

# PCR(qPCR & RT-PCR) THEORY, OPERATION AND TROUBLESHOOTING

9<sup>th</sup> - 13<sup>th</sup>  
MARCH 2026

## Course overview

This 5-day training is designed to provide a solid understanding of specific topics through presentation and laboratory work. Participants will gain significant experience in the performance of laboratory techniques taught in this PCR training. Through integrated learning methods, utilizing hands-on training to reinforce lecture material, participants will be able to apply information learned into applications in their own laboratories.

During this training participants will learn the process of amplification by learning theory and techniques for PCR. Following this training, participants will be able to perform PCR reaction in their own laboratories, troubleshoot experiments, design primers and determine reaction conditions. We will cover critical requirements for amplification, thermostable DNA polymerases, reverse transcriptase reactions, cloning of PCR products, primer design and mutagenic PCR.

## Suitability

This course is suitable for researchers, scientists, laboratory analysts, graduate students and postgraduate students who have a background in cell/molecular biology, biochemistry, biotechnology and those who are interested in learning more about PCR operation.

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Day 1 9-03-26	EVENTS
09:00 – 10:00 am	<b>Orientation and Climate Setting.</b>
10:00 – 10:30 am	<b>Introduction to PCR: Principles and Applications</b> <ul style="list-style-type: none"> <li>Central dogma and nucleic acid basics</li> <li>Concept, mechanism &amp; components of PCR reaction (template, primers, polymerase, buffer, dNTPs)</li> <li>PCR applications (pathogen detection, gene expression, genotyping)</li> <li>Overview of endpoint PCR, qPCR and RT-PCR evolution and their possibilities</li> </ul>
10:30 – 11:00 am	<b>TEA- BREAK</b>
11:00 – 12:30 p.m	<b>PCR Chemistry and Reaction Design</b> <ul style="list-style-type: none"> <li>Primer, probe design (TaqMan, SYBR Green, molecular beacons) and specificity</li> <li>Avoiding Primer-dimer formation</li> <li>Optimization of MgCl<sub>2</sub>, dNTPs, enzyme, and buffer concentrations</li> <li>Contamination control, good pipetting practices &amp; Use of master mixes</li> </ul>
12:30 – 14:00 p.m	<b>LUNCH - BREAK</b>
14:00 – 16:30 p.m	<ul style="list-style-type: none"> <li>Sample preparation and DNA extraction</li> </ul>
Day 2 10-03-26	
09:00 – 10:30 am	<ul style="list-style-type: none"> <li>Cycle optimization (temperature &amp; time).</li> <li>Amplicon analysis by agarose gel electrophoresis, including gel preparation, band visualization, documentation, and gel image analysis</li> </ul>

10:30 – 11:00 am	<b>TEA- BREAK</b>
11:00 – 12:30 p.m	<b>Introduction to Real-Time PCR (qPCR)</b> <ul style="list-style-type: none"> <li>Real-time PCR instrumentation overview</li> <li>Fluorescent chemistries (SYBR Green, TaqMan probes)</li> <li>Quantification approaches (absolute vs. relative), Instrument platforms and detection technologies</li> </ul>
12:30 – 14:00 p.m	<b>LUNCH - BREAK</b>
14:00 – 16:30 p.m	<ul style="list-style-type: none"> <li>Reaction setup and operation in qPCR software</li> <li>Calibration and standard curve generation, and amplification efficiency</li> </ul>
<b>Day 3 11-03-26</b>	<b>EVENTS</b>
09:00 – 10:30 am	<b>Hands-On qPCR set up and Data Analysis</b> <ul style="list-style-type: none"> <li>qPCR set up and operations</li> <li>Programming cycling and amplification profiles</li> </ul>
10:30 – 11:00 am	<b>TEA- BREAK</b>
11:00 – 12:30 p.m	<ul style="list-style-type: none"> <li>Data acquisition, threshold settings &amp; Analysis of amplification, melt curves, and Cq values</li> <li>Generating standard curves and efficiency calculations</li> </ul>
12:30 – 14:00 p.m	<b>LUNCH - BREAK</b>
14:00 – 15:30 p.m	<b>Reverse Transcription PCR (RT-PCR)</b> <ul style="list-style-type: none"> <li>RNA extraction and quality/integrity assessment</li> <li>Reverse transcription enzymes and protocols/reaction setup</li> </ul>
<b>Day 4 12-03-26</b>	
09:00 – 10:30 am	<ul style="list-style-type: none"> <li>One-step vs. two-step RT-PCR workflows</li> <li>Quantitative RT-PCR applications (gene expression profiling)</li> <li>RT Priming methods, RNA integrity and DNase treatment</li> </ul>
10:00 – 10:30 am	<b>TEA- BREAK</b>
11:00 – 12:30 p.m	<b>Quantification, Normalization, and Data Validation</b> <ul style="list-style-type: none"> <li>Reference genes and relative quantification (<math>\Delta\Delta Cq</math> method)</li> <li>Replicates and statistical reliability</li> <li>Data export and analysis using qPCR software, Interpretation of gene expression results &amp; Reporting and documentation standards</li> </ul>

12:30 – 14:00 p.m	<b>LUNCH - BREAK</b>
14:00 – 15:30 p.m	<b>Troubleshooting and Quality Assurance in PCR</b> <ul style="list-style-type: none"> <li>Troubleshooting failed reactions: no bands, multiple bands, weak signal, no amplification</li> <li>Addressing contamination, primer-dimers and inhibition</li> <li>Controls: positive, negative, NTC &amp; Preventing false positives/negatives</li> </ul>
<b>Day 5 13-03-26</b>	<b>EVENTS</b>
09:00 – 10:30 am	<ul style="list-style-type: none"> <li>Quality assurance and internal QC practices</li> <li>Documentation per ISO 15189/17025 standards</li> <li>Internal and External quality controls</li> </ul>
10:30 – 11:00 am	<b>TEA- BREAK</b>
11:00 – 12:30 p.m	<b>Instrument Operation, Calibration, and Maintenance</b> <ul style="list-style-type: none"> <li>Thermocycler models and calibration, verification &amp; the procedures</li> <li>Temperature uniformity testing</li> <li>Preventive maintenance and service logs</li> <li>Safety, biosafety and equipment handling protocols</li> </ul>
12:30 – 14:00 p.m	<b>LUNCH - BREAK</b>
	<b>Review, Assessment and Certification</b> <ul style="list-style-type: none"> <li>Review session and Q&amp;A</li> <li>Case study analysis and group discussion</li> </ul>
	<b>Closing ceremony and issuance of certificates</b>

Deadline: 28<sup>th</sup> Feb 2026  
**9<sup>th</sup> - 13<sup>th</sup> MARCH 2026**

**Cost Kes. 125,000.00  
or USD 1,200.00  
exclusive of taxes**

**KISUMU**

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