The Piacentino Lab Handbook

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Welcome, Expectations & Conduct

Welcome to the Lab

We're excited to have you on our team! Our group is committed to maintaining an exciting, supportive, educational, and productive environment, and we expect you to play your part in keeping this atmosphere thriving. We hope you will learn a lot about cell and developmental biology, build new skills at the bench and at the computer, and contribute new knowledge in these areas to the greater scientific community. Along the way, we hope you will have fun with your research projects and form new friendships and colleague networks that will serve you long after you have left the lab.

This lab handbook will forever be a work in progress which is designed to help Piacentino lab members at all stages by providing information on what we expect of one another, how we conduct ourselves, how the lab runs, how to get help, and more. *Please help us maintain this resource*: let Mike know if you have any additions, clarifications, or corrections to suggest!

To make sure everyone is on the same page when they join our group, new lab members are expected to read and understand this handbook, and discuss concerns with Mike. In addition, we hope that this handbook will come in handy later, so if you're wondering about something, check this document out for answers or pointers to new resources! And if you have a question about something NOT in the handbook, ask Mike, and we can work together to add it for the future.

This lab handbook was inspired by the lab handbooks of many great labs, most notably the Aly, Barber, Blind, and Gilbert labs. We want to thank these groups for generously sharing, and for leading our community in building supportive research groups dedicated to clear communication. If you're writing your own lab handbook, we welcome you to take inspiration from ours (with appropriate citations).

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Values and Expectations

Mission Statement

We in the Piacentino lab aim to empower one another to grow as scientists and as human beings while seeking to understand how the developing embryo constructs our adult form. Specific projects combine chicken embryology with rigorous live imaging, molecular biology, and biochemistry to determine how cellular lipids drive cell migration in development and disease. We foster a community built on curiosity, compassion, inclusivity, and determination.

Why do our work?

- Increased understanding of normal development helps recognize, prevent, and treat congenital anomalies
- Conserved mechanisms from development impact homeostasis, aging, and disease

- Training in interdisciplinary science unlocks future innovation

Our Lab Values:

1. Integrity - be honest, truthful, and reliable
2. Curiosity, enthusiasm, and engagement
3. Producing high-quality results through hard work, patience, and determination
4. Empowering one another to reach new heights and maintain a growth mindset
5. Being inclusive and respectful of one another's experiences

Science is hard, but it's also fun. We want to make sure that everyone experiences a positive, engaging, hostility-free, challenging, and rewarding lab environment. To maintain that environment, we all seek to embody these values and operate under the following expectations of ourselves and one another:

Everyone

- Always strive for your best – today's best might look different from yesterday or tomorrow, and it is the same for everyone else around
- Behave professionally, responsibly, reliably, and with integrity
- Put in the time and effort needed to move your project forward – a strong PhD typically takes more than 40 hours of effort per week, though this varies at different phases of the project
- Take ownership of your project – find the question that interests you, build/draw a mechanistic model to explain your data, identify the assumptions it makes, and design experiments to test those assumptions
- Take ownership of your own experience and education – keep up to date with the literature, with your training, and with developing new skillsets
- Work with urgency, but work carefully and ask for help
- Support your fellow lab mates when they need help
- We expect you to make mistakes, we also expect you to admit and learn from them
- Keep honest and detailed records of your experiments, their analyses, and results
- Respect your fellow lab mates – their strengths and weaknesses, their passions and motivations, respect their desire for quiet if they need it, and for support and a kind ear when they need it. Respect their culture, their values, their beliefs, and their identities.
- Report areas where you may be struggling or if you witness tension or hostility in the lab
- Attend and participate in relevant meetings
- Give feedback candidly and with the intention to help, not to hurt
- Receive feedback with gratitude and appreciation – it's a gift to be shown how to improve
- Challenge me (Mike) when I'm wrong or when your opinion is different, and treat the rest of the lab to your unique expertise
- Be a good lab citizen – work safely, be tidy, do your lab chores, lock the doors and turn off the lights at the end of the day, etc.

Principal Investigator – Mike

"Everyone" above, and I promise to also…

- Support you and your research (scientifically, financially, emotionally)
- Help you develop your paper and grant writing skills
- Provide constructive feedback in a timely manner
- Be available in person and via email/Slack on a regular basis
- Share my perspective on where the lab is going, where the field is going, and tips on surviving and thriving in academia
- Support your career development by introducing you to other researchers in the field, promoting your work at talks, writing recommendation letters, and letting you attend conferences as often as schedules/finances permit
- Help you prepare for the next step of your career, wherever it may be

- Care for and prioritize your physical and emotional well-being
- Adhere to the JHU policies on mentorship across all career stages

Postdocs

“Everyone” above, and you will be also expected to…

- Develop your own independent line of research
- Work to build a distinguishing research identity that is *yours* and recognized from outside
- Don’t wait for opportunities/ideas, but rather actively seek them out and nourish them
- Help train and mentor students in the lab when they need it
- Present your work at departmental events, at other labs (if invited), and at conferences
- Apply for grants (e.g. NIH F32, NIH K99). Though I will only hire you if I can support you for at least one year, it’s in your best interest to get experience writing grants.
- Apply for jobs (academic or otherwise) when you’re ready, and discuss with Mike the steps you plan to take toward identifying these jobs and developing the necessary skillset

Graduate Students

“Everyone” above, and you will be also expected to…

- Develop your thesis research – this should represent a significant contribution to our scientific knowledge. You will work closely with Mike in developing this research project and overseeing its progress, but ultimately you are expected to take ownership of this body of work and its execution.
- Help mentor undergraduate students and more junior graduate students when they need it
- Present your work at departmental, regional, and national events
- Apply for grants (e.g. NSF GRFP, NIH F31, NIH F99/K00). It’s in your best interest to get experience writing grants.
- Think about what you want for your career (research or teaching in academia, industry, scientific writing, something else), and talk to Mike about it to make sure we both identify the right training you’ll need to prepare for that career
- Make sure you meet all departmental and program deadlines (exams, thesis, committee meetings) – and make sure Mike is aware of them!
- After completing your core curriculum, prioritize your research – coursework is important, but ultimately your research project is what earns your PhD and prepares you for your next career stage.
- Adhere to the JHU Mentorship Commitments of Faculty Advisors and PhD Students

Lab Staff

“Everyone” above, and you will be also expected to…

- Work on your own part-time research project (developed with Mike’s help)
- Help new lab members adjust to the lab by answering whatever questions you can
- Help with onboarding and getting new members familiar with our systems
- Organize and maintain databases for common lab protocols, image storage, and analyses
- Maintain laboratory stocks – monitor inventory levels; prepare solutions and reagents when running low; order new reagents, consumables, and equipment as needed; maintain laboratory equipment and train new users
- Maintain lab safety protocols for the lab (writing, renewal), archives of old paperwork and forms related to lab SOPs, orders, shipments, etc.
- Be in the lab on a regular basis – your presence is needed in the lab, so you are expected to hold a *predictable* schedule

Undergraduate and High School Students

“Everyone” above, and you will be also expected to…

- Develop a weekly schedule by talking to your lab mentor – you should be coming in every week and scheduling enough time to get your work done
- Help your mentor perform experiments, or work on your independent project if relevant
- Always ask if you are unsure of something
- Commit to at least: one full academic year plus one full time summer, or two full academic years
- Show up and be engaged – read the literature, think about our science, participate in lab meetings, etc

Code of Conduct

Harassment and Discrimination

Our lab, and Johns Hopkins, are environments that must be free of harassment and discrimination. Every lab member must abide by the Johns Hopkins University policies on harassment and discrimination, found at the Office of Institutional Equity. You can also learn about JHU's Sexual Misconduct Policies and Procedures. Please read these policies now.

Mike is committed to ensuring the lab is a safe, friendly, and accepting environment for everyone. We will not tolerate any verbal or physical harassment or discrimination on the basis of gender, gender identity and expression, sexual orientation, ability/disability, physical appearance, body size, race, or religion. We will not tolerate intimidation, stalking, following, unwanted photography or video recording, sustained disruption of talks or other events, inappropriate physical contact, and unwelcome sexual attention. Finally, it should go without saying that lewd language and behavior have no place in the lab, including any lab outings.

If you feel you are being harassed or discriminated against or notice someone else being harassed, please alert Mike immediately. If Mike is the cause of your concern, please reach out to the Cell Biology department director (Andrew Ewald) or another trusted faculty member of Cell Biology or your graduate program.

