



LABYRINTH

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SPONSORS: B. Cantrell, J. Standeven

Lambert iGEM

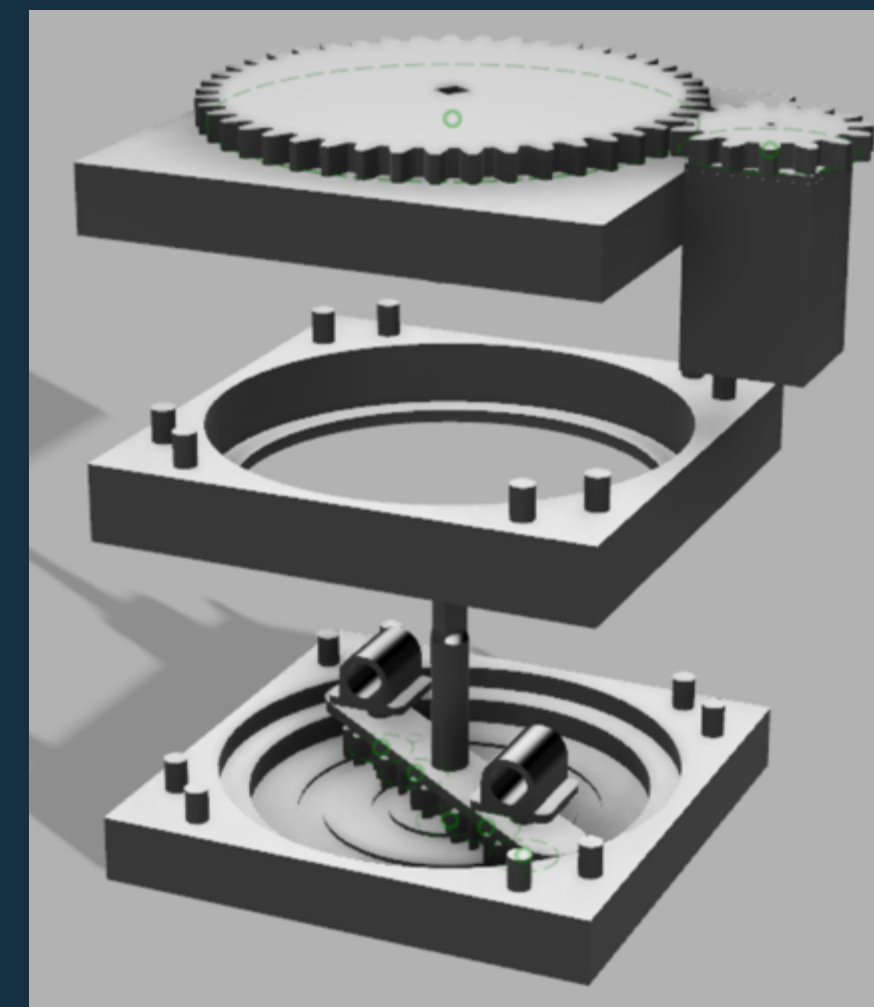
EXTRACTION: FILTER & BEAD HOMOGENIZER

MICROMESH - Filter

Our 2-dollar, multi-mesh, modular helminth egg filter for feces and soil samples isolates helminth eggs from other particles.

