

Human Practices

Our project meets a need!

We turned to experts in various fields (doctors, scientists, ethics professors) to assess our project. They gave us insight which we integrated to improve the points, highlighted in **green**.

Teaching the public

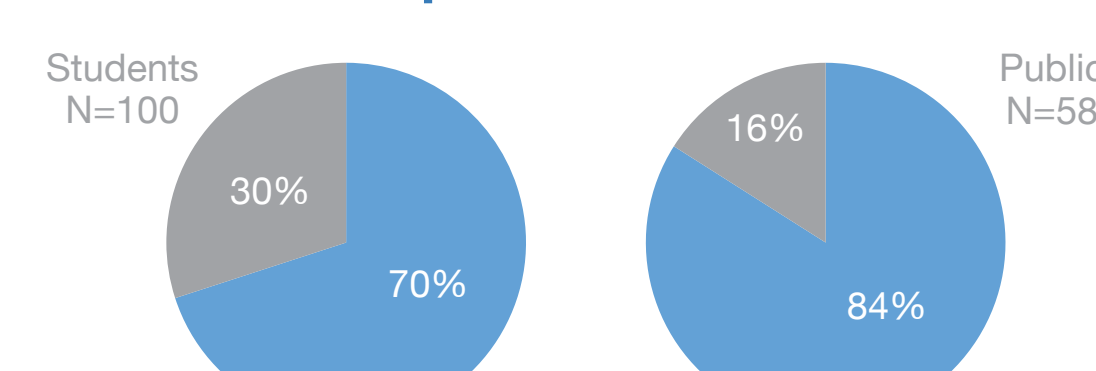


Through fairs, youth educational meet-ups and university open-days, we:

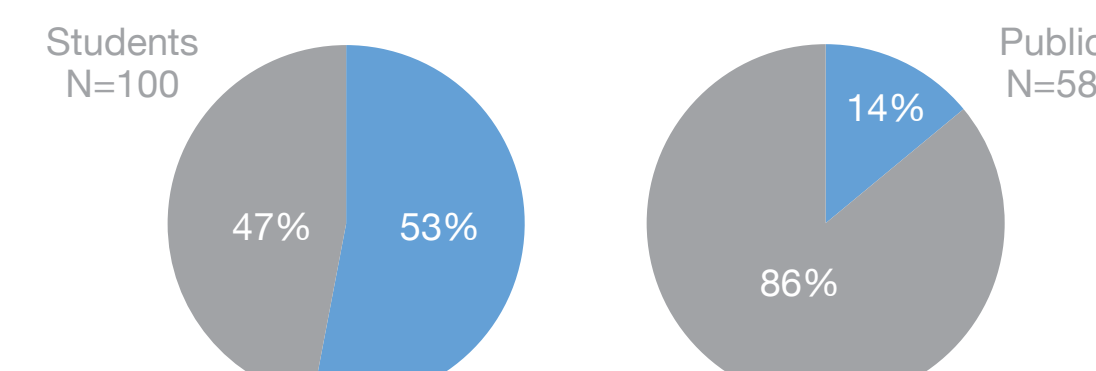
- raised awareness about antibiotics use
- explained synthetic biology and working principles of home-diagnostics devices
- let people experiment with printed paperstrips

- ✓ **Target choice**
- ✓ **Less than \$1 per test**
- ✓ **Portability**
- ✓ **Stability on paper**

Is the public comfortable with our product?



Do you know about synthetic biology?

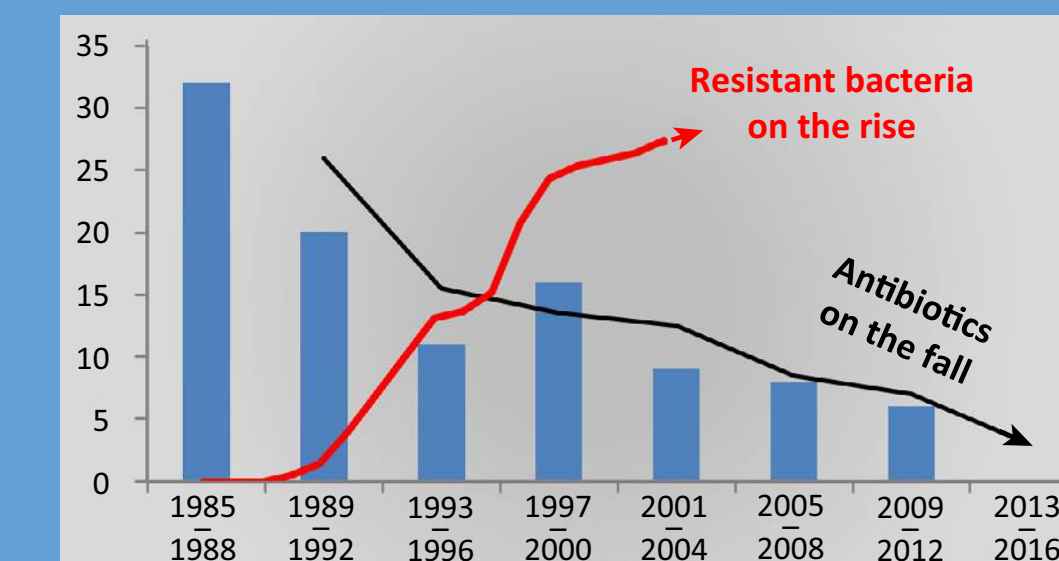


Would you be comfortable using a home-diagnostic device for viral and bacterial infections?

