

Supplemental Figure 1

Cloning and sequencing of the splice region of ABCA4 hybrid dual vectors

The retinas of mice injected with ABCA4 hybrid vectors were harvested, the RNA extracted (RNeasy, 74104, Qiagen, Hilden, Germany) and reverse transcribed (Superscript III First-Strand Synthesis system, 18080-051, Invitrogen,). The region in which splicing is expected to occur was amplified by PCR with primers P13: 5'-CCACACATCCTGTGCTTCG-3' and P14: 5'-GACCAAGGTCAGCCAGCG-3' using Taq DNA Polymerase (NEB) and the resulting product was cloned into a plasmid vector (TOPO™ TA Cloning™ Kit, K45001, Invitrogen, Carlsbad, CA). Plasmid inserts of individually isolated clonal colonies were submitted to Sanger sequencing and analyzed with VectorNTI software package (Invitrogen).

Splice
junction
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GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 1 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 2 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 3 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 4 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 5 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 6 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 7 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 8 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 9 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
Clone 10 GAATGAAGAGGCTCAGGACCTATCAGGTGGCATGCAGAGAAAGCTGTCGGTTG
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Supplemental Figure 1. Sequence analysis of the full length ABCA4 RNA produced by the ABCA4 hybrid dual vector *in vivo*. 10 random colonies were analyzed by Sanger sequencing and aligned. The position of the Splice junction between 5' and 3' vector is marked with an arrowhead. Top line is the wild type reference sequence of ABCA4.