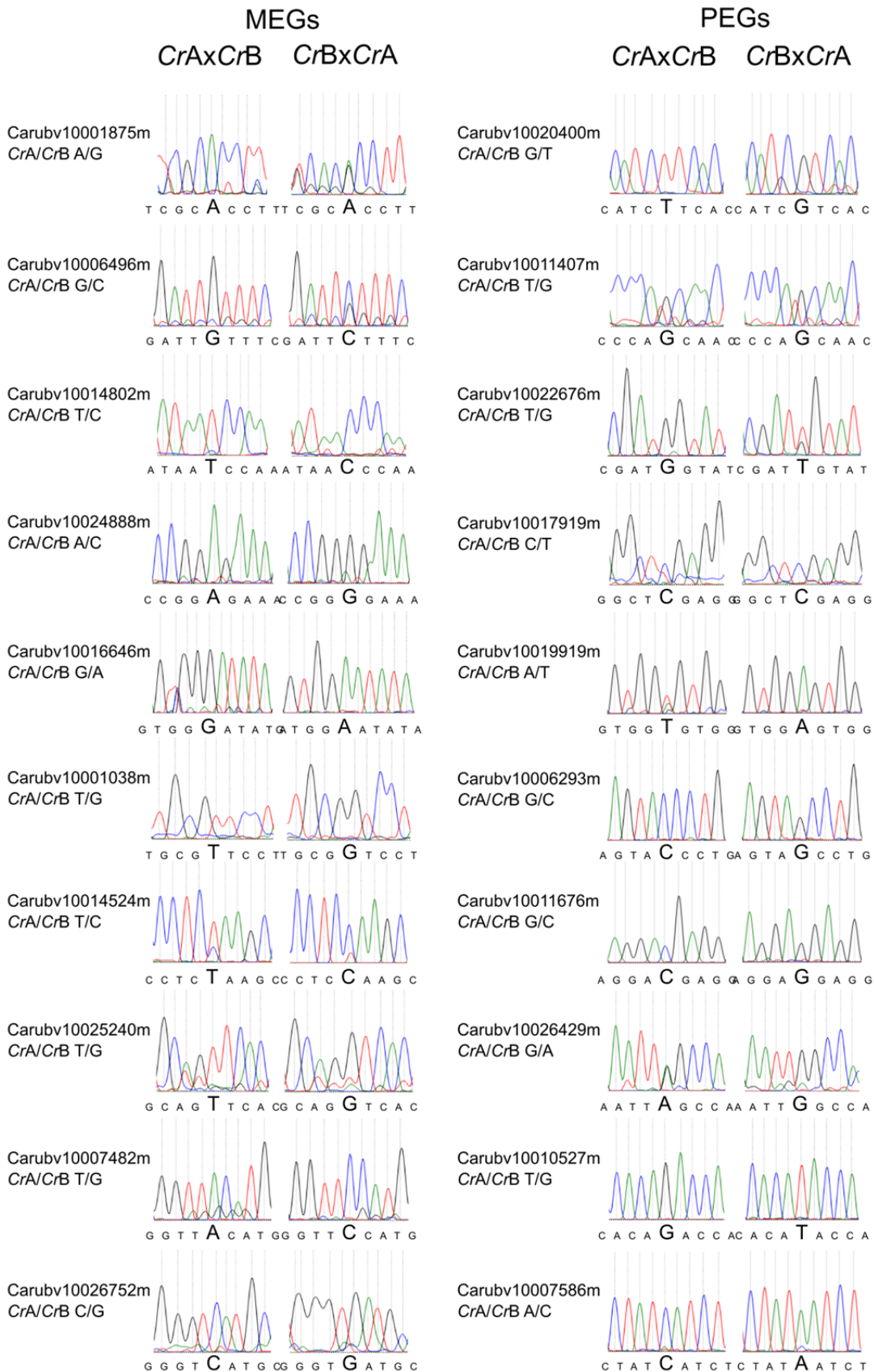
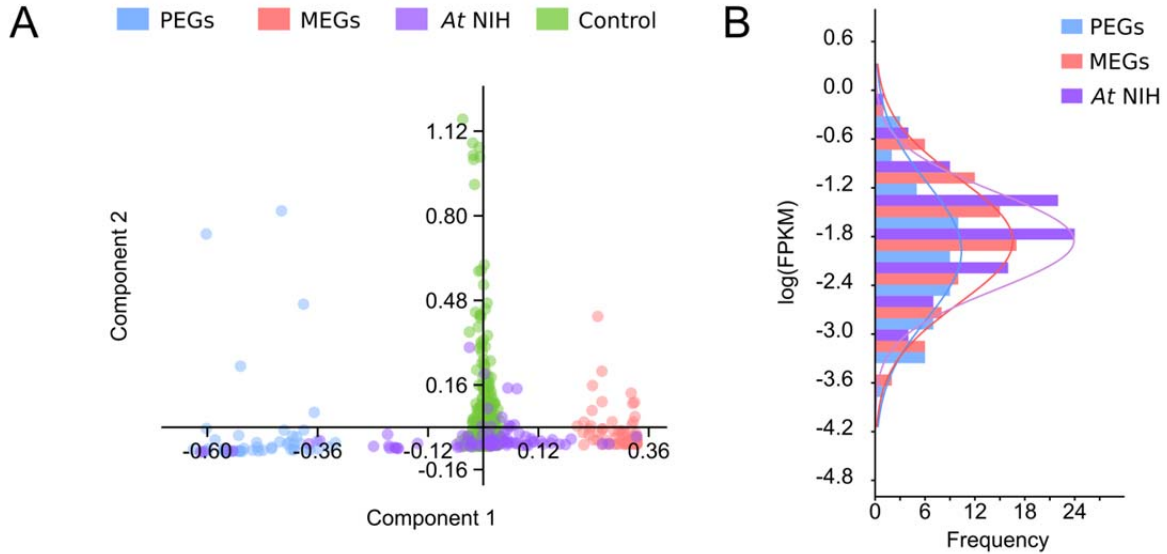


