

PHS 675

Special Topics: Analysis of RNA-Sequencing Data

Fall 2026

Syllabus

Course Directors:

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Course Description:

This is a special topics course in the analysis of data generated by RNA-Sequencing. The course will include bulk RNA-Seq and single-cell/single-nucleus RNA-Seq. Other topics may be included according to availability of time and student interest. The course is aimed at graduate students in the Biomedical Sciences program and is designed to provide the skills to analyze and interpret RNA-Seq data they may generate during their research.

Analysis topics will include:

- Bulk RNA-Sequencing:
 - data-preprocessing,
 - quality assurance,
 - building count tables by alignment or quantification,
 - differential expression analysis,
 - gene set, pathway, and network analysis.
- Single-cell and single-nucleotide RNA-Sequencing:
 - data preprocessing and QC
 - clustering (based on cell identification and dimensional reduction)
 - differential gene expression analyses
 - pathway analyses

Credits:

This is a 3 credit hour, 15-week course which will primarily be conducted by directed self-study.

Required Text:

There is no required text for this course. Online teaching materials will be provided by the instructors.

Class meeting times and location:

The class will meet Tuesdays and Thursdays from 10-11:15 in BBSC 102.

Academic Calendar:

For beginning, ending, and add/drop dates, see the [Marshall University Academic Calendar](https://www.marshall.edu/academic-calendar/) (URL: <https://www.marshall.edu/academic-calendar/>).

Campus Carry Policy:

University Policy, UPGA-12 (Campus Carry Policy) derives its authority from West Virginia State law, including the Campus Self-defense Act (W. Va. Code § 18B-4-5b). It pertains to the exercise of Concealed Carry on Marshall University's campus, except in designated areas, by individuals with a valid permit to Conceal Carry.

Individuals who choose to Conceal Carry are responsible for knowing and understanding all applicable federal, state, and local laws and Marshall University Board of Governors Rules, University Policies, and Administrative Procedures. University Policy, UPGA-12 applies to areas of campus and buildings that are directly under the possession or control of Marshall University.

Concealed Handguns are not observable to others and must be holstered and concealed on the body of the permit holder or in a personal carrier, such as a backpack, purse, or other bag that remains under the exclusive and uninterrupted control of the permit holder. This includes wearing the personal carrier with a strap, carrying or holding the personal carrier, or setting the personal carrier next to or within your immediate reach at all times. If your participation in class activities impedes your ability to maintain constant control of your Handgun, please make alternate arrangements prior to coming to class.

Faculty Offices:

NOTICE: University Policy, UPGA-12 (Campus Carry Policy) defines Sole Occupancy Offices as areas that may restrict Concealed Carry. Please be aware that offices of all faculty for this course are Sole Occupancy Offices and this statement serves as notice that concealed weapons or handguns are not permitted in these office. Additionally, these offices are in a secure area of campus which is protected as it houses scientific laboratory equipment, and concealed weapons or handguns are not permitted in this area. If you plan to attend a meeting in these offices, secure your weapon or handgun appropriately before you arrive.

Prerequisites:

Students should have an understanding of basic statistics, including statistical inference, hypothesis testing, and interactions, and experience with R and R Studio, up to the level of BMR 617. Experience with command-line Linux is useful but will be taught as needed.

Objectives and Outcomes:

Upon successful completion of this course, students will be able to:

- Perform data preprocessing and quality control of raw data from RNA Sequencing
- Build count tables of RNA-Seq data
- Identify differentially expressed genes between experimental conditions, among one or more experimental variables.
- Perform gene set, pathway, and network analyses given the results of an RNA-seq experiment and a set of suitable pathways and/or networks.
- Perform data preprocessing and quality control of raw data from single-cell RNA sequencing
- Identify cell-specific and dimensional reduction-based clusters
- Perform differential gene expression and analysis and pathway analyses in scRNA-Seq data
- Be familiar with software tools and databases, including but not limited to FastQC, multiQC, HISAT2, HTSeq, DESeq2, GSEA, GO, Kegg, Reactome, MSigDB, and Cytoscape.

Grading and assessment.

