

1. TAIL PCR

TAIL PCR first round

Stage 1 (x1 cycle)	95 °C 3 min
Stage 2 (x5 cycles)	95 °C 30 sec, 62 °C 1 min, 72 °C 30 sec
Stage 3 (x1 cycle)	95 °C 3 sec, 25 °C 3 min, * 72 °C 30 sec
Stage 4 (x15 cycles)	95 °C 10 sec, 68 °C 1 min, 72 °C 30 sec 95 °C 10 sec, 68 °C 1 min, 72 °C 30 sec 95 °C 10 sec, 44 °C 1 min, 72 °C 30 sec
Stage 5 (x1 cycle)	72 °C 5 min

*10% ramp (~0.34 °C per second)

TAIL PCR second round

Stage 1 (x1 cycle)	95 °C 3 min x1 cycle
Stage 2 (x5 cycles)	95 °C 10 sec, 64 °C 1 min, 72 °C 30 sec
Stage 3 (x15 cycles)	95 °C 10 sec, * 64 °C 1 min, * 72 °C 30 sec 95 °C 10 sec, * 64 °C 1 min, * 72 °C 30 sec 95 °C 10 sec, * 44 °C 1 min, * 72 °C 30 sec
Stage 4 (x5 cycles)	95 °C 10 sec, 44 °C 1 min, 72 °C 2 min, 72 °C 30 sec
Stage 5 (x1 cycle)	72 °C 5 min

*30% ramp (~1 °C per second),

Adapted from Liu et al (1995)

2. Amplification of sgRNA insert in T-DNA

Stage 1 (x1 cycle)	95 °C 5 min
Stage 2 (x35 cycles)	95 °C 30 sec, 52 °C 15 sec, 72 °C 10 sec
Stage 3 (x1 cycle)	72 °C 5 min

3. Amplification of Nos-Kan insert in T-DNA

Stage 1 (x1 cycle)	95 °C 5 min
Stage 2 (x35 cycles)	95 °C 30 sec, 46 °C 15 sec, 72 °C 30 sec
Stage 3 (x1 cycle)	72 °C 5 min

4. Amplification of amplicons for NGS sequencing

Stage 1 (x1 cycle)	98 °C 30 sec
Stage 2 (x35 cycles)	98 °C 10 sec, 65 °C 30 sec, 72 °C 12 sec
Stage 3 (x1 cycle)	72 °C 2 min

Table S6. PCR cycles used for analysis of plant samples. Reaction volumes: 1=20 µl; 2,3 = 25 µl; 4 = 50 µl.