

Chen Lab

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Welcome to the Chen Lab!

We are a research lab in the Biochemistry and Molecular Biology Department at Colorado State University. Our research projects are led by [Primary Investigator](#), Dr. Chaoping Chen, and focus on characterizing the structure and functional mechanism of HIV-1 precursor protease.

Undergraduate Students

Hello to all undergraduate students interested in working in our lab!

Dr. Chen readily accepts self-motivated and intellectually curious undergraduates into her lab as research assistants. If you have ever considered biochemical research as a career, or if science fascinates you, the sooner you start in a lab the better. Getting involved in research as an undergrad provides you with hands-on experience that complements what you will be learning in your science courses. It also gives you a leg up when applying to graduate school or for a job in industrial science. Even if you decide that research is not the career for you, the experience will help you critically examine significant problems in our society and give you the means to solve them.

Undergraduate research is all about getting experience in a lab as early as you can to build your repertoire of techniques. Some primary investigators (PI's) will only let you wash dishes your first year, but depending on your availability, willingness to learn, and past experience, you can start out with your own project in the Chen Lab. Dr. Chen perfectly balances being available to show you procedures and answer questions while letting you be autonomous with a relevant project.

If you are interested in working in the Chen Lab, use this website as a resource to learn about research in general and find out specifically what we do and who we are. Feel free to email Dr. Chen with any questions about joining our lab group.

Graduate Students

Welcome, rotating graduate students!

Whether this is your first rotation at CSU or your last, use this website as an introduction to the research we do in the Chen Lab. It will familiarize you with our particular areas of investigation as well as provide details about our procedures and equipment which might be different from what you are used to. Browse the [Publication](#) section to understand the background of our research and be sure to contact Dr. Chen to set up a meeting to discuss the project you will be working on for the next three months.

Useful Resources:

Dr. Chaoping Chen, PI
Email: Chaoping.Chen@colostate.edu
Location: Molecular & Radiological Biosciences, room 233 (office) and room 227 (lab)
Office Phone: 970-491-0726
Lab Phone: 970-491-0791

Amanda Purnell, [HURS](#) Coordinator and Associate Director of the Office for Undergraduate Research and Artistry
[Apply for HURS](#)
Email: Amanda.Purnell@colostate.edu
Location: TILT building, room 145
Office Phone: 970-491-2185

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[Campus Maps](#)
[Building Floor Plans](#)
[CSU Student, Faculty, and Staff Directory](#)
[Undergraduate Research at CSU](#)
[PubMed](#)
[Celebrate Undergraduate Research & Creativity \(CURC\)](#)

Research Background

HIV-1 virions pack efficiently for their journey into the cell. They contain all components necessary for self-replication—from entry to repackaging of new viral particles—in a very small space. HIV accomplishes all this with just ten genes contained on a single strand of RNA.

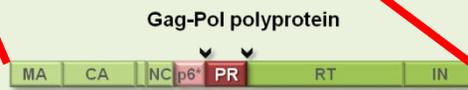
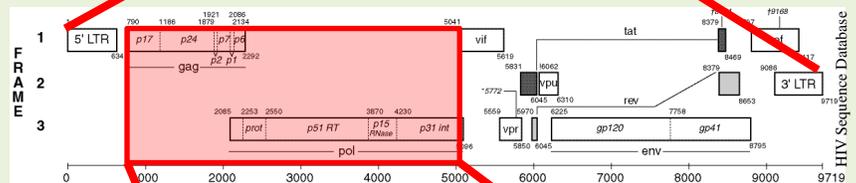
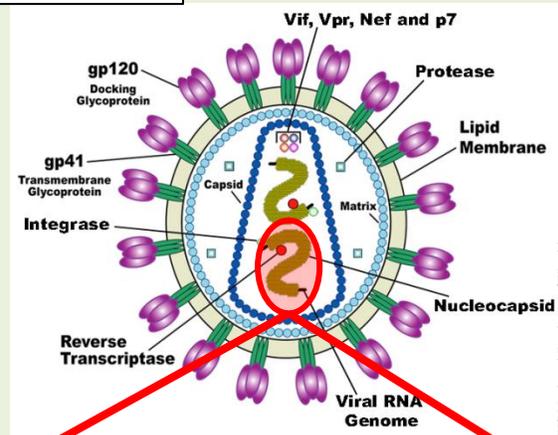
Our research concentrates on two of these genes, *Gag* and *Pol*, and the proteins they produce. *Pol* contains the information for HIV protease (abbreviated PR on diagrams), which is necessary to cleave the large **polyproteins** produced into individual functional products. Without protease activity, HIV would not be able to produce infectious **virions**, which is why it is a proven therapeutic target.

In order for HIV protease to become an active (mature) protease it must first cleave *itself* out of the Gag-Pol polyprotein. This reaction in which the precursor (immature) protease cleaves itself from its polyprotein precursor is called **autoprocessing**, and our lab focuses on characterizing the autoprocessing mechanism and developing novel autoprocessing inhibitors.

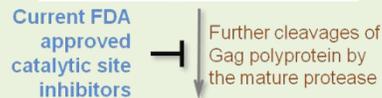
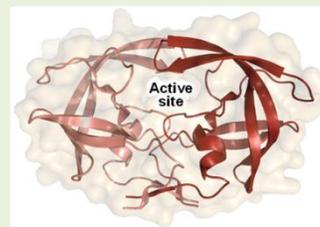
Most HIV drugs on today's market target the mature protease. With its high mutation rate, HIV is notorious for negating the activity of these normal protease inhibitors—one changed amino acid can regain protease function—which is why patients require a cocktail of multiple inhibitors to prevent drug resistance. By targeting autoprocessing, we hope to inhibit HIV one step *before* the release of the mature protease.

