

Competing endogenous RNA (ceRNA) in a non-model animal: Non-coding RNAs respond to heat stress in rainbow trout (*Oncorhynchus mykiss*) through ceRNA-regulated mechanisms



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1 Introduction

- High summer temperatures are the biggest threat to rainbow trout farming.
- Non-coding RNAs (ncRNAs) in rainbow trout liver may play a regulatory role through the competitive endogenous RNA (ceRNA) network in response to heat stress.



ceRNA network construction;

2 Method

Validation of targeting relationship between LOC110485411, hsp90ab1, and novel-m0007-5p;
Measurement of LOC110485411 and hsp90ab1 mRNA and protein expression under heat stress by transfection with novel-m0007-5p;



Measurement of LOC110485411 and hsp90ab1 mRNA expression levels under heat stress by small interfering RNA (siRNA) transfection.

3 Result

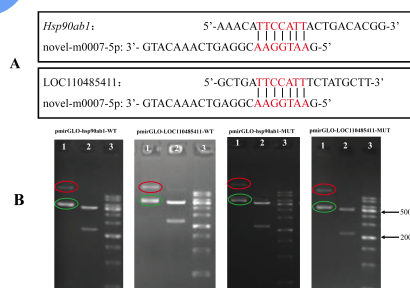


Fig. 2. Studies of the targeting relationship between novel-m0007-5p, hsp90ab1, and LOC110485411. (A) Schematic representation of the target binding sites of hsp90ab1, LOC110485411, and novel-m0007-5p (B) Validation of recombinant vector digestion (SpeI and XhoI). Lane 1: Red indicates empty vector self-conjugated before digestion, green indicates recombinant vector before digestion; lane 2: vector and target fragment after digestion; lane 3: 1-kb marker

Result

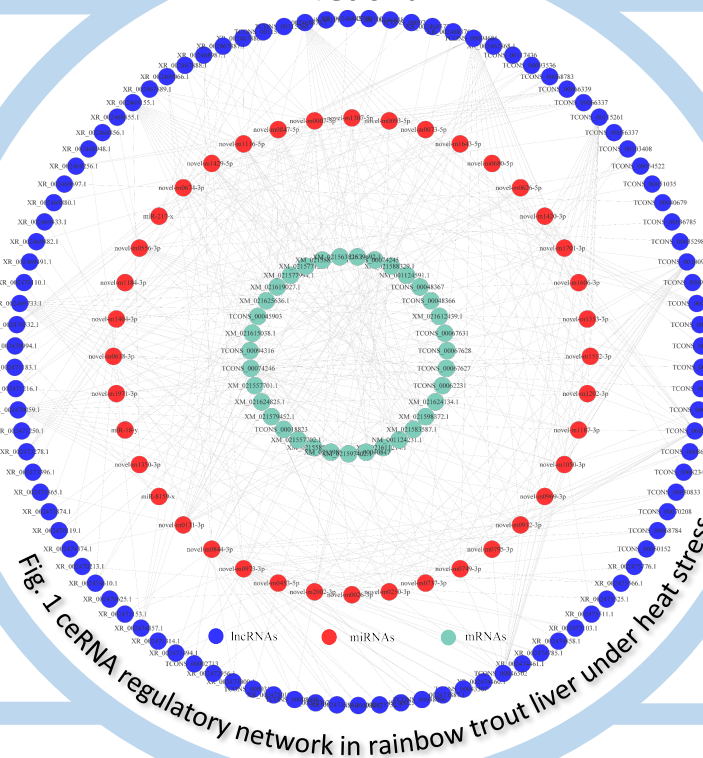
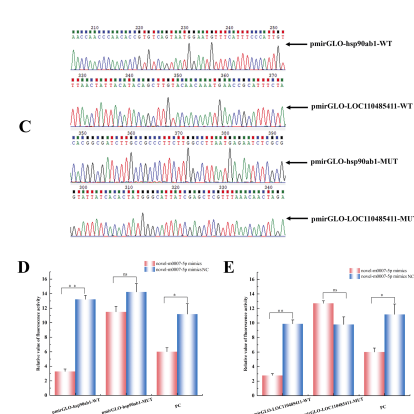


