Principles of Genetic Analysis I



Genetics is an experimental science. MGY314H is a laboratory course in prokaryotic (bacterial) and eukaryotic (yeast) genetics; you will perform several experiments over the 12-week period. Students will work in teams of 2 (sometimes 3) to carry out a variety of crosses, mutant hunts, and phenotypic characterization in bacteria, phage, and yeast, and learn to analyze and interpret the genetic data that you obtain. During this course, you will generate mutants, deduce gene function from phenotypic analysis, identify genetic suppressors, characterize mutant alleles (dominant or recessive), perform meiotic segregation analysis, order genes in a genetic pathway (epistasis analysis) and generate genetic interaction profiles. Most of your time will be in the lab, with some tutorials and pre-lab lectures to discuss experimental results and to supplement your understanding of genetics.

The emphasis in MGY314H is to learn the fundamental concepts of genetics: mutation, complementation, recombination, genetic suppression and regulation (epistasis)--notably, how to apply the tools of genetic analysis and how to interpret them. The models we use in this course are *Escherichia coli*, the best studied gram-negative bacterial species that reproduce asexually, and *Saccharomyces cerevisiae* (also known as baker's or brewer's yeast), the best characterized eukaryotic model that reproduces through both asexual (mitotic) and sexual (meiotic) cycles. *E.coli* and budding yeasts are often the models of choice in the study of more harmful bacterial/fungal species because many principles of their biology are generally applicable, and both have contributed much to our understanding of the core principles of inheritance and genetic interaction. Finally, both organisms are broadly used as workhorses for molecular biology (cloning, expression, genetic interactions), and much of the original genetics defined in *E. coli* and budding yeast has led to important tools for diagnosis and scientific research.

Date, Time and Location:

Thursdays, 1:10 - 5 pm. Medical Sciences Building (MSB), Rm 3280, 3282 & 3377

First class will include an organizational meeting, location will be communicated in Quercus

You must submit proof of completion of the <u>EHS923 Biosafety for Undergraduates</u> prior to the first lab (refer to Quercus) & bring a Lab coat/ safety glasses. Bring a personal lock for the hallway lockers as those of you assigned to a level 2 lab will need to keep your belongings outside the lab due to biosafety regulations (personal clothing/bags cannot be kept in the level 2 space).

Instructors:

Part 1 (prokaryotic genetics):

Prof. Jessica Hill Department of Molecular Genetics Medical Sciences Building, room 7253 (1 King's College Circle) email: jessica.hill@utoronto.ca

Part 2 (eukaryotic genetics):

Prof. Brigitte (Bri) Lavoie (Course coordinator) Department of Molecular Genetics MaRS West Tower, room 1514 (661 University Ave) email: brigitte.lavoie@utoronto.ca

Prerequisites: BIO230H/BIO255H, BIO260H/HMB265H (or equivalents)

Familiarity with foundational concepts in genetic analysis and terminology is expected (review your second year genetics notes!). Applied knowledge of basic chemistry concepts (high school /intro chemistry courses) is expected, notably good working knowledge of aqueous solutions, pH, dilutions from concentrated stocks ($M_1V_1=M_2V_2$) as well as serial dilutions, along with the interconversion of units of measurement (M, mM, uM, nM, pmol/ul = uM etc).

Learning objectives:

The fundamental tools of genetic analysis are **Mutation (genes, alleles)**, **Complementation, Recombination, Genetic Suppression, Genetic Enhancement and Genetic Interaction (Epistasis)**. In this course, learners will carry out weekly experiments with risk level 1 organisms to demonstrate these concepts, generate figures/tables of their results, critically analyze the data they have produced, and clearly and concisely communicate their findings in written lab reports/worksheets.

On a weekly basis, students will formulate hypotheses/predictions based on pre-existing knowledge/scientific models on genetic topics covered in pre-lab readings & lectures in the lab manual. Comprehension and lab-readiness will be assessed with short on-line quizzes that include the generation of protocols/workflows for each lab. Data presentation, interpretation and scientific thinking will be assessed through worksheets and lab reports.