We look at aptamers, small RNA or DNA molecules that bind to certain molecules with high specificity, to bind precursor protease and inhibit autoprocessing. Usually, aptamer studies are done once the structure of the target molecule is identified, but little is known about the structure of HIV precursor protease. Though both the immature and mature protease are enzymatically active, results from our studies suggest that they have different conformations. Since little research has been done on immature protease, it is our challenge to characterize its structure and the mechanism by which it autoprocesses in

HIV-1 Virion



Mature protease dimer



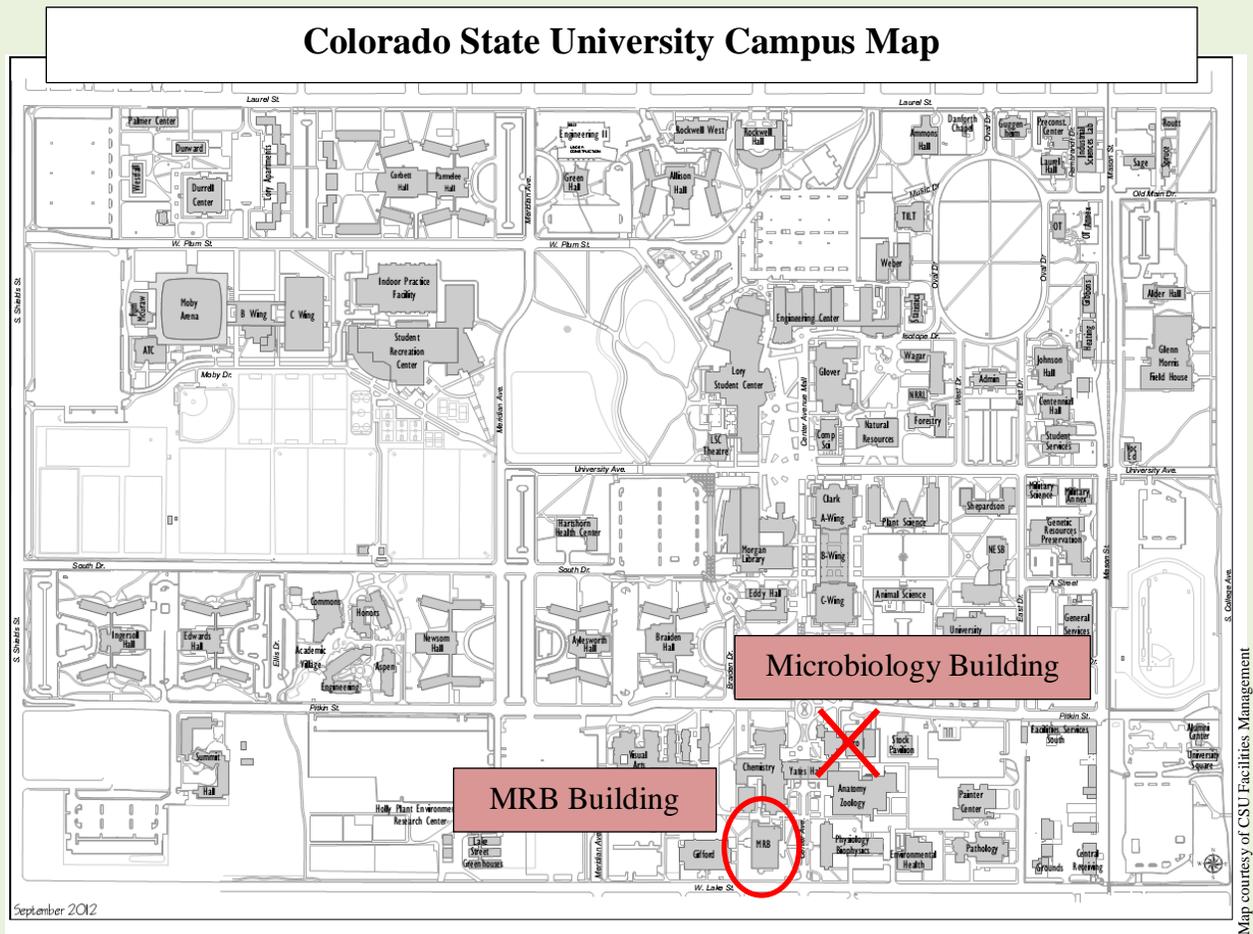
Infectious virions

Diagram by Dr. Chaoping Chen & Molly Plehaty

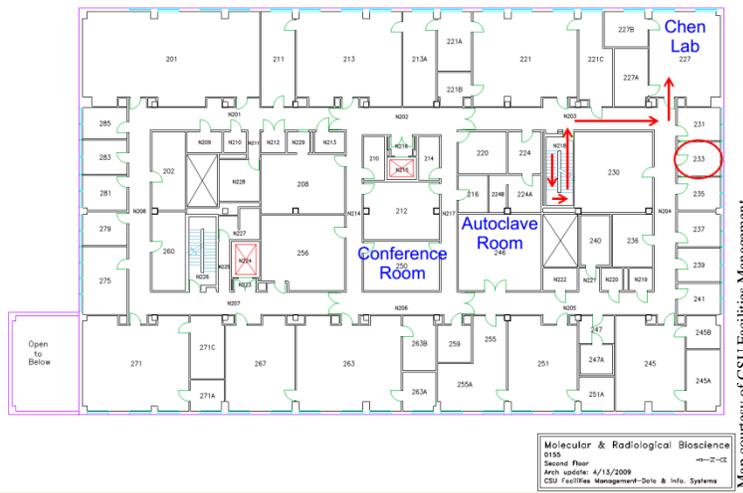
order to inhibit it effectively.

Finding the Lab

Our lab, located in the Molecular & Radiological Biosciences (MRB) building (not the Microbiology building!) might be hard to find your first time. The numbering system in MRB can also prove somewhat tricky, so we have provided some maps to help you locate the lab (room 227) and Dr. Chen's office (room 223.) Larger maps can be found [here](#).

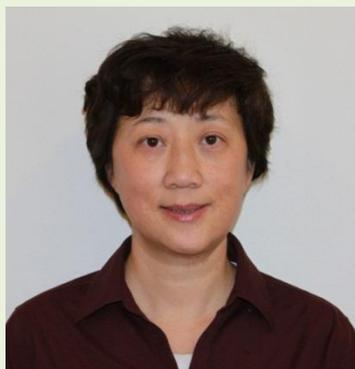


Molecular & Radiological Biosciences Building Map



People

Primary Investigator:



Dr. Chaoping Chen, Ph.D.

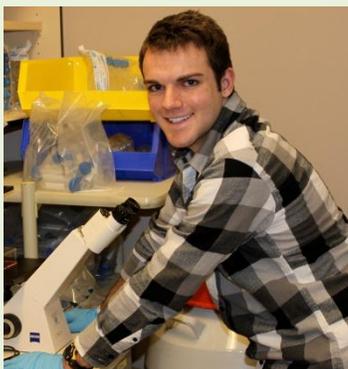
Biography: Dr. Chen is the [PI](#) of our lab and is an Associate Professor at Colorado State University. She has degrees in multiple molecular biology fields from around the world: a BS in Biochemistry and an MS in Genetics from universities in China, a Ph.D. in Molecular Virology from Purdue University, and Post-Doc training in Retrovirus Assembly at the University of Pittsburgh. Her research focuses on a new approach to targeting HIV autoprocessing for anti-HIV drug development and has shown that the immature protease is enzymatically different from the mature form.

Best piece of advice: “Don’t be afraid to ask—it makes you look smart plus we don’t bite ☺.”

Best “horrible” lab mistake: She recently made a mistake when doing a western blot transfer: after checking that the membrane and gel were in the correct orientation and that the electrodes were connected correctly to the apparatus, she neglected to check that the other ends of the electrodes were in the power supply according to the color code. It only takes one wrong step to ruin an entire experiment and even the best and brightest still make mistakes sometimes.

Current Undergraduate Research Assistants:

Chris Counts

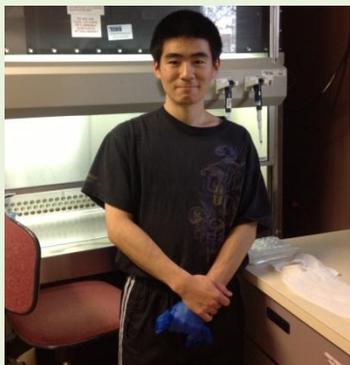


Biography: Chris is a senior majoring in Biomedical Science and Anthropology, and this is his 4th year working in the Chen Lab. In that time he has collaborated with researchers at other universities in a project analyzing [HIV-1 precursor protease](#) amino acid covariance. This statistical method has allowed him to identify two protease residues not in the catalytic site of the protease that are involved in [autoprocessing](#) regulation. At the moment Chris is working on the manuscript for this exciting discovery. He recently received the prestigious Marshall Scholarship which will allow him to pursue an advanced degree in Public Health in the United Kingdom. His interests in global health and medical anthropology led him to live in Tanzania for five months where he helped with different projects

related to public health.

Best piece of advice: “My best piece of advice to incoming lab members is to write down EVERYTHING you do in your lab notebook. This will make your life a lot easier down the road when you need to present your data or if an experiment goes wrong.”

Best “horrible” lab mistake: He accidentally added 10 microliters of a solution in an experiment when he was supposed to use 10 milliliters. (See the chart in the [Tips & Tricks](#) section for a handy guide to metric prefixes and conversions.)



Satoshi Machihara

Biography: Satoshi is a Biochemistry and Molecular Genetics Major with a Chemistry Minor senior at CSU currently working on obtaining both a Master’s and Bachelor’s Degree. He started in the lab his freshman year at the same time as Chris and vividly remembers their first day when Dr. Chen handed them a 0.5mL tube of [SELEX](#) product to characterize an [aptamer](#) for their project. It was an intimidating experience—not knowing half of what she explained, what they were given, or where to start—but several years later he is still here successfully characterizing that aptamer for HIV protease autoprocessing inhibition.

Best piece of advice: “I’ve seen other freshmen enter the lab and though they don’t get dropped right into the deep end as Chris and I were, I really want to help people not feel helpless when they are here—whether it’s their first day, fifth week, or tenth month. Also, don’t be too intimidated. Good luck and see you in the lab.”

Best “horrible” lab mistake: He switched the enzyme buffer and loading buffer when doing a restriction digest which neutralized the enzyme and ruined his entire reaction.



Molly Plehaty

Biography: Molly is a Biochemistry major who has been in the Chen Lab for three years. During that time she has worked on projects involving both HIV autoprocessing and Flavivirus capping enzyme inhibition. She spent the past few summers working in a lab at the University of Colorado Denver medical campus as part of their schizophrenia research group. Her favorite part of that internship was getting to work with brains (human, rat, and mouse) on a daily basis.

Best piece of advice: “Watching other people go through a procedure helps you process the information even better than reading the protocol or hearing them describe what to do. While you are observing them, write down the details of every move they make; noting minor deviations from the written protocol and locations of the different reagents so later on you can duplicate everything exactly.”

Best “horrible” lab mistake: She accidentally added [PBS](#) to her [MINI-prep](#) instead of Buffer PB, almost losing many long hours of hard work because of that one-letter difference. (For future reference, PB and PBS are drastically different reagents.)



Rachel Sauer

Biography: Rachel is a junior Biomedical Sciences major and became a pro at [cloning](#) by helping one of our previous graduate students with his project. She is currently on hiatus from the lab because she is juggling a full academic schedule, a job, and a shadowing opportunity at a local hospital. Like Molly, she gets very excited about brains and dissections.

Best piece of advice: “Don’t be discouraged because you don’t know everything! When you first get here you won’t understand most of what’s being said during lab meetings, but that’s normal. It’s impossible to absorb the huge amount of information we throw at you right away, but time and

repeated exposure will help.”

Best “horrible” lab mistake: When making some [SDS gels](#) she forgot to add the [APS](#) to polymerize the gel and sat there for half an hour waiting for the gel to solidify before she realized her mistake.



Brittany Kemp

Biography: Brittany, the newest undergraduate addition to our lab family, arrived earlier this semester as a freshman Biochemistry major with a concentration in Health and Medical Sciences. Currently on the Pre-Med track, she someday wants a career in pediatrics but is open to wherever science takes her. Dr. Chen recruited Brittney as the “official cloning assistant” for now which means she prepares cells with specific DNA for Dr. Chen to clone in subsequent experiments. She hopes to start on her own project within the next year and really let her research experience take off. Originally from a small rural community in southeastern Colorado, she had no prior experience coming into the lab but loves being able to learn

new things every day and apply knowledge from her classes to hands-on experiments.

Best piece of advice: “My advice for anyone who wants to do research—and even someone who doesn’t—is to get in a lab as soon as possible. Whether or not you have lab experience or understand the scientific jargon, try to engage yourself in the conversation and you’ll surprise yourself by what you’ll cabbage on to. Don’t be afraid of mistakes—trust me, you’ll make them—but certainly learn from them. There will be days when your negative control is contaminated, you completely lose a sample without any idea of its possible location, or you make SDS-Gels that don’t polymerize; as long as you have an open mind research will be one of the most beneficial experiences you encounter.”

Best “horrible” lab mistake: Somewhere between the lab and the shaker room down the hall one of her samples mysteriously disappeared.

Very Important Lab Members:



R2-D2

Biography: R2 the Liquid Nitrogen Tank has been with the lab for a long time and tirelessly keeps our [cell lines](#) frozen at temperatures around -196°C day in and day out. As our expert on liquid nitrogen he boasts being able to accommodate more than 90L of it at a time. Every couple of months two strong lab members take him on a vacation down to the chemistry

stock room to refill, rejuvenate, and read the Far Side comic books that the attendant always provides.

Best piece of advice: When dealing with liquid nitrogen, always wear the large orange autoclave gloves to protect your hands and arms. It's cold enough to burn your skin, be careful!

Best "horrible" lab mistake: One time he threw the lab into turmoil when they discovered he was almost completely dry so they ran him down to the stock room where he got an emergency fill-up. Fortunately the cell lines hadn't thawed which would be disastrous to the lab's work.

Lab Alumni:

Liangqun "Lillian" Huang, Ph.D. (Post-Doctoral Research Assistant)

Srinivasa Bodeda (Grad Student)

Anna Gaber ([Rotating Grad Student](#))

Matt Schoffield (Rotating Grad Student)

Jordan Spiedel (Rotating Grad Student)

Meg Basila (Rotating Grad Student)

Current Member Resources

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[Terms & Abbreviations](#)

[Tips, Tricks, & Habits to Form](#)

[Equipment](#)

Lab Rules:

1) Understand what you are doing.

This rule can be rephrased as, “Ask questions! *Ask questions!* ASK QUESTIONS!” When you start working in the lab you will be running experiments that are relevant to the group’s research so it becomes vital to know what’s going on. You won’t know everything at first—that would be impossible—so that’s why there are always people around to answer questions and show you where to find things and how to do things.

2) Write *everything* down.

Minor details can make the difference between success and failure of an experiment, so writing down exactly what you do will help you and others to repeat it later on.

3) Be honest.

Everyone makes mistakes, and owning up to them sooner rather than later will serve you better than pretending nothing went wrong. This way the situation can be remedied instead of causing further harm down the road. This rule also applies to academic honesty and the interpretation of your results. In molecular biology research, even negative outcomes are informative and often lead to new discoveries and different ways of seeing a problem. Fudging data is not only dishonest but counterproductive to your project and the work of those who might base their research on your results.

4) Clean up after yourself.

This rule may sound like something a nagging mother would say, but it is vitally important to keep the lab space tidy and open for the next person to use. Contamination—especially in the [biosafety cabinet](#)—can be a big problem and set any projects involving the contaminated equipment and reagents back by a week or more. Cleaning up after yourself is more than just a common courtesy, it is a necessity to keep the lab running smoothly.

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Terms & Abbreviations

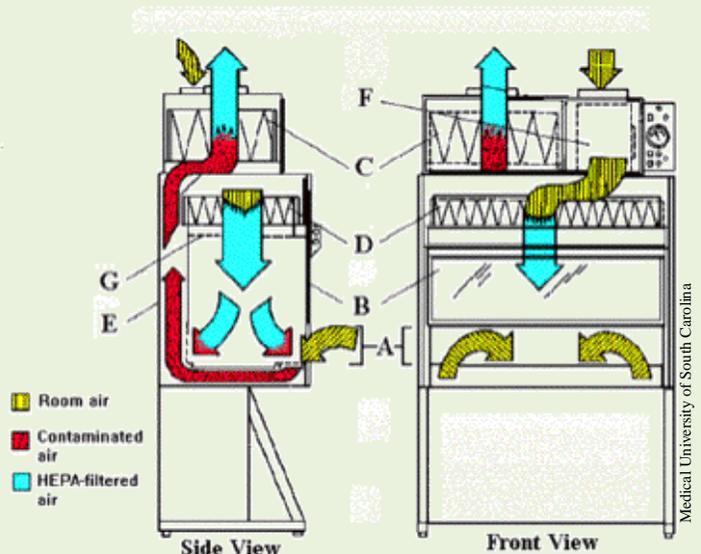
1. **Acrylamide**—ingredient in SDS gels that polymerizes in the gel. Wear gloves when working with this as it is a neurotoxin!
2. **Aliquot**—smaller division of a large amount (usually for convenience.)
3. **APS**—abbreviation for Ammonium Per Sulfate: a source of free-radicals that accelerates polymerization of the acrylamide in SDS-PAGE gels.
4. **Aptamer**—short sequence of RNA or DNA that inhibits a target molecule or structure by binding to it with high specificity.
5. **Autoclaving**—sterilization process that uses extreme heat and pressure to kill any organisms on glassware or in solutions used in the lab.



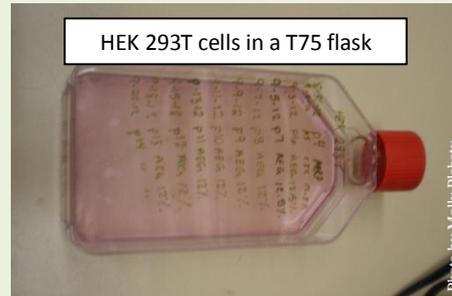
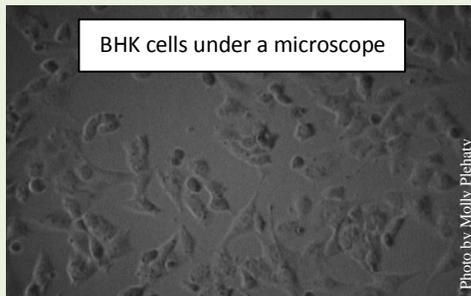
Autoclave tape is placed over the seal of each piece before autoclaving. The stripes will turn black to indicate that the autoclaving has been completed.



6. **Autoprocessing**—step in which the immature HIV protease cleaves *itself* from the precursor strand to turn into the mature form.
7. **Biosafety Cabinet**—sterile environment created by circular air currents and special filters in which we work with cells. In your safety training you will see it abbreviated as “BSC” but we commonly refer to it as the “hood.”

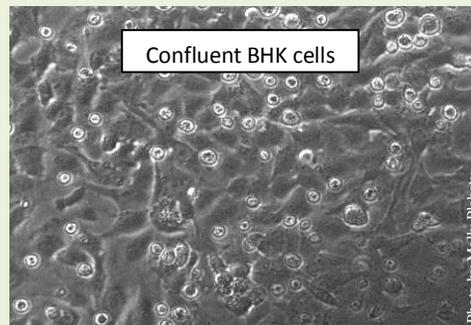


8. $C_1V_1=C_2V_2$ —common formula used for mixing reagents at the right ratios, it stands for (Concentration of initial solution)(Volume of initial solution) = (Concentration of final solution)(Volume of final solution.)
9. **Cell lines**—immortalized cells (usually mammalian) that come from different organisms and sources.

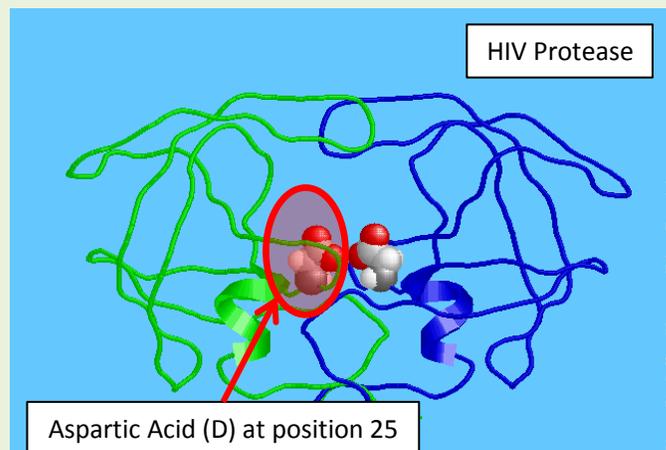


Example: BHK cells are Baby Hamster Kidney cells and HEK 293T cells are Human Embryonic Kidney cells from a specific line. Different cell lines have different morphologies and properties that make them suited to different experiments.

10. **Chemically competent cells**—cells that have been treated with chemicals to make their membranes more permeable and capable of taking up DNA.
11. **Cloning**—process of inserting recombinant DNA into bacterial cells and using them to express the gene(s) of interest in large quantities.
12. **Confluent**—cell growth has completely covered the surface available and cells are crowded. Cells at complete confluence have passed their exponential growth phase.



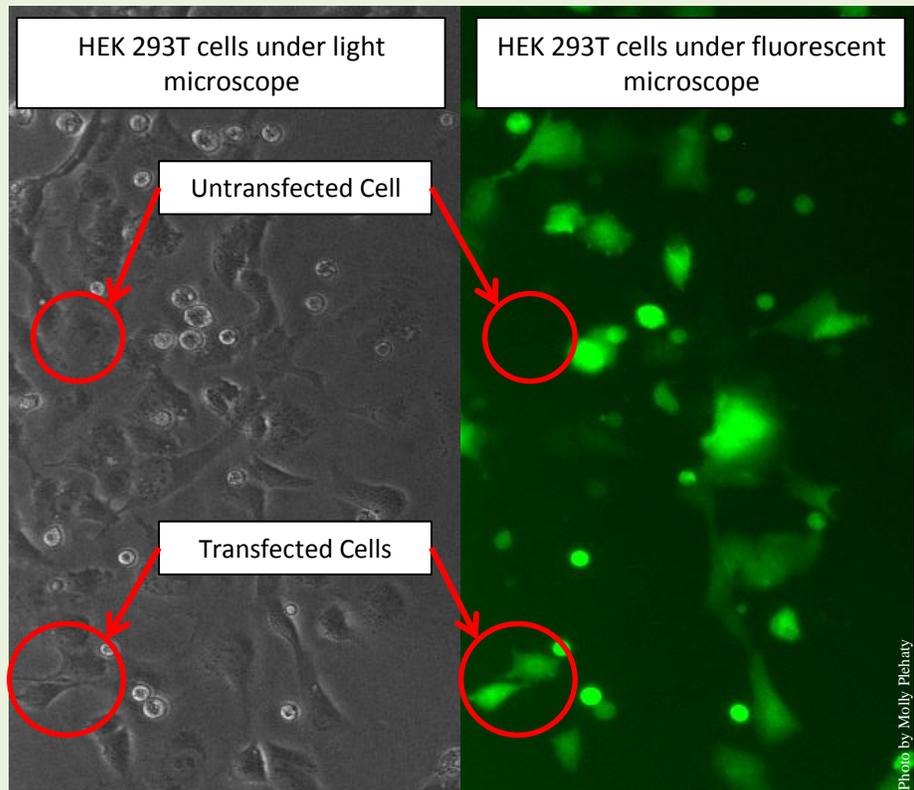
13. **D25N**—induced mutation in HIV precursor protease that changes the negatively charged Aspartic Acid residue at position 25 to a neutral Asparagine. This mutation occurs on the catalytic site of the protease and completely shuts down autoprocessing.



14. **DMEM-C**—bright pink liquid cell food that has all the essentials for happy tissue cultures: salts, amino acids, vitamins, and glucose. The “C” stands for “complete” which indicates 10% [FBS](#) has been added. Often shortened to just “media.”
15. **EtOH**—common abbreviation for ethanol, C₂H₅OH. 200 proof alcohol is 100% EtOH and is usually diluted to a 75% solution with [nanopure water](#) for cleaning purposes.
16. **FBS**—abbreviation for Fetal Bovine Serum, this is a component of blocking buffer for a western blot that increases the specificity of the antibodies by binding to non-specific proteins.
17. **Fume hood**—ventilated cabinet used for working with chemicals that give off hazardous fumes.

18. **GFP**—abbreviation for Green Fluorescent Protein and is used in the lab as a fluorescent tag to check for transfection efficiency.

GFP is one of the most common fluorescent proteins used in today’s research. The GFP gene can be attached to a [plasmid construct](#) containing a gene of interest. When the plasmid is [transfected](#) into cells, the cell machinery expresses the GFP along with the gene of interest and researchers can see how effective the transfection was by looking at the cells under a fluorescent microscope. More green cells means that more plasmids made it into the cells and were expressed successfully.



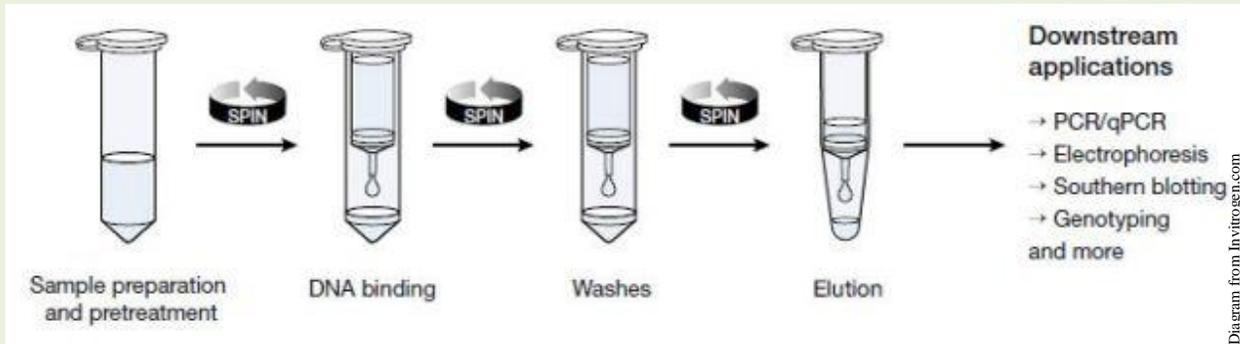
19. **Glycerol stock**—bacteria that express a gene of interest preserved in glycerol and stored at -80°C.
20. **H69D**—induced mutation in HIV precursor protease that changes the positively charged Histidine residue at position 25 to a negatively charged Aspartic Acid. This mutation, like the D25N mutation, abolishes autoprocessing behavior.

21. **HURS**—Honors Undergraduate Research Scholars program at CSU that helps place students in research labs.
22. **Incubator**—cabinet kept at 37°C with 5% carbon dioxide to provide the optimal environment for cell growth.
23. **kDa**—abbreviation for kilodaltons, a unit of measurement for atomic mass commonly used to describe the size of proteins or microRNAs on a western or northern blot respectively.

24. **Millipore water**—water that comes from the small tap on each sink in the lab. It is partially filtered and is sufficient for a final rinse when cleaning glassware.



25. **MINI/MIDI-prep**—method used to extract and purify DNA from bacterial cells. The amount of sample determines the size (MINI or MIDI) of the purification columns used.

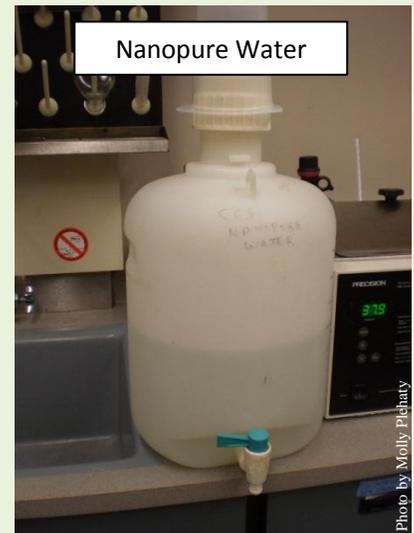


26. **Nanopure water**—purest water available in the lab and is free of all ions and impurities and used to make solutions or as a final rinse for western blot containers that need to be very clean. The main supply is from a special filter in the autoclave room and there are large jugs of it around the lab for everyday use. Sometimes abbreviated as dH₂O.

27. **PBS**—abbreviation for Phosphate Buffered Saline, a common salt solution used in many protocols.

28. **Pipettes**—instruments used in a lab that are capable of measuring precise amounts of liquid. Sizes range from 0.1µl on a P2 pipette to 50+mL on a serological pipette. (See “Pipetting” section on the [Tips & Tricks](#) page.)

29. **Plasmid** —small circular bacterial DNA into which genes of interest have been inserted. These constructs are then inserted into bacterial cells by transformation so that the genes of interest are expressed and the products can be purified.



30. **Polyprotein**—large protein comprised of several smaller proteins with separate biological functions.

Gag-Pol polyprotein



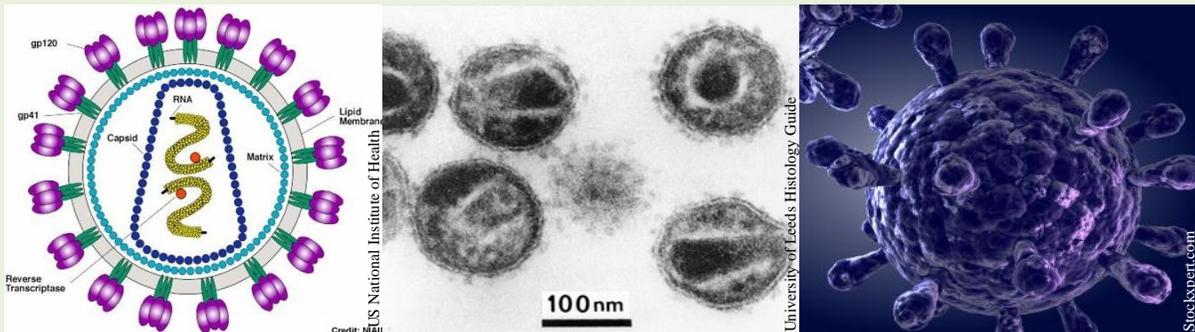
31. **Post-Doc**—common term for a “post doctoral research assistant” who has received a Ph.D. but who needs more experience and a greater depth of knowledge before achieving professorship.

32. **Precursor protease**—immature form of HIV-1 protease that has not cleaved itself from the polyprotein.

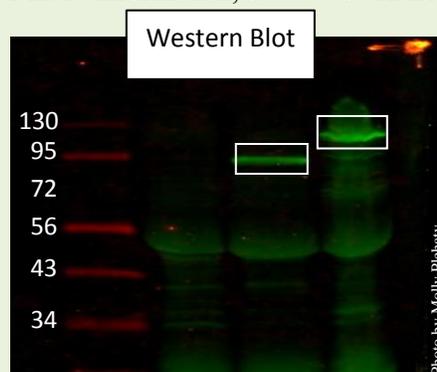
33. **Protease inhibitor**—molecule that prevents proteases from chopping up proteins. It is sometimes abbreviated as “PI,” so be sure not to confuse it with the PI of a lab (see below.)

34. **Primary investigator**—head researcher who leads the lab, writing grants and proposals and deciding where the research is headed. Also called the “PI” of a lab.

35. **Resolving gel**—high percentage (usually 8-15%; depends on protein size) SDS-PAGE gel layer below the stacking gel that makes up most of the gel and separates the proteins by size.
36. **Rotating graduate student**—first-year graduate student in the Department of Biochemistry and Molecular Biology who spends 3 months working in a lab on a project, gives a presentation of their results to the faculty, and moves to a new lab. Rotations give the students a broad scope of the departmental research and allow them to find the best fit for working in a lab.
37. **SDS-PAGE gels**—abbreviation for Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis gels. These gels are used to separate denatured proteins by size so that they can be visualized.
38. **SELEX**—abbreviation for Systematic Evolution of Ligands by Exponential Enrichment. It is the process used to identify [aptamers](#) that bind and inhibit specific molecules.
39. **Stacking gel**—low percentage (usually 4%) SDS-PAGE gel layer at the top of the gel that collects the protein samples at the same height before they go into the resolving gel.
40. **TEMED**—abbreviation for Tetramethylethelenediamine, one of the ingredients in an SDS gel that helps with polymerization. It smells like rotting fish, so be sure to close the bottle quickly after use.
41. **Transfection**—technique in molecular biology research in which a DNA plasmid of interest is taken up by a mammalian cell and the genes expressed by the cell's transcription/translation machinery. (For more information, see “Transfecting” on the [Equipment](#) page.)
42. **Virion**—viral particle including the genetic material surrounded by a capsid.



43. **Western blots**—technique in molecular biology research used to determine the presence and size of proteins of interest with high specificity. (For more information, see “Western Blotting” on the [Equipment](#) page.)



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Tips, Tricks, and Habits to Form

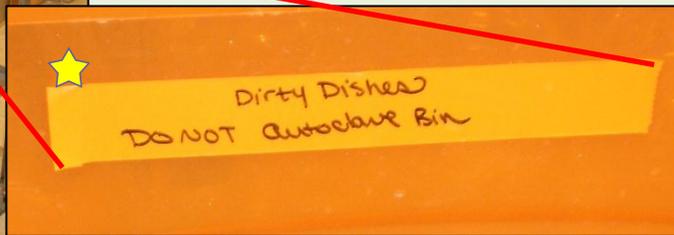
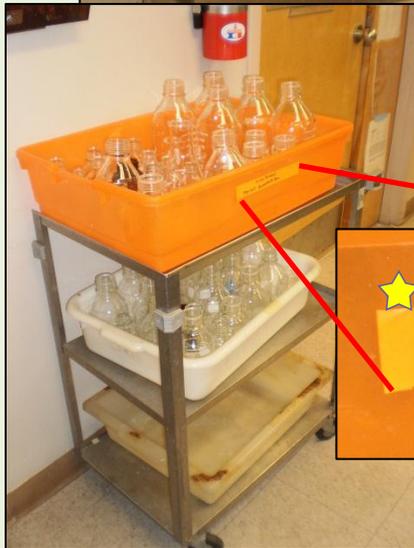
Mouse over the gold stars on each picture for helpful advice about common lab practices.

Wearing Gloves



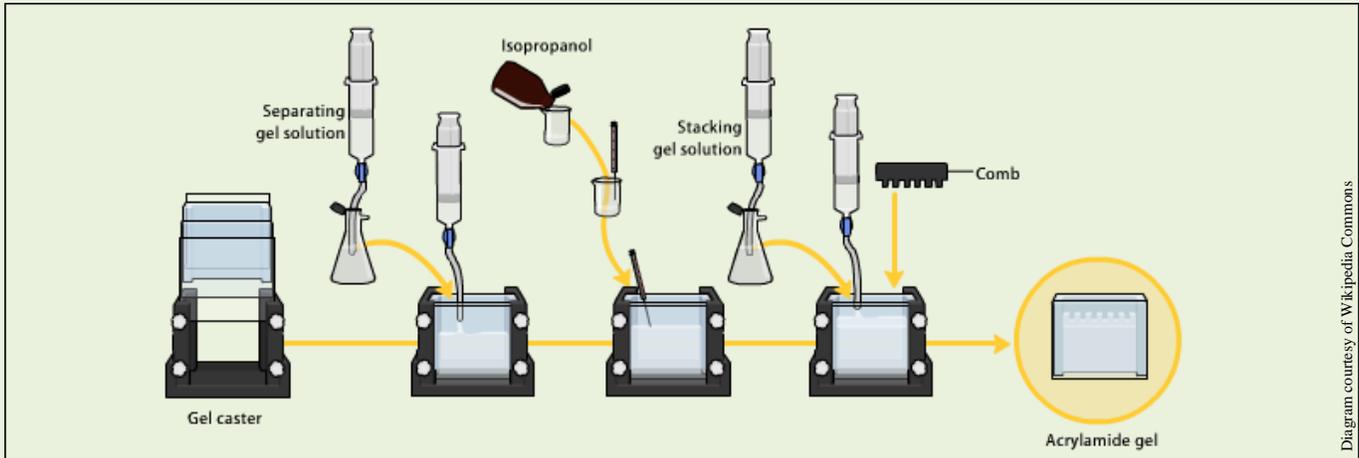
Photos by Molly Plehatty

Autoclaving/Dishwashing

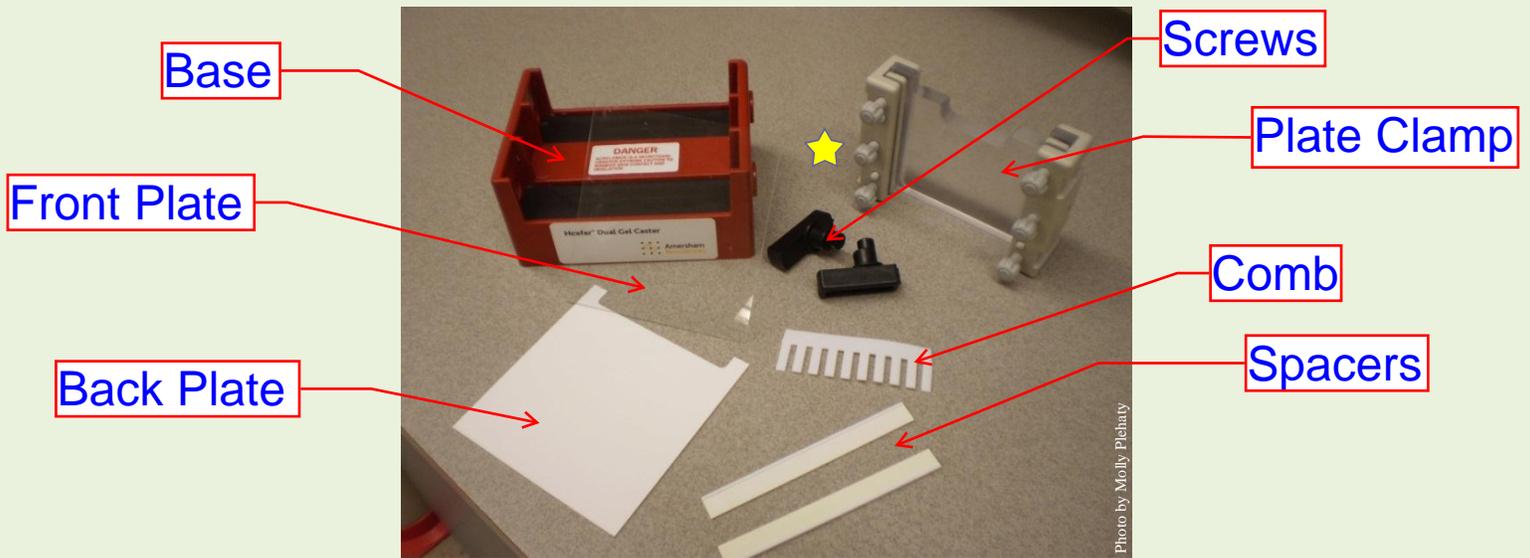


Making SDS Gels

A quick guide to making gels for SDS-PAGE:



Gel-making cassette components:



Assembled cassette:



Working in the Hood/Cell Culture Room



Photos by Molly Plehatty

Handy Guide to Metric Prefixes & Conversions

Symbol	Prefix	Multiplication Factor	
E	exa	10^{18}	1,000,000,000,000,000,000
P	peta	10^{15}	1,000,000,000,000,000
T	tera	10^{12}	1,000,000,000,000
G	giga	10^9	1,000,000,000
M	mega	10^6	1,000,000
k	kilo	10^3	1,000
h	hecto	10^2	100
da	deka	10^1	10
d	deci	10^{-1}	0.1
c	centi	10^{-2}	0.01
m	milli	10^{-3}	0.001
μ	micro	10^{-6}	0.000,001
n	nano	10^{-9}	0.000,000,001
p	pico	10^{-12}	0.000,000,000,001
f	femto	10^{-15}	0.000,000,000,000,001
a	atto	10^{-18}	0.000,000,000,000,000,001



Chart from Nanotechnology Now

Molarity Conversions

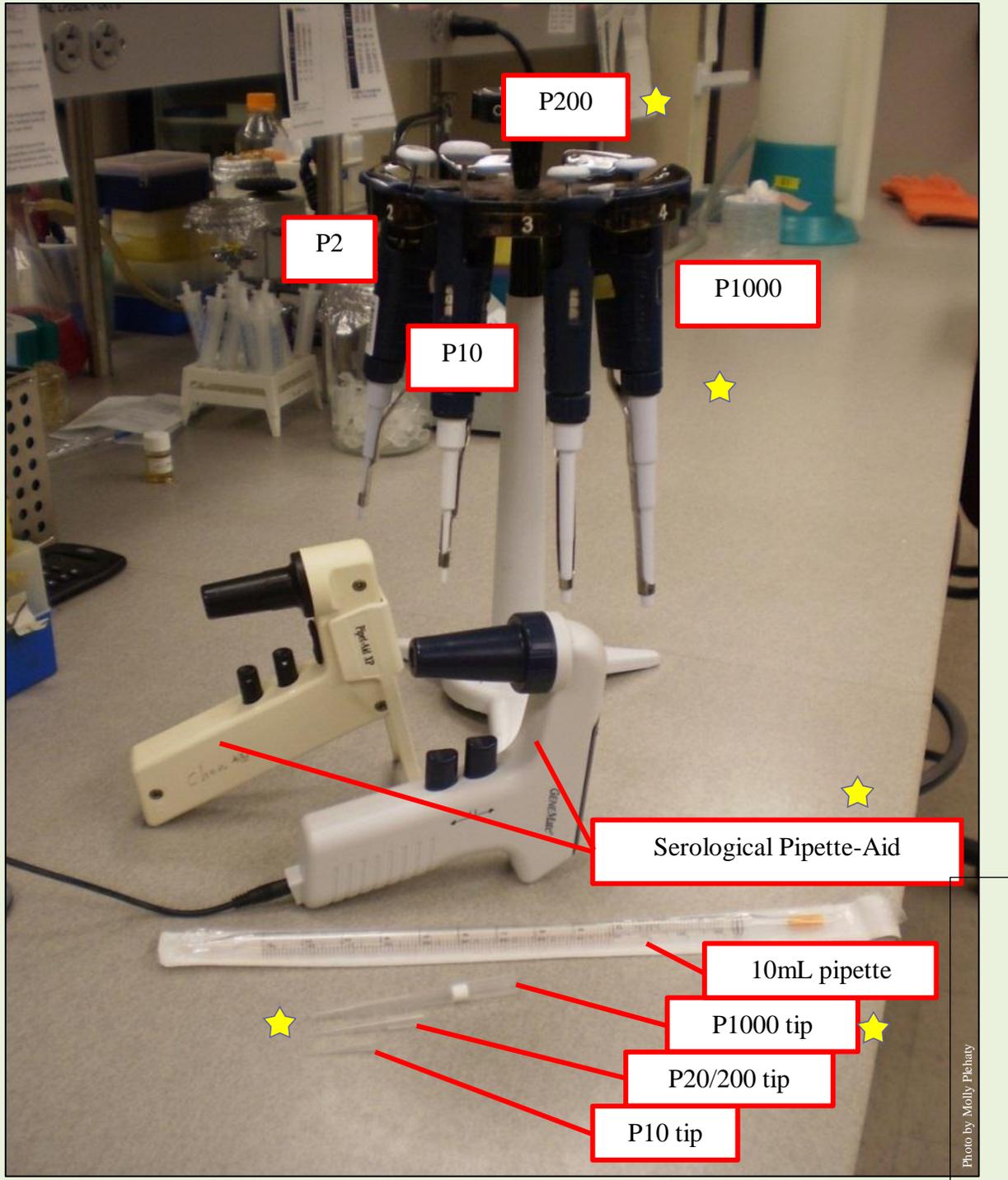
M	mol/L	mmol/mL	μmol/μL
mM	mmol/L	μmol/mL	nmol/μL
μM	μmol/L	nmol/mL	pmol/μL
nM	nmol/L	pmol/mL	fmol/μL

Chart by Molly Plehaty

Saving Samples ★



Pipetting



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Equipment

[Autoclave](#) ➤



[Biosafety cabinet](#) ➤



[Vortex](#) ➤



[Centrifuge](#) ➤



[Gel casting box](#) ➤



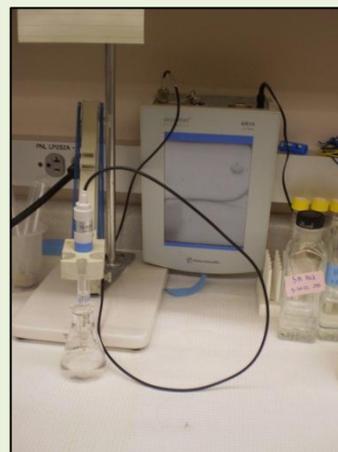
[Dishwasher](#) ➤



[Voltage Supply](#) ➤



[pH meter](#) ➤



Photos by Molly Plehary

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Publications

This section contains a list of relevant papers and other documents that provide more detail about the research being done in the Chen Lab:

- [Flexible catalytic site conformations implicated in modulation of HIV-1 protease autoprocessing reactions](#)
- [Autoprocessing of human immunodeficiency virus type 1 protease miniprecursor fusions in mammalian cells](#)
- [Cysteine 95 and other residues influence the regulatory effects of Histidine 69 mutations on Human Immunodeficiency Virus Type 1 protease autoprocessing](#)
- [Modulation of human immunodeficiency virus type 1 protease autoprocessing by charge properties of surface residue 69](#)