OUTCASST

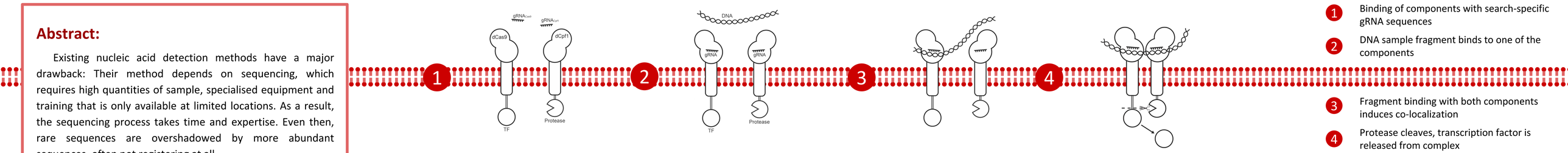
Out-of-cell **C**RISPR **A**ctivated **S**equence-specific **S**ignal **T**ransducer

Abstract:

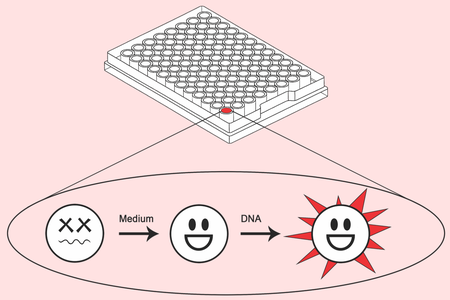
Existing nucleic acid detection methods have a major drawback: Their method depends on sequencing, which requires high quantities of sample, specialised equipment and training that is only available at limited locations. As a result, the sequencing process takes time and expertise. Even then, rare sequences are overshadowed by more abundant sequences, often not registering at all.

The OUTCASST two-component system hopes to alleviate these limitations, opening the possibility of quick diagnoses based on cell-free DNA. Two proteins are expressed to the membrane of the mammalian HEK-293T cells. One is a Cas9-fusion and the other contains Cpf1. Both proteins can be given a guide RNA that makes it bind to a specific, user-chosen, complementary sequence.

When both proteins bind a DNA fragment from a sample, they co-localize, so that a transcription factor is released intracellularly which then induces an intracellular reporter mechanism such as a fluorescence signal.



1. Binding of components with search-specific gRNA sequences
2. DNA sample fragment binds to one of the components
3. Fragment binding with both components induces co-localization
4. Protease cleaves, transcription factor is released from complex

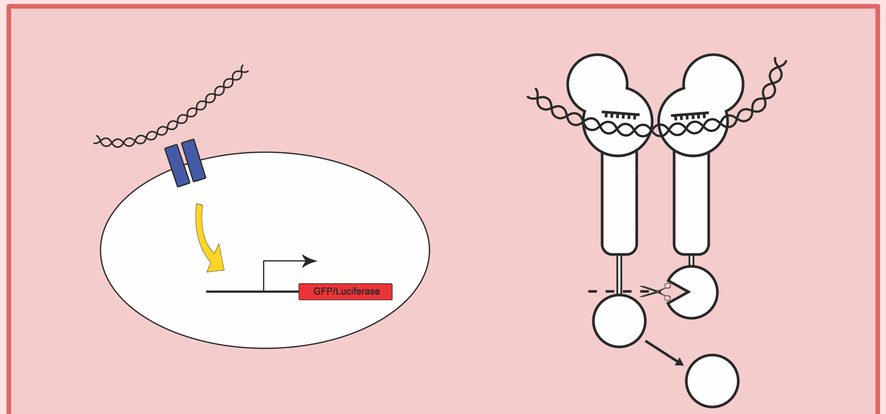


Test-kit design:

Implementation of the OUTCASST system in cells that can be dried for storage and later revived for use allows for easy and safe logistics.

Ideal cells for this come from the *Polypedilum vanderplanki* insect. Its cells can be dried in approximately 96 hours and revived by adding liquid medium. After 1 hour of incubation, the cells are once more vital and ready for use. Even in their dormant state, they will remain functional for up to 250 days.