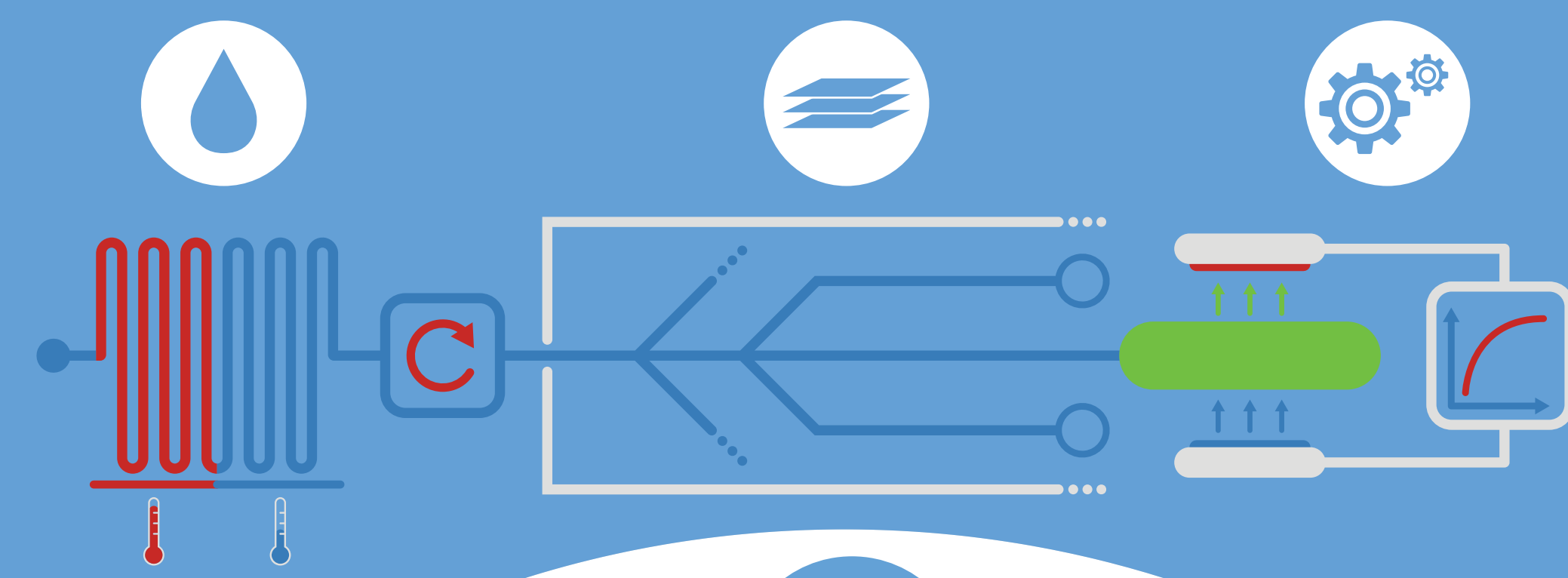
Overview

To prevent further spread of antibiotic resistance, we designed an affordable, rapid point-of-care test for infectious diseases to distinguish viral and bacterial pathogens: CascAID. The modules of this device enable extraction, amplification and detection of target RNA sequences to fulfil the A.S.S.U.R.E.D. criteria.

Antibiotic resistance crisis: a major health threat



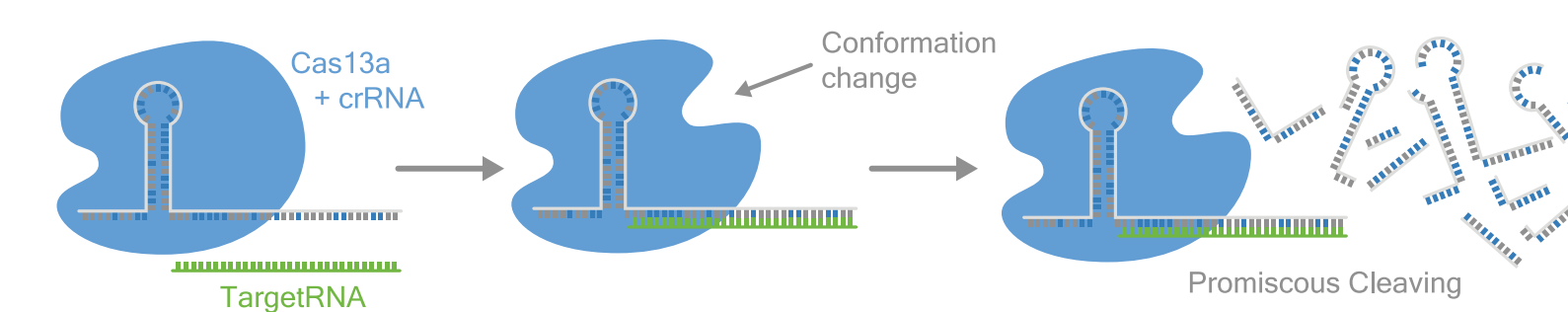
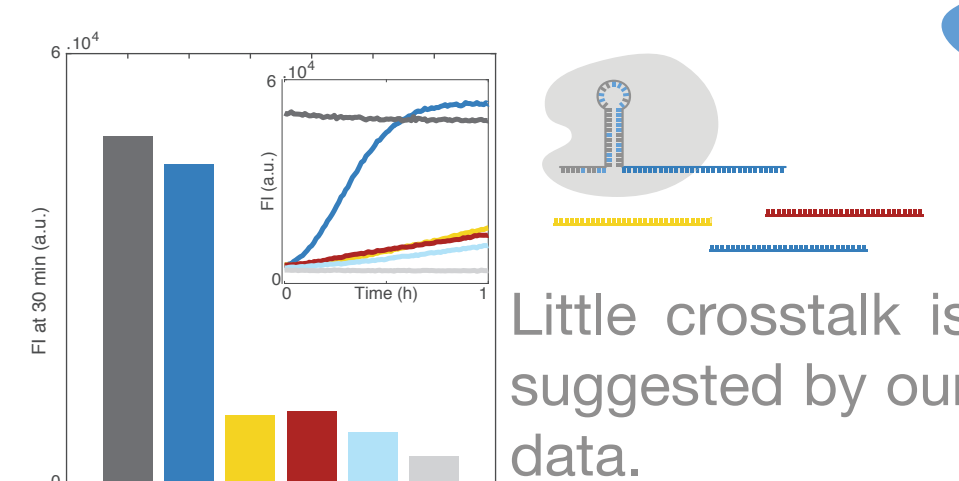
Because antibiotics are prescribed unnecessarily, bacteria grow resistant and simple infections are on the rise.



Cas13a

fighting the antibiotic crisis

Cas13a is a CRISPR-associated protein which recognizes RNA sequences, with the help of a complementary CRISPR RNA (crRNA). It then becomes an unspecific RNase. Its single-nucleotide specificity makes it ideal for differentiating bacteria and viruses, by recognizing a unique 28-nucleotides sequence.

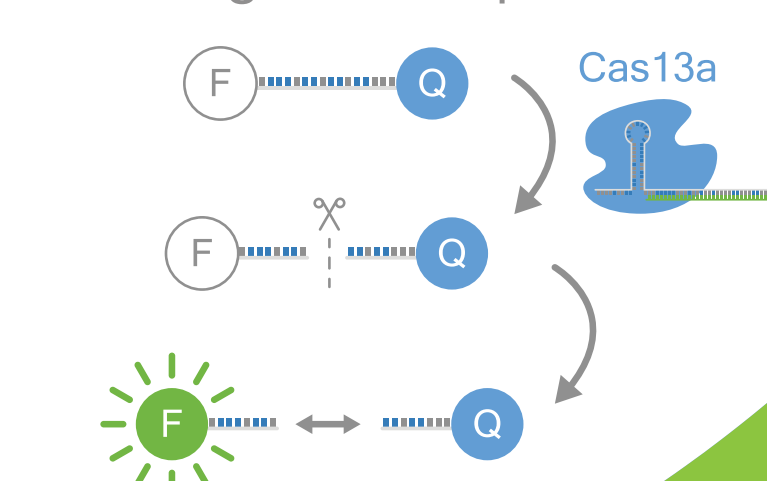


The antibiotic resistance crisis

This mechanism allows a fast detection of viral or bacterial pathogens and clear differentiation between them.

Readout reaction

Fluorescence of RNase Alert rises strongly after cleavage of the quencher.

