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Two new PCR based polymorphic markers in bin 5.09

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In our effort to positionally clone a gene located near the telomere of the long arm of chromosome 5, we have developed two PCR based co-dominant polymorphic markers in bin 5.09. Figure 1 shows CDPK DEL, a marker derived from a 21 bp deletion found in a putative calcium dependent protein kinase (Gen Bank Accession DV541158) located at Sbi.0.23295 in sorghum and LOC_Os02g58520 in rice. Figure 2 shows BZIP DEL, a marker derived from a 24 bp deletion found in a putative bZip transcription factor (Gen Bank Accession CK370734) located at Sbi.0.23306 in sorghum and LOC_Os02g58520 in rice. PCR products for both

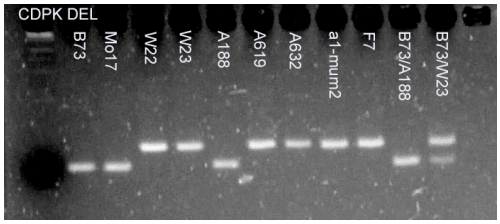

Figure 1. CDPK DEL.

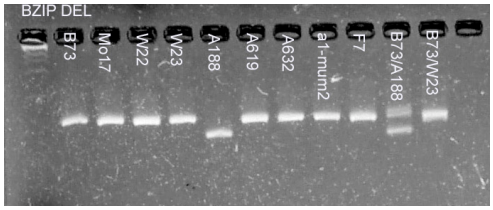

Figure 2. BZIP DEL.

markers are ~150 bp for the larger and ~130 bp for the smaller fragment and can be clearly resolved on a 4% APEX gel. In both figures several common inbreds, the *a1-mum2 Mutator* minimal line and two equal mixtures of DNA demonstrate the markers are polymorphic and co-dominant.

Primers:

CDPK DEL forward
TGATCCCAGGCCCAGCGATGC

CDPK DEL reverse
CGACAGGGCGATGCTGTTGCTGCTG

BZIP DEL forward
CAGCTGAGCCTGAGCGGCTGCAGC

BZIP DEL reverse
CGCCGAGCGTGAGCGACAGGAGAGG

PCR Conditions: Both use standard PCR reaction mixture with the addition of glycerol to a final concentration of 6% and DMSO to a final concentration of 3%.

Thermal Cycler Program:

1) 94ºC	2 min	1 cycle
2) 94ºC	30 sec	
3) 66ºC	30 sec	
4) 72ºC	30 sec	repeat 2-4 for 35 cycles
5) 72ºC	5 min	1 cycle