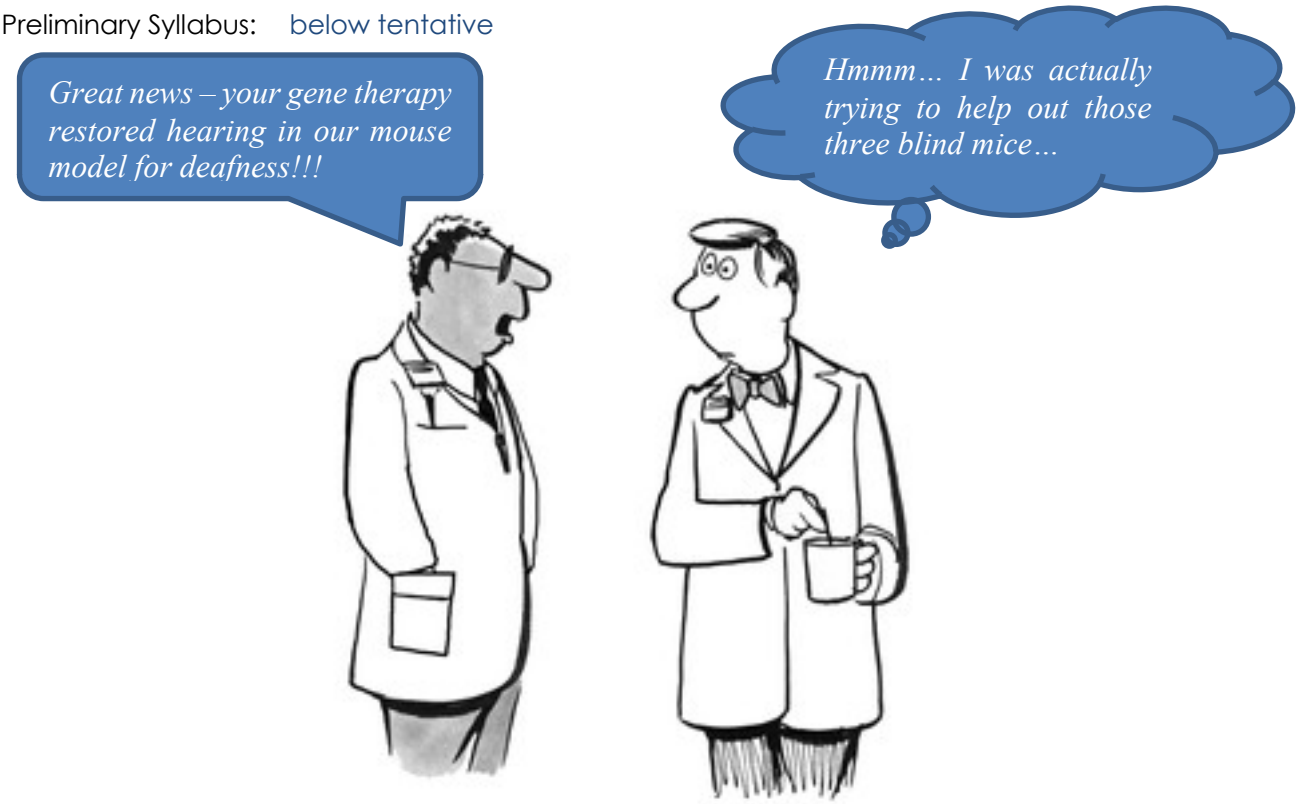
USC University of Southern California

599L: Techniques in Viral Vector Engineering

Units:	2
Lecture Day - Time:	Mondays, 4:00 - 5:50 pm
Lecture Location:	MCB 204
Lecture Location:	MCB 253 (meet in MCB 204)
Zoom:	Meeting ID: ###, Passcode: ### (only for return to remote instruction)

Instructor:	**Jennifer Treweek**
Office:	MCB 240A, Zoom
Office Hours:	TBD (or anytime by email request with > 24h advance notice)
Contact Info:	jtreweek@usc.edu, (213) 821 – 3478

Preliminary Syllabus: below tentative



The importance of engineered viral vectors in effective gene therapies.