Students will learn to plan, execute, interpret, and publish (lab reports) genetic experiments, and will demonstrate the concepts of rigorous analysis as well as the effective presentation of data (scientific figures and tables) to convey their findings. Learners will also learn to work effectively and safely in a genetics lab and must complete <u>EHS923 Biosafety for Undergraduates</u> prior to working in the lab. All students are expected to demonstrate safe lab practices during every lab.

Part 1--Bacterial genetics (first 6 weeks).

Bacteria are prokaryotic organisms (haploids) lacking a nucleus--they reproduce asexually by binary fission and exchange genetic information through horizontal gene transfer mechanisms including conjugation, transduction and transformation. Historically, much of what we know about gene structure and function has come from using simple, fast-growing organisms (including bacteria and phages). During the first 6 weeks of the lab, learners will perform multi-week experiments and will become proficient in sterile and safe handing techniques of risk level 1 organisms, dilutions, pipetting and plating of bacteria, all core skills for molecular biology practiced in nearly every wet lab.

Experiment 1: Mutation and genetic suppression.

• <u>Learning concepts:</u> Genetic nomenclature, mutations, revertants, suppressors and phage growth

Experiment 2: Mutant hunt-- generating and isolating mutations in E.coli that affect lactose metabolism and sensitivity to phage infection

• <u>Learning concepts</u>: mutagenesis strategies, transposon mutagenesis, genetic selection and genetic screens, genetic complementation

Experiment 3: Control of gene expression and use of genetic reporters

• Learning concepts: gene induction and repression, gene structure, reporter genes,

Part 2--Eukaryotic genetics (last 6 weeks).

The much-loved eukaryotic organism budding yeast (of bread/ beer & wine fame--what's not to love?) is our genetic model of choice for the last 6 weeks of the lab. Unlike bacteria, many genes/pathways found in larger eukaryotes (like us) are conserved in yeast. In addition, and also unlike bacterial (haploid) cells, budding yeast have both haploid and diploid states that grow mitotically, and diploids can be induced to undergo meiosis to regenerate the haploid state. In this last module, we'll be performing 6 different experiments, some of which can be completed in one afternoon while others will take several weeks to complete. Through these experiments designed to illustrate universal genetic concepts (mutation, complementation, recombination, genetic interaction (synthetic enhancement and epistasis/suppression), learners will gain both practical bench skills as well as real-world experience designing molecular genetic experiments (and constructing the necessary strains) as well as interpreting and presenting genetic data.

Experiment 1: Genetic analysis of yeast cell fate determination. How do cells from the same organism adopt different fates?

• <u>Learning concepts:</u> genetic nomenclature, genetic locus, marker genes, using complementation for genetic selection, yeast haploid and diploid life cycles, crosses/sexual reproduction, inferring genotype from phenotype, inferring gene function from mutants, making and testing scientific models.

Experiment 2: Synthetic Genetic Analysis. Many phenotypes arise through multi-gene interactions (where the single mutations alone exhibit little to no discernible phenotype). Interactions between gene deletions can be negative (synthetic enhancement) or positive (genetic suppression or epistasis).

• <u>Learning concepts:</u> multi-gene traits, synthetic genetic interactions, sophisticated genetic selection and counter-selection strategies, synthetic/heterologous marker genes, systematic generation of double mutant combinations, sporulation (meiotic segregation), light microscopy.

Experiment 3: Yeast sex change. Unlike organisms that have different sex chromosomes (like X and Y in humans, mice, flies...), the identity of yeast cells is determined by a single MATING TYPE locus on chromosome III. A programmed gene replacement event causes haploid yeast cells to change their fate via a gene conversion event.

• <u>Learning concepts</u>: gene repression and induction, GAL promoters/GAL4-dependent transcription, gene silencing, homothalloism, gene conversion, double strand break repair, homologous recombination, chemical transformation, genetic complementation, marker genes

Experiment 4. Ordering genes in a genetic pathway. G-protein coupled receptors transmit extracellular signals to the cell through evolutionarily conserved MAP Kinase signal transduction pathways. How do we know where each gene functions? By using combinations of gain-of-function and null alleles with distinguishable phenotypes, the order of gene function in a pathway can be determined through epistatic relationships.

