

Transgene verification (GD library)

For transgene verification extract genomic DNA from two flies per line

- put the flies into a tube/96 well plate
mash with a pipette with yellow tips containing 50 μ l squishing buffer
(10 mM Tris, 1mM EDTA, 25 mM NaCl and 200 μ g/ml fresh Proteinase K)
- incubated at 37°C for 30 min, then at 95°C for 3 min
(to destroy the Proteinase)
- spin for 5 min at 4000 rpm
- move supernatant to a new tube/96 well plate
- PCR reactions set up with one specific primer (the left primer that was used for cloning) and one common primer (hsp7_2: GAGGCGCTTCGTCTACGGAGCGAC, SV2: CACAGAAGTAAGGTTCCCTTCACAAAGATCC)
- 34 cycles of 94°C for 30 sec, 59° for 30 sec and 72° for 1 min 30 sec
- 1.5 mM MgCl₂, 200 μ M dNTPs (each), 0.5 μ M each primer, 0.25 μ l Taq and 1 μ l genomic DNA in a total reaction volume of 50 μ l
- 10 μ l of the PCR reaction load on a 2 % agarose gel
- control: product length = sequence length + 200 bp