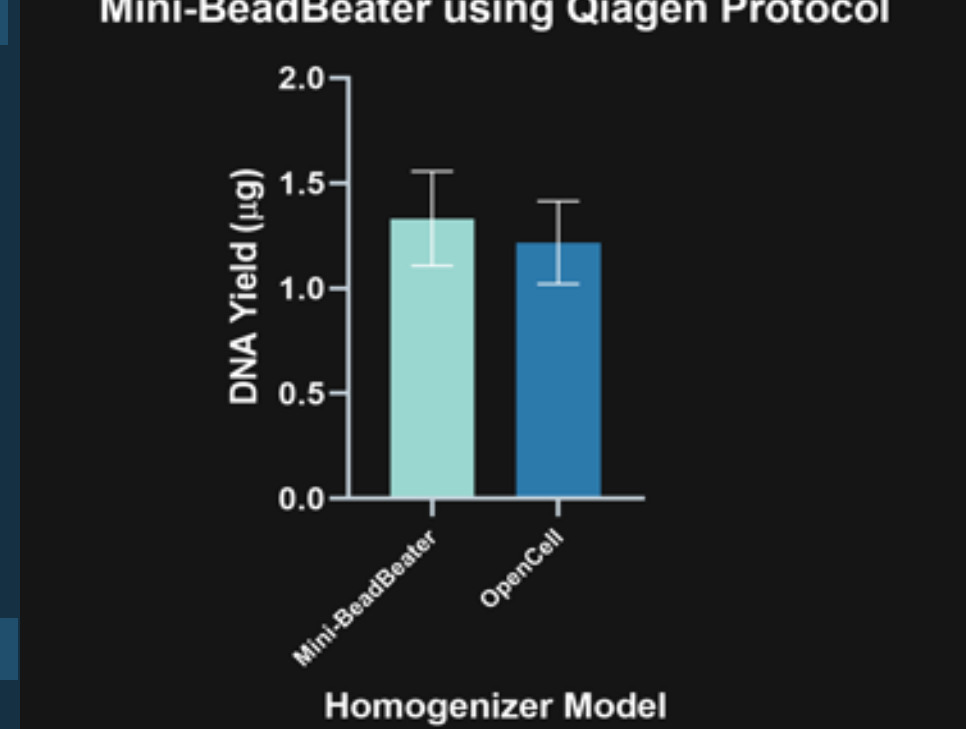
OPENCELL - Homogenizer System

Our low-cost system uses epicyclic gearing to shear the rigid chitin layer within the helminth egg and produces usable DNA within minutes. OpenCell transforms into a centrifuge and vortexer.

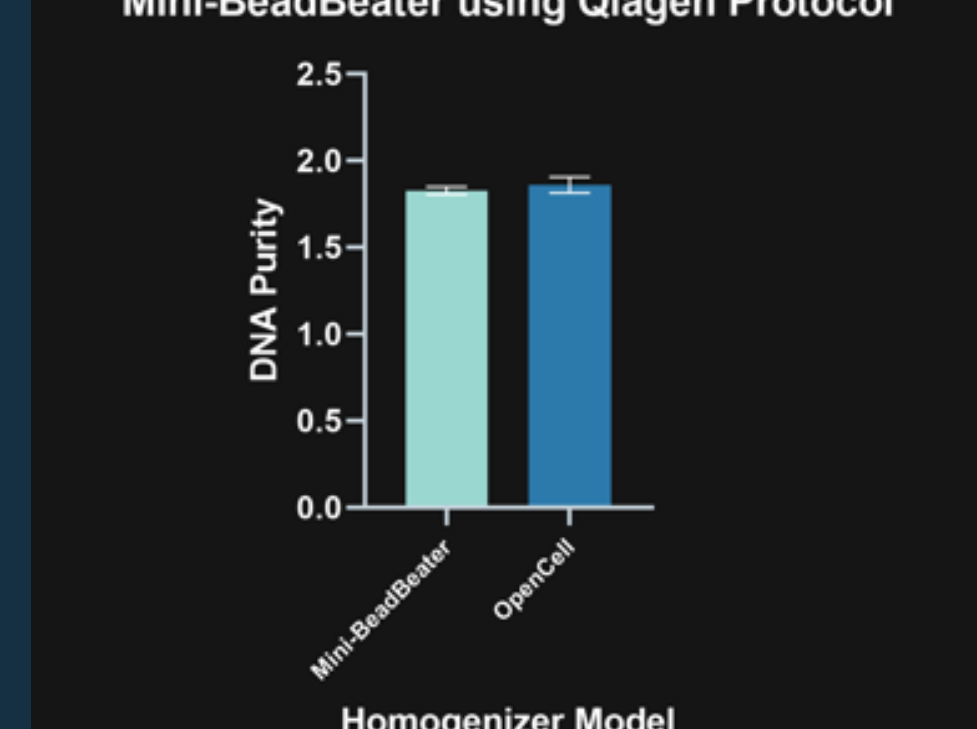


Inside OpenCell casing

Spinach DNA Yields from OpenCell vs BioSpec Mini-BeadBeater using Qiagen Protocol



Spinach DNA Purities from OpenCell vs BioSpec Mini-BeadBeater using Qiagen Protocol



Experimental Spinach DNA Yields from OpenCell vs. BioSpec Mini Beadbeater

Experimental Spinach DNA Purities from OpenCell vs. BioSpec Mini Beadbeater

Homogenization Method	Yield	Purity (A260/A280)
Vortex Mixer-Qiagen Garnets	0.135	0.370
OpenCell-Zirconium Beads 100 Micron	0.276	1.620
OpenCell-Qiagen Garnets	0.690	1.770
OpenCell-Qiagen Garnets after PCR Purification	0.560	1.740

MODELING

PURPOSE

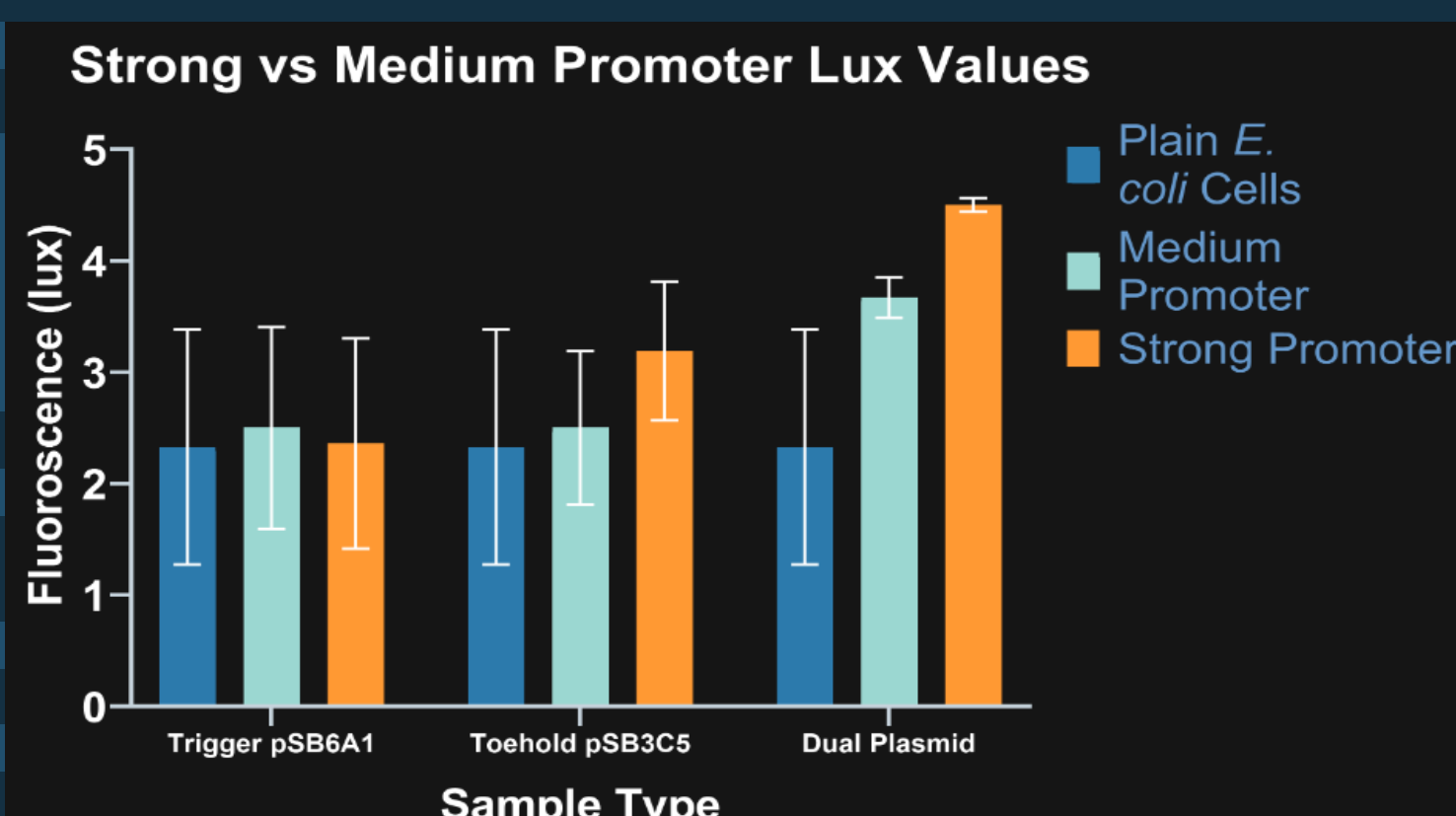
In the presence of a trigger RNA sequence, the toehold will unfold, allowing for translation of the reporter. A "leaky" promoter thus induces the reporter gene in the absence of the trigger and produces false positives.

IMPROVEMENT

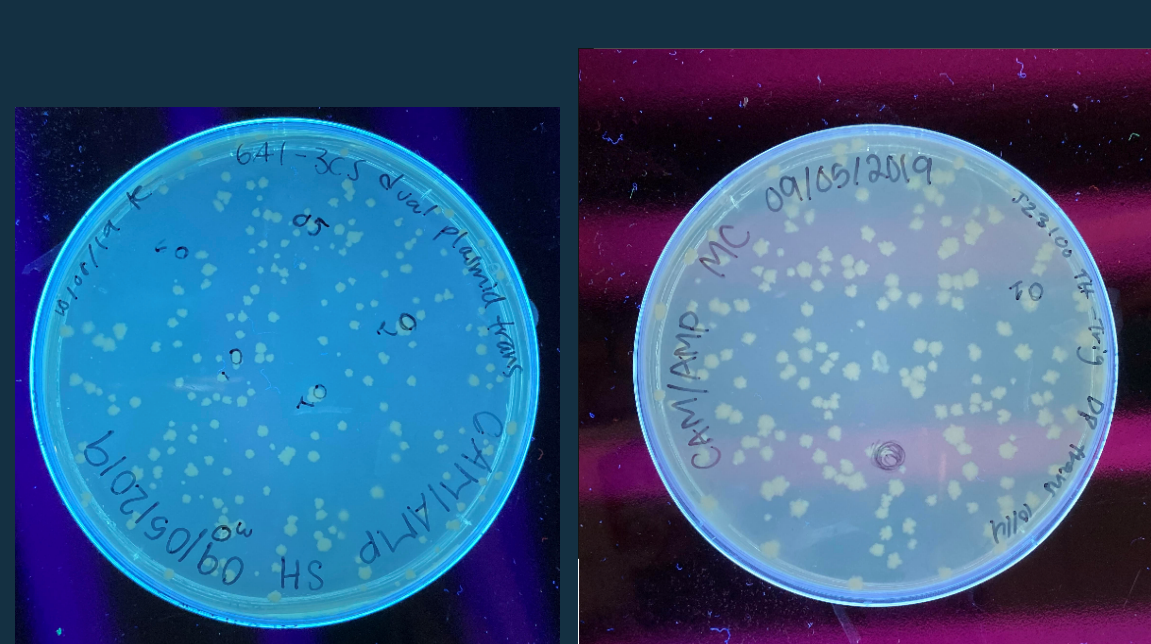
In our 2018 project, the J23100 promoter caused "leakiness" in our T7 toehold biosensor. LABYRINTH replaces its promoter with the weaker J23106 of the same Anderson series, thus, preventing leaky expression.

MODELING (TUNING)

