

Manipulating Gene Expression in Transgenic Zebra Fish Using RNAi

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Abstract

RNA interference (RNAi) is a sequence specific gene silencing process that occurs at the posttranscriptional stage. The effectors of RNAi are short (21-23 nucleotides) double-stranded RNA (dsRNA) molecules --- small interfering RNAs (siRNA). The guide strand of the siRNA serves as a template for the RNA-induced silencing complex (RISC) to recognize and cleave a complementary messenger RNA (mRNA), which is then rapidly degraded and thus the gene expression is blocked. This technique has already become a powerful tool for gene functional studies in many organisms, including plants, flies, mice and humans. A common method to induce sustained RNAi expression is to introduce plasmids that synthesize short hairpin RNAs (shRNAs) using Pol III promoters. However, the drawbacks of non-cell specificity and off-target silencing have limited its applications. An alternative way to deliver RNAi is to use Pol II driving shRNA expression. Many attempts have been made to test the potential of Pol II-shRNA mediated silencing in mice and human cells, but very few have been done with zebrafish. We plan to utilize a *fli-1* promoter (endothelial cell specific Pol II promoter) to drive microRNA-based shRNA expression in the transgenic zebrafish. Our goal is to make a stable shRNA transgenic construct to silence the target gene throughout generations in zebrafish.

Introduction

➤ The zebrafish (*Danio rerio*) is a commonly used model organism in molecular genetics research. The advantages of using zebrafish include optically clear and easily-manipulated embryos, high fecundity, and the availability of a complete genomic sequence. We have successfully established RFP/GFP converted transgenic zebrafish by Cre-loxP action.

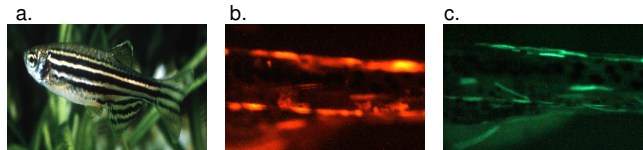


Fig 1. Zebrafish: a. wild type; b. pBa/RFP/loxP2/GFP/SBI/R transgenic red fish; c. converted pBa/RFP/loxP2/GFP/SBI/R green fish

➤ *Cre-loxP* is a site-specific recombination system (SSR). A target fragment flanked by *loxP* sites will be recognized and then deleted from the genome by *Cre-recombinase* (*Cre*). A temperature controlled heat-shock promoter from the HSP70 gene can be used to drive conditional *Cre* gene expression following heat shock.

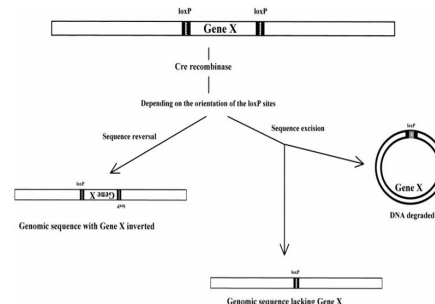


Fig 2. Schematic of Cre-mediated recombination of a fragment (*GeneX*) flanked by *loxP* sites [2].

➤ RNAi has become a popular experimental tool for loss-of-function genetics. Many studies have been performed on RNA polymerase III promoters controlling short hairpin RNAs (shRNAs) and RNA polymerase II promoters mediating microRNA-based shRNAs in transgenic organisms, including plants, flies, mice, and humans.

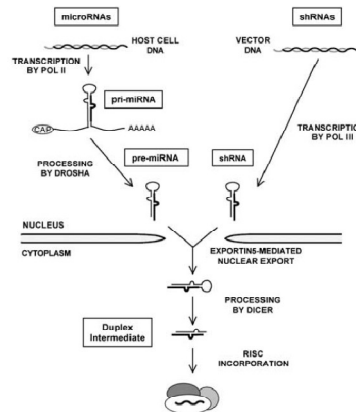


Fig 3. miRNAs are initially transcribed by polII as long pre-miRNAs and then cleaved by nuclear RNase III enzyme Drosha to give a hairpin intermediate. In contrast, shRNAs are directly transcribed by polIII. [3]

Objective

- Establish stable transgenic line of pHsp70-Cre (Dr. Ryan Thummel) and pFli1-EGFP (Dr. Ekker) zebrafish;
- Construct a Fli1 promoter driving microRNA-based shRNA (flanked by two loxP sites) targeting GFP and reversibly express shRNA when crossed with pHsp70-Cre zebrafish.
- Replace GFP shRNA with other shRNAs for further gene functional studies *in vivo*.

Materials and Methods

1. Stable transgenic lines of pHsp70-Cre and pFli1-EGFP were obtained as gifts from Dr. Ryan Thummel and Dr. Ekker.

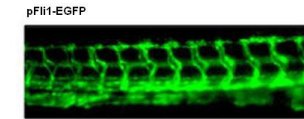


Fig 4. pFli1-EGFP transgenic zebrafish (picture taken by Dr. Allen Albig)

2. Plasmid of pTolflil1epEGFPDest was kindly provided by Dr. Lawson. This is a short version of pFli1-EGFP plasmid with similar Fli1 expression patterns.

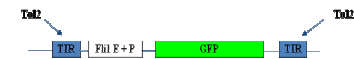


Fig 5. Schematic graph of pTol2/Fli1/EGFP construct

3. Based on the structure of pTolflil1epEGFPDest, we plan to introduce a short Fli1 intron fragment including microRNA-based shrank behind Fli1 promoter and flank the shrank with two loxP recombinant sites (on same orientation) which can be recognized by Cre resulting in reversible conversion to inhibit shrank function. Also, EGFP will be replaced by RFP as the marker.

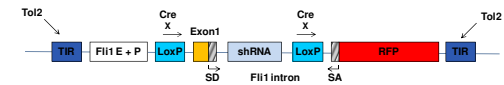


Fig 6. Schematic graph of pTol2/Fli1/shRNA/loxP2/RFP construct SD: splicing donor; SA: splicing acceptor.

4. Use *egfp* as target gene (pFli1-EGFP transgenic fish) to design shGFP construct for *gfp* expressing-inhibition test. After introduction of Cre (cross with pHsp70-Cre fish), the shGFP fragment should be inverted and GFP expression will be recovered in the next generation. If this works successfully, we plan to replace shGFP with other shRNAs for further tests.

Acknowledgment

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References

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