

# Real-time PCR internship (20 hrs.)

Real time PCR or qPCR, is an advance form of polymerase chain reaction that allows for the simultaneous amplification and quantification of specific DNA sequences.

To measure specific gene expression of different control/treatment samples, first the interest target molecules have to be extracted.

## 1. EXTRACTIONS

This is the first step in the process and involves extracting the desired molecules (DNA, RNA, or protein) from the samples.

### A. DNA

- i. Strawberry DNA extraction via reagents
- ii. Tissue DNA extraction via TRIzol reagents
- iii. Tissue DNA extraction via Kit

### B. RNA

- i. Tissue RNA extraction via TRIzol reagents
- ii. Tissue RNA extraction via Kit

### C. Protein

- i. Tissue protein extraction via TRIzol reagents

## 2. cDNA SYNTHESIS

This is the process of converting RNA into complementary DNA (cDNA) using reverse transcriptase.

### A. Converting RNA to cDNA via kit and PCR

### B. PCR setup and running

## 3. REAL TIME PCR

This involves using a real-time thermal cycler to monitor the PCR reaction as it progresses, allowing for more accurate and precise quantification of the target DNA sequences.

### A. Real time PCR setup and optimization: Design primers and optimize PCR conditions.

- B. Real Time PCR reaction setup and running: Prepare samples and run Real Time PCR reactions.
- C. Data analysis: Analyze the Real Time PCR data to quantify target DNA sequences.

#### 4. PCR

This is the process of amplifying specific DNA sequences using a thermal cycler.

- A. PCR setup and running

#### 5. NANODROP

This is a spectrophotometer that can be used to measure the concentration and purity of the extracted molecules.

- A. Verify the quantity and purity of molecules by the spectrophotometer

#### 6. GEL ELECTROPHORESIS

This is a technique used to separate DNA fragments by size, which can be used to visualize the results of the PCR reaction.

- A. DNA gel electrophoresis
  - i. Agarose gel preparing and stain it by safe stain
  - ii. Preparing the samples by adding DNA to loading dye
  - iii. Run the gel electrophoresis and visualize it by UV light

#### 7. DATA ANALYSIS

- A. This involves analyzing the data from the Real Time
  - i. Increased or decreased fold change
- B. This involves post PCR analysis
  - i. Gel electrophoresis bands
  - ii. Melting curves

After registration, more information about reference materials and videos you need to study and watch, will send your email.