Catalogue Description

Following on the recent success stories of virus-vectored vaccines and of FDA-approved gene therapies that make use of recombinant viral vectors, this course will provide a hands-on learning experience for the design, production, and screening of clinically relevant viral vectors. Students will first be provided with a broad overview of virus-based therapeutics and gene delivery vehicles, from inactivated viruses and virus-like particles that can serve as effective vaccines, to viruses that have been rigorously engineered to serve as vectors for safe and noninvasive gene delivery in biomedical research and in clinical practice. After this introduction, we will delve more deeply into the rise of adeno-associated viruses (AAVs) as one of the leading viral vectors of choice for both basic research and gene therapy applications. The instructor will then guide students through the entire AAV production pipeline within her lab, from initial capsid engineering and AAV genome construction, to the generation and purification of high-titer virus suitable for *in vivo* administration. The course will conclude with a critical discussion of the current limitations of AAVs in their use as vectors for gene therapy, as well as the exciting new disease treatment possibilities that engineered AAVs may help to address.

Although this 2-unit course, which is split between lecture and in-person lab demos, can be taken as a standalone course on viral vector engineering, it has been designed to integrate seamlessly with a concurrent 2-unit classroom course (BME 599: Viral Immunology & Viral Vector Engineering) that broadly covers the principles that underlie basic virology, viral immunology, and virus-associated pathology in humans; as well as the theory behind the development of virus-inspired therapeutics and viral vectors for gene transfer. Although this latter course will introduce the topic of adeno-associated viruses (AAV) and provide context for the application of AAV vectors to gene therapy, BME 599L will take a much deeper dive into how AAV vectors are used "in practice". The hands-on training in AAV production will be particularly relevant to students wishing to adopt the use of AAVs in their own graduate research, as well as to students planning to apply to industry jobs in the big pharma or gene therapy arenas.

Learning Objectives

Students will learn:

1) the core principles of virology, with a focus on viral replication and infectivity – as these are traits that must be genetically modified so as to safely apply viruses to human gene delivery applications
2) the basic concepts of how the human immune system recognizes viruses, and how such recognition can be avoided in the case of viral vectors
3) the different virus-based and vector-based platforms that have been pioneered for basic research as well as for combatting disease in humans, including
    a. active vaccination strategies (e.g., inactivated or live-attenuated virus vaccines, virus-like particle vaccines, viral vectored vaccines, etc.)
    b. immunoengineering strategies (e.g., viral vector delivery of immunomodulators or immune system components)
    c. viral vectors for connectomics and basic research
    d. FDA-approved gene therapies that use viral vectors for gene transfer
4) All aspects of AAV engineering and production for *in vivo* applications, including:
    a. recombinant AAV genome design
    b. capsid engineering to refine critical AAV vector parameters such as cell-type specificity, transduction efficiency, tissue-targeting, and transgene carrying capacity
    c. the high-titer preparation and rigorous purification of AAV vectors for *in vivo* use
    d. routes of administration and dosing parameters for effective gene delivery
    e. important considerations and current limitations in the use of AAV vectors in human gene therapy and disease treatment

Course Outcomes

1) Apply course teachings to brainstorming novel biomedical applications for viral vectors across the entire spectrum of disease diagnosis, prevention, and treatment.
2) Interpret and critique data from published journal articles on viral vector engineering and on vector-based gene delivery.

3) Collaborate with classmates on proper laboratory technique for handling biohazardous reagents and for producing biologics with high purity so that they may be used safely *in vivo*.

4) Use engineering/math to perform quantitative assays for measuring complex virological processes – such as viral infectivity, viral replication, viral load or titer, etc.

5) Summarize the current state of FDA-approved viral vectored therapeutics: identify safety concerns as well as unmet clinical needs.

6) Develop the laboratory skillset required for future research and work opportunities in viral vector engineering and in gene therapy.

Recommended Preparation

Previous coursework in one or more of the following is a plus: lecture as well as lab courses in molecular/cell biology, coursework in immunology, virology, biochemistry, genetics, or neuroscience a plus.

Prerequisite(s): none

Co-Requisite(s): course(s) that must be taken prior to or simultaneously

Concurrent Enrollment: students must enroll in online lab safety courses offered by USC EH&S, including General Lab Safety (GLS), Principles of Biosafety, Trojan Learn: PPE, and Viral Vector Training (VVT). Bloodborne Pathogens (BBP) is optional. Students will be led through lab-specific biosafety training by the instructor during class time.

