CM581A2: NEXT GENERATION SEQUENCING PLATFORMS AND LIBRARY GENERATION

Fall 2015

Instructors:

Coordinator: Carol Wilusz, Associate Professor MIP, CMB
Instructor: Dan Sloan, Assistant Professor, Biology, CMB
Instructor: Mark Stenglein, Assistant Professor, MIP, CMB

Required Texts and Materials:

No required texts. Students will require access to LabArchives Electronic Lab Notebook which can be purchased for $15 per semester.

Description:

Graduate students from both biological and computational/mathematical fields will be trained in the theoretical and experimental aspects of next generation sequencing experiments with a focus on the Illumina platform.

Objectives:

Students will be able to:

- Tell the history of DNA sequencing, highlighting the technological advances that have allowed rapid, large-scale sequencing of genomes and transcriptomes.
- Describe the differences between Sanger sequencing, sequencing-by-synthesis (e.g. Illumina) and direct sequencing (e.g. PacBio, Oxford Nanopore)
- Describe in detail the steps to generate a library of DNA fragments for sequencing starting from isolation of genomic DNA including quality control, fragmentation, adapter ligation and amplification
- Highlight how preparation of libraries from total RNA differs from the protocols used for genomic DNA
- Describe how and why rRNA is removed from total RNA prior to RNA-seq
- Explain how and why strand-specific RNA sequencing is employed
- Describe differences in library preparation for eukaryotic versus prokaryotic nucleic acids
- Tell how enrichment strategies and barcoding work, and how they can reduce the costs of DNA sequencing and analysis
- Give details on the steps of an Illumina sequencing reaction and tell how fluorescent output is generated and converted to sequence information
- List multiple applications of NGS sequencing and how library preparation should be modified for each
- Perform library preparation and submit samples for Illumina sequencing
- Troubleshoot common problems arising during library preparation and sequencing

Pre-requisites:

Life sciences BS degree with solid background in molecular biology, or CM000 Introduction to Nucleic Acids (experimental course) or equivalent.

Credit Hours: 1 Credit

Class Breakdown:

This is a 1CR lab class offered over 4 weeks with 8 hr per week of contact time. This course is designed to require an average of 4 hours per week of outside work (preparation, problem sheets, reading, online lectures, quizzes etc). Students with weaker biology backgrounds may need to spend more time preparing for class and/or completing homework.

Teaching Strategies:

Labs, presentations on theory behind experiments, quizzes, problem sets, online lectures, practice through problem solving.

Evaluation:

Laboratory Notebook	50%
Online Quizzes	10%
Problem Sets	15%
Attendance/Participation	5%
Final Exam	20%
TOTAL	100%

Grading Scale:

A	90-100
B	80-89
C	70-79
D	60-69
F	59 & below

This class does not issue grades of + or –

WEEK 1

Day 1

PRESENTATION: Introduction to CM581A2 (30 min)
- Overview, objectives, expectations, grading, academic integrity

Introduction to LabArchives Electronic Lab Notebook (20 min)

Introduction to NGS (50 min)
- History of DNA Sequencing
  - Sanger Sequencing (Gel, Capillary)
  - Transition to NGS
- Applications and Limitations of NGS
  - WGS, RNA-Seq, CHIP-Seq, Metagenomics
- Brief overview of Illumina Sequencing-by-Synthesis including library generation

LAB: Isolation of genomic DNA from a prokaryote
- Lysis, centrifugation, RNAse treatment, column purification (~ 1hr 40 min)

HOMEWORK: LabArchives Set-Up (30 min)

Isolation and Quality Control of Genomic DNA (1 hr video lecture online)
- DNA isolation from prokaryotic, eukaryotic cells
  - Lysis
  - RNA removal
  - Organic Extraction & Precipitation
- Quantification & Quality Control:
  - Fluorometric
  - qPCR
  - Nanodrop (limitations)

Quiz 1 (30 min)

Day 2

LAB: Quality control and quantification of genomic DNA (1 hr)
- Fluorometric quantification
- Nanodrop

DNA fragmentation and Quality Control (2hr)
- Covaris, Tapestation

PRESENTATION: Library Generation for Illumina Genome Sequencing (1hr)

Part 1: DNA isolation & fragmentation (30 min)
- Covaris, Enzymatic, Nextera Transposons

Quality Control
- TapeStation, Bioanalyzer, qPCR, nanodrop, fluorometry

Part 2: Adaptors, Amplification (30 min)

Size Selection
- SPRI-beads, Gels, PippinPrep

Pre-run QC and Quantification
- Gels, TapeStation, Bioanalyzer, qPCR, nanodrop, fluorometry
- Cloning and Sanger Sequencing

