Supplementary material

Long-range RT-PCR of *Zbed6* and *Zc3h11* transcripts in mouse C2C12 cells

Materials and Methods

Long-range RT-PCR. First strand cDNA synthesis was performed with a long range reverse-transcriptase RevertAid Premium kit (Fermentas, Lithuania), according to manufacturer's instructions, using 5 μg total RNA with a gene specific primer (ZC1as, 5'-CTCTTTCGTTAATGGCAAATGGTTC) starting 60 nt upstream of the poly-adenylation site. PCR were performed using the KAPA HiFi HS polymerase (Kapa Biosystems, South Africa) and the following cycles: 95°C for 3 min; 98°C for 20 s, 56°C for 15 s, and 72°C for 8 min (4 cycles); 98°C for 20 s and 72°C for 8 min (36 cycles); 72°C for 3 min. Initial PCR was performed with a sense primer (ZCx2s with *Sal*I site underlined, 5-GATACGTCGACCGCAGCTAAGAAAAGAGAAAGACGTG) located close to the presumed transcription start, as judged by the UCSC Genome Browser (http://genome.cse.ucsc.edu/), and an anti-sense primer (ZCx2as with *Not*I site underlined, 5'-CATGCGGCCGCGTTGAACACAAATCATTGACTAATACAG) located 35 nt upstream of ZC1as. PCR with oligos ZCx2s-ZCx2as gave a very robust band at ~4.5 kb that was shown by direct sequencing to be the Zc3h11a transcript. Smearish DNA of higher molecular weight were seen on the gel, but an attempt to clone these fragments was unsuccessful. We postulated that a potentially much larger Zbed6 transcript would be underrepresented after amplification due to competition with the smaller Zc3h11a product. We therefore performed PCR to obtain the 5'- and 3'-part of the Zbed6 transcript in separate reactions, so that the reactions would not be affected by Zc3h11a-amplicons. The 5'-part of the Zbed6 transcript was obtained with ZCx2s and the anti-sense primer KFxx1as (*Not*I site underlined; 5'-CATGCGGCCGCCTCAAAGCTTCCATTGCTTCCAG), situated in the coding part of *Zbed6*, and the 3'-part was obtained with sense primer KFxx1s (*Sal*I site underlined; 5'-GATACGTCGACCAAGAATTCCAAAATGATCACCAG, situated in the coding part of *Zbed6*, and ZCx2as. The PCR products were gel purified, cloned and sequenced. The sequence gap between products ZCx2s-KFxx1as and KFxx1s-ZCx2as, were filled with *Zbed*6 sequence from Markljung et al.[1]; a sequence that originated from the same C2C12 cell line.

1. Markljung E, Jiang L, Jaffe JD, Mikkelsen TS, Wallerman O, Larhammar M, et al. ZBED6, a novel transcription factor derived from a domesticated DNA transposon regulates IGF2 expression and muscle growth. PloS Biol 2009; 7:e1000256.