Grades will be assigned on a standard percentage scale:

A	At least 90%
B	At least 80% and less than 90%
C	At least 70% and less than 80%
D	At least 60% and less than 70%
F	Less than 60%

Assessment will be by graded assignments. We will aim to assign one homework assignment (which should take 1-2 hours) each week. There will be one major project assigned during week 6 (due on or after September 24th) and one major project assigned during finals week (due on or after December 10th). Homework will account for 20% of the final grade; the projects will account for 40% each.

Generative Artificial Intelligence (AI) Policy for use in this course:

Generative AI is permitted in this course in a limited fashion, and must be attributed. Students must understand and be able to explain satisfactorily any work they submit. This includes any computer code submitted and any narrative or writing submitted. Instructors may ask students to explain material they have submitted; copying and pasting from Generative AI output without being able to explain the content of the material submitted will not be accepted.

University Policies:

By enrolling in this course, you agree to the University Policies. Please read the full text of each policy (listed below) by going to [MU Academic Affairs: University Policies](https://www.marshall.edu/academic-affairs/policies/). (URL: <https://www.marshall.edu/academic-affairs/policies/>)

- Academic Dishonesty Policy
- Academic Dismissal Policy
- Academic Forgiveness Policy
- Academic Probation and Suspension Policy
- Affirmative Action Policy
- Pre-Finals Week Policy
- Inclement Weather Policy
- Sexual Harassment Policy- Title IX prohibits the harassment of students based on sex, which includes pregnancy, childbirth, and related conditions. This includes that students will not be penalized for taking medically necessary leave related to pregnancy, childbirth, or related conditions. Marshall's Title IX Office may be contacted at TitleIX@marshall.edu
- Students with Disabilities (Policies and Procedures)
- University Computing Services Acceptable Use Policy

Attendance Policy:

As a graduate-level class, it is assumed that students understand the benefit of class attendance. Absences will not be penalized directly, but students are highly unlikely to perform well in the assignments if they are not present in class for the discussions.

As a matter of etiquette, students should inform the instructor(s) in advance, whenever possible, if they are unable to attend class.

Illness or incapacity in any other form which prevents students being able to complete an assignment by the deadline should be negotiated with the instructor responsible for the assignment. Absences guaranteed to be excused by the undergraduate excused absence policy (UPAA-2) which prevent a student from completing an assignment in the allocated time will result in reasonable extra time being allowed for that assignment.

Course schedule:

Week	
1	Introduction. The central dogma of biology, sequencing, and RNA sequencing. Terminology, including "Expression Profiling", "Differential Expression", and "Pathway analysis". Quick start: Differential expression from a precomputed count table.
2	Quick start: Visualization of a differential expression experiment: PCA plots, sample cluster plots, MA-plots, volcano plots, and heatmaps. Pathway analysis with GSEA and Reactome.
3	Introduction to Linux. Quality assessment with FastQC and multiQC.
4	Alignment with HISAT2 and generating counts with HTSeq count. Transcript quantification estimation with Salmon and/or Kallisto.
5	The details of DESeq2: the design model, low expression cutoff thresholds, statistical testing, and contrasts. Working with multiple experimental variables and batch effects.
6	Pathway analyses. Pathway databases including Reactome, Kegg, GO, and MSigDB. Gene Set Enrichment Analysis: Ways to rank genes, permutation testing, and leading edge genes.
7	Other pathway analyses: overrepresentation analysis and gene network analyses. Cytoscape, protein-protein interaction databases, network clustering techniques.
8	Introduction to single-cell/single-nuclei RNA sequencing Quick start: Generate UMAP plots from prepared data and perform cell identification
9	Quick start: Analysis of a pre-computed single-cell data set
10	Data preprocessing and filtering (barcode-based demultiplexing, transcriptome alignment, barcode counting and UMI counting, normalization and quality control) using Cell Ranger and Seurat
11	Clustering (based on cell identification and dimensional reduction) using SingleR and Seurat. Cell cycle phase scoring and PCA.
12	Cell identification, layer processing, PCA on integrated data, dimensional reduction
13	Differential gene expression analysis for single-cell data
14	Pathway analyses for single-cell data
15	Descriptive overview of other sequencing experiments: ATAC-Seq, Cut-And-Run, and Methyl-Seq in both bulk and single-cell experiments. Spatial sequencing technologies. Multiomic approaches.