in Vitro

Volume II, Issue I

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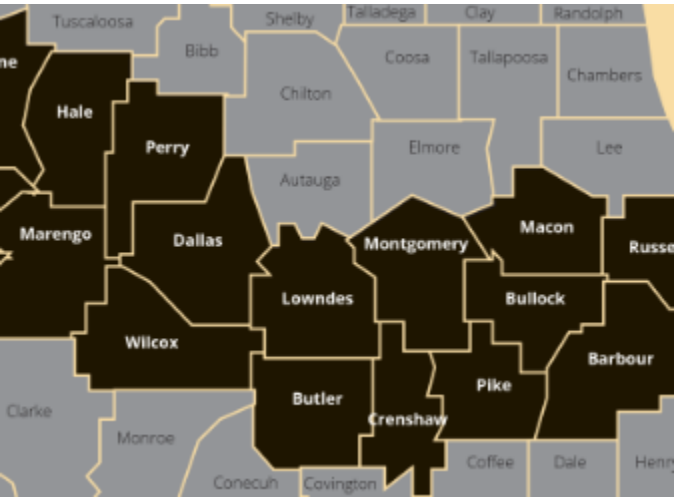

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asfainvitro@gmail.com

Lab Basics

It's your first day in the (biology) lab…. what are all these machines!?

So, you've just started your ASFA senior research project. After going through a lengthy and arduous lab safety training course, you step foot into your new home for the next few months. In this article, we'll introduce a few common microbiology lab techniques and tools to help you get up and running!

- Conrad Feng & William Peng

Cell Culture

Besides all the tedious paperwork, working with live animals is a very complicated and lengthy process that requires a lot of resources. So, where can we begin? Cells! More specifically, in vitro experimentation.

At the beginning of any great in vitro experiment is cell culture. That is, cells removed from an animal or plant are grown in an artificial environment. When isolated, these initial cells are called the primary culture. Typically, these cells will proliferate in a medium (Dulbecco's modified eagle medium is commonly used) with some sort of supplement (in many cases, fetal bovine serum). Each day, these cells will proliferate, and you will change the medium to resupply the cells with fresh supplements. Eventually, when the cells reach confluence, the cells have to be subcultured (passaged) by transferring them to a new vessel with fresh growth medium to provide more room for growth.

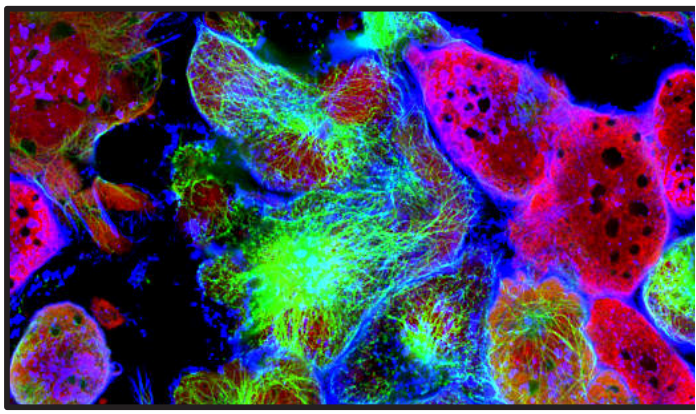
Once you have enough cells, other more unique experiments specialized towards a hypothesis can be utilized.

IF / IHC

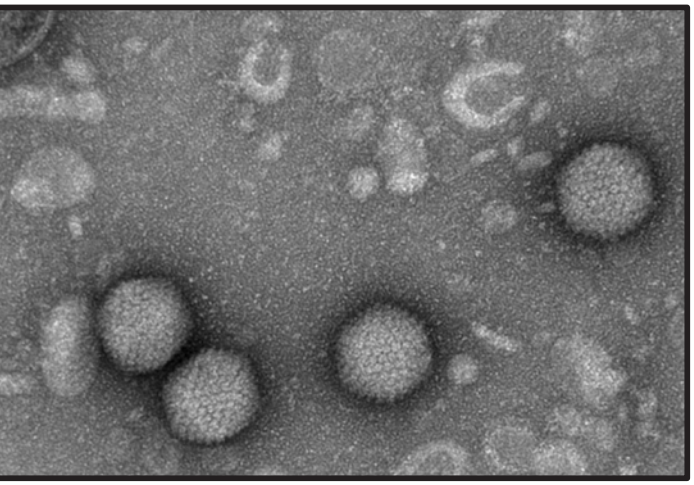
Two of the cooler protocols– immunofluorescence (IF) and immunohistochemistry (IHC)– produce beautiful and colorful images of your cells. These protocols typically involve fixing your cells with a formaldehyde solution followed by blocking and two or three antibody incubations. The primary antibody will bind to the protein of interest, and the secondary antibody will bind to the primary antibody. The secondary antibody will commonly be conjugated (connected to) with a fluorescent dye (think green fluorescent protein, GFP), which emits fluorescence at a specific wavelength.

Contrasting with IF, IHC uses antibodies conjugated to an enzyme, such as horseradish peroxidase, to deposit color. The underlying antibody-binding concepts are the same, but they differ in the way they produce color.

You'll be doing a lot of washing steps!



Transfection & Transduction

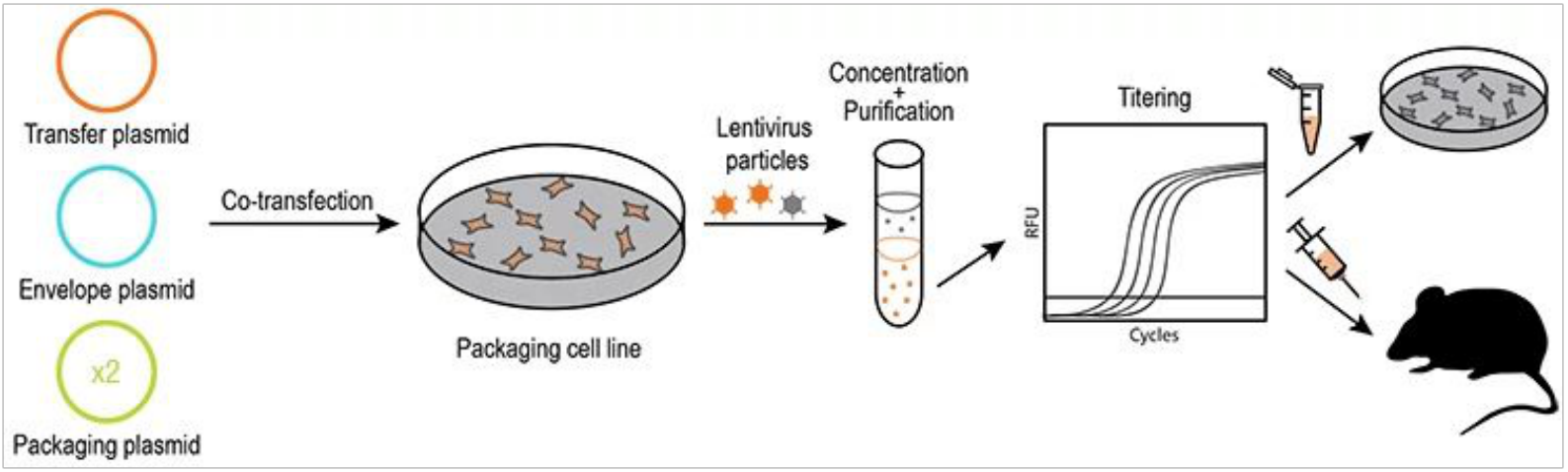


Transfection:

When working with cells, we often want to mess with their gene expression. We can express or overexpress a gene by introducing a plasmid into a cell's nucleus. Conversely, we can knock down a gene (reduce its expression) using siRNA. Both these approaches typically use a cationic lipid transfection reagent (usually Lipofectamine) to envelop the nucleic acid, forming a complex that enters the cell through endocytosis. These methods are temporary–gene expression eventually returns to normal, generally after a couple of weeks.

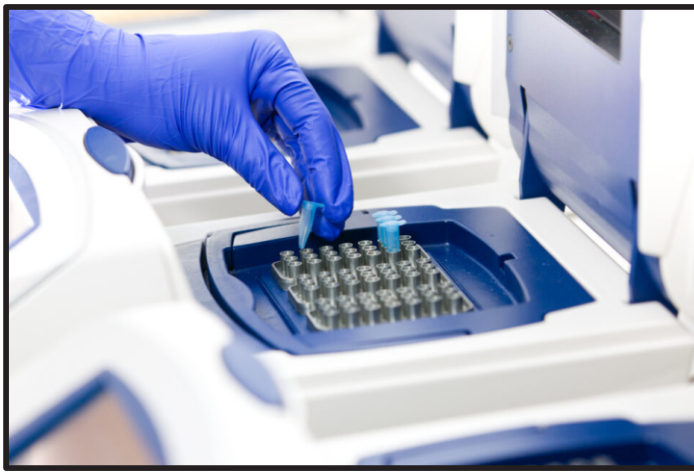
Transduction:

As opposed to transfection, transduction protocols use viral vectors to permanently alter gene expression. Common viral vectors are retroviruses and lentiviruses, which integrate their genetic information into the genome, and Sendai viruses, which are non-integrating. These protocols will include a viral packaging step where specific cell lines (commonly HEK293T) are transfected with viral genes and a plasmid construct containing the gene of interest. The virus will then self-assemble with the custom plasmid and enter the culture medium, where it can be collected and frozen for later use. Transduction itself is easy: just add some virus-containing medium to your cells!



PCR & qPCR

There's nothing more cliché than PCR in a biology lab. PCR is an acronym for polymerase chain reaction. Essentially, we're making copies of a DNA sequence using the same machinery that living cells use to duplicate their DNA. This means that you'll be adding DNA polymerase, ligase, and free nucleotides (dNTPs, or deoxynucleotide triphosphates) to your isolated DNA solution.

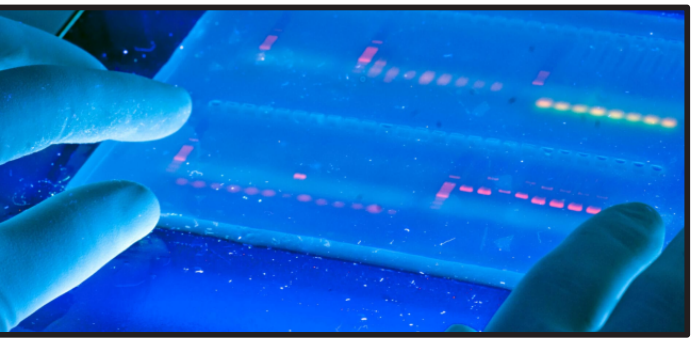


Inside the thermocycler, the mixture will be cycled through different temperatures to facilitate the reaction. If you're working with RNA, you'll first have to convert it into cDNA (complementary DNA) using the reverse transcriptase enzyme.

What if we want to compare two groups and quantify the change of a certain gene between the two? We can't use PCR since it only tells us if a gene is present. To quantify the changes in gene expression, qPCR measures the amount of DNA produced in real-time. That is, qPCR uses fluorescent markers that emit a detectable signal proportional to the amount of DNA amplified during each cycle.

By comparing the threshold cycle (Ct) value from different samples, we can quantify the differences in gene expression between two groups. Lower Ct values indicates higher initial amount and thus higher gene expression, and vice versa. A housekeeping gene (one that stays consistant between two experimental groups) normalizes the expression level. That is, by comparing Ct values of a target gene to the housekeeping gene, we can account for any variation in sample preparation or input DNA amounts, ensuring more accurate and reliable quantification of gene expression.

Gel Electrophoresis



Arguably just as familiar as PCR, gel electrophoresis is used to separate biological macromolecules (typically DNA and protein) by size. This allows for the identification or validation of knockouts, isolation of specific protein or DNA segments, and is a step in many other biological and genetic assays.

Gel electrophoresis uses an electric field and a gel. Since DNA has a negative charge from its phosphate groups, it will travel towards the anode (positive). Larger fragments will travel slower than smaller ones. A "ladder" composed of DNA/protein fragments of known sizes is used to determine the sizes of your samples.

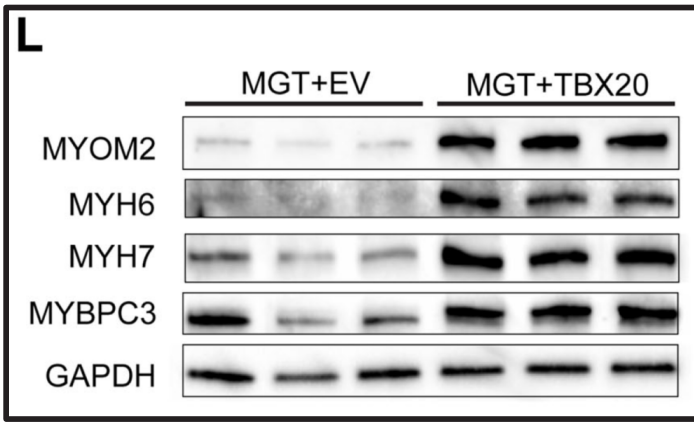
Protein gels work similarly, but with a few extra steps. Heat is used to denature the proteins, a reducing agent is used to break up disulfide bridges, and a detergent is used to coat the unraveled protein with a uniform negative charge. The rest of the steps are the same!

Western Blots

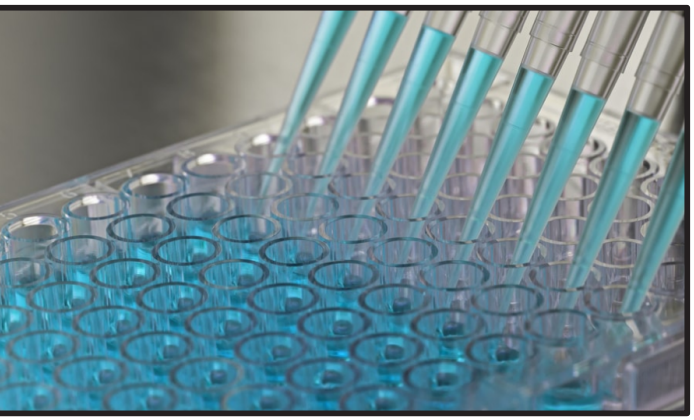
Western blotting is like a combination of gel electrophoresis and immunohistochemistry. Used to identify or measure specific proteins in a mixture, western blotting combines protein gel electrophoresis (specifically SDS-PAGE) and enzyme-conjugated antibodies to visualize proteins.

The unique part of western blotting comes after running the gel, when the proteins are transferred to a nitrocellulose membrane. The membrane is then blocked (sometimes with milk), the primary antibody is added, and the secondary enzyme-conjugated antibody is added. Horseradish peroxidase is probably the most common enzyme. Once you add the enzyme's substrate to the membrane, you'll be able to visualize your gel!

Just like IF and IHC, expect many washing steps! These are needed to reduce background and get a clear image.



ELISA

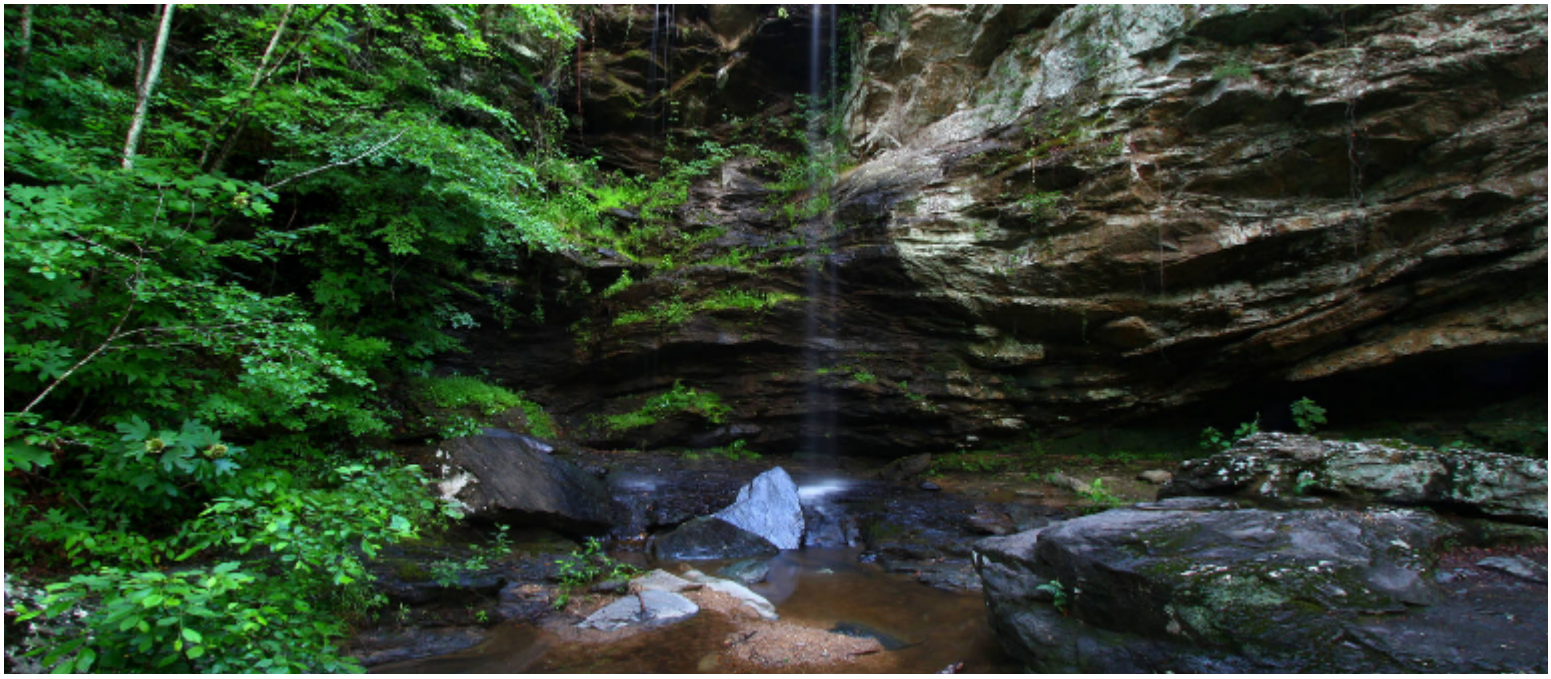


Western blotting is great for visualizing proteins; however, just like PCR, it lacks the capability of precisely quantifying protein levels. So, ELISA, or the enzyme linked immunosorbent assay, is used for accurate detecting and quantification of a specific protein in a mixture.

Generally, ELISAs are performed in 96 or 384-well polystyrene plates, which are coated with an antigen solution. These plates are then usually incubated for the antigens to bind to the plate. After that, blocking agents are used to eliminate the possibility of non-specific binding of other proteins. Conditional media containing antibodies specific to the antigens is added. Then, a second antibody linked with an enzyme "tag" (commonly horseradish peroxidase!) is added as it binds to the antibody that's already attached to the antigen. Once again, when you add the enzyme's substrate, the tag might produce a color change, fluorescence, or chemiluminescence, which can be measured using a microplate reader.

This is just a small preview into the world of celllular research! Eventually, you will come across even more complex and intriguing devices you can use for more niche experiments. For example, maybe you want to stretch cells to simulate hypertension (cyclic straining) or understand ion channels using patch-clamp electrophysiology. Either way, a good understanding of the these fundamental lab techniques and AP Biology will serve you well while you're working for your lab.

If you have any questions or would like to know more, feel free to email cfeng@asfaschool.org or wpeng@asfaschool! ■



Black Belt Equity

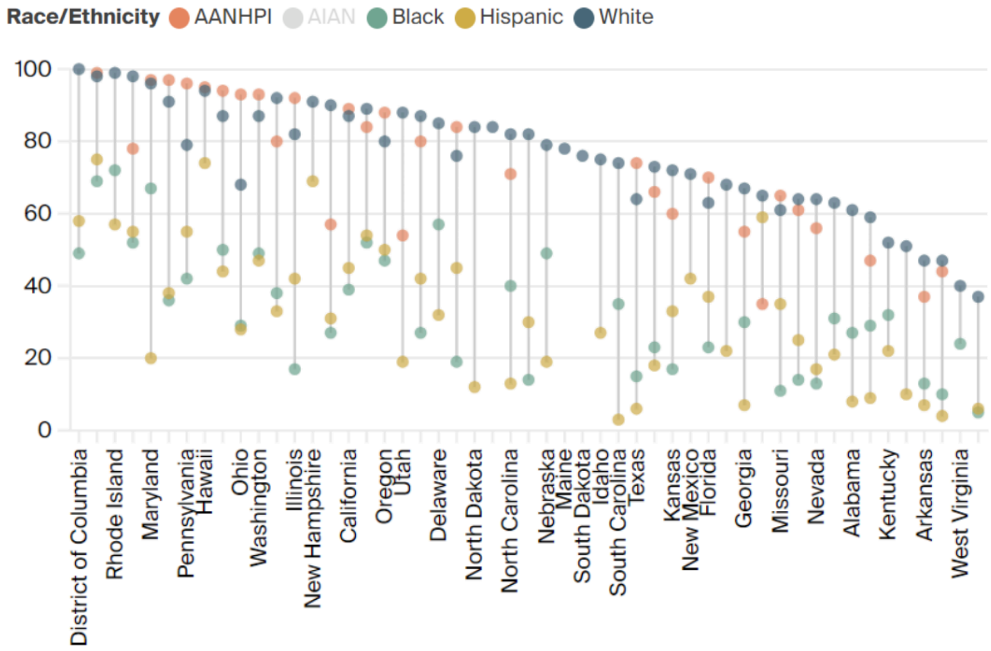
By Ian Shen

Healthcare equity is one of the most pressing issues in the United States. Despite the urgency of this issue to communities across the country, metrics that measure healthcare performances of states often mask inequalities faced by smaller, minority communities. According to the Commonwealth Fund 2024 State Health Disparities Report, which scores health system performance for racial and ethnic groups within states, significant health and healthcare disparities exist between White, Black, Hispanic, American Indian, and Alaska Native communities in almost all states.

According to another report by the Commonwealth Fund, Black and American Indian / Alaskan Native people are more likely to die from conditions that are treatable with high quality healthcare, further emphasizing the widespread effect that inequities in access to high quality healthcare have on minority communities.

The Black Belt is a region in the United States defined by its dark and fertile soil, making it ideal for growing cash crops. Historically, this region's economic and political power relied heavily on the labor of enslaved African Americans. Over time, the economic influence of the Black Belt declined, but the region remained predominantly African American. As a result, the socioeconomic challenges faced by these individuals after slavery have continued to affect the region through high poverty rates, and limited access to healthcare and education. Today, the Black Belt still faces many of the systemic inequalities.

Health system performance scores, by state and race / ethnicity



Scores are based on the percentile distribution of each group's final composite z-score across all indicators/dimensions; each figure is rank-ordered by highest state performance for the noted population. Gray dots represent the highest score achieved in each state by any of the five groups (if no gray dot is visible, the highlighted group has the top score). The 50th percentile represents tvhe median health performance score among all the groups measured. AANHPI = Asian American, Native Hawaiian, and Pacific Islander. AIAN = American Indian and Alaska Native

When analyzing health outcomes of individuals in the Black Belt, it becomes apparent that health inequalities are present in the region. When compared with the rest of the country, rural Southern African Americans in the Black Belt counties suff

-er from higher rates of mortality, premature death, and lower life expectancy. Why is this the case? Systemic inequities in healthcare in the Black Belt region can be attributed to three key factors: historical, economic, and legislative barriers.

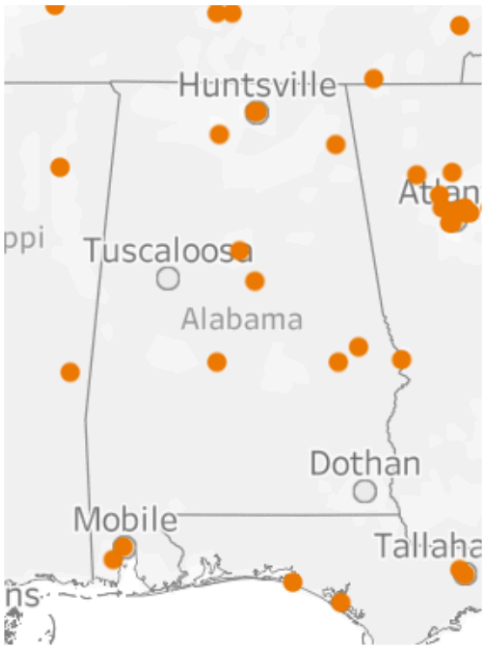
Due to the Black Belt's historically African American population, the region has suffered from systemic racism that influences policy. For example, many states in the Black Belt have historically resisted the expansion of Medicaid, a decision that has often been linked to "racial bias and resentment." This leaves many low income, minority individuals without health insurance, amplifying existing health issues. Economic challenges are also increasingly present in the Black Belt, where poverty rates are usually higher than the national average. This leads to large proportions of people who are uninsured, resulting in delays and financial stress. Additionally, current policy lacks incentive for healthcare practitioners to practice in the Black Belt, leading to closures of hospitals in Black Belt counties. Current legislation also lacks funding for these rural clinics, causing further closures that only add to the geographic barriers people in the Black Belt face when seeking healthcare.

In order to address the social determinants of these inequalities, targeted interventions and policy are needed. However, when designing these solutions, a social justice approach must be taken. In this context, social justice is the pursuit of an equitable distribution of healthcare resources across all communities regardless of racial, economic, or geographical differences. For example, this would look like policy reforms, investment in local infrastructure, and community based initiatives that target the communities that need it most.

However, in order to correctly identify and execute a solution, scientific bodies must work together with the concept of social justice. To combat the historical, economic, and legislative challenges, fields such as epidemiology, economics, as well as political science must be employed.

Epidemiologists could conduct targeted surveillance to help identify and map the prevalence of diseases specific to the Black Belt. Additionally, epidemiologists can aim to reduce the effect of Black Belt specific diseases by studying the environmental and genetic factors in the region. Economists analyze and study the financial barriers that prevent people from seeking and receiving care, in order to allocate resources equitably. Political scientists focus on creating and implementing policies to close the gap between healthcare access and quality through reforming existing policies. However, even though previously mentioned scientific fields play a large part in finding a solution for this issue, countless disciplines come together to address healthcare inequities in the Black Belt. The efforts made by these scientists are guided by social justice principles, including integrative research, community engagement, and equitable data collection.

Currently, there are several community health initiatives that exist in the Black Belt, including mobile clinics, telemedicine, and training for providers who these regions to practice. For example, launched with a 3.9 million dollar grant, the Central Alabama Neighborhood Health Initiative focuses on increasing COVID-19 vaccinations and testing, improving health literacy, and improving patient experience in Elmore, Montgomery, and Pike counties. This initiative works with local communities to identify areas at the highest risk for health disparities and develops health literacy plans aimed to solve them. Many institutions and organizations also have dedicated mobile clinics. For example, Alabama A&M University, University of Alabama Birmingham, and the Opelika Neighborhood's mobile clinics provide vaccination, chronic illness screening, women's health screening, and general screening services to communities all around the state. Additionally, some scholarships provided by the Blue Cross Blue Shield of Alabama require that recipients practice in underserved areas after completing residency. However, in order to improve upon these programs, steps must be taken to expand Medicaid coverage, and determine a viable funding mechanism to support mobile clinics and telehealth while still investing in permanent infrastructure, like hospitals.



Maps of mobile clinics in Alabama

Despite many efforts to bridge the gap in healthcare equity between the Black Belt and the rest of the country bringing about various levels of success, keeping social justice as a priority will not only improve the health outcomes of residents living in the region, but ensure that future generations will never have to experience these inequities. ■

Inside a mobile clinic

Alumni Interviews

Eli Mrug

Introduction: Class of 2024

I want to pursue a career in Psychiatry and Bioinformatics, so I am most interested in studying Neuroscience and Bioinformatics in college

Give a brief summary of your research

I found attracting regions for parabolic and attracting fixed points of polynomials. Basically, If you have a complex polynomial f(z), The repeated application of f on a given initial value will almost always approach a (possibly infinite) fixed point of the function under enough iterations. My project is about determining when you can say with certainty that the iterate of the initial point will for sure go to each fixed point. I did this for the two simpler types of fixed points, namely attracting (f(z)=z, |f'(z)|<1) and parabolic (f(z)=z, f(z)=e^2πit for rational t)

What inspired you to do your research project?

I've been interested in working with fractals for a longer time, and in the past have made a fractal rendering app that allowed you to search through many Mandelbrot and Julia sets. The main limiting factor in producing images was the time needed to render each one, so I wanted to develop good optimizations for such renders.

What's your favorite part of doing research?

I like being able to discover new things, mainly. The idea of learning or coming up with something that hasn't done before is just very attractive to me since you get to be the one that determines what you can do and there is no set path.

What have you learned during your research? Have you made any mistakes? How did you overcome them?

I've learned it's very important to start very early on research. I myself had to jump across multiple different fields before I found one that appealed enough to me (or really even would work as a research project in the first place). I guess this was my main mistake—I wasn't fully sure what I wanted to do so it took me a bit longer to settle on my final project idea. However, I'd say it was worth it, since the topic I ended up with was very interesting to me and I enjoyed working on it a lot. And I think that's the most important part of doing research—make sure to have fun!

What's some of your best advice for upcoming high school researchers?

Make sure you are doing something you're invested in, and try to get as much control as you can in your project! It's harder to do in biology related subjects where clearance is more tedious to get, but being able to determine what your own research project is about makes you more invested and more enthusiastic in completing it. It also gives you more experience with creating such projects, which will always be helpful if you want to go into academia.

Alumni Interviews

Cynthia Liu

Introduction: Class of 2024

I'm interested in pursuing a pre-med track and would like to become a pediatrician in the future.

Give a brief summary of your research

My senior research project is titled "The Effects of TLR7 Stimulation on Mitochondrial Complex I Activity of Autoimmune B Cells." Essentially, my project aims to determine if pathways that cause autoimmune responses are linked with energy production in B cells of mice with systemic lupus erythematosus (SLE), an autoimmune disease.

What inspired you to do your research project?

I've always been interested in immunology, and I specifically wanted to research autoimmune diseases. When I found out that 9 out of every 10 adults with lupus are women, I was hooked and became intrigued about the causes of this disparity. I reached out to a lab currently studying lupus, which led to my senior research project!

What's your favorite part of doing research?

My favorite part of doing research was seeing the hours of experimentation come together into tangible data that actually made sense. It was really rewarding to finally have results at the end of all the long procedures.

What have you learned during your research? Have you made any mistakes? How did you overcome them?

I definitely made a lot of mistakes during my research. Sometimes, the errors weren't even things I could control, and my lab had to reorder materials multiple times so I could repeat the procedures to get better data. However, because of all the trials, I learned to problem-solve through every step of protocols to pinpoint sources of error.

What's some of your best advice for upcoming high school researchers?

Research a topic you're truly interested in, not just something you think would make a "successful" project. The process will be so much more enjoyable if you're passionate about what you're studying, and it will show in your presentations. Find good mentors, COMMUNICATE with them (make an effort to stay in contact!), and don't get discouraged if things don't always go as planned—research is a long process but usually well worth it.

Department Interviews

Ms. Lugemwa

What do you love most about our department?

I get to teach passionate students. I enjoy working in a diverse community. There is a lot you learn when you work in a diverse community. I am always learning something new.

As a long standing faculty member, how has M/S grown since you've been here

The department has changed in many ways. When I came in, the department had almost 150 students but now they are about 90 students. We were half the school! Now as the other departments grow, we have to limit our numbers. Research was not a requirement for graduation, this is a plus for our students. Computer Science and the Seventh grade have been added. Extra STEM activities have raised our standards in the state.

Where did the bus come from?

I was teaching Honors Algebra II, Trigonometry and also precalculus in one class, so I moved at a fast pace. My co-worker , the late Ms. Cantwell always said that my class was a bus and her class, Chemistry, was a Helicopter. We both moved very fast to cover our courses. So I named my class the Bus. Students often found it funny and would come and tell me "the bus left them" or "The bus was crashing them!", "The bus needs to stop for gas" "we have survived the bus" .... and I would say "No parrots in the bus."

Your courses are very intensive classes; any advice for struggling students?

"Stay in the bus" and "Do not fall out!" At the end of the year 'It is the Party Bus."

What is your favorite subject other than a Math / Science subject?

I love to travel and learn about different cultures. I enjoy the outside, hiking... I enjoy gardening but I do not know why the insects get to my food before I get to it! I do not like to add insect repellants!

Favorite food / beverage?

There is always time for a hot cup of tea!

Upcoming Events

Mu Alpha Theta Tapping – Sep. 19, 2024
ASFA Open House – Oct. 19, 2024
ASFA CS Olympics – Oct. 26, 2024
Robotics In-Reach – Nov. 6, 2024
ASFA Math Tournament – Jan. 18, 2025
Robotics In-Reach – Feb. 5 , 2025
CARSEF – Mar. 1, 2025
ASFA MS Audition – Mar. 15, 2025
MS Senior Research Symposium – Apr. 10, 2025

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