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Quantitative PCR analysis to assess gene expression changes in hyperglycemic larval zebrafish

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We use this protocol and it's working

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Abstract

Summary:

To assess hyperglycemia in larval zebrafish, we analyzed the expression of genes implicated in glucose metabolism, such as insulin, insulin receptor, glucagon, and phosphoenolpyruvate carboxylase (pepck), using quantitative PCR (qPCR). We analyzed gene expression following treatment of Tg(*ins:NTRmCherry*) transgenic larvae either with 0.5 % DMSO (controls) or 10 mM metronidazole (see Protocol 2) for β-cell ablation at 3 and 8 days post fertilization (dpf).

Diabetic Complication:

Neuropathy

Materials

MATERIALS

- RNeasy Mini Kit **Qiagen Catalog #**74104
- oliog (dT) or random hexamer primers **Integrated DNA Technologies, Inc. (IDT)**
- SuperScript®III First-Strand Synthesis System for RT-PCR **Life Technologies Catalog #**18080-051
- SYBR Green Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix **Agilent Technologies Catalog #**600882
- ROX reference dye (included in Brilliant III master mix) **Agilent Technologies Catalog #**600882
- Mx3000P QPCR System **Stratagene Catalog #**Mx3000P

Primers:

Gene name	Gene symbol	5'-3' forward primer	5'-3' reverse primer
insulin	*ins*	ccc ttt atc tgg tct gtg gc	ttc ctt atc agc tcg gca tg
phosphoenolpyruvate carboxylase	*pepck*	gag aac agc acc atc ctc ag	tca ccg ttt tac tct cca cac
glucagon a	*gcga*	aag act tcg ttc agt ggc tc	tgg gtt gtc cgg att tta gc
insulin receptor a	*insra*	ata aga atg atc ggg agt gtg g	tgc ttg cat gag gat gga c
Elongation factor alpha (control gene)	*ef1a*	tct aca aat gcg gtg gaa tcg	gag caa tgt caa tgg tga tac c

Note:

QIAGEN (RRID:SCR_008539)

Troubleshooting

1 Preparation of mRNA from larval zebrafish. Pool 10 zebrafish larvae and isolate RNA according to the RNeasy Mini Kit (QIAGEN) manual. Elute final mRNA in 30 µl of RNase-free water.

2 Preparation of cDNA from mRNA. Prepare cDNA from isolated mRNA according to the SuperScript®III First-Strand Synthesis System (Life Technologies) using either oligo(dT) or random hexamer primers.

3 Preparation of qPCR mix (according to the Agilent manual for preparation of the Brilliant III Ultra- Fast SYBR® Green QPCR Master Mix):

Dilute the reference dye 1:500 using nuclease-free PCR-grade water. Prepare the experimental reactions by combining the components of the reagent mixture in the order listed in the table below. Prepare a single reagent mixture for replicate reactions (plus at least one reaction volume excess) using multiples of each component.

Reagent Mixture

Nuclease-free PCR-grade water to bring final volume to 20 µl (including cDNA)
10 µl of 2× SYBR Green QPCR Master Mix
x µl of upstream primer at optimized concentration (200–500 nM)
x µl of downstream primer at optimized concentration (200–500 nM)
0.3 µl of diluted ROX reference dye

Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes. Add 0.5-50 ng (x µl) of cDNA to each reaction to bring the final reaction volume to 20 µl.

We amplified each transcript using the following PCR conditions:

1) 95ºC 3 minutes

2) **40 cycles**
95ºC 15 seconds
60ºC 20 seconds,

3) **Melting curve**
95ºC 1 minute
60ºC 30 seconds
95ºC 30 seconds

Potential Pitfalls:

4 Inconsistent qPCR results: Always use master mixes and the suggested reference dye (ROX reference dye, Life Technlogies, USA). Include pre-mix of triplicate reactions with sample and primers. Alternatively, utilize the Taqman system (Life Technologies).