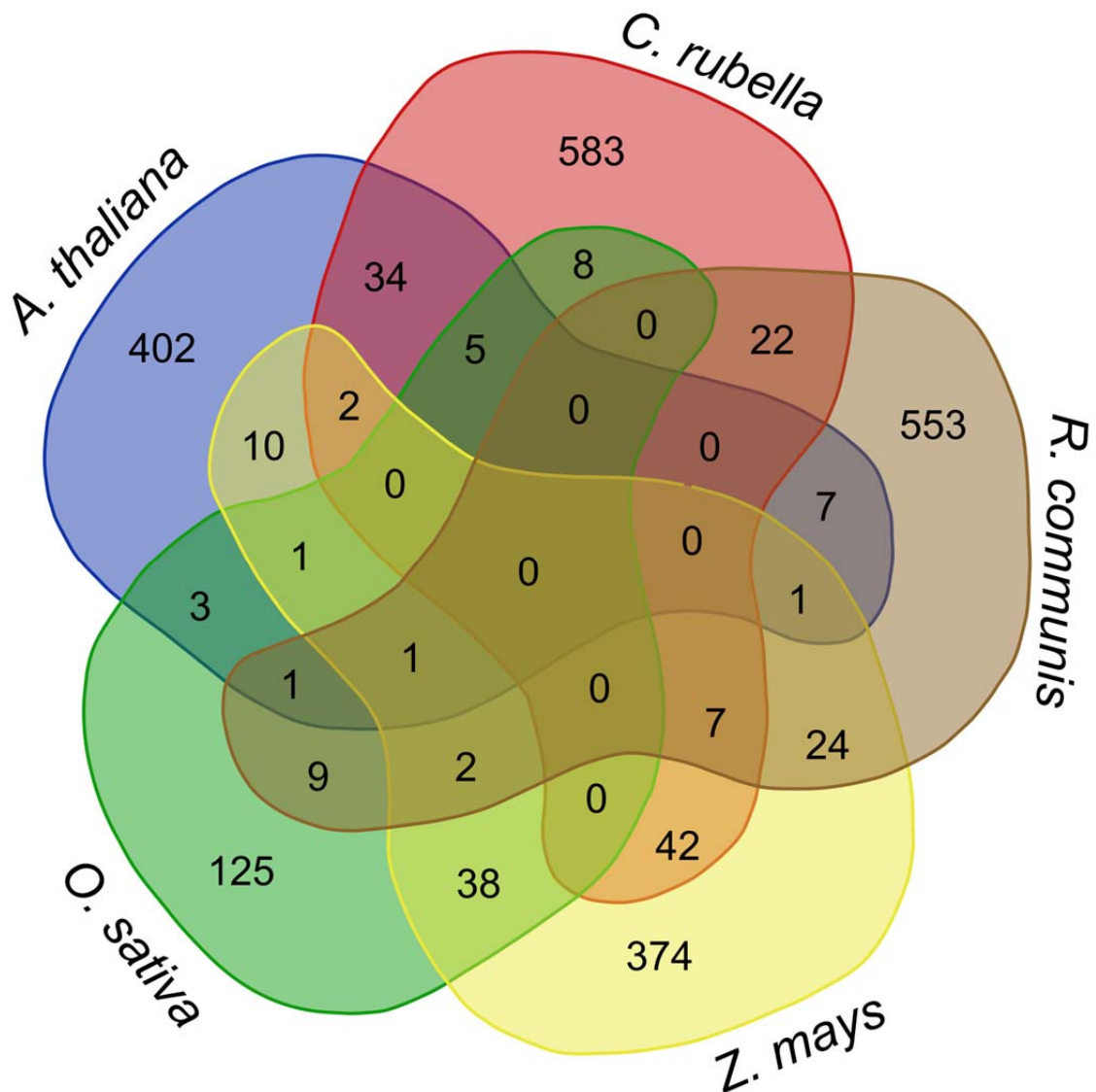
Supplemental Figure 1. Analysis of hybrid seed development. (A) Percentage of seeds at defined developmental stages at 6 days after pollination (DAP). **(B)** Cleared seeds at the indicated developmental stages. **(C)** Germination of hybrid seeds derived after reciprocal crosses.



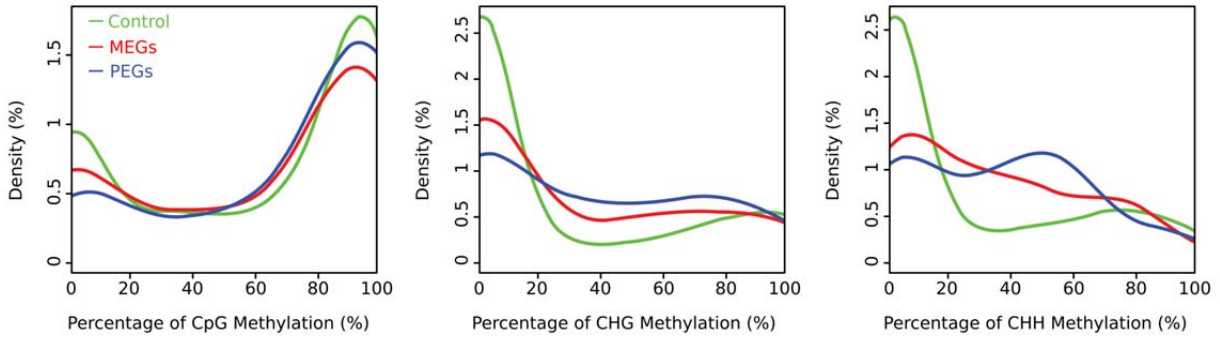
Supplemental Figure 2. Confirmation of 10 selected MEGs and PEGs by Sanger sequencing of RT-PCR products. The Sanger sequences of RT-PCR products of 10 randomly selected MEGs PEGs confirm the next generation sequencing results. The first two MEGs are imprinted only in one direction of the cross.