Diversity, Equity, Inclusion and Belonging (DEIB) Statement

As a researcher, a mentor, and a member of the LGBTQ+ community, Mike is dedicated to fighting for diversity, equity, inclusion and belonging. Race, ethnicity, gender, age, religion, language, abilities/disabilities, sexual orientation, gender identity, political affiliation, and socioeconomic status should not be barriers to the STEM community or grounds for discrimination. As such, the Piacentino lab welcomes people and viewpoints from all backgrounds. Only when everyone feels a sense of belonging and support from their community can our scientific and human endeavors reach their peak ingenuity and excellence.

Scientific Integrity and Research Ethics

The lab and Johns Hopkins University are committed to ensuring research integrity and take a hard line on research misconduct. Fabrication, falsification, and plagiarism are not tolerated. Any allegations of misconduct will be handled swiftly. The JHU Research Integrity Policies are posted on the Office of Research Integrity website. In addition, you will be required to participate in institutional training on the responsible conduct of research as an undergraduate, graduate student or postdoc; the precise training will depend on funding source and career stage, please see the Responsible Conduct of Research ("RCR") program information.

The deeper problem is that some people feel pressured to commit research misconduct in the first place. If you are feeling like your work doesn't measure up to others around you or need to publish more, publish faster, or publish in higher impact journals, reach out and talk to Mike. These feelings are normal, and something everyone experiences at various stages in their career – but it is *NEVER* an excuse to fabricate, falsify, or plagiarize. You joined the lab because you're curious about the world and you want to find out how things really work. Science is about uncovering and sharing the truth, or as close as we can get to it with the available methods. Engaging in research misconduct is a disservice to you and to the field. It risks your career and the careers of your colleagues. It is not worth it. Don't do it.

Rotating in the Lab

Big Picture

Rotations are a two-way street – you want to know if our lab is a good fit for you, and we want to know if you are a good fit for our lab. Here's what we're evaluating:

1. **Your demonstrated interest and aptitude for the research and field.** Show us your independent perspective on how your project fits into other work in the lab and in the field. What would you do next? If you want to join the lab for your thesis, what question would you like to answer for your project? Independently reading is the most important way to gain this insight and can be honed by actively participating in lab meetings and seminars, informal discussions in the lab, and one-on-one meetings with Mike.
2. **Your independent drive to get the work done.** We want to see your individual efforts to take ownership over your project and drive it through i) researching the field, ii) designing rigorous experiments and analyses, iii) exploring alternative methods to answer your question, and iv) looking for ways to troubleshoot stalled experiments. You're being evaluated on how you push a project and your work, not on if your specific experiment works right away. Troubleshooting is 99% of science, so embrace the frustration and work to figure out what needs to be changed before you try again. We're here to troubleshoot with you, so ask someone for help if you get stuck. This will help you learn what kind of support you'll get from the rest of our team going forward.
3. **Your ability to integrate with the team.** You are responsible for your project but also contributing positively to the lab environment. Treat others in the lab with respect and integrity. Communicate early and often to avoid confusion and conflict. Ask for help when you need it and offer help to others. Be the kind of person you want to work with.

Rotation Expectations

1. A few weeks before your rotation, please provide Mike with the anticipated start and end dates of your rotation, your current schedule outside of lab (classes, etc), any forms that need to be completed for your program, and any deadlines for submission of end-of-rotation grades/presentations/reports/etc.
2. We ask you to follow our lab Code of Conduct, and to uphold our listed expectations of *Everyone* and *Graduate Students* on the Welcome, Expectations & Conduct page.
3. During your rotation, you will have one-on-one meetings with Mike every two weeks (or more frequently), which we will schedule as a standing time once you start – see the Individual MWMs section of the handbook. You may also be directly supervised by a more senior lab member, with whom you should also be communicating frequently.
4. During your rotation, you will attend lab meetings, and near the end of your rotation, you will present briefly (20-30 min) about what you tried to accomplish during your rotation.

General Logistics

Last updated August 21, 2026

Lab Hours

Our primary goal is productivity and reliability rather than strict hours. While specific schedules are flexible, regular presence in the lab is important for collaborative learning, effective mentorship, and successful experimentation. Full-time lab members typically dedicate at least 40 hours per week; undergraduate students commit a minimum of 8 hours weekly and should plan to stay with the lab for two academic years, or one year plus a summer full-time. Undergraduates may not work alone without prior approval from Mike.

Flexibility works both ways – experiments sometimes demand unusual schedules or extended hours, but occasional work-from-home days are acceptable when tasks are suitable. If you plan to work from home, notify Mike in advance via Slack or email and ensure it doesn't conflict with meetings or collaborative activities.

Everyone should generally aim to be present during peak hours (~10 am - 4 pm), when possible, to facilitate interaction. Lab staff (technicians, managers) are expected to keep more regular hours (approximately 8 hours/day, 5 days/week), with limited exceptions approved by Mike. Graduate students and undergraduates must provide their class schedules to Mike (and mentors, if applicable) at the start of each term. Balancing coursework and research responsibilities effectively is expected.

General Points

- If you are sick, stay home, notify Mike, reschedule your commitments, and delegate essential tasks as needed.
- You aren't expected to regularly work evenings, weekends, or holidays, but occasional exceptions may be necessary for experiments or deadlines.
- Mike may communicate outside regular hours; immediate responses aren't expected unless specifically indicated for urgent deadlines.

Extreme Weather or Campus Closures

In case of campus closure or delays due to weather or other emergencies, prioritize safety and work from home as needed. Scheduled meetings will switch to Zoom unless otherwise indicated.

Lab Responsibilities & Maintenance

Sharing space means sharing responsibilities. Keeping the lab stocked, organized, and clean is everyone's job. Responsibilities are outlined in our Responsibilities Document, which we review and rotate every six months to evenly distribute tasks and provide everyone with diverse lab experiences. Please regularly monitor your assigned tasks, frequently check common stocks, and proactively add items to the "jobs" board when running low. If you're unsure how to perform a task, it's your responsibility to request training from the previous person in charge.

Cleanliness is a shared priority. Always leave common spaces cleaner than you found them, and clean and store all equipment and reagents after use. While your individual workspace organization style is flexible, common reagents must never be left out overnight, and shared areas must always be cleared immediately after use.

Twice a year, we dedicate one lab meeting slot to a comprehensive deep-cleaning session. Full participation is expected, ensuring our workspace remains clean, functional, and safe for everyone.

Time Off

You have sick leave and vacation time – use it! You're not expected to be in lab on University holidays, though experimental needs may occasionally require otherwise. If you observe religious holidays not recognized by the University calendar, please speak with Mike to arrange alternate time off.

For planned absences, email Mike as early as possible, ideally with at least three weeks' notice. If you'll be out for more than a couple of days, make a plan to cover your responsibilities and support any mentees. Your assigned lab chores remain your responsibility – coordinate coverage with lab mates before you leave.

For unplanned absences due to illness or emergencies, notify Mike via Slack or email as soon as you're able and share an estimated return date. If you're unable to arrange coverage yourself, let Mike or the lab know so we can step in. If it's a true emergency and you're unsure how long you'll be out, that's okay – just keep us in the loop.

Family leave is available for births, adoptions, serious illness, or deaths in your family. Let Mike know as early as you can so we can make a plan to support your projects and mentees during your leave.

- Staff Sick Leave policy
- Staff Vacation policy

Attire

Regular Lab Work

Casual outfits and an outrageous personal style can be a lot of fun, and I support dressing however you are comfortable for routine lab work! But I care about both your safety and the contamination of sensitive experiments.

Your safety requires that you always wear closed-toed shoes that cover the tops of your feet while in the lab. Shorts and skirts must cover your knees when sitting. You cannot wear shirts that expose your back or midriff in the laboratory (or throw on a lab coat or sweatshirt). Lab coats, gloves, protective eyewear, and other personal protective equipment should be worn as lab safety standards and SOPs require.

To prevent experimental contamination, lab members with long hair should tie hair back and/or wear a disposable cap for sensitive experiments (e.g. tissue culture, RNA work). If wearing short sleeves, wear a lab coat for sensitive work. Work with eggs can also get messy and gloves and lab coats are great at keeping your clothing clean.

When Representing the Lab

Please be mindful that you are representing the lab when you attend meetings and present your work outside the lab! Professional attire can be confusing, and there is no "one right way" to present yourself professionally. I encourage you to find a style that allows you to feel comfortable and like yourself – no need to be a slacks-and-button-down person if that's not your style.

