FRSC 422 (3 cr.)   **Advanced Sequencing Methods**

Course Catalog Description: Theory and application of DNA sequencing technology including Sanger sequencing, pyrosequencing, and massively parallel sequencing and their uses in forensic DNA analysis. Five lecture/laboratory hours. Prerequisites: FRSC 420 Body Fluid Analysis and MATH 237 Elementary Biostatistics or permission of instructor.

Learning Objectives: Students will gain theoretical understanding and laboratory experience with good lab practice, sample handling and preparation, implementing quality assurance and quality control processes, advanced sequencing techniques, data analysis and statistics applied to real world forensic DNA testing.

Course Purpose and Goals: Students will gain theoretical and hands-on experience with advanced DNA sequencing techniques including validation and reporting. Students will work in small groups to solve forensic problem using one or more of the following techniques: Sanger sequencing, pyrosequencing, and massively parallel sequencing. Attendance is required.

Required Text: None

Reference Texts: Next-Generation Sequencing Data Analysis, by Xinkun Wang. Boca Raton: CRC Press, 2016, 258 pp.

Lab Fees: A lab fee request will be submitted. A lab fee of $100 will be charged for this course. This fee will pay for sequencing supplies.

Course Requirements and Grading:

Course Evaluation:
Seminar Summaries
Journal Club
Project Proposal
Research Project Report
Oral Presentation
Poster Presentation
Exam
Surveys

Graded Assignments-Details:

Seminar Summaries: Attend in person or watch pre-recorded NGS seminar and report upon content. Graded for effort and completion.

Journal Club: Students will participate in Journal Club paper discussions in class on assigned papers. Graded for effort and insight.

Research Proposal: Groups of students will prepare a project proposal complete with a detailed explanation of the number and type of samples, controls, standards, procedures or kits to be used, and research questions. Proposals will be graded by the instructor using a critique rubric. Grading will be based upon study design, thoroughness, and quality of written work.

Research Project Report: Students will prepare a *Journal of Forensic Sciences* quality research report complete with Abstract, Keywords, Introduction, Materials and Methods, Results and Discussion, Conclusion, References, Tables and Figures based upon the group project goals and data. Grading will be based upon thoroughness, accuracy, and quality of written work using a rubric.

Oral Project Presentation: Students will present a seminar on the research project report. The total duration (presentation and questions) will be 15 minutes per group. Presentations will be graded by the instructor and other faculty members in attendance using a critique rubric. Grading will be based upon content provided on slides, content delivery and ability to answer questions correctly.

Poster Project Presentation: Students will present a poster on the research project. The presentation will occur during the Poster Session scheduled for the week before final exams. Presentations will be graded by the instructor and other faculty members in attendance using a critique rubric. Grading will be based upon content provided, content delivery and ability to answer questions correctly.

Exam: Each student will be evaluated on their responses to essay questions on a take home Exam. Students will be given one week to complete the exam. Grading will be based upon thoroughness, accuracy, and quality of written work using a rubric.

Surveys: Students will be given validated surveys to evaluate if course learning objectives were met. Surveys will be graded on completion, thoughtfulness and insight.

Grading Policy:

Assessment Item	Points
Seminar Summary 1	25
Seminar Summary 2	25
Journal Club 1	25
Journal Club 2	25
Project Proposal	50
Research Project Report	200
Oral Project Presentation	200

Poster Project Presentation	100
Exam	300
Surveys	50
Research Paper	Not Applicable
Course Total	1000

Grades:

Letter grade will be assigned based on the following:

93% points and above = A

90-92.9% = A-

87-89.9% = B+

83-86.9% = B

80-82.9% = B-

77-79.9% = C+

70-76.9% = C

67-69.9% = D+

60-66.9% = D

Less than 60% = F

Schedule:

Date	Topic	Reading / Assignment
Week 1	History of Human Identity Approaches History of Sequencing for Human DNA Typing: STRs and SNPs Sanger, SNaPshot, Pyrosequencing, Massively Parallel Sequencing (MPS) also termed Next Generation Sequencing (NGS) Project Topic and Group Pairings	
Week 2	Overview of NGS on MiSeq FgX, Sequencing by synthesis SWGDAM Internal Validation, ForenSeq validation Watch pre-recorded MPS seminar #1 on forensic application Research Question and Project Design Development with your group and draft submitted for feedback	Seminar Summary 1
Week 3	Watch pre-recorded MPS seminar #2 on forensic application Research Question and Project Design Revisions and Submission	Seminar Summary 2 Project Proposal
Week 4	Sample Acquisition and Preprocessing Journal Club #1 (Jäger AC et al., Developmental validation of the MiSeq FGx Forensic Genomics System for Targeted Next Generation Sequencing in Forensic DNA Casework and Database Laboratories. FSI: Genetics 28 (2017) 52–70.)	Journal Club 1
Week 5	DNA extraction DNA quantitation	
Week 6	ForenSeq PCR1/Amplify and Tag Targets to create MPS library ForenSeq PCR2/Enrich Targets to attach indexes and adaptors	

	Creating a project in MiSeq ForenSeq software	
Week 7	PCR cleanup/Purify Libraries Library normalization Gel analysis to detect DNA amplicon production Operation of MiSeq FgX, Run wash protocol	
Week 8	Multiplexing/Pool Libraries, thaw cartridge, denature and dilute libraries, load samples, MiSeq FGx run 1 Post-run wash and MiSeq FGx run 2 **Take home Exam** distributed	
Week 9	Did my run work? Run feedback files from MiSeq FGx instrument Instruction on Data analysis on using UAS software: Sequence, Graphs and Charts, Phenotype and Ancestry	**Exam Due**
Week 10	Journal Club #2 (Kulstein G et al., As solid as a rock comparison of CE- and MPS-based analyses of the petrosal bone as a source of DNA for forensic identification of challenging cranial bones. Int J Legal Med (2018) 132:13-24.) Project data review and analysis and making graphs and tables	Journal Club 2
Week 11	Project data review and analysis and writing the final report	
Week 12	Peer Review of final report Preparing a poster presentation	
Week 13	Draft Poster Presentation, Project Report Due Preparing an oral presentation	**Report**
Week 14	**Poster Presentations** Draft Oral Presentation Slides	**Poster Presentation**
Week 15	**Final Exam Week: Oral Project Presentations**	**Oral Presentation & Surveys**

Policy on Attendance: There will be a direct correlation between student performance and classroom and lab attendance. Attendance will be taken and participation will be recorded. Students who miss a class are responsible for obtaining all missed materials and information and performing all laboratory steps. Students who fail to submit an assignment or deliver a presentation on the appropriate date due to a University-sanctioned reason (with appropriate documentation) will be allowed to arrange a make-up opportunity directly with the instructor for full credit. In the case of non-University-sanctioned absences, a late penalty of 5% per day will be applied to the grade of the make-up work. For more information, access the University Attendance Policy: https://catalog.towson.edu/undergraduate/academic-policies/class-attendance-absence-policy/.

Disability Statement:

Towson University is committed to providing equal access to its programs and services for students with disabilities, in accordance with section 504 of the Rehabilitation Act of 1973 and the Americans with Disabilities Act of 1990. Students with disabilities should consult with the Towson Accessibility and Disabilities Services (ADS) 7720 York Road, Suite 232-235, 410-704-2638 (Voice), 410-704-4423 (TDD) or tuads@towson.edu, to learn about how to arrange for any

appropriate accommodations. It is the student's responsibility to let the instructor know when he/she is a student with needs in this area. Students who expect that they have a disability but do not have documentation are encouraged to contact ADS for advice on how to obtain appropriate evaluation. A memo from Disability Support Services (DSS) authorizing your accommodations will be needed before any accommodation can be made.

Diversity Statement:

The students, faculty, and staff at Towson University represent a diverse and vibrant community of learners and scholars. As a community, we value the unique contributions of each individual and promote active participation in all aspects of the learning process by each community member. Towson University values diversity and fosters a climate that is grounded in respect and inclusion, enriches the educational experience of students, supports positive classroom and workplace environments, promotes excellence, and cultivates the intellectual and personal growth of the entire university community. Your instructor strives to create a classroom environment built upon the principles of mutual respect and support. Toward this end, all members participating in this course are expected to demonstrate respect for all other members of the class. Should you feel that you are experiencing a negative environment related to diversity issues or cultural sensitivity, we encourage you to speak with your instructor or to contact the Chemistry Department's Diversity Liaison, Dr. Cynthia Zeller, czeller@towson.edu. See also https://www.towson.edu/fcsm/departments/chemistry/diversity.html. For further information regarding the diversity and inclusion policies of Towson University, please see the Towson University Commitment to Diversity, the Fisher College of Science and Mathematics Diversity Action Website, and the Chemistry Department Diversity Action Plan.

Policy on Academic Dishonesty: All types of academic dishonesty are deplorable and severe consequence actions will be taken. All students must familiarize themselves with the TU policies on academic integrity and will be held accountable for all standards outlined in this policy. Note that academic dishonesty includes (but is not limited to) cheating and allowing another student to cheat. Resubmission of previous work is also a violation of the academic integrity policy under definition 2.G. Every student found in violation of the university's academic integrity policy will be penalized, possibly including a grade of F for the course. The Towson University Code of Conduct prohibits "all forms of dishonesty including cheating (and) plagiarism." Plagiarism is copying the words of another or the use of ideas of another without proper citation. Plagiarism can result from copying an entire document to inappropriate paraphrasing. In order to avoid plagiarism, the use of words or ideas of another without proper citation, it is imperative to consciously think about what you have read and what you are trying to write. In scientific literature, we do not normally use direct quotes from the primary resources that we are using to gather our information. It is important to paraphrase the ideas and conclusions obtained from the primary literature and rewrite them in your own words. One method to assure that you will not plagiarize is to take notes on each of your primary resources and then write the paper based on your notes, not from the references. Your paper will summarize the works of others, not directly quote from them. If you have any questions about plagiarism and correct citations see

https://towson.libguides.com/c.php?g=530232 for further examples and methods to correct the problems. The consequences of cheating or plagiarism will be a failing grade of 0 points for the assignment and may result in failure of the course. Plagiarism, fabrication, falsification, cheating, complicity in academic dishonesty, abuse of academic materials, multiple submissions of the same work or part thereof for multiple courses/assignments, will not be tolerated and will result in a failing grade of 0 for that assignment and may result in failure of the course.

Course Repeat Policy: "Students may not repeat a course more than once without prior permission of the Academic Standards Committee."

Bibliography

Books:

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Wang X. (2016) <u>Next-Generation Sequencing Data Analysis</u>, Boca Raton, FL: CRC Press, 258 pp. ISBN: 9781482217889

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