Background Notes Needed for My ScienceFair

Dec 21st 2024 - Dec 24th 2024

CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

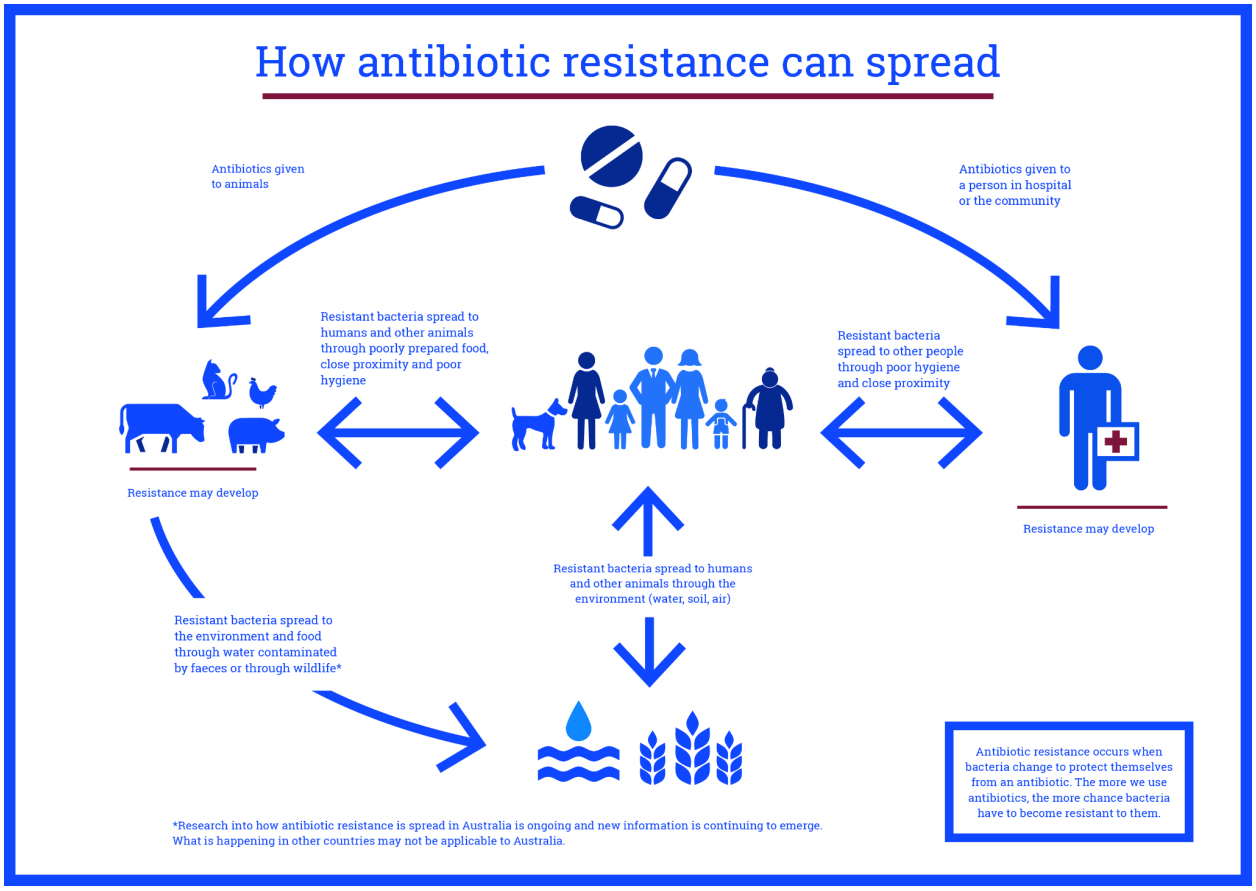
- What is CRISPR
- It is a technology that can edit genes.
- CRISPR uses a guild RNA to find a specific DNA sequence that needs to be edited

Why is CRISPR Important

- It's a technology that can change the world
- It can modify genes and can help fight diseases and even cancer more effectively.
- Now it's cheaper and more effective than it has ever been
- It has the potential to transform medicine for both the treatment and prevention of many diseases as a cheap and effective solution
- By creating a guild RNA scientists can target and edit virtually any gene in a living organism

Important Facts

- CRISPR is a defense mechanism found in bacteria that stores segments of viral DNA (called "spacers") and uses this information to recognize and defend against similar viruses in future infections.
- The 2 primary components of CRISPR-cas9 are: There are short repetitive snippets of DNA *Sequences called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
- And CAS proteins *CRISPR-associated proteins
- During an infection a snippet of viral DNA is inserted into the CRISPR region as new “spacers”, this happens naturally in bacteria even if there isn’t an infection present just as an adaptive immune response over time.
- CRISPR sequences are transcribed into RNA which then guilds CAS9 to a DNA sequence, binding and matching to it allowing for precise targeting and editing
- RNA binds with a special protein, CAS9, which will scout for an existing DNA match of the virus and can recognize if it breaks in again
- After DNA is cut the cell will try to repair it.
- Typically proteins called nuclei trim the broken ends and bring them back together (the process is called non-homologous end joining)
- This process however is prone to mistakes.



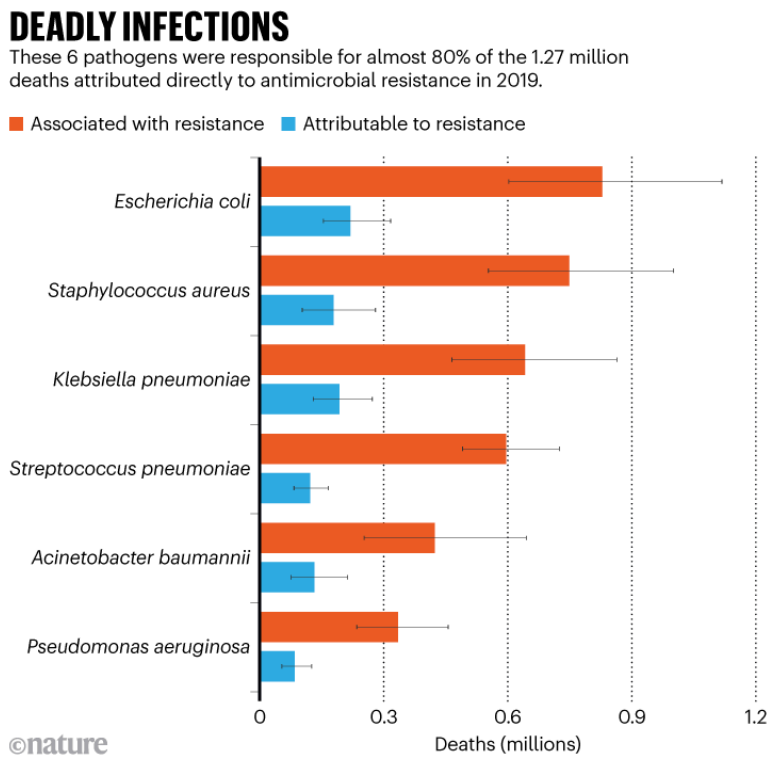
Dec 26th 2024

How it works in the lab

- Scientists take a guide RNA and put it in the gene they wanna modify
- After attaching it to CAS9, it gets directed to the target gene and cuts the gene in a specific location
- After the cut, the cell repair mechanism can either introduce mutations or be used to introduce a new line of DNA for gene editing
- By having a guild gene in the CAS9 protein, scientists can target specific sequences for any type of editing

Fun facts

- People are eating CRISPRised food
- An attempt in China has been made to make a gene-edited baby (though it did cause legal repercussions)
- The stated aim of the project was to make individuals immune to HIV (ridding them of the protein CCR5)



Dec 28th 2024 - Dec 30th 2024

Definitions:

CRISPR - A Technology used to edit genes

DNA - A self-reciprocating material in all organisms, the main constituent of chromosomes. It is the carrier of genetic information.

RNA - A molecule responsible for biological processes. Its role is to maintain, regulate, and add to genes.

CAS9 - A specialized enzyme that acts as a defense mechanism that acts like scissors cutting DNA in specific locations.

Gene expression - The process by which the information in a gene is used to produce a functional product, typically a protein. It involves two main steps:

- **transcription** (DNA is copied into mRNA)
- **translation** (mRNA acts as a messenger and delivers info on how to build proteins at the ribosome).
- This process is tightly regulated to ensure proper cellular function.

This process is tightly regulated to ensure proper cellular function.

During gene expression, all life employs the same genetic code.

Differences in organisms happen because of different arrangements of nucleotides.s

- Organisms are different from one another because of differences in nucleotide sequence.ce
- We can figure out the relationship species have with each other by studying the gene.

Cellular machinery - the complex system that keeps a cell working properly

Genome - All inherited genetic instructions

Humans have 2 similar sets of chromosomes with each one having around 3 billion nucleotides.

Jan 1st, 2025 - Jan 5th

Key Research:

Topics I'm going over

- CRISPR
- Antibiotic resistance
- Synthetic biology/bio-circuits
- Biotechnology

Why I chose this:

- Antibiotic resistance is becoming a serious problem as 1.2 million people die from it every year. The ability to get rid of the gene responsible for resistance could save millions.

Background info:

- Antibiotic resistance happens when bacteria evolve to survive antibiotics
- CRISPR can already edit genes in organisms
- Synthetic bio circuits act as a control panel for CRISPR

Synthetic Bio circuit info

- Bio circuits are biological systems that can be programmed to do specific tasks (detecting genes, controlling gene expression in microorganisms)
- They’re engineered systems made from biological components

- Work similarly to an electrical circuit but instead of wires and resistors it uses biological molecules

Bio circuits in antibiotic resistance

- Bio circuits can be designed to detect resistant genes in a microorganism, which once identified could use the help of CRISPR to disable these genes.
- Their purpose is to be sensors that detect bad genes.

Intro Info

- By 2050 Antimicrobial resistance could be the cause of 50 million deaths worldwide.e
- Antimicrobial resistance is when a microorganism becomes immune toantibioticsi.cs
- It is the biggest threat to global health.
- Pharmaceutical companies have stopped researching antibiotics because they are no longer as profitable as other drug.s
- Once 1 bacteria is resistant, it shares that gene with other bacteria even frodifferentff species

Hypothesis Info

If we're able to apply CRISPR-based bio-circuits to resistant microorganisms then we'll be able to neutralize antibiotic resistance because by using synthetic bio circuits we can precisely target and disable resistant genes that are responsible for antimicrobial resistance.

Key research:

- Scientists have engineered bio-circuits that can sense the presence of antibiotics and trigger a response, such as producing an antimicrobial compound or turning on a resistance gene to outcompete harmful bacteria.

Examples of Successful Bio-circuit

- bio-circuits have been engineered in bacteria to sense the presence of toxins and release antidotes. This means we can create bio-circuits that respond to the presence of antibiotic-resistant bacteria.

Random research that may be useful

- Bacterial strain - a different version of the same species
- Promoters - A bio circuit component that can detect antibiotics in a person's system, can act asana on and off for CRISPR
- E.Coli is the most prominent killer
- E.coli’s resistant gene is called blaTEM
- Bacterial DNA – a circular chromosome plus plasmids
- gRNA is a version of RNA that acts as a guide for CRISPR to direct where to make its genetic cuts

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Methodology (simplified)

- Step 1: Design bio-circuit

Use a genetic sequence that encodes a CRISPR system, and combine it with a promoter that triggers the CRISPR system when in the presence of specific antibiotics.

- Step 2: Choose a Bacterial Strain

Select a bacterial strain that has known antibiotic resistance. For example, E. coli strains resistant to the antibiotic ampicillin or tetracycline.

- Step 3: Genetic Engineering

Using CRISPR, insert or modify the bacterial genes to create a promoter "switch" that will respond to antibiotics by activating our CRISPR system.

- Step 4: Test the Circuit

Grow the engineered bacteria in media containing antibiotics. Measure how the bio-circuit responds—whether the bacteria remain resistant or the bio-circuit helps neutralize resistance.

Limitations

- Rapid bacteria evolution - Bacteria can evolve quickly, which could cause mutations that could let them bypass CRISPR
- Detection failure - It could fail to detect certain bacteria allowing them to spread.
- Price - The Cost simply may be too much compared to antibiotics

Feedback loop - Controls how much neutralizing enzymes CRISPR produces

By cutting DNA sequences responsible for causing resistance, CRISPR can disable genes responsible for resistance inside bacteria. With its origins rooted inside the immunological defense mechanism of bacteria, CRISPR mixed with our technological advancements can direct Cas-9 to a particular spot within one’s genome. When Cas-9 cuts the problematic gene in the right location, the cell will no longer be able to function as it used to, preventing bacteria from producing the proteins required for resistance. My project revolves around this concept if we’re able to disable the gene in a bacteria responsible for resistance, then the bacteria's ability to produce proteins deteriorates, causing them to be vulnerable once again.

Jan 29th 2025:

1. Abstract

Antibiotic resistance is projected to kill 10's of millions of people in the years following up to 2030, becoming one of the leading causes of death worldwide. My research mainly focuses on how to beat antibiotic resistance by combining 2 revolutionary tools, CRISPR & bio-circuits. CRISPR-based bio-circuits allow for automation and effectiveness that's much more viable than manipulating CRISPR by hand. This method offers a 1-time solution to neutralizing resistance in a bacterial strain, almost eliminating the need for mass antibiotic research and production. The way my proposed circuit works is CRISPR is responsible for the actual gene editing (using Cas9 proteins to edit genes), while the bio circuit identifies which gene is causing resistance by identifying specific genetic markers in nucleotides. After the resistant gene has been found, the bio circuit activates CRISPR and "guides" it to the correct gene to make changes. My study is focused on the methodology of how to build the bio circuit and the information surrounding it.

Jan 5 2025 - Jan 8th 2025

2. How do Bacteria strains become resistant?

Bacterial infection phases	
2.1. Initial exposure	When bacterial strains are initially introduced to antibiotics, the drug targets specific bacterial processes the organism needs to survive, such as inhibiting/preventing cell wall synthesis or protein production. Since it's the first time this bacteria has been exposed to the antibody, it works like a charm, inhibiting and killing the target bacteria effectively. However, because of the genetic variation in bacteria within a population, some bacteria may be able to survive and gain resistance. Because bacteria reproduce asexually, their daughter cell will be given their resistance to antibiotics as well.

2.2. Acquiring Resistance	There are multiple ways a bacterium may acquire resistance, my research covers the 3 most common ways they do this: **Genetic Mutation** - Because of environmental factors like radiation or chemicals, an error in DNA replication may happen. This could cause random new traits to appear, mutations like this may or may not cause more resistant genes to appear **Binary Fission** - This is the process in which bacteria reproduce asexually. When this process is in motion, bacterial DNA duplicates and divides into 2 genetically identical daughter cells. Even though this process had intentions to create clones, mutations during the replication process can begin to introduce genetic variation over time Transformation - It's a form of horizontal gene transfer where a bacteria picks up free DNA fragments from its environment (mostly from dead or lysed bacteria). If the foreign DNA is similar enough, homologous recombination will allow DNA to integrate into the bacterium's genome. Possibly giving the bacterium resistance or other survival mechanisms. Regardless of which way bacteria get resistance, conjugation (transfer of resistant gene via bacterial contact) & transduction (transfer of resistant genes via bacteriophages) allows bacteria to spread their resistant genes to other bacteria, making the drug once meant to kill bacteria useless

2.3 Selection Pressure	Selection pressure occurs when antibiotics are overused or misused (e.g., not completing the full treatment or using antibiotics for viral infections). It's basically when bacteria susceptible to antibiotics are killed off while the resistant bacteria survive. Impact? Because of drugs killing the susceptible bacteria, the resistant ones receive more opportunities to multiply and become the dominant strain. Essentially, the antibiotic acts as a filter, only letting resistant bacteria reproduce and survive.
2.4. Spread of Resistance	Bacteria aren't just idle, they transfer to humans the same way COVID did (human contact, environmental contamination, global travel, etc). As more and more people start to catch the infection, it makes the likelihood of catching the infection in an everyday setting higher, most likely causing slower recovery times and treatments that may not be effective.
2.5. Multidrug resistance (MDR)	As the name suggests, multidrug resistance (MDR) is when bacteria develop resistance against multiple antibiotics. After using multiple drugs to try killing an already resistant bacterial strain, there's a small chance it could gain immunity to other drugs as well. These bacteria often use a combination of different mechanisms to develop resistance, possibly creating its version of the same antibiotic, altering and changing its target. MDR is one of the biggest problems of doctors since they have limited options when it comes to curing this. In many cases, doctors use final-resort antibiotics, which are more expensive, less effective, and have stronger side effects.
2.6. Potential Pandemic	In severe cases, a pandemic may begin if resistant bacteria can transfer from person to person quickly. In this stage, resistant bacteria will be in control of how fast it spreads, as without medication that works, there wouldn't be any way to progress the infection.

Jan 7th 2025 - Jan 9th 2025

3. What impact does Antibiotic Resistance have on the world?

3.1 Impact Statistics

- Currently, around 1.2 million people die annually from antibiotic resistance
- Around 10 million people are expected to die annually from antibiotic resistance by 2050
- Treatment of Antibiotic resistance takes longer to treat, which could cause even more complications in a patient's health
- Surgeries, cancer treatments, organ transplants, or those with chronic illnesses have a higher risk of complications since their immune system is weak
- With healthcare as poor as it is right now, Antibiotic Resistance will become a burden to countries all over the world
- Crops and agriculture suffer from new diseases constantly infecting crops
- The global economy is on its way to lose 100 trillion by 2050 due to reduced labor forces
- People will start to doubt the healthcare system and become scared as incurable diseases take over the world

Figure 3.1.1: Of Antibiotic Resistance Spreading Phases

Jan 10th 2025 - Jan 13th 2025

4. Why do current solutions not work?

To fend off antibiotic resistance, we have a couple of methods to keep it under control like:
- Constantly making new antibiotics
- Vaccination
- Limitation of antibiotic use

However, these solutions have slowly become less valuable. This is why:

4.1. Profit/Loss Ratio:
In the 1900s, when medicine had just started to become a thing, there was minimal genetic variation between bacterial strands, making 1 vaccine that cured everyone with that disease. This caused companies to want to create and research vaccines as it came with a huge reward. However, as we started to get past the year 2000, bacterial strands of the same population began to have genetic variation. This caused the 1 cure fits all antibiotics to become impossible, leading companies to slow down and even stop research and production of antibiotics, as depending on the version of each strain, there will need to be another cure to be made. With the slowed-down development of medicine, many people won't be able to get the help they need.

4.2. Complexity of Resistance:
Bacteria, though small, are highly complex, and able to adapt and develop new ways to survive treatment. Making the bacteria harder to eliminate.

4.3. Global Disparities:
Because people in different parts of the world have different access to healthcare, some countries will struggle to manage resistance effectively or even detect it.

Jan 14th & Jan 16th - 19th 2025

5. CRISPR & Biocircuits

5.1. Useful terms

- 5.1.1 CRISPR - Gene editing tool used to precisely modify a piece of DNA
- 5.1.2 gRNA (**guide RNA**) - a short synthetic RNA molecule used in the CRISPR system-based genome editing
- 5.1.3 Cas9 - A protein derived from a bacterial immune system that's used as a tool to cut and modify DNA
- 5.1.4 Promoter - DNA sequence that begins the transcription of genes
- 5.1.5 Repressor - Protein that inhibits gene expression by binding to a promoter or operator region
- 5.1.6 Feedback loop - Amplifies a certain biological pathway or inhibits it, based on the outputs of a process that influence its own activity

5.2. What is CRISPR?

Clustered regularly interspaced short palindromic repeats, otherwise known as CRISPR, is a gene editing tool that will revolutionize healthcare. Originating from the adaptive immune system of bacteria, CRISPR allows organisms to "remember" viral infections that were fought before to create a more effective counterattack. Using modern technology, we have repurposed this natural mechanism for precise gene editing in various organisms.

The CRISPR system primarily consists of:

5.2.1. Guild RNA (gRNA) - Responsible for guiding the Cas9 protein to the DNA within a genome that needs editing. By custom-designing RNA sequences that replicate the target DNA sequence, RNA can match with resistant DNA, signaling to Cas9 where to edit.

5.2.2. Cas9 Protein - It's an enzyme that acts as molecular scissors, editing/cutting DNA in the needed location. When the DNA is cut, natural cellular repair mechanisms can disable, replace, or correct faulty genes. This gives CRISPR the power to change basically anything within a living organism's genome.

5.3. What are Bio-Circuits?

Biocircuits are essentially the wiring inside living cells. They are designed to control and program biological functions within living cells. Biocircuits can respond to their environment by detecting the presence of molecules, allowing them to create a biological response accordingly. The main functions of bio circuits are:

- Sensing capabilities - They sense any threats within their environment by detecting genetic or environmental signals, such as antibiotic presence or the existence of resistant genes
- Response Activation - Once the threat is detected, they trigger responses to activate CRISPR to edit and disable target genes
- Control Systems - To program bio circuits and ensure they work, it can include regulatory mechanisms (e.g. Feedback loops, promoters, etc) to control the extent and timing of responses

Adding on to this some key components that ensure these functions are completed include:

- Sensors - Responsible for detecting molecular signals like antibiotic presence or gene expression levels
- Logic Gates - Processes information based on predefined conditions (e.g. activating CRISPR only when a threat is present)
- Actuators - Triggers responses within bacteria to combat antibiotic resistance (e.g. Gene editing, protein synthesis, cell death, etc)

Biocircuits, in my project, essentially act as a control panel for CRISPR. They can detect antibiotic-resistant bacteria and activate CRISPR when necessary. The biocircuit is a key system needed to increase the effectiveness of CRISPR.

5.4. How CRISPR-Based Bio-Circuits Work

CRISPR's modification system requires a specific sequence of steps to be followed in order to function. If these steps aren't done then CRISPR won't be able to edit genes precisely.
This is the following process:

1. Gene Identification - Firstly, to ensure CRISPR edits the right gene, we need to know which gene is responsible for the resistance. This could be done by using gRNA, as its job is to essentially scan for resistant sequences within bacteria genes. Once the target sequence is found we can edit it.
2. DNA Binding - Now the nucleic sequence has been found, the gRNA will begin to bind to the complementary DNA, forming a stable complex.
3. Cas9 Editing - Because of the gRNA binding to the DNA, Cas9 will know where to edit the gene without making mistakes. Cas9 can stop resistance by either cutting, replacing, or binding to the DNA.
4. Cellular Dysfunction - If the DNA was cut then the bacterial cell senses something wrong, and tries to fix the break. This ends up disrupting the resistant gene or completely removing the harmful genes entirely. However, sometimes but very rarely, a bacteria with a strong repair system could potentially repair its DNA to be resistant again. On the other hand, if Cas9 binds to the DNA, the cell will sense nothing wrong and will simply lose its resistance as RNA polymerase won't be able to read the gene, preventing it from being expressed.
5. Cellular Response - Now once the antibiotics are reintroduced into a person's body, these bacteria will no longer have resistance.

Jan 21st 2025 - Jan 27th 2025

6. Creating a Theoretical CRISPR Based Bio-Circuit - (Methodology)

Now that all the info we need has been researched we can develop CRISPR-based bio-circuits to actually neutralize antibiotic resistance. The following are the steps and processes I came up with

6.1. Step 1: Choose the Bacteria Strain You Want to Target

Choosing which bacteria you want to edit is crucial since we need to know what the problematic genes are that we need to edit. For this project, I used E.coli due to its significant impact on the world and its good documentation/research. We also know the resistant gene of E.coli is called blaTEM, being responsible for resistance against ampicillin and tetracycline.

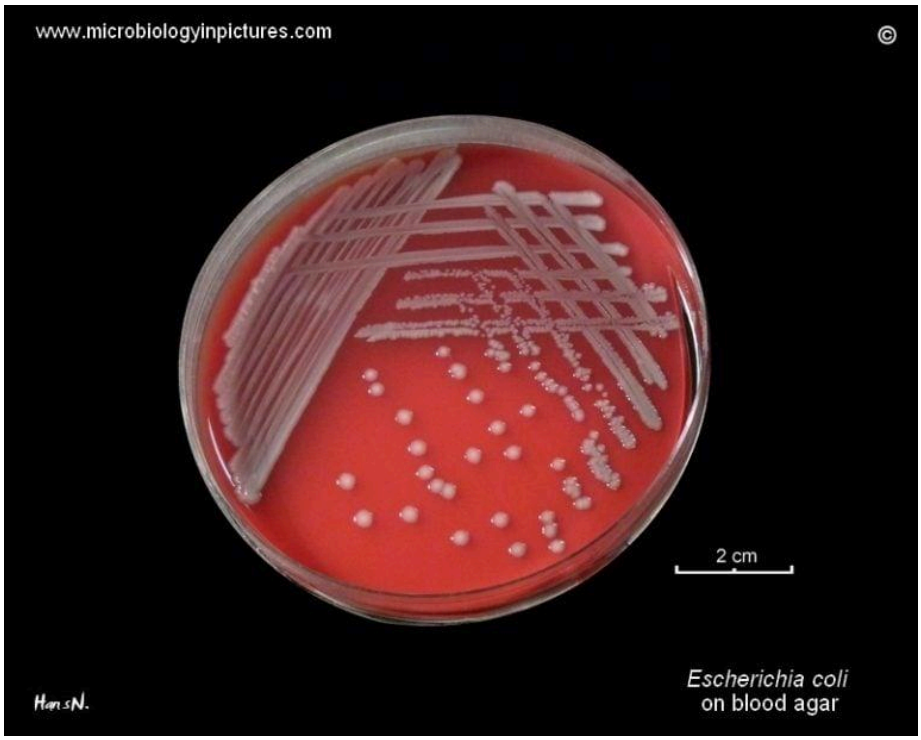

Figure 6.1.1: Of Escherichia Coli Culture on Blood Agar

6.2. Step 2: Designing the Bio Circuit

Bio circuits are very complex and designing them would take various biological components. Here are the basic ones:

1. Sensors
   - Create a genetic sensor capable of detecting blaTEM gene sequences within E.coli
   - Add gRNA to recognize, then bind to blaTEM
   - The sensor should be tested to make sure it can differentiate between resistant and non-resistant E.coli strains

2. Promoter
   - Have the promoter activate only in the presence of antibiotic-resistant E.coli
   - Must transcribe the CRISPR-cas9 system reasonably to the quantity of blaTEM genes detected

3. Feedback loops - Regualtes CRISPR's responses
   - Positive feedback loops ensure CRISPR stays activated until the resistant gene is eliminated
   - Negative feedback loops add safety; preventing unnecessary gene editing when blaTEM is gone

4. Repressors - Controls activation
   - Act as a fail-safe mechanism, inhibiting CRISPR activation when resistance isn't detected
   - Basically makes sure the right genes are modified

6.3. Step 3: Implement The Circuit

After we've made sure the bio circuit can identify blaTEM, it must neutralize resistance by activating CRISPR. For it to work properly, we need to make sure it can:

Correctly Identify genes - The gRNA will only bind to the resistant gene within E.coli & not anything else

Bind to DNA & Activate Cas9 - gRNA should be able to guild Cas9 to the resistant gene site

Deactivate/Disrupt Genes - Cas9 should be able to edit blaTEM and render it useless

Have a Positive Outcome - Make sure that the E.coli is once again resistant to antibiotics

6.4. Step 4: Test & Modify

Once experiments have been conducted and the circuit has been created, we need to test it thousands of times to make sure it works & is safe:

1. In-Vitro Testing
   - After placing our CRISPR-based bio-circuit into E.coli cultures, observe gene editing efficiency using PCR and sequencing techniques
   - Test to confirm that E.coli has lost its antibiotic resistance after treatment

2. Check For Mutations
   - Analyze the bacteria's genome to ensure potential mutations or other unintended effects are avoided
   - Make sure it is only blaTEM that's being edited

3. Add Finishing Touches
   - Adjust the mechanics of the circuit if deemed necessary (e.g promoter sensitivity, modify feedback loops, etc)

Jan 27 - Jan 28th 2025

7. Pros & Cons of Antibiotic Resistance

Antibiotic resistance is a problem that concerns not only us but the entire world. However, with CRISPR on the rise, it is expected to neutralize antibiotic resistance completely. But like all good things, there are both positive and negative sides to this gene editing tool. On the positive side, since these bio-circuits remove antibiotic resistance from bacteria, they remove the need for operations to continue creating new antibiotics, which is costly and time-consuming. Furthermore, their automation provides real-time bacterial detection, making treatment much more effective. Not to mention that CRISPR-based bio-circuits can be adapted to the resistant genes of multiple bacterial strains, allowing for a long-term, targeted, versatile solution.

However, there are further challenges that need to be addressed. For example, delivering the CRISPR circuit to the resistant bacteria remains a technical problem we still struggle with, as bacteria might have the ability to evade genetic editing. Some scientists have concerns about target editing, which could potentially lead to unprecedented consequences. Finally, to be able to implement CRISPR on a large scale, we still need extensive testing & research, requiring a large sum of money (mostly from funds), making CRISPR a technology that most likely won't be implemented within the near future. Just as space exploration leads to technological advancements, researching CRISPR will unveil new technologies & innovations that will positively impact health for generations to come

Timeline

Nov 21st	● Heard about the science fair coming up and thought I should participate ● Put it as a will do it in my calendar
Dec 10th	● Over the past couple of weeks, I decided that antibiotic resistance would be the problem I would want to solve ● Brought up the idea of CRISPR-based bio-circuits to my teacher and she liked it
Dec 21st - Dec 24th	● Did some background research on CRISPR ● Mostly copy pasted and looked over info I had taken from over a year ago on CRISPR, with some original info I researched for ofc
Dec 26th	● Again just reviewed & pasted info from last year, then pasted a

	diagram from the internet along with wrote some original info
Dec 28th - Dec 30th	● Actually started learning more about my topic ● Got some cool definitions and their descriptions ● Read some articles which I didn't write notes about, just read them to add information to my mind
Jan 1st - Jan 5th	● Here I decided what I was gonna go over & went more in-depth with what I know ● Had a set outline with what I wanted to do and stuck to it ● Researched a lot in 5 days and learned a lot
Jan 5th - Jan 8th	● Today I saw what my friends notes looked like and got jealous, so I decided to try hard and make my info look really nice ● It took 3 days but I finally finished the phases of antibiotic resistance. ● Read a LOT
Jan 7th - Jan 9th	● As a break, I just looked at what impact antibiotic resistance would have on the world
Jan 10th - Jan 13th	● Still felt lazy so just went over why current solutions don't work ● Had school work to finish but lowkey snooze past em
Jan 14th & Jan 16th - Jan 19th	● Started to try with deadlines coming up ● Went over EVERYTHING (bio-circuits, CRISPR, combination, biological components, grammar, etc)
Jan 21st - Jan 27th	● With deadlines so close I had to hurry up ● Looked at multiple sources, but most barely have info on bio-circuits ● Eventually found sources with what I need and got a lot done in a short amount of time ● Finished writing about the other steps and simply relaxed while I write 6.4 even though it still hurt my brain
Jan 27th - Jan 28th	● Wrote pros and cons of Antibiotic resistance ● Had upcoming tests so I focused on those for a bit
Jan 29th	● Wrote Abstract to contribute something today
Jan 31st - Feb 3rd	● Realised the video was due quickly and had to rush that ● Actually, I sort of lazed off until the last minute when I realized I had to do it ● That was probably one of my biggest blunders since my video was very rushed and I didn't incorporate all my information ● Wrote this part of the entry a few days after lol
Feb 3 ~	● Just pasted my research into the website and ordered my trifold ● Chilled for a while and now ima begin making my trifold (Its like march now)

Scholarly Articles

- **Nouri, Ahmad, et al. "CRISPR-Based Platforms for Antibiotic Resistance: Detection and Therapeutic Approaches." *Frontiers in Bioengineering and Biotechnology*, vol. 10, 2022, https://www.frontiersin.org/articles/10.3389/fbioe.2022.869206/full.**
- **Zhang, Wei, et al. "Antibiotic Resistance and Its Mechanisms: New Insights into an Old Problem." *Scientific Reports*, vol. 11, 2021, https://www.nature.com/articles/s41598-021-96735-4.**
- **Yan, Ming, et al. "A Novel dCas9-Based Gene Regulation System with Enhanced Targeting Efficiency." *Nature Communications*, vol. 13, 2022, https://www.nature.com/articles/d41586-022-00228-x.**
- **"Dead Cas9 (dCas9): Concept, Functions, and Applications." *Genetic Education*, https://pmc.ncbi.nlm.nih.gov/articles/PMC9356603/.**
- **"Antibiotic Resistance Mechanisms: Understanding the Evolution of Bacterial Defenses." *PubMed Central*, https://pmc.ncbi.nlm.nih.gov/articles/PMC8549092/.**
- **"CRISPR and Bio-Circuitry: A New Era of Genetic Engineering." *PubMed Central*, https://pmc.ncbi.nlm.nih.gov/articles/PMC6152938/.**
- **"Genetic Circuit-Based CRISPR Systems for Targeted Gene Editing." *PubMed Central*, https://pmc.ncbi.nlm.nih.gov/articles/PMC1091604/.**
- **"Gene-Targeting Strategies for Antimicrobial Resistance." *PubMed Central*, https://pmc.ncbi.nlm.nih.gov/articles/PMC10573330/.**

Government and Health Organization Reports

- **Centers for Disease Control and Prevention. "About Antimicrobial Resistance." *CDC*, https://www.cdc.gov/antimicrobial-resistance/about/.**
- **Centers for Disease Control and Prevention. "Causes of Antimicrobial Resistance." *CDC*, https://www.cdc.gov/antimicrobial-resistance/causes/index.html.**
- **Government of Canada. "About Antimicrobial Resistance." *Public Health Agency of Canada*, https://www.canada.ca/en/public-health/services/antimicrobial-resistance/about.html.**
- **World Health Organization. "Antimicrobial Resistance." *WHO*, https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.**

News Articles and Reports

- **"Stanford Explainer: CRISPR Gene Editing and Beyond."** ***Stanford News*****, 2024,** https://news.stanford.edu/stories/2024/06/stanford-explainer-crispr-gene-editing-and-beyond#how-CRISPR**.**
- **"More than 3.9 Million Deaths from Antibiotic-Resistant Infections."** ***Institute for Health Metrics and Evaluation (IHME)*****,** https://www.healthdata.org/news-events/newsroom/news-releases/lancet-more-39-million-deaths-antibiotic-resistant-infections**.**
- **"Antimicrobial Resistance (AMR)."** ***The Lancet*****, vol. 403, no. 10429, 2024,** https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(24)01867-1/fulltext **.**

Educational and Research Resources

- **"Promoter."** ***National Human Genome Research Institute*****,** https://www.genome.gov/genetics-glossary/Promoter**.**
- **"Plasmid."** ***National Human Genome Research Institute*****,** https://www.genome.gov/genetics-glossary/Plasmid**.**
- **"Targeting Antibiotic-Resistant Microbial Infections with Synthetic Biology."** ***Integrated DNA Technologies*****,** https://www.idtdna.com/pages/education/decoded/article/targeting-antibiotic-resistant-microbial-infections-with-synthetic-biology**.**

Multimedia and Video Sources

- **YouTube. "CRISPR Gene Editing Explained."** ***YouTube*****, uploaded by Kurzgesagt – In a Nutshell,** https://youtu.be/ZvhFeGEDFC8**.**
- **"Medicine Pills and Tablet Macro View."** ***Stock Video*****,** https://dm0qx8t0i9gc9.cloudfront.net/thumbnails/video/Vd3bj2jPe/videoblocks-medicine-pills-and-tablet-macro-view-of-ph**.**

Additional Scientific Database Sources

- **"Antibiotic Resistance Trends."** ***PNAS*****, 2025,** https://www.pnas.org/doi/10.1073/pnas.2411919121**.**
- **"Mechanisms of Antibiotic Resistance."** ***Molecular Cancer*****,** https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01925-5**.**

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For my project I utilized generative AI tools to help enhance my project in various ways. While every foundational idea, research, and insight was a product of my hard work, AI helped structure and organize my work in a more absorbable fashion, and also wrote links in MLA format. I have ensured all info collected is from reliable sources and my trifold presentation hopes to reflect my understanding of my topic. AI is revolutionary and is a tool that must used appropriately, and that is exactly how I used it. All sources I have used have now been properly sourced, including any AI I may have used.