Photos, Videos, and Social Media

Please respect your lab mates' privacy – photos and videos should only be taken with their explicit verbal consent. Always ask before recording, and again before sharing any images on social media. Everyone has different comfort levels with being photographed or having their name and image posted online, and that's okay.

If you're excited to share a fun or candid lab moment, go for it – just be sure everyone in the photo is on board. When sharing lab life on social media, remember: you're representing both the Piacentino Lab and Johns Hopkins. Share only appropriate,

safe, and respectful content, and avoid posting anything that includes unpublished data. If you're unsure, check with a colleague or Mike.

Want to contribute to the lab website? Upload your favorite lab photos to our shared Google Drive folder – we update the site periodically with images from the team!

Equipment

We use a lot of specialized equipment or shared work stations during the execution of experiments. These include embryology stations, microscopes, cryostats, centrifuges, thermocyclers, etc. We use a Google Calendar for each piece of equipment and ask that you sign up in advance by making an event on the appropriate calendar with your name in the title. Be respectful of others and only book the time you'll need to complete your task, and honor that timeline to the best of your ability. If you are running late or finish early, communicate with folks before or after you to help them get the most out of their own experiments.

Organization

Calendars

We keep a few Google calendars to help stay organized with lab events, relevant seminars, one-on-one meetings with Mike, and equipment sign ups. Please see the onboarding checklist and request to be invited to the relevant calendars listed.

Please USE the calendars! When you're planning an experiment, book an entry on each piece of equipment for the time you'll need it. Please stick to that booking when possible or cancel/adjust in advance. Be respectful to other lab members who may want to use the instrument before or after you, and if something comes up and you can't use your time, let the lab know so they can hop on instead!

Lab Stocks

We have many lab stocks – plasmid DNAs, primers, antibodies, enzymes, etc. These are meant to be shared amongst members of the lab and exist in centralized boxes in the freezer. It is EVERYONE'S responsibility to protect the integrity of these stocks and maintain our records and organization to enable rapid recovery of any of these items. With anything needing aliquoting (e.g. antibodies), please aliquot upon receiving, label, and store appropriately.

- **Plasmid DNAs** – plasmid stocks should be digitally logged in Benchling under our file named "Plasmid Stock Boxes." This should link to the sequence map with appropriate nanopore sequence results. Maintain the Benchling entry to reflect the organization in the freezer, and tubes in this box are to preserve into the future. Do NOT use the stock as your working preparation for experiments – instead, take a small amount to transform and grow your own miniprep/maxiprep. If the stock is getting low, replace it with a fresh aliquot of your most recent prep after first confirming its sequence integrity. When generating a new plasmid, sequence it fully and make a well annotated map in Benchling. Then add the link to this map in the Plasmid Stock Boxes entry, and place an aliquot of the plasmid in the corresponding position for the future.
- **Primers** – centralized boxes hold our primer stock tubes and are maintained at 100 µM unless otherwise noted. Take a small aliquot to make a working dilution for your personal boxes and return the primers to their appropriate spots in these boxes. When receiving new primers, add them to the Benchling spreadsheet called "Primer Stock Boxes" and put the tubes in the corresponding cells in the freezer.

Meetings & Communication

We use several different types of meetings in the Piacentino Lab, each with a different purpose. The goal is to make good use of our time together while creating opportunities for scientific discussion, project planning, professional development, and lab community.

The basic distinction between our two most frequent meetings is:

> **Lab meeting is where we work on the science. MWM is where we manage the work.**

General Meeting Expectations

You are expected to attend all regular lab meetings and MWMs, or let Mike know in advance if you will be unable to attend. During meetings, everyone is expected to pay attention while others are speaking, actively participate by asking questions and offering constructive feedback, and be receptive to feedback when it is offered.

You may use your computer for meeting-related activities such as taking notes, viewing data, or presenting. Doing unrelated computer work during meetings is not acceptable.

If unavailable to attend meeting in person, please log in through Zoom.

Weekly Lab Meetings

We hold a weekly lab meeting in the Piacentino Lab. Each meeting consists of two parts.

Lab Business

(~10 min) We briefly discuss matters related to lab operations, including news, stocks and ordering, safety and cleanliness, scheduling, and events. Bring up problems that affect the group, including **major experimental hurdles that might reflect a systemic issue** (e.g., possible media, enzyme, antibody, or equipment problems); issues that are primarily individual should generally be discussed with Mike separately.

Research Discussion

(~60-80 min) The lab is divided into two groups that alternate presenting each week, so each person presents every other week. Each person will have approximately **20 minutes** (~10 minutes presentation and ~10 minutes group discussion) to focus on **one or two active research goals**.

We are shifting this portion of lab meeting from a seminar-style format toward a **round-table, work-in-progress format**. The goal is to spend more of our group time thinking together about the science: experimental rationale and design, raw data, analysis, interpretation, and how we ultimately turn the work into a clear figure and conclusion.

At the end of the semester, we'll do a round of more formal 25-minute seminar-style presentations as an opportunity to practice telling the complete story of your project and synthesize together the insights you gained over the semester.

What should we bring?

You do **not** need to give a status update on everything you are working on. Instead, choose **one or two works in progress** where discussion with the group would be useful. Depending on where the project is in its development, this could include:

- **Question & rationale:** What are you trying to understand, why is it important/makes sense to ask this question now, and why does the experiment address that question?
- **Experimental design:** What are you planning to do and how? What are the critical controls, comparisons, and readouts? What would different outcomes mean?
- **Pilot/raw data:** What did you actually observe? Always bring the raw data ready to flip through – not just a summary or representative image.
- **Analysis:** How are you quantifying or interpreting the data? Are there alternative ways we should analyze it?
- **Interpretation:** What do the results tell us, and what do they *not* tell us?
- **Figure development:** How would you present the result in a paper or presentation? What should the figure show, and what conclusion should the reader take away?
- **Next experiment:** Given what we now know, what is the most informative next step?

A single experiment may therefore appear in lab meeting **two to three times** over its lifetime: when we are designing it, when we have pilot or preliminary data, and when we are analyzing the completed experiment and developing the final figure.

What should we take away?

A successful lab meeting discussion is **not** one where you demonstrate that you have been busy or have everything figured out. It is one where the group leaves with a better understanding of the experiment and its implications than when we started. Come prepared to **show your work**:

1. **Explain the scientific question and rationale clearly.**
2. **Show the actual data or experimental plan**, rather than simply describing what happened.
3. **Identify the parts you are uncertain about.**
4. **Ask specific questions where you want input from the group.**
5. **Be prepared to revise your interpretation, analysis, or experimental plan based on the discussion.**

You do not need to have a polished story or finalized figures; bringing work to the group early enough for us to influence the experiment is an important part of this format.

Individual MWMs

Mike holds 30-minute 1:1 meetings (or “Mornings with Mike”) every other week with each member of the lab. MWMs are our time to **step back from the experimental details and think strategically about your work**. We will use this time to:

- Keep track of the **overall progress of your projects**
- Discuss **priorities** and competing demands
- Decide what deserves your attention next
- Discuss **timelines** and longer-term goals
- Address professional development and other career-related topics
- Identify obstacles that require Mike’s input or intervention
- Make sure your work remains aligned with the broader goals of the project and lab

MWMs are **not intended to be a second lab meeting**. Detailed discussion of experimental rationale, raw data, analysis, figure development, and technical decisions should generally happen in group lab meeting, where the whole lab can contribute. If a technical issue affects a priority or decision, we can discuss it in an MWM, but we should avoid spending significant MWM time troubleshooting an experiment that would benefit from group discussion.

MWM Agenda

The MWM agenda is primarily a **project-tracking and planning tool**. It should give both of us a quick picture of where things stand and provide a record of what we agreed should happen next. Please prepare an agenda in advance and send it to Mike

at least 12 hours before your meeting. After the meeting, send an updated version incorporating any changes or decisions we made. The agenda should contain three sections:

Business to discuss

- Anything you want to discuss with me, such as conferences/workshops, upcoming talks or committee meetings, professional development, or other issues where you need a decision, feedback, or input.
- Include anything that may affect your priorities, timeline, or ability to make progress.

In the past two weeks I did…

- Briefly describe the major things you worked on and the important outcomes or developments.
- Focus on information needed to understand the current state of your projects.
- You do **not** need to provide a detailed account of experiments, data, or analyses that have already been or will be discussed in lab meeting.