- <u>Learning concepts:</u> reporter gene assay, gain-of-function and loss-of-function alleles, epistasis analysis, genetic pathways (biosynthetic vs switch-regulatory), MAP kinase signal transduction.
- Genome database utilization, gene structure, oligonucleotide primer design, mutagenesis (error-prone PCR), de novo screen design.

Experiment 5. Meiotic segregation analysis (Sexual reproduction). Much of the work of a geneticist is to characterize mutants found in genetic screens, as well as generate strains with novel combinations of mutations to test specific hypotheses. Meiotic analysis is commonly used to determine whether a phenotype of interest derives from a single gene mutation (or through multigene effects), to map the genomic position of mutants, and to validate mutants generated through transformation and recombination through linkage with known genetic markers nearby. As meiosis independently assorts alleles of different genes, it is a powerful tool for the generation novel double (triple, quadruple etc) mutants to test hypotheses.

- <u>Learning concepts</u>: sexual reproduction/meiotic segregation, dominant and recessive alleles, single gene versus multi-gene traits, complementation, recombination, genetic linkage and mapping
- Mendel's rules of segregation (of alleles) and independent assortment

Experiment 6. Genome engineering. Modern technologies for genome engineering introduce a double strand break in the genome to create recombinogenic ends. In higher organisms, these ends are preferentially repaired by an error-prone end joining mechanism. In budding yeast (and mouse ES cells), double strand breaks are preferentially repaired by an error-free process called homologous recombination that can target exogenous DNA ends to the yeast genome. In this experiment, learners will perform high efficiency transformation with different types of vectors (integrating, CEN-based and episomal) and determine what types of DNA repair products lead to yeast transformation (marker gene expression).

• <u>Learning concepts:</u> DNA repair mechanisms--homologous recombination (HR) versus non-homologous end joining (NHEJ), high efficiency transformation, bi-directional genetic information flow, alternative products of genetic recombination.

Lab Manual / Textbooks: MGY314 has an on-line lab manual available on Quercus that contains hyperlinks to additional readings from the published literature. A genetics textbook however will be necessary to review and understand the biology and concepts behind the experiments you will perform. It also helps to keep your lecture notes from BIO260/HMB265 handy as well as those from MGY340. While there is no required textbook, some recommended supplementary texts are:

- Molecular Genetics of Bacteria, 4th edition, Snyder, L., Peters, J.E., Henkin, T.M., & Champness, W. American Society for Microbiology (ASM) Press, 2013;
- Hartwell et al, Genetics, from Genes to Genomes, McGraw Hill;
- Hawley, RS & Walker, MY. *Advanced Genetic Analysis: Finding Meaning in a Genome* (2009) Blackwell Publishing, ISBN 978-1-4054-0336-7 (a classic read!)
- Meneely, P. (2020) *Genetic Analysis, Genes, Genomes & Networks in Eukaryotes*, Oxford Univ Press.

Most labs will include a tutorial and/or pre-lab lectures posted to Quercus to help with more specialized background and concepts.

<u>StarGenetics</u>: During the eukaryotic genetics section, we will make use of an on-line Mendelian Cross Simulator developed at MIT. The StarGenetics software runs on JAVA and can be downloaded onto your own personal computer (Mac or PC) or accessed in person or remotely through the Sid Smith Computer labs (remote access will allow the software to work on any platform including tablets/phones). <u>http://star.mit.edu/genetics/index.html</u>

40%

15%

MGY314H 2023 Marking Scheme

- Lab Reports
 - Lab Participation 5%
 - Pre-lab quizzes 10%
 - Midterm (1 hr, in class, October 13)
 - Final exam (2 hr)

- 30% (20:80 prokaryotic:eukaryotic genetics)
- Deadline to drop MGY314H: Nov. 6, 2023

Lab reports (excluding group reports handed in during the lab):

"Normally, students will be required to submit their course essays to the University's plagiarism detection tool for a review of textual similarity and detection of possible plagiarism. In doing so, students will allow their essays to be included as source documents in the tool's reference database, where they will be used solely for the purpose of detecting plagiarism. The terms that apply to the University's use of this tool are described on the Centre for Teaching Support & Innovation web site (https://uoft.me/pdt-faq)." If students choose to opt out, they should let their instructor know well in advance of submitting their paper. Ideally, they should communicate this during the first class, when the instructor is reviewing the course outline.