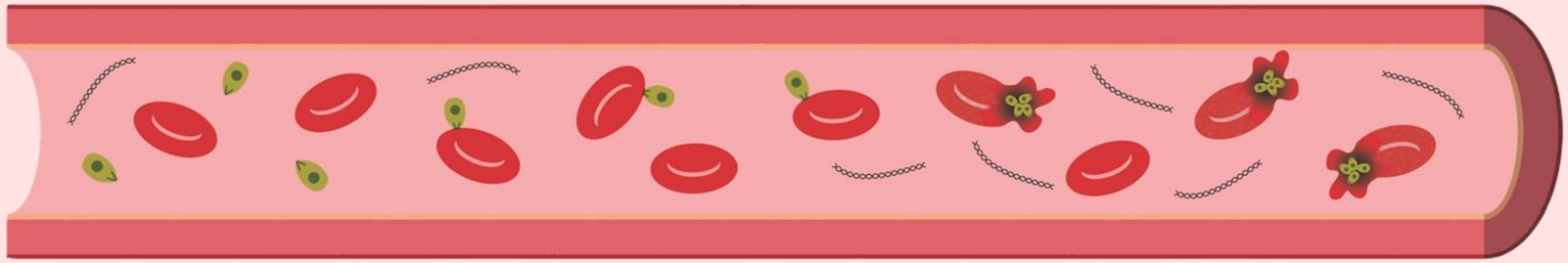
This way, the cells can be prepared and dried in specialised labs. These dried cells can be sealed and shipped all over the world only to be revived and used where they are needed, even in an area with few medical facilities.



Current DNA detection mechanisms require Polymerase Chain Reaction (PCR) amplification steps to enhance sample concentrations. They result in large datasets that can only be interpreted with bioinformatics tools. This process takes time and is error sensitive. Ideally, the diagnosis of diseases happens quickly and with as few intermediate steps as possible.

The advantages of the OUTCASST approach are:

1. **Simplicity**. The cells simply need to be acclimatized and revived for use. They can be prepared by addition of the relevant guide RNA for the user's intentions. Read-out of the output is also quite straightforward as the hardware to measure fluorescence signals is widely available.
2. **Modularity**. The same cell-line can be used for different tests, depending on the gRNA that is added. As such, multiple tests can be performed from the same batch of cells. High-throughput testing is thus a possibility.
3. **Sensitivity**. The sensitivity of the OUTCASST system overcomes the need for DNA amplification prior to detection, thus resulting in fewer false calls. Quantification of the output signal can be used for estimation of target sequence concentration.

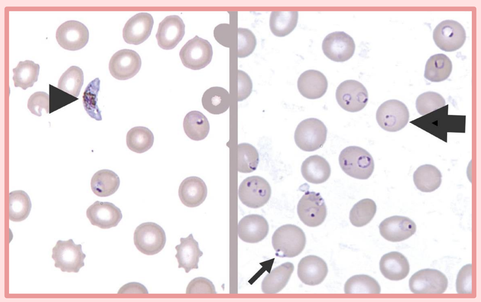


Malaria, a difficult disease:

Malaria is a disease that can be caused by five species of the Plasmodium protozoa. It is very common around the equator. Effective use of anti-malarial medication depends on the infecting organism and severity of symptoms. Of the five species, *Plasmodium falciparum* is the most deadly.

As with all infections diseases, drug resistance is of great concern. Therefore, in regions where the disease is common, it would be best to determine which species is responsible for the disease before treatment starts. That way, a more specific treatment can be used, reducing the pathogen's exposure to the general treatment, thus reducing the risk of resistance development.

Proper treatment usually results in complete recovery but proper treatment relies on accurate and precise diagnosis and that is a lot harder to achieve.



Information source:

https://en.wikipedia.org/wiki/Diagnosis_of_malaria#Over-_and_misdiagnosis

Image source:

Racsa LD, Schechter MC, 2015. Diagnosing *Plasmodium falciparum* in a teenage traveler by peripheral blood smear. *Blood*. 126; 8: 1042.

The trouble with diagnosis:

Current malaria diagnosis happens by microscopic examination of blood using blood films. These techniques do not perform well in an early stage of infection. Antigen-based tests also exist but have the same problem.

Methods that use PCR to detect the parasite's DNA can detect malaria early in infection but these are too expensive and require too much expertise and materials to be widely used. Areas that cannot afford laboratory testing often start malaria treatment for fever symptoms, resulting in overdiagnosis.

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