In the next two weeks I plan to…

- Describe what you plan to accomplish, including the major steps and why they are the priority.
- Identify the **one or two highest-priority goals** for the next two weeks.
- Flag anything that may prevent you from accomplishing those goals or where you need input from Mike.
- We will use this section to adjust priorities and make sure your planned work is realistic and appropriately focused.

Please Come Prepared With

1. **Your MWM agenda**, sent at least 12 hours in advance.
2. **Your notebook and raw data up-to-date and organized**, so that the work discussed in your MWM can be readily traced back to the underlying experiments.
3. **A clear understanding of your current priorities and what you plan to accomplish next and why.**

You are responsible for maintaining your notebook and data organization; **routine inspection by Mike is not part of the MWM process**. Raw data should be organized and available on the server, but routine review of raw data, detailed analysis, and technical troubleshooting belong primarily in lab meeting. We will **periodically audit documentation** to make sure our system is working.

What Does a Successful MWM Look Like?

At the end of the meeting, we should have a shared understanding of:

- Where your projects stand
- What matters most right now
- What you will focus on next
- Why those things are the priority
- What decisions, feedback, or support you need from me

The goal is not simply to report what you have been doing. It is to **make sure you are spending your time on the right things and have a clear, realistic path forward.**

In Short

Lab meeting: *Let's work through the science together.*

MWM: *Let's make sure you're working on the right things.*

Other Meetings

Journal Club & Reading Roundtables

We hold a weekly journal club with the Miao Lab, where we discuss papers in developmental biology. See the “LabMeeting_JournalClub” folder on Google Drive for the schedule and presentation guidelines; everyone should volunteer to present approximately **2-3 papers per year**.

On some weeks without a formal journal club, we will instead hold a **Reading Roundtable**, where everyone briefly shares something they have been reading or thinking about and explains why it is interesting or relevant to their research.

Professional Development

On some weeks without a formal journal club, we will use the meeting for **professional development**, including topics such as CV development, writing abstracts or papers, preparing talks and posters, and broader career skills. Please let Mike know if there are topics you would like to discuss.

Career Development

In addition to our regular MWMs, Mike will meet annually with each lab member to discuss **career goals, Individual Development Plans (IDPs), and progress toward those goals**. This provides dedicated time to think beyond immediate project priorities and consider longer-term professional development.

Cell Migration Meeting

The Devreotes, Robinson, and Iglesias labs host a joint **Cell Migration meeting on Thursdays from 10:30 AM-12:00 PM** in our conference room. If your project is aligned with cell migration questions, this is a valuable opportunity to get feedback from researchers outside our lab and to practice presenting your work to a broader scientific audience.

Office Hours

In addition to scheduled meetings or me wandering through the lab, if my door is open, feel free to step in and ask to talk. I’ll say yes as much as possible, though I may let you know I only have a few minutes or ask you to return later.

If the door is closed, I am either gone, in a meeting, or need to work undisturbed, so please email/Slack instead of knocking.

If you need more time with me (we run out of time during our MWM, you want to discuss something in a larger dedicated time block, etc), reach out on Slack or email and we’ll find a time.

Communication

Communication is key to maintaining a supportive and productive environment, so we all need to practice clear communication skills. It is critical to identify what you are trying to get across, voice it clearly and appropriately, and then be receptive to feedback. We also should practice active listening to fully appreciate what our colleagues are saying, then respond respectfully and helpfully after the speaker is finished.

While a lot of communication will happen in person, there are a million and one ways to communicate digitally, and each have their strengths and weaknesses. Below are a few rules/guidelines for how we communicate as a lab and which formats are used for which reasons.

Slack

We have a lab Slack workspace where most of our day-to-day communication takes place. Please ask your mentor to invite you to join this workspace and have this resource available for communication on your computer (and mobile or tablet, if you choose). We use Slack for most quick communication between lab members like:

- “Does anyone know where to find extra slide boxes?”
- “We’re running low on P1000 filter tips and need to order more”
- “Look at how beautiful this embryo is!”
- “We need more gloves, I added them to the order form to be triggered!”

Slack is great for direct messaging between two or more people, and we have several lab-wide channels covering general information, cool papers, lab events, etc. We will make channels as needed to facilitate communication between folks working on a common project. We should all be attentive to Slack messages throughout the workday and try to respond in a prompt manner. At the same time, we must respect one another’s desire to “turn off” at the end of the day and disable notifications or close Slack.

Emails

Emails are our main mode of digital communication with the greater community – the department, the institute, the world. We will use emails occasionally, most frequently when communicating with folks outside our Slack group.

Text messaging

Texts are a great and fast way to communicate, but they may not be so great for everyone. Some may like to keep texting for personal communications with friends and family, while others may like to use texting for everything. Before texting other lab members, ask them their preferences for text messaging and respect their requests. For example, Mike would prefer you to reach him by Slack (high priority) or email (low priority), and only text or call his cell phone for special cases or emergencies.

How to reach Mike

I get a lot of emails, and I’m sure you do too – I’ve turned off my email notifications for this reason. This means, if you email with an urgent request, I might not see it promptly. Instead, please send me a Slack message (either @Mike or DM) and I’ll respond more quickly. It is helpful for me to compartmentalize my “lab attention” in Slack, while my email inbox reflects more outward communications. I have my Slack notifications on my phone to automatically go silent at nights – this means I’ll get notifications for urgent requests during the day but might not see or respond to a late-night request until the next day.

You can expect me to respond to your Slack or email within one business day. If I need more time to reply in detail, I’ll respond to say that I got your message and will provide a timeline on when I’ll be able to respond more completely. I expect the same courtesy from you – please try to respond to Slack messages and emails within one business day as well, and if you need more time, acknowledge the request and give an estimated timeline. Please respect people’s desires to work on their own schedules – if someone messages after hours, feel free to leave it until your next business day unless you’re working toward a deadline.

If you have a true emergency, in-lab emergency contact information is posted throughout the lab – see the Biosafety chapter for what to do in a safety emergency.

Rigor, Reproducibility & Data Management

Rigor

Rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, execution, analysis, interpretation, and reporting. This is achieved by careful record keeping and clear reporting, as well as considering the results of our experiments from all angles, and making sure our conclusions stand up to criticism and replication.

Replicates are critical in this effort – we don't want to draw conclusions from an observation that happened once, or on one day. Instead, we need to ensure it holds true over multiple biological and technical replicates. Our convention in the laboratory is that cell-based experiments are performed ≥ 3 independent biological replicates (on three independent days), and resulting datasets include ≥ 10-20 cells analyzed per biological replicate. For chick embryo-based experiments, we collect ≥ 6 independent biological replicates (embryos) generated from ≥ 3 independent days, with more replicates needed to observe subtle phenotypes.

Our standard is that an experiment is not considered completed until:

1. At least three independent biological replicates are performed and reported in your notebook
2. Supporting raw and analyzed data, as well as all code/analytical methods, are organized and saved to our shared server as well as your external hard drive
3. A final, publication-quality figure that can go straight into a manuscript is assembled and presented at lab meeting

Data Reproducibility

Science rests on reproducibility. You should be able to take a paper, acquire all the reagents, follow their protocols, and achieve the same results. If you cannot, it suggests that either you or the original author made mistakes in conducting the experiment, or there is some uncontrolled variable that has not been accounted for. Extended from this, if given a raw dataset, you should be able to reproduce any analyses exactly and achieve identical results. If you can't reproduce the results, it suggests that either you or the original author made errors in the analysis, and the results can't be trusted. To ensure reproducible results, experimental and analysis protocols must be thoroughly documented!

When conducting an experiment, you should transcribe the full protocol into your notebook, or load the protocol (from our digital resources) into your electronic lab notebook, and annotate it as you go through the experiment with anything you may have changed (intentionally or accidentally). Note the exact volumes you used. Note any brands that may have changed. Note if you let an incubation run long. If you make substantial changes to a protocol, rewrite it and add it to the protocol notebook for others to use. Remember, if you change a protocol, make notes within it about *why* you changed it so that others can determine which protocol version is appropriate for their experiment.

When conducting analysis, the pipeline must be organized and well-documented. To meet these goals, you should take extensive notes on *each step* of your analysis pipeline. This means writing down how you did things every step of the way (and the *order* that you did things), from any pre-processing of the data, to running models, to statistical tests. Additionally, your code should also be commented, and commented clearly. We all know what it's like to sit down, quickly write a bunch of code to run an analysis without taking time to comment it, and then having no idea what we did a few months down the road. Comment your code so that every step is understandable by an outsider.

