

4. Real Time PCR and data analysis workshop

Course details

Lecture 1: Central dogma of molecular biology

- Introduction to important macromolecules: DNA, RNA & Protein
- Replication, transcription and translation-overview

Lecture 2: Polymerase Chain reaction

- Introduction and history
- Importance and advantages
- PCR concept, PCR process and PCR program preparation
- PCR reaction components and their function
- PCR reaction set up
- PCR controls and troubleshooting
- PCR machine: software and hardware function

Lecture 3: Primer designing

- Important considerations for primer designing
 - Melting temperature
 - DNA stability
- Primer designing with online tools

Lecture 4: Reverse Transcriptase Polymerase Chain Reaction (RTPCR)

- Introduction and importance
- RNA extraction and quantitation
- DNase treatment,
- Function and protocol
- CDNA synthesis concept and protocol
- CDNA reaction setup
- RTPCR introduction and advantages
- Concept and protocol

Lecture 5: Traditional verses Realtime PCR

- Limitation of traditional PCR
- Advantages of Realtime PCR
- Realtime PCR detection chemistries
 - SYBR Green- concept & mechanism
 - TaqMan prob-concept & mechanism

Lecture 6: qRTPCR experimental setup and workflow

- Program preparation
- Plate map preparation
- Reaction setup
- Controls and troubleshooting

Lecture 7: MIQE guidelines

- Minimum requirements for qRTPCR for publication
- Technical controls

- Biological controls
- PCR efficiency

Lecture 8: qRTPCR data analysis

- Standard curve preparation
- PCR efficiency calculation
- Gene expression data analysis using $\Delta\Delta C_t$ method