	0	0	1
55	Hypothetical protein [Arabidopsis tha	gi|9665102	82 kDa	WAHR	0	0	0	2	2	3,9%	2	0	0	0	1	0	0	0	1	3	4	5,6%	6
56	meprin and TRAF homology domain-	gi|18402593	43 kDa	WAHR	0	0	0	2	4	5,5%	3	0	0	0	1	0	0	0	1	0	0	0	1
57	fructose-bisphosphate aldolase, put	gi|15231715	39 kDa	WAHR	0	0	0	0	0	0	1	2	3	7,3%	3	0	0	0	1	2	2	7,3%	3
58	VPS46.1 [Arabidopsis thaliana], gi|21	gi|15220819 (-	23 kDa	WAHR	3	4	17%	0	0	0	1	2	2	12%	4	0	0	0	1	0	0	0	1
59	60S ribosomal protein L18 (RPL18C) [	gi|15241061	21 kDa		0	0	0	0	0	0	1	2	4	12%	4	0	0	0	1	0	0	0	1
60	PDC3 (PYRUVATE DECARBOXYLAS	gi|15240952	64 kDa	WAHR	0	0	0	2	2	3,4%	2	0	0	0	1	0	0	0	1	0	0	0	1
61	WD-40 repeat family protein [Arabido	gi|30695804 (	120 kDa	WAHR	0	0	0	0	0	0	1	2	2	1,9%	2	0	0	0	1	0	0	0	1
62	PIP1B (plasma membrane intrinsic pro	gi|145331417	32 kDa	WAHR	0	0	0	0	0	0	1	2	2	6,3%	2	0	0	0	1	0	0	0	1
63	nuclear transport factor 2 (NTF2) fam	gi|30697452 (	49 kDa	WAHR	0	0	0	0	0	0	1	2	2	5,0%	2	0	0	0	1	0	0	0	1
64	clathrin heavy chain, putative [Arabic	gi|30681617	193 kDa	WAHR	0	0	0	2	2	1,5%	19	2	2	1,5%	23	0	0	0	1	0	0	0	1
65	60S ribosomal protein L22-2 (RPL22	gi|15230008	14 kDa	WAHR	0	0	0	0	0	0	1	2	2	20%	2	2	2	20%	3	0	0	0	1
66	nuclear RNA-binding protein, putativ	gi|145334757	32 kDa	WAHR	0	0	0	0	0	0	1	2	2	9,0%	2	0	0	0	1	0	0	0	1
67	s-adenosylmethionine synthetase lik	gi|110740085	43 kDa	WAHR	0	0	0	2	2	6,4%	2	2	2	6,4%	2	0	0	0	1	0	0	0	1
68	PIP3 (PLASMA MEMBRANE INTRINS	gi|15236267 (	30 kDa		0	0	0	2	2	11%	2	2	2	11%	2	0	0	0	1	0	0	0	1
69	AHA1 (ARABIDOPSIS H+ ATPASE 1);	gi|15224264 (	104 kDa	WAHR	0	0	0	0	0	0	1	3	3	3,0%	3	0	0	0	1	0	0	0	1
70	ribosomal protein L9, putative [Arabid	gi|21554945	22 kDa	WAHR	0	0	0	2	2	5,7%	2	0	0	0	1	0	0	0	1	0	0	0	1
71	AtRPN1a/RPN1A (26S proteasome re	gi|18399399	98 kDa		0	0	0	0	0	0	1	2	2	2,8%	2	0	0	0	1	0	0	0	1
72	ACT8 (ACTIN 8); structural constituen	gi|15222075 (	42 kDa	WAHR	0	0	0	2	2	6,1%	3	0	0	0	1	0	0	0	1	0	0	0	1
73	Eukaryotic initiation factor 4A, putativ	gi|21555870	47 kDa	WAHR	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	2	2	5,8%	3
74	ubiquinol-cytochrome C reductase c	gi|15236811	15 kDa		0	0	0	0	0	0	1	0	0	0	1	2	2	17%	3	0	0	0	1
75	SUS2 (ABNORMAL SUSPENSOR 2)	gi|15220049 (	278 kDa	WAHR	0	0	0	0	0	0	1	3	3	1,4%	3	0	0	0	1	0	0	0	1
76	AT3g53030/F8J2_200 [Arabidopsis	gi|15010662 (-	59 kDa	WAHR	0	0	0	3	3	8,5%	3	0	0	0	1	0	0	0	1	0	0	0	1
77	HSP70B (heat shock protein 70B); A	gi|15219109	71 kDa	WAHR	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	2	3	4,3%	6
78	ATMLP-300B (MYROSINASE-BINDI	gi|15228214	32 kDa	WAHR	0	0	0	2	2	5,0%	2	0	0	0	1	0	0	0	1	0	0	0	1
79	porin, putative [Arabidopsis thaliana]	gi|15242146	30 kDa	WAHR	0	0	0	2	2	7,7%	2	0	0	0	1	0	0	0	1	0	0	0	1
80	U2A' (U2 small nuclear ribonucleopro	gi|15218274 (-	28 kDa		0	0	0	2	2	8,0%	2	0	0	0	1	0	0	0	1	0	0	0	1
81	uracil phosphoribosyltransferase, put	gi|15232108	54 kDa	WAHR	0	0	0	2	2	9,9%	2	0	0	0	1	0	0	0	1	0	0	0	1
82	PAB4 (POLY(A) BINDING PROTEIN 4	gi|15227815 (-	72 kDa	WAHR	0	0	0	0	0	0	1	2	2	3,6%	2	0	0	0	1	0	0	0	1
83	BZO2H3 (ARABIDOPSIS THALIANA	gi|30691973 (	34 kDa		2	3	17%	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
84	putative acetyl-coA dehydrogenase	gi|110739702	92 kDa	WAHR	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	2	2	3,5%	3
Hits with one specific peptide support																							
85	F-box/FBD/LRR-repeat protein	gi|22326934	At5g22610	no	54 kDa	WAHR														1	11	2%	
86	HTA6; DNA binding [Arabidopsis thali	gi|15238549	At5g59870	no	16 kDa	WAHR														1	2	6%	
87	HOG1 (HOMOLOGY-DEPENDENT G	gi|15236376 (	At4g13340	V	53 kDa	WAHR						1	2	2%									
88	dynamin-like protein B (DL1B) [Arabid	gi|30695480	At3g61760	V	68 kDa	WAHR						1	6	7%									
89	VPS60.1 [Arabidopsis thaliana]	gi|12322781 (-	At3g10640	V	25 kDa	WAHR						1	2	5%									

Supplemental Table S2 Overview of the primer pairs used for cloning of the AtVPS2.2-GFP construct and the Y2H and BiFC interaction analyses.

