Two new PCR based polymorphic markers in bin 5.09
--Slewinski, TL; Braun, DM

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.](image1)

![Figure 2. BZIP DEL.](image2)

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
TGATCCAGGCCCAGCGATGC

CDPK DEL reverse
CGACAGGGCGATGCTGTTGCTGCTG

BZIP DEL forward
CAGCTGAGCCTGAGCGGCTGCAGC

BZIP DEL reverse
CGCCGAGCGCTGAGCGACAGGAGAGG

**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:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94°C</td>
<td>2 min</td>
<td>1 cycle</td>
</tr>
<tr>
<td>2</td>
<td>94°C</td>
<td>30 sec</td>
<td>1 cycle</td>
</tr>
<tr>
<td>3</td>
<td>66°C</td>
<td>30 sec</td>
<td>1 cycle</td>
</tr>
<tr>
<td>4</td>
<td>72°C</td>
<td>30 sec</td>
<td>repeat 2-4 for 35 cycles</td>
</tr>
<tr>
<td>5</td>
<td>72°C</td>
<td>5 min</td>
<td>1 cycle</td>
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