Fig. 1 ceRNA regulatory network in rainbow trout liver under heat stress

3 Result



(C) Sanger sequencing peak diagram of recombinant vector (D) Dual luciferase analysis of the targeting relationship between novel-m0007-5p and hsp90ab1 (E) Dual luciferase analysis of the targeting relationship between novel-m0007-5p and LOC110485411

3 Result

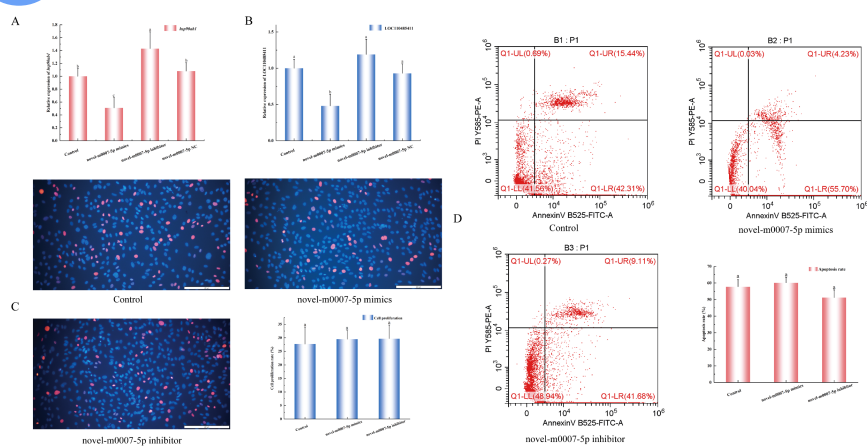


Fig. 3. Effect of novel-m0007-5p on hsp90ab1 and LOC110485411 mRNA expression in rainbow trout liver cells. Effect of novel-m0007-5p on hsp90ab1 (A) and LOC110485411 (B) mRNA expression in the cells. (C) Effects of novel-m0007-5p on rainbow trout hepatocyte proliferation. Bar graphs indicate the cell proliferation rate in each group. (D) Effects of novel-m0007-5p on rainbow trout hepatocyte apoptosis. Bar graphs indicate the apoptosis rate in each group.

Conclusion: LOC110485411 and hsp90ab1 can bind competitively to novel-m0007-5p via 'sponge adsorption' and that interference with LOC110485411 affects hsp90ab1 expression.

3 Result

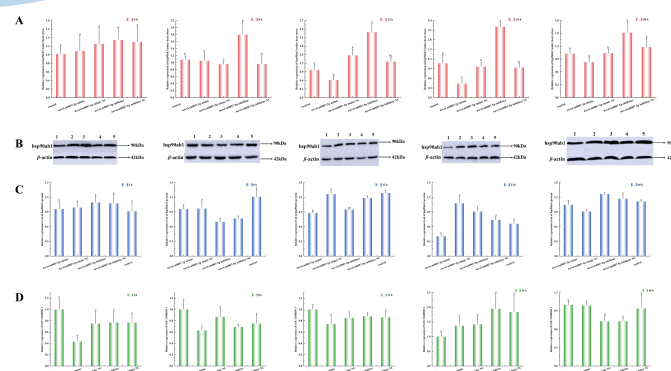


Fig. 4. Effect of novel-m0007-5p on hsp90ab1 and LOC110485411 expression under heat stress. Effect of novel-m0007-5p on hsp90ab1 mRNA expression. (A) and hsp90ab1 protein expression. (B) under heat stress. (C) Quantitative analysis of protein expression grayscale values. (D) Effect of novel-m0007-5p on LOC110485411 expression under heat stress.

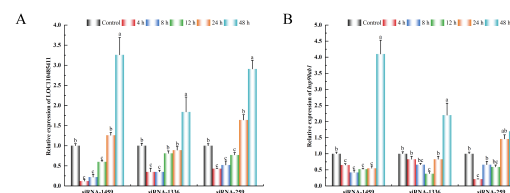


Fig. 5. Effect of siRNA transfection on LOC110485411 expression (A) and hsp90ab1 mRNA expression (B) under heat stress.