# LAMP Assay Enabled Mosquito Classification Device

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# Malaria has a large, negative impact worldwide

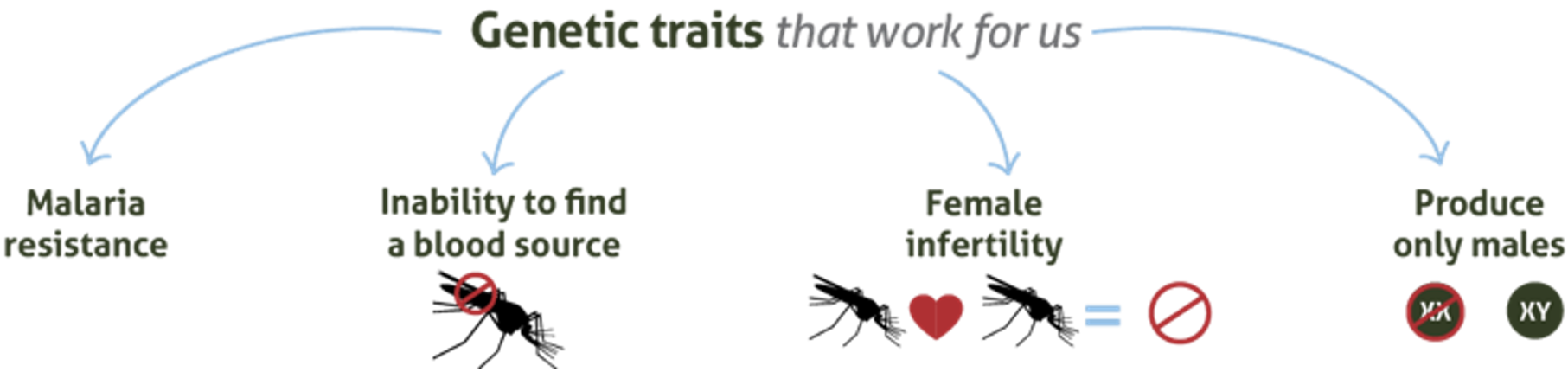
Image from: https://targetmalaria.org/why-malaria-matters/

# Who is Target Malaria?

- Non-profit research consortium
- Goal to develop and share technology for malaria control


Image from: https://targetmalaria.org/where-we-operate/

# Gene Drive: Mosquito Control Mechanism




- Gene drives are transgenes
- Promising gene drive would:
  - reduce carrier populations
  - increasing parasite resistance

Image from: https://targetmalaria.org/our-work/

# Engineering Need: Field Enabled Assay

- Target Malaria has started early field studies
- **Challenge**: transport of samples to centralized labs
- **Currently**: field solutions are limited
  - 1-4 samples at a time
  - Rely on inconsistent/disposable components
  - Focus on either imaging or assay

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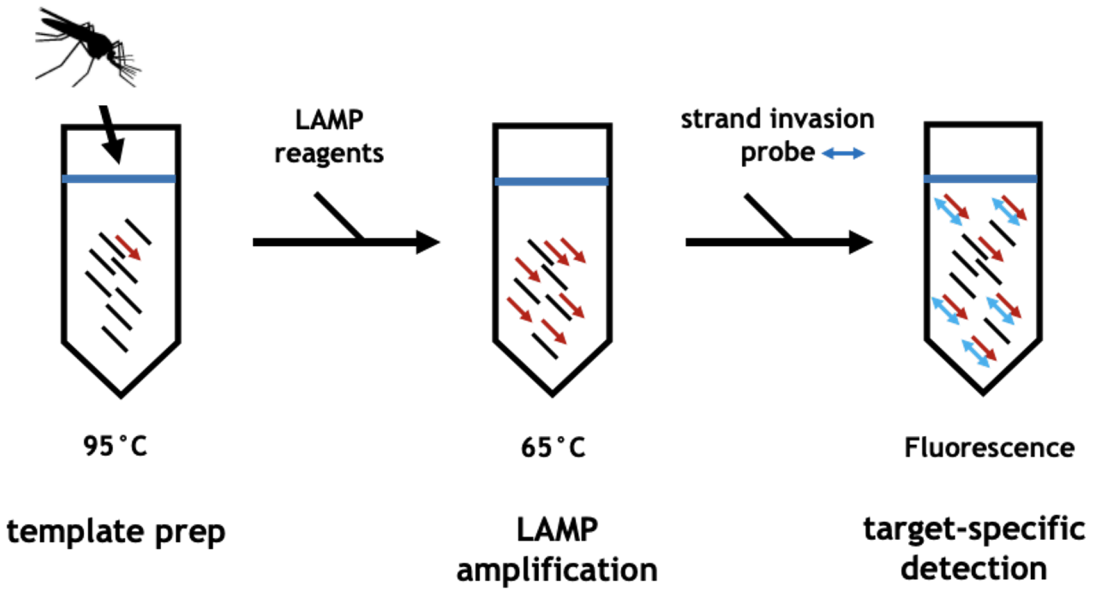
**Need:** field enabled detection of transgene spread in mosquito populations with higher throughput

# Field Enabled Assay: LAMP

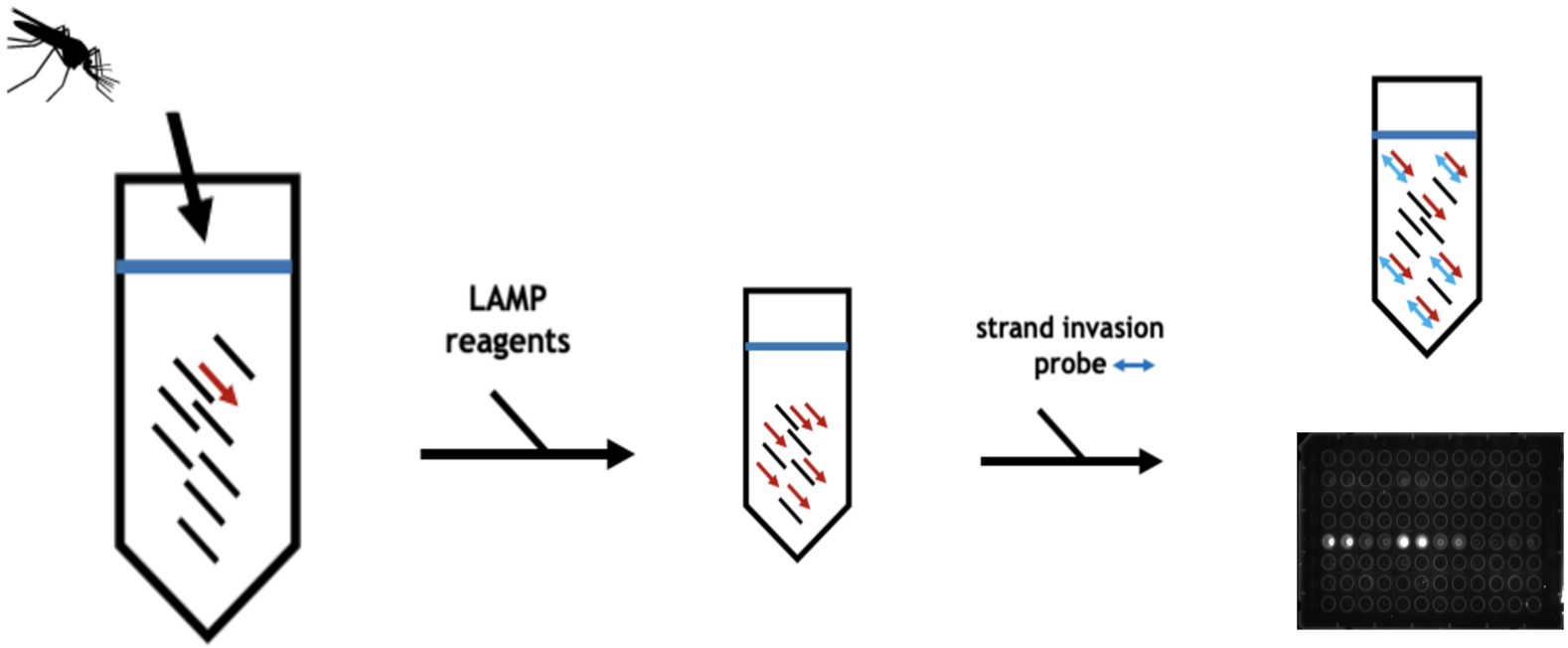
- **L**oop mediated isothermal **Amp**lification
- Modified PCR
  - Amplifies DNA at constant temperature
  - Target specific fluorescent readout
  - Robust



# Design Constraints

| | |
|---|---|
| Operating conditions | • Shock resistant<br>• 10-40℃<br>• Robust |
| Usability | • Minimal training<br>• Remote use<br>• Universal Design |
| Engineering Standards | • Easily sterilized<br>• Enables commercial production<br>• Includes controls |

# Workflow and Specifications

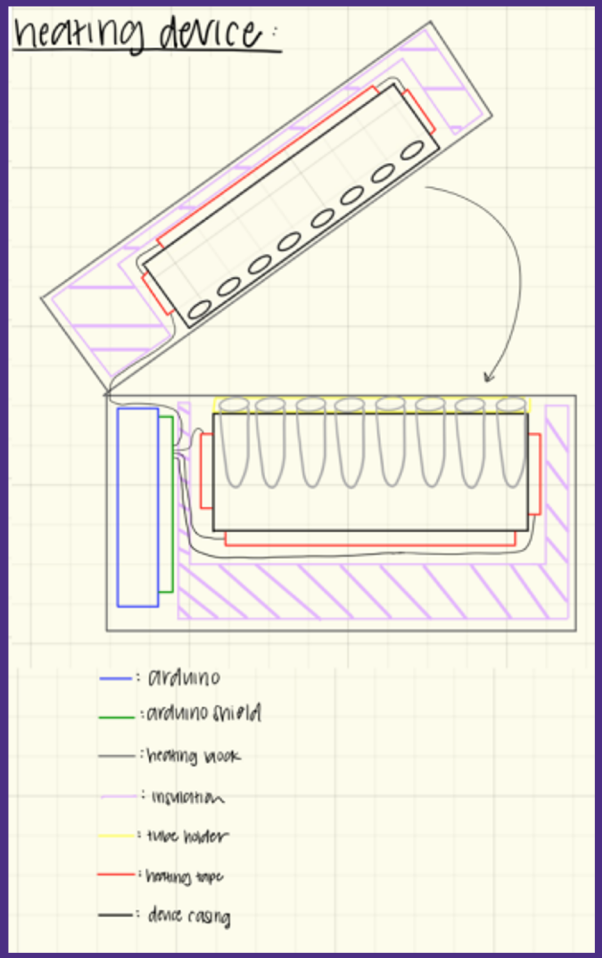


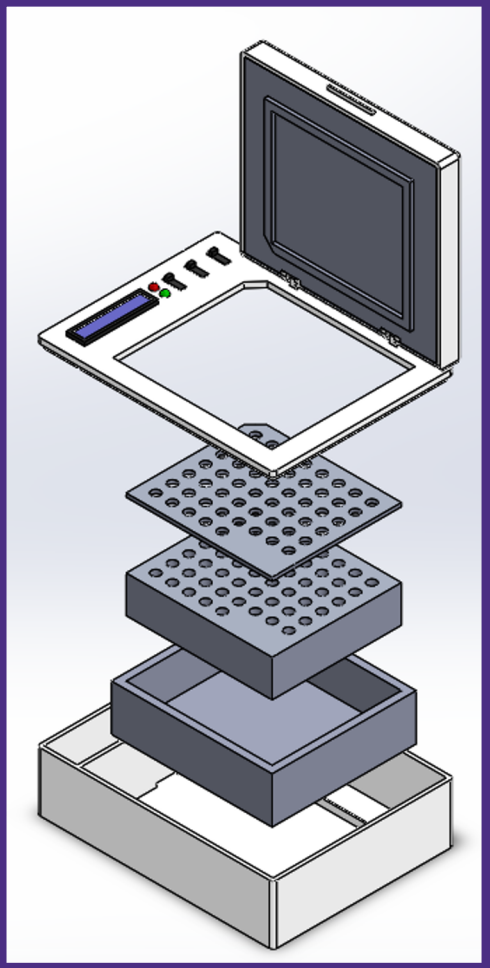

**Template prep**

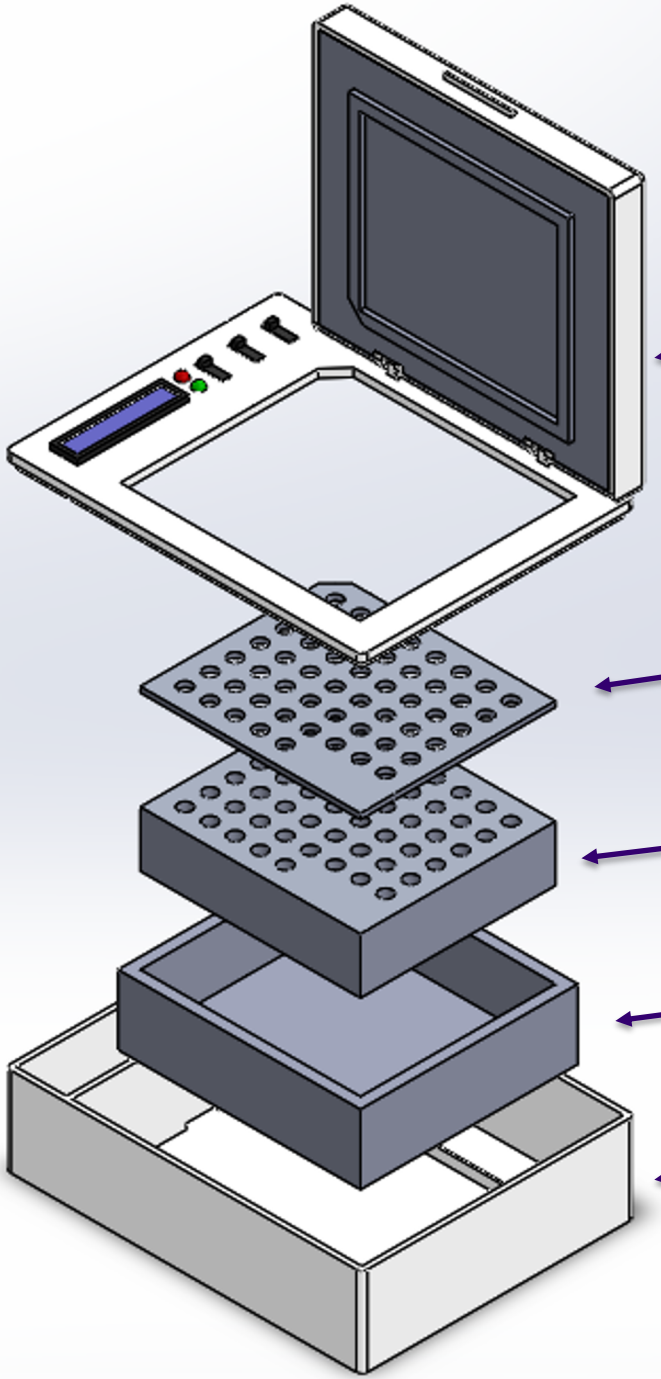
**LAMP Assay**

**Fluorescence and imaging**

# Heating Module Design





Lid and user interface

Sample handling tray

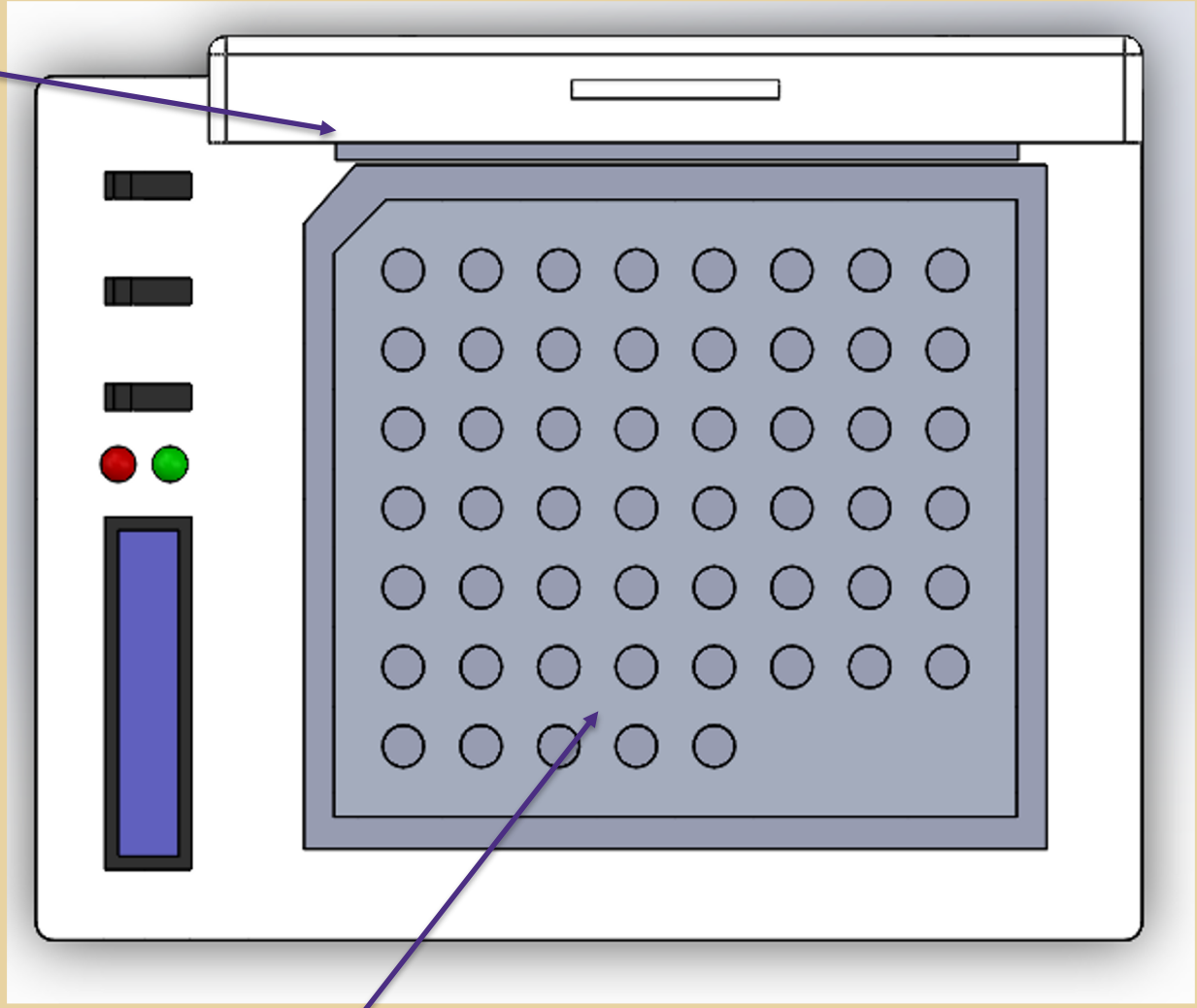
Interchangeable heating block

Integrated heating block

32 x 24.5 cm footprint

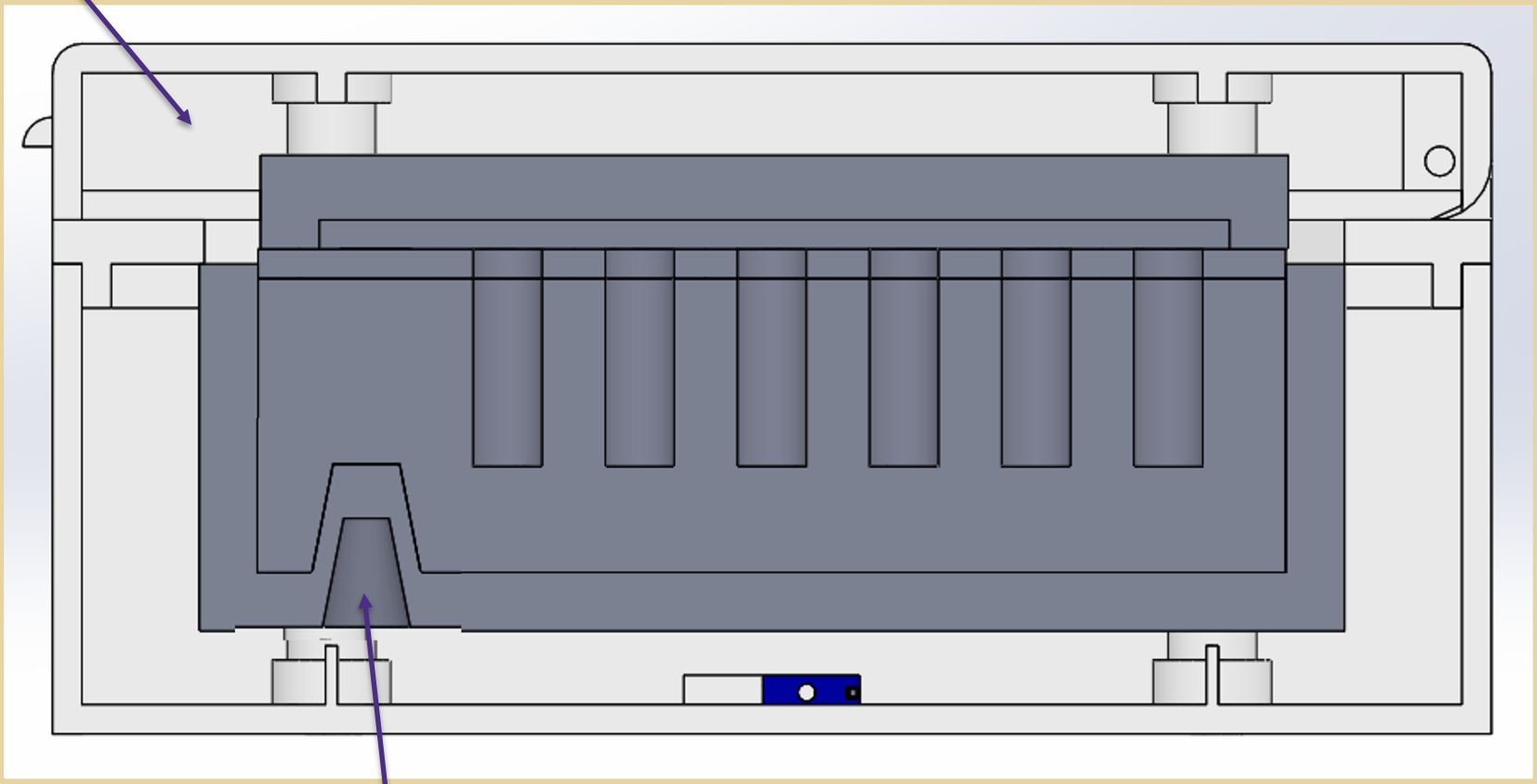
Heat conductive lid

Simple User Interface



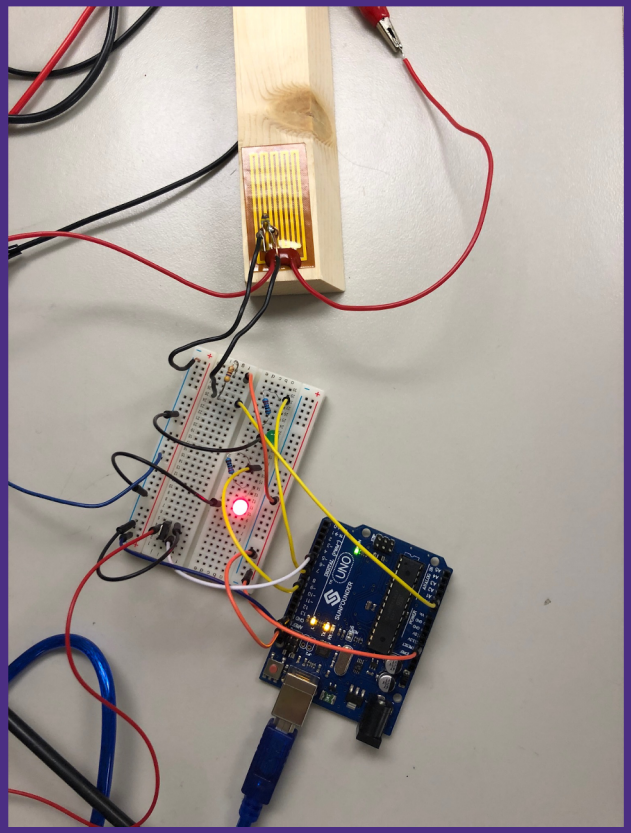
48 samples + 5 controls per cycle

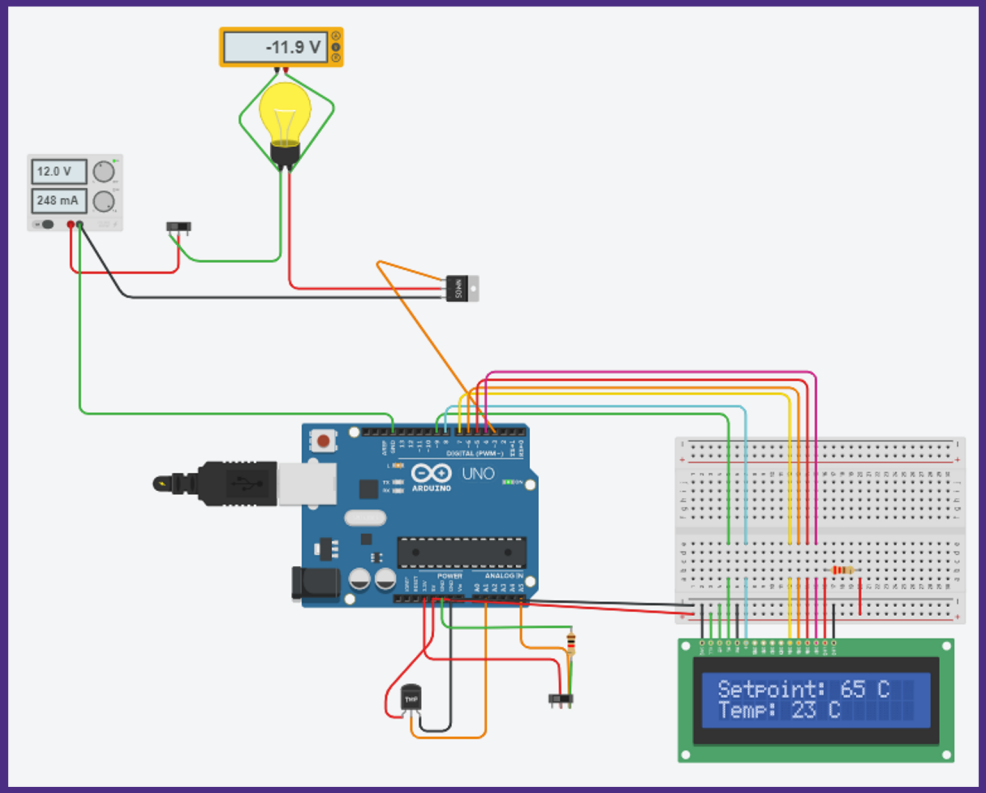
Room for insulation



Space for thermocouple and temperature sensor

# Heating Circuit Design

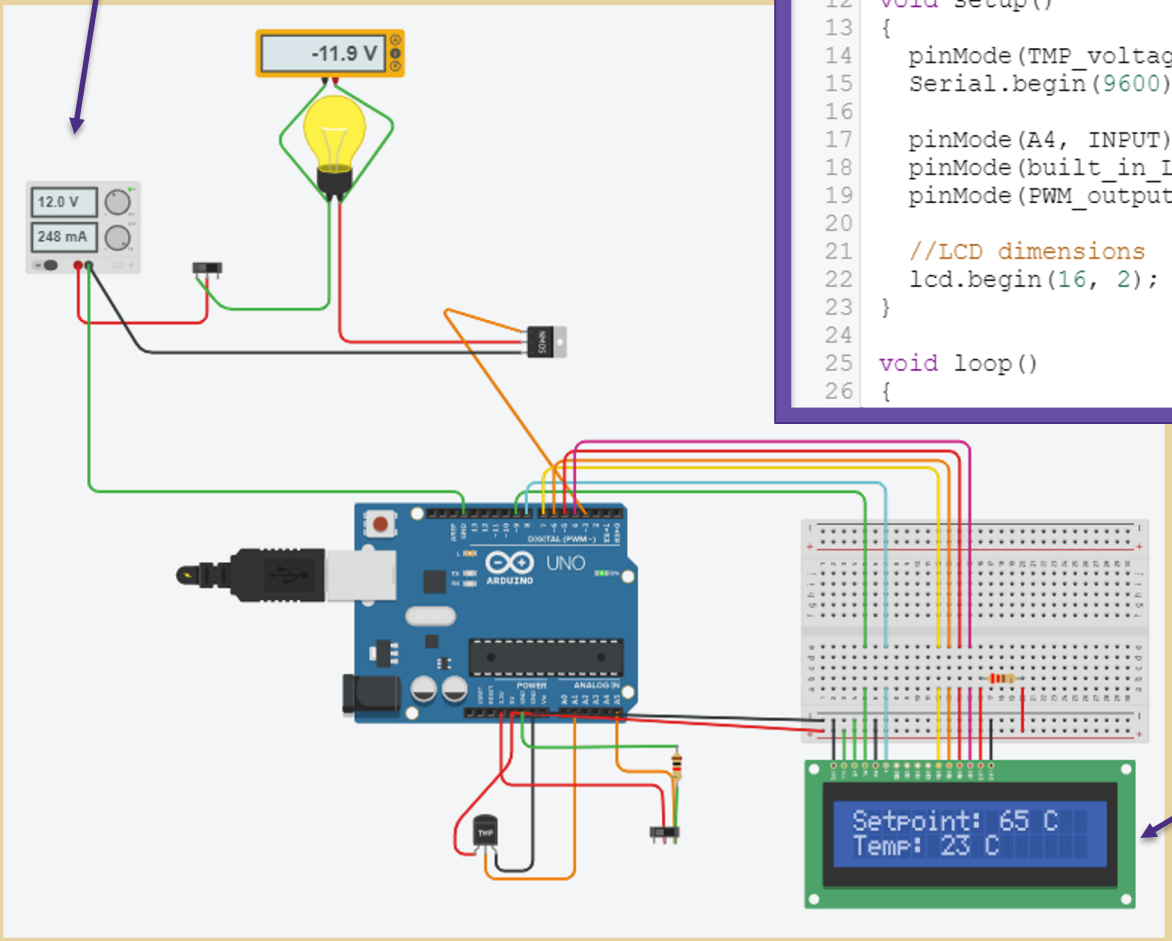




12 Volt power supply

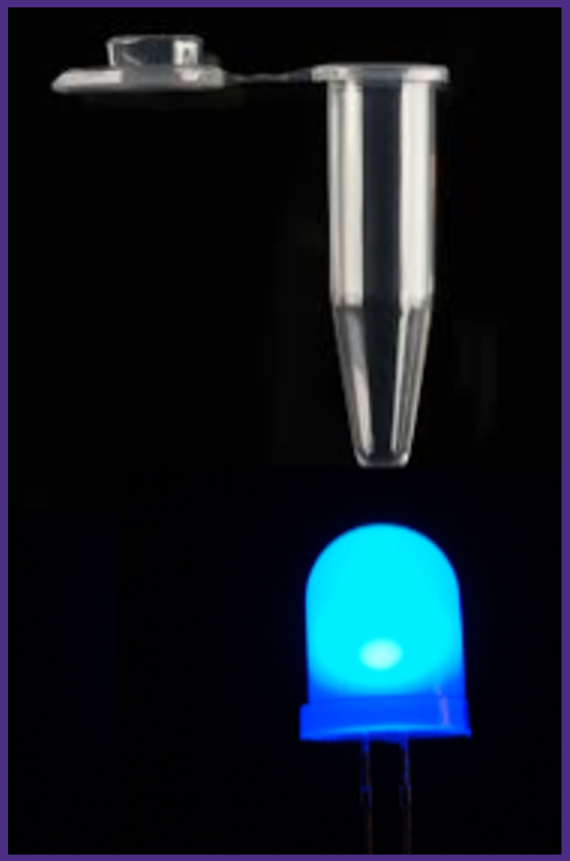
```
#include <LiquidCrystal.h>
LiquidCrystal lcd(9, 8, 7, 6, 5, 4);

int temperature = 0;
int TargetTemperature = 0;
int PWM_strength = 0;

//Define the output Pin
#define PWM_output  3
#define built_in_LED  13
#define TMP_voltage  A1
void setup()
{
  pinMode(TMP_voltage, INPUT);
  Serial.begin(9600);

  pinMode(A4, INPUT);
  pinMode(built_in_LED, OUTPUT);
  pinMode(PWM_output, OUTPUT);

  //LCD dimensions
  lcd.begin(16, 2);
}

void loop()
{
```

Arduino program for PWM control

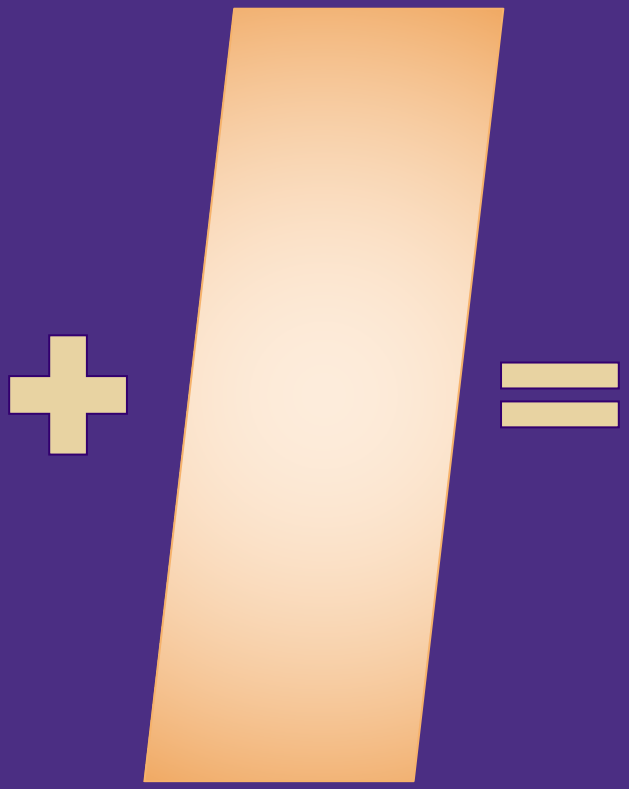



LCD for User Interface

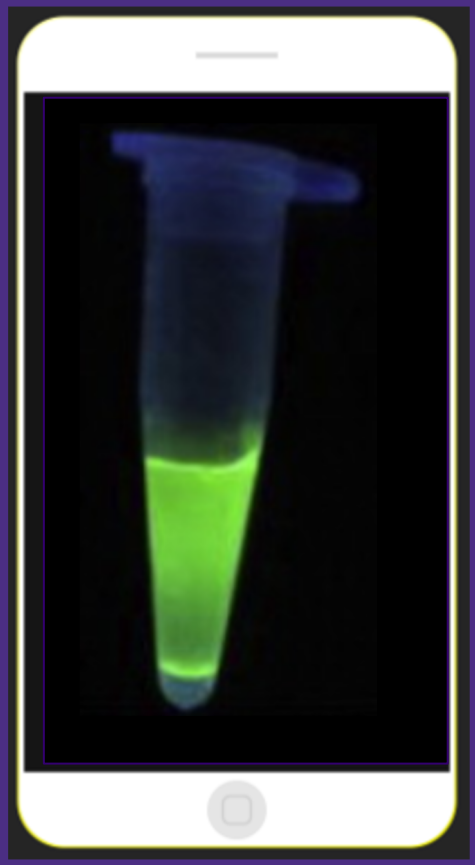
# Approach to Imaging Module



Sample Excitation with LED array

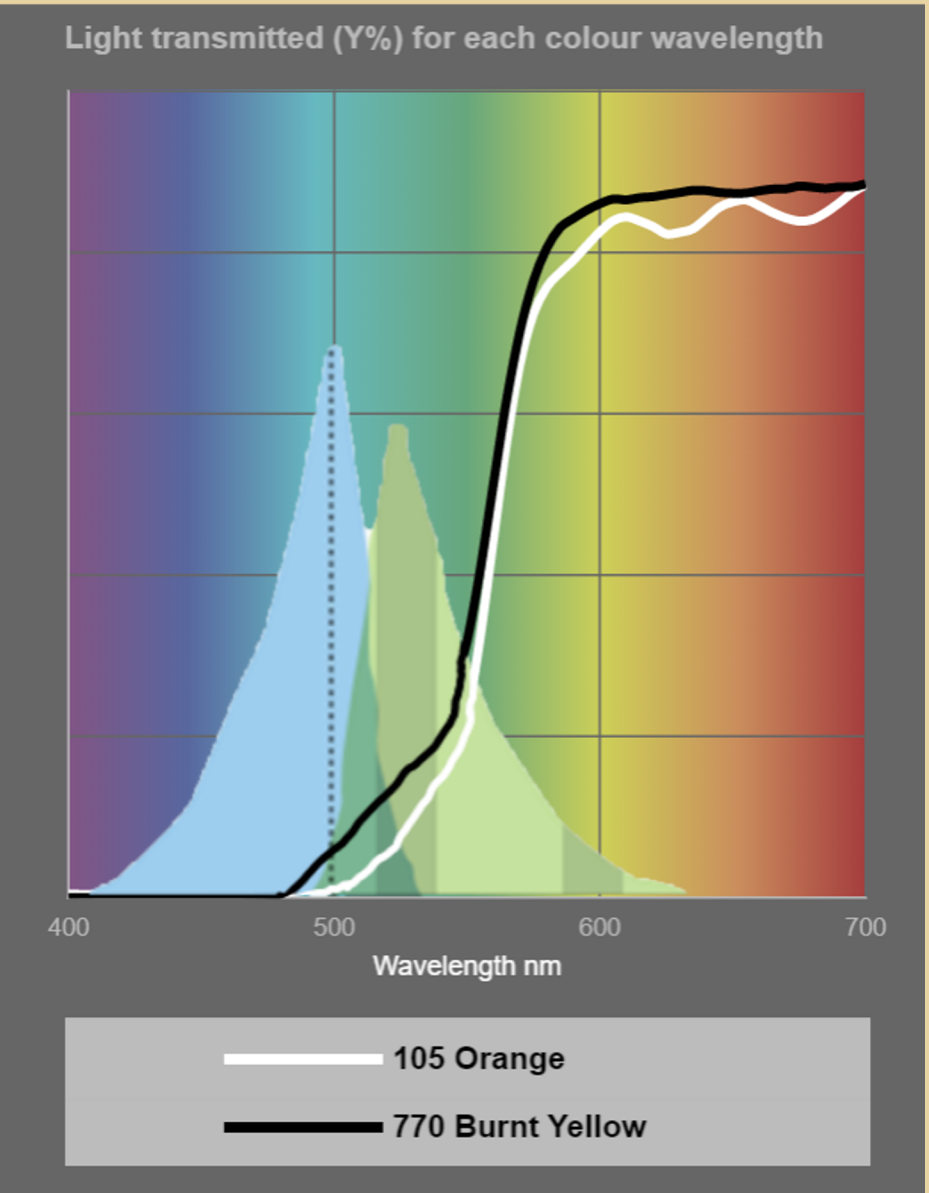


High-pass filtering



Signal Detection

# Filter and Fluorophore Selection

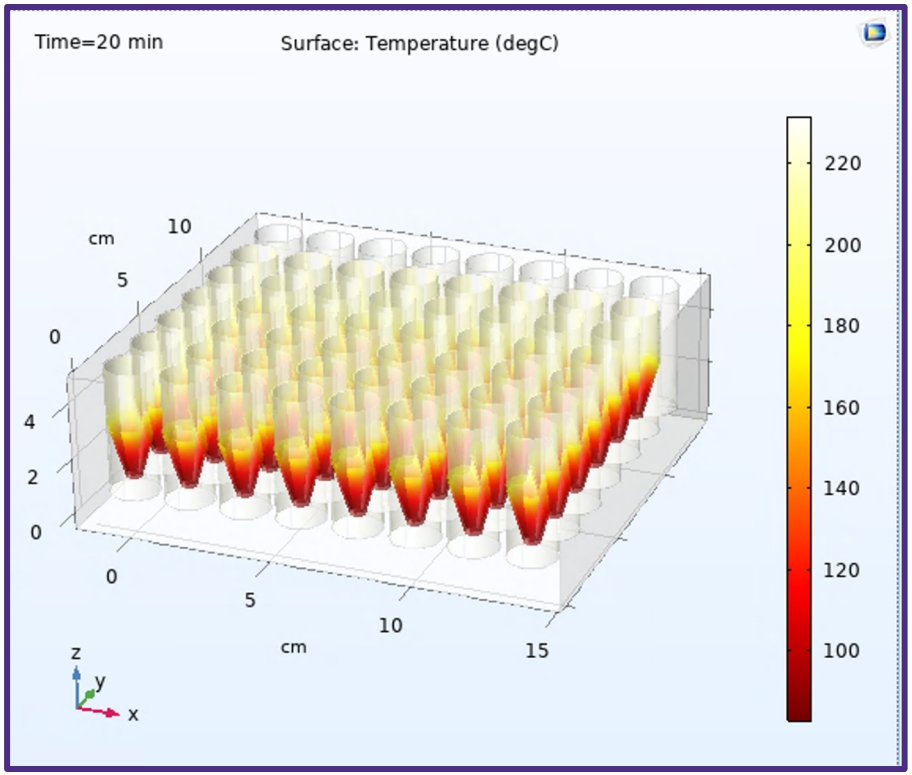



- Maximize Area under curve (AOC) for Emission
- Minimize AOC for Excitation

# Test Methods

## Implemented:

- COMSOL Modeling of heating dynamics
  - Time to temp
  - Heat distribution
  - Power requirements
  - Material selections
  - Priority to tests that design decisions
- CAD
  - Interference detection



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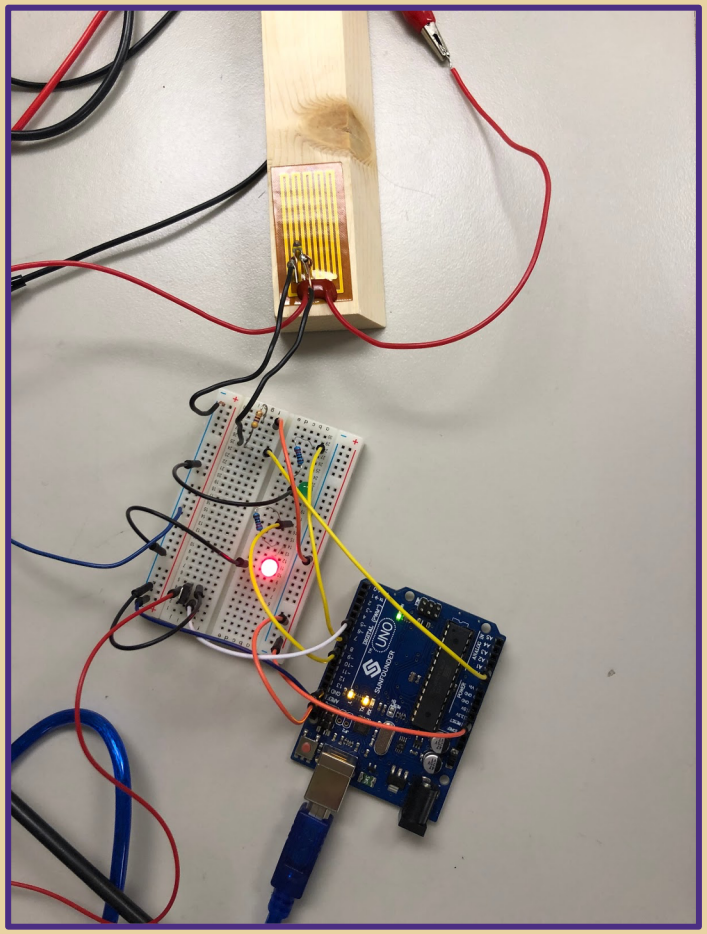
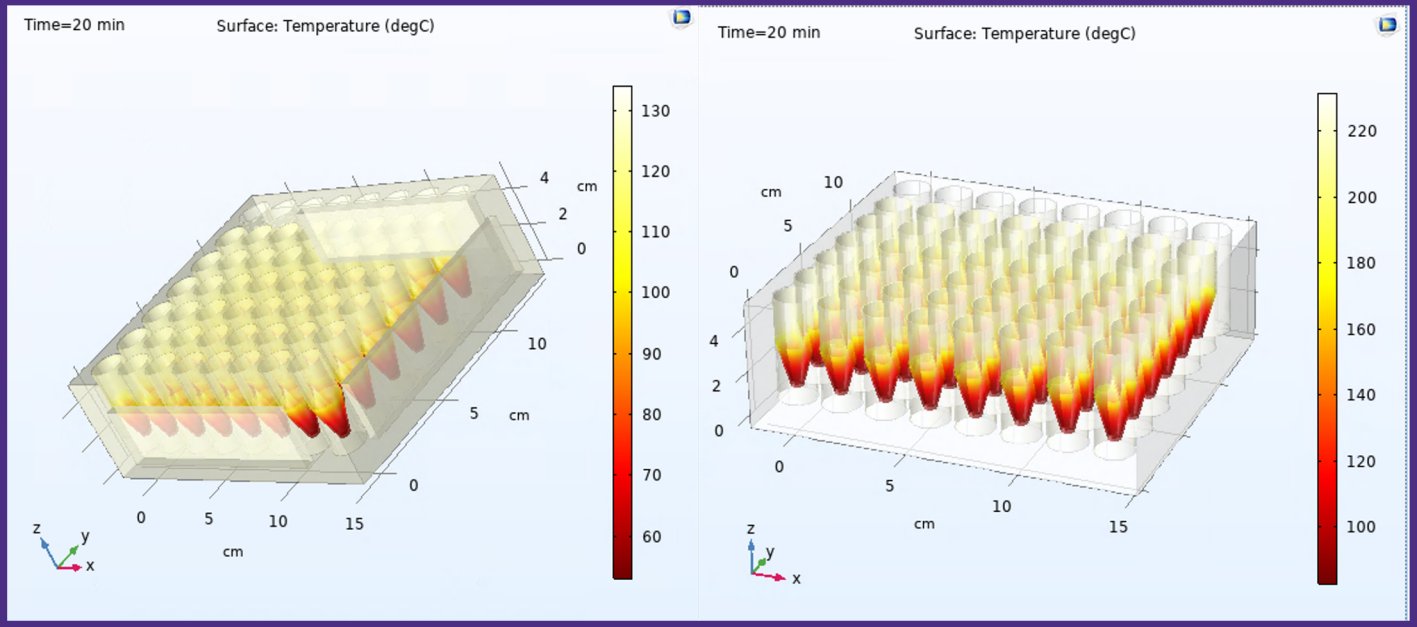


**Planned**

- Image Capture Testing
  - Dilution series for detection threshold
  - Varied arrangements
    - Excitation lighting
    - Image capture
  - Multiple fluorescence imaging
- Heating Block Testing
  - Physical validation of time to temp
  - Validation of temperature control

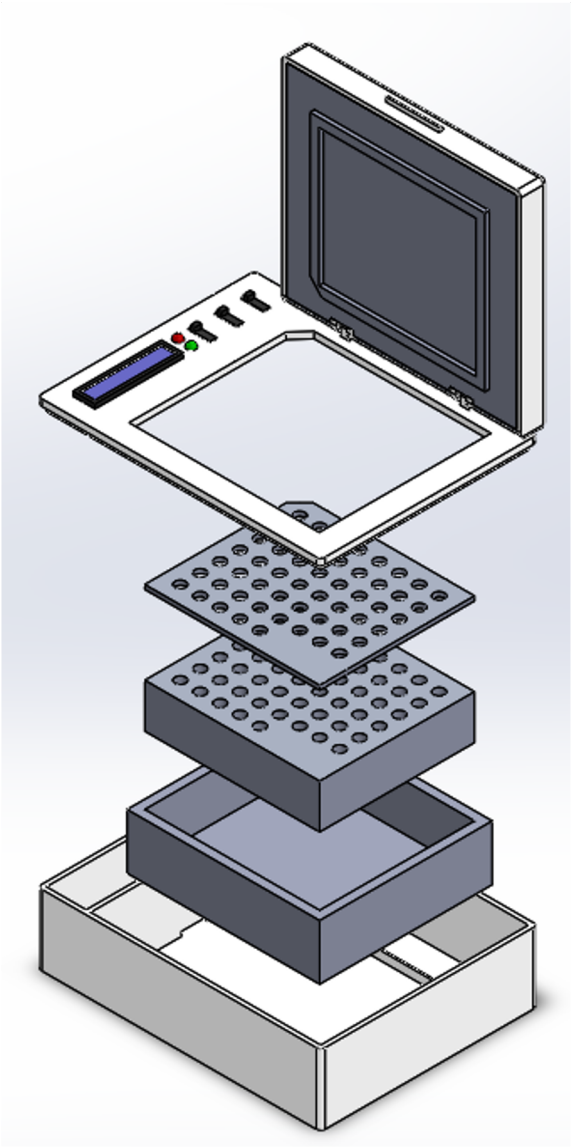
# Test Results

- Characterized behaviors of heating block in silico
  - Time to operating temperature
  - Power requirements
- Uncovered relationships that drove design decisions
  - Heating orientation
  - Material selection



# Conclusion

- Developed the Heating Module
- Identified Imaging Approach
- Outlined tests for Engineering Analysis
- Future work including:
  - BWB Handoff
  - Developing Imaging Module
  - Field Testing with Target Malaria



# Thank you!

- Thank you to our mentors Dr. Monnat and Jamie Hernandez for your guidance
- Thank you to our friends at Target Malaria for your help and feedback
- Thank you to Dr. Taylor and Dr. Neils for your support inside and outside class
- Thank you to all our talented classmates for making a great cohort
- Thank you to our friends and family for your support

# Questions?

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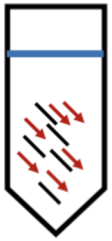
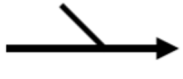
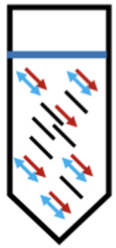
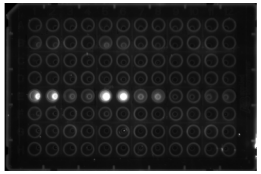
|  |  |  |  |  |  |  |
| **Step Description** | DNA Release | Sample Prep and Transfer | LAMP Assay | Sample Prep and Transfer | Sample Imaging | Image Analysis |
| **Module and Configuration & Modification** | Heating Module<br>95 C<br>15 min<br>1.5 mL<br>48 Tubes<br>Heating Block | Change Heating Module Configuration for 0.5mL tubes | Heating Module<br>65 C<br>1-2 Hours<br>0.5 mL<br>>48 Tubes<br>Heating Block | Transfer to imaging Module | Take image of samples | |

# References


- James, Anthony A. “Gene-Drive Technology Must Be Developed in a Safe, Ethical Way.” STAT, 9 July 2018, www.statnews.com/2018/07/10/gene-drive-technology-safety-ethics/.
- “Recommendations for Safe and Ethical Testing of Gene Drive Mosquitoes to ReduceMalaria Transmission in Africa.” American Journal of Tropical Medicine and Hygiene, FNIH, 7 June 2018, fnih.org/news/announcements/AJTMH-publishes-recommendations-safe-and-ethical-testing-gene-drive-mosquitoes.
- Target Malaria, https://targetmalaria.org/
- Velders, A.H., Schoen, C. & Saggiomo, V. Loop-mediated isothermal amplification (LAMP) shield for Arduino DNA detection. *BMC Res Notes* 11, 93 (2018). https://doi.org/10.1186/s13104-018-3197-9

# Normal Inheritance

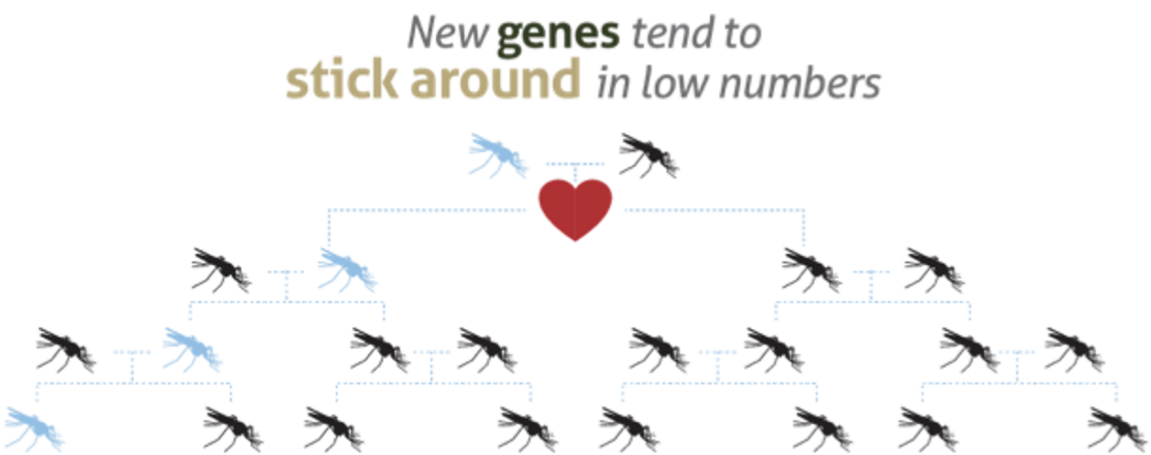
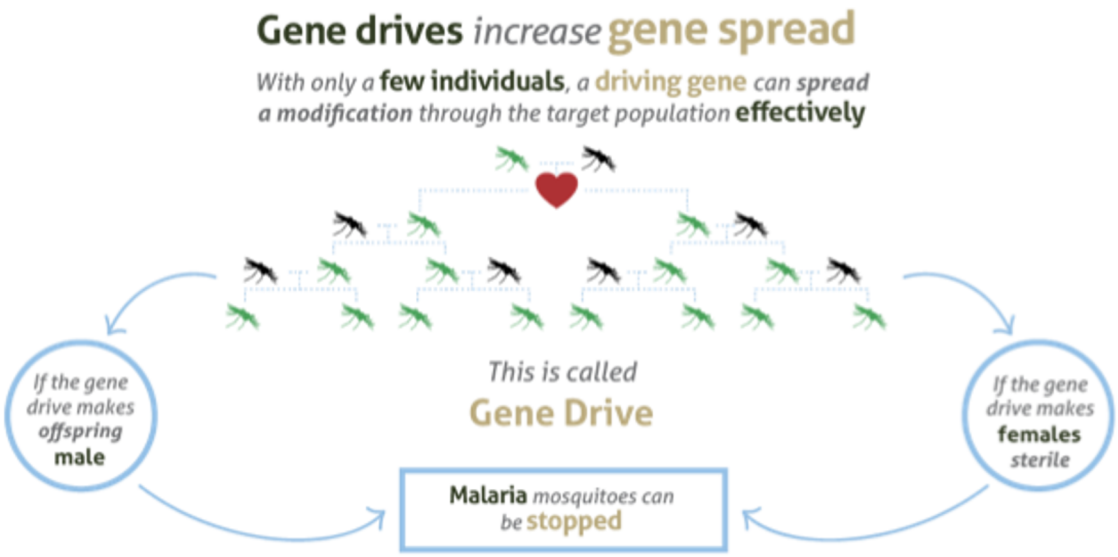



Image from: https://targetmalaria.org/our-work/

# Gene Drive

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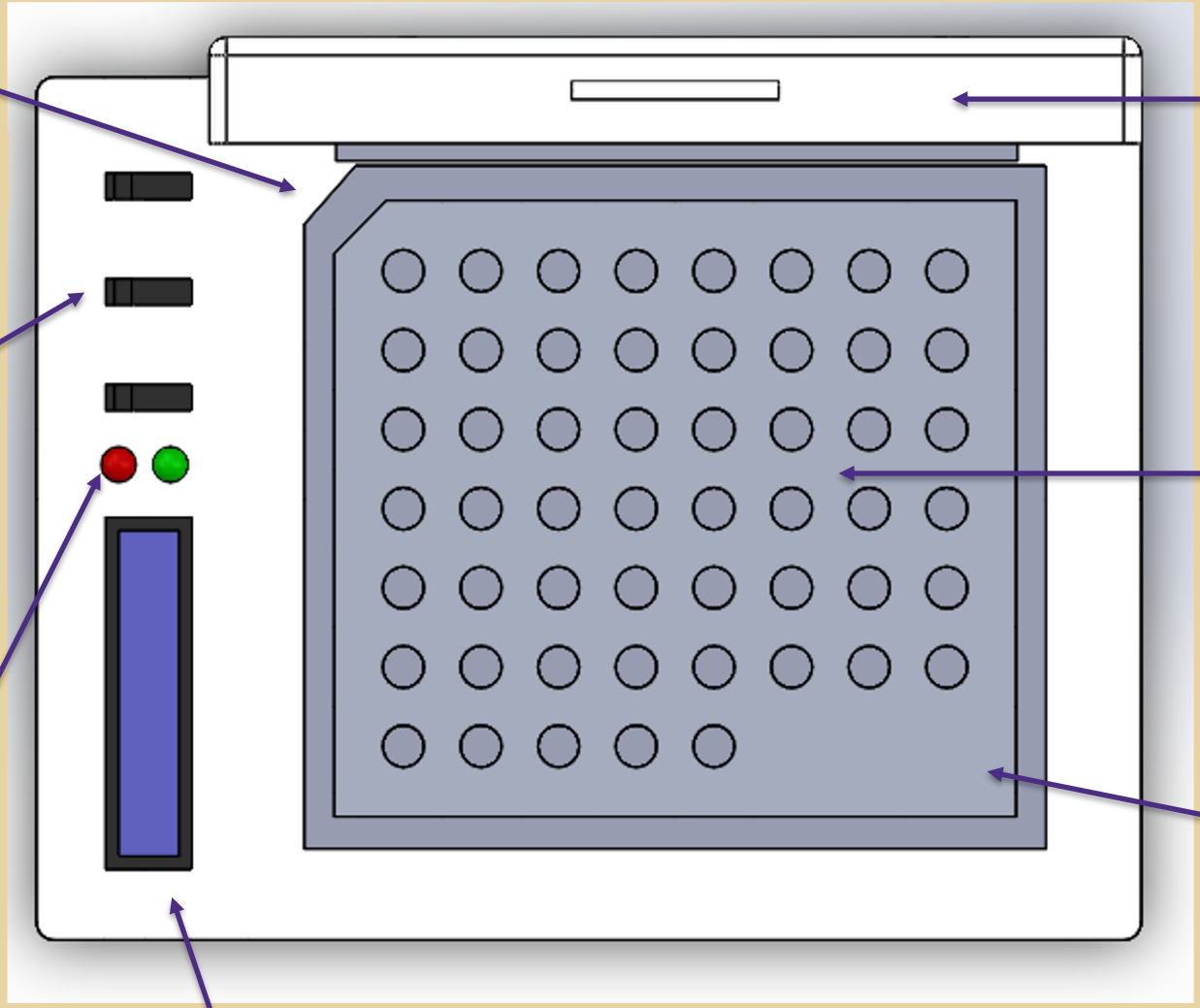
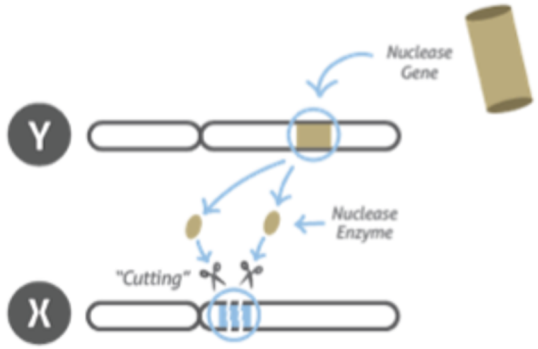

Asymmetry notch
Lid to prevent condensation on sample lids
Control Panel for Power, Temp Setpoint, and program
48 samples + 5 controls
Removable inner block for different tube sizes
LEDs for visual Indicator of device status
LCD Display

# Strategy 1: Sex Biasing

A nuclease gene that produces a nuclease enzyme is placed on the Y chromosome. It recognises the key sites on the X chromosome, cutting through them and leaving the chromosome fragmented.

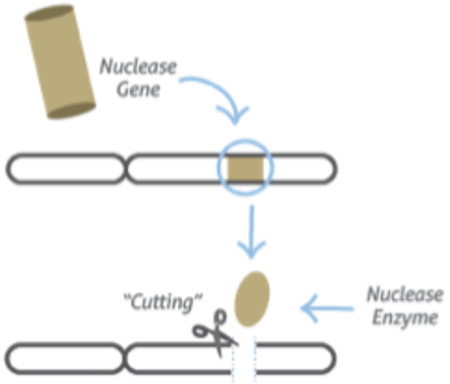



The Y chromosome remains intact.

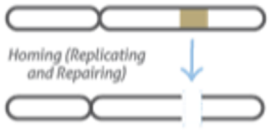
Image from: https://targetmalaria.org/our-work/

# Strategy 2: Reducing Female Fertility

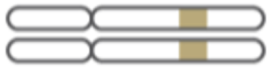
*During the production of eggs and sperm, the gene produces an enzyme that* **cuts** *the unmodified chromosome and* **"homes"** *into the female fertility gene.*




*As the second chromosome repairs itself, it* **copies** *the genetic material from the modified chromosome.*




*In the cells that make the eggs and the sperm,* **both chromosomes** *now carry the* **nuclease gene.**



*With* **both copies** *of the chromosome carrying the gene, all sperm and eggs will receive a copy of the nuclease and the modification has a* **100% chance** *of being passed to offspring.*

Image from: https://targetmalaria.org/our-work/

# Ethical Considerations

- Implementation of the gene drive as it spreads new genes throughout a population
  - Serious implications if not properly managed
  - Limit spread outside the region
  - Minimize impact outside the region
  - Concern for global extinction
- Gene drive management and regulation outline a multiphase methodology to evaluate safety and efficacy of the gene drive product
  - World Health Organizations publication in 2014, *The Guidance Framework for testing genetically modified mosquitoes*
  - Gene drive report released by the National Academies of Science, Engineering and Medicine

# Engineering Need: Field Enabled Assay

- Need method of detecting the spread of transgene in wild mosquito populations
- Transport of samples back to the lab
  - requires **additional logistics/ planning**
  - **potential loss of samples**
  - Often fields sites are remote
- Study of samples on the field
  - **reduce transportation costs**
  - **prevent loss**
  - allow for **immediate results**

# Technology Gap for Large Scale Projects

- Current field technologies for LAMP assays are **limited to 1-4 samples at a time**
- Many rely on **inconsistent/disposable** components
- Existing methods focus on **either** imaging or assay
- Other methods of transgene detection require **centralized facilities**