Recommended Preparation: Previous coursework in one or more of the following is a plus: molecular/cell biology (lecture and lab), immunology, virology, biochemistry (lecture and lab), genetics, or neuroscience.

Course Notes

Copies of lecture slides, video-recorded lectures, suggested and mandatory reading material, and other class information will be posted on Blackboard (Bb) or Design2Learn (D2L) course website.

Technological Proficiency and Hardware/Software Required

For remote instruction, students will be required to use an internet-enabled device with browser capabilities, such as a laptop. The course will be delivered in-person (DEN accessibility, if applicable) or potentially by Zoom, depending on the evolving pandemic situation. Bb or D2L will be used for important class announcements, written assignment submission, claiming JC articles, and student discussion board posts.

Required Readings and Supplementary Materials

The course will not be taught chapter-by-chapter from a single textbook; instead, students will be assigned peer-reviewed journal articles. Occasionally, a single chapter from Principles of Virology (see below) will be assigned, depending on overall student comprehension. Finally, students may be referred to portions of the following textbooks for additional background on a given topic:

- Principles of Virology ("POV", 4th or 5th Ed.) by Jane Flint, Vincent R. Racaniello, Glenn F. Rall, *et al.* (4th Ed. available electronically through USC library)
- Molecular Biology of the Cell, by Bruce Alberts *et al.* (4th Ed. available electronically through USC library and on NCBI)
- Cellular and Molecular Immunology (8th Ed.), by Abul K. Abbas, Andrew H. H. Lichtman, Shiv Pillai, Saunders (available electronically through USC library)

Students should read the assigned materials (e.g., journal articles, textbook excerpts flagged as mandatory, etc; posted on Bb or D2L) before the class meeting in which they are assigned. This will ensure that students are prepared to actively *listen* during lecture (as opposed to feeling the need to transcribe every word that the professor says) and that students can engage in productive discussions of more advanced topics.

Description of Assignments

Lab Worksheets: Throughout the semester, students will need to complete 4-5 lab worksheets on the basic theory and methodological details of lab demos. Herein, the lab worksheets will consist of: short-answer questions that pertain to the reasoning behind a particular step of the experimental methods, fill-in-the-blank questions on

procedural details, short calculations, and/or brief summary statements on the lab results and data analysis. Each lab worksheet will be distributed at the start of the designated class session; it is to be completed by-hand and turned-in to the instructor at the end of class. No late turn-ins will be accepted. Students may collaborate on these lab worksheets, but answers must be THEIR OWN (i.e., directly copying another student's work, such as the short-answer to a question or the exact mathematical steps to a calculation, is prohibited).

In-Class Work: When not in-lab, class time will often be used for journal article discussion; herein, it is crucial that students read the journal article before class in order to participate in this discussion. The majority of class time will be spent actively engaged in laboratory demos (or in pre-lab lecture about the procedural steps, or post-lab wrap-up and discussion about the results and next steps). Because lab demos cannot be repeated for absent students and because lab demos are ordered sequentially to build upon one another, regular attendance is "highly recommended" (i.e., mandatory). Thus, if a student knows in advance that he/she/they will be absent on the day of a lab demo for an important occasion or non-emergency situation (at the discretion of the instructor), notify the instructor by email as soon as possible (≥ 2 weeks beforehand) to discuss possible accommodations. Except under the scenario of health/family emergency or pre-excused absence, NO MAKE-UP WORK FOR LAB DEMOS OR FOR LAB WORKSHEETS WILL BE ACCEPTED.

Participation: The overall participation grade will be based on measures of engagement, including preparation for and participation in class discussions on assigned reading; as well as active engagement during lab demos. Participation will be assessed over the course of the term on an informal point-scale: no credit (0) = frequently absent from class, (1) = regularly attends class, (2) = regularly attends class and demonstrates intellectual engagement (e.g., through asking/answering questions in-class or during lab demos, through active participation in lab demos, through one-on-one meetings with the professor to discuss course topics (i.e., if the student has reservations about speaking up in-class, one-on-one meetings with the professor may offer a less intimidating alternative); always turns in lab worksheets, wherein he/she/they goes the "extra mile" in their completion). Participation during Week 15's Final Project Oral Presentations is mandatory, and the participation grade will be weighted to reflect whether students in the audience ask/answer questions during each presenter's talk or during the Q&A part.

