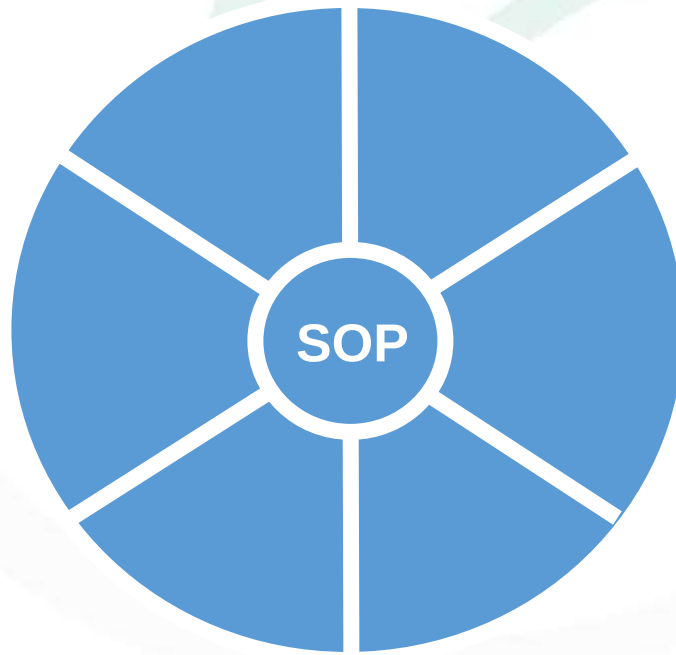
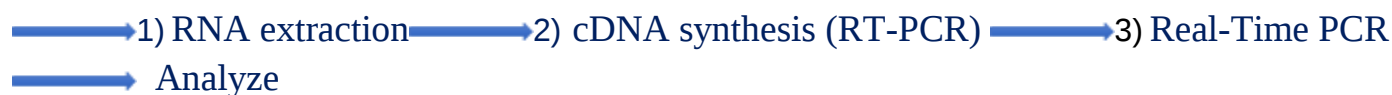


## *Real Time polymerase chain reaction (RT-PCR)*



## Workflow



\* 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<br>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<br>It is stored at room temperature                                                                                                                                                 |
| SYBR Green PCR master mix | Applied Biosystems-Catalog No- 4309155<br>It is stored at room temperature                                                                                                                                          |
| RNase                     | Thermo Scientific-Catalog NO: EN0531<br>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-dimer formation
- Predicted hairpins in the amplicon
- Free tools at: <http://primer3.ut.ee>

##### Step2:

Prepare materials as below and start pipetting

Real-time PCR reaction mixture per tube

- |                                  |                       |
|----------------------------------|-----------------------|
| - cDNA sample 1 $\mu$ L          |                       |
| - Master mix (2X) 10 $\mu$ L     |                       |
| - Primer Forward 0.05 $\mu$ L    | Total Vol. 20 $\mu$ L |
| - Primer Reverse 0.05 $\mu$ L    |                       |
| - DEPC-treated water 8.9 $\mu$ L |                       |

Note: Place Master Mix on 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  $2 \times 2$  times as much. After N cycles we shall have  $2^N$  times 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.

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شناسنامه سند: CoreLab.108

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|---------------|---------------------------------------------------------------------------------|
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| تاریخ صدور    | ۱۳۹۹/۷/۲۲                                                                       |
| 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                                                                   |

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