Important: It is easy to get behind in updating and maintaining our lab records. This problem snowballs if you don't address it promptly. Make a habit of making sure your notes are up to date at least weekly. The longer it goes undocumented, the more likely you are to miss something and end with results that cannot be reproduced.

Data Management

Data management is critical to research. Below are a few of our main practices to maintain good experimental records and data management.

Notebooks

We use Benchling for our lab notebooks. This is a powerful and free online platform that allows project organization, note booking, and DNA analysis. Mike or your mentor will invite and orient you with the Piacentino Lab Benchling group.

Ask to see:

- Making an entry from scratch or from an experiment template
- Navigating DNA analysis
- Our lab's Plasmid and Primer Stock Databases

We also have paper notebooks and notepads available for pen-to-paper record keeping, but please translate into a more accessible digital format on Benchling. Just let Mike know if you have any concerns about using Benchling and keeping a digital notebook.

File Naming

Most of our raw data collection will be microscopy images, which should use the file name structure described below. Importantly, separate metadata categories by an underscore (_), and separate information within a category with a semicolon (`;`). This structure and labeling order allows us to rapidly parse and filter data during analysis and visualization – careful naming up front will save a lot of time for future you!

Format:

`Date_Treatment;Dose_Label/ChannelName1;2;3;etc_SampleNumber_Stage_Objective_OtherImageMetadata`

Example:

`20230828_pCIApoD;2.5ugul_BF;Pax7;H2B-RFP;Snai2;DAPI_Emb3_8ss_10x_stack`

Key:

- **Date:** Using YYYYMMDD format, enter the date the experiment started – generally electroporation or transfection date, *not* imaging date. We should be able to search this date in your notebook to find more experiment details.
- **Treatment;Dose:** Describe the treatment – generally the experimental construct name with a dose separated by a semicolon (`;`).
- **Label/ChannelName:** Describe what each channel shows, and in the order collected on the microscope. Brightfield (BF) and DIC are common transmitted light labels, and fluorescent channels for immunohistochemistry or HCR should indicate the protein/gene detected. Use the order of channel collection so that you can easily associate channels in ImageJ/FIJI with the appropriate label name.
- **SampleNumber:** Which sample is this in your experiment? Generally, this will be Embryo (Emb), Explant (Expl), or Field of View (FOV), immediately followed by a number. We start with #1 for the first sample of a given dose within an experiment.
- **SampleStage:** What stage is the sample? When possible, use somite staging (ss) as it is more specific and informative than Hamburger-Hamilton (HH) staging.
- **Objective:** What objective was used? Generally 5x, 10x, 20x, or 40x.
- **OtherImageMetadata:** Use this position if there is other identifying information – e.g. when collecting a Z-stack through an embryo, you can append "_stack" in this position, and replace with "_MIP" if you produce a maximum intensity projection file.

Organization

Within an experiment's data folder, there will be a few iterations of analysis and data formats to keep track of. Make sure you sync any analyses across your data management systems – see the "3-2-1" system below. One general example is as follows:

- Folder describing the experiment (e.g. "Sox9KD_SMPD3Enh3Activity" – everything pertaining to the effect of Sox9 knockdown on SMPD3Enh3 activity)
  - Experiment folder name: YYYYMMDD (experiment start date) and details as above
    - "czi" – folder containing the raw microscopy data, often in .czi format from Zeiss microscopes
    - "jpgs" – folder containing jpeg exports of raw data – useful for quick flipping through what each image shows
    - "measurements" – folder containing .csv files that include measured numbers from our images
    - "rois" – folder containing saved ImageJ/FIJI ROI files and exported images showing ROI positions
  - FIJI Export Macro – save the version of code used to produce the jpeg exports for this experiment
  - FIJI Analysis Macro – save the version of code used to produce the ROIs and measurements for this experiment
  - Data Analysis Workbook – R, Python, or Excel file where you analyzed the data
  - Final analyzed data table (.csv format)
  - "raw_source_data" – folder compiling all the measurement files from the experiment; this allows a "one stop source" of numbers for analysis
  - Extras: graphs or representative images that encapsulate the results of the experiment

Example experiment data folder:

Storage

It is critical to store data in a format that is Findable, Accessible, Interoperable, and Reusable (FAIR). "Lost" data is as good as the experiment having never been performed, and this can result from a hard drive failure when the data is stored in one place, or data can be "lost" because the labeling is unclear and we can't trace back the experiment's details. Further, sustained good Data Management practices is a requirement for our federal funding.

During the project: We use a 3-2-1 storage plan: 3 copies, in at least 2 physical locations, over more than 1 type of device. This is achieved by storing your data on your individual computer, on lab-provided external hard drives, and on our lab's Network Attached Storage (NAS) server. Everyone is required to update and sync their data across these three platforms on a monthly basis, and more frequently is better. Never trust that data on a microscope computer will still be there after you walk away – this is NOT a long term data storage solution, and these are prone to crash; take your data with you when you're done imaging and get it into your 3-2-1 system!

After the project: While we are publishing a project, we will deposit our data in a globally-accessible format. This takes two main shapes:

1. **GitHub:** we will publish all code used for analyzing images, processing and visualizing results, and the source data used in our lab's GitHub repo.
2. **JHU Research Data Repository:** we will compile all raw data (everything on GitHub AND all raw images, sequence data, etc included in the study analyses) and publish this in the Hopkins Research Data Repository. Here, professional staff will curate and keep this data Findable, Accessible, Interoperable, and Reusable (FAIR), make it available to the world, and assign a permanent DOI for the project.

After your time in the lab: When you leave the lab, we need to maintain your data in-house. Make sure you sync to the server and hard drives before you leave and turn in your hard drives to Mike during off-boarding.

Authorship

We follow the APA guidelines for authorship credit:

> “Authorship credit should reflect the individual’s contribution to the study. An author is considered anyone involved with initial research design, data collection and analysis, manuscript drafting, and final approval. However, the following do not necessarily qualify for authorship: providing funding or resources, mentorship, or contributing research but not helping with the publication itself. The primary author assumes responsibility for the publication, making sure that the data are accurate, that all deserving authors have been credited, that all authors have given their approval to the final draft; and handles responses to inquiries after the manuscript is published.”

In general, the trainee leading a project will be first author on resulting publications, and Mike will be last author. Lab members who contribute meaningfully over the course of the project may be included as co-authors, with authorship order determined in consultation with all contributors. If a project is handed off to another person before completion, first authorship will likely transfer to the individual who brings the study through to publication.

If you leave the lab with unfinished work, discuss with Mike whether you plan to continue leading the project through writing and potential revisions, or whether those responsibilities should be reassigned. In that case, authorship may be revisited – retaining first authorship will depend on how much remains to be done to bring the work to publication.

In collaborative projects with other labs, final author placement may vary. For example, an external PI may be listed last, with Mike second-to-last, and Piacentino Lab contributors in middle-author positions. Authorship expectations for collaborations will be discussed at the outset and re-evaluated as the project progresses.

Discussing authorship early is helpful, but research is dynamic – roles and contributions can shift. Authorship should be revisited regularly and discussed openly. If you have questions or concerns about your authorship status on any project, talk to Mike – transparency and fairness are essential.

Laboratory Conduct & Biosafety

Introduction

Working in a research laboratory requires being aware of the different types of hazards we use or have in the lab, how to handle them safely, and what to do in the event of a spill or an exposure incident. For the most part, we follow the Johns Hopkins Safety policies, and much of this material is covered in your required safety trainings. Additional biosafety information is available in the Biosafety in Microbial and Biomedical Laboratories (BMBL) handbook, currently on its 6th edition. We function at BSL-1 and BSL-2 levels, so we encourage you to review these chapters in the BMBL.

We do have a few unique standard operating procedures (SOPs) that describe how we work in the lab. These are detailed in the following sections and will be taught to you by Mike and/or your primary laboratory mentor. The most important thing here is to ask your mentor, ask Mike, or ask the Biosafety office if you have *ANY* questions. We want to all stay safe and keep the lab a secure and fun place to work, so *IF YOU ARE UNSURE*, *ASK SOMEONE*.

Safety Training

Stay up to date with your required safety training. Online training courses can be found on the Hopkins Medicine HSE training page. Please take each of the following training courses, and any additional courses relevant to your project or that you think may help you operate safely.

Required of each Piacentino Lab member:

- Laboratory Safety/Biosafety Levels I & II (virtual, instructor-led)
- Bloodborne Pathogens Orientation (online, user-driven)
- Fire, Hazard Communication and Construction Safety (online, user-driven)
- Chemical Waste Management (online, user-driven)

After completing these trainings, keep an eye out for notifications in your email for training refreshers. Safety procedures change as we develop new ways to mitigate risk, so it is important to stay up to date and renew our training periodically. Some of these courses require re-training every year or two.

