

Introduction to Bioinformatics and Computational Biology

2021-02-16

Contents

1	Course information	11
1.1	Contributors	11
2	Introduction	13
2.1	Brief history of bioinformatics	14
2.2	Should I take this course?	14
2.3	Course information	14
2.4	Lab 1	14
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	20
5.2	DESeq2 variance stabilization	20
5.3	Multiple hypotheses testing and False Discovery Rate	20
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	26
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
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 ChIP-seq	31
11.2 BWA and MACS	31
11.3 ChIP-seq QC	31
11.4 TF interactions (motif)	31
11.5 TF target genes (expression integration)	31
12 Epigenetics, DNA Methylation	33
12.1 Epigenetics	33
12.2 DNA methylation	33
12.3 Promoter function	33
12.4 Gene body function	33
12.5 Enhancer function	33
12.6 Repetitive region function	33
12.7 Early cancer detection	33

13 Histone Modifications , Chromatin Accessibility	35
13.1 Nucleosome positions	35
13.2 Histone modification	35
13.3 Promoters (bivalent)	35
13.4 Genes (K36me3, new genes)	35
13.5 Enhancers (K27ac)	35
13.6 Super-enhancers	35
13.7 DNase-seq	35
13.8 ATAC-seq	35
14 Long Range Chromatin Interactions	37
14.1 Chromatin interactions	37
14.2 HiC	37
14.3 HiC contact map	37
14.4 HiC normalization	37
14.5 Fractal globule	37
14.6 Loops	37
14.7 Domains	37
14.8 Compartments	37
14.9 Phase separation	37
15 Hidden Markov Model	39
15.1 Intro to HMM	39
15.2 Pb1: Forward & backward procedure	39
15.3 Pb2: Viterbi algorithm	39
15.4 Pb3: Parameter estimation	39
15.5 HMM application	39
16 Module II Review	41
16.1 Module II Review	41
16.2 Practive Questions	41

<i>CONTENTS</i>	7
17 SNP and GWAS	43
17.1 SNP and LD	43
17.2 Family-based vs case-control association studies	43
17.3 GWAS studies and catalog	43
17.4 GTEx and eQTL	43
18 GWAS and Epigenomics	45
18.1 Find tissue / cell type	45
18.2 Identify causal SNPs and genes	45
18.3 Predict phenotypes	45
19 Single-cell RNA-seq (1)	47
19.1 Intro to scRNA-seq	47
19.2 Smart, Droplet, microwell, SCI-based	47
19.3 QC	47
19.4 Normalization	47
19.5 Imputation	47
19.6 Dimension reduction	47
19.7 Clustering	47
19.8 t-SNE and UMAP	47
20 Single-cell RNA-seq (2)	49
20.1 Annotate scRNA-seq clusters	49
20.2 Differential expression	49
20.3 Batch effect removal	49
20.4 Pseudotime	49
20.5 Overload 10X	49
20.6 Other applications (CITE-seq, multi-seq, spatial transcriptomics)	49

21 scATAC-seq	51
21.1 Intro to scATAC-seq	51
21.2 Sample and cell QC	51
21.3 Dimension reduction, clustering & visualization	51
21.4 Differential peaks and annotations	51
21.5 Integration with scRNA-seq	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 TCGA expression	57
24.2 Tumor subtypes	57
24.3 Survival analysis	57
24.4 GoF Oncogenes and LoF TS	57
24.5 Chromatin regulator mutations in cancer	57
24.6 DNA methylation and CIMP	57
25 Targeted Therapy, Drug Resistance, Compound and Genetic Screens	59
25.1 Hallmarks of cancer	60
25.2 Chemo vs targeted therapy	60
25.3 Drug resistance	60
25.4 Synthetic lethality	60

<i>CONTENTS</i>	9
25.5 Precision medicine	60
25.6 Tumor (bulk vs scRNA-seq), mice, cell lines	60
25.7 Compound screens	60
25.8 Genetic screens	60
25.9 Tumor heterogeneity	60
26 Cancer Immunotherapy (1)	61
26.1 Systemic immunotherapy	61
26.2 Personalized immunotherapy	61
26.3 HLA and neoantigens	61
26.4 Tumor immune deconvolution	61
26.5 T cell signaling (PD1/PDL1, etc)	61
26.6 Other immune-cells (scRNA-seq)	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.