Final Project: A Final Project will be completed in lieu of a "Final Exam". Students must compose a "Specific Aims" page for an NIH grant proposal (i.e., the Final Project written report, 20% grade) on a viral vector engineering project (e.g., novel strategy for engineering new vectors or virus-based platform, novel application of a viral vector to a biomedically relevant topic, etc). The written report must be uploaded to the course website assignment link (D2L or Bb) before the end of the scheduled final exam time period. The 5-minute "elevator pitch" for his/her/their Final Project (i.e., the Final Project oral presentation, 5% grade) will be presented on the last day of class. Additional details and a rubric for grading of this Final Project will be provided (please see Bb or D2L). SORRY, NO MAKE-UPS OR LATE TURN-INS WILL BE ACCEPTED – PLAN ACCORDINGLY!

Assignment Submission Policy

Submission guidelines: For all written/at-home assignments (e.g., Final Project Oral Presentation slides – saved as a PDF, Final Project Written Report – saved as a PDF or DOC, EH&S training certificates – bundled into a single PDF file), a single file should be uploaded to the assignment link on the Bb or D2L site by the due date and time. All Lab Worksheets will be completed by hand in-class, to be submitted directly to the professor at the end of that class session. No late turn-ins will be accepted – plan accordingly.

Late Policy for Assignments: Late Lab Worksheets will only be accepted in cases of extremely extenuating circumstances (e.g., family or health emergency); under non-emergency situations, there will be no make-ups for missed Lab Demo attendance or Lab Worksheet submission. Planned absences (e.g., sports, conference travel, interviews, and other non-emergency situations, etc) must be communicated to the professor at least two weeks in advance in order to arrange for make-up work, at the discretion of the instructor. In the event of an unexcused absence, no grade or feedback will be provided on assignments submitted late. EH&S training certificates must be submitted before the start of Week 8 (i.e., before 12:00 AM on February 27th 2023) for full credit; late or incomplete submissions will result in NO or partial credit.

Assessment and Grading Policies

Grading Timeline: Lab Worksheet grades are provided within one week of their completion. All EH&S training certificates must be uploaded to the assignment link on D2L or Bb as a single file before Week 8 for full credit; only

50% of maximum credit will be given for late turn-in; 0% or no credit will be given for a partial submission. *Students are encouraged to discuss their performance and approximate grade at any point during the semester with the instructor during office hours or by individual appointment*.

Regrade Policy: All regrading requests are due within one week of their return. The requester must email Prof. Treweek about this regrade, providing a clear explanation for the regrade and attaching the original graded assignment.

Grading Breakdown: (assignment-specific rubrics will accompany individual assignment instructions)

Assessment Tool (assignments)	**% of Grade**
Lab Worksheets (5)	50
Safety training (4 EH&S classes, 1 lab training session)	15
Participation	10
Final Project (oral – 5%, written – 20%)	25
TOTAL	100

Grading Scale: Final letter grades are not assigned based on absolute percentage values, but they are curved to generate a reasonable grade distribution (e.g., Z-score, with mean and standard deviation of approximately 88 ± 12). Students can expect their final grades to loosely align with the following scale:

A	95-100
A-	90-94
B+	87-89
B	83-86
B-	80-82
C+	77-79
C	73-76
C-	70-72
D+	67-69
D	63-66
D-	60-62
F	59 and below

Additional Policies

Technology Policy: During class, devices should only be used to participate in activities guided by the instructor or for note-taking. Use of devices for other purposes (email, web-surfing, social media, A/V-recording) is not permitted, wherein any non-academic use of such devices that distracts the instructor or students will result in no credit for in-class work for the day. Photographing or audio/video-recording of lecture material and/or slides is strictly prohibited, as is uploading course content to third-party sites for viewing and/or distribution.

