Microinjections:

We directly used the extracted Miniprep DNA (average concentration ca. 150–200 ng/µl) to mix with delta23 helper plasmid DNA and directly (without any further purification) microinjected Drosophila embryos with this DNA.
Borosilicate glass capillaries GC120TF-10 from HARVARD Apparatus were pulled on a Suttner P-97 micropipette puller and opened on a Narishige EG-400 micropipette grinder. A Eppendorf FemtoJet with a micromanipulator mounted on a Zeiss Axiovert 200 Inverted Microscope was used for microinjections.
Drosophila embryos were collected every 30 min, dechorionated with 50% Chlorox-bleach and collected on a membrane filter using an All-glass vacuum filtration unit from Sartorius. On the membrane the embryos were arranged in short lines of 45 eggs. Each line was picked up with a 24 X 24 mm coverslip that had been coated with glue (extracted from Scotch sticky tape with heptane) before. The coverslip was stuck onto a microscope glass slide with a drop of water and put for drying into a 12 cm Petri dish filled with silica gel at 18°C for 20 minutes. After drying the embryos were covered with 10S Voltalef oil and microinjected. The coverslips with the injected embryos were placed into 5 cm Petri dishes which collectively were put in a bigger plastic box together with moisturized paper towel. After two days at 18°C the coverslips were removed from the Petri dishes and shoved (without getting rid of the oil before) into fly food vials supplemented with yeast paste. About two weeks later at 25°C the hatching adult flies are collected (on average 18.5% of lined up embryos survived to adulthood). The adults are split into max 4 groups (ordered by sex) of at least 3 animals per group and crossed out to w1118 flies in bigger fly food bottles in order to obtain as many progenies as possible.

Transgene verification

For transgene verification we extracted genomic DNA from two flies per line in 96 well plates using a low quality high throughput protocol:
Two flies per line were collected into a 96 well plate and mashed with a 8-channel pipette with yellow tips containing 50 µl squishing buffer (10 mM Tris, 1mM EDTA, 25 mM NaCl and 200 µg/ml fresh Proteinase K). After mashing was finished, the

plates were incubated at 37°C for 30 min, then at 95°C for 3 min (to destroy the Proteinase) and finally the solid remnants were spun down using the plate centrifuge and discarded.

PCR reactions in 96 well plates are set up with one specific primer (the EcoRI primer that was used for cloning) and one common primer (hsp7_2: GAGGCGCTTCGTCTACGGAGCGAC) binding to the promoter region of the transgene. The reactions (34 cycles of 94°C for 30 sec, 59° 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. 10 μl of the PCR reaction were then loaded on a 2 % agarose gel.