

slc4a1^{tr217}**Nature of the mutation**

The *tr217* allele contains a single T-to-A point mutation that creates a new canonical splice acceptor site (Paw et al., Nature Genetics 34: 59-64, 2003).

Genotyping assay

Genotyping of the *tr217* allele is based on the dCAPS assay (**d**erived **C**leaved **A**mplified **P**olymorphic **S**equences; Neff *et al.*, The Plant Journal 14(3): 387-392, 1998). In this assay, a restriction enzyme recognition site that includes the single nucleotide polymorphism (SNP) is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The PCR product modified in this manner is then subjected to restriction enzyme digestion and the presence or absence of the SNP is determined by the resulting restriction pattern.

To genotype the *tr217* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *tr217* mutation create a BfaI restriction enzyme site in the amplified product. The BfaI site is not present in the PCR product derived from the WT DNA template.

Primers:

SLC_09d: 5' TGC TGG TTA AAT GTG ATC TTG **CT** 3'

SLC_12: 5' AGA AAA AGT TAA ATG ACT TCC TTC CA 3'

PCR program (55_30_30):

1. 94°C for 3 min
2. 94°C for 30 sec
3. 55°C for 30 sec
4. 72°C for 30 sec
5. Go to step 2 (above) for 39 cycles
6. 72°C for 5 min
7. 8.0°C hold
8. END

Product size: 209 bp

Digestion of the PCR product with the BfaI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	209 bp
PCR product containing the mutation	cleaved	187 bp and 22 bp

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