



**Supplemental Figure 3:** Comparing the locus structure between primary transformants (T1), selected on hygromycin, and marker-free progeny plants *via* Southern blot analysis. Panel A: Original allele: theoretical length of the band indicative for a direct repeat with nptII-2 (▨) and hpt (▨) probe: 3,085 bp; for an inverted repeat (RB) with the nptII-2 probe: 2,630 bp; for an inverted repeat (LB) with the hpt probe: 3,540 bp. DNA from primary transformants was digested with *SspI* and probed with nptII-2 probe to obtain information around RB. The probe was washed away and the blot was reprobed with hpt probe to obtain information around the LB. Lane 1: SDS-1-2; lane 2: SDS-1-14; lane 3: SDS-1-21. Panel B: Recombined allele: theoretical length of the band indicative for a direct repeat 1,641 bp; for an inverted repeat (RB) digested with *SspI*: 2,630 bp; for an inverted repeat (LB) digested with *BstNI*: 1,862 bp. DNA from marker-free T4 plants was the same as used in the Southern blot analysis displayed in figure 5. To obtain information around the RB, DNA was cut with *SspI* and probed with nptII-2 probe. For obtaining information around the LB, DNA was digested with *BstNI* and probed with nptII-2 probe. Lane 4: SDS-1-2-H3-FC1; lane 5: SDS-1-14-H5-FC2; lane 6: SDS-1-21-H5-FC1. P\*: germline-specific promoter (AP1 or SDS).