AfricaCDC
Centres for Disease Control and Prevention

Safeguarding Africa's Health

Module W04

Wet-lab Foundational Course

Introduction to Basic PCR Techniques

NGS Academy for the Africa CDC

ASLM
AFRICAN SOCIETY FOR LABORATORY MEDICINE

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Pan African Bioinformatics Network for H3Africa

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Module W04

Basic Molecular Techniques for NGS Implementation

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Module last updated:
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Number of sessions	5–6
Total learning time	1–1.5 days
Target audience	Wet laboratory personnel (i.e., scientists, laboratory technicians, etc.)
Format	Lectures, videos, practicals/tutorials
Level of the module	Introductory

Contributors

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Recommended prerequisite module(s)

- Module G06. Introduction to Genomic Sequencing and NGS
- Module W01. Introduction to Biosafety and Biosecurity
- Module W02. Basic Laboratory Requirements and Operational Setup for NGS Implementation
- Module W03. Introduction to Fundamental Laboratory Skills and Calculations

Module description

Previous modules established key foundations for NGS implementation: module W01 introduced laboratory biosafety and biosecurity principles, module G06 explored NGS methodologies and applications, module W02 covered laboratory requirements and operational setup, and module W03 addressed fundamental laboratory calculations and skills. Building on these foundations, this module advances into practical molecular techniques required for NGS applications, as it applies to pathogen genomic surveillance. Processes from nucleic acid extraction and PCR applications to NGS are covered. Furthermore, the practical aspects of quality control procedures, various analytical platforms, and both Oxford Nanopore and Illumina NGS workflows are explored, enabling participants to perform accurate pathogen detection, characterization, and outbreak monitoring. In this module, participants are introduced to the following topics and/or concepts:

- Different nucleic acid extraction methods[1] for NGS applications
- The fundamentals principles of spectrophotometry (i.e., Nanodrop) and fluorometry (i.e., Qubit) for nucleic acid quantification
- Interpreting different nucleic acid ratios
- The applications of capillary electrophoresis and Agilent TapeStation in nucleic acid analysis
- Overview of the fundamental principles of PCR
- The basic workflow of a typical PCR experiment
- Different PCR types (such as conventional PCR, qPCR, RT-PCR, digital PCR and multiplex PCR) and their applications in an NGS workflow
- A comparison between (NGS) and qPCR technologies
- The setup and preparation of PCR reactions
- The role and application of high proofreading Taq polymerases in PCR
- Different types of primers and their applications in NGS
- Primer design for standard versus multiplex PCR
- Key principles of different electrophoresis methods for separating and analysing PCR products
  - Gel electrophoresis, capillary electrophoresis and automated electrophoresis (i.e., Agilent Tapestation)
- Overview of these electrophoresis techniques
- The significance of nucleic acid fragment size selection in an NGS workflow
- PCR optimization, troubleshooting and quality control metrics
- Sample collection, storage, preparation, and quality control for mNGS
- Oxford Nanopore Technologies (ONT) Workflow for mNGS:
  - mNGS protocols
  - Nucleic sequencing with the MinION Flow Cell
- Illumina Workflow for mNGS:
  - mNGS protocols
  - Library preparation and quality control for mNGS
  - Nucleic sequencing with iSeq and MiSeq platforms
- Sequencing run/data quality control

Module learning outcomes

On completion of this module, the participants will have a basic knowledge of, or will be able to:

- Discuss the fundamental principles of spectrophotometry and fluorometry
- Explain how spectrophotometric (Nanodrop) and fluorometric (Qubit) methods are used to accurately quantify nucleic acids
- Describe and interpret the significance of different nucleic acid ratios in assessing nucleic acid purity and quality
- Explain how capillary electrophoresis versus Agilent TapeStation can be used to perform quality control checks on nucleic acids
- Explain the fundamental principles of Polymerase Chain Reaction (PCR) and the role of key components such as DNA templates, primers, nucleotides, and Taq polymerase.
- Explain the importance of high proofreading Taq polymerases for NGS
- Explain the differences between conventional PCR, qPCR, and RT-PCR, including their respective uses in detecting, quantifying, and analysing nucleic acids.

1 Note, pathogen-specific extraction methods are covered in the various pathogen surveillance courses

- Understand the concept of ct value
- Extract high-quality DNA or RNA using a specified method, understanding the pros and cons of each technique.
- Prepare a complete PCR reaction mix with minimal error and successfully set up the reaction for amplification.
- Analyse PCR products using gel electrophoresis and understand the role of staining and visualisation in detecting nucleic acids.
- Apply different types of PCR (e.g., quantitative PCR, RT-PCR, qPCR) in research and diagnostics.
- Design a set of primers for targets and/or target regions

Module assessments

Module practical: Practical assessment available
Module quiz: Assessment questions available on the ASLM platform

