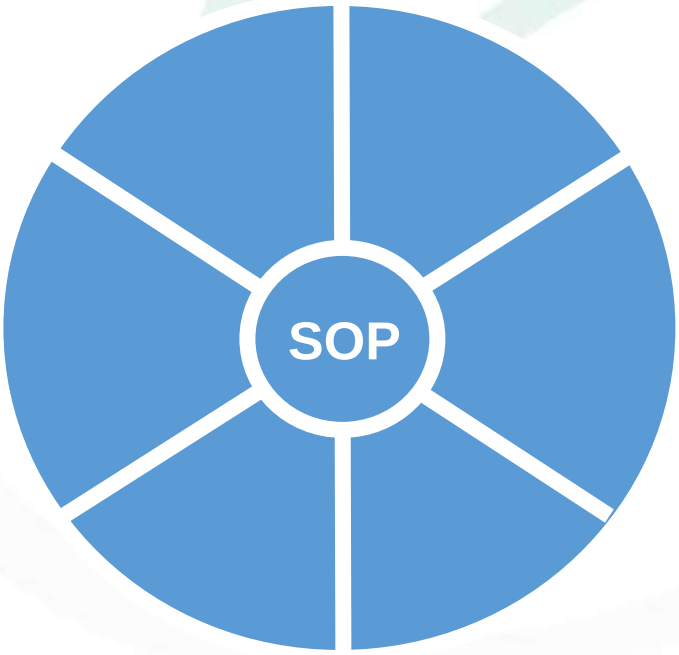
آزمایشگاه جامع تحقیقات
دانشگاه علوم پزشکی تبریز

Real Time polymerase chain reaction (RT-PCR)



Workflow

1) RNA extraction → 2) cDNA synthesis (RT-PCR) → 3) Real-Time PCR → Analyze

* A real-time PCR is a laboratory technique in molecular biology that it is an advanced form of the Polymerase Chain Reaction (PCR). It provides multiple copies of a rare piece of DNA. Changing temperature will control the activity of thermostable polymerase to working faster.

Specimen

Type: RNA and DNA Extraction from Blood and Tissue (Samples must be stored in a -80°C freezer)

Materials

Reagents (including catalog No. and storage conditions)

Oligonucleotide Primers	Sigma-Aldrich-Catalog NO - 25322-68-3 We recommend keeping oligonucleotides at 4 °C for short-term use (stable for approximately 1 year) and at -20 °C for long-term storage (stable for approximately 2 years).
Total RNA and DNA master	Sigma-Aldrich-Catalog NO- M7501 It is stored at room temperature
SYBR Green PCR master mix	Applied Biosystems-Catalog No- 4309155 It is stored at room temperature
RNase	Thermo Scientific-Catalog NO: EN0531 It is stored at -20°C.temperature

Equipment and supplies:

96 well Micro plate

Optical tube

cap strips

primers and probes

spin

strip racks

Safety (Warning and Biohazard consideration):

- Identify potential biohazards, chemical hazards and unsafe situations
- Know where to get safety and emergency information about chemicals in the lab
- Protect yourself and students from chemicals and potential biohazards
- Maintain a safe lab environment

Procedure:

Detailed procedure

Step1:

First design your primers using the following eSource.

Find your genome from: http://www.ncbi.nlm.nih.gov/genome.

Principles of designing primer.

-Optimal amplicon length 18-25 bp

-Predicted primer-dimmer formation

-Predicted hairpins in the amplicon

-Free tools at: http://primer3.ut.ee

Step2:

Prepare materials as below and start pippeting

Real-time PCR reaction mixture per tube

- cDNA sample 1µL
- Master mix (2X) 10 µL
- Primer Forward 0.05 µL — Total Vol. 20 µL
- Primer Reverse 0.05 µL
- DEPC-treated water 8.9 µL

Note: Place Master Mixon ice.

Step3:

-Centrifuge micro tubes.

-Make sure that all bubbles are removed. *Denaturation* (Room Temp -95)_ *Annealing* (95-50)_ *Extension* (50-72)

Step4:

Set-up your plate.

Cycles of real-time PCR:

1.50°C 2 min, 1 cycle

2.95°C 10 min, 1 cycle

3.95°C 15 s 60°C 30 s 72°C 30 s, 40 cycles

4.72°C 10 min, 1 cycle

Note: Annealing temperature will be different in each primer.

Data analysis

The amount of DNA theoretically doubles with every cycle of PCR (exponential reaction). The reaction finally tails off and reaches a plateau. After two cycles we have 2 ×2 times as much. After N cycles we shall have 2Ntimes as much.(Real-time graphs are for Miss Malekian, pharmacy student at Tabriz University of medical sciences

References:

-Varkonyi-Gasic E, Wu R, Wood M, Walton EF, Hellens RP. Protocol: a highly sensitive RT-PCR method for detection and quantification of microRNAs. Plant methods. 2007 Dec;3(1):1-2.

- Shaw AE, Reid SM, Ebert K, Hutchings GH, Ferris NP, King DP. Implementation of a one-step real-time RT-PCR protocol for diagnosis of foot-and-mouth disease. Journal of virological methods. 2007 Jul 1;143(1):81-5.

شناسنامه سند: CoreLab.108

نام سند	پروتکل استاندارد Real-Time PCR
تاریخ صدور	۱۳۹۹/7/22
نام کامل فایل	پروتکل استاندارد Real-Time PCR
شرح سند	روش انجام استاندارد Real-Time PCR واقع در آزمایشگاه جامع تحقیقات را شرح می‌دهد.
تهیه کننده	حسین محمدزاده