Safe Laboratory Conduct

Some general rules in lab:

- Always work in a safe manner and with a professional attitude
- No food or drink, or storage of food/drink for human consumption is allowed in lab
- No animals or plants are allowed in the laboratory unless they are part of the work being performed or approved by Mike and the Biosafety office
- No handling cosmetics, contact lenses, earrings, or other personal items that may introduce hazardous materials into the body/face
- Wash your hands after performing any and all laboratory procedures
- Wear appropriate clothing and PPE in the laboratory and while performing work
- Keep our lab clean, tidy, and disinfected
  - Keep an organized bench with clearly labeled solutions
  - Tidy any common space you use so it is safe for the next user
  - Wipe down/decontaminate all workspaces following experiments, and always decontaminate the area following a spill

- All incidents which result in an injury to any lab members or visitors *must* be appropriately documented and reported – contact Mike immediately following any incident

Laboratory Attire & PPE

The purpose of appropriate laboratory attire and PPE is to protect our skin and bodies from exposure to hazardous materials.

Shorts, miniskirts, or any apparel that does not cover the skin above the knee when seated shall NOT be worn in the laboratory without appropriate overprotection (a buttoned lab coat that extends past the knee). Open-toed shoes, sandals, or shoes made of loosely woven materials are not permitted when working in the lab.

PPE are an additional layer of protection that should always be worn as appropriate to the laboratory task. Most of our work falls under Biosafety Level 1 (BSL-1) classification and should be performed while wearing disposable gloves.

Our tissue culture work (in WBSB 104A) and working with chicken eggs that have not been confirmed *Salmonella*-clean fall under BSL-2 classification. When working in these systems, wear disposable gloves, a lab coat, and safety goggles when producing aerosols.

When working with cryogenic hazards (liquid nitrogen, dry ice), additional PPE is required including cryogenic gloves, impermeable shoes and apron, goggles, and a face shield.

Dispose of your gloves after use, and never touch your computer, doorknobs, or common areas while wearing gloves. Always remove your gloves before leaving the lab, or remove one glove to handle doors if transporting materials in the other gloved hand.

Hazards in the Lab

This lists the most significant hazards we keep in the Piacentino lab. If you notice something not on this list, let Mike know, and we'll add it! Before using any of these items, please seek appropriate training from Mike and/or your designated lab mentor.

Physical Hazards

- **Sharp hazards**: glass (including broken beakers, flasks, slides, and coverslips), needles (glass microinjection needles and syringe needles), dissecting tools (forceps, scissors, and knives), razor blades
- **Electrical hazards**: all major lab equipment must be connected directly to a power outlet, no power strip; all power strips must be elevated >12 inches off the floor in case of flooding; do not daisy-chain electrical cables; cover any cables that cross a walk-space, otherwise it's a tripping hazard
- **Compressed gas cylinders**: these can become a rocket if they fall over and/or have a broken regulator; always keep compressed gas strapped to a secured surface using chains or straps rated for this purpose; always use a cart to transport gas cylinders
- **Cryogenic hazards** (liquid nitrogen and dry ice): appropriate PPE (especially for liquid nitrogen) includes covering all skin, cryogenic gloves, impermeable shoes and apron, goggles and face shield. Liquid nitrogen and dry ice can pose an asphyxiation hazard since they can displace oxygen rapidly – work with cryogenics in open spaces with good ventilation, NEVER use or put these items in a cold room, and NEVER seal cryogenics in an air-tight container, as it will become explosive
- **Laser hazards**: many microscopes use laser lines to excite fluorescent samples; misuse of lasers can result in physical damage, can start a fire, and can result in blindness

Chemical Hazards

Safety Data Sheets (SDS) are found in the lab safety binder, and can be accessed online. Below are the chemical hazards we house and use in the laboratory, and their respective hazards:

- **Fixatives**: paraformaldehyde (flammable, corrosive, irritant, acute toxicity, chronic health hazard); glutaraldehyde (corrosive, chronic health hazard, acute toxicity, aquatic toxicant)
- **Acids and bases**: hydrochloric acid (corrosive); acetic acid (corrosive, flammable); sodium hydroxide (corrosive)
- **Alcohols**: ethanol (flammable, irritant, acute toxicity); isopropanol (flammable, irritant, acute toxicity); methanol (flammable, acute toxicity, chronic health hazard)
- **Other**: phenol (corrosive, acute toxicity, chronic health hazard); chloroform (acute toxicity, chronic health hazard); ethidium bromide (acute toxicity, chronic health hazard, carcinogen)

Biological Hazards

- **Biosafety Level 1 (BSL-1) hazards**: *E. coli* K12 (DH5α) strains; recombinant DNA molecules handled in or extracted from *E. coli* that cannot infect or integrate into cells; chicken eggs that are *verified* as *Salmonella*-clean (supplied by UConn Poultry)
- **Biosafety Level 2 (BSL-2) hazards**: human-derived or non-human primate-derived cell lines (including U-2 OS cells); any recombinant DNA molecules used in tissue culture experiments; chicken eggs that are **UN**verified as *Salmonella*-clean

Biohazard SOPs

This section details the primary biohazards we use in the lab, how to handle and dispose of them safely, and how to clean up spills or messes with these agents. We have IBC approval for these procedures, and documentation of our risks and handling procedures. Please read the material below and also look at our registered protocols that are printed in our lab safety binder.

E. coli SOP (BSL-1)

We perform routine molecular biology work using *E. coli* bacterial strains (like DH5α) to amplify our plasmid DNAs. These experiments can be performed safely at your laboratory bench following standard BSL-1 practices. Importantly:

PPE: When working with bacterial cultures, wear gloves at all times. If performing a procedure that produces aerosols, wear safety glasses to protect from splashes into your eyes. When finished working, dispose of your gloves and any contaminated materials in the red-bagged biohazard box and wash your hands.

Disposal: Any bacterial waste must be disposed of as biohazardous materials.

- Solids (including LB plates, gloves, pipet tips, serological pipets, disposable culture tubes, etc) are disposed of in red-bagged biohazard boxes for subsequent pickup and incineration. Before tossing your LB plates, please wrap them in parafilm to contain the bacteria.
- Liquids produced during bacterial work (including bacterial cultures, LB and SOC broths, etc), are inactivated by incubation with bleach (10% final concentration) for 30 minutes. After inactivation, liquid waste can be rinsed down the sink with excess water.

Cleanup and disinfection: After working with bacterial cultures, always disinfect your workspace (surfaces, tools, etc) with 70% ethanol and paper towels. In the event of a large spill, follow the Spill Clean Up protocol below. When finished cleaning, dispose of your gloves, and wash your hands.

Chicken Egg SOP (BSL-2)

We frequently receive fresh fertilized chicken eggs which will be incubated to desired embryonic stages in the lab, manipulated at the designated embryology spaces, then cultured either *ex ovo* or *in ovo*. You will get hands-on training as is appropriate for your research project. Here we detail the primary safety concerns.

The primary concern with chicken egg material is the potential for *Salmonella* transmission, which can pose a human health risk if ingested; *Salmonella* is designated as a BSL-2 biohazard. We receive fertilized eggs from distributors that are part of the National Poultry Improvement Plan (NPIP) – a program committed to diminishing the spread of Pullorum Disease. Under this program, these distributors maintain farming practices free of *Salmonella*, as well as Pullorum Typhoid and Avian influenza. Certification is provided with each shipment and will be verified upon receipt.

PPE: When handling egg materials, always wear gloves and a lab coat. If producing aerosols or at risk of splashing, also wear eye protection. A lab coat is optional but encouraged if you can confirm the eggs are *Salmonella*-clean. We don't want to spill eggs on our clothing! To protect your workspace and make cleanup easy, always cover your working area with absorbent bench paper. When finished working, dispose of your gloves and any contaminated materials in the red-bagged biohazard box and wash your hands.

Disposal: Experiments with unfixed egg/embryo material will be performed over benchtops covered with disposable bench paper to absorb any spills, and the bench paper will be collected into red-bagged biohazard boxes. At the end of each experiment, all bagged egg waste will be frozen at -20°C and stored in the freezer in WBSB 104 until disposal. When 3-4 experiments worth of frozen egg waste are accumulated, we will transfer these bags into a fresh, red-bagged biohazard box which will be removed for incineration through standard biohazard waste streams.