Communication Policy: To promote independence and critical thinking, students are encouraged to work through the following process for obtaining answers to questions on course content and policy *before contacting the instructor*. (1) consult the course syllabus and course policies. If you do not find the answer you need, (2) consult a classmate directly or through D2L/Bb "Discussion" boards. If you are still not satisfied with the answer, (3) review recent lecture slides and announcements posted on D2L/Bb for class updates, (4) email/ask your TA (if applicable), (5) ask the instructor at office hours. Finally, after you have exhausted these methods, (6) email the instructor. In your email, please indicate the steps you have undertaken to seek the answer. Assuming that you have followed the

aforementioned criteria (steps 1-5), your question will be answered within 48 hours between 9am-5pm, but response may be delayed on the weekend or holidays. Please use USC email for all correspondence with the section TA and instructor, and list the course ID (and section, where applicable) in the subject line. The instructor does not respond to questions pertaining to assignments during the 24 hours before an assignment due date. Emails that require a long response (at the discretion of the instructor) will not be answered over email. Instead, the student will be directed to office hours.

Office Hours: *Students are strongly encouraged to take advantage of office hours.* Herein, a preset time and location for office hours (OH) will be identified at the beginning of second session of the class (after compiling student input on timing). Given the ongoing COVID-19 situation in LA county, OH will likely be held in a hybrid fashion, both online via Zoom (meeting ID and password will be provided by email) and in-person by advance request.

For Zoom OH or for scheduling a meeting outside of normal office hours, students are to email jtreweek@usc.edu at least 24-hr in advance to request a 10-min, 20-min, 30-min, or 60-min time block on a given day. Professor Treweek will then give each student a specific time to log-in to the Zoom meeting (or to arrive at her office). This will ensure that no students are caught waiting indefinitely in the Zoom waiting room for "admission" into weekly OH, and it allows all students to have privacy during OH for discussing a personal matter/sensitive issue/grades during OH. To reiterate, Professor Treweek is happy to meet outside of normal OH, however she can only accommodate requests if they are made > 24-hour in advance.

Attendance Policy: When a class session includes lab demos, in-person attendance and active participation is mandatory and will only be excused in case of an emergency, at the discretion of the instructor. For absence due to non-emergency situations, the student must notify the instructor at least 2 weeks in advance, and whenever possible make-up work will be arranged (at the discretion of the instructor). Make-up work will likely take the format of a rigorously evaluated written report – hence, it behooves the student to attend class.

Policies on teamwork: Collaboration is not only permitted but it is also highly encouraged when students are completing Lab Worksheets. This includes the discussion of concepts, exchange of information, and soliciting feedback. Depending on course enrollment, Lab Worksheets and the Final Project may be completed in groups of 1-2 students (TBD). *However, each student is responsible for contributing to and for fully understanding the work product that their group submits*. This class has a no-tolerance policy on academic integrity violations – direct copying of a fellow students' work, transcribing or "paraphrasing" online/published resources without proper attribution, and misrepresenting one's own intellectual contribution on an assignment are all forms of cheating. Review USC's Integrity Policies (see below, and http://www.usc.edu/student-affairs/SJACS/docs/AcademicIntegrityOverview.pdf and http://www.usc.edu/student-affairs/SJACS/docs/GradIntegrity.pdf), as they will be strictly enforced. Violations of this policy will result in an automatic F in the class and filing of an academic misconduct report to the Office of Student Conduct.

Course Syllabus (Tentative)

	Topics/Daily Activities	Deliverables*
Week 1	Review of course plan and policies; Introduction to viral vectors for gene delivery: examples and scientific/clinical usage of viral vectors	
Module 1: Viral Vectors – Usage, Design, and Production		
Week 2	*Martin Luther King Holiday*	
Week 3	Strategies for the production of replication deficient/incompetent viral vectors; Background on Adeno-associated viral vectors (AAVs)	***Read assigned POV textbook chapters***
Week 4	Design of the AAV genome, parameters and considerations for transgene cargo and regulatory elements	***AAV review paper***
Week 5	Properties of different AAV serotypes, capsid selection	***AAV properties***
Week 6	Overview of the AAV production, purification, and titering protocol Lab tour and biosafety training by the instructor	***AAV production***
Week 7	*President's Day Holiday*	
Week 8	Monday - Lab Demo: Cell confluency Wednesday – Lab Demo: Triple Transfection technique	***Lab Worksheet #1***
Week 9	Monday - Lab Demo: Virus Harvest Wednesday – Lab Demo "day 2" Virus Harvest	***Lab Worksheet #2***
Spring Recess	*No class*	
Module 2: AAV Purification and Administration		
Week 10	Methods for Virus Purification, Titering, and Assay for Infectivity	***Read assigned POV textbook chapters***
Week 11	Monday - Lab demo: Titering	***Lab Worksheet #3***
Week 12	AAVs: Routes of administration, and corresponding efficiency and specificity of biodistribution, cell entry, and transgene expression	
Week 13	Monday - Lab demo: retro-orbital injection, direct brain injection	***Lab Worksheet #4***
Week 14	Discussion of Capsid Engineering and AAV Screening methods – CREATE, m-CREATE	***CREATE, M-CREATE***
Week 15	Final Project: student oral presentations	***Lab Worksheet #5***
Finals Week	Final Project: written report due	

