**Midi prep for injection into fly embryos**

Inoculate 50 ml LB medium o/n at 37°C, 250 rpm (Ampicillin for pGem-T Easy, SMG3 and pHer).

Spin 35 ml bacteria in autoclaved plastic screw cap bottles for 15 min at 6.000 rpm and 4°C.

Discard supernatant and invert tube to drain remaining liquid on a paper towel.

Re-suspend bacteria in 5 ml P1 in the same plastic vial and vortex until the pellet is re-suspend.

Add 5 ml P2, close the screw cap, invert several times, and proceed to the next step within 5 min!

Add 5 ml P3 (NOT N3!), close the screw cap, and invert 10x.

Cenetrifuge for 25 min at 11.000 rpm and 4°C.

Meanwhile, equilibrate a Tip-100 column with 5 ml QBT.

Place a funnel with ½ Kimwipes onto the opening of the Tip-100 column.

Pour plasmid containing supernatant through the Kimwipes and funnel into the equilibrated Tip 100.

Wash 2x with 10 ml QC.

Tape the Tip-100 over an autoclaved 15 ml Corex glass vial.

Elute the DNA with 5 ml QF.

Add 3.5 ml Isopropanol to the eluted DNA.

Close the Corex vial with Parafilm, invert 10x, take away the Parafilm, and wipe off drops with Kimwipes.

Centrifuge for 30 min at 11.000 rpm and 4°C.

Mark the area of the DNA pellet (purple pen), decant and invert the vial on a paper towel.

Add 350 µl sterile MQ water and dissolve the DNA pellet by vortexing.

Collect the plasmid DNA into a 1.5 ml Eppendorf tube.

Add 35 µl 3M of Na-Acetate (pH 5.5).

Add 875 µl pure EtOH and invert 10x.

Centrifuge at maximum speed in the cold room for 20 min.

Remove supernatant and wash with 300 µl 70% EtOH by inverting the tube 10x.

Centrifuge for 10 min at maximum speed (RT is OK).

Remove supernatant and centrifuge for 1 min.

Remove all EtOH.

Dissolve the DNA in 50-100 µl EB, measure the OD, and add EB to a final concentration of 1 g/l.