Cleanup and disinfection: Clean up after each experiment by collecting all disposable materials into your egg waste bag. Wipe down the work area with 70% ethanol and paper towels to prepare the workspace for the next user. Small spills will be cleaned with paper towels and water first to pick up as much egg material as possible. Then disinfect the area with 70% ethanol (ethanol will fix liquid egg material to the bench if not wiped up with water first) and paper towels. Contaminated materials will be bagged, and frozen with the remaining experiment material. In the event of a large spill, follow the Spill Clean Up protocol below. When finished cleaning, dispose of your gloves, and wash your hands.

Working with Fixatives SOP

Preparing stocks and fixing tissues

We frequently fix embryonic tissue with hazardous fixatives such as paraformaldehyde and glutaraldehyde. Spills and inhalation during use can pose serious risks to your health, and appropriate measures are required to mitigate these risks.

When to follow this: Always **work in the chemical fume hood** when…

1. Making 4% Paraformaldehyde (PFA) stocks, as heating solutions to promote dissolving of PFA powder releases strong hazardous fumes
2. Fixing embryos/tissue – bring your live tissue to the fume hood and pour PFA into your desired dishes. Add your embryos to the liquid in the hood and leave to fix. Return to the hood for the first wash after fixation (collecting fixative and the first wash into appropriately labeled bottles), then you can remove your fixed tissue to return to the embryology station for dissection.

PPE: When handling hazardous chemicals, always **work in a chemical fume hood** (one in WBSB 104 and another in WBSB 108), and wear gloves and a lab coat. The hood is designed to ventilate hazardous fumes so that you do not inhale them. This is especially important when fixatives are heated (e.g. during preparation of 4% paraformaldehyde (PFA) stocks). Discard your gloves after working with fixatives.

Disposal: See Lab Waste Handling & Disposal, *Hazardous Liquids (Chemical)*. Notably, fixatives cannot be poured down the drain and must be collected in labeled containers stored in our Satellite Accumulation Area (SAA) within the fume hood. After fixing tissue, discard fixative waste and the first "non-hazardous" wash into the appropriately labeled waste bottle.

Cleanup and disinfection: Clean up after each experiment by removing all your materials from the fume hood and leaving the space open and clear for the next user. Wipe down the work area with 70% ethanol and paper towels. When finished cleaning, dispose of your gloves, and wash your hands.

Tissue Culture SOP (BSL-2)

We grow cells in tissue culture in WBSB room 104A. These cell lines include chicken-derived tissues (like DF-1 fibroblasts), but also some cell lines from human-derived samples (like U-2 OS osteosarcoma cells). Whenever working with human-derived materials there is an elevated concern for possible exposure to bloodborne pathogens. As such, we treat all active tissue culture material as a BSL-2 biohazard. Mike or your lab mentor will train you in safe tissue culture procedures before you work alone with these materials.

PPE: When working in WBSB 104A on any tissue culture experiment, wear gloves and a lab coat. Handle all materials within the Biosafety cabinets (BSC) with the airflow vents activated. If operating outside of the BSC, wear safety goggles to protect against splashes. When finished working, dispose of your gloves and any contaminated materials in the red-bagged biohazard box and wash your hands.

Disposal: Any tissue culture waste must be disposed of as biohazardous materials.

- Solids (including plates, gloves, pipet tips, serological pipets, tubes, etc) are disposed of in red-bagged biohazard boxes for subsequent pickup and incineration.
- Liquids produced during tissue culture work (including media and buffers, resuspended cells, etc), are inactivated by aspiration into a vacuum flask and incubation with bleach (10% final concentration) for 30 minutes. After inactivation, liquid waste can be rinsed down the sink with excess water.
- We frequently perform immunohistochemistry for visualization of cells from tissue culture experiments. For these experiments, fix cells in the BSC with an appropriate volume of 4% paraformaldehyde (generally 200-1000 µL) for 10 minutes at room temperature. Fixed cells are effectively inactivated and can be taken out of the BSC for subsequent labeling and imaging.

Cleanup and disinfection: After completing your work in the BSC, remove all materials and disinfect the surfaces and tools with 70% ethanol and paper towels. Close the BSC sash, turn off the airline, and turn on the UV lamp for 30 minutes to disinfect the cabinet. Bleach and dispose of all liquid waste as described above, remove your PPE and dispose of gloves in the red-bagged biohazard bins. Wash your hands before leaving the tissue culture area.

Lab Waste Handling & Disposal

It is our responsibility to protect not only ourselves and our teammates but the Johns Hopkins community and beyond. This means being aware of how we dispose of materials in the lab to prevent chemical hazards or biohazards from leaving our controlled environment and posing a risk to others and the environment. While we know what we are using and their inherent risks, it is *essential* that we take waste disposal seriously and treat all waste produced in our lab as a potential chemical or biohazard risk. Below we detail how to handle the major classes of waste you will encounter and how to safely dispose of these materials. If you're unsure, what should you do? *ASK SOMEONE!*

Solids: Almost all solids we produce in the lab must go into the red-bagged biohazard boxes. These boxes are taken for incineration to ensure no release of biohazardous materials. All gloves, soiled paper towels, rigid plastics (tubes, plates, tips, serological pipets, etc), chicken eggs and embryos, tissue culture supplies, ethidium bromide-stained gels, and even autoclaved solids must be disposed in this box. Never remove the red bag liner from the box.

When the box is 70% full:

- Tape the red bag shut
- Cover the box with the included cardboard lid and seal it with tape
- Label the top with "Piacentino Lab, WBSB 104" and the date
- Place the box in the hallway for collection
- Immediately set up a new red-bagged biohazard box

Uncontaminated paper/plastic products and sharps (needles, razor blades) are the exception and do not go in the red-bagged biohazard box. Uncontaminated paper/plastic products (catalogs, journals, notebook paper, cardboard boxes, and empty pipet tip boxes) can be disposed in clearly labeled recycling containers. If you're not sure if it became contaminated, treat it as if it is a hazard and put it in the red-bagged biohazard box.

Sharps: Sharps waste have their own dedicated container – smaller, red plastic sharps containers with appropriate biohazard labels. Any sharps to be disposed (needles, razor blades, etc.) should be placed in a plastic sharps disposal container. NEVER reach into a sharps container.

When the sharps container is filled to the indicated line:

- Close the lid of the sharps container
- Label with "Piacentino Lab, WBSB 104" and the date
- Place the box in the hallway for collection
- Immediately set up a new sharps container

Non-Hazardous Liquids: We will produce a variety of liquid wastes, the majority of which are non-hazardous. These include water, buffer solutions used in immunostaining, in gel electrophoresis, etc. These solutions can be disposed of down the drain with plenty of water.

Hazardous Liquids (Chemical): Several solutions we use routinely in lab contain hazardous chemicals that CANNOT be poured down the drain. These include fixatives (paraformaldehyde, glutaraldehyde), methanol, chloroform, phenol, etc. When working with these hazardous liquids (e.g. fixing embryos, performing phenol/chloroform extractions, etc), work in the chemical fume hood until the samples have been sufficiently washed out of hazardous chemicals and collect used liquid in our Satellite Accumulation Area (SAA) in the chemical hood in WBSB 104. Add your solution to the appropriate waste container of matching waste. DO NOT MIX chemical waste. Keep waste containers in a secondary container (tub underneath them), and make sure they are clearly labeled with the contents. We will dispose of filled chemical waste bottles during the next chemical waste collection on the ground floor of PCTB (room G06 from 1-2pm on Wednesdays). Before bringing down for drop-off, fill out and print the chemical waste form.

Hazardous Liquids (Biohazard): We work with a few liquids that classify as a biohazard: 1) chicken egg waste, 2) media from bacterial work, and 3) liquids from tissue culture work. Chicken egg waste should be frozen at -20°C and disposed of in a fresh red-bagged biohazard box; do not package with any materials that can puncture, like pipet tips. Liquids produced from bacterial or tissue culture work must be inactivated before disposal. To inactivate these liquids, add bleach to a 10% final concentration and incubate for at least 30 minutes. After this incubation, the biohazards are effectively inactivated, and this waste is now safe to rinse down the sink with plenty of water.

If you are *EVER* unsure of how to dispose of something, ask Mike, ask a more senior lab member, or ask the Biosafety office.