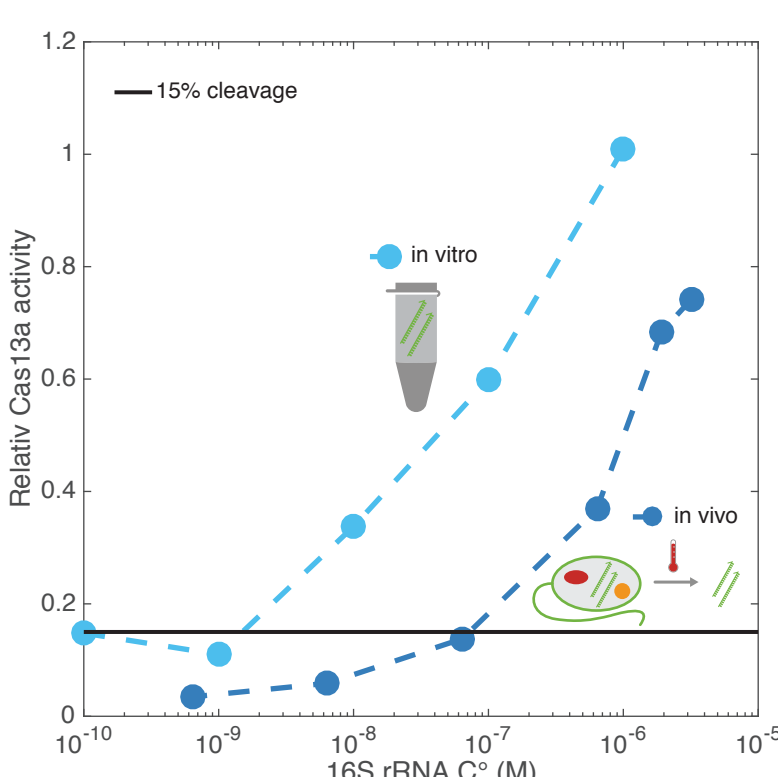


crRNA	fluorescence	no fluorescence
E.coli	+	-
Norovirus	+	-
HCV	+	-
E.coli	-	+
Norovirus	-	+
HCV	-	+
no target	-	+

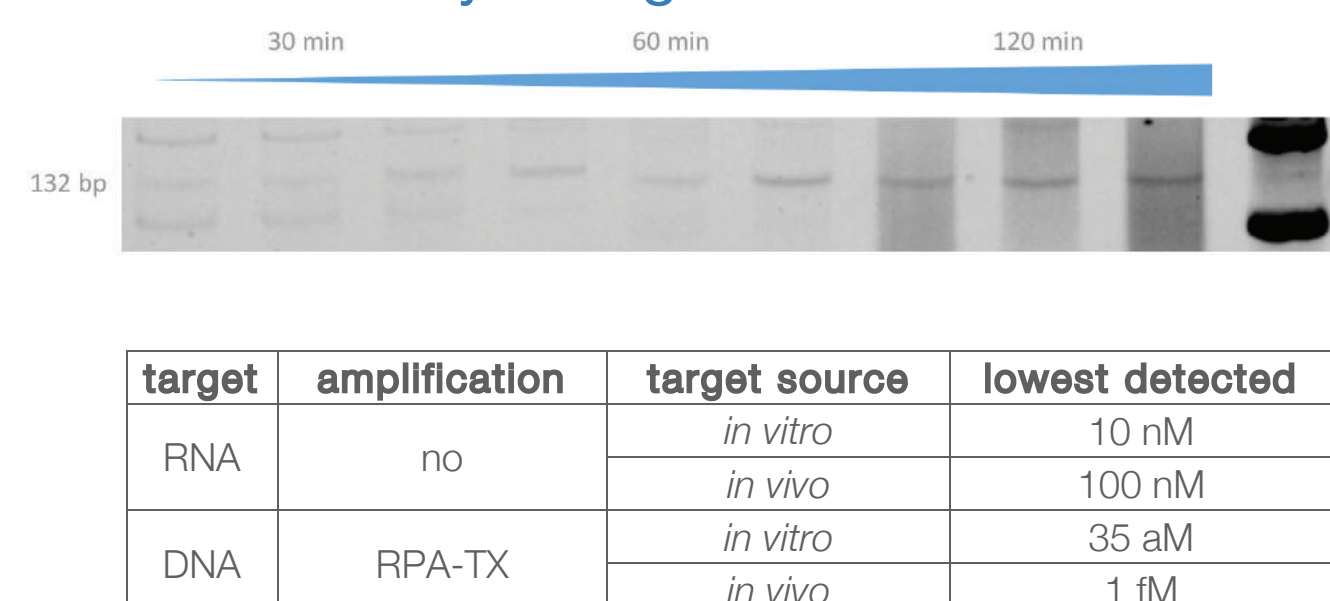
We clearly differentiated bacteria and viruses

Sample Processing

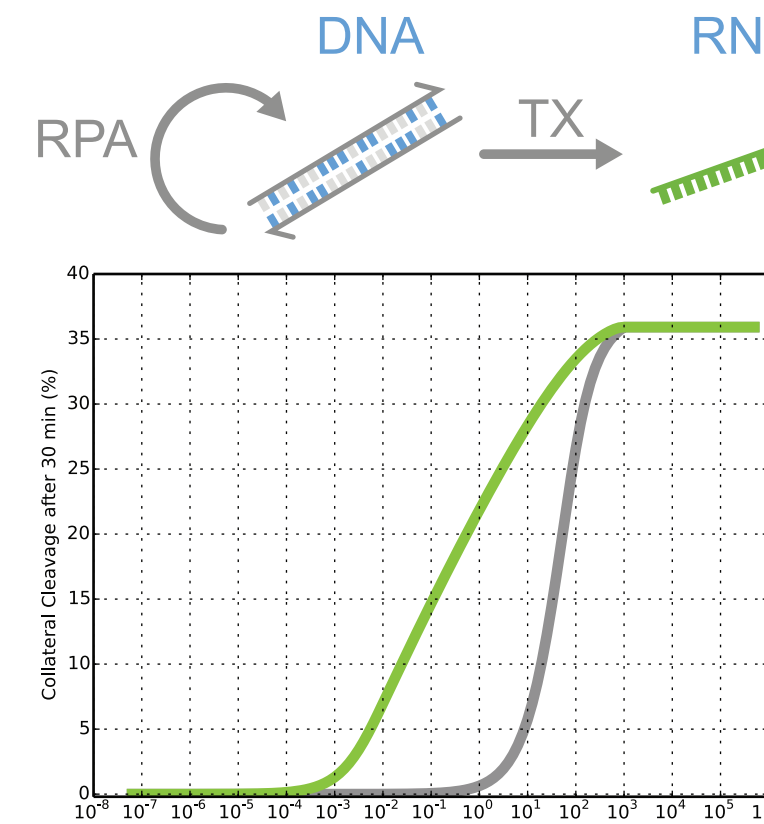
- ✓ **Sensitive: 35 aM**
- ✓ **Functional on paper**
- ✓ **Modular and integrated**



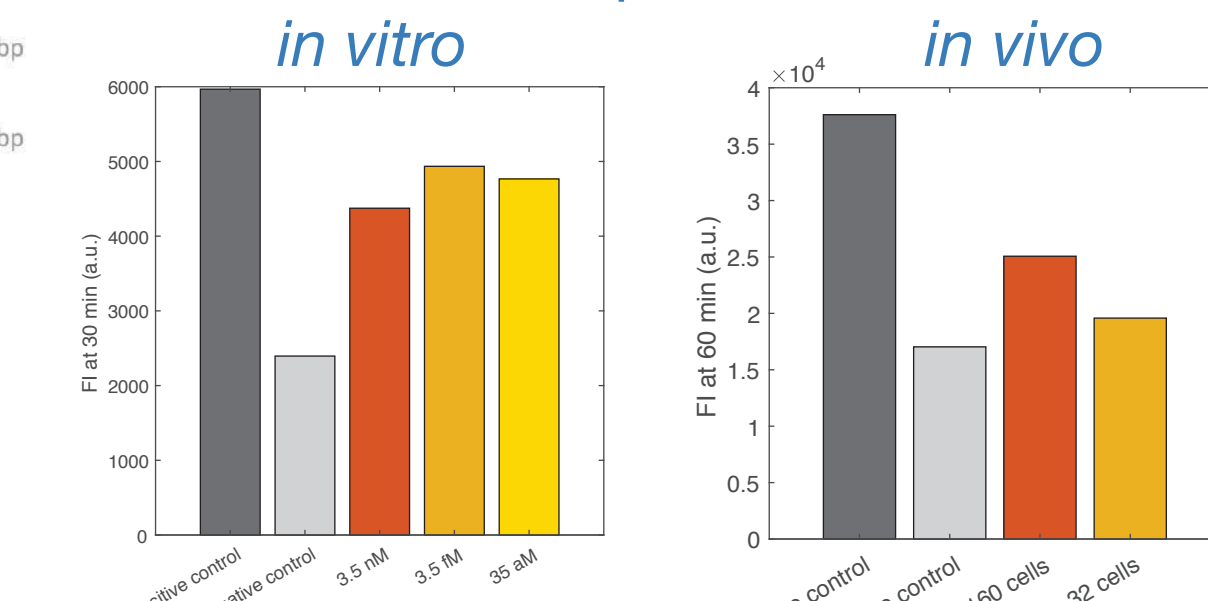
Gel for in vivo lysis + gel for RPA-TX



Model



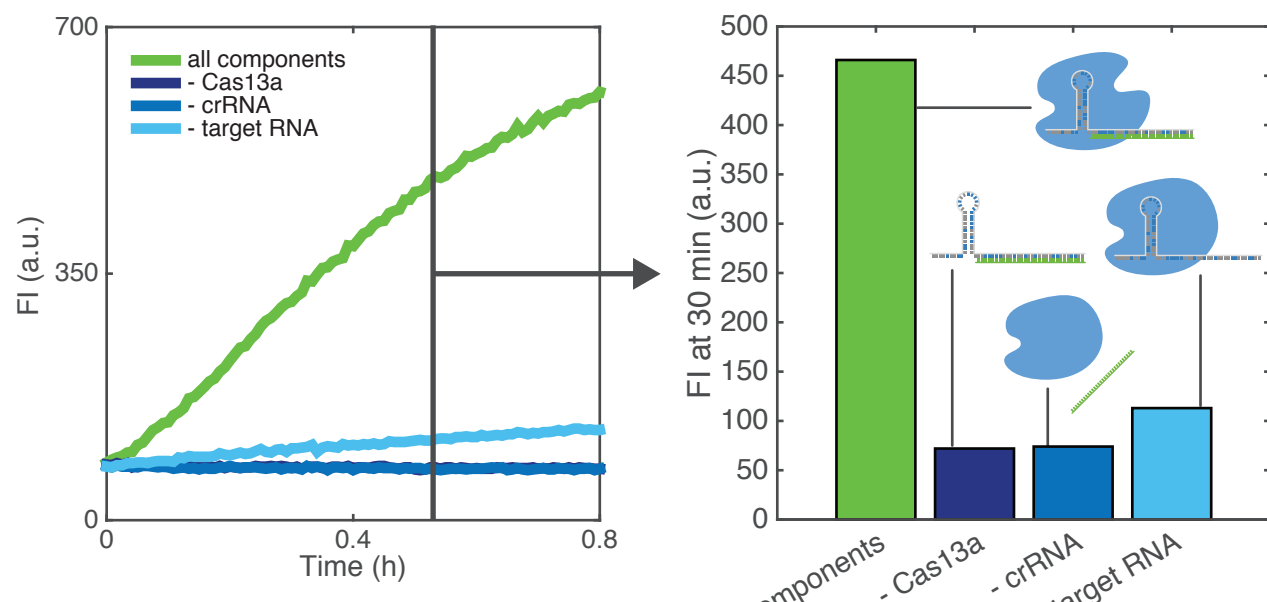
Detection after amplification:



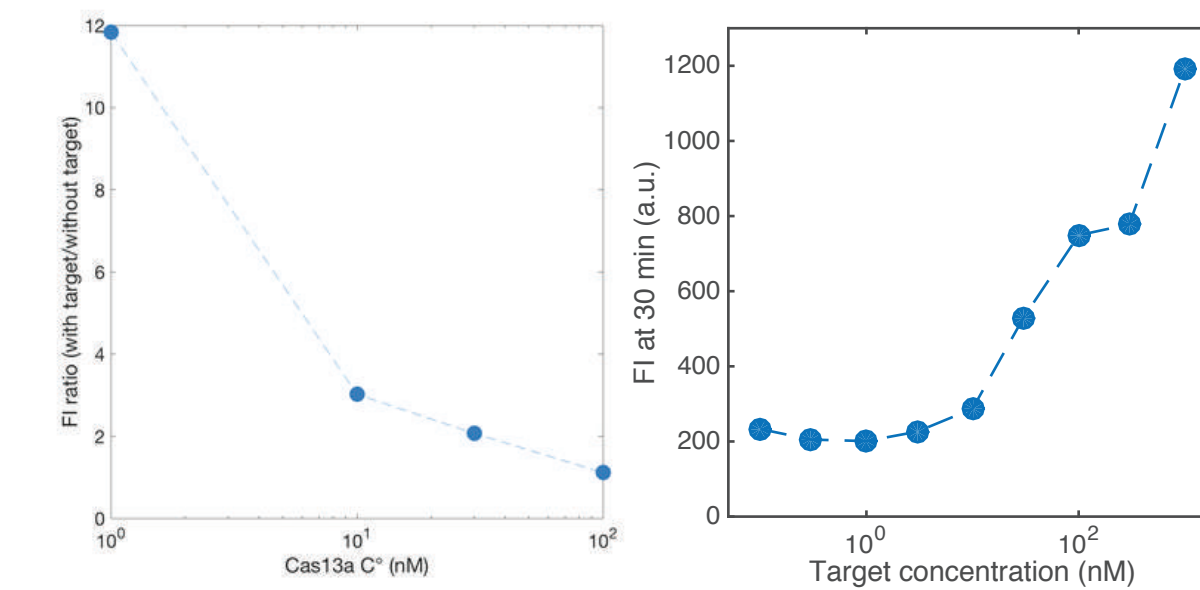
Cas13a Characterization

- ✓ **Sensitive and Specific**
- ✓ **Rapid: readout in < 30 minutes**
- ✓ **Robust: across experimenters**
- ✓ **Universal: any target sequence**

Functionality of Cas13a

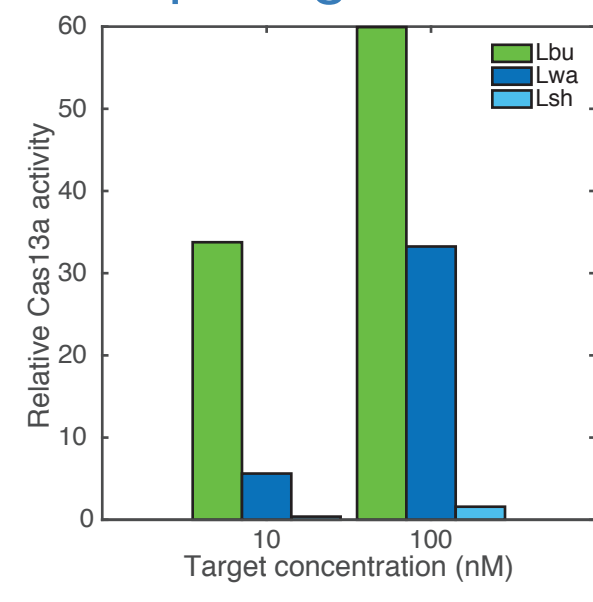


Sensitivity vs. speed

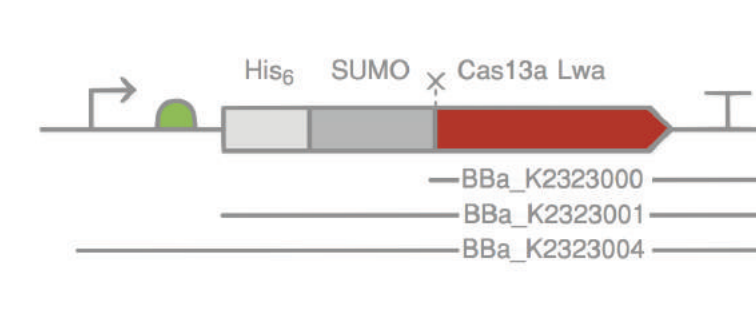


Cas13a sensitivity goes down with increasing concentration, while the kinetics of cleavage go up. We chose a compromise concentration of 10 nM Cas13a. The detection limit was then 10 nM target RNA.

Comparing different Cas13a

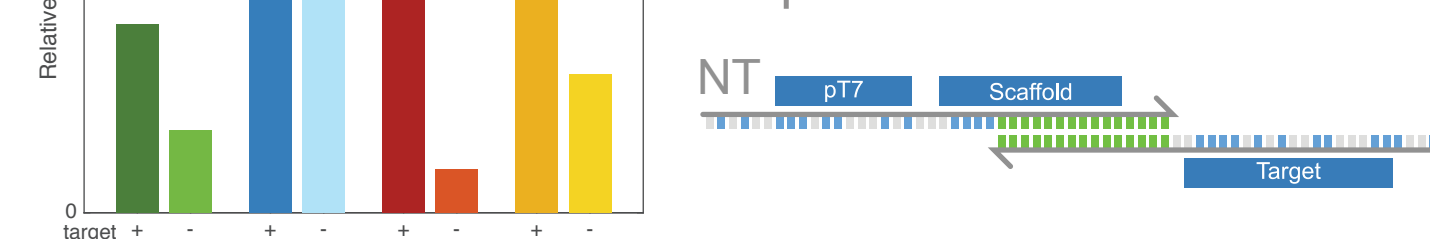


Lwa Biobrick



Fast prototyping of targets

We can change the crRNA sequence to match different targets with our split DNA template:

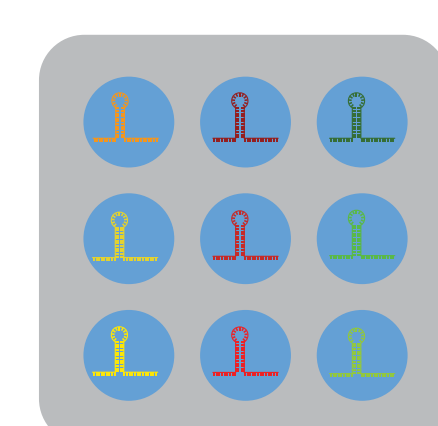
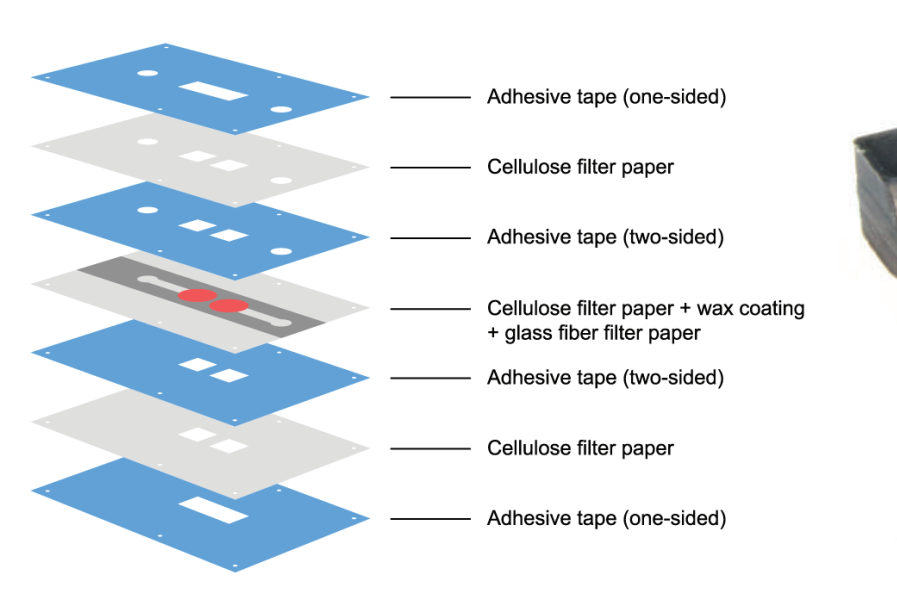


According to WHO:

- E. coli* - 3rd most critical multiresistant strain
- B. subtilis* - test for gram positive bacteria
- Norovirus - main cause for gastroenteritis
- HCV - 399 000 deaths per year

Paperstrip

Paperstrips make our test independent from expensive lab equipment, easy to use and storable. It also allows for multiplexing using paperfluidics. This allows for densely-packed, detector-independent readout, similar to a QR-code.



Paper support optimization

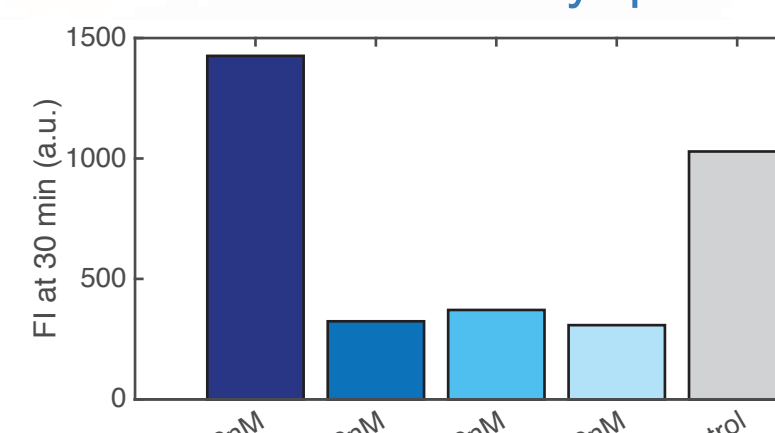
We used the design-test cycle and the "keep it simple" principle to choose our paper support:

- nitrocellulose; most available but autofluorescent and toxic to Cas13a
- glass fiber paper; functional when treated with BSA



Lyophilization of Cas13a

We built a sample holder where the paperstrip is held. It has two windows to analyze a blank and the sample. It can be clipped into our fluorescence detector, "Lightbringer".



- ✓ **Affordable**
- ✓ **Distributable**
- ✓ **Scalable: paperfluidics**

Results

We successfully used **Cas13a** in a fluorescence-based assay on paper to distinguish viral and bacterial RNAs with a **detection limit of 10 aM**. We built the **most affordable and sensitive fluorescence detector** in iGEM, to our knowledge.

Cost calculation

disposable components	cost per test
processing chip	0.310 \$
paper strip (incl. enzymes & chemicals)	0.460 \$

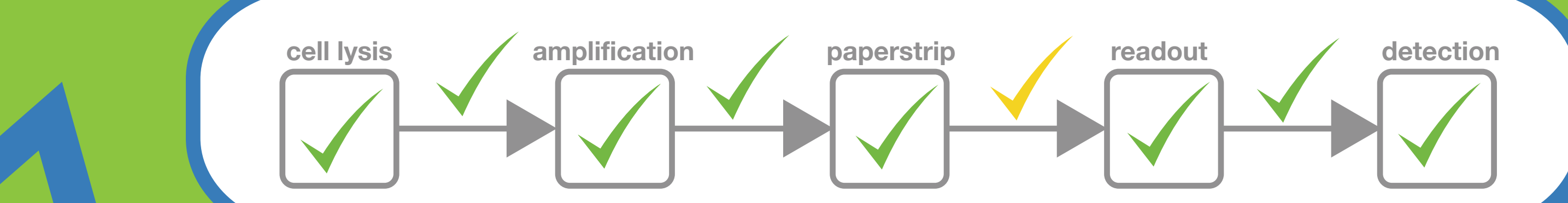
reusable components

cost per test	
fluorescence detector	0.014 \$
processing unit	0.026 \$
pressure supply	0.007 \$
energy supply	0.006 \$
Raspberry Pi	0.030 \$
Total	0.853 \$

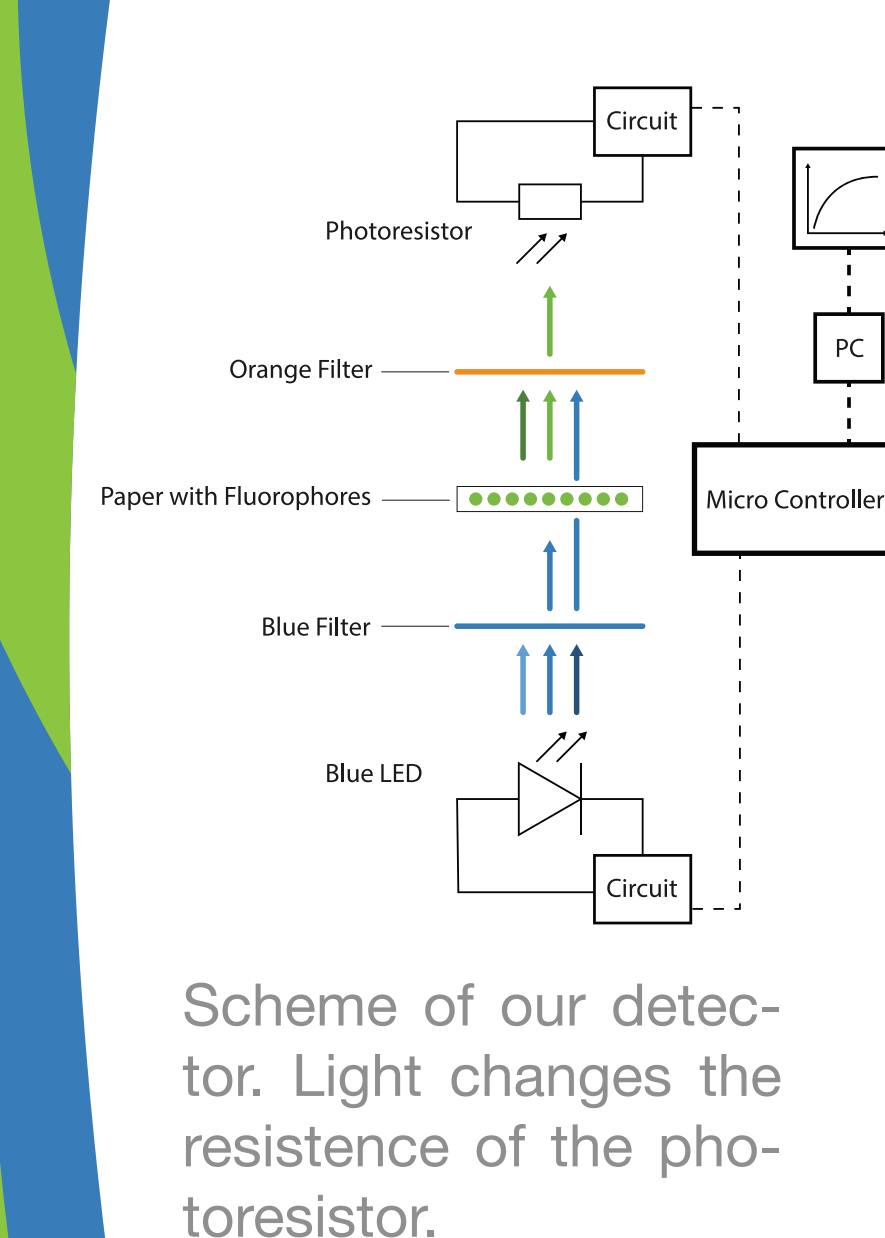
1 at 1000 tests

WHO criteria fulfilled

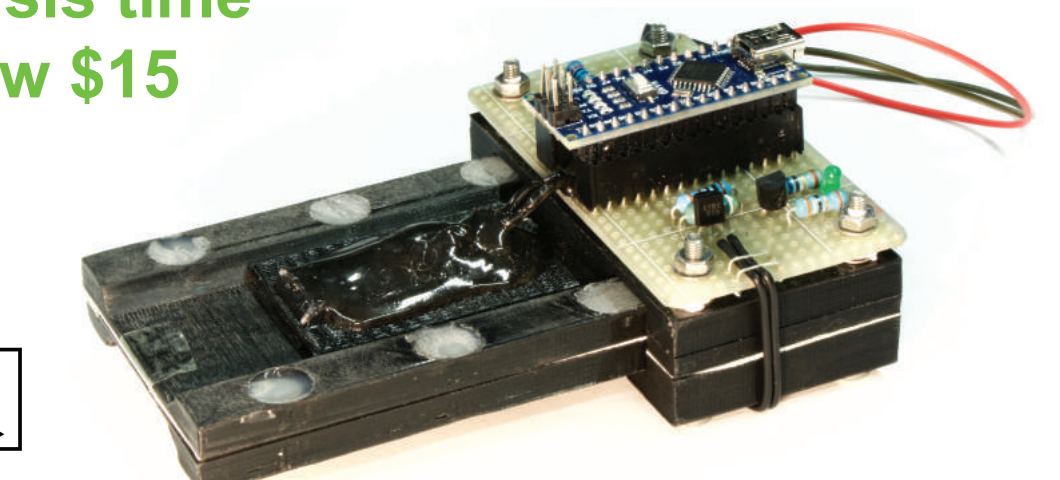
Criteria	Value
Affordable	\$0.85 per test
Sensitive	10 aM
Specific	strong target orthogonality
User-friendly	easy setup
Rapid & Robust	30 min detection time
Equipment-free	no power outlet
Deliverable	fits in your pocket



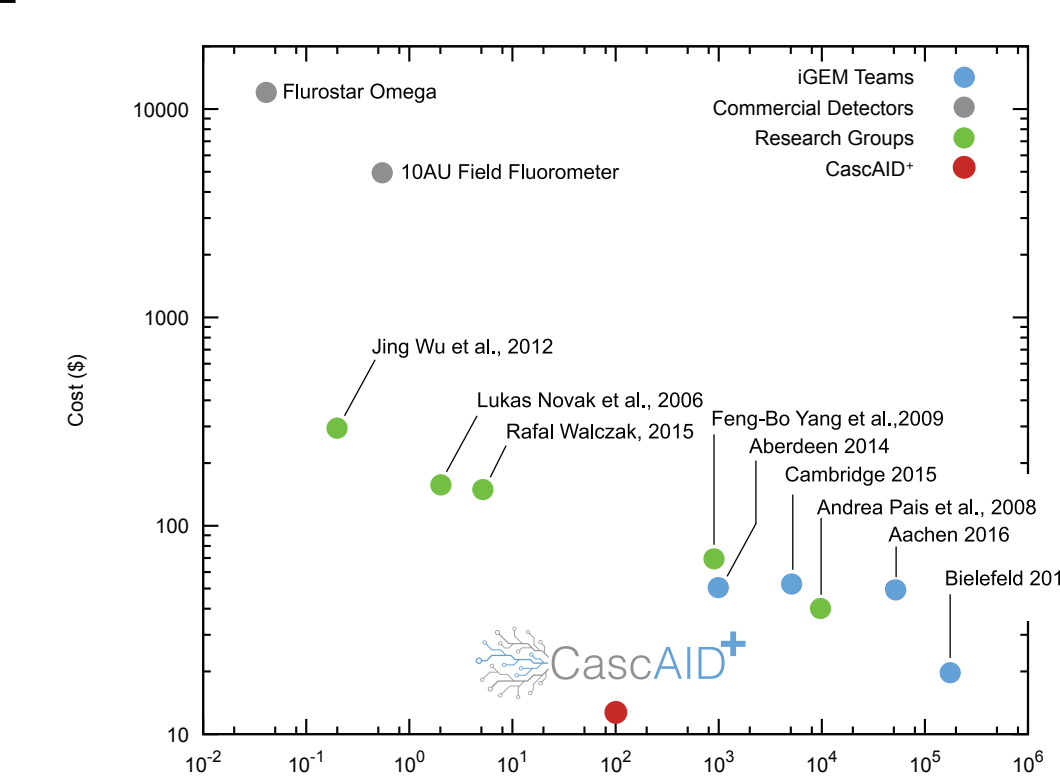
- ✓ **100 nM fluorescein detection limit**
- ✓ **< 30 min analysis time**
- ✓ **total cost below \$15**
- ✓ **open source**



Scheme of our detector. Light changes the resistance of the photoresistor.

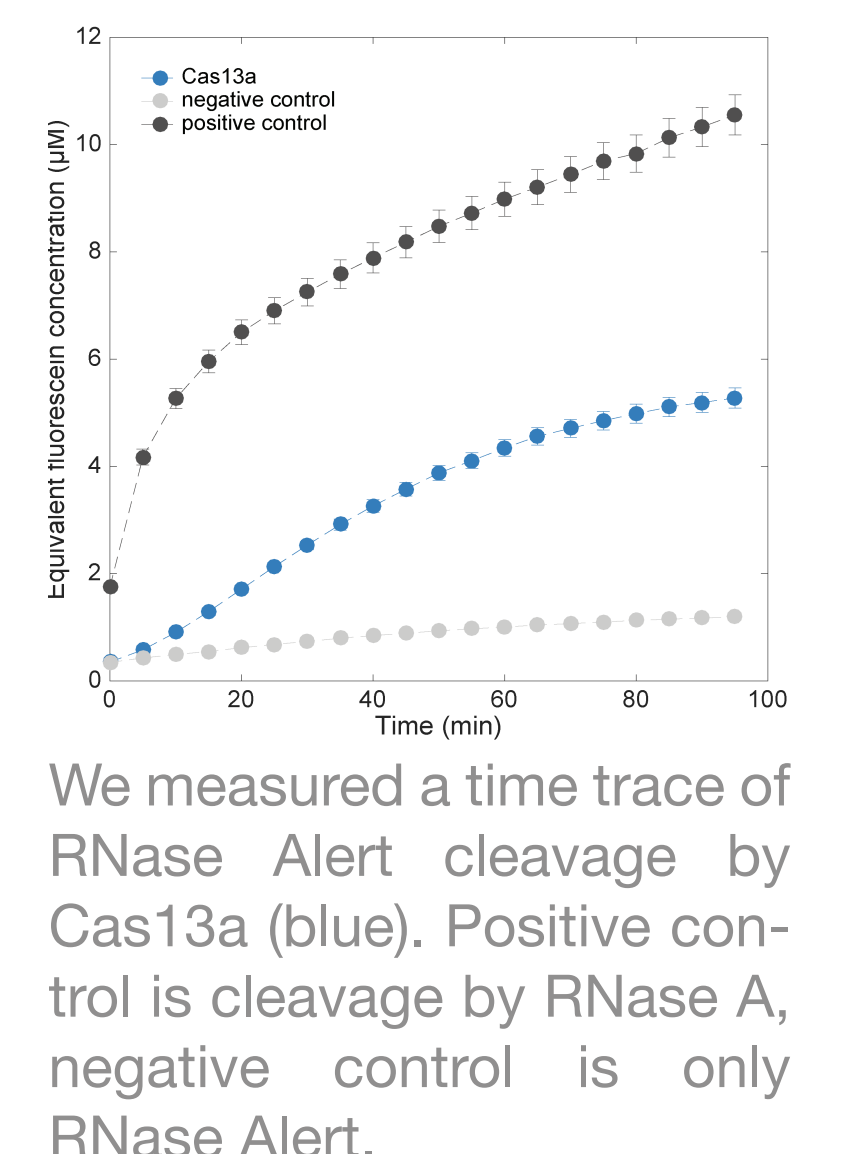


Universal, portable and open source detector for fluorescence measurement.

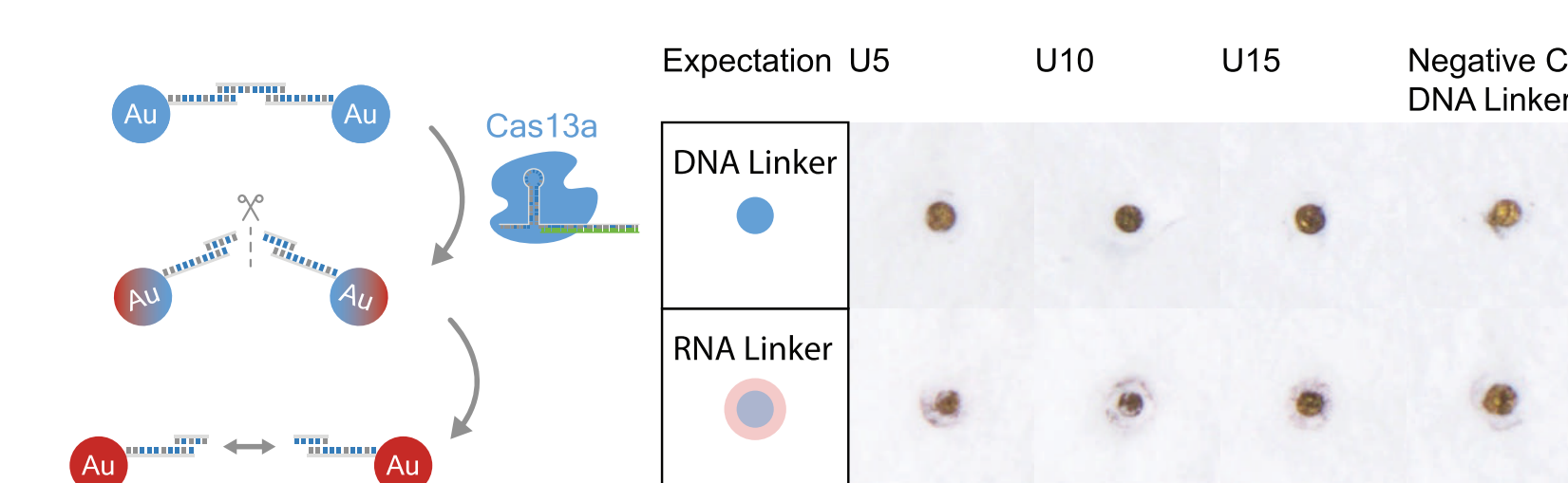


Not only is our detector the most sensitive and least expensive one ever built by an iGEM team, it convinces with a price of less than 15 \$ at a detection threshold of 100 nM fluorescein.