1.1 Contributors

Xiaole Shirley Liu (lead instructor)

Joshua Starmer

Martin Hemberg

Ting Wang

Feng Yue

Ming Tang

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

8.6 KNN

8.7 Decision trees

8.8 Random forest

8.9 SVM

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 ChIP-seq

11.2 BWA and MACS

11.3 ChIP-seq QC

11.4 TF interactions (motif)

11.5 TF target genes (expression integration)

Chapter 12

Epigenetics, DNA Methylation

12.1 Epigenetics

12.2 DNA methylation

12.3 Promoter function

12.4 Gene body function

12.5 Enhancer function

12.6 Repetitive region function

12.7 Early cancer detection

Chapter 13

Histone Modifications , Chromatin Accessibility

13.1 Nucleosome positions

13.2 Histone modification

13.3 Promoters (bivalent)

13.4 Genes (K36me3, new genes)

13.5 Enhancers (K27ac)

13.6 Super-enhancers

13.7 DNase-seq

13.8 ATAC-seq

Chapter 14

Long Range Chromatin Interactions

14.1 Chromatin interactions

14.2 HiC

14.3 HiC contact map

14.4 HiC normalization

14.5 Fractal globule

14.6 Loops

14.7 Domains

14.8 Compartments

14.9 Phase separation

Chapter 15

Hidden Markov Model

15.1 Intro to HMM

15.2 Pb1: Forward & backward procedure

15.3 Pb2: Viterbi algorithm

15.4 Pb3: Parameter estimation

15.5 HMM application

Chapter 16

Module II Review

16.1 Module II Review

16.2 Practive Questions

Chapter 17

SNP and GWAS

17.1 SNP and LD

17.2 Family-based vs case-control association studies

17.3 GWAS studies and catalog

17.4 GTEx and eQTL

Chapter 18

GWAS and Epigenomics

18.1 Find tissue / cell type

18.2 Identify causal SNPs and genes

18.3 Predict phenotypes

Chapter 19

Single-cell RNA-seq (1)

19.1 Intro to scRNA-seq

19.2 Smart, Droplet, microwell, SCI-based

19.3 QC

19.4 Normalization

19.5 Imputation

19.6 Dimension reduction

19.7 Clustering

19.8 t-SNE and UMAP

Chapter 20

Single-cell RNA-seq (2)

20.1 Annotate scRNA-seq clusters

20.2 Differential expression

20.3 Batch effect removal

20.4 Pseudotime

20.5 Overload 10X

20.6 Other applications (CITE-seq, multi-seq, spatial transcriptomics)

Chapter 21

scATAC-seq

21.1 Intro to scATAC-seq

21.2 Sample and cell QC

21.3 Dimension reduction, clustering & visualization

21.4 Differential peaks and annotations

21.5 Integration with scRNA-seq

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 TCGA expression

24.2 Tumor subtypes

24.3 Survival analysis

24.4 GoF Oncogenes and LoF TS

24.5 Chromatin regulator mutations in cancer

24.6 DNA methylation and CIMP

Chapter 25

Targeted Therapy, Drug Resistance, Compound and Genetic Screens

25.1 Hallmarks of cancer

25.2 Chemo vs targeted therapy

25.3 Drug resistance

25.4 Synthetic lethality

25.5 Precision medicine

25.6 Tumor (bulk vs scRNA-seq), mice, cell lines

25.7 Compound screens

25.8 Genetic screens

25.9 Tumor heterogeneity

Chapter 26

Cancer Immunotherapy (1)

26.1 Systemic immunotherapy

26.2 Personalized immunotherapy

26.3 HLA and neoantigens

26.4 Tumor immune deconvolution

26.5 T cell signaling (PD1/PDL1, etc)

26.6 Other immune-cells (scRNA-seq)

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