RT-qPCR information

**Experimental design**

Experimental group: 35 paired of Papillary thyroid cancer tissue; Control group: 35 paired of normal thyroid tissues.

**Sample information**

The sample tissues were collected from surgery, immediately frozen, immobilized less than 24 hours, storage at -80℃.

**Nucleic acid extraction**

Extract the sample RNA with Trizol, and finally add 100µl of RNase-free water to dissolve the RNA.

DNase I digests the DNA in the sample RNA:

|  |  |
| --- | --- |
| Component | dosage |
| Template (RNA) | 10µg |
| RNase Inhibitor | 4µl |
| DNase I buffer | 10µl |
| DNase I | 10µl |
| DEPC treats H2O | to 100µl |
| Mix 37℃ 30min |

**RNA agarose gel electrophoresis validation**

1) Weigh 0.45g of agarose into an Erlenmeyer flask, add 4.5ml of 10×MOPS buffer and 39.5ml of DEPC water to it, and dissolve in a microwave oven.

2) When it is cooled to about 60 degrees Celsius, add 1ml formaldehyde, 5ul GelRed, and shake well (to avoid bubbles). Pour into the gel plate and solidify for 30 minutes.

3) Take 4μl of each RNA sample, add 2μl of 6×RNA electrophoresis loading buffer, mix well, and add all the liquid to the sample well of denaturing gel.

4) Electrophoresis at 120V for 25min. Observe with a gel ultraviolet analyzer, take pictures and save.



RNA extraction product agarose gel electrophoresis figure

**Reverse Transcription**

The procedure for reverse transcription of RNA to cDNA is summarized in the following table:

|  |  |
| --- | --- |
| Component | dosage |
| Template (RNA) | 1µg |
| Reverse transcription primer T18 (50µM) | 1.0µl |
| DEPC treats H2O | to 12µl |
| Mix, 70℃ for 5min, immediately ice bath |
| 5×buffer | 4.0µl |
| dNTP（10mM） | 1.0µl |
| RNase Inhibitor（MBI） | 0.5µl |
| M-MLV | 1.0µl |
| Make up DEPC water to 20µl |
| 42℃ 60min，70℃ 15min |
| Chill on ice for 5 minutes |

**qPCR primers**

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer** | **sequence (5'to3')** | **Base number** | **Product length** |
| **GAPDH** | F：TGAAGGTCGGAGTCAACGG | **19** | **226bp** |
| R：TCCTGGAAGATGGTGATGGG | **20** |
| **TFF3** | F：GGGCTGCTGCTTTGACTCCA | **20** | **238bp** |
| R：TCGTTAAGACATCAGGCTCCAGAT | **24** |

**qPCR experimental protocol**

Take 0.2ml thin-walled PCR tubes and number them respectively. Add dye-containing Premix Taq 12.5ul, 10uM primer mixture 0.75ul to each tube, corresponding cDNA 1ul each, one tube without template is used as a negative control, and each tube is filled with water to 25ul. Summarized as follows:

|  |  |
| --- | --- |
| Component | dosage |
| Template（cDNA） | 1.0µl |
| 10μM primer F/R | 0.75µl |
| Premix Taq | 12.5µl |
| ddH2O | To 25µl |

**PCR product validation**: After mixing, place it in a PCR machine at 95℃ for 5min pre-denaturation, 95℃ 10s→60℃ 30s→72℃ 30s, 40cycles, 4℃ pause. Electrophoresis at 120V voltage for 20min, after the electrophoresis is over, take pictures on the gel UV analyzer



M: DL2000; 1:GAPDH; 2:GAPDH negative control; 3:TFF3; 4:TFF3 negative control;

**REAL TIME PCR experiment data**: Take Roche 384-well plates and number them respectively. The system is summarized as follows:

|  |  |
| --- | --- |
| Component | dosage |
| Template（cDNA） | 1.0µl |
| 10μM primer F/R | 0.3µl |
| Premix Taq | 5µl |
| EvaGreen | 0.5µl |
| ddH2O | to 10µl |

Mix well and place in a fluorescent quantitative PCR machine.

After 95°C 5min pre-denaturation, 95°C 10s→60°C 30s (fluorescence detection), 40 cycles. Melting curve 65℃-95℃.

**GAPDH melting curves**



**TFF3 melting curves**

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**Data analysis**: Analyze the results with the relative quantitative 2-ΔΔCT method, subtract the Ct value of the housekeeping gene of each sample from the Ct value of the target gene of each sample to obtain delta Ct; subtract the value of the control group from delta Ct to obtain delta delta Ct. Use 2-ΔΔCT to calculate the expression change of the target gene in each sample. Statistical methods for result significance was used with SPSS version 23.0.

**Reagents, consumables and primers used in the experiment**

1. Trizol Invitrogen, USA

2. M-MLV reverse transcriptase Bao Bioengineering (Dalian) Co., Ltd.

3. DNase I Fermentas, USA

4. RNase Inhibitor Fermentas, USA

5. 6×Loading Dye Fermentas, USA

6. dNTP(10mM) Bao Biological Engineering (Dalian) Co., Ltd.

7. Premix Taq™ (Ex Taq™ Version 2.0) Bao Biological Engineering (Dalian) Co., Ltd.

8. DL2000 DNA Marker Bao Biological Engineering (Dalian) Co., Ltd.

9. RL 6,000 RNA Marker Fermentas, USA

10. Agarose Biowest, Spain

11. RNase-free H2O Bao Biological Engineering (Dalian) Co., Ltd.

12. EvaGreen Dye Biotium, USA

13. Isopropanol (Analytical Pure) Beijing Chemical Reagent Company

14. Anhydrous ethanol (analytical grade) Beijing Chemical Reagent Company

15. Chloroform (Analytical Pure) Beijing Chemical Reagent Company

16. Formaldehyde (Analytical Pure) Beijing Chemical Reagent Company

17. 50xTAE Bao Biological Engineering (Dalian) Co., Ltd.

18. 10×MOPS Beijing Chemical Reagent Company

19. GelRed American biotium company

20. Real Time PCR 384-well plate Roche, Switzerland

21. 10µl/200µl/1000µl Tips AXYGEN, USA

22. 1.5ml /0.5ml centrifuge tube AXYGEN, USA

**Instruments used in the experiment**

1. Type 2720 PCR instrument ABI Company, USA

2. system electrophoresis Mupid (Mupid 2plus) electrophoresis tank TAKARA Japan

3. image system (EUV-LDUV) gel imaging system Korea Biotech, Korea

4. Centrifuge (MICRO 17TR) Centrifuge Hanil Corporation, South Korea

5. Ultra-clean workbench Suzhou Purification Company

6. 2.5µl/10µl/100µl/200µl/1000µl pipettes Eppendorf, Germany

7. Eppendorf epMotion 5070 Automatic Dosing Workstation Eppendorf, Germany

8. Autoclave (LS-B50L) Sterilizer Jiangyin Binjiang Company, China

9. Freezer Big (DW-40L262) refrigerator Qingdao Haier Company

10. Mixer vortex (Voltex-Genie2) vortex mixer SI company, USA

11. oven dry(DHG-9240A) Shanghai Yiheng Company

12. Balance (BT-214) balance Denver company, USA

13. Roche LightCycler® 480II real-time fluorescent quantitative PCR system Roche, Switzerland