

Introduction to Bioinformatics and Computational Biology

2021-04-14

Contents

1	Course information	11
1.1	Contributors	11
2	Introduction	13
2.1	Brief history of bioinformatics	13
2.2	Should I take this course?	13
2.3	Course information	13
2.4	Lab 1	13
3	High throughput sequencing	15
3.1	Three generations of sequencing technologies	15
3.2	FASTQ and FASTQC	15
3.3	Early sequence alignment (1 with 1)	15
3.4	Sequence search algorithms (1 with many)	16
3.5	Borrow-Wheeler Aligner (many with many)	16
3.6	Alignment output	16
4	RNA-seq Quantification	17
4.1	Introduction to RNA-seq experiment	17
4.2	RNA quality control and experimental design	17
4.3	Alignment	17
4.4	RNA-seq QC	17
4.5	RNA-seq expression index	17
4.6	RSEM and Salmon	17

4.7	RNA-seq read distribution	17
4.8	Lab 2	17
5	Differential expression, FDR, GO, and GSEA	19
5.1	DESeq2 library normalization	19
5.2	DESeq2 variance stabilization	19
5.3	Multiple hypotheses testing and False Discovery Rate	19
5.4	DESeq2 gene filtering	20
5.5	Gene Ontology (GO analysis)	20
5.6	Gene Set Enrichment Analysis (GSEA)	20
5.7	DESeq2 tutorial	20
6	Clustering	21
6.1	Heatmap and clustering quality	21
6.2	Hierarchical cluster	21
6.3	K means cluster	21
6.4	Pick K and consensus clustering	21
6.5	Batch effect removal	21
6.6	Lab3	21
7	Dimension Reduction	23
7.1	Principal Component Analysis: idea behind PCA.	23
7.2	Principal Component Analysis: PCA applications.	23
7.3	Multidimensional Scaling (MDS)	23
7.4	Linear discriminant Analysis (LDA)	24
8	Classification	25
8.1	Introduction	25
8.2	Supervised learning	25
8.3	Cross validation	26
8.4	Regression	26
8.5	Regularization	26
8.6	KNN	26

<i>CONTENTS</i>	5
8.7 Decision trees	26
8.8 Random forest	26
8.9 SVM	26
8.10 Lab 4	26
9 Module I Review	27
9.1 Module I review	27
9.2 Analysis Scenario 1	27
9.3 Analysis Scenario 2	27
10 Transcription Factor Motif Finding	29
10.1 Transcription regulation	29
10.2 Motif representation	29
10.3 EM	29
10.4 Gibbs sampler	29
10.5 Gibbs intuition	29
10.6 Motif finding in eukaryotes	29
10.7 Known motif database	29
11 ChIP-seq, Expression Integration	31
11.1 Motif finding in eukaryotes, and ChIP-seq	31
11.2 MACS and ChIP-seq QC	31
11.3 Identify TF interactions from ChIP-seq motifs	31
11.4 TF target genes and expression integration	31
11.5 Lab 5	31
12 Epigenetics, DNA Methylation	33
12.1 Intro to DNA Methylation	33
12.2 DNA Methylation Pattern and Function	33
12.3 DNA Methylation in Diseases	33
12.4 Techniques to Measure DNA Methylation	33

13 Histone Modifications , Chromatin Accessibility	35
13.1 Nucleosome Positioning	35
13.2 Introduction to Histone Modifications	35
13.3 Infer Transcription Factor Binding from Histone Mark Dynamics	35
13.4 Using Histone Marks to Infer Gene Functions	35
13.5 Introduction to DNase-seq and ATAC-seq	35
13.6 Infer TF from Differential Genes Using LISA	35
13.7 Caution on DNase/ATAC-seq footprint analysis	35
13.8 Summary of Epigenetics and Chromatin	36
13.9 Lab 6	36
14 Hidden Markov Model	37
14.1 Markov Chain	37
14.2 Hidden Markov Model	37
14.3 Hidden Markov Model Forward Procedure	37
14.4 Hidden Markov Model Backward Procedure	37
14.5 HMM Forward-Backward Algorithm	37
14.6 Viterbi Algorithm	37
14.7 Baum Welch Algorithm Intuition	37
14.8 HMM Bioinformatics Applications	37
15 HiC	39
15.1 Introduction to Chromatin Interaction and Organization	39
15.2 Methods to Investigate 3D Genome Organization	39
15.3 Topologically Associating Domains	39
15.4 TAD Function and Loop Anchors	39
15.5 Chromatin Compartments	39
15.6 Computational Methods to Call Chromatin Loops	39
15.7 Variations of Chromatin Interaction Technologies	39
15.8 Resources for Exploring 3D Genomes	39
15.9 Lab 7	40