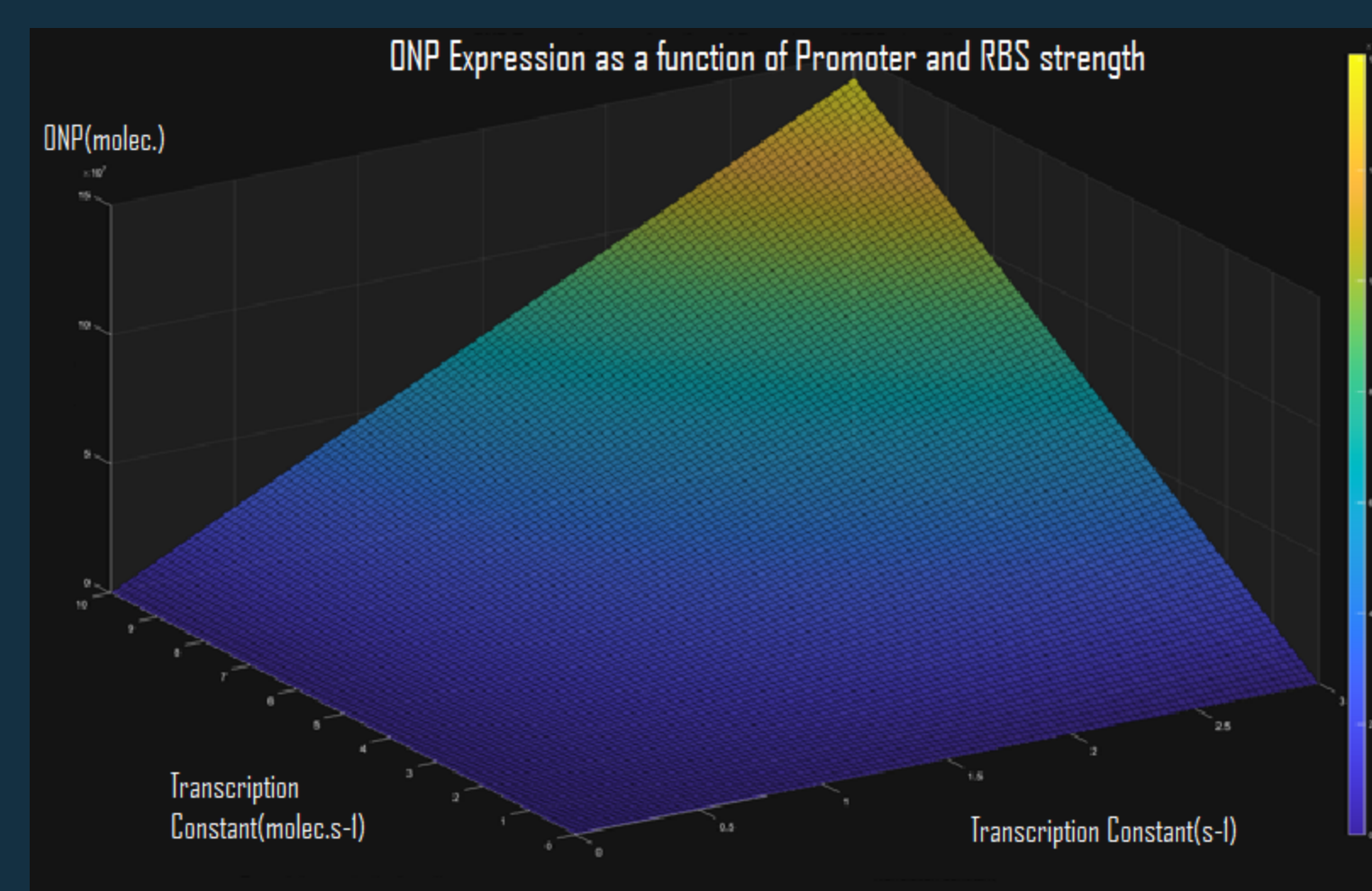
An ordinary differential equation model simulates the reactions taking place during the cleavage of ONPG and a multivariate regression model correlates the strength of the promoter and RBS to expression. Experimental fluorescence data was taken to characterize strength of expression within the 2018 and 2019 toehold systems.



Lux values between BBa_J23100 and BBa_J23106 toehold systems



BBa_K2974101 and BBa_K2974700 Dual Plasmid transformations with BBa_K2550001, respectively



3D graph of ONP expression as a function of promoter and RBS strength

ACKNOWLEDGEMENTS

RESO

SnapGene®

GT

IDT
INTEGRATED DNA TECHNOLOGIES

Boehringer
Ingelheim



ACHIEVEMENTS

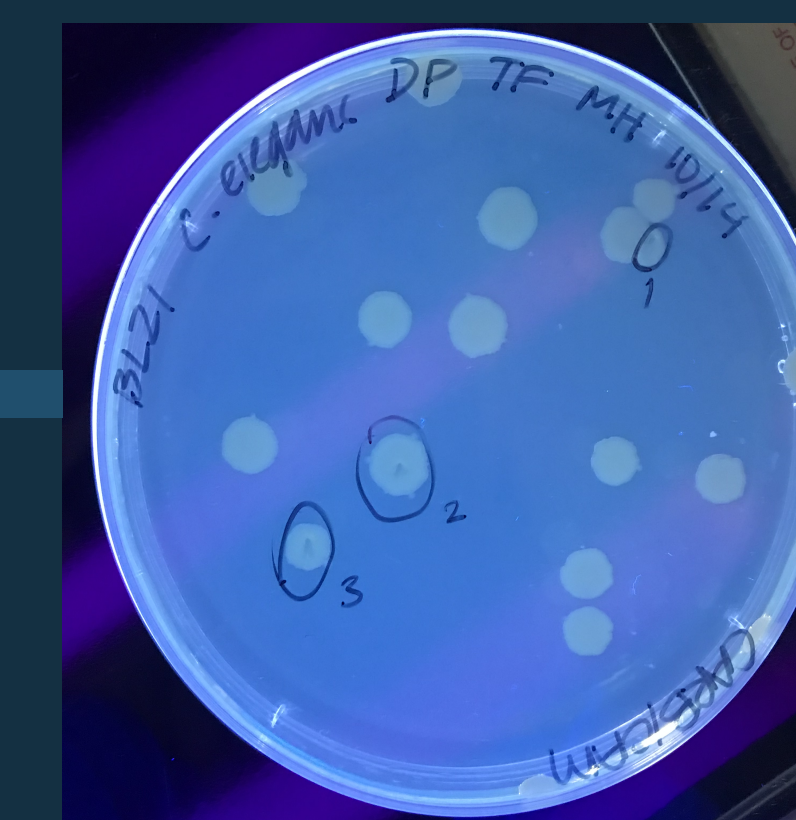
Characterized: BBa_J10050-58, BBa_J23115, BBa_J0035, BBa_K2550001, BBa_K2550000
Validated: BBa_K2974101, BBa_K2974316, BBa_K2974400, BBa_K2974310, BBa_K2974410, BBa_K2974700

PROOF OF CONCEPT: *C. ELEGANS* TOEHOLD

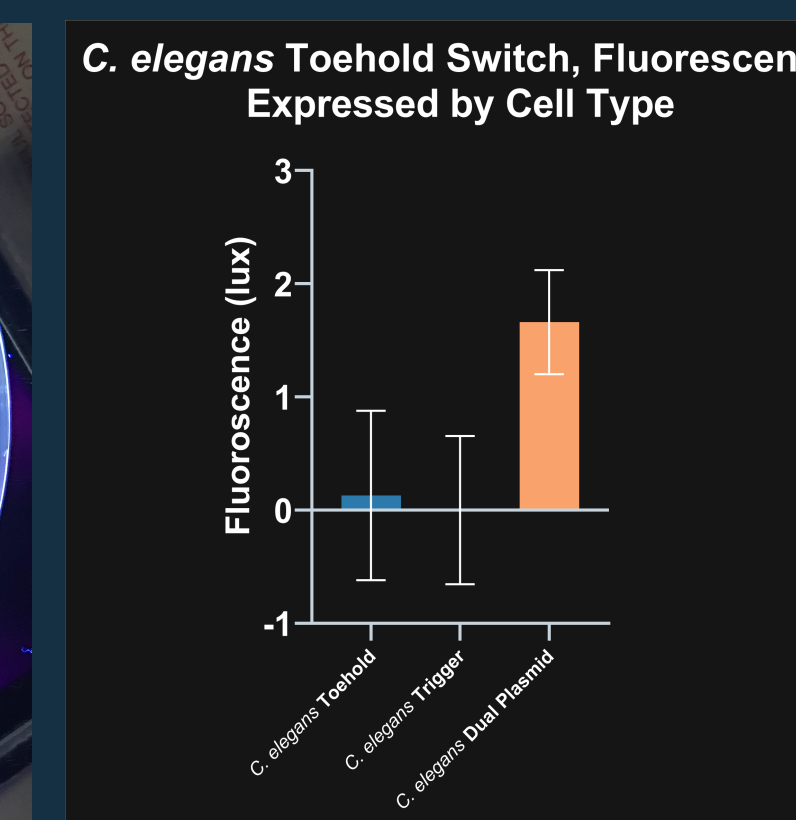
PURPOSE

We used *C. elegans* as a model organism and NUPACK software to design a toehold specific to the lin-4 gene pre-mRNA primary transcript, F59G1.6. The toehold is assembled in pSB3C5 and the trigger in pSB1A3.

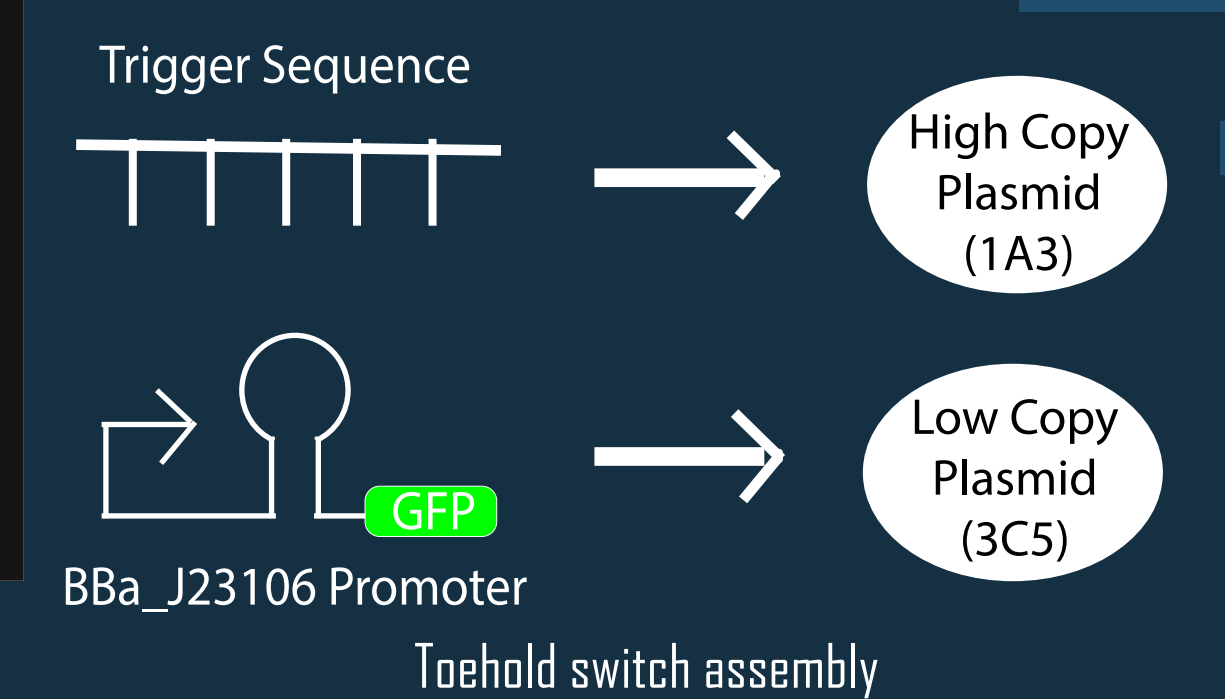
When the switch is in the presence of the trigger, the toehold will unbind, exposing the RBS and allowing for downstream expression of the GFP reporter.



BBa_K2974316 Dual-plasmid transformation with BBa_K2974400



FluoroCents lux values of *C. elegans* Toehold Switch



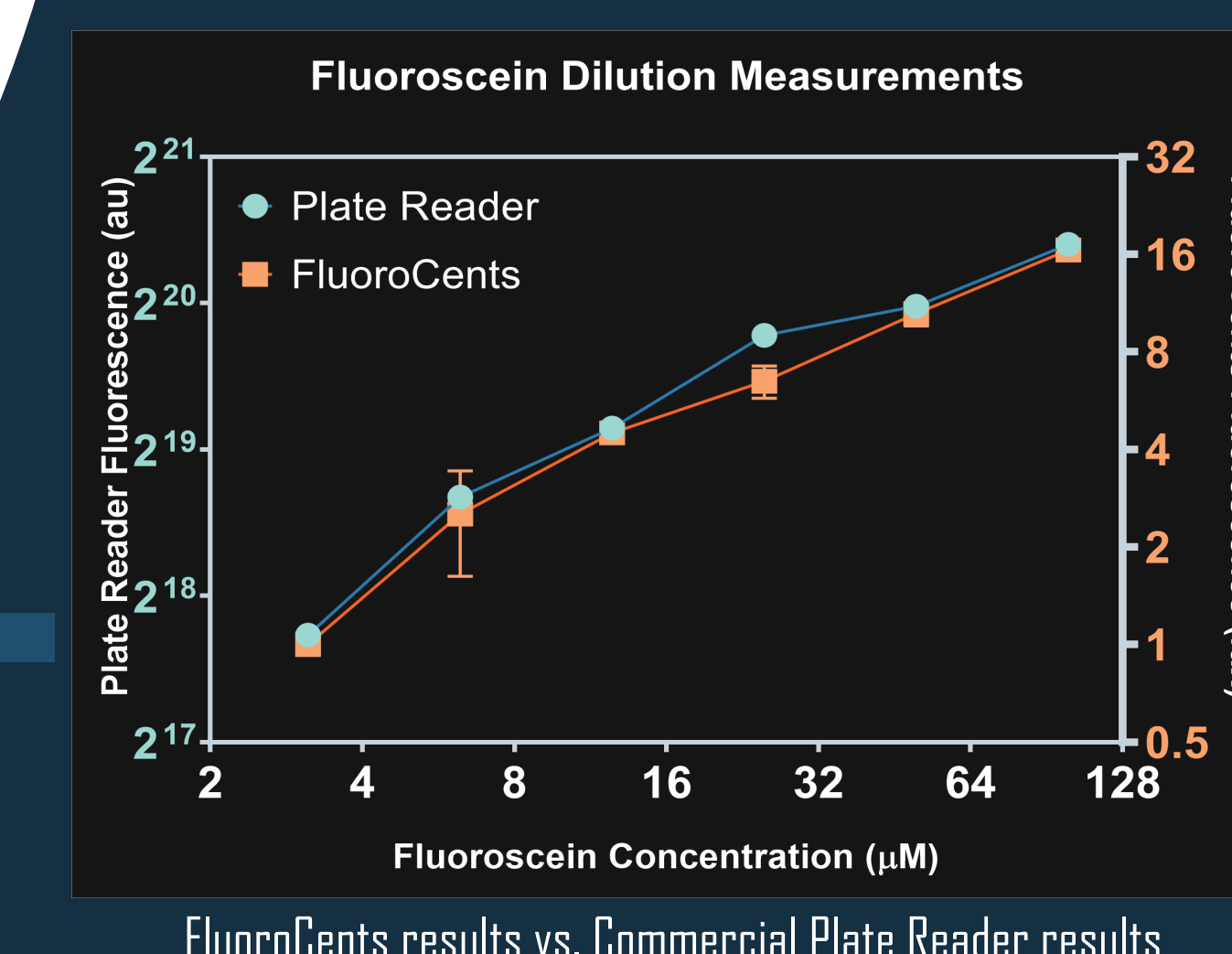
QUANTIFICATION: FLUOROCENTS

PURPOSE

Our low-cost fluorometer consists of a hardware case to hold the sample. The sample is attached to the ambient light sensor on Android phones. The device utilizes two filters positioned perpendicularly for calibration, similar to a typical fluorometer.

ADVANTAGES

The FluoroCents android app measures the lux value from a sample to determine the concentration of helminth egg DNA. FluoroCents costs less than \$2 to create and does not require constant electricity.



FluoroCents results vs. Commercial Plate Reader results



Assembled FluoroCents system

HUMAN PRACTICES

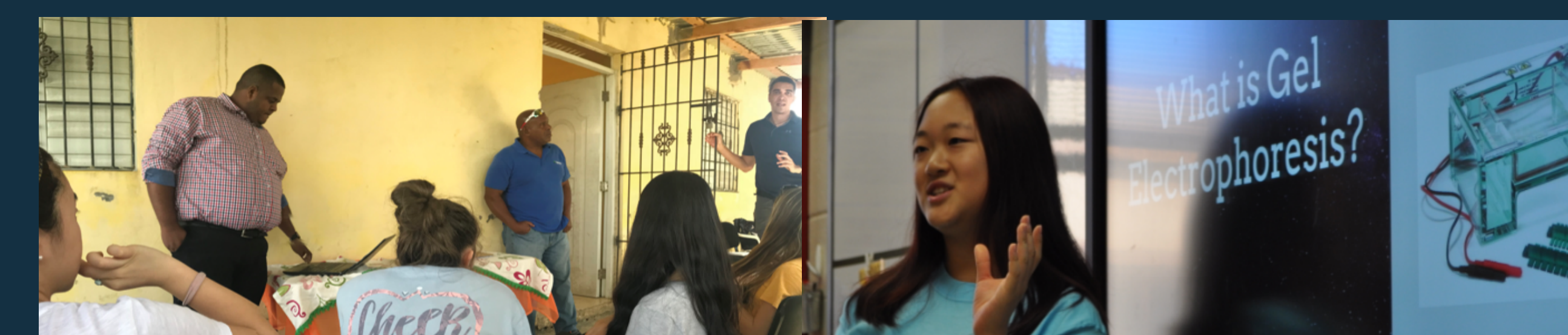
INTEGRATION

Dominican Republic

Dr. Martinez emphasized the need for frugal diagnostics and inspired us to focus on soil-transmitted helminths.

Georgia Tech

We created the Zin-Q Android app as a companion to their zinc biosensor. Our toehold switch and hardware devices were designed and optimized in their labs.



Dr. Rainier Martinez at Dominican Republic

Presentation at Biotech Summer Camp

Thirst Project

The Thirst Project helped maximize the impact of our marketing campaigns through social media.

Boehringer Ingelheim

Experts from Boehringer Ingelheim provided feedback on the feasibility and development for LABYRINTH.



QR Code for Lambert iGEM Wiki!

Donate to our page in the Thirst Project to fund a well in Eswatini!

