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| **EXPERIMENTAL DESIGN** |  |
| Definition of experimental and control groups | Female tick tissues (ovaries, salivary glands, midguts and fat bodies), whole adult male, eggs and larvae. |
| Number within each group | 6-10 each tissue (mix) |
| Four ovaries para RNAi: each tissue was evaluated separately. |
| **SAMPLE** |  |
| Description | Total RNA was extracted using the TRIzol Reagent (Invitrogen) following manufacture’s protocol. |
| Volume/mass of sample processed | Homogenization of samples was performed in eppendorf tube and pestle using 1 ml of TRIzol Reagent per 50 mg of tissue. |
| Microdissection or macrodissection |
| Processing procedure | All samples was colected, placed in TRIzol and inmediately frozen at -70ºC. The purified RNA was dissolved in DEPC-Treated Water and stored at -70°C. |
| If frozen - how and how quickly? |
| **NUCLEIC ACID EXTRACTION** |  |
| Procedure and/or instrumentation | TRIzol Reagent (Invitrogen). We exactly followed manufacture’s protocol. |
| Name of kit and details of any modifications |
| Source of additional reagents used | Chloroform (Merck); 2-propanol (Merck); Ethanol (Merck); DEPC-Treated Water (Ambion) |
| Details of DNase or RNAse treatment | 1 µg of RNA was treated with 1 U of Dnase I (Invitrogen) in a 10 µl final volume reaction. Digestion of DNA was achieved with 15 minutes incubation at room temperature. The reaction was stopped with 1 µl of 25 mM EDTA solution (Invitrogen) following 10 min incubation at 65°C for inactivation. |
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| Contamination assessment (DNA or RNA) | PCR using RNA and actin primers was done. No amplification band was obtain for any samples. |
| Nucleic acid quantification | Spectrophotometry (Amersham Biosciences, Pharmacia Biotech, ultraspec 1000). |
| Instrument and method |
| Purity (A260/A280) | Done by 260/280 determination (value 1.70-1.90) |
| RNA integrity method/instrument | Done by 260/280 determination (value 1.70-1.90) and agarose gel electrophoresis |
| RIN/RQI or Cq of 3' and 5' transcripts | Not applicable |
| Inhibition testing (Cq dilutions, spike or other) | Standard curves dilution from 50 ng to 500 ng |
| **REVERSE TRANSCRIPTION** |  |
| Complete reaction conditions | 3.0 - 5.0 μg of total RNA to reaction volume of 20 µl |
| Amount of RNA and reaction volume |
| Priming oligonucleotide (if using GSP) | Not applicable |
| and concentration |
| Reverse transcriptase and concentration | Superscript III reverse transcriptase (Invitrogen) with Oligo-dT20 primer (Invitrogen) |
| Temperature and time | 65°C for 5min; 50°C for 60min; 70°C for 15min |
| **qPCR TARGET INFORMATION** |  |
| Sequence accession number | 40S ribosomal: EW679928 / RmGRP: KY271084 |
| Amplicon length | 40S ribosomal (106 pb), RmGRP (115 bp) |
| *In silico* specificity screen (BLAST, etc) | PrimerExpress 3.0 software (Applied Biosystems, Foster City, USA). |
| Location of each primer by exon or intron (if applicable) | Not applicable |
| What splice variants are targeted? | Not applicable |
| **qPCR OLIGONUCLEOTIDES** |  |
| Primer sequences | 40S ribosomal: forward: 5´ACGACCGATGGCTACCTCCTCCGC´3; reverse: 5´TGAGGCGAACCTGGTTGTGCTGAGCG´3 |
| RmGRP: forward: 5’CTTGCCCCCTGAGTCCTACA'3; reverse: 5’TTCCACAGTGCAGGCTTCAA'3 |
| Location and identity of any modifications | Not applicable |
| **qPCR PROTOCOL** |  |
| Complete reaction conditions | Reaction volume: 25 μl |
| Reaction volume and amount of cDNA/DNA | cDNA: 100 ng (RNAi: 300 ng) |
| Primer, (probe), Mg++ and dNTP concentrations | Primer: 1.0 μM |
| Polymerase identity and concentration | Platinum®SYBR®Green qPCR SuperMix kit (Invitrogen) / RNAi: Go-Taq qPCR Master Mix (Promega) |
| Buffer/kit identity and manufacturer | Platinum®SYBR®Green qPCR SuperMix kit (Invitrogen) / RNAi: Go-Taq qPCR Master Mix (Promega) |
| Additives (SYBR Green I, DMSO, etc.) | Kits with SYBR Green |
| Complete thermocycling parameters | 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. |
| Manufacturer of qPCR instrument | Applied Biosystems Step One Plus / RNAi: Rotor Gene® Q (Qiagen) |
| **qPCR VALIDATION** |  |
| Specificity (gel, sequence, melt, or digest) | Melt curve and agarose gel electrophoresis |
| For SYBR Green I, Cq of the NTC | Cp NTC : 32.13 |
| Standard curves with slope and y-intercept | Ovaries - 40S: -3.55 (slope); 24.9 (y-intercept)/ RmGRP: -3.44 (slope); 26.2 (y-intercept) |
| PCR efficiency calculated from slope | Ovaries - 40S: 91.29% / RmGRP: 95.3% |
| Confidence interval for PCR efficiency or standard error | Ovaries - 40S: ±0.083 / RmGRP: ±0.635 |
| r2 of standard curve | 0varies: 40S: 0.998 / RmGRP: 0.889 |
| Linear dynamic range | In average, linear dynamic range was considered taking into account the linearity of the standard curves. |
| Cq variation at lower limit |
| Evidence for limit of detection | Standard curves |
| If multiplex, efficiency and LOD of each assay. | Not applicable |
| **DATA ANALYSIS** |  |
| qPCR analysis program (source, version) | Relative Expression Software Tool (REST -MCS©, version 2) (Pfaffl et al., 2002) |
| Cq method determination | The threshold is determined using the Amplification-based Threshold method. |
| The threshold is used to specify Cq values of samples |
| Results of NTCs | The signal of the amplification plot was very late (Cq>34) and therefore there was a high Cq value difference between the negative control and all the cDNA sample. |
| Justification of number and choice of reference genes | Reference gene 40S Ribosomal was chosen by stable gene expression (housekeeping gene), as indicated by previous publications (Pohl et al., 2008; Fabres et al., 2010) |
| Description of normalisation method | mRNA expression was analyzed by comparative Ct method, which was normalized using the 40S ribosomal gene. The relative amount of RmGRP produced per unit of 40S was calculated for each sample. |
| Number and stage (RT or qPCR) of technical replicates | qPCR reactions were performed in duplicate |
| Repeatability (intra-assay variation) | Variance: 0.183± 0.428 |
| Statistical methods for result significance | One-way ANOVA and Tukey’s post-test |
| Software (source, version) | GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA) |