<i>CONTENTS</i>	7
16 Module II Review	41
16.1 Module II Review	41
16.2 Module II Analysis Scenarios	41
17 SNP and GWAS	43
17.1 SNP, LP, and Association Studies	43
17.2 GWAS Studies and eQTL Analysis	43
17.3 Lab 8	43
18 GWAS and Epigenomics	45
18.1 Intro Functional Annotate GWAS	45
18.2 GWAS Functional Enrichment	45
18.3 Find Causal SNPs	45
18.4 Predict disease risk	45
19 Single-cell RNA-seq (1)	47
19.1 Intro to scRNA-seq	47
19.2 scRNA seq techniques	47
19.3 scRNA seq preprocessing and QC	47
19.4 Cleaning up expression matrix	47
20 Single-cell RNA-seq (2)	49
20.1 scRNA seq dimension reduction	49
20.2 Clustering and projections	49
20.3 Pseudo time and RNA velocity	49
20.4 Clustering by genotype and CITE seq	49
21 scATAC-seq	51
21.1 Single-Cell ATAC-seq Technique	51
21.2 Single-Cell ATAC-seq Pre-Processing and QC	51
21.3 Single-Cell ATAC-seq Analysis	51
21.4 scATAC-seq Downstream Analyses and scRNA-seq Integration .	51
21.5 Lab 9	51

22 Module III Review	53
22.1 Module III Review	53
23 Cancer Genome Sequencing , Mutation analyses	55
23.1 Intro to TCGA	55
23.2 Cancer mutation characterization	55
23.3 Cancer mutation patterns	55
23.4 Tumor purity and clonality	55
23.5 Interpret tumor mutations	55
23.6 Find cancer genes	55
23.7 Summary and future	55
24 Cancer Subtyping, Survival Analyses	57
24.1 Tumor Subtypes	57
24.2 Survival analysis	57
24.3 Oncogenes and Tumor Suppressor Mutations	57
24.4 Cancer Epigenetics	57
25 Targeted Therapy and Precision Medicine	59
25.1 Introduction to Targeted Therapy and Precision Medicine	59
25.2 Resistance to targeted therapy	59
25.3 Model system chemical and genetic screens	59
25.4 Overcoming resistance to targeted therapy	59
26 Cancer Immunotherapy (1)	61
26.1 Intro to Cancer Immunotherapy	61
26.2 HLA and Neoantigen Presentation	61
26.3 Immune Cell Infiltration in Tumors	61
26.4 T Cell Receptor Repertoires in Cancer Immunology	61
26.5 Lab 10	61

27 Cancer Immunotherapy (2)	63
27.1 TCR analysis	63
27.2 BCR analysis	63
27.3 Microbiome	63
27.4 Immunotherapy response biomarkers	63
27.5 Targeted therapy as immune-modulators	63
27.6 Epigenetic therapy as immune-modulators	63
28 CRISPR Screens	65
28.1 CRISPR and KO	65
28.2 CRISPRa and CRISPRi	65
28.3 CRISPR design and outcome	65
28.4 CRISPR screens & DepMap	65
28.5 CRISPR screen analysis	65
28.6 CRISPR screens in drug response	65
28.7 CRISPR screens in immunology	65
28.8 Enhancer CRISPR screen	65
28.9 CRISPR screens + scRNA-seq	65
29 Module IV Review and Course Review	67
29.1 Module IV Review	67
29.2 Course Review	67

Chapter 1

Course information

This is the course material for STAT115/215 BIO/BST282 at Harvard University.

All the YouTube videos in this course are organized under the 2021 STAT115 playlist.

1.1 Contributors

Xiaole Shirley Liu Harvard University and Dana-Farber Cancer Institute

Joshua Starmer StatQuest

Ting Wang Washington University

Feng Yue Northwestern University

Ming Tang Dana-Farber Cancer Institute

Martin Hemberg Wellcome Sanger Institute

Gad Getz Harvard University and Broad Institute

Yang Liu

Bo Yuan

Jack Kang

Scarlett Qian

Jiazhen Rong

Phillip Nicol

Maartin De Vries

We thank many colleagues in the community, who helped Dr. Liu in prepare the STAT115/215 BIO/BST282 course over the years. Some of the lecture slides acknowledged their contributions, but these contributors are not individually acknowledged here.