*Deliverable due date: reading (hyperlink provided with topic title) must be completed before class on the week that it is listed; lab worksheets are distributed at the start of class, for turn-in before the end of that same class period.

Statement on Academic Conduct and Support Systems

Academic Conduct:

Plagiarism – presenting someone else's ideas as your own, either verbatim or recast in your own words – is a serious academic offense with serious consequences. Please familiarize yourself with the discussion of plagiarism in SCampus in Part B, Section 11, "Behavior Violating University Standards" policy.usc.edu/scampus-part-b. Other forms of academic dishonesty are equally unacceptable. See additional information in SCampus and university policies on scientific misconduct, policy.usc.edu/scientific-misconduct.

Support Systems:

Counseling and Mental Health - (213) 740-9355 – 24/7 on call
studenthealth.usc.edu/counseling
Free and confidential mental health treatment for students, including short-term psychotherapy, group counseling, stress fitness workshops, and crisis intervention.

National Suicide Prevention Lifeline - 1 (800) 273-8255 – 24/7 on call
suicidepreventionlifeline.org
Free and confidential emotional support to people in suicidal crisis or emotional distress 24 hours a day, 7 days a week.

Relationship and Sexual Violence Prevention and Services (RSVP) - (213) 740-9355(WELL), press "0" after hours – 24/7 on call
studenthealth.usc.edu/sexual-assault
Free and confidential therapy services, workshops, and training for situations related to gender-based harm.

Office of Equity and Diversity (OED)- (213) 740-5086 | Title IX – (213) 821-8298
equity.usc.edu, titleix.usc.edu
Information about how to get help or help someone affected by harassment or discrimination, rights of protected classes, reporting options, and additional resources for students, faculty, staff, visitors, and applicants. The university prohibits discrimination or harassment based on the following *protected characteristics*: race, color, national origin, ancestry, religion, sex, gender, gender identity, gender expression, sexual orientation, age, physical disability, medical condition, mental disability, marital status, pregnancy, veteran status, genetic information, and any other characteristic which may be specified in applicable laws and governmental regulations. The university also prohibits sexual assault, non-consensual sexual contact, sexual misconduct, intimate partner violence, stalking, malicious dissuasion, retaliation, and violation of interim measures.

Reporting Incidents of Bias or Harassment - (213) 740-5086 or (213) 821-8298
usc-advocate.symplicity.com/care_report
Avenue to report incidents of bias, hate crimes, and microaggressions to the Office of Equity and Diversity |Title IX for appropriate investigation, supportive measures, and response.

The Office of Disability Services and Programs - (213) 740-0776
dsp.usc.edu
Support and accommodations for students with disabilities. Services include assistance in providing readers/notetakers/interpreters, special accommodations for test taking needs, assistance with architectural barriers, assistive technology, and support for individual needs.

USC Support and Advocacy - (213) 821-4710
uscsa.usc.edu
Assists students and families in resolving complex personal, financial, and academic issues adversely affecting their success as a student.

Diversity at USC - (213) 740-2101
diversity.usc.edu
Information on events, programs and training, the Provost's Diversity and Inclusion Council, Diversity Liaisons for each academic school, chronology, participation, and various resources for students.

USC Emergency - UPC: (213) 740-4321, HSC: (323) 442-1000 – 24/7 on call
dps.usc.edu, emergency.usc.edu
Emergency assistance and avenue to report a crime. Latest updates regarding safety, including ways in which instruction will be continued if an officially declared emergency makes travel to campus infeasible.

USC Department of Public Safety - UPC: (213) 740-6000, HSC: (323) 442-120 – 24/7 on call
dps.usc.edu
Non-emergency assistance or information.