Genotyping *Slco1c1-BAC-CreERT2*

(*organic anion transporter 1c1; Oatp14; Slc21a14; solute carrier organic anion transporter family, member 1c1; Slco1c1; Slco1c1-CreER^{T2}; Tg(Slco1c1-icre/ERT2)^{1Mrks}*)

JDW 7/18

MGI: 5301361

Reference: TAK1 in brain endothelial cells mediates fever and lethargy. Ridder DA, Lang MF, Salinin S, Röderer JP, Struss M, Maser-Gluth C, Schwaninger M. J Exp Med, 2011. PMID: 22143887

Note: A BAC (RP24-85B20) that harbors the mouse Slco1c1 locus with an 80-kb 5'-upstream and an 81-kb 3'-downstream region was modified by homologous recombination to insert a cassette encoding a codon-improved Cre recombinase (iCre), a mutated ligand-binding domain of the human estrogen receptor (ERT2), and an ampicillin resistance cassette flanked by two FRT sites. The construct was linearized and electroporated into heat-induced EL250 bacteria harboring the BAC. Clones with the recombinant BAC were induced with l-arabinose to express FLP recombinase, which resulted in deletion of the ampicillin resistance cassette. The modified genomic fragment containing the iCreERT2 knockin at the ATG of the Slco1c1 gene was separated from the BAC backbone by NotI digestion and subsequent purification with Sepharose CL4b Column (GE Healthcare). The DNA was microinjected into the B6D2F1 hybrid mouse pronuclei. Transgenic offspring was identified by genotyping PCR with following primers: Rec1_UB5, 5'-CTCGAGGAAGTTCCTATTCTC-3'; and Rec1_DB3, 5'-TCTCTGTCTCCTCTGCTTATC-3'.

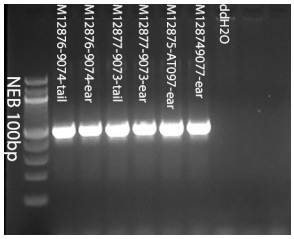
Primers:

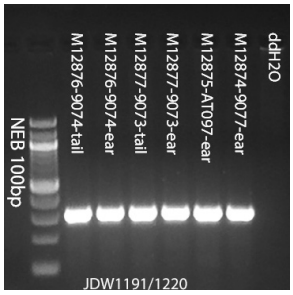
JDW 1190 (Fwd1, in Slco1c1 promoter):	5' GCTATTCATGTCTTGGAAGCC
JDW 1191 (Fwd2, in Slco1c1 promoter):	5' TAGGGTCTCGATGGCAGGATTCG
oJDW 1220 (REV1, unclear where this is):	5' CAGGTTCTTCCTGACTTCATC

= 550 or 350 bp (oJDW 1190+1220 = ~550 bp; oJDW 1191 + 1220 = ~350 bp)

**Internal control primers that target murine IL2 (can multiplex with above 1190/1220 primers):
JDW 565 (oIMR7338): 5' CTA GGC CAC AGA ATT GAA AGA TCT
JDW 566 (oIMR7339): 5' GTA GGT GGA AAT TCT AGC ATC ATC C

= 324 bp





Reaction Conditions:

10x CL buffer	(Qiagen)	2.5µl
Q solution	(Qlagen)	2.5µl
dNTPs	(10mM each stock)	0.5µl
Sclo1c1-FWD	(20mM stock)	0.5µl
Cre2-REV	(20mM stock)	0.5µl
DNA		1µl
Taq	(Qiagen)	0.25µl
ddH_2O		17.25µl

95ºC – 3 minutes
95ºC – 30 seconds
56ºC – 30 seconds | **X** 30 Cycles
72ºC – 45 seconds
72ºC – 5 minutes
16ºC – forever

From: Endothelial LRP1 transports amyloid-β_{1-42} across the blood-brain barrier. Storck SE et al., JCI, 2015.

Note that these FWD primers do not work with usual CreER reverse primer,
JDW 83 (Cre2-Rev): 5' CCTGTTTTGCACGTTCACCG

Also, our Cre generic primers (from the Groves lab protocol), do not amplify from Cre cassette, suggesting some sort of codon optimization that diminishes hybridization by the primers.