Supplementary Information File 4. Description sheet of the genotyping assay for the *SLC12A6* NC_006612.3(XM_014109414.2): c.178_181delinsCATCTCACTCAT variant.

Description:

The wild type (wt) allele contains "ATGA", while the variant type (vt) allele contains "CATCTCACTCAT". The PCR assay uses 3 primers to simultaneously amplify both alleles (primer sequences below). The forward Fwt-primer contains "ATGA" (in bold) and can only bind to the wt-allele. The forward Fvt-primer contains "CATCTCACTCAT" (in bold) and can only bind to the vt-allele. It also contains a 16-bp tail at its 5'-side (in italic) to enlarge the fragment of the vt-allele. The reverse R-primer binds to both alleles. The resulting fragments of 126 bp (wt) and 157 bp (vt) can be amplified by PCR (conditions below) and easily distinguished from each other after agarose gel electrophoresis (gel picture below).

Primer sequences (IDT, Leuven, Belgium):

Cfam*SLC12A6*_Fwt: 5'-CCGCAGTGAGCCT**ATGA**GTGAGATGTCTGGAG-3'
Cfam*SLC12A6*_Fvt: 5'-*TAAATATAATAAATAT*AAACAAGCCGCAGTGAGCCT**CATCTCACTCAT**-3'
Cfam*SLC12A6*_R: 5'-CCTCCGTCTTTCTACTCACCCTCGGTAACGTC-3'

PCR mix (VWR International, Oud-Heverlee, Belgium):

5.7 µl H_2O
1.0 µl 10x Key buffer
1.0 µl Primer mix (5 µM each primer)
0.2 µl dNTPs (10 mM each nucleotide)
0.1 µl TEMPase HS DNA Polymerase
2.0 µl DNA (10 ng/µl)
10.0 µl Total volume

PCR program (S1000 thermal cycler, Bio-Rad Laboratories, Nazareth, Belgium)

14'40" - 95°C
00'20" - 95°C]
00'20" - 70°C] x30
00'40" - 72°C]
02'00" - 72°C
HOLD – 15°C

Gel picture (3% agarose, Gentaur, Kampenhout, Belgium)

L: Hyperladder V (Gentaur, Kampenhout, Belgium)
NTC: no template control