Transgene verification (KK library)

Each transformant line was verified for the presence of the correct UAS-IR construct by first amplification by PCR and then sequencing. For the PCR amplification genomic DNA from two flies per line was extracted. Flies were collected into a 96 well plate and mashed with an 8-channel pipette with a yellow tips containing 50 μl squishing buffer (10 mM Tris, 1mM EDTA, 25 mM NaCl and 200 μg/ml fresh Proteinase K). Then the plates were incubated at 37°C for 30 min, at 95°C for 3 min (to destroy the Proteinase K) and spun down in the plate centrifuge. PCR reactions were set up in the 96 well plates with the universal forward primer (as for cloning) and specific reverse primer SV2 or HSP7 in the vector. The reactions (38 cycles of 94°C for 30 sec, 60° for 30 sec and 72° for 1 min 30 sec) were done in the presence of 1.5 mM MgCl2, 200 μM dNTPs (each), 0.5 μM each primer, 0.25 μl Taq (Eppendorf, 5 u/μl) and 1 μl genomic DNA in a total reaction volume of 50 μl. For sequencing the PCR reactions were purified with the ExoSAP IT (USB) according to the manufacturer protocol.

Verification primers

ETU GCGCGAATTCTGGCGCCCCTAGATG
BTU GCGCGGATCCTGGCGCCCCTAGATG
GTU GCGCAGATCTTGGCGCCCCTAGATG
MTU GCGCCAATTGTGGCGCCCCTAGATG

SV2 CACAGAAGTAAGGTTCCTTCACAAAGATCC
HSP7 AAGCAAAGTGAACACGTCGCTAAGCG