**Genomics Training Program**

Genomics Core Facility

Hamad Bin Khalifa University (HBKU)

**Hands-on training in the Next Generation Sequencing technology**

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Prepared by: Dr Khalid Ouararhni – Genomics Core manager (HBKU)

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**Training Overview:**

Next-Generation Sequencing "NGS is an advanced sequencing technology that offers ultra-high throughput, scalability, and speed sequencing of nucleic acids in biological systems at a level never before possible.  Today's complex genomics questions demand a depth of information beyond the capacity of traditional DNA sequencing technologies. NGS has filled that gap and become an everyday tool to address these questions. The next generation sequencing can adreess complex biological problems in different biomedical fields such as cancer biology, metabolic disorders and neurological diseases.

This course will provide an overview of different NGS applications, all methodology aspects of NGS library preparation, NGS sequencing technology and bioinformatics data analysis. Participants will receive hands on training  on a range of NGS methodologies in the laboratory. Techniques include quantification and quality assessment of the nucleic acid, NGS library preparation and sequencing.

**Teaching Methods:**

- • Lectures.
- • Hands-on training in the laboratory.

**Assessment:**

At the end of the training, each student must provide a laboratory report. Typical components of the report are the summary, introduction, material, methods, data, and discussion/conclusion.

**Laboratory Supervisors:**

- • Dr Khalid Ouararhni
- • Dr Thasni Abdi Azis
- • Ms Somayyeh Abbasi

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**Programme**:

The proposed experiments will be performed at HBKU core genomics lab

**Day 1**

- Explore the genomics core facility and take a tour of the different research centers and facilities in QBRI.
- Mastering Pipetting Techniques.
  - Good practice in the use of different tabletop instruments

**Day 2**
**Lecture:** Introduction to genomics (Sample processing)

**Lab training:**

- Quantifying RNA (spectrophotometer / Qubit fluorometer)
- Bioanalyzer setup (QC)
- Bioanalyzer evaluation

**Day 3**
**Lecture: Library preparation**

**Lab Training:**
- Purify and Fragment Messenger RNA
  1. Capture mRNA
  2. Clean Up mRNA
  3. Fragment and Denature mRNA
- Synthesize First Stranded cDNA
- Synthesize Second Stranded cDNA
  1. Generate cDNA
  2. Clean Up cDNA

**Day 3**
**Lab training:**

- Adenylate 3' Ends
- Ligate Anchors
- Bead Clean Up Fragments
- Amplify Library
- Bead Clean Up Library

**Day 4**
**Lab training:**

- Amplify Library
- Bead Clean Up Library

**Day 5**
**Lab training:**

- Library quantification
- Bioanalyzer setup (QC)
- Bioanalyzer evaluation
- Samples pooling
- Dilute Library to the starting Concentration

**Day 6**
**Lab training:**

- Samples pooling
- Dilute Library to the starting Concentration

**Day 6**
**Lecture: Sequencing – 1h**

**Lab Training:**
- Sample sheet preparation
- NextSeq 2000 Setup
- Load library and starting sequencing run

**Day 7**
**Lecture: Analysis with DRAGEN**

- FastQ Generation
- Data analysis

**Day 8**
**Lab training: Sanger Sequencing**

- PCR Clean-up
- Sanger Sequencing

**Day 9**

- Data analysis

**Day 10**

Equipment:

- Qubit Fluorometer
- NanoDrop 2000
- Agilent 2100 Bioanalyzer
- Thermocycler system
- Centrifuge
- NextSeq2000 system
- Shaker

**Reagents:**

- NextSeq2000 (100 cycles)
- PhiX
- Truseq Stranded mRNA library
- TruSeq RNA single indexes A or B
- SuperScript II Reverse Transcriptase
- AMPureXPbeads
- Qubit dsDNA HS Assay Kit
- Bioanalyzer High Sensitivity DNA Analysis
- Bionalyzer RNA 6000 Nano Kit