Fig. S6

Forward primer

Reverse primer

Genome DNA

Target site

PCR

Forward primer

FM primer

RM primer

Mutated site

Mutated site

3’ UTR with mutated target site
Figure S6. Scheme of fusion PCR for generating 3’UTRs with mutated target sites. Wildtype target sites on 3’UTRs were indicated in red and mutated ones were in green (Fig. 4A). First, full length 3’UTRs of predicted targets with wildtype target sites (red) were amplified from genomic DNA using the Forward and Reverse primers. Then two primers (FM and RM) with overlapping sequences and carrying mutated target sites (green) were used for the next PCR step. The Forward primer and RM primer were used as a pair, and the FM primer and Reverse primer as another pair. The resulting two PCR products, which carry the mutated site and overlapping sequence, were purified from agarose gels and used as templates for the last overlapping PCR. Full 3’UTRs carrying mutated sites were obtained in the end. (All primers used here can be found in Table S4).