Late penalties for term work: 10% will be deducted per day up to 2 days late, after which the work will not be accepted. Reports more than 2 days late will receive a mark of 0. Students requiring accommodation due to unforeseen emergencies should contact their TA as soon as possible and provide appropriate documentation to the course coordinator (Bri Lavoie).

<u>Absence declaration</u>: If, for any reason, you are unwell, please declare your absence on <u>ACORN</u> and communicate with your TA/instructor. This will ensure that we can make every effort to provide needed academic accommodations to support you. **If you will miss a term test, please contact your instructor preferably before but no later than 48H after the test after declaring your absence on <u>ACORN</u>. Students remain responsible for meeting course requirements as determined by your instructors.**

• <u>https://www.acorn.utoronto.ca/Links to an external site.</u>

Workload: The lab periods are scheduled as 4 hours per week (1 - 5 pm Thursdays). This will allow time to perform the experiments and to include sessions to discuss data and sample problems, and for occasional tutorials. Some weeks will take the full 4 hours; others will be shorter. This is the nature of research – some days are longer and shorter than others, depending on the experiments, and we, as researchers, must accommodate our schedules to the requirements of these experiments. On occasion, you will be required to come to the lab for short periods outside of the Thursday lab time slot.

Students work in pairs and perform experiments jointly unless instructed otherwise. You must prepare for each lab by reading the appropriate section in this lab manual/watching the pre-lab lecture on-line <u>before</u> you come to the lab. In addition, there may be pre-lab readings assigned. If you are prepared, you will be able to complete each exercise in the time allotted. Your pre-lab quiz will test you on concepts and preparation for that week's lab (see section E below).

Students will be required to submit a report on each of the experiments completed in class (see instructions below). Lab reports are usually due one week after the experimental results have been obtained (see Quercus for due dates and late penalties)--some of these will be individual reports, others will be group reports (to promote discussion between partners!) and some will be in-class worksheets. It is expected (encouraged!) that lab partners will discuss their data with each other (and it's fine to discuss with other students in class but be aware that you can have different strain arrays).

Quercus: We will use Quercus to post information, handouts, lab report instructions, marks & other useful information like assignment due dates, & to communicate with you. You will also submit your lab reports electronically via the site. The Quercus course page is updated frequently and should be considered the final word should any discrepancies with this document arise.

<u>**Preparation and Participation:**</u> As above, you are required to prepare ahead of time for each lab session, which means reading the relevant sections of the lab manual and any additional material (papers/JOVE videos/pre-lab talks) posted on Quercus.

Short on-line pre-lab quizzes posted to Quercus are due before the lab starts—these are designed to verify that you've done your pre-lab work, and to help you think through the lab and practice any calculations needed—for instance, how may plates will you need? which experiments have incubations (during which you can do other things?), how many samples will you have? what experiments do you need to do and in what order will you perform them? what temperature incubators/water baths do you require etc.

You will also upload your own abbreviated version of the experimental protocols for each lab--this will serve as your plan of action. It can be handwritten or typed out--and you should aim to keep it as brief as possible yet containing all the information you need so you don't need to refer to the lab manual (which contains so much extra explanation that it's not great as an in-lab protocol). Because MGY314 has no formal lecture component, there is independent study on your part required to review genetic concepts from your 2nd year courses and to read about the biology of the systems involved. The recorded pre-lab lecture posted on Mondays is designed to help you review the theoretical genetic concepts as well as to highlight some aspects of the lab including details about the experimental practicalities and data interpretation. If you've done your reading/watched the video and are still confused--ASK your TA or instructors! We are here to help.

Looking forward to meeting you all in the lab!

Prof Bri Lavoie, course coordinator

Prof Jessica Hill