HOMEWORK: LabArchives Recordkeeping (30 min)

Sequencing Platforms (1hr online video)
- Illumina Sequencing By Synthesis
  - How it works
  - Differences between MiSeq and HiSeq and NextSeq
- Other technologies – advantages and limitations
  - Current technologies – Pacbio, Ion Torrent

Coming Soon - Oxford Nanopore

Quiz 2 (30 min)

WEEK 2

Day 1

LAB: Adapter Ligation, Amplification, Size Selection (2-3 hr)
- Nextera tagmentation reactions
- Post-Nextera cleanup
- Setup and run adapter build out PCRs

PRESENTATION: Optimizing your NGS Experiment (1 hr 40 min)
- Cost and Time Considerations
  - Library Prep
  - Sequencing
  - Analysis
- Making Mapping Reads Easier
  - Read length
  - Single end versus paired end reads
  - Mate pairs
- Controlling Coverage
  - Barcoding and Pooling Strategies
    - Moleculo
- Troubleshooting
  - Low complexity libraries
  - Read length
  - Errors
  - Amplification Bias
    - Polymerase choice
    - # of cycles
    - Avoiding amplification
  - Misidentification of barcodes
    - Double barcoding
  - Contamination

HOMEWORK: LabArchives Recordkeeping (30 min)

Problem Set 1 (1 hr 30 min)

Day 2

LAB: Library size selection and pooling (3 hr)
- Size select libraries (by gel).
- Quantification of individual libraries for pooling (by qubit)
- Pool libraries. Cleanup pool.
- Additional PCR of final pool may be necessary depending on concentration…

PRESENTATION: RNA-Seq (50 min)
- RNA isolation and quality control
- rRNA depletion
  - poly(A) selection
  - RiboZero
- Fragmentation
  - Physical, Enzymatic, Chemical, Nextera
- Reverse transcription
- Adapter Ligation, Amplification, Size Selection, QC (same as for DNA, so brief)

Strand-specific RNA seq

PRACTICE: Problem Set 1 (50 min)

HOMEWORK: Quiz 3 (30 min)
Problem Set 1 (30 min)
Reading: (1hr)
Mamanova *et al*. Target-enrichment strategies for next-generation sequencing. Nat Methods. 2010; 7(2):111-8 PubMed PMID: 20111037.

WEEK 3

Day 1

LAB: Quality Control and quantification of final Libraries (2 hr)
- Kapa quant of final pooled library
- Gel of final pooled library (?)
- Preparation of sample sheet for MiSeq

PRESENTATION: Target Enrichment (1hr)
Enrichment vs subtraction
- Enrichment methods
  - Capture-based enrichment
    - Exome sequencing
    - [polyA capture]
  - PCR-based enrichment (amplicon sequencing)
    - 16S rRNA seq and the microbiome
  - Affinity-based enrichment
    - RIP-Seq/ChIP-Seq
    - Ribosome profiling
- Subtraction methods
  - [RiboZero]
  - RNaseH-mediated depletion
  - DSN-seq/hydroxyapatite (for normalization)

PRACTICE: Problem Set 2 (30 min)

HOMEWORK: LabArchives Record Keeping (30 min)
Quiz 4 (30 min)
Problem Set 2 (1hr)

Day 2

LAB: Illumina MiSeq Laboratory Visit (2hr)

PRESENTATION: Experimental Design and Validation of Results (50 min)
Importance of planning ahead - defining the experimental question and analysis strategy
Controls – yes, you need them! Examples
Replicates - Biological versus technical replicates
Validation of NGS results

PRACTICE: Problem Set 2 (50 min)

HOMEWORK: Reading:
- Sims *et al*. Sequencing depth and coverage: key considerations in genomic analyses. Nat Rev Genet. 2014; 15(2):121-32. PMID: 24434847.
- Fang Z, Cui X. Design and validation issues in RNA-seq experiments. Brief Bioinform. 2011;12(3):280-7. PMID: 21498551

Problem Set 2: (30 min)

Quiz 5: (30 min)

WEEK 4

Day 1

PRESENTATION: Applications of NGS (1hr 40 min)
Variant detection
Structural variation detection
Microbial (16S) metagenomics
Shotgun metagenomics
Genotyping-by-sequencing
Tn-Seq
Host/Pathogen sequencing
3C-Seq
RAD-seq & reduced representation
Small RNA-seq

PRACTICE: Practice Exam Questions (1hr 40 min)

HOMEWORK: Prepare for Final Exam
1hr review lecture and quiz provided online

Day 2

FINAL EXAM: Open Book Final Examination (3hr)

Course Survey: (20 min)