Sharps Handling

We use several sharps in the lab routinely, including needles (metal and glass), glass slides, coverslips and bottles, razor blades, and dissecting tools. Sharps can pose a threat because they can puncture or cut our skin. While this is unpleasant, it also can introduce biohazards into our bodies or make us more susceptible to future exposures. Here are some tips for using sharps safely:

- Minimize the handling of all sharps
- Never recap needles or use any technique that involves pointing a needle or sharp toward the body (yours or another's)
- Do not remove a used needle from a syringe or a blade from a handle – dispose of the whole item in the sharps bin
- Shearing, breaking, or bending of needles is prohibited
- Promptly dispose of your sharps in the appropriate containers
- NEVER jam sharps into a container

- NEVER insert your hand or fingers into a sharps container – if it is necessary to open and retrieve something from a sharps container, call HSE at x5-5918
- Use a utensil such as tongs, forceps, or dustpan and broom to pick up any broken glass, needles, or sharps that have fallen on the floor or another surface

Needlesticks & Exposure

All needlesticks and exposures to sharps must be reported immediately by calling the Needlestick Hotline 5-STIX (955-7849) for the East Baltimore Campus. An incident report must be filed. Specific details concerning the type of sharp and the actual occurrence should be listed to identify practices or types of equipment which may need to be modified.

Problems involving a particular type of sharp or procedure that may cause exposure to blood-borne pathogens should be brought to the attention of Health, Safety and Environment (5-5918).

Spill Clean Up

Spills happen, and usually, we can take care of them quickly and safely. When working with chemicals or biohazards, we need to take extra precautions to decontaminate the spill area before returning to work.

Take this time to identify where our lab spill kit is located: PPE (gloves, lab coats, goggles/face shield), broom and dust pan, absorbent pads, autoclave bags, red-bagged biohazard boxes, bleach or other disinfectant and container for mixing.

For Biological Spills

Small (<50 mL)

1. Remove contaminated clothing and PPE and place in biohazard box or set aside for decontamination.
2. Replace any removed PPE and put on a lab coat, double gloves, and eye protection.
3. Cover spill with absorbent materials saturated with fresh decontamination solution (10% bleach). Spread the bleach around the outside of the spill and work inward, covering an area that extends greater than the visible spill area.
4. Allow decontamination to sit for 30 minutes, then collect these materials.
5. Dispose of all cleanup materials in the biohazard box.
6. Remove lab coat, eye protection, and gloves; wash hands thoroughly.
7. Notify Mike.

Large (>50 mL)

1. Alert the room and isolate the area from traffic. If potentially hazardous aerosols have been generated, leave the area immediately and let the aerosols settle and building exhaust clear the area for 20-30 minutes. Otherwise, without risking exposure, surround the spill with absorbent material to contain spreading.
2. Remove contaminated clothing and PPE and place in biohazard box or set aside for decontamination.
3. If you can clean the spill without help, replace any removed PPE and put on a lab coat, double gloves, and eye protection.
4. Cover the perimeter of the spill and then work inward to cover the spill itself with a decontamination solution (10% bleach) – cover the area with absorbent materials, larger than the actual spill to catch any unseen splatter.
5. Wait 20-30 minutes, then dispose of materials in a biohazard box.
6. Remove lab coat, eye protection, and gloves, and wash hands thoroughly.
7. Notify Mike.

For Chemical Spills

1. **EVALUATE** the spill: are the materials innocuous, corrosive, flammable, toxic or explosive? Identify all materials by common or chemical name, estimate how much is spilled, and evaluate the degree of danger to yourself, the lab, and equipment/property.
2. **CONTAIN** the spill: utilize any action to prevent the spilled material from spreading and causing increased damage.
3. **EVACUATE** the area if the spill cannot be contained, or if the spilled material produces irritating odors, flammable or explosive vapors.
4. **COMMUNICATE** to the rest of the lab that there is a spill, and notify Mike.
5. **CLEAN** up the spilled materials:
    - Innocuous materials can be cleaned up by us in the lab using paper towels or absorbent pads.
    - Spills of acids, bases or flammables and mercury can be cleaned up by lab personnel with proper PPE and neutralizers/absorbents.
    - Spills of toxic or explosive materials, or large spills of corrosive or flammable materials, should be handled by HSE – call the Haz-Techs at x5-4444 and have this information ready: your name and phone number, precise location of spill, exact description of what spilled (state any compounds that may form toxic compounds), any steps you have taken to control the spill, and any injuries that have occurred.
6. **DISPOSE** of all contaminated materials.
7. Consult HSE at x5-5918 with any questions regarding chemical or biological spills and spill cleanup.

Emergency contact numbers (fire, medical emergency, security, biological/chemical exposure and spill lines) are posted physically throughout the lab – familiarize yourself with their locations as part of your onboarding.

Checklists

Onboarding Checklist

There are many systems to engage with when starting in the lab, including but not limited to the list below. Please let Mike know if you encounter something else important that belongs on this Onboarding Checklist for the future!

- [ ] Read this lab handbook and raise any questions/concerns with Mike
- [ ] Get access to the lab:
  - [ ] Pick up an ID badge from the Card Office (Nelson Harvey Building, Room #108)
  - [ ] Email our department's senior administrator to introduce yourself and request a key to our lab (WBSB 104) – "cc" Mike on this email
- [ ] Tap into lab digital resources:
  - [ ] Request invitation to Slack
  - [ ] Request invitation to the Google Drive Folder (important files, protocols, etc) and familiarize yourself with the "PiacentinoLab_Master_Log" file
  - [ ] Request invitation to the Google Calendars, including Lab Events, Seminars, Birthdays (add your birthday so we can celebrate you!), and Equipment (e.g. Electroporation Station #1, PCR Block, etc.)
  - [ ] Request access to the Piacentino Lab Server
  - [ ] Make an account in Benchling and request access to the Piacentino Lab
  - [ ] Request invite to the Piacentino Lab LabSuit and get familiar with our inventory/ordering pipeline
- [ ] Prepare for group meetings by reviewing (on Google Drive > LabMeeting_JournalClub):
  - [ ] Lab Meeting Agenda and Expectations document
  - [ ] Weekly Research Question Guidelines
- [ ] Register for and/or take all required **Health and Safety** trainings (see Laboratory Conduct & Biosafety)
- [ ] Review the Laboratory Conduct & Biosafety chapter again, and familiarize yourself with the location of safety features including:
  - [ ] Eyewash stations and emergency showers
  - [ ] Fire extinguishers, fire alarms, and evacuation routes
  - [ ] Spill kits and decontamination solutions
  - [ ] Waste containers (biohazards, sharps)
- [ ] Register for and/or take all required **Human Resources** training (see onboarding emails from HR) – these trainings cover important topics including inclusive work environments, harassment policies, research ethics, and beyond
- [ ] Send Mike a picture and information about yourself (include pronouns and a brief bio) for the people page
- [ ] Fill out the Work Style Questionnaire and discuss your answers with Mike
- [ ] *Optional:*
  - [ ] Get a paper lab notebook from Mike's office
  - [ ] Set up a tool kit for embryo work
  - [ ] Get access to the stationary closet

Offboarding Checklist

- [ ] Data storage
  - [ ] Backup all your data and lab documents across your external hard drives and in the server
  - [ ] Walk Mike through your data organization on the server
  - [ ] Turn in your hard drives to Mike. If you need a hard drive, ask Mike.

- [ ] Data analysis
  - [ ] Make sure all your analyzed data (image measurements, code and Prism files) are clearly organized and accessible, backed up both on external hard drives and the server
- [ ] Notebooks and records
  - [ ] Ensure your notebook and any lab documents are up to date and detailed, show Mike
- [ ] Samples – make sure they are clearly labeled
  - [ ] Show Mike where your samples are stored (4°C, -20°C, -80°C, LN2)
- [ ] Return lab key and tools (if applicable)
- [ ] What do you wish you knew before starting your time with us?

Work Style Questionnaire

The **Piacentino Lab Work Style Questionnaire** – fill this out and discuss your answers with Mike as part of onboarding.

1. The one thing I need most from a mentor/supervisor is: *(explain)*
2. The top three things that motivate me are:
3. These are the ways I receive feedback best:
4. I do my best to work well with everyone, but these are a few things that I find difficult to work with:
5. When I am upset or struggling in the workplace, this is how I tend to express it:
6. My three main strengths as a scientist and colleague that help me meet my goals are:
7. Three areas in which I could improve as a scientist and colleague in order to meet my goals are:
8. Colleagues and supervisors can best support me in the following ways:
9. My favorite lab snacks are: / My favorite lab beverages are: / My dietary restrictions/preferences are:
10. *(Optional)* Last, I think you should know that: