





Our Inspiration.

People on the move

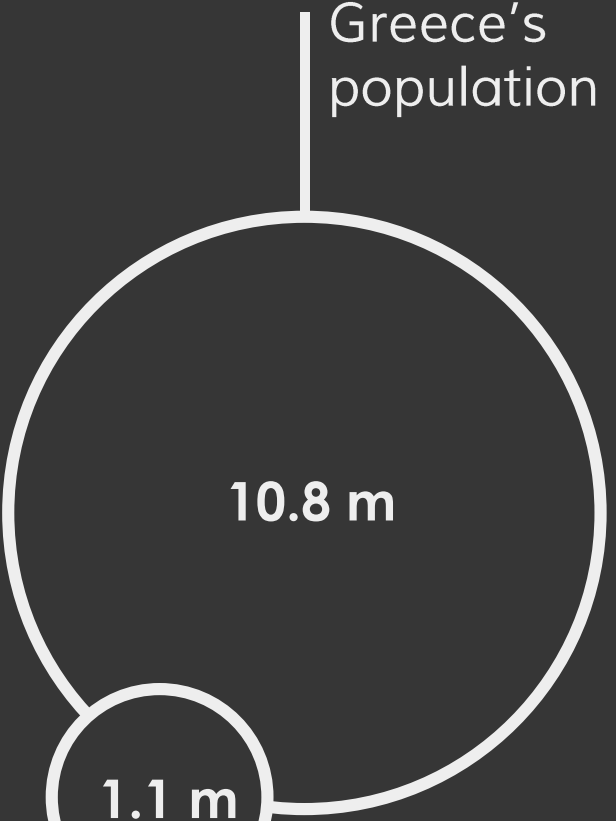
70.8m Forcibly displaced
people worldwide



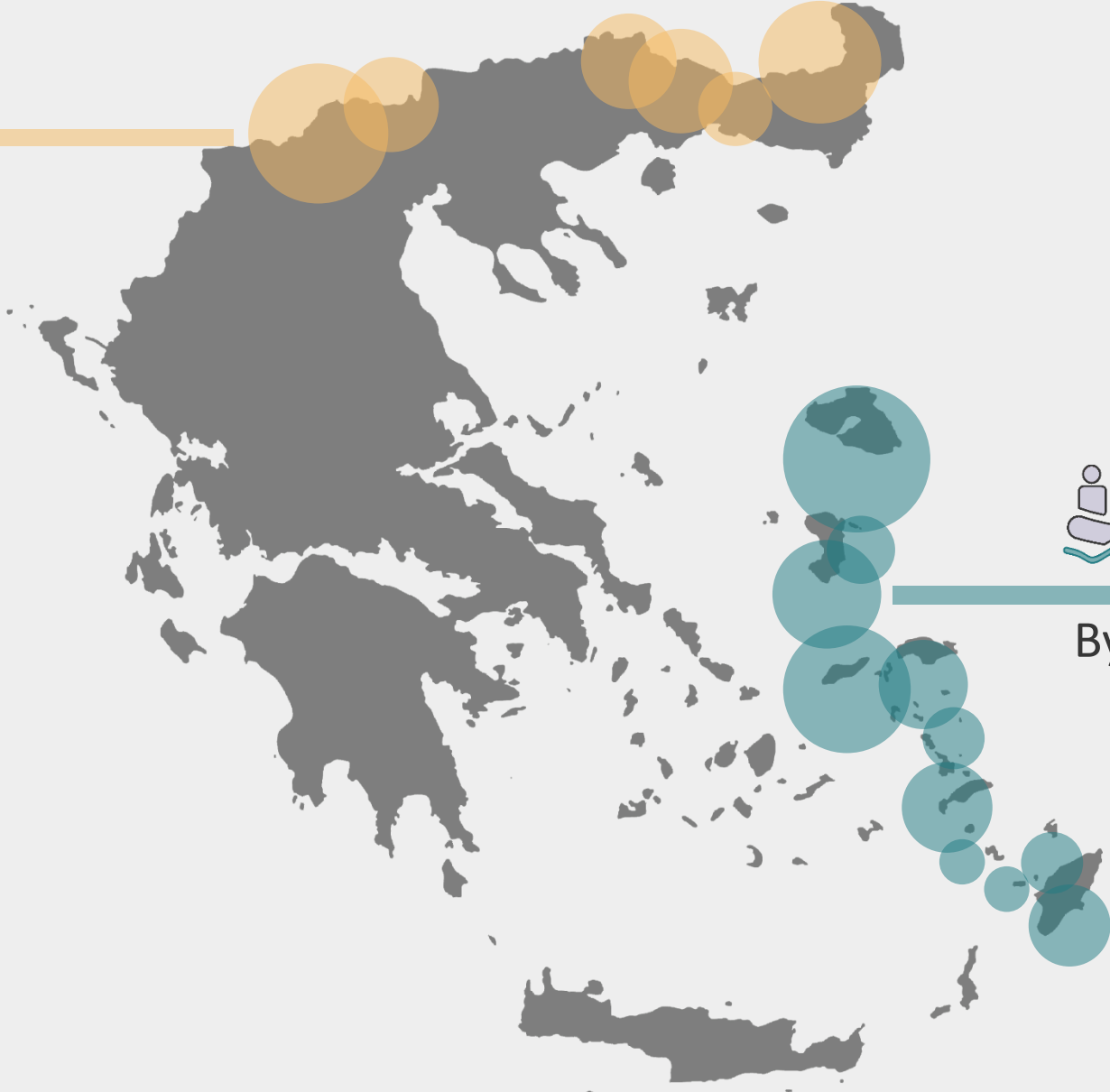
People on the move



People on the move



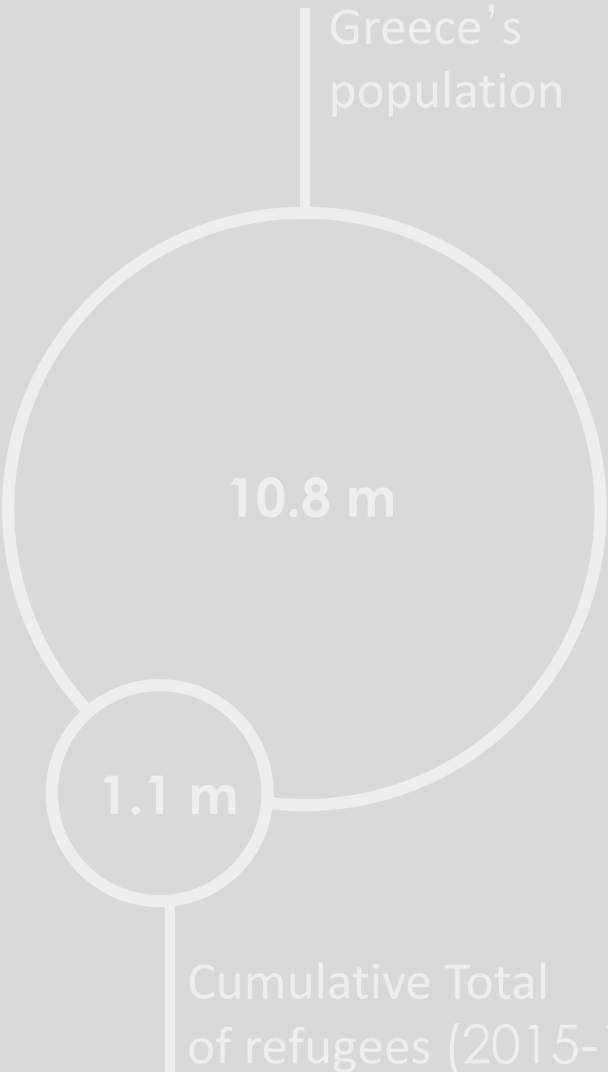
By Land



By Sea

Our inspiration.

People on the move



By Land

Limited access to healthcare



By Sea

Our inspiration.

A lethal disease

Tuberculosis (TB)

1 of **10**
causes of death
worldwide

*Mycobacterium
tuberculosis*



Active
or
Latent

A sneaky killer



36%

of people infected
with TB were left

undiagnosed

in 2017

WHO, TB Global Report (2018)

**We present you a new era of TB
diagnostics**



DYSSSEE

Simple. Safe. Universal.



The **type** that is important to be detected for screening purposes is **active TB**.

Dr. Simona Karampela



ΓΕΝΙΚΟ ΝΟΣΟΚΟΜΕΙΟ
ΝΟΣΗΜΑΤΩΝ ΘΩΡΑΚΟΣ ΑΘΗΝΩΝ
Η ΣΩΤΗΡΙΑ

A large, solid orange square is positioned on the left side of the image, serving as a background for the text.

Project Design.

Our Considerations

Biological
sample



Urine

Biomarker



IS6110 gene

Safety



Cell free
system

2 MODULES

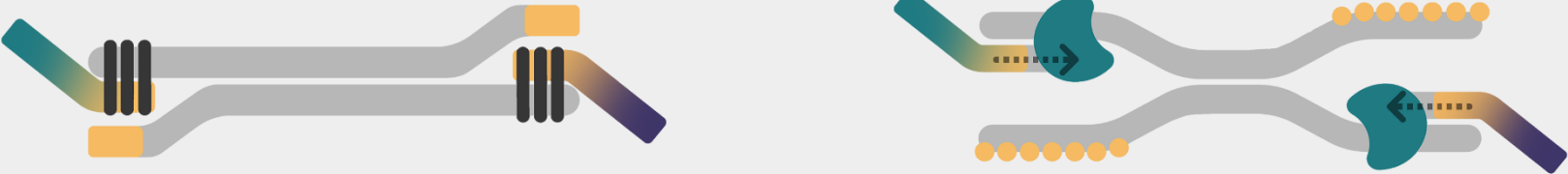


**DETECTION
MODULE**

**READOUT
MODULE**

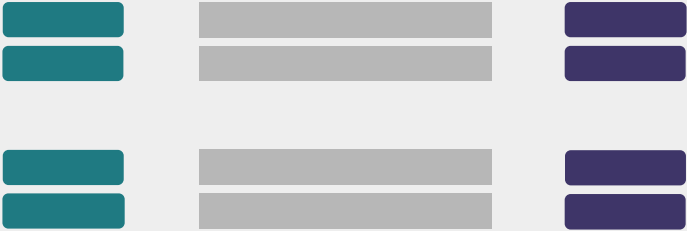
DETECTION MODULE

Recombinase Polymerase Amplification (RPA)

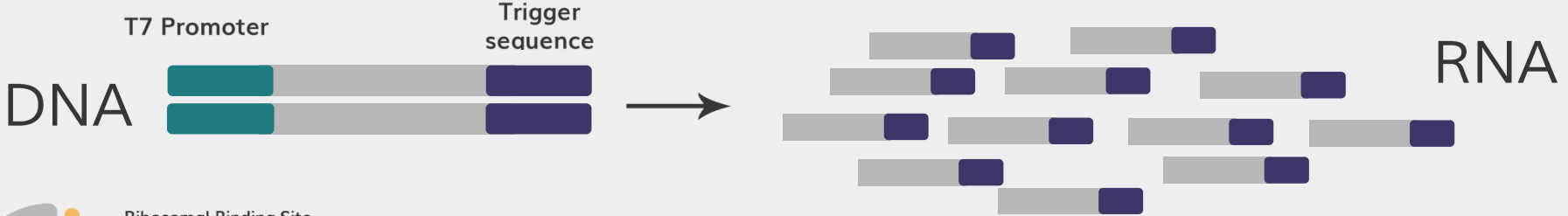


T7 Promoter

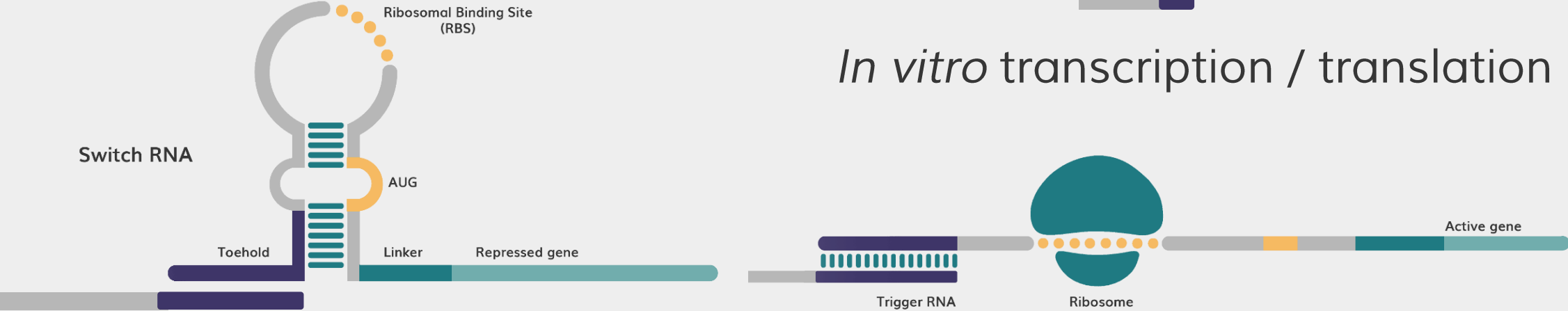
Trigger sequence



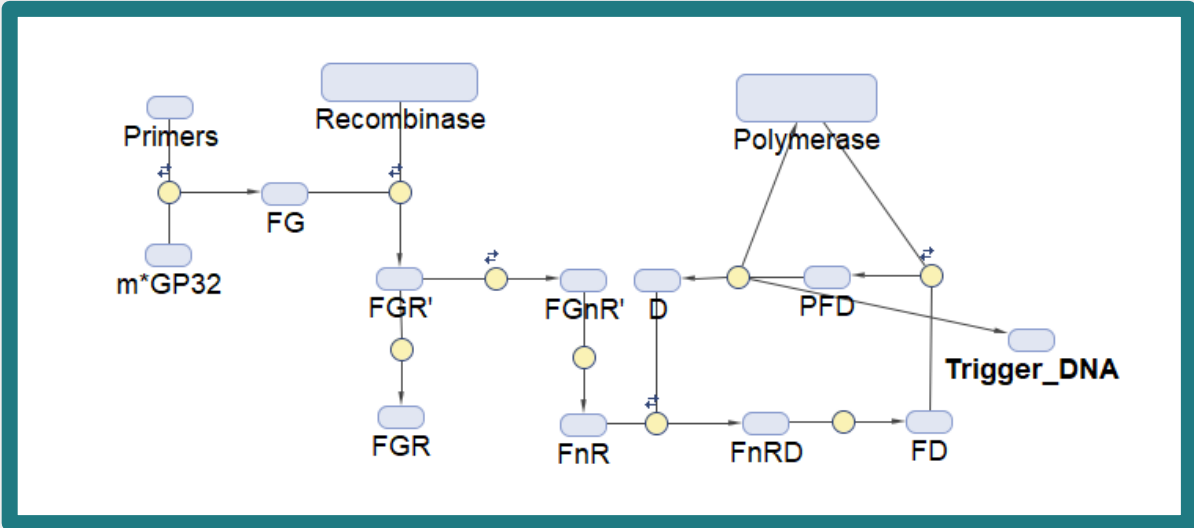
READOUT MODULE



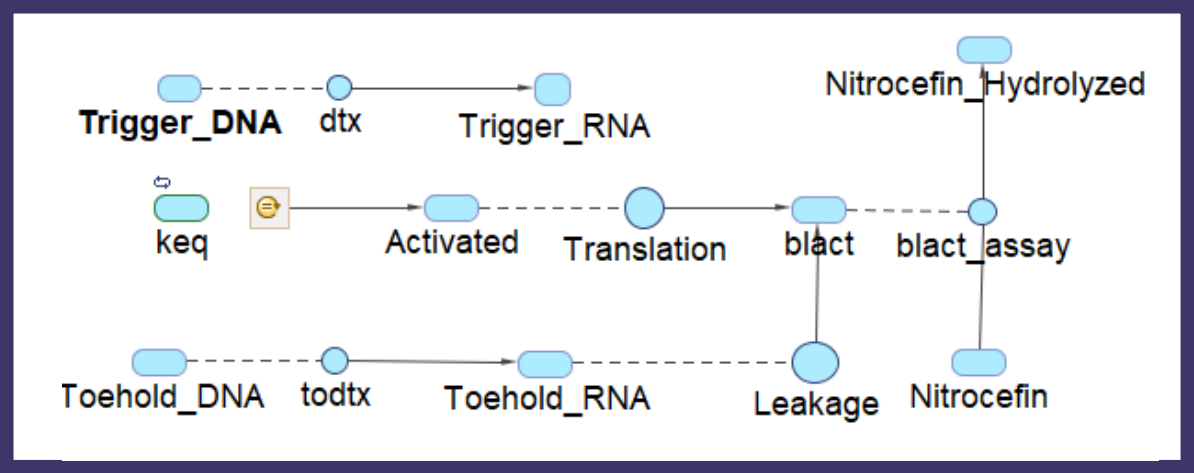
In vitro transcription / translation



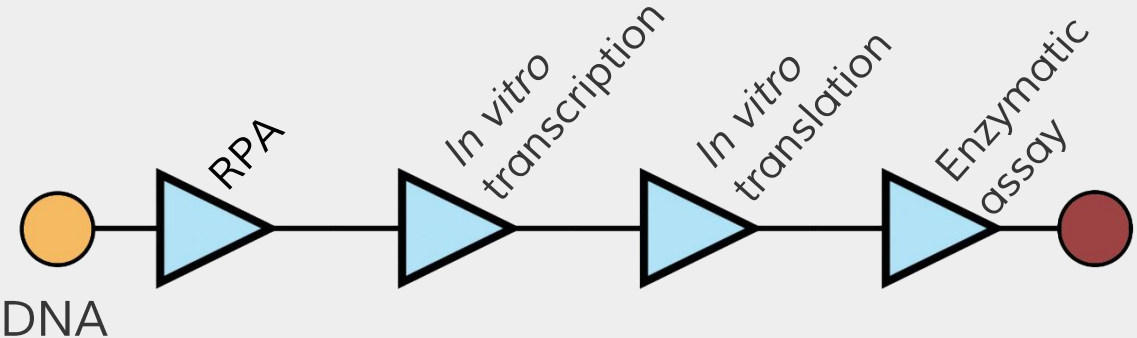
2 MODULE SYSTEM



DETECTION MODULE.



READOUT MODULE.

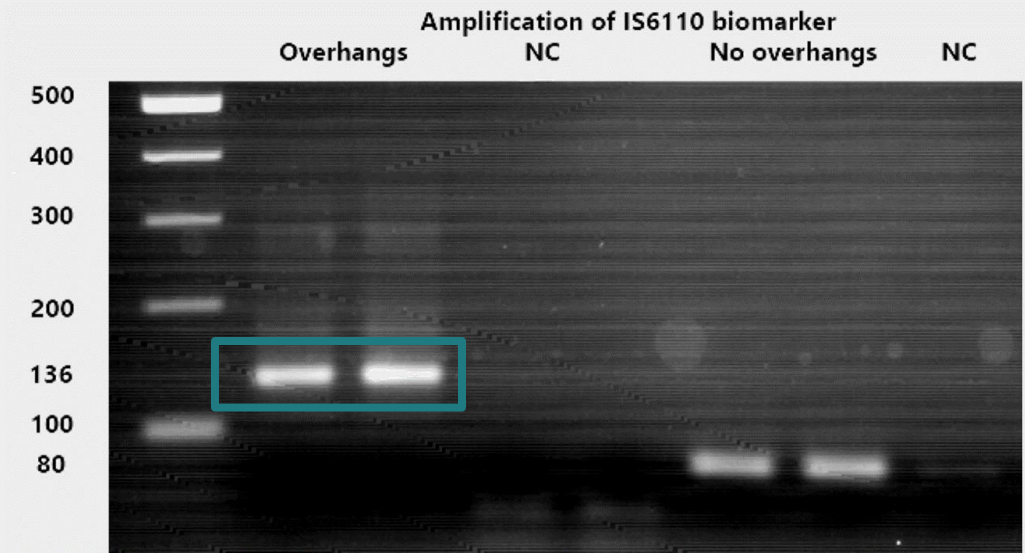


A large teal square is positioned on the left side of the slide, partially overlapping the text.

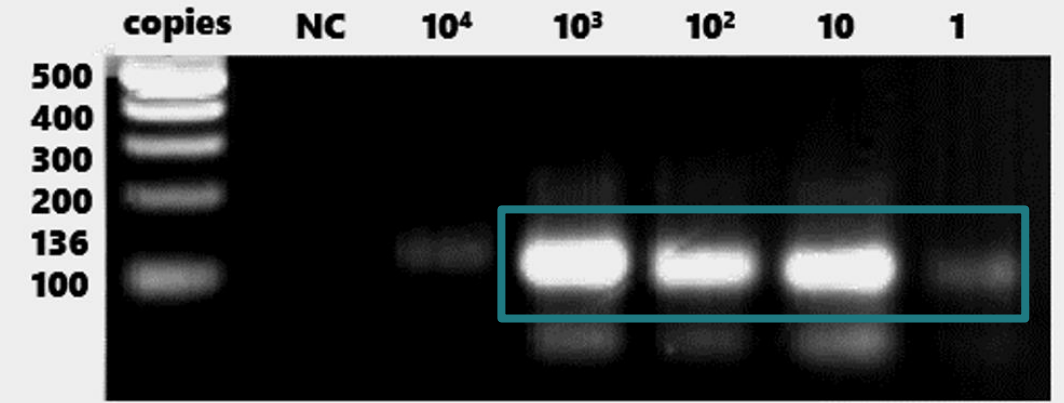
Results.

DETECTION MODULE

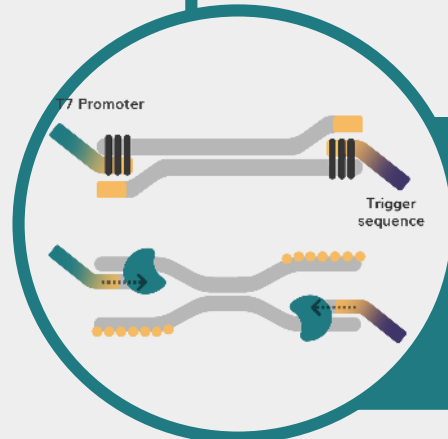
Validating the sensitivity



PCR amplification



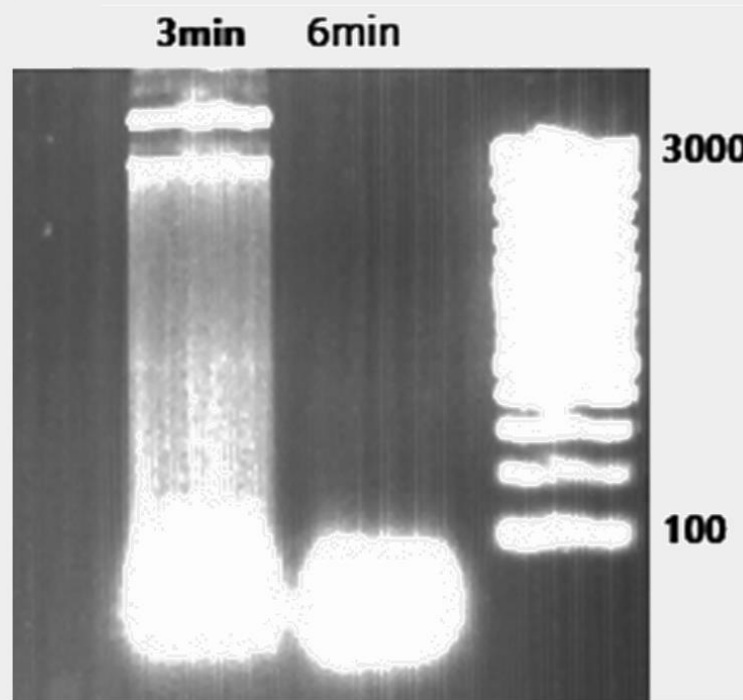
RPA amplification



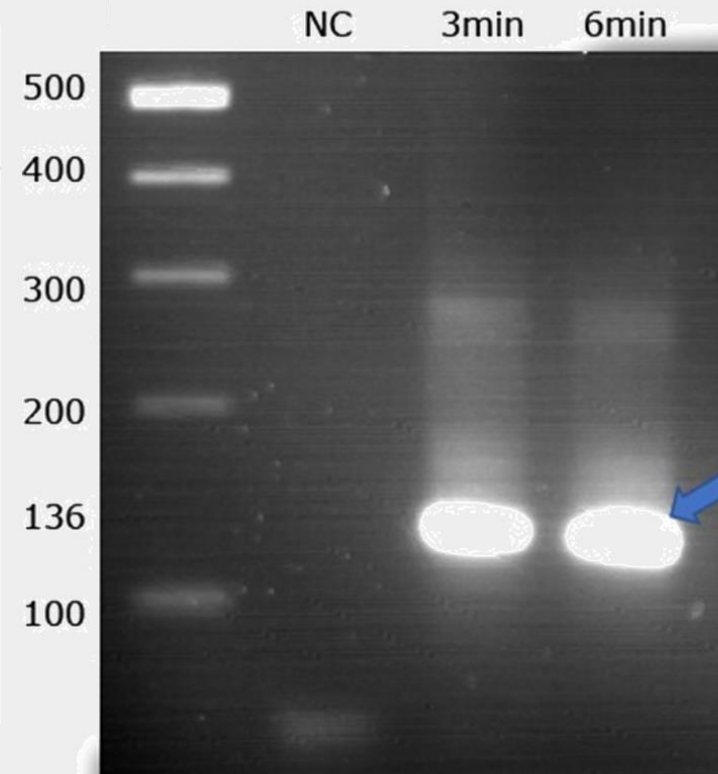
Optimal Conditions
5 minutes | 42 °C

DETECTION MODULE

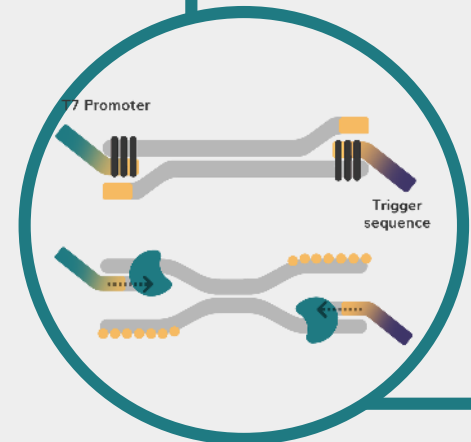
Validating the functionality



Random fragmentation of IS6110 using DNase1



Amplification of the fragmented part

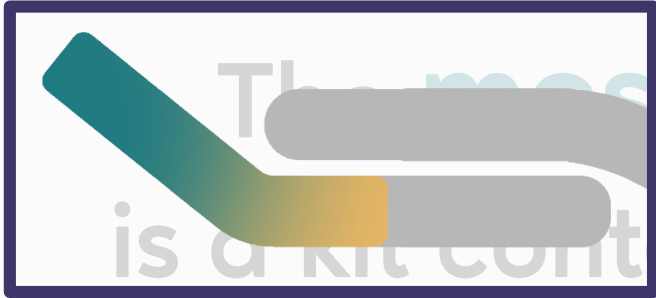




The **most needed tool** in field
is a kit containing diagnostic tests
not only for TB but for **other diseases**
commonly appeared as well.

Dr. Apostolos Veizis



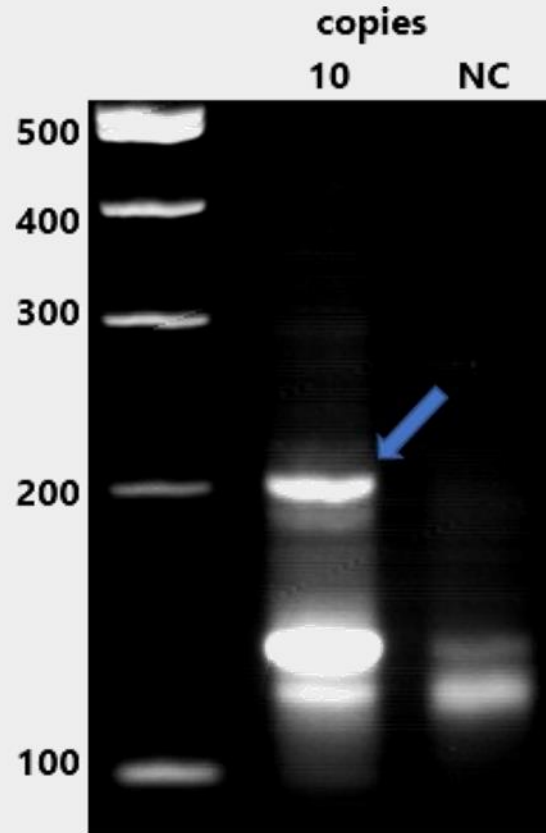


The most needed in the field is a kit containing diagnostic tests not only for TB but for other diseases commonly appeared as well.

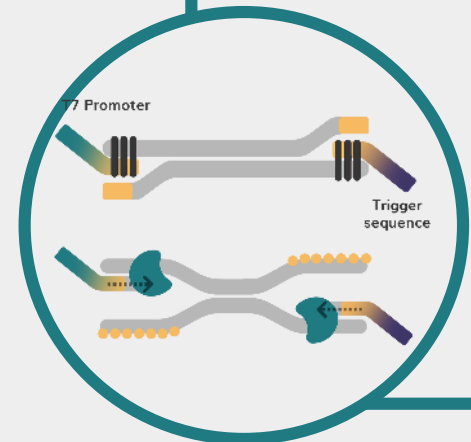
Dr. Apostolos Veizis

DETECTION MODULE

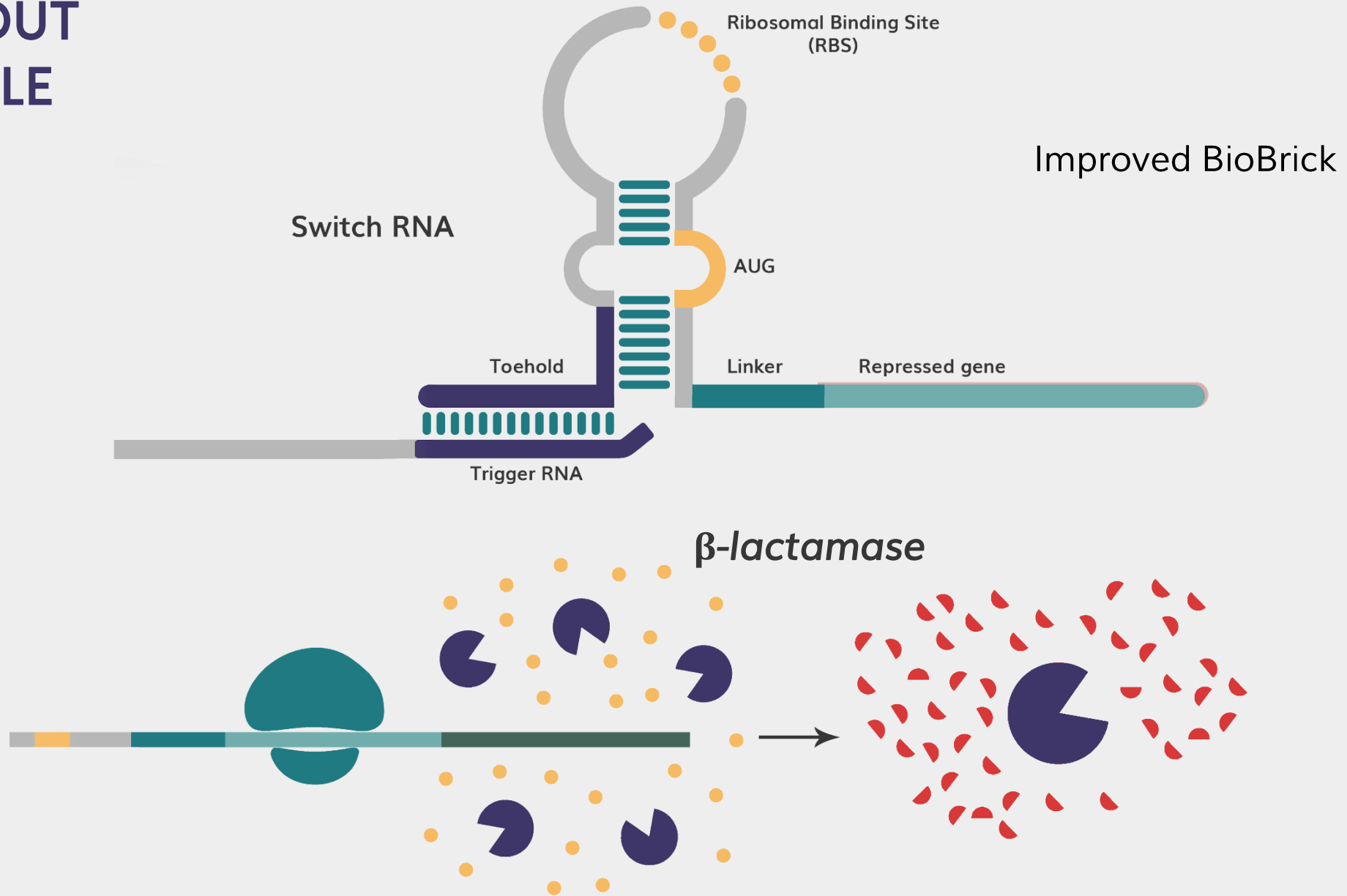
Validating the universality



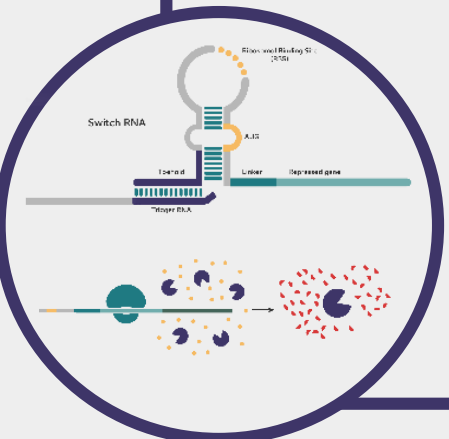
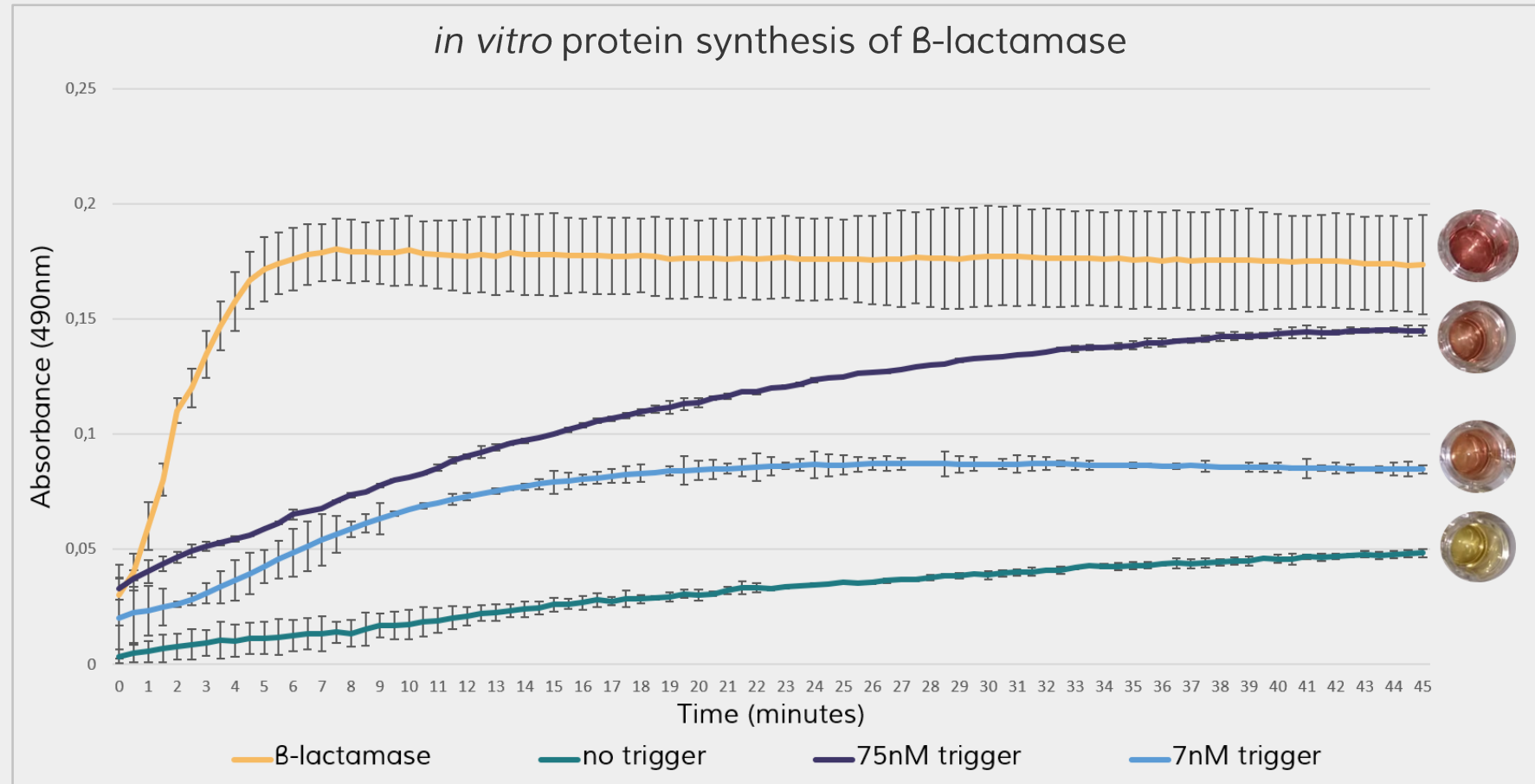
Detection of an HBV genome fragment using RPA



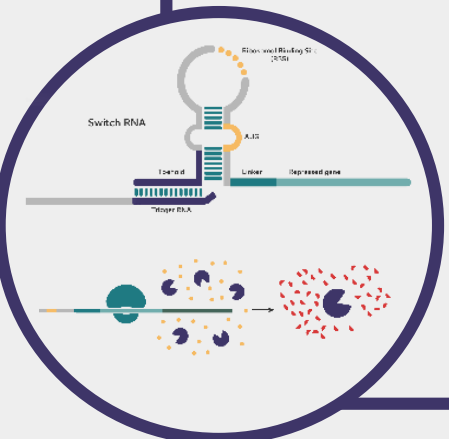
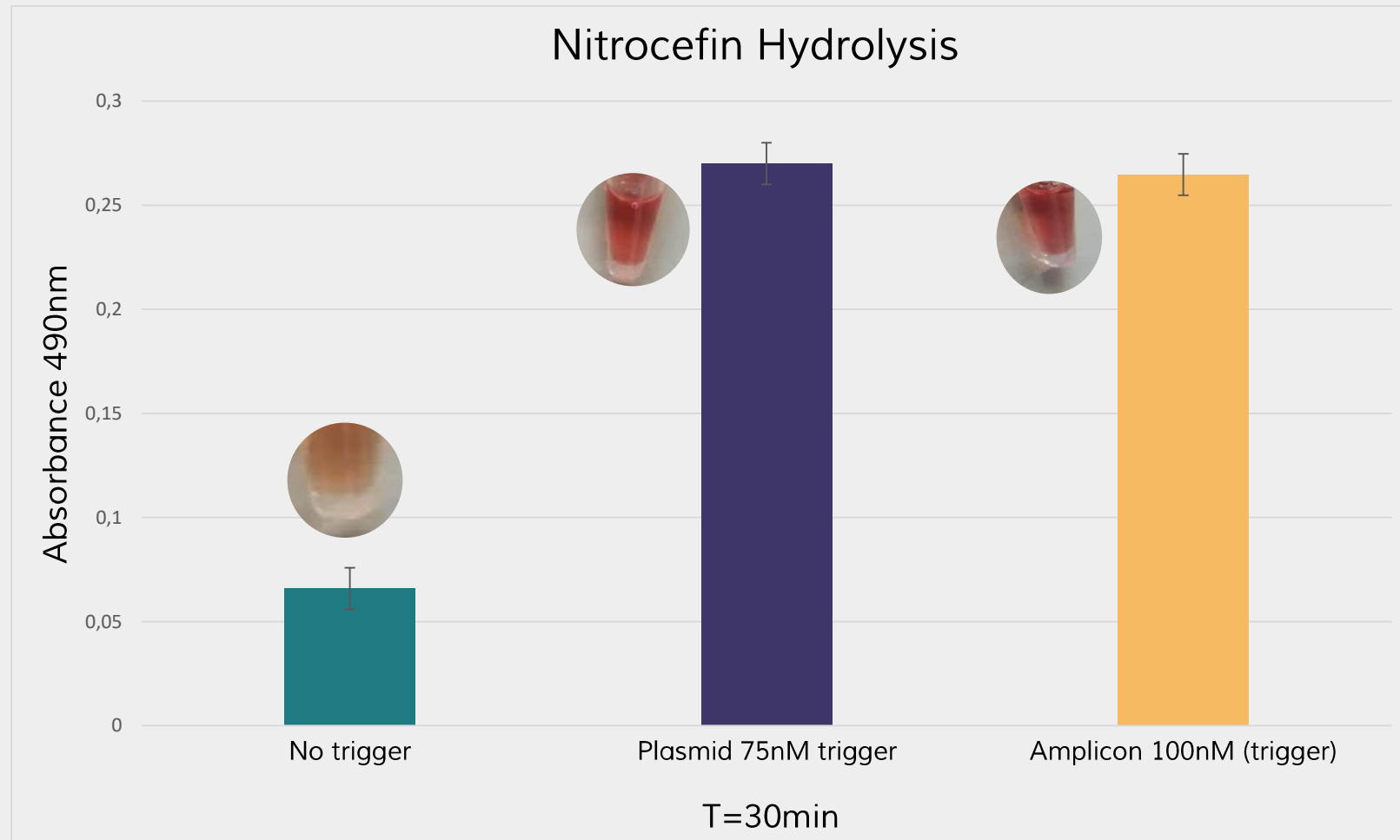
READOUT MODULE



READOUT MODULE Validation

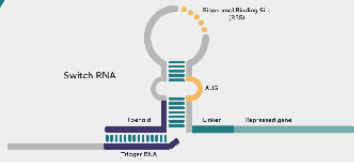


READOUT MODULE Validation



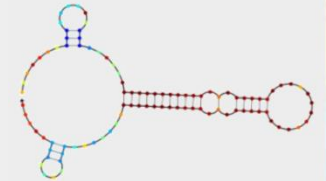
READOUT MODULE

Engineering new toehold switches

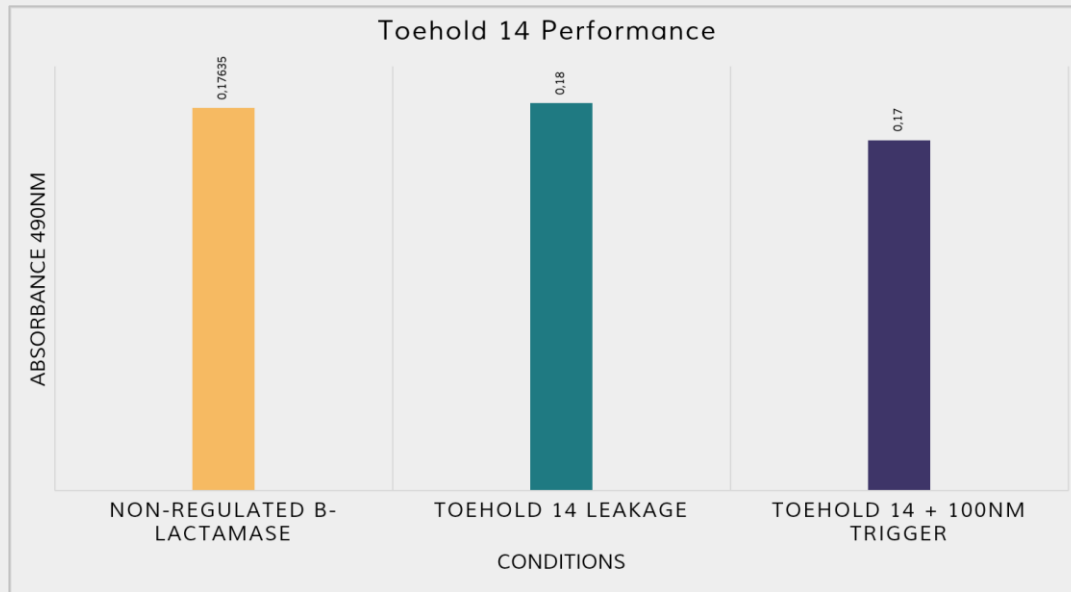


hyperthermophile
organisms

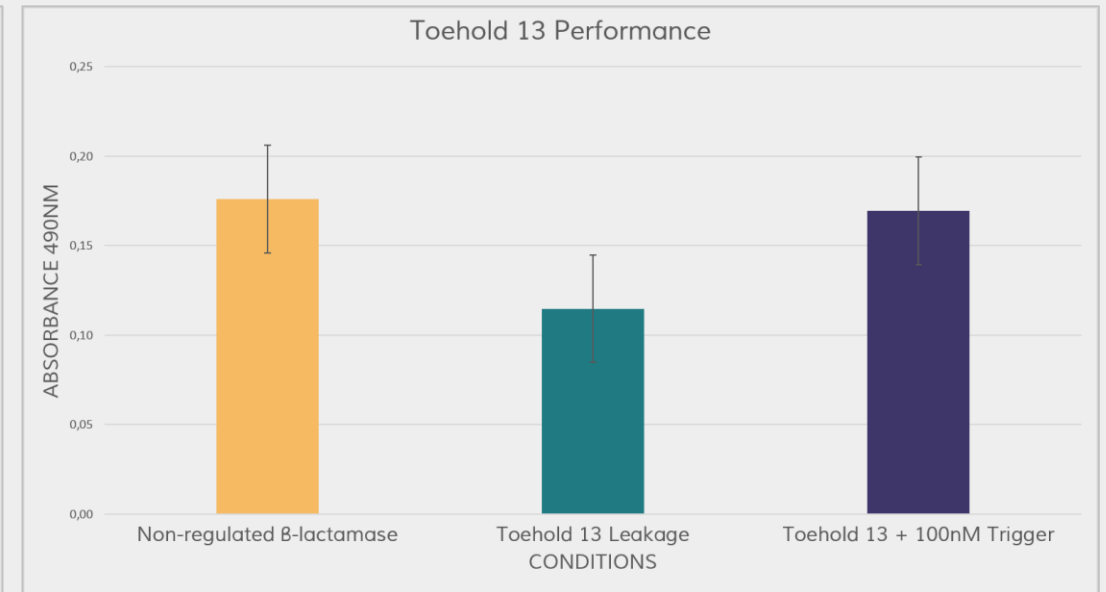
NUPACK



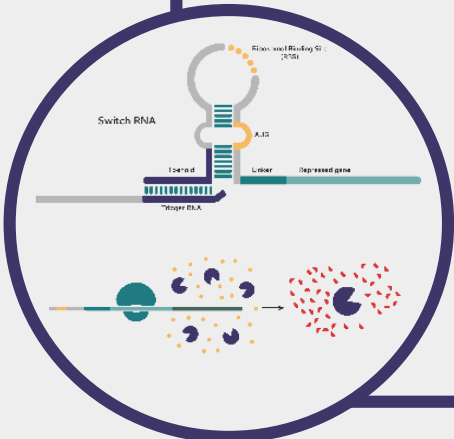
READOUT MODULE Validation



Toehold 14



Toehold 13





READOUT

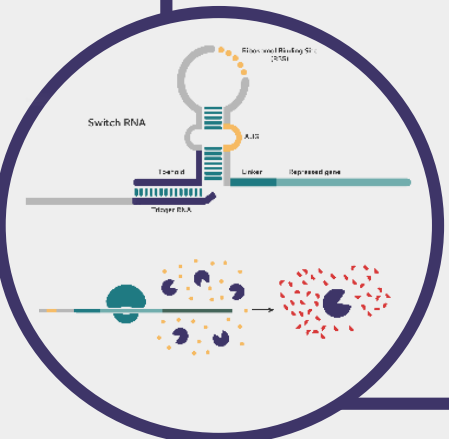
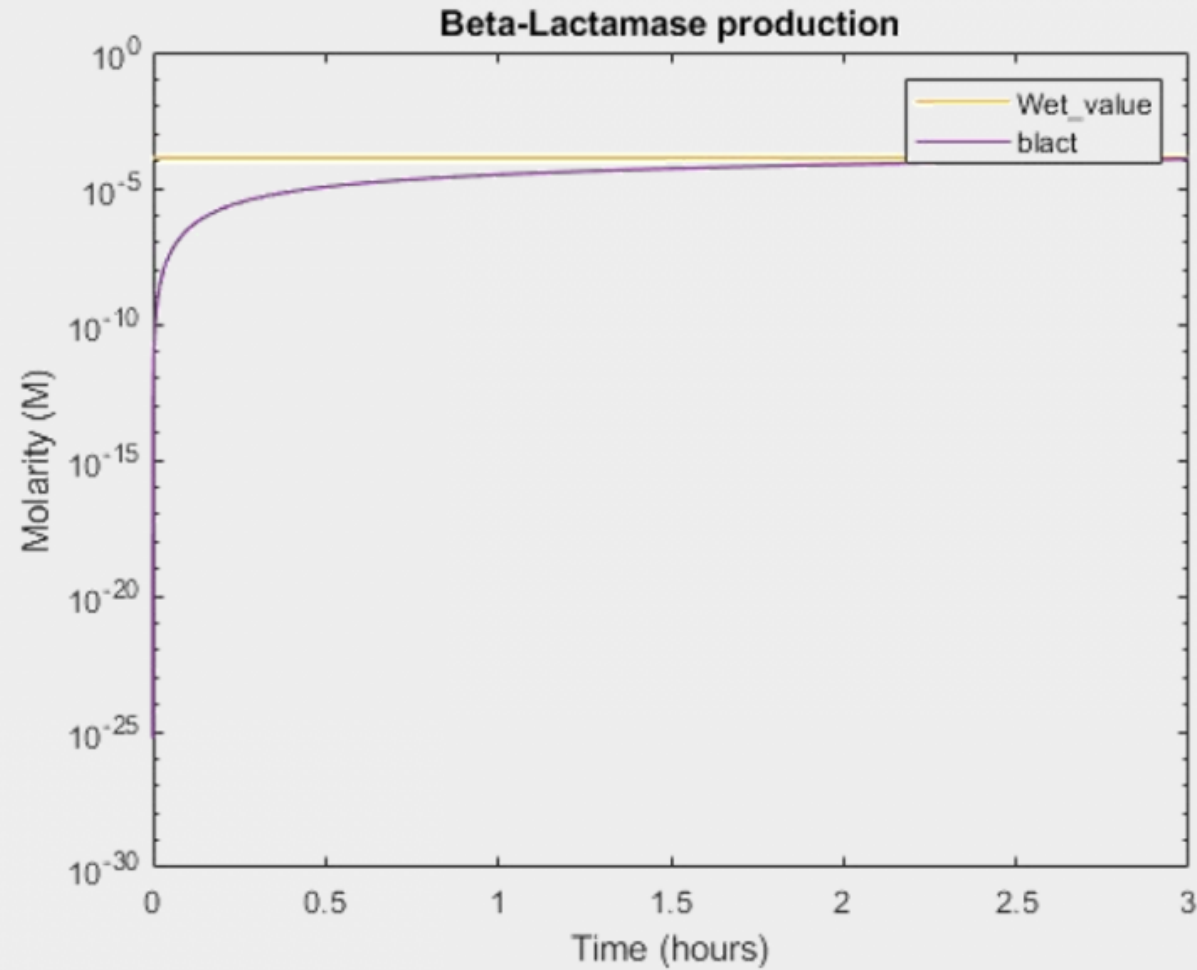
MODULE

Modeling

Results

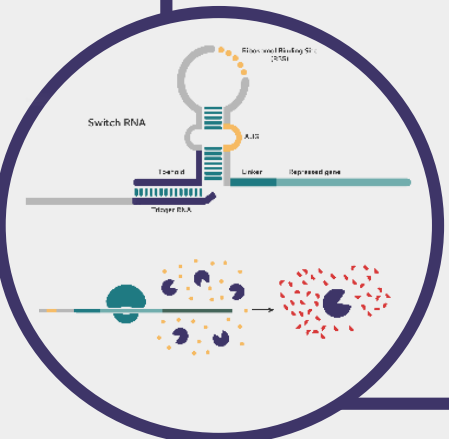
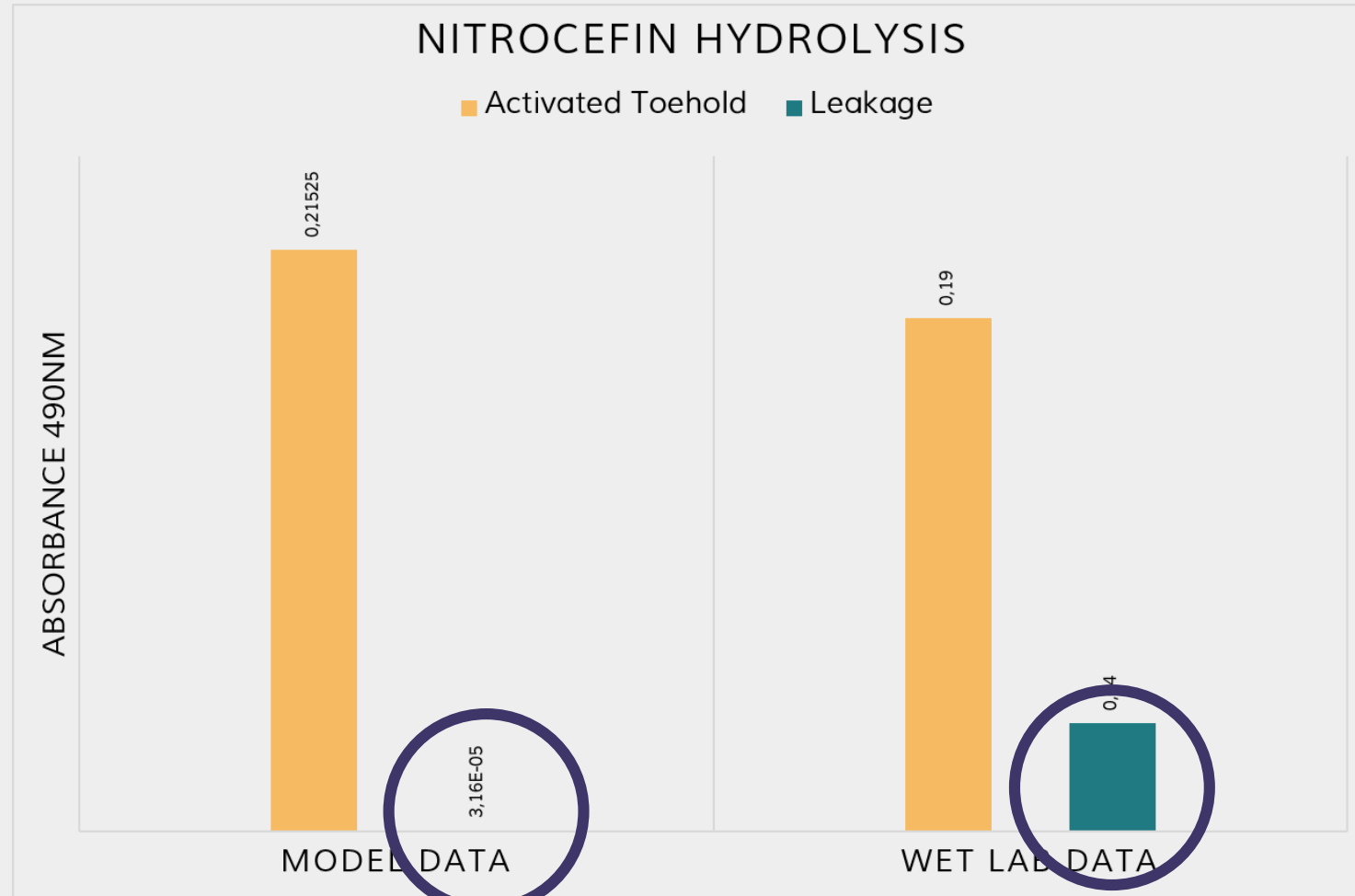
READOUT MODULE

Minimum Time Prediction

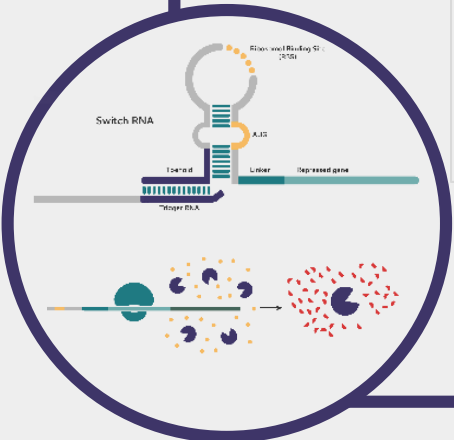
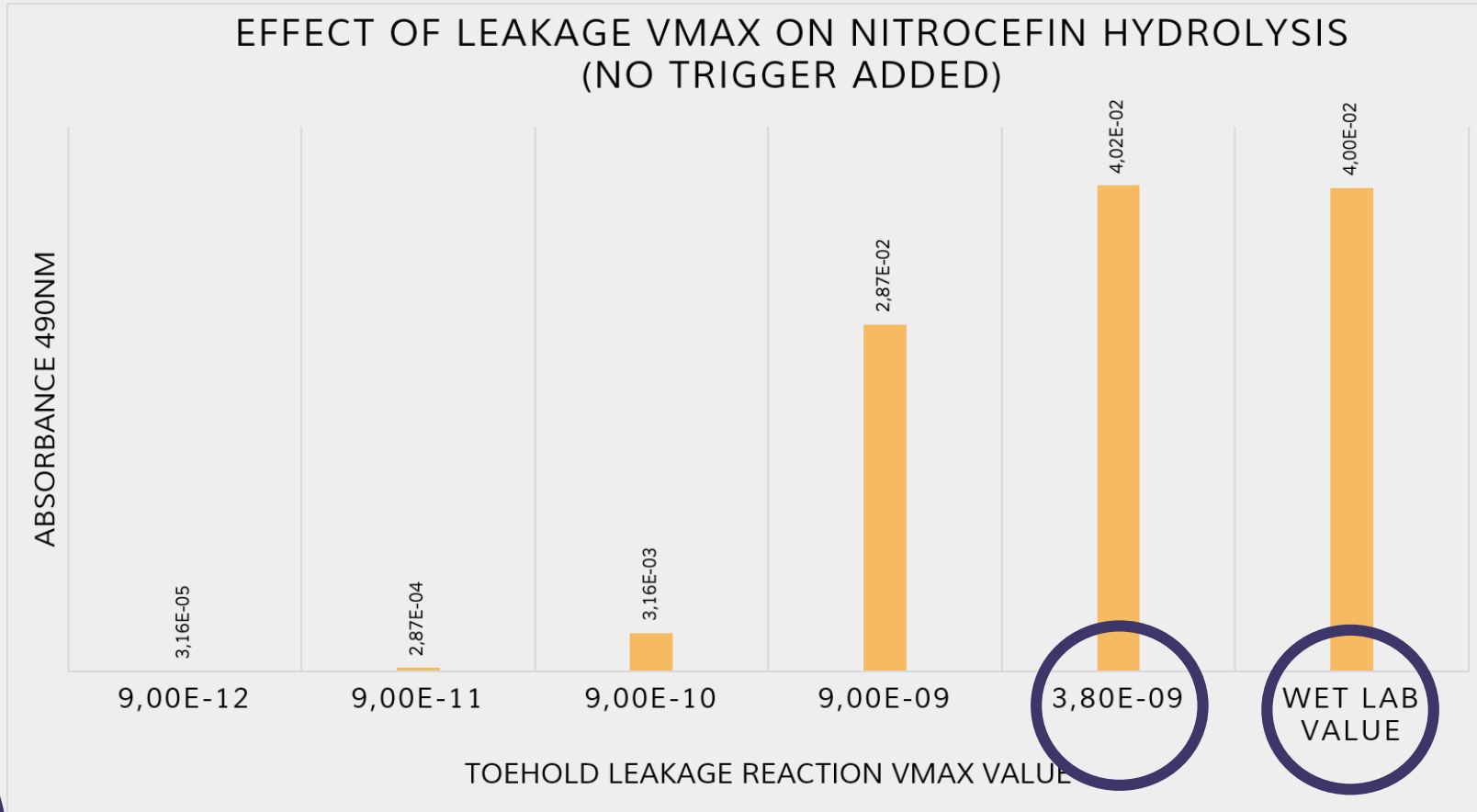


READOUT MODULE

Minimum Time Prediction

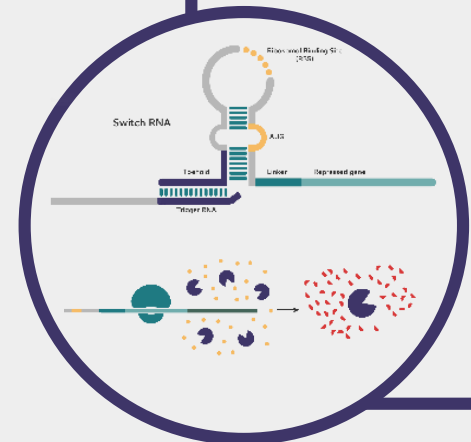
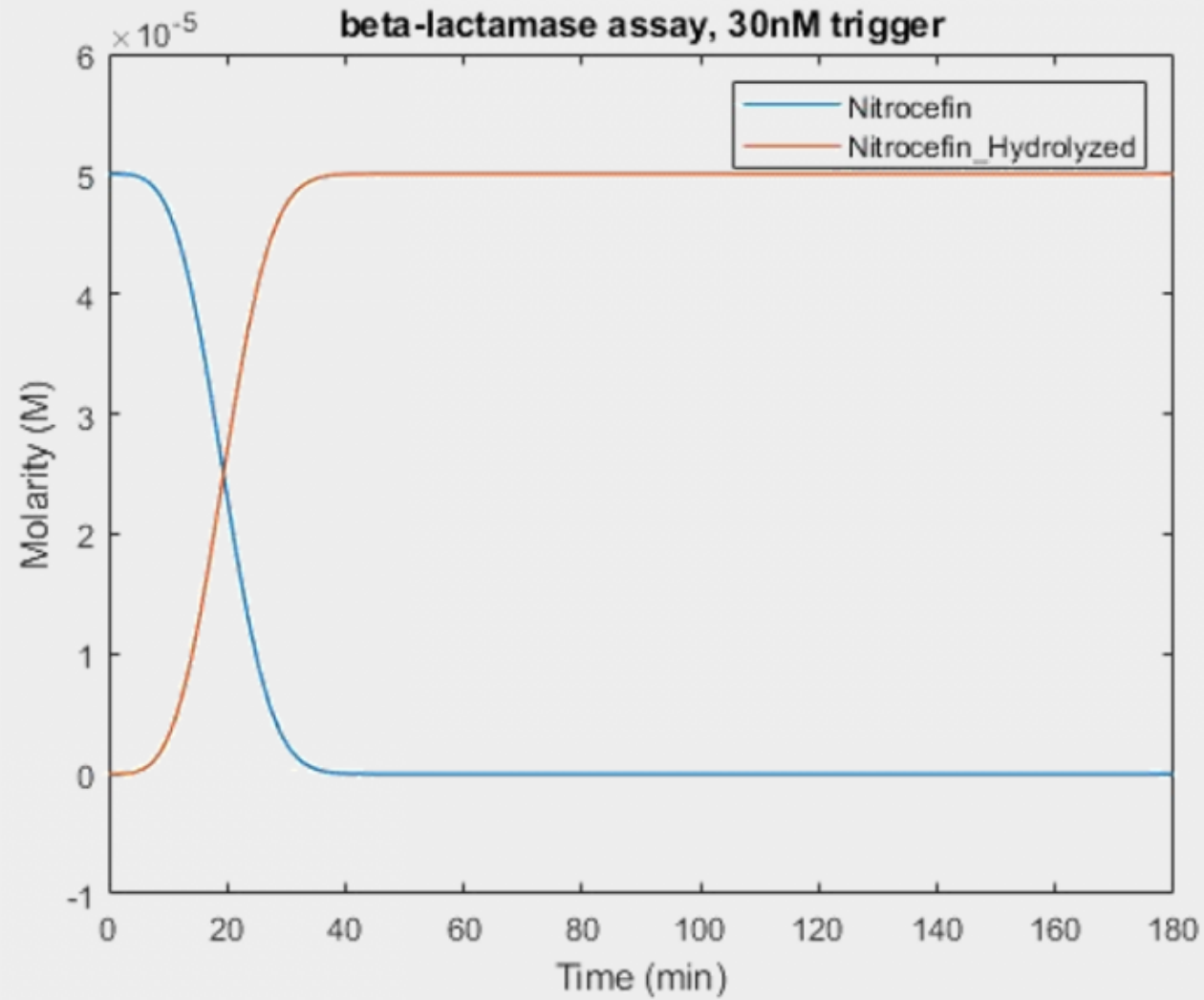


READOUT MODULE Calibration

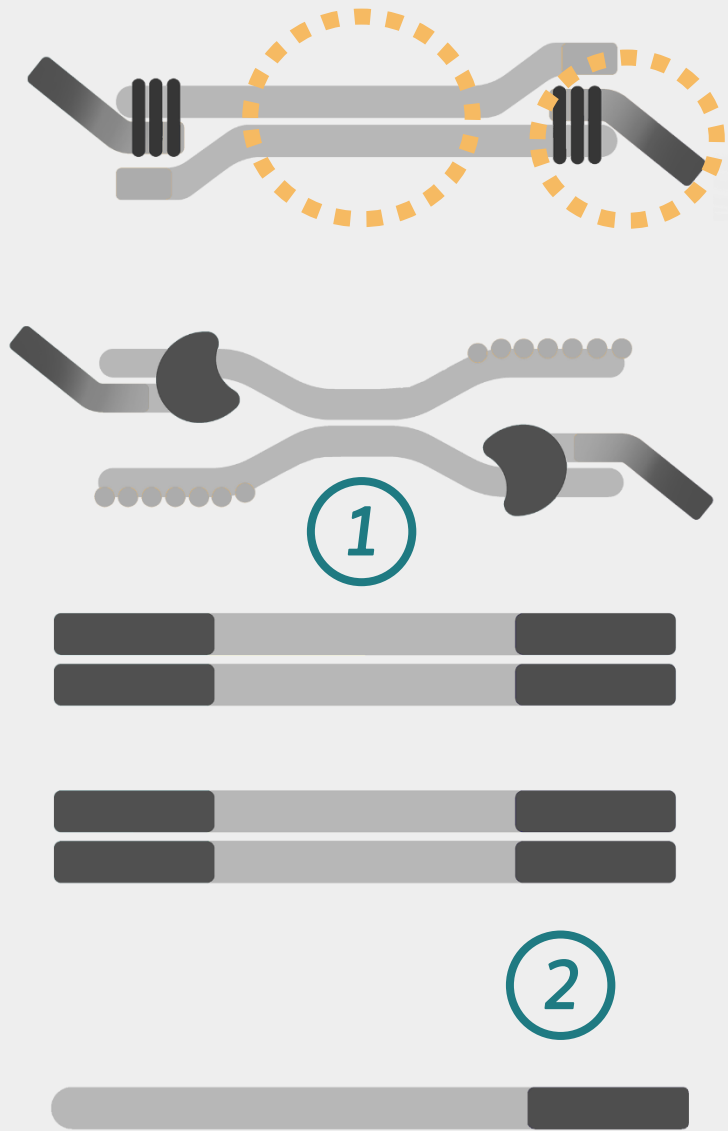


READOUT MODULE

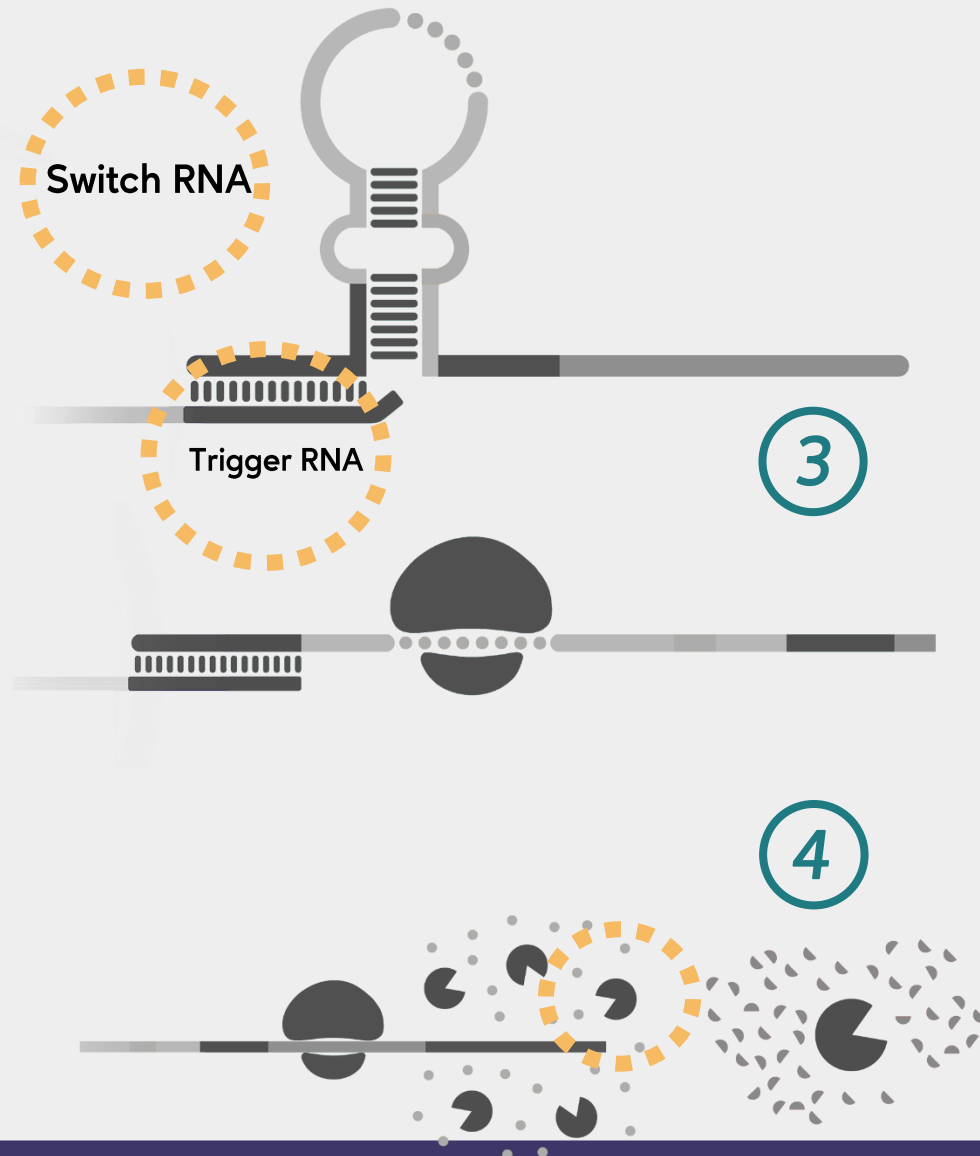
Minimum Time Prediction



DETECTION MODULE



READOUT MODULE



2 modules
system

4 signal
amplifiers

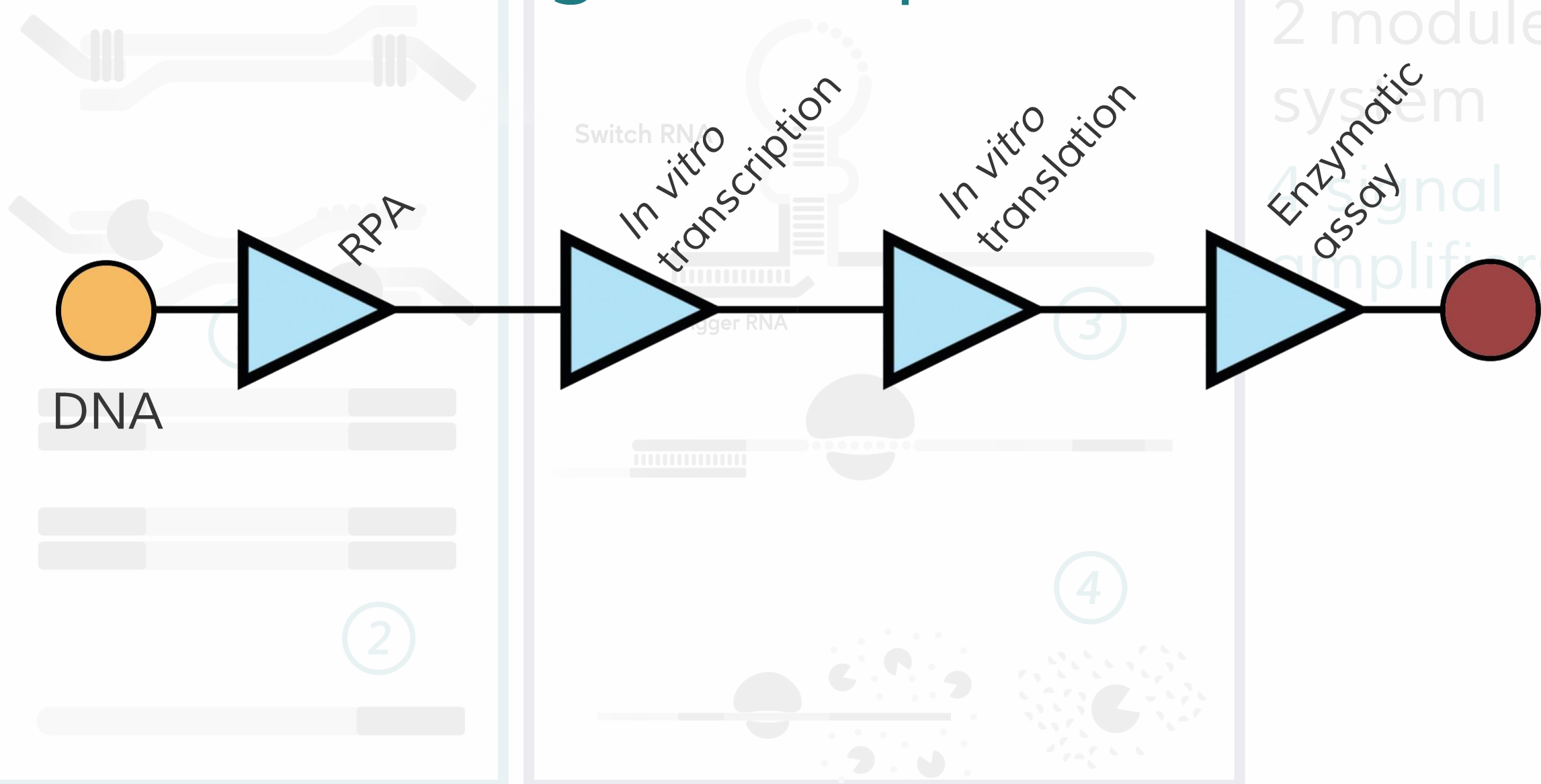
1 copy of
DNA

18 new
BioBrick
parts

DETECTION MODULE

READOUT MODULE

4 signal amplifiers





Beyond the Lab.

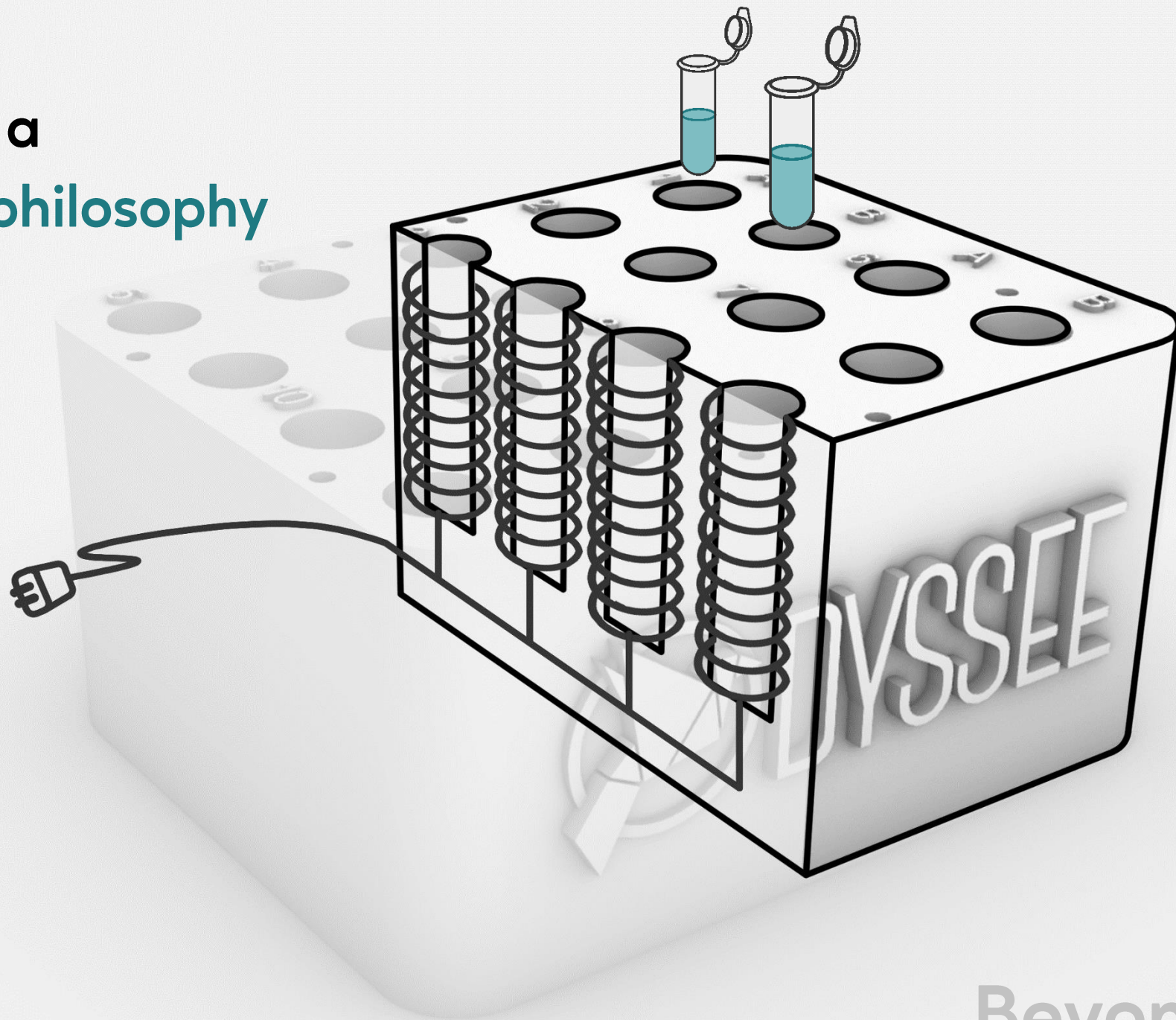
Application



Reception & Identification Centres

Triage test
a screening test

Welcome to a
2 tubes philosophy



Beyond the Lab.

Deployment at a refugee facility



Beyond the Lab.

Deployment at a refugee facility



Beyond the Lab.

Product guide



**Making
our system
accessible**

Arabic دليل المنحج

French Fiche produit

Urdu پروڈکٹ گبئیٹ

Beyond the Lab.

Healthcare model



Inclusive,
intersectoral &
multidisciplinary
approach to
migration

Beyond the Lab.

Entrepreneurship

reaching
key populations



H.F.R.I.
Hellenic Foundation for
Research & Innovation



Beyond the Lab.



Education &
Public
Engagement

Beyond the Lab.

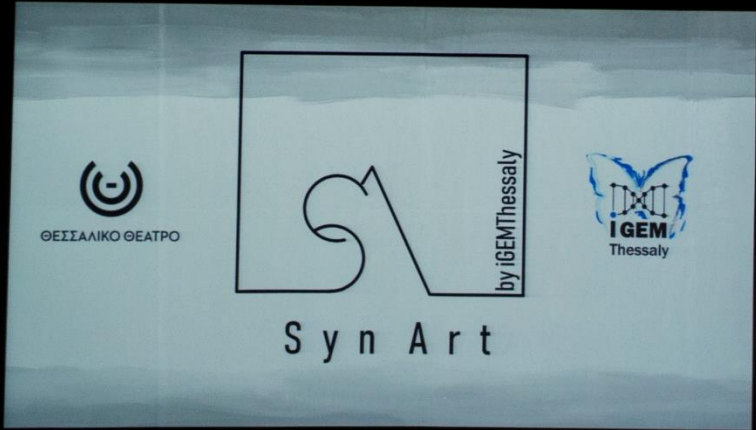


SynArt
by
iGEM Thessaly

SynArt
by
iGEM Thessaly



SynArt
by
iGEM Thessaly



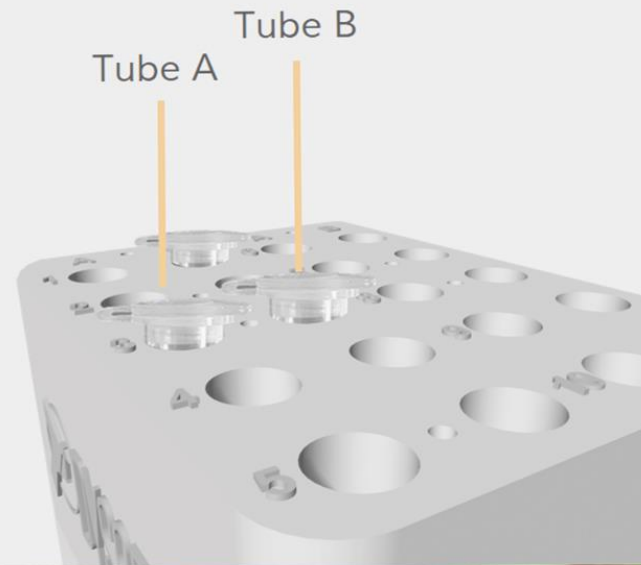
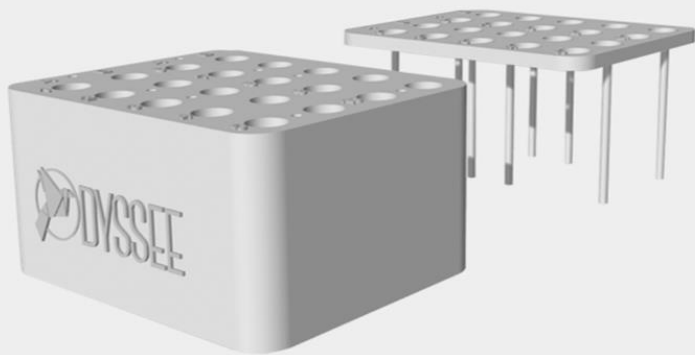
SynArt
by
iGEM Thessaly



Implementation



Welcome to the
2tubes Philosophy
Simple. Safe. Specific.



Science without borders



First Greek Meetup



First Greek Meetup

Supported by | IKY | EFOM | OMIC ENGINE



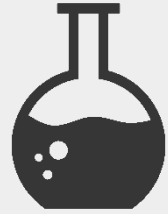
First Greek Meetup



First Greek Meetup



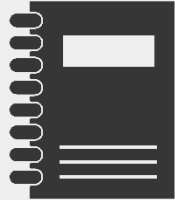
Achievements



Create a **universal platform**



Design of **Biosafety**



Develop a **healthcare model**



Explore **entrepreneurship**



Promote **science without borders**



Maria

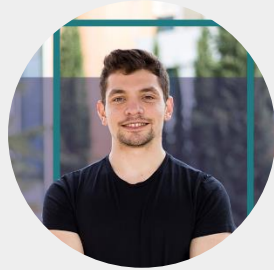


Afroditi

The people behind **ODYSSEE**



Athina



Nick



Vasiliki



Eleftheria



Nikoleta



Leandros



Xenia



Theodore

Thank you!



Pls, Instructors & Advisors

Laboratory of Plant &
Environmental Biotechnology

Laboratory of Molecular
Biology & Genomics



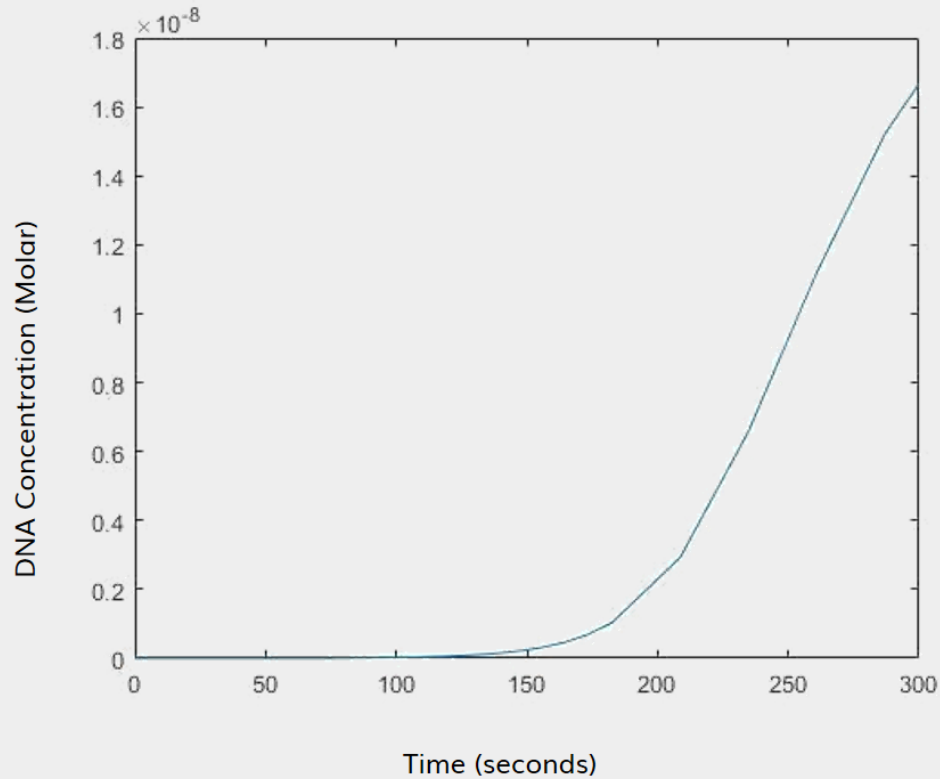
Lab Supplies
Scientific



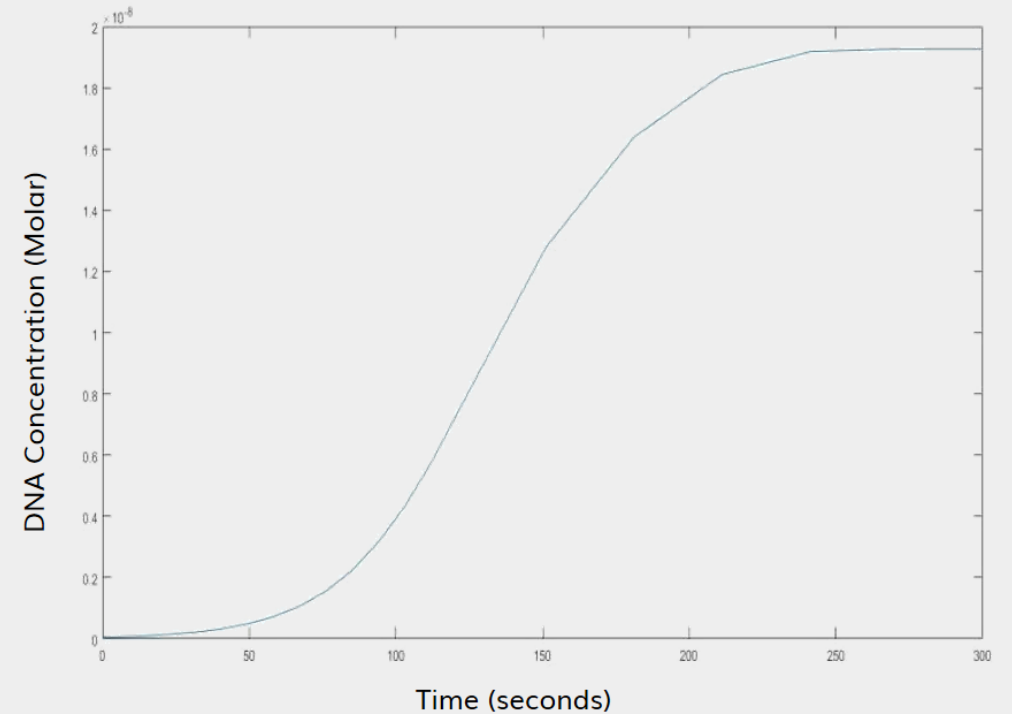
NOVARTIS



DNA Amplification Modeling Results

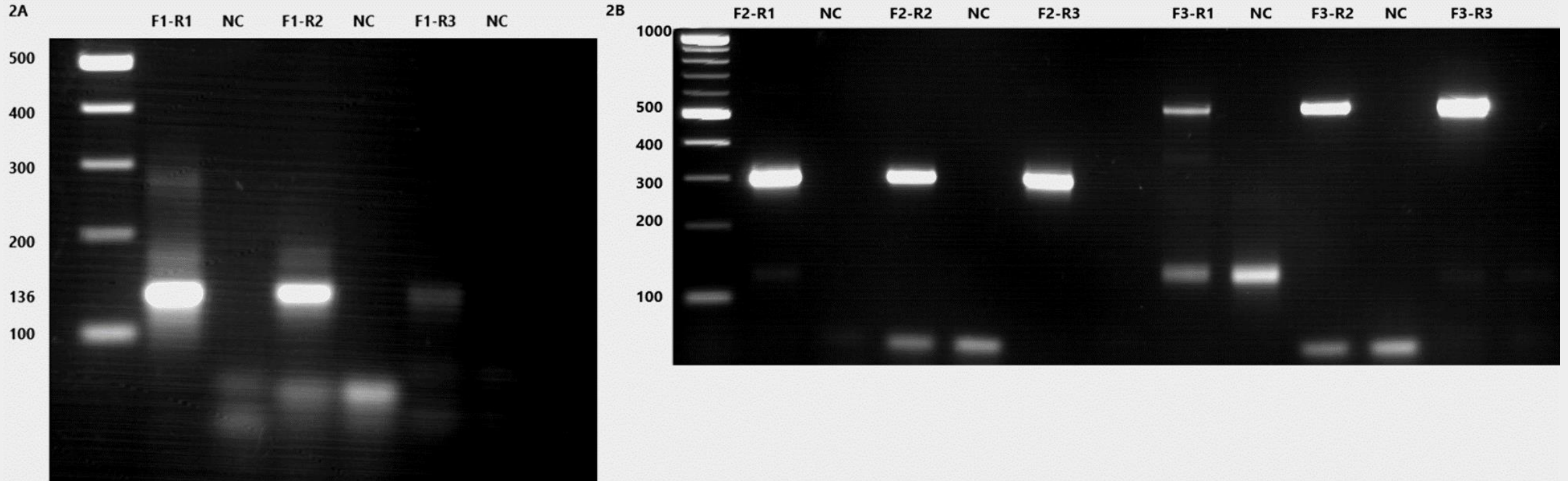


Concentration of amplified DNA (M) using $2.94 \times 10^{-17} \text{ M}$ as DNA input during time (sec)



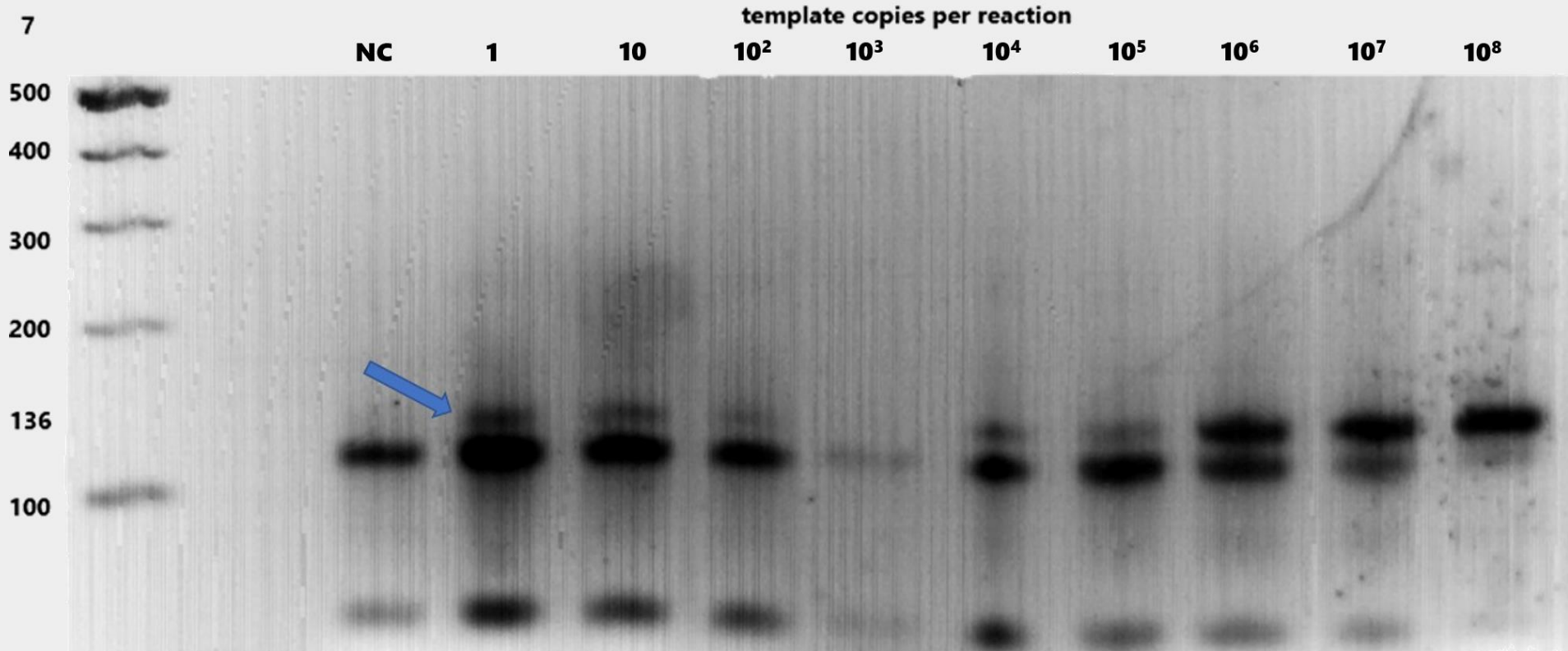
Concentration of amplified DNA (M) using $5.8 \times 10^{-11} \text{ M}$ as DNA input during time (sec)

Primers Screening



A) Primer screening of 3 primer combinations with the 1st Forward primer, which correspond to ~136bp amplified product lengths and different annealing lengths for the reverse primers (32, 36 and 39 bp annealing). **B)** Primer screening of 6 primer combinations with the 2nd and 3rd Forward primer, which correspond to ~306 and 556bp amplified product lengths and different annealing lengths for the reverse primers (32, 36 and 39 bp annealing).

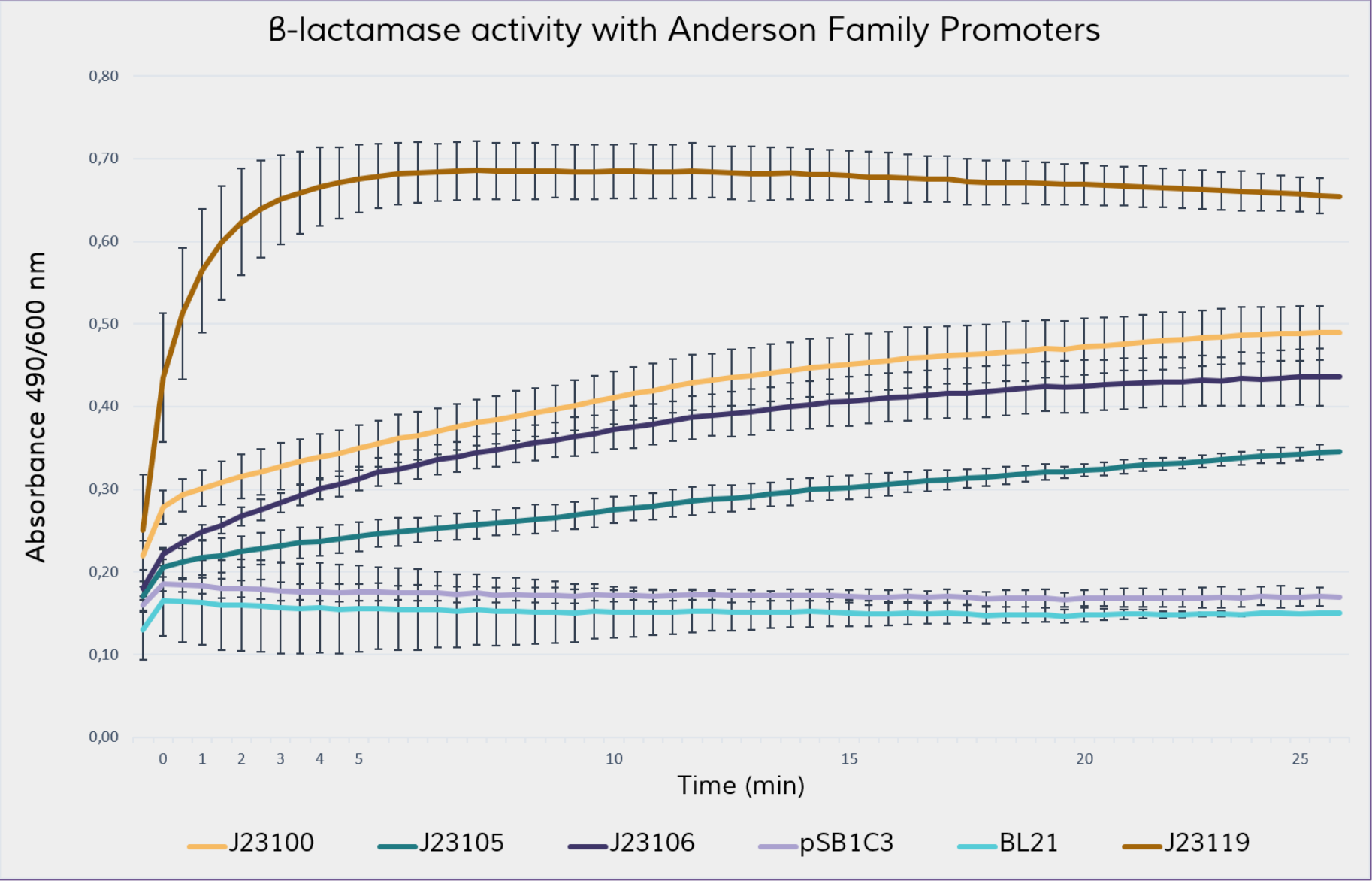
RPA Amplification



Amplification reaction with serial dilutions of our DNA template

Characterization

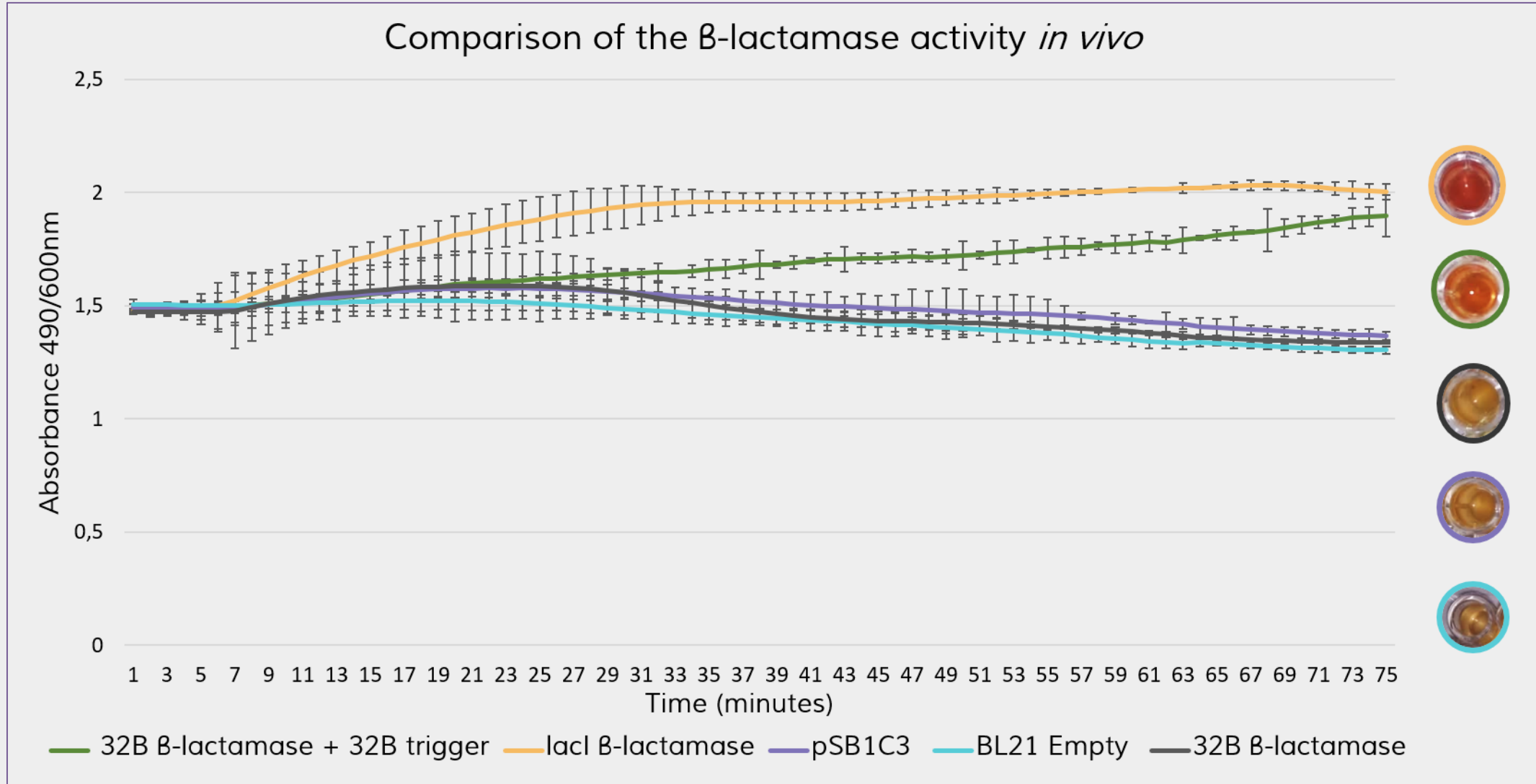
BBa_I757010



Improvement

BBa_K1189007

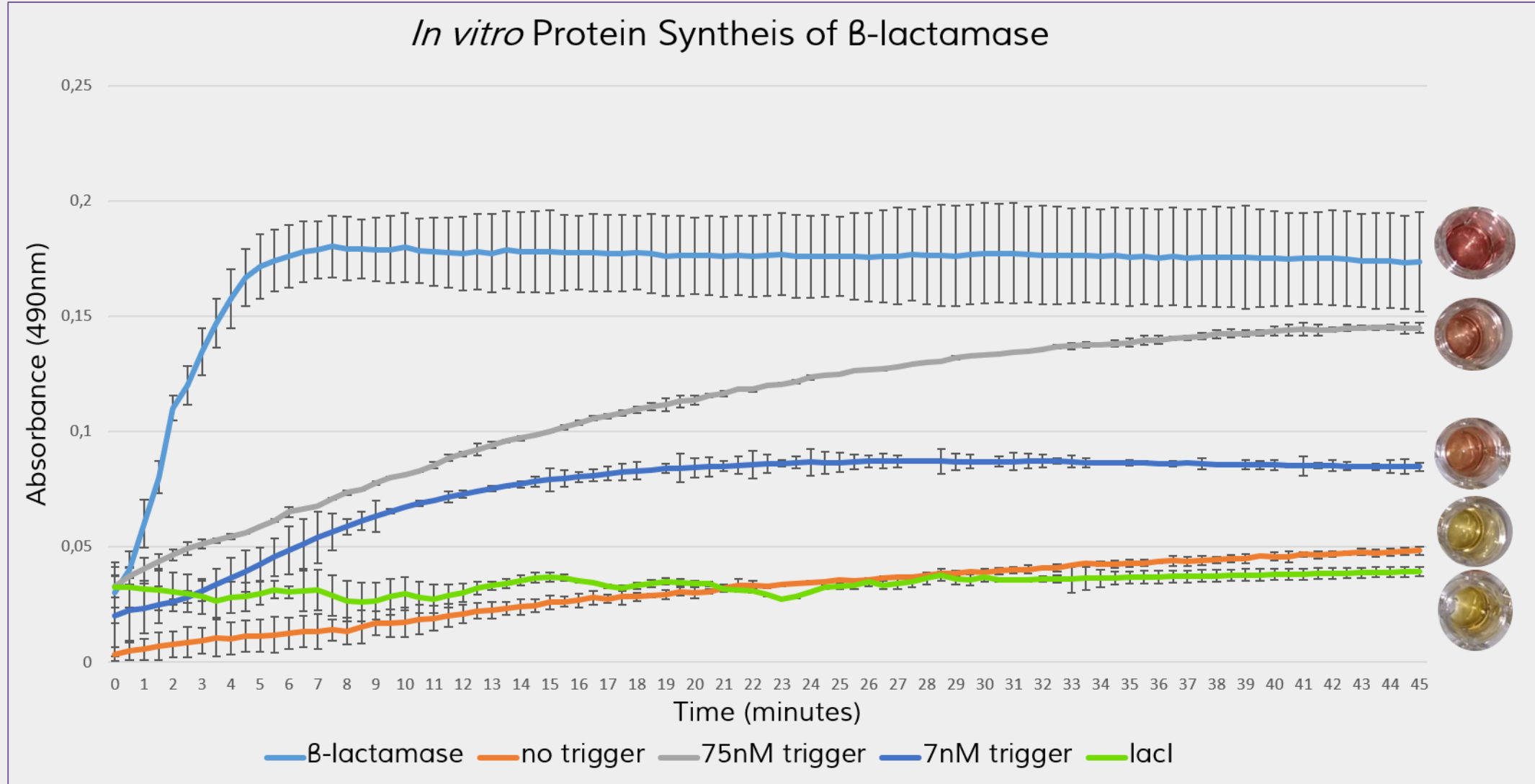
In vivo



Improvement

BBa_K1189007

In vitro



Existing Solutions

Smear Microscopy

Description

Microscopic examination of smears prepared from sputum or other biological specimens to detect acid fast bacilli after staining with acid-containing.

Benefits

- Cost-effective
- Easy to perform
- Highly specific

Limitations

- Low sensitivity (20 – 80%)
- Results are seldom on the same day.

XPRT/RIF

A real-time quantitative PCR assay for *Mycobacterium tuberculosis* -complex DNA. It amplifies part of the *rpoB* gene that contains mutations that cause rifampicin resistance (a critical first-line drug).

- High sensitivity & specificity (89% and 99%, respectively)
- Detection of TB and resistance, simultaneously
- Time efficient (results <2h)
- Universal Gene Xpert platform for diagnosing other diseases.

- Need for uninterrupted power
- Expensive instrumentation
- Predominantly implemented in centralized laboratories
- Limited affordability
- Need for annual recalibration of the instrument
- Requires trained personnel
- Inability to distinguish between live and dead bacilli.
- Sensitive to high temperatures

Existing Solutions

Lipoarabinomannan (LAM) Test

A lateral flow assay for Lipoarabinomannan (a component of the mycobacterial cell wall) detection in urine of patients with TB.

- Result in 25 minutes
- Cost-effective (<3 USD)
- Urine based test

- Low sensitivity (56%) & specificity
- The test may be used to assist the diagnosis of TB in HIV positive patients and TB presumptive patients who have a CD4 count less or equal to 100 cells/L (WHO policy guidance 2015)

Tuberculin Skin Test (TST)

Immune response - based screening method performed by an intradermal infection of a purified protein derivative from MTB, tuberculin, into the skin of the forearm.

- Cost effective
- Diagnosis of latent TB

- Recent Bacillus Calmette Guirin (BCG) vaccination history limits the specificity of TST
- Positive in exposure to nontuberculous mycobacteria (NTM)
- Dependent on the immune status of the patient
- Requires two visits at a health care provider within 48 to 72 hours.
- Cannot separate latent infection from active disease

Interferon Gamma Release Assays (IGRAs)

Measurement of a person's immune reactivity to *M. tuberculosis*. White blood cells from most persons that have been infected with *M. tuberculosis* will release interferon - gamma (IFN-g) when mixed with antigens derived from *M. tuberculosis*.

- Less cross-reactivity with BCG than with TST
- Diagnosis of latent TB
- Available results within 24 hours

- Complex infrastructure
- Need of highly skilled staff
- Cannot separate latent infection from active disease.
- Blood samples must be processed within 8-30 hours after collection, while white blood cells are still viable.

Medal Criteria

- ✓ Register for iGEM, have a great iGEM season, and attend the Giant Jamboree.
- ✓ Competition Deliverables
 - Team Wiki
 - Team Poster
 - Team Presentation
 - Judging Form
 - Project Attribution
 - Safety Forms
 - Registry Part Pages
 - Project Inspiration
- ✓ Attributions
- ✓ Project Inspiration and Description
- ✓ Characterization/Contribution: we have successfully added quantitative experimental characterization data for the β -lactamase coding sequence [BBa_I757010](#), under the control of different promoters from the Anderson family ([BBa_J23100](#), [BBa_J23105](#), [BBa_J23106](#) and [BBa_J23119](#)).

- ✓ Validated part: we have successfully created a variety of new BioBrick Parts of our own design that are related to our project and work as expected. Our validated parts are [BBa_K2973011](#) , [BBa_K2973009](#) and [BBa_K2973006](#).
- ✓ Collaborations
- ✓ Human Practices: we have created the SynArt events, interactive games for students and conducted several speeches to academic and non-academic events. Taking education in a deeper level, we introduced science to groups with limited access to education; refugee children and prisoners. .

- ✓ Integrated Human Practices: we contacted various stakeholders and implemented their opinion into our project.
- ✓ Improve a Previous Part: we have successfully created a new BioBrick Part that has a functional improvement of an existing BioBrick Part. The part we improved is [BBa_K1189007](#) by altering the regulatory system upstream the coding sequence and make it functional *in vitro*. The new Biobrick Part is [BBa_K2973007](#).
- ✓ Model Your Project
- ✓ Demonstration of your work

Past iGEM TB projects

Team iGEM	Drawbacks	Advantages of ODYSSEE	Advantages of the other projects
<p>Paris Bettencourt 2013 (Uses CRISPR/Cas9 as detection method)</p>	<p>Uses sputum as a sample:</p> <ul style="list-style-type: none"> • Many people are unable to produce sputum (especially children). • It's difficult to process • Doesn't include an amplification method. So, the sensitivity of the test decreases. <p>Biosafety warning: Uses alive mycobacteria for the detection method</p>	<p>Uses urine as a sample:</p> <ul style="list-style-type: none"> • Non invasive method • Easily accessible sample <p>Involves RPA, a very sensitive and fast amplification method.</p> <p>Urine samples don't contain alive mycobacteria, but only DNA fragments of them.</p>	<p>Detects antibiotic resistance.</p> <p>A fast and cheap method.</p> <p>Result is visible with the naked eye</p>

Past iGEM TB projects

Team iGEM	Drawbacks	Advantages of ODYSSEE	Advantages of the other projects
Pune 2017	<p>Detection method is based on the cell cycle of the Mycobacterium Tuberculosis (48h).</p> <p>Not a fast method (under 48 hours).</p> <p>Doesn't specify which sample they are using.</p> <p>Biosafety warning: Uses alive mycobacteria for the detection method</p>	<p>Detection method is based on the presence of the IS6110 gene in urine samples.</p> <p>Detection method lasts under 1 hour.</p> <p>Urine samples doesn't contain alive mycobacteria, but only DNA fragments</p>	<p>Result is visible with the naked eye.</p> <p>A cheap method.</p>

Past iGEM TB projects

Team iGEM	Drawbacks	Advantages of ODYSSEE	Advantages of the other projects
SCSU New Haven 2016 (Uses VOCs as biomarker)	<p>Their detection method wasn't tested.</p> <p>Not a reliable biomarker.</p>		<p>Result is visible with the naked eye.</p>
Marseille 2019	<p>Uses sputum as a sample:</p> <ul style="list-style-type: none"> • Many people are unable to produce sputum (especially children). • It's difficult to process • Biosafety warning: Uses alive mycobacteria for the detection method 	<p>Uses urine as a sample:</p> <ul style="list-style-type: none"> • Non invasive method • Easily accessible sample • Urine samples don't contain alive mycobacteria, but only DNA fragments of them. 	

Cost Analysis

Components	Manufacturer	Cost/reaction (€)
RPA kit	TwistDx	2,60
Magnesium acetate	TwistDx	(included in RPA)
PURExpress	New England Biolabs	4,97
Nitrocefin	Cayman	0,30 (40µl)
Consumables		
Disposable pasteur pipette (3ml)	Anadrasis MED	0,019
Disposable microcentrifuge tubes 2 ml (x2)	Anadrasis MED	0,0122
DIY centrifuge	-	0,20
Eye drop tubes (x2)	Syndesmos	0,20
Syringe 0,5ml	Syndesmos	0,020