Chapter 2

Introduction

2.1 Brief history of bioinformatics

2.1.1 Protein structure wave

2.1.2 Gene expression wave

2.1.3 Genome sequencing wave

2.1.4 Big data challenge from sequencing

2.2 Should I take this course?

2.2.1 Bioinformatics vs computational biology

2.2.2 Is this class for me?

2.3 Course information

2.3.1 Logistics

2.3.2 X Shirley Liu lab introduction

2.4 Lab 1

2.4.1 Introduction**2.4.2 Introduction to R****2.4.3 Introduction to Bash****2.4.4 Getting started with Cannon**

Chapter 3

High throughput sequencing

3.1 Three generations of sequencing technologies

First generation sequencing is Sanger sequencing. It is the technology that was used to obtain the first human genome sequence.

Second generation sequencing is also called next generation sequencing (NGS) and is the start of high throughput sequencing. It is what scientists use most often nowadays, and Illumina is the market leader. Most of the rest of this course will cover data analysis using second generation sequencing.

Third generation sequencing is single-molecule sequencing. There are many new technologies still under active development, although none has reached market penetration.

3.2 FASTQ and FASTQC

NGS generates FASTQ files. FASTQC is an computational approach to evaluate the quality of your NGS data.

3.3 Early sequence alignment (1 with 1)

In the early days (1970s), scientists were not worried about having to align too many sequences. They wanted to find the best alignment between two

sequences. Many bioinformatics courses start with learning these, although it is not the main focus of our course. We included two videos in case you are interested.

The Needleman-Wunsch algorithm is the earliest algorithm to find the alignment between two sequences and score their similarity.

When two sequences are long, and only a portion of them can align well with each other, the Smith-Waterman algorithm can find the best local sequence alignment. It is still considered the best alignment approach, although it is slow.

3.4 Sequence search algorithms (1 with many)

With more and more sequences available in the public in the 1980s, scientists were interested in finding whether their newly sequenced string has been sequenced before in the public database. Therefore, the fast search algorithm BLAST was developed, using one sequence as the query to find similar sequences from a database.

3.5 Borrow-Wheeler Aligner (many with many)

With NGS, scientists need much faster search (aka mapping) algorithms in order to align the millions of sequences to the reference genome. The current best algorithm is called Borrow-Wheeler Aligner or BWA.

In order to understand BWA, we first need to introduce Borrow-Wheeler transformation and LF mapping

The basic idea of Borrow-Wheeler alignment

3.6 Alignment output

NGS raw data is in FASTQ. Alignment gives you SAM (alignment) or BAM (binary version of SAM) files which contain the sequence information in FASTQ and the mapping locations. BED file is the simplest, although there is information loss.

Chapter 4

RNA-seq Quantification

4.1 Introduction to RNA-seq experiment

4.2 RNA quality control and experimental design

4.3 Alignment

4.4 RNA-seq QC

4.5 RNA-seq expression index

4.6 RSEM and Salmon

4.7 RNA-seq read distribution

4.8 Lab 2

4.8.1 STAR tutorial

4.8.2 RSeQC tutorial

4.8.3 RSEM/Salmon Tutorial

Chapter 5

Differential expression, FDR, GO, and GSEA

DESeq2 is a popular and accurate computational algorithm to detect differential gene expression from RNA-seq data. It includes many elegant quantitative considerations, such as:

- Normalize the gene read counts by library size and composition
- Model gene read counts with negative binomial distribution
- Use hierarchical modeling to stabilize the gene variance
- Use Benjamini-Hochberg to calculate control for false discovery rate of calling differentially expressed genes
- Filter lowly expressed genes to reduce the number of hypotheses to be tested

5.1 DESeq2 library normalization

5.2 DESeq2 variance stabilization

5.3 Multiple hypotheses testing and False Discovery Rate

5.4 DESeq2 gene filtering

5.5 Gene Ontology (GO analysis)

5.6 Gene Set Enrichment Analysis (GSEA)

5.7 DESeq2 tutorial

Chapter 6

Clustering

6.1 Heatmap and clustering quality

6.2 Hierarchical cluster

6.3 K means cluster

6.4 Pick K and consensus clustering

6.5 Batch effect removal

6.6 Lab3

6.6.1 PCA tutorial

6.6.2 Clustering tutorial

6.6.3 Combat tutorial

6.6.4 DESeq2 Tutorial

6.6.5 DAVID/GSEA Tutorial

Chapter 7

Dimension Reduction

RNA-seq samples have tens of thousands of genes, although many genes might not vary much between samples and many others have correlated gene expression. Dimension reduction techniques aim to reduce the dimension of representing each sample with tens of thousands of genes to much fewer dimensions, e.g. 2 to 100.

7.1 Principal Component Analysis: idea behind PCA.

PCA / SVD automatically outputs PC1, PC2, PC3, etc, with earlier PCs capturing the highest level of variability in the original data. Each PC is a linear combination of raw gene expression, and is orthogonal to all other PCs.

7.2 Principal Component Analysis: PCA applications.

PCA is a widely used method to project samples with high dimensions (e.g. with gene expression data) onto two dimensions for better visualization. It is an intuitive way to identify sample clusters, and identify batch effect.

7.3 Multidimensional Scaling (MDS)

MDS can use differential ways to calculate pair-wise distance, then use lower dimensions to satisfy the pair-wise distance. PCA is a special case of MDS.

7.4 Linear discriminant Analysis (LDA)

LDA is not only a dimension reduction method, but also a supervised machine learning method.

Chapter 8

Classification

8.1 Introduction

Imagine you have RNA-seq of a collection of labeled normal lung and lung cancer tissues. Given a new sample of RNA-seq from the lung with unknown diagnosis, will you be able to predict based on the existing labeled samples and the expression data whether the new sample is normal or tumor? This is a sample classification problem, and it could be solved using **unsupervised** and **supervised** learning approaches.

Unsupervised learning is basically clustering or dimension reduction. You can use hierarchical clustering, MDS, or PCA. After clustering and projection the data to lower dimensions, you examine the labels of the known samples (hopefully they cluster into separate groups by the label). Then you can assign label to the unknown sample based on its distance to the known samples.

Supervised learning considers the labels with known samples and tries to identify features that can separate the samples by the label. Cross validation is conducted to evaluate the performance of different approaches and avoid over fitting.

StatQuest has done an amazing job with machine learning with a full playlist of well organized videos. While the full playlist is worth a full course, for the purpose of the course, we will just highlight a number of widely used approaches. They include logistic regression (this is considered statistical machine learning), K nearest neighbors, random forest, and support vector machine (these are considered computer science machine learning).

8.2 Supervised learning

8.3 Cross validation

8.4 Regression

8.5 Regularization

8.5.1 Ridge regression

8.5.2 LASSO regression

8.5.2.1 LASSO tutorial in R

8.6 KNN

8.7 Decision trees

8.8 Random forest

8.9 SVM

8.10 Lab 4

8.10.1 K-Nearest Neighbors tutorial

8.10.2 Regression/Ridge/LASSO Tutorial

8.10.3 Logistic Regression Tutorial

8.10.4 Support Vector Machine Tutorial

8.10.5 Random Forest Tutorial

Chapter 9

Module I Review

9.1 Module I review

9.2 Analysis Scenario 1

9.3 Analysis Scenario 2

Chapter 10

Transcription Factor Motif Finding

10.1 Transcription regulation

10.2 Motif representation

10.3 EM

10.4 Gibbs sampler

10.5 Gibbs intuition

10.6 Motif finding in eukaryotes

10.7 Known motif database

Chapter 11

ChIP-seq, Expression Integration

11.1 Motif finding in eukaryotes, and ChIP-seq

11.2 MACS and ChIP-seq QC

11.3 Identify TF interactions from ChIP-seq motifs

11.4 TF target genes and expression integration

11.5 Lab 5

11.5.1 MACS Tutorial

11.5.2 ChIP-seq QC Tutorial

11.5.3 TF Motif Finding Tutorial

11.5.4 TF Collaborator Tutorial

Chapter 12

Epigenetics, DNA Methylation

12.1 Intro to DNA Methylation

12.2 DNA Methylation Pattern and Function

12.3 DNA Methylation in Diseases

12.4 Techniques to Measure DNA Methylation

Chapter 13

Histone Modifications , Chromatin Accessibility

13.1 Nucleosome Positioning

13.2 Introduction to Histone Modifications

13.3 Infer Transcription Factor Binding from
Histone Mark Dynamics

13.4 Using Histone Marks to Infer Gene Func-
tions

13.5 Introduction to DNase-seq and ATAC-seq

13.6 Infer TF from Differential Genes Using
LISA

13.7 Caution on DNase/ATAC-seq footprint
analysis

13.8 Summary of Epigenetics and Chromatin

13.9 Lab 6

13.9.1 ChIP-seq Expression Integration

13.9.2 Cistrome-GO Tutorial

13.9.3 ATAC-seq Analysis and LISA Tutorial

Chapter 14

Hidden Markov Model

14.1 Markov Chain

14.2 Hidden Markov Model

14.3 Hidden Markov Model Forward Procedure

14.4 Hidden Markov Model Backward Procedure

14.5 HMM Forward-Backward Algorithm

14.6 Viterbi Algorithm

14.7 Baum Welch Algorithm Intuition

14.8 HMM Bioinformatics Applications

Chapter 15

HiC

- 15.1 Introduction to Chromatin Interaction and Organization
- 15.2 Methods to Investigate 3D Genome Organization
- 15.3 Topologically Associating Domains
- 15.4 TAD Function and Loop Anchors
- 15.5 Chromatin Compartments
- 15.6 Computational Methods to Call Chromatin Loops
- 15.7 Variations of Chromatin Interaction Technologies
- 15.8 Resources for Exploring 3D Genomes

15.9 Lab 7

15.9.1 BS-seq and Bismark Tutorial

15.9.2 Tutorial on Associating DNA Methylation with Expression

15.9.3 HiC Analysis Tutorial

Chapter 16

Module II Review

16.1 Module II Review

16.2 Module II Analysis Scenarios

Chapter 17

SNP and GWAS

17.1 SNP, LP, and Association Studies

17.2 GWAS Studies and eQTL Analysis

17.3 Lab 8

17.3.1 HW4 FAQ & cooler

17.3.2 Pikachu&HiGlass

17.3.3 HMM

Chapter 18

GWAS and Epigenomics

18.1 Intro Functional Annotate GWAS

18.2 GWAS Functional Enrichment

18.3 Find Causal SNPs

18.4 Predict disease risk

Chapter 19

Single-cell RNA-seq (1)

19.1 Intro to scRNA-seq

19.2 scRNA seq techniques

19.3 scRNA seq preprocessing and QC

19.4 Cleaning up expression matrix

Chapter 20

Single-cell RNA-seq (2)

20.1 scRNA seq dimension reduction

20.2 Clustering and projections

20.3 Pseudo time and RNA velocity

20.4 Clustering by genotype and CITE seq

Chapter 21

scATAC-seq

21.1 Single-Cell ATAC-seq Technique

21.2 Single-Cell ATAC-seq Pre-Processing and QC

21.3 Single-Cell ATAC-seq Analysis

21.4 scATAC-seq Downstream Analyses and scRNA-seq Integration

21.5 Lab 9

21.5.1 MAESTRO tutorial

Chapter 22

Module III Review

22.1 Module III Review

Chapter 23

Cancer Genome Sequencing , Mutation analyses

23.1 Intro to TCGA

23.2 Cancer mutation characterization

23.3 Cancer mutation patterns

23.4 Tumor purity and clonality

23.5 Interpret tumor mutations

23.6 Find cancer genes

23.7 Summary and future

Chapter 24

Cancer Subtyping, Survival Analyses

24.1 Tumor Subtypes

24.2 Survival analysis

24.3 Oncogenes and Tumor Suppressor Mutations

24.4 Cancer Epigenetics

Chapter 25

Targeted Therapy and Precision Medicine

- 25.1 Introduction to Targeted Therapy and Precision Medicine
- 25.2 Resistance to targeted therapy
- 25.3 Model system chemical and genetic screens
- 25.4 Overcoming resistance to targeted therapy

Chapter 26

Cancer Immunotherapy (1)

26.1 Intro to Cancer Immunotherapy

26.2 HLA and Neoantigen Presentation

26.3 Immune Cell Infiltration in Tumors

26.4 T Cell Receptor Repertoires in Cancer Immunology

26.5 Lab 10

26.5.1 TCGA exploration

26.5.2 LIMMA on microarray data

26.5.3 Survival analysis

Chapter 27

Cancer Immunotherapy (2)

27.1 TCR analysis

27.2 BCR analysis

27.3 Microbiome

27.4 Immunotherapy response biomarkers

27.5 Targeted therapy as immune-modulators

27.6 Epigenetic therapy as immune-modulators

Chapter 28

CRISPR Screens

28.1 CRISPR and KO

28.2 CRISPRa and CRISPRi

28.3 CRISPR design and outcome

28.4 CRISPR screens & DepMap

28.5 CRISPR screen analysis

28.6 CRISPR screens in drug response

28.7 CRISPR screens in immunology

28.8 Enhancer CRISPR screen

28.9 CRISPR screens + scRNA-seq

Chapter 29

Module IV Review and Course Review

29.1 Module IV Review

29.2 Course Review