1. Introduction

The international Genetically Engineered Machine (iGEM) competition is the largest synthetic biology competition for collegiate students. It aims to combine and create novel biological (genetic) parts, which can be incorporated in an organism, thereby equipping it with new functions. Our work will be presented at the Giant Jamboree to the international scientific community in Boston in November 2017. An iGEM project consists of lab work, modelling, policy & practice and public outreach with results being documented on a wiki page.

This year, the team will try to produce an easy-to-use device to identify common antibiotic resistance genes in bacteria samples. According to the World Health Organization, antibiotic resistance development is one of the biggest threats to global health, food security and development today [1]. An accelerating factor in this problem is that roughly 80% of the antibiotics are being used in agriculture, of which a significant portion is already ineffective due to previously acquired antibiotic resistance. To decrease unnecessary use of antibiotics in agriculture, we aim to create an affordable, user-friendly diagnostic tool able to detect antibiotic resistance genes.

The key element to this diagnostic tool is a recently discovered variant of a protein from the CRISPR/Cas system, known as Cas13a/C2c2 [2]. Upon recognition and cleavage of a target RNA, Cas13a undergoes a conformational change and is activated. Subsequently, it engages in collateral cleavage of non-targeted RNAs, so all RNA in vicinity of the Cas13a protein is cut, allowing for conversion of the target recognition into an optical readout. By using Cas13a with different target sequences we are aiming to identify resistance to different antibiotic types in bacteria.

In our reference [2], the optical readout upon target recognition has been achieved by adding Cas13a to a solution containing RNA labeled with a fluorophore and a quencher. When Cas13a is activated, the RNA is cut and the fluorophore no longer quenched, giving a fluorescence signal. Currently, we are developing new methods to convert the target recognition into a readout visible for the eye. Naturally, all methods involve cleavage of RNA, combined with different mechanisms such as bioluminescence [3].

For increased shelf life and easy transport and storage of the diagnostic tool, we are investigating the use of intrinsically disordered tardigrade proteins (TDPs). They allow other proteins to retain their function after desiccation and subsequent rehydration. In our case, the TDPs will be added to Cas13a, stabilizing it. To avoid having to purify both proteins separately, we are engineering bacteria that package both proteins in Outer Membrane Vesicles (OMVs). This also resolves the issue of having to lyse the producing bacteria, which increases the overall output [5], [6].

On the final device, OMVs containing both Cas13a and TDPs in sufficient conditions will be mixed with collateral RNA and then dried. The device will be in the form of either a paper microfluidic device or a well-plate, both allowing identification of several different antibiotic resistance genes.

2. Contact information

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3. Regulations

The materials will be handled cautiously wearing appropriate gloves and always working according good safety and laboratory practices. ML-1 safety requirements will be followed, and all the team members are obliged to follow four mandatory safety tests (Biological safety ML-1, General Safety TNW Zuid, Lab safety and basic Laser safety) before entering any lab.

The project will be done according the **GGO IG 15-027 permit** from the Department of Bionanoscience.

The work described is according to the Dutch GMO laws, under **'inschalingsartikel 5.2i inperkingsniveau MLI'.** For requirements, see appendix 1.

4. Biological safety

The aim of the 2017 TU Delft iGEM team is to make dryable cells with an additional a diagnostic function, using the microorganism *Escherichia coli.* The *E. coli* strains used are non-infectious and non-pathogenic and are scaled under biosafety level 1, see table below.

Host species	Strains	Biosafety level
Escherichia coli	MG1655	1
	BL21	1
	DH5a	1
	TOP10	1
	JW0729-3*	1

*This strain is part of the Keio Collection (a set of precisely defined, single-gene deletions of all nonessential genes in *Escherichia coli* K-12 BW25113) [7]. It will be acquired from Wageningen and assistance for registration in the labservant biosafety module is needed.

All materials will be provided by the department of Bionanoscience. Before working with biological material, all students will have taken a Biological safety ML-1 course. Students that have no Life Science & Technology background or have no experience in working with microorganisms will be under guidance by supervisor Esengül Yildirim for at least four weeks to learn good VMT practices.

The genes would be carried by the standard plasmids according to the iGEM rules (BioBrick Plasmid Backbone), and they would be introduced into *E. coli* via DNA transformation. The table below shows all inserts that are going to be used for the project, their native organism and function. The backbones in which the genes will be inserted will be one of three available iGEM backbones carrying different antibiotic resistance genes: pSB1C3, pSB1K3, pSB1A3.

Insert name	Donor organism	Function
CAHS 106094	*Hypsibius dujardini*	Tardigrade desiccation tolerance
CAHS 77611	*Hypsibius dujardini*	Tardigrade desiccation tolerance
CAHS 94205	*Hypsibius dujardini*	Tardigrade desiccation tolerance
CAHS 107838	*Hypsibius dujardini*	Tardigrade desiccation tolerance
SAHS 33020	*Hypsibius dujardini*	Tardigrade desiccation tolerance
SAHS 53582	*Hypsibius dujar dini*	Tardigrade desiccation tolerance
SAHS 64681	*Hypsibius dujardini*	Tardigrade desiccation tolerance
SAHS 68234	*Hypsibius dujardini*	Tardigrade desiccation tolerance
LwCas13a	*Leptotrichia wadei*	Part of defense mechanism against hostile RNA
TolR	*Escherichia coli K12*	Binds outer membrane to cytoplasmic membrane

The genes will be combined with promoters as well as the iGEM prefix and suffix restriction sites and then synthesized by IDT.

The main experiments that are going to be performed for introducing the new genes, and the assays for testing the outcome of the experimentations, are:

- Transformation
- Restriction with enzymes
- Purification of nucleic acids
- Precipitation of nucleic acids
- Protein purification
- Drying experiments
- Lyophilisation
- Ligation
- PCR
- DNA gels
- Microscopy (confocal, SEM, TEM)
- Spectroscopy
- Papermicrofluidics
- Optional: characterization work in ML-1 after cloning processes have been concluded

In the last stages of the project, we may characterize our device by analysing cow faeces. Since the bacteria in the sample are unknown and potentially harmful, this characterization will be carried out in the ML-1 lab after all cloning processes have been concluded.

6. References

[1] „WHO | Antibiotic resistance“, *WHO*. [Online]. Verfügbar unter: http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/. [Zugegriffen: 28-Mai-2017].

[2] J. S. Gootenberg *u. a.*, „Nucleic acid detection with CRISPR-Cas13a/C2c2“, *Science*, Bd. 356, Nr. 6336, S. 438–442, Apr. 2017.

[3] R. Arts, I. den Hartog, S. E. Zijlema, V. Thijssen, S. H. E. van der Beelen, und M. Merkx, „Detection of Antibodies in Blood Plasma Using Bioluminescent Sensor Proteins and a Smartphone“, *Anal. Chem.*, Bd. 88, Nr. 8, S. 4525–4532, Apr. 2016.

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[5] C. Schwechheimer und M. J. Kuehn, „Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions“, *Nat. Rev. Microbiol.*, Bd. 13, Nr. 10, S. 605–619, Okt. 2015.

[6] J. L. Baker, L. Chen, J. A. Rosenthal, D. Putnam, und M. P. DeLisa, „Microbial biosynthesis of designer outer membrane vesicles“, *Curr. Opin. Biotechnol.*, Bd. 29, S. 76–84, Okt. 2014.

[7] T. Baba *u. a.*, „Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection“, *Mol. Syst. Biol.*, Bd. 2, S. 2006.0008, Feb. 2006.

Appendix 1 - inschalingsartikel 5.2i inperