###### bacteriaBudding yeastPrinciples 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), 3rd floor dept teaching labs**

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

You must submit proof of completion of the [EHS923 Biosafety for Undergraduates](https://q.utoronto.ca/courses/279492/modules/items/3835828)  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):

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###  Prof. Jessica Hill

###  Department of Molecular Genetics

###  Medical Sciences Building, room 7253 (1 King’s College Circle)

###  email: [jessica.hill@utoronto.ca](file:///Users/jhill/Library/Mobile%20Documents/com~apple~CloudDocs/Documents/6.%20MGY314/Lab%20manual/jessica.hill%40utoronto.ca)

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### 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](file:///Users/jhill/Library/Mobile%20Documents/com~apple~CloudDocs/Documents/6.%20MGY314/Lab%20manual/brigitte.lavoie%40utoronto.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 ( M1V1=M2V2) as well as serial dilutions, along with the interconversion of units of measurement (M, mM, uM, nM, pmol/ul = uM etc).

### Ancillary Fees 2025: $26.25

### 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[EHS923 Biosafety for Undergraduates](https://q.utoronto.ca/courses/279492/modules/items/3835828) 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.**

* Learning concepts: 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**

* Learning concepts: 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?

* Learning concepts: 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).

* Learning concepts: 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.

* Learning concepts: 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.

* Learning concepts: 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 multi-gene 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.

* Learning concepts: 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).

* Learning concepts: 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 **lab manual (file 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;
* **Miller, DE, Miller, AL & Hawley, RS (2023). *Genetic Theory and Analysis: Finding Meaning in a Genome* Wiley Publishing, ISBN 978-1-118086926 (Required text for MGY340!)**

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

#### StarGenetics : 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).

**MGY314H 2023 Marking Scheme**

* Lab Reports 40%
* Lab Participation 5%
* Pre-lab quizzes 10%
* Midterm (1 hr, in class, October 9) 15%
* Final exam (2 hr) 30% (20:80 prokaryotic:eukaryotic genetics)
* **Deadline to drop MGY314H: Nov. 11, 2025**

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

A core learning objective of MGY314 is clear scientific writing and appropriate referencing (which is something generative AI tools like ChatGPT are notoriously poor at). These are skills that are developed iteratively through practice, receiving and incorporating feedback. Many scientific journals have prohibited the use of generative AI tools in the writing of scientific papers (would *you* trust something that is known to make up facts???) and as such learning to summarize/cite the relevant literature accurately, present results clearly and succinctly as well as discuss the implications of your results are skills every scientist needs.   In MGY314, you may not use artificial intelligence tools to generate new material for lab reports, including writing or graph construction: ***copy/pasting text generated by generative AI tools and passing it off as your own work is plagiarism and constitutes an academic violation*.**  Please review the MGY314 policy on generative AI tools (at the end of this document).

**“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 & Flex points:** Most lab reports will be due at midnight the Thursday following completion of the experiment.  This allows you to complete your work in the lab with lots of time to confer with your lab partner/ ask any last minute questions of your TA during the lab.   10% will be deducted per day for submissions 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.

**Flex points** are available for you to use to gain extra time to complete reports.  At the start of the semester, **each student will be allotted 12 flex points** **to use as you see fit**.  **Each point is worth 4H of extra time to extend deadlines, no questions asked.** You will need to track your own usage and indicate it in a little table on each report title page (the TAs will keep track too).  You can choose to pool all 12 points and blow them in one go for 48H of extra time, or you can use them a couple at a time to give yourself a bit of extra time when things get really busy (ie midterm season!).  There's no judgement here--use them as you see fit.  They count in addition to regular extensions granted through accessibility services.  For reports where both partners collaborate, if you both extend the deadline, you will both need to use your flex points (they are not additive).  If one partner is ready by the deadline, just submit your part of the report to your TA (give a copy to your lab partner) and your partner can merge the full report and submit your and their part as a whole when they are done.  Don't worry about the late notice given automatically by Quercus, we will override this manually after accounting for flex points/accomodations.

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).

**Absence declaration:** If, for any reason, you are unwell, please declare your absence on [ACORN](https://www.acorn.utoronto.ca/) 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** [ACORN](https://www.acorn.utoronto.ca/)**.**  Students remain responsible for meeting course requirements as determined by your instructors.

