dsRNA injection

How it works

RNA interference offers a possibility for rapid analysis of gene function. We developed and standarized direct dsRNA injection method into embryos carrying GFP transgenes expressed in a tissue-specific manner.

We can provide GFP lines expressed in the embryonic muscle (MHC-GFP) in the heart (Tin-GFP, Him-GFP) and in the CNS (Elav-GFP) so that the analyses of phenotypes in these tissues can be performed. Other tissue-specific GFP lines provided by customers can also be used.

To determine effects of attenuation of gene function we inject 100 embryos at blastoderm stage (ventrally, when mesoderm and CNS are targeted). dsRNA of 400-500 nt is synthesized on PCR-generated T7-flanked DNA templates using T7 RNA Pol (see protocols). To avoid off target effects, two distinct templates are generated and two distinct dsRNAs are injected for a single gene.

As a result of injection we determine the percentage of lethality and we document the changes in GFP pattern in embryos with phenotypes (approximately 10 photos per gene and/or time lapse analysis if requested). CELL® - Optigrid and confocal scanning microscopy is used for documentation.

Usually dsRNA injection and documentation of phenotypes for one gene takes 1 week. Design, synthesis of primers and production of dsRNA probes take additional 2 weeks.

For RNAi screen purposes (large number of genes to be tested) contact our platform staff .

There are two levels of dsRNA services we propose:
Basic

Customer provides dsRNA probes, which are injected and receives documentation of phenotypes.

Non-Profit academic users - 200€ / gene

Extended

Customer indicates the gene of interest and we are in charge to design, produce and inject dsRNA and document the phenotypes.

Non-Profit academic users - 300€ / gene

MHC-GFP - muscle-specific GFP line used for testing muscle gene function, time lapse experiment showing muscle contraction