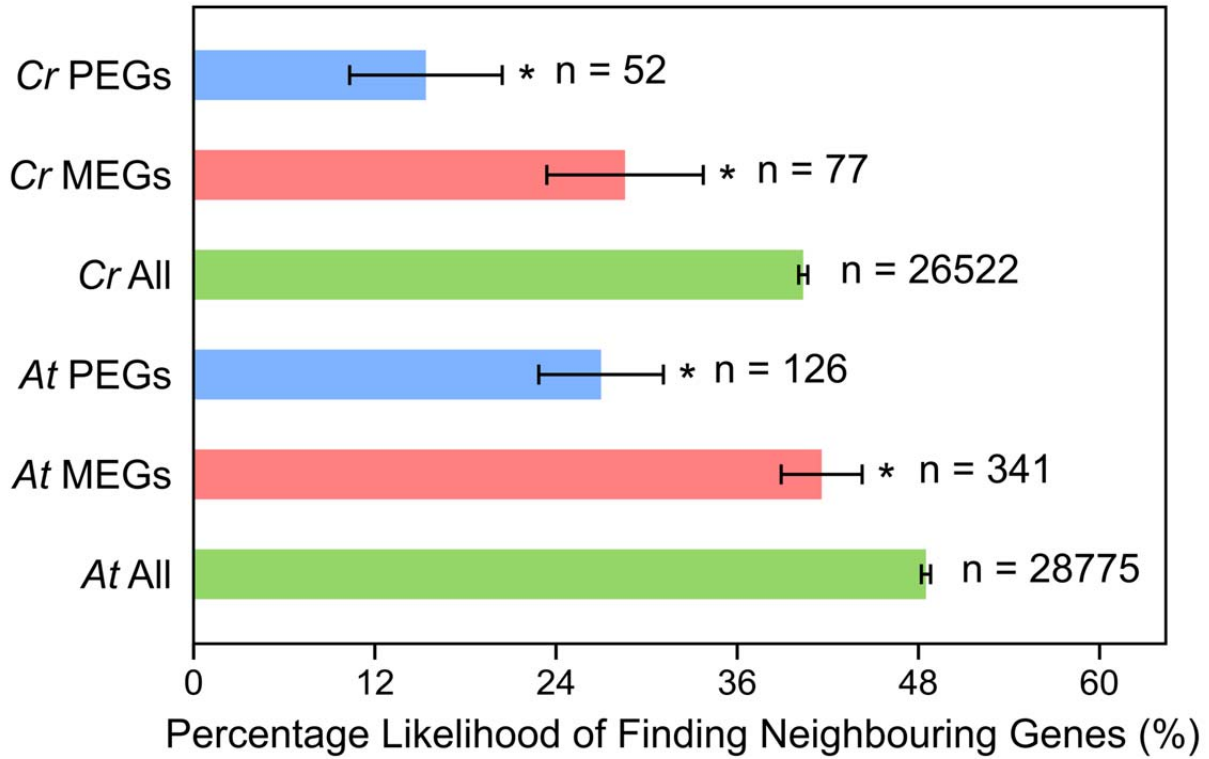
Supplemental Figure 3. Principal Component Analysis (PCA) scatter plot for different categories of imprinted and non-imprinted genes in *C. rubella*. (A) PCA using maternal informative allele to total informative allele ratio (M/T) and FPKM data for each gene. At NIH: Homologs of imprinted *A. thaliana* genes that have been classified as non-imprinted in *C. rubella*, Control: Non-imprinted control. (B) log expression level (FPKM) of different categories of genes. At NIH: Homologs of imprinted *A. thaliana* genes that have been classified as non-imprinted in *C. rubella*. Expression level of all genes in these three categories is not significantly different (Mann-Whitney pairwise test, $p\text{-val} > 0.10$).



Supplemental Figure 4. Cross comparison of imprinted genes in five different species. Gene sets used in the comparison are limited to those genes where homologs have been identified in *A. thaliana* (blastn, e-value 1e-6). All homologs with a maximum e-value of 1e-6 were included in the comparison. Data of imprinted genes in different species are derived from the following sources: *A. thaliana* (Hsieh et al., 2011; Wolff et al., 2011; Gehring et al., 2011; Pignatta et al., 2014), rice (Luo et al., 2011), maize (Waters et al., 2013), and castor bean (Xu et al., 2014).



Supplemental Figure 5. Frequency distribution of methylated regions within flanking regions of imprinted genes compared to control genes. Flanking regions (1 kb) of MEGs, PEGs and endosperm expressed control genes were binned (100 bp) and the methylation level in each sequence context was determined for each bin. Only bins with at least five cytosines were considered. The bin with the highest methylation value for each gene was used to calculate the frequency methylation distribution. Genes in the control group were selected based on the presence of SNPs and detectable expression in the endosperm.



Supplemental Figure 6. Likelihood of genes being located within 500 bp proximity of MEGs, PEGs or all genes of *C. rubella* (*Cr*) and *A. thaliana* (*At*). Whiskers represent one standard deviation. Asterisks mark significantly decreased likelihood (hypergeometric test, $p\text{-val} < 0.05$) of finding genes in the proximity of imprinted genes compared to all genes of the corresponding species.

Supplemental Table 1. Properties of sequencing libraries used in the experiment.

Genomic Library	Mapped Reads to the Reference	Average Coverage	Reads Mapped to Annotated Genes
Cr A	60,952,946	43.22	33,330,305
Cr B	45,540,269	32.45	24,498,833
Transcriptomic Library	Mapped Reads to The Reference	Average Reads Mapped per Genes	Reads Mapped to Annotated Genes
Cr Whole Seed RNA Control*	65,672,150	845.94	61,978,839
Cr A x Cr B Rep 1	118,995,845	1797.37	101,109,365
Cr B x Cr A Rep 1	119,297,773	1868.99	108,871,275
Cr A x Cr B Rep 2	137,143,848	1668.66	127,591,292
Cr B x Cr A Rep 2	135,108,495	1651.77	125,937,105

* from replicate 1 Cr x Cr (Rebernig et al., 2015).

Supplemental Table 2. Enriched GO categories of conserved imprinted genes in *C. rubella* (Cr) and *A. thaliana* (At)

*: p-values (p-val) have been corrected using Benjamini-Hochberg method.

GO Category	Description	Cr. p-val*	At. p-val*
Conserved MEGs			
GO:0045449	P regulation of transcription	0.0094	0.002
GO:0019219	P regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	0.0094	0.002
GO:0031323	P regulation of cellular metabolism	0.0094	0.002
GO:0003700	F transcription factor activity	0.0094	0.002
GO:0006350	P transcription	0.0094	0.002
GO:0019222	P regulation of metabolism	0.0094	0.002
GO:0051244	P regulation of cellular physiological process	0.0102	0.0026
GO:0050794	P regulation of cellular process	0.0102	0.0026
GO:0030528	F transcription regulator activity	0.0102	0.0026
GO:0050791	P regulation of physiological process	0.0102	0.0026
GO:0050789	P regulation of biological process	0.0149	0.0043
Conserved PEGs			
GO:0008757	F S-adenosylmethionine-dependent methyltransferase activity	0.0406	0.0087
GO:0006259	P DNA metabolism	0.0406	0.0018
GO:0006325	P establishment and/or maintenance of chromatin architecture	0.0406	0.0018
GO:0006323	P DNA packaging	0.0406	0.0018
GO:0007001	P chromosome organization and biogenesis (sensu Eukaryota)	0.0406	0.0018
GO:0051276	P chromosome organization and biogenesis	0.0406	0.0018
GO:0008168	F methyltransferase activity	0.0406	0.0249
GO:0016741	F transferase activity, transferring one-carbon groups	0.0406	0.0249

Supplemental Table 3. Primer sequences used for validation of imprinted genes.

All primer pairs were designed to amplify a single locus.

Gene ID	Homolog	SNP Position	Cr A Genotype	Cr B Genotype	Forward Primer	Reverse Primer
MEGs tested for Sanger sequencing validation						
Carubv10001875m	AT4G00220.1	16515343	TT	CC	ATGAGCGGCAGCGGA ATAAACCC	GAGACGCAGCCGTAGATAG G
Carubv10006496m	AT4G17870.1	8892373	GG	CC	ATGCCTTCGGAGCTAA CACC	AATTCGTGAGCCTGTGCTCT
Carubv10014802m	AT2G04240.2	9694159	TT	CC	CAGAAGACCACCAGC TTTCTC	CAAATAACTCTCTTTCTTGC AC
Carubv10024888m	AT2G34880.1	9637481	TT	CC	ATCGAAATTTCAAAGA CGCCTA	ATGTTTTCTCATCGCCCTCTC
Carubv10016646m	AT3G47950.1	7747627	CC	TT	TATACCTGCAAGTCAG TATTATCAG	GACCATGGAGCGTTCTCTGT GCG
Carubv10001038m	AT4G09650.1	12711790	TT	GG	CAAACACTACTGCATCT GGTTC	CATGTGCTTGCCATTAGCAG
Carubv10014524m	AT3G14770.1	5063987	AA	GG	CTGGTCATGATCGAAG GATGG	TAAAACCGCGATGAAAGGT C
Carubv10025240m	AT2G23580.2	1179435	AA	CC	AAAGGTTTGTGCTCGT CCAT	CTAGAGGGCGATCAGGTGT C
Carubv10007482m	AT5G40430.1	16106240	TT	GG	AAAACGTGGGCTACTC TCGATG	TCATCATCATCCACGGGTTT
Carubv10026752m	AT5G61430.1	10979408	GG	CC	AATCGCTTGTGGGAT GAAG	GAGAAGCAGGGCACGTAGA C
PEGs tested for Sanger sequencing validation						
Carubv10020400m	AT1G70560.1	10073861	CC	AA	CGCTGATTTCACTCTC ACCA	ATGATCTAGAGGTCAATGCT TC
Carubv10011407m	AT1G17770.1	6158407	TT	GG	CTCATTTGTTTCGGGT GCTGG	GTCCTGCAATTAGCCTCAGC
Carubv10022676m	AT2G32370.1	8528371	AA	CC	GAATACTGAACGTAC GATCG	ACAGCTTCTCGGAGCCCTAT G
Carubv10017919m	AT3G29575.4	3677370	GG	AA	TGAGTAGGACTTGTTT GATGC	CCAGAGGAACCACTTCCAG A
Carubv10019919m	AT5G39550.1	7693760	TT	AA	TGTTCAAGTTCCTTGC GACG	CAGTCAAAGTCACATCAGCC TC
Carubv10006293m	AT4G31900.2	3284332	CC	GG	TTGCTGAGGCAGAAC ATGAC	CAGCTACAAAGCTCGCTCCT
Carubv10011676m	AT1G28370.1	9749192	CC	GG	TCAACAACAACAACCT CGGA	TAGTTCTCAGGTGGAGGAG GG
Carubv10026429m	AT5G57180.3	9331482	CC	TT	GTCTTCCCAAACCCA ATTAG	TTCCTCGTTGAGATTGCGAT GGATG
Carubv10010527m	AT1G32540.3	11453189	AA	CC	GTACAAGTGGACAGA GCCAGTTAG	TCACATCAGGAGTCGTGCTC
Carubv10007586m	AT4G14580.1	10545469	AA	CC	TATACTGCACCGGAAG TGATTG	ATCAGACCGAACCTCAAGA G