* [https://www.acorn.utoronto.ca/Links to an external site.](https://www.acorn.utoronto.ca/)

### 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 our 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 *before* 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 relevant concepts and preparation for that week’s lab (see 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. Collaborative reports must indicate on the title page the contributions of each lab partner (for instance, AB wrote the introduction; CD made the graphs etc--but you are expected to discuss your results as a team and agree on the interpretation). **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). Science is a team sport and explaining / discussing / helping your classmates is collaborative learning, and we are all for it. This type of collaboration is not an academic violation, in contrast to copying an old lab report or any form of someone else's work (including generative AI). If someone explains to you how to do a calculation and then you can do it yourself independently, that's learning.

### 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 (as pdfs) via the site. The Quercus course page is updated frequently and should be considered the final word should any discrepancies with this document arise.

### Preparation and Participation: 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. Having thought this through beforehand allows you to check that you have all the reagents you need to complete your tasks, that the incubators/water baths are at the correct temperature, that there are enough plates...

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, with schematics or typed out: up to you. But 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 in fact rather poor 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 lectures posted on Mondays are 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, please ASK your TA or instructors. We are here to help. And for having the "stickability" to read through this entire document, email me with "got polio? me neither, thanks science" in the header to be entered into a draw for a genetics related prize. You deserve it.

Looking forward to meeting you all in the lab!

 Profs Bri Lavoie & Jessica Hill, your friendly neighborhood instructors

**MGY314 Generative AI Policy**

**What is Generative AI and what are Large Language Models?**

Generative AI is powered by very large machine learning models that are pre-trained on vast amounts of data (like the contents of the Internet) to create images, text, conversations, etc. A subset of Generative AI is Large Language Models (LLMs), which are trained on up to trillions of words to learn language patterns and grammar, then generate responses based on the learned language patterns. Some LLMs you may be familiar with include ChatGPT, PerplexityAI, Bing, etc. For the remainder of this section, the term “artificial intelligence tools” typically refers to LLMs.

**What are some of the considerations for use of artificial intelligence tools?**

 There are some important shortcomings to consider when using artificial intelligence tools. The outcome you get from artificial intelligence tools are only as good as the data you train the system with. That is, the responses from artificial intelligence tools demonstrate biases that are found on the internet including racial and gender biases and cultural stereotypes. Furthermore, artificial intelligence tools are prone to hallucination – *making up facts*. ChatGPT, for example, was trained on data from September 2021 and earlier, meaning that it has no knowledge of recent events. Caveat emptor!

 Of course, there are benefits to using artificial intelligence tools. You could, for example, use one to verify your calculations, ask for advice on how to set up a data table or potentially present your data. These are skills, however, that you must develop yourself and can expect to be tested on – use artificial intelligence tools for verification of your work, not to do the work itself!

 Finally, an LLM like ChatGPT is quite effective at editing text you have written for clarity. This may be an especially valuable feature for those of you whose first language is not English, but using generative AI to create text that you then copy is plagiarism and constitutes an academic violation.  There are also AI tools that can support you in summarizing and citing papers, but they cannot perform these tasks to the level of quality required in this course.

**What is the policy for the use of Generative AI in MGY314?**

*A core learning objective of MGY314 is clear scientific writing and appropriate referencing (which is something generative AI tools are notoriously poor at). These are skills that are developed through practice, by receiving and incorporating feedback (often through several rounds). Many scientific journals have prohibited the use of generative AI tools in the writing of scientific papers (would you trust something that is known to make up facts???). Learning to summarize/cite the relevant literature accurately, present results clearly & succinctly as well as discuss the implications of your results are foundational skills.*

* You may not use artificial intelligence tools to generate new material for lab reports, including writing or graph construction—***copy/pasting text generated by LLMs and passing it off as your own work is plagiarism and constitutes an academic violation*.**
* You may, however, use artificial intelligence tools to:
	+ Edit sections of your lab report for clarity (spell check, grammar check etc)
	+ Suggest ways to present your data (including generating data tables)
	+ Check calculations, as applicable.
* Note that you are 100% responsible for any inaccuracies in submitted work. If AI hallucinates (making up references is a common issue) and you don't fix it, then it's on you. Please reach out if you have any questions!