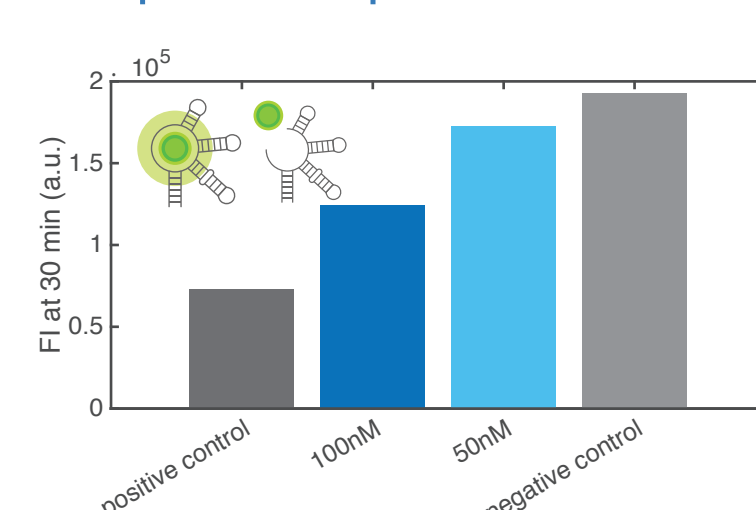
Detector



- ✓ **4 alternative readouts**
- ✓ **Functional AuNPs, Spinach-Aptamer**
- ✓ **Easy to interpret**

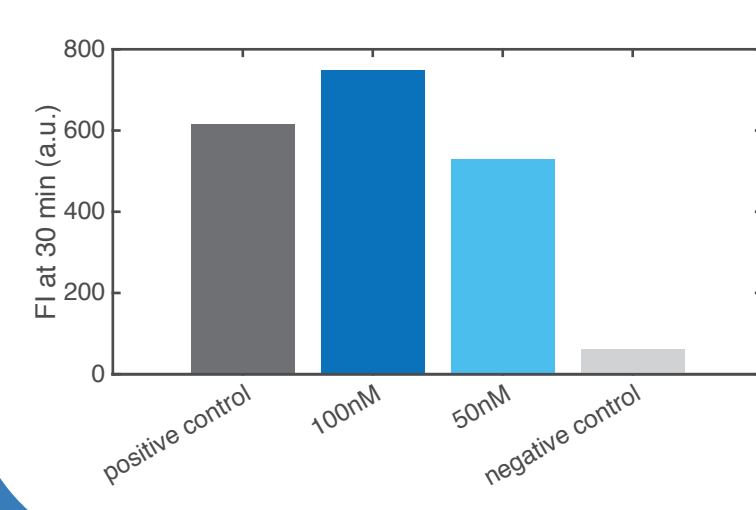


Spinach Aptamer



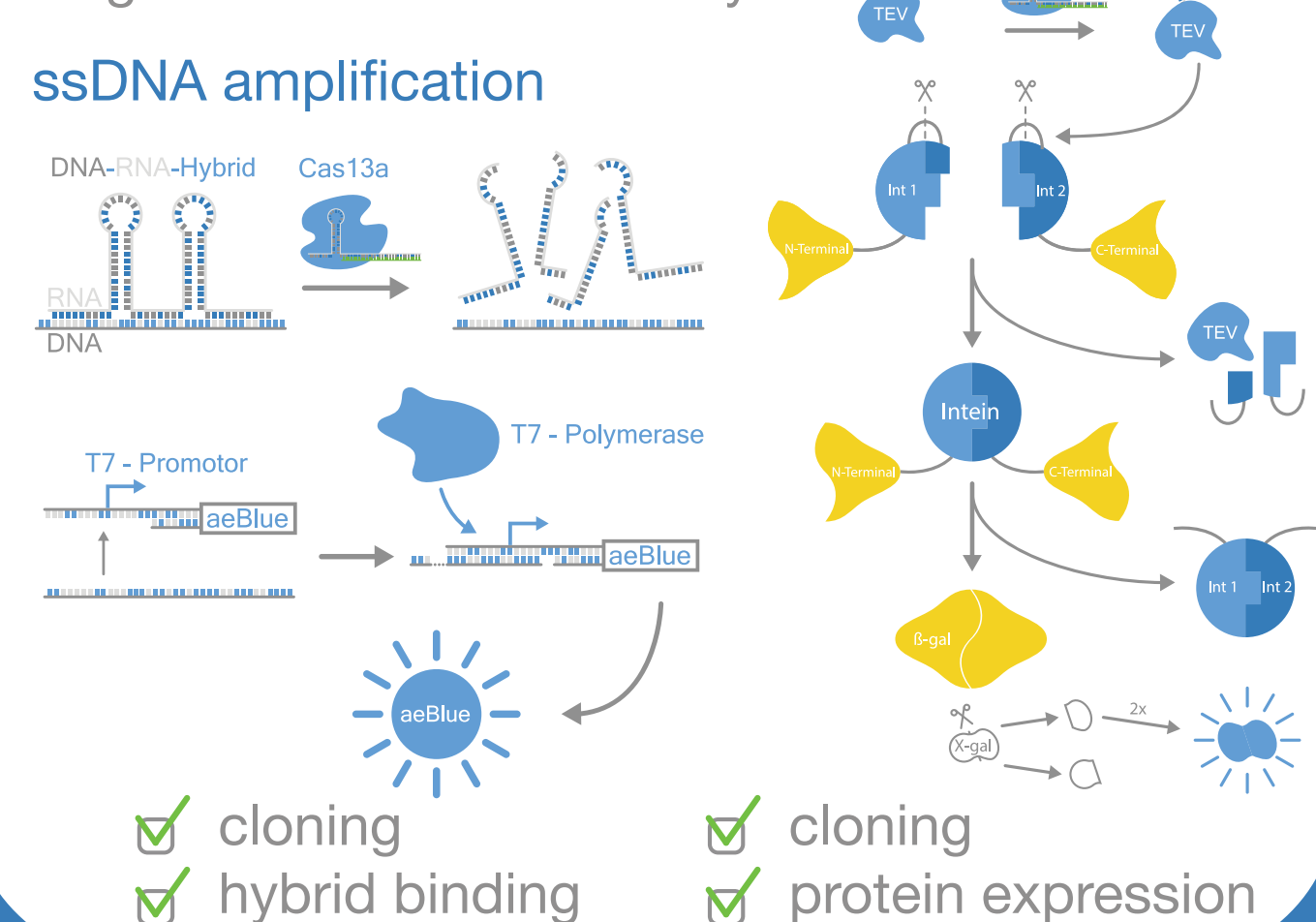
The spinach aptamer increases the fluorophore signal upon binding. Activated Cas13a is then detected by a decrease in fluorescence, when the RNA binding site is cleaved.

RNase Alert



RNaseAlert detection is shown for comparison.

We designed additional colorimetric readouts that include an amplification step, increasing the detection sensitivity.



References

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Who did the work

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Sponsors