Primer name	Primer sequence
5g44560-5Bam	5`-AAGGATCCAAAGTGCTAGCCATCTAC
5g44560-3Bam	5`-CTGGATCCACGTATTGTGTTTGCATGAC
5g44560_GFP_SacI-pm	5`-TAGTTGAGCTCCGTTCTGTAATATTCG
5g44560_GFP_NcoI_R	5`-TATTCCATG GATCGTCGTAGCGAAGCCA
At5g44560_Y2HR	5`-CAGGATCCTCAGATTCGTCGTAGCGA
At5g44560_Y2HF	5`-CAGAATTCATGAACATTTTCGAGAAGAAG
At5g22950_Y2HR	5`-GAGGATCCTTAGGATCTAACTTTAGCGAG
At5g22950_Y2HF	5`-CACATATGGAGAGAGTGATGAACATC
At2g06530_Y2HR	5`-GACCCGGGTCACATTTTTCTAAGGTTATCC
At2g06530_Y2HF	5`-GACATATGATGAATTCAATCTTCGGAA
At1g03950_Y2HR	5`-TCGAATTCCTATCTAAGCGCCGCCAA
At1g03950_Y2HF	5`-CAGAATTCATGAACATCTTCACTAAGAAAC
At1g17730_Y2HR	5`-CTGGATCCTTAACCTCTGGCTTTAAGCT
At1g17330_Y2HF	5`-TCGAATTCATGGGTAATACAGATAAGCTG
At1g73030_Y2HR	5`-TAGGATCCTTATCCTCTGGCTTTAAGCT
At1g73030_Y2HF	5`-GAGAATTCATGGGTAACACAGATAAGC
At3g45000_Y2HR	5`-GAGGATCCCTATGATCTAACATTGGCTAG
At3g45000_Y2HF	5`-CTCATATGACTATTAAAAGTTTGCTTTCAG
At5g09260_Y2HR	5`-CACCCGGGTTCAAGCTTCCAAAGGTTCTTC
At5g09260_Y2HF	5`-GACATATGGGGAATTTATTCGTGAAG
At3g10640_Y2HR	5`-CTGGATCCTTAACCCCGGAGAGAAGCT
At3g10640_Y2HF	5`-TAGAATTCATGAGGAGAGTTTTCGGCGCGA
At5g63880_Y2HR	5`-CTGGATCCTCAAGCCGGCAAACCTTC
At5g63880_Y2HF	5`-CAGAATTCATGGGGAATTTGTCGTGAAG
At4g29160_Y2HR	5`-TAGGATCCTTAGAGGGCCATCTC
At4g29160_Y2HF	5`-TAGAATTCATGATGAATCGGCTATTCGA
At2g19830_Y2HR	5`-GAGGATCCTTAGAGAGCCATCTCAGCT
At2g19830_Y2HF	5`-CTCATATGTTTATGAATCGGCTATTCGGGA
At5g04850_Y2HR	5`-CAGGATCCTTAGCCCCGAAGTGATGCA
At5g04850_Y2HF	5`-CAGAATTCATGAAGAGAATCTTTGGAGCGA
5g44560_BamHI_15_start	5`-CGCCACTAGTGGATCCATGAACATTTTCGAGAAG
5g44560_XmaI_15_end	5`-ATGGGTACATCCCGGGGATTCGTCGTAGCGAAGC
1g73030_BamHI_15_start	5`-CGCCACTAGTGGATCCATGGGTAACACAGATAAGC
1g73030_XmaI_15_end	5`-ATGGGTACATCCCGGGTCCTCTGGCTTTAAGCTC
1g17730_BamHI_15_start	5`-CGCCACTAGTGGATCCATGGGTAATACAGATAAGCTG
1g17730_XmaI_15_end	5`-ATGGGTACATCCCGGGACCTCTGGCTTTAAGCTCG
4g29160_BamHI_15_start	5`-CGCCACTAGTGGATCCATGATGAATCGGCTATTC
4g29160_SalI_15_end	5`-TACCCTCGAGGTCGACGAGGGCGATCTCAGCCT