Module resources

- ONT Video - How to extract high-quality DNA and RNA
- MRI Global | RNA Extraction using the QIAamp Viral RNA Mini Kit (Qiagen)
- NIH | NLM Nucleic Acid Extraction and Enrichment
- US CDC | Videos - Basic Molecular Biology: Nucleic Acid Extraction
  - Organic Extraction
  - Liquid Phase Extraction
  - Column-Based Extraction
  - Magnetic Bead-Based Extraction
- NIH | NLM: Article - A new, simple, highly scalable, and efficient protocol for genomic DNA extraction from diverse plant taxa
- WIPO | Methods and reagents for enrichment of nucleic acid material for sequencing applications and other nucleic acid material interrogations
- BioNetwork | Video - How does a spectrophotometer work?
- Bio-Rad | Video - How To Perform DNA Quantitation Using a Spectrophotometer
- Edmerls | Video - Describe the instrumentation of Fluorometry?
- Protocols.io | GitHub - DNA Quantification using the Qubit Fluorometer
- Thermo Fisher Scientific | Video - Quantitation: Comparing Qubit fluorometer to the Nanodrop One UV/Vis absorbance
- Plos One | Article - Comparison of DeNovix, NanoDrop and Qubit for DNA quantification and impurity detection of bacterial DNA extracts
- Promega Corporation | Fluorometric DNA Quantification with the QuantiFluor® ONE dsDNA System
- Diagnostech | Agilent TapeStation Applications Jan2021 Video Lecture - Sample QC and DNA/RNA applications

- Thermo Fisher Scientific | Article - Interpretation of Nucleic Acid 260/280 Ratios
- Biology Lectures | Nanodrop Ratios Explained
- Thermo Fisher Scientific | Video - PCR reaction types and applications
- NIH | NLM: Article - Research Techniques Made Simple: Polymerase Chain Reaction (PCR)
- USDA APHIS | PCR Resources - Video Series
- Illumina | A comparison between (NGS) and qPCR technologies
- US CDC | Video - Basic Molecular Biology: PCR and Real-Time PCR – Principle of PCR
- CHS Protocols | Polymerase Chain Reaction
- Seeding Labs | Video - How To: PCR Calculations
- ASM | Video - Connecting the Genome to Molecular Diagnostics
- Addgene | Video - How To: Design Primers for PCR
- Thermo Fisher Scientific | Video - Designing PCR and Sanger Sequencing Primers
- University of Glasgow | Molecular Biology Explained
  - Restriction Mapping
  - PCR Primer Design
- NIH | NLM - Workshop Resources: How BLAST Works & Using Web BLAST Effectively
  - Slides
  - Video
- SnapGene | Video - Create Primers and Simulate PCR in SnapGene
- IDT | Video - Design PCR Primers in a Region with PrimerQuest
- IDT | Video - How to use the IDT OligoAnalyzer™ Tool
- BMH Learning | Video - Degenerate PCR | Degenerate Primers
- Henrik's Lab | Video - cDNA Synthesis Protocol by Reverse Transcription
- NIH | NLM: Article - NGS-PrimerPlex: High-throughput primer design for multiplex polymerase chain reactions
- Aakechin | GitHub - NGS-PrimerPlex
- Bioconductor | Designing and analyzing multiplex PCR primers with openPrimeR
- SCFBM | Article - PrimerView: high-throughput primer design and visualization
- Wingolab | GitHub - MPD: Multiplex PCR Design
- Wellcome Connecting Science Learning and Training | Video - How to set up a PCR
- FAO of The UN | Video - PCR Master Mix preparation and RT-PCR
- Hands-on DNA | Video - Preparing the PCR primer stock and mixes
- Seeding Labs | Video - Gel Electrophoresis Buffers for Nucleic Acids
- Thermo Fisher Scientific | Video - Understanding real-time PCR terminology
- Thermo Fisher Scientific | Video - Phases of real-time PCR and why they're important
- Thermo Fisher Scientific | Video - What are Ct values in real-time PCR?
- NIH | NLM: Article - Electrophoresis
- NIH | NLM: Article - Agarose Gel Electrophoresis for the Separation of DNA Fragments
- Wellcome Connecting Science Learning and Training | Video - How to run an agarose gel
- US CDC | Video - Basic Molecular Biology: Nucleic Acid Extraction – Gel Electrophoresis
- Protocols.io | Gel Electrophoresis Protocols
- Protocols.io | Capillary Electrophoresis Protocols
- OpenUCT | Manual of SDS-PAGE and Immunoblotting Techniques
- ScienceDirect | Article - Analysis of therapeutic nucleic acids by capillary electrophoresis

- Diagnostech | Agilent TapeStation Applications Jan2021 Video Lecture - Sample QC and DNA/RNA applications
- BioRxiv | Article - A comparison of DNA stains and staining methods for Agarose Gel Electrophoresis
- NIH | NLM: Article - A low-cost smart system for electrophoresis-based nucleic acids detection at the visible spectrum
- US CDC | Video - Basic Molecular Biology: PCR and Real-Time PCR – RT-PCR Fluorescent Probe-Based Detection
- Nicole Lantz | Video - How to Interpret a Gel
- NIH | NLM: Article - Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies
- CZ Biohub | Sample collection, storage and preparation for mNGS
- CZ Biohub | Sample QC
- Oxford Nanopore Technologies (ONT) Workflow:
  - ONT | Loading samples onto a Oxford Nanopore Technologies MinION Flow Cell
  - ONT | Starting the Oxford Nanopore Technologies MinKNOW Control Software
  - ONT | Oxford Nanopore Technologies mNGS protocols
- Illumina Workflow:
  - Illumina | iSeq 100 Loading Guide
  - Illumina | MiSeq Loading
- CZ Biohub | Sequencing run/data quality control

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