

Morpholino injections in Tribolium:

(Gregor Bucher, Martin Klingler 8/2002)

A Morpholino oligo against the *giant* gene was designed to cover both possible starting ATGs (oligo sequence: 5'CCATCGCAAATTCTGCTTTTCCAT-3').

Injection of 1mM and 0.66 mM concentrations (in injection buffer) resulted in premature termination of development in all embryos. With lower concentrations (0.4 and 0.2 mM), the portion of fully differentiated embryos (32%) and cuticles displaying phenotypes (42% of differentiated embryos) was similar as in embryonic RNAi experiments.

Order morpholino from Gene-Tools (see design advice at www.gene-tools.com)

Stock solution: 2 mM (about 16,5 mg/ml) in H₂O (example: if total morpholino yield was 300 nMol: dissolve in 150 ul)

Dilute stock solution for injection: 1:5 to 1:20 in injection buffer (potassium phosphate 20mM, sodium citrate 3mM, pH7.5; Fire, Xu et al. 1998)

Injection procedure:

It is hard to say, how much volume exactly we inject into Tribolium eggs. Unlike Drosophila, the Tribolium embryo volume cannot be reduced by drying, and some injected fluid inevitable leaks out again. Maybe, 1/20th egg volume was injected... For more details about the egg handling procedure see our "Tribolium Germline Transformation" protocol

(Zebrafisch reference for morpholino concentrations: Nasevicius et al., Nature 2000: Stock solution: 8mM (about 65 mg/ml) in H₂O, they injected 0,09 - 3 mg/ml in Inj.Puffer. Effektive Dosis: 4 - 9 ng je Fisch)