

Supplementary Data**Discrimination among rice varieties based on rapid detection of single nucleotide polymorphisms by a newly developed method, mass spectrometric cleaved amplified polymorphic sequence (MS-CAPS) analysis****Hideyuki Kajiwara^{1*}, Masayuki Yamaguchi², Hideki Sato², Hiroyuki Shibaie³**

Table S1. Applicability of MS-CAPS analysis. Four cases are considered. In case 1, the cultivars are distinguished by the presence or absence of a PCR product. In case 2, the cultivars are distinguished by the presence or absence of products after digestion with a restriction enzyme. In both cases, CAPS analysis using gel electrophoresis shows different bands. In case 3, if there is more than one nucleotide inserted/deleted, the peak obtained in the analysis would reflect a mass difference of greater than 330, because the average molecular weight of a nucleotide is approximately 330. Only large insertions/deletions can be detected by CAPS analysis. In case 4, if products obtained after PCR and enzymatic reactions differ in composition, the difference can be detected as a mass difference by MS-CAPS analysis (Table S2), but not by conventional CAPS analysis. To show differences between cultivars, suitable enzymes must be considered when designing primers.

Case	Difference between cultivars	Observation in MS-CAPS
1	Sequence amplified or not amplified by PCR	Presence or absence of peak
2	Amplicon digested or not digested by enzyme	Presence or absence of peak
3	Amplicons differ in length because of insertion or deletion of nucleotide(s)	Peak differences reflect a mass difference of approx. 330 per nucleotide
4	Amplicons differ in nucleotide composition	Differences of peaks reflect mass differences ranging from 9.02 to 40.00.

Table S2. Mass differences between nucleosides. Mass of mono isotope was used for calculation. Although dUTP was used in PCR amplifications when UDG was going to be used in MS-CAPS analysis, it was removed by UDG digestion.

Nucleoside (Molecular weight)	Adenine (135.13)	Cytosine (111.10)	Guanine (151.10)	Thymine (126.11)
Adenine	0.00	24.03	-15.97	-9.02
Cytosine	-24.03	0.00	-40.00	-16.00
Guanine	15.97	40.00	0.00	24.99
Thymine	-9.02	16.00	-24.99	0.00