Mosquito-Borne Pathogen Detection and Transmission System.

A booklet by students of iGEM Montpellier: Océane, David, Lada, Shaswati, Jordi, laure and Chaimaa

https://2023.igem.wiki/montpellier/

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INTRODUCTION

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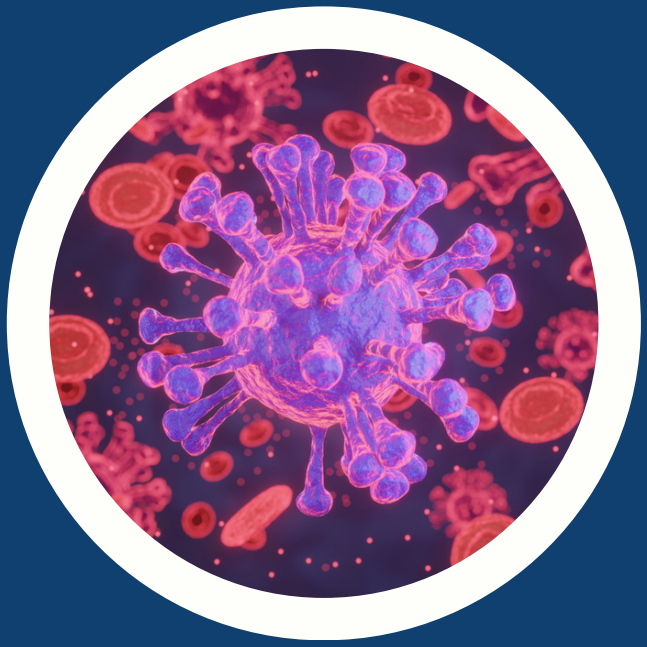

Greetings everyone! We extend a warm welcome to all. We are an assembly of devoted students specializing in the realm of Biological Sciences here in Montpellier. Our enthusiastic involvement finds its purpose in the international arena of Synthetic Biology, where we engage as contenders in the renowned IGEM competition. As we embark upon this journey, it marks the sixth occasion when the Montpellier team immerses itself in this exhilarating endeavor. With a resolute spirit, we aspire to exceed our prior achievements.

Diversity is our hallmark, as our team members hail from diverse backgrounds and contribute their unique strengths to our collective effort. Allow us to introduce ourselves :

Meet **Océane** : A dynamo of activity within our team, Océane is a versatile force in the wetlab domain. Her skill set spans a broad spectrum, encompassing protocol selection, modeling, and crafting our team's Wikipedia page. In the face of challenges, Océane's steadfast commitment and determination remain unshaken.

Introducing **David** : A constant presence in our laboratory, David's knowledge is a beacon guiding us through the intricacies of our tasks. A wellspring of assistance, he tackles inquiries with enthusiasm. Steering the wetlab ship, David oversees investigations, procures necessary materials, delves into bioinformatics, and consistently extends a helping hand.

Meet **Lada** : Fluent in the language of both wetlab work and team management, Lada is a pivotal member. Collaborating closely with David and Océane, she navigates the laboratory's challenges. Administration finds its anchor in her capable hands, and she contributes actively to our online presence through social media engagement and human practices considerations.

Say hello to **Shaswati** : Crossing borders, Shaswati joins us from India, bringing a wealth of experience. In her role as co-responsible for modeling, human practices, and social media, she adds a global perspective to our team's endeavors, enriching our solutions.

Presenting **Jordi** : Our bioinformatician par excellence, Jordi dons multiple hats by alternating between bioinformatics and wetlab roles. His contributions bridge these worlds, further solidifying our holistic approach.

Meet **Laure** : A creative mind and adept communicator, Laure is instrumental in shaping our social media presence and crafting engaging wiki content. Her touch adds vibrancy to our team's narrative.

Introducing **Chaima** : Our financial steward, Chaima takes on the responsibility of managing our fiscal aspects, ensuring our journey remains well-supported.

As we stand united in this pursuit of Synthetic Biology excellence, we invite you to follow our journey, celebrate our accomplishments, and share in our discoveries. Together, we are poised to conquer new frontiers and make our mark in the world of IGEM.

WHAT EXACTLY IS **SYNTHETIC BIOLOGY?**

At its core, synthetic biology is the discipline that delves into the manipulation and reconstruction of preexisting biological processes to yield novel outcomes. Take, for instance, the idea of coaxing bacteria to manufacture enzymes capable of breaking down plastics, thereby facilitating environmental cleanup. This domain of scientific exploration is relatively nascent and is currently basking in the limelight due to its remarkable potential and boundless possibilities.

Imagine the prospect of generating meat without relying on animal sources or concocting entirely new pharmaceutical agents. These feats, seemingly plucked from the realm of science fiction, are, in fact, within the realm of feasibility courtesy of synthetic biology. This field's capacity to spark innovation knows no bounds, heralding a future brimming with transformative breakthroughs.

THE ISSUE AT HAND

But why are we sharing all of this with you? Perhaps you've observed a surge in mosquito populations in your vicinity, particularly those dwelling close to stagnant water sources near homes. These mosquitoes belong to the Tiger mosquito species, scientifically known as Aedes albopictus, originating from South-Eastern Asia. An intriguing twist of fate, propelled by the global trade of tires, led these insects to find a new home in Europe. Because of climate change, they've not only adapted well but also thrived here. These Tiger mosquitoes gravitate towards warmth and elevated humidity levels, often finding an idyllic breeding ground in a simple garden pond in the South of France.

Beyond their vexing presence, these mosquitoes carry a more sinister reputation as disease vectors. Here's the crux of the matter: viruses are no strangers to international travel. The lightning pace at which the Covid-19 pandemic swept across the globe serves as a vivid reminder of this fact. It's a scenario we all earnestly wish to avert. Our team is currently channeling its efforts towards comprehending and combating two specific viruses: West Nile and Chikungunya.

DELVING INTO VIRAL REALITIES

In our quest to grapple with these pressing issues, it's imperative to explore the intricacies of the viruses we confront. Here's a closer look at the specifics of some noteworthy viruses: Dengue, Chikungunya, Zika, West Nile, and Usutu.

1. Dengue Virus (DENV):
Dengue fever, caused by the Dengue virus, is a mosquito-borne disease prevalent in tropical and subtropical regions. The virus is transmitted primarily by the Aedes mosquito species, especially Aedes aegypti. Symptoms range from mild to severe, including high fever, severe headache, joint and muscle pain, and, in extreme cases, hemorrhagic fever or dengue shock syndrome. *1,2*

2. Chikungunya Virus (CHIKV):
Chikungunya is another arbovirus transmitted by Aedes mosquitoes. It leads to a disease characterized by fever, severe joint pain, muscle pain, rash, and fatigue. The name "chikungunya" derives from an African word meaning "to become contorted," reflecting the excruciating joint pain it causes. Though rarely fatal, it can lead to prolonged discomfort.*1 2*

3. Zika Virus (ZIKV):
The Zika virus gained global attention due to its association with severe birth defects when pregnant women are infected. Aedes mosquitoes again serve as vectors. Zika can cause mild flu-like symptoms, but the primary concern lies in its potential to cause microcephaly and other neurological disorders in infants born to infected mothers. *1,2*

4. West Nile Virus (WNV):
West Nile virus is transmitted primarily through infected mosquitoes, particularly those of the Culex genus. It can lead to West Nile fever or more severe neuroinvasive diseases like encephalitis or meningitis. While many infected individuals exhibit no symptoms, severe cases can result in long-term neurological issues. *1,2*

References: *1.* WHO Reports, https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
2. CDC Reports: https://www.ecdc.europa.eu/en

5. Usutu Virus (USUV):
Usutu virus belongs to the Flavivirus genus, akin to West Nile virus. It circulates among birds and is transmitted by mosquitoes. In humans, it can cause mild flu-like symptoms, but severe neurological complications have been reported, particularly in immunocompromised individuals. *1,2*

Understanding these viruses is vital to our mission of tackling disease transmission and safeguarding public health. Through our endeavors, we aspire to contribute to the greater understanding of these pathogens and devise innovative strategies to mitigate their impact.*1,2*

UNVEILING THE TIGER MOSQUITO: **ORIGINS, LIFE CYCLE, AND HAUNTS**

Origins:

The Tiger mosquito, scientifically known as Aedes albopictus, originates from the tropical and subtropical regions of Southeast Asia. Its name aptly derives from the striking black and white stripes that adorn its body, resembling the patterns of a tiger.*3*

Refrence: *3.* Goubert, C., Minard, G., Vieira, C. et al. Population genetics of the Asian tiger mosquito Aedes albopictus, an invasive vector of human diseases. Heredity 117, 125–134 (2016). https://doi.org/10.1038/hdy.2016.35

Life Cycle:

The life cycle of the Tiger mosquito, like many mosquitoes, comprises four distinct stages: egg, larva, pupa, and adult. *3*

1. Egg: After a blood meal, a female Tiger mosquito lays her eggs in small containers of water or other moist environments. These eggs are characterized by their unique ability to survive periods of desiccation, enabling them to withstand dry conditions until they are submerged in water again. *3*

2. Larva: Upon hatching, the egg releases a larva that resides in the water. These larvae breathe through a snorkel-like structure called a siphon and feed on microorganisms present in the water. They go through several molting stages as they grow. *3*

3. Pupa: The mature larvae enter the pupal stage, which is a transition period before adulthood. During this phase, the pupa rests at the water's surface, resembling a comma shape. Pupae do not feed but are still mobile and responsive to disturbances. *3*

4. Adult: Once the pupal stage concludes, the adult Tiger mosquito emerges from the water. Female mosquitoes require a blood meal to develop and lay their eggs, while males mainly feed on nectar. The adults' lifespan varies, but they are capable of transmitting diseases if they are carriers. *3*

Where to Find:

Tiger mosquitoes exhibit adaptability and a propensity for urban and suburban environments. They prefer warm and humid climates, as well as areas with stagnant water sources. Common locations for Tiger mosquito habitats include:

- Containers: Any water-holding container, such as flower pots, buckets, discarded tires, and bird baths, can become a breeding ground.
- Gardens: Gardens with ornamental plants, pools, and fountains can provide ideal sites for mosquito breeding.
- Unused Items: Unused items that collect water, like old toys, containers, and trash, can inadvertently harbor mosquito larvae.
- Rain Gutters: Clogged rain gutters can accumulate water, offering a prime breeding location.
- Tree Holes: Natural tree holes can serve as breeding sites for Tiger mosquitoes. *4*

References: *4*. https://cisr.ucr.edu/invasive-species/asian-tiger-mosquito

Preventing the proliferation of Tiger mosquitoes involves eliminating standing water in and around your living space. By addressing potential breeding sites and employing appropriate mosquito control measures, we can help curb the population and mitigate the risks associated with disease transmission.

PROJECT OVERVIEW

Our project centers on a groundbreaking device that combines technology and biological insights to spot pathogens carried by mosquitoes. This device not only detects harmful pathogens but also instantly links up with epidemiologists and researchers. This connection allows them to access crucial data, enabling quick actions and informed choices to combat diseases.

We're making steady progress through a series of experiments involving both living organisms and controlled environments. Our approach employs the advanced CRISPR-Cas9 system. Our initial steps involved crafting synthetic DNA sequences designed specifically for the Chikungunya virus (ChikV). These sequences served as blueprints to create guide DNAs. The resulting DNA was amplified, verified through gel electrophoresis, and its concentrations meticulously measured. This DNA was then transformed into RNA for detection using a fluorescent probe. Our upcoming stages involve further DNA amplification, followed by transcription into RNA. Integrating Cas RX and the detector molecule, Biotin-FAM, will enable us to accurately detect pathogens. For our in-vivo experiments, we're collaborating to obtain deactivated virus samples. These combined with CasRX and Biotin-FAM will help us identify viruses within our living model. This multi-faceted approach, uniting cutting-edge genetics with collaborative virology expertise, forms the core of our pathogen detection system.

Choosing Cas Rx:
We picked Cas Rx over Cas13 for a reason. Cas Rx has unique strengths that make it shine in pathogen detection and editing. It boasts accuracy and efficiency, backed by studies that highlight its potency. Cas Rx excels at precisely targeting and editing RNA molecules, a vital capability for pathogen analysis. *5*

Moreover, Cas Rx is exceptional at avoiding off-target effects, a common concern in genetic work. Unlike Cas13, which targets RNA over DNA, Cas Rx minimizes the risk of unintended changes to unintended RNA sequences. This reduces the chance of unintended outcomes, adding confidence to our pathogen detection efforts.CasRX did not work according to the plan, but it did give us better understanding of the protein purificatiion technique.

Refrences: 5Puig-Serra P, Casado-Rosas MC, Martinez-Lage M, Olalla-Sastre B, Alonso-Yanez A, Torres-Ruiz R, Rodriguez-Perales S. CRISPR Approaches for the Diagnosis of Human Diseases. Int J Mol Sci. 2022 Feb 3;23(3):1757. doi: 10.3390/ijms23031757. PMID: 35163678; PMCID: PMC8836363.

Key Objectives:

Here are the main goals we're working towards:

1. Design specific DNA sequences for Chikungunya virus using advanced tools.
2. Create corresponding guide DNAs based on the designed DNA sequences.
3. Amplify synthesized DNA through PCR and confirm through gel electrophoresis.
4. Measure concentrations of amplified DNA samples.
5. Transform designed DNA into RNA, a crucial step.
6. Produce Cas RX for the Sherlock CRISPR-Cas9 system.(
7. Employ Cas RX and Biotin-FAM for accurate detection.
8. Collaborate for deactivated virus samples for in-vivo experiments.
9. Use these inactivated viruses with CasRX and Biotin-FAM to detect the virus in a living model.
10. Fuse advanced genetic techniques with virology expertise for a robust pathogen detection system.

Impact of Our Project:

By merging technology and biology, our project aims to transform mosquito-borne disease surveillance and control. Swift transmission of pathogen data empowers experts to respond promptly to potential outbreaks, take preventive measures, and save lives. Our device advances the understanding of mosquito-borne diseases and fosters global collaboration to combat their impact.

In short, our Mosquito-Borne Pathogen Detection and Transmission System epitomizes innovation and collaboration. It's a crucial tool in the ongoing fight against mosquito-borne diseases.

Do not hesitate to contact us by email at: igem.assomtp@gmail.com or in our social media networks(Instagram,Facebook)

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