

Supplement 15

Primersequences / Methods

Generation of variants for in vitro analysis

QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent #210518) was used to generate variants from IMAGE clone 3918690 using following primers:

| variant | forward primer | reverse primer |
|-----------|-------------------------------------|-------------------------------------|
| c.709G>A | acagaaaactgcacatctcaccgagatgaggatgg | ccatcctcatctcggtgagatgtgcagttttctgt |
| c.893T>C | caggccctacctcagaagcaggatgcttgttttca | tgaaaacaagcatcctgcttctgaggtagggcctg |
| c.1321A>C | ctccatttcttcgcctgctgttcaccttctttgg | ccaaagaaggtgaacagcagggcgaagaaatggag |

Cloning for cell culture experiments

N-terminally Flag tagged wild type (WT) and mutant SLC20A1 cDNA of human origin were cloned into pcDNA3 plasmid backbone (Clontech) using the following primers:

forward:
5' ATCTAAGCTTGCCACCATGGACTACAAAGACGATGACGACAAGATGGCAACGCTGATTACCAGTACTACAG 3'
reverse:
5' ATCTGGGCCCTCACATTCTGAGGATGACATATC 3';

Whole-mount Zebrafish In Situ Hybridization (WISH)

cDNA plasmids for the preparation of antisense and sense probes for pax2a, evx1, slc20a1a, and slc20a1b were generated by PCR from zebrafish poly-T embryonic cDNA using the following primers:

| gene | forward primer | reverse primer |
|----------|--------------------------------------|------------------------------------|
| pax2a | CATCATgtcgcagcgttctaacaggcacatcc | CATCATgcggccgcgatcgctatccgttcaaagc |
| evx1 | CATCATctcaggacaagagacagcgtctggc | CATCATggatccggtccgtgtcctgttaaaatgg |
| slc20a1a | CATCATctcaggaaagctgagcgttcattgttactg | CATCATgagctcccgaacggatggtttcgctga |
| slc20a1b | CATCATctcgcagacctgcaaccaggatccaaga | CATCATgagctcggagacagaacgtctgaactc |

Re-sequencing of SLC20A1 in Individuals with BEEC

Primers used for sequencing of SLC20A1:

| name | primer sequence | name | primer sequence |
|------------|--------------------------|-------------|-------------------------|
| SLC20A1-2F | GCTCGGTTTCTGTGCCGTAG | SLC20A1-7R | AAGCCTCCTCACTACTGCAG |
| SLC20A1-2R | TTATGCACAAAATGGAACCAACC | SLC20A1-8F | TACCTGCAGTGGTGAGGAGG |
| SLC20A1-3F | GGGTGAGCAAATTTGTGTCATGG | SLC20A1-8R | TACCTGCAGTGGTGAGGAGG |
| SLC20A1-4R | GGCCGCATACCTATACACCAAT | SLC20A1-9F | TTCTGTTGTGTATGTTGGGGTTC |
| SLC20A1-5F | ATTGGCACTATAATTCCCAAACC | SLC20A1-9R | GTCTGACCAAAATGTTTAAGGGC |
| SLC20A1-5R | AGCGTACACTAGCCTGCAGC | SLC20A1-10F | AGGAAACTGGGCATCTGTGG |
| SLC20A1-6F | GTCCCCAGAGGAGGGTTTAC | SLC20A1-10R | ACCATTTGAAAGACACACATTGG |
| SLC20A1-6R | TAAAAACAGTTTGGACAACAAACC | SLC20A1-11F | TCTTGTCCAGGGGTCTGGG |
| SLC20A1-7F | GGTGGAGGAAAGGAGACGTG | SLC20A1-11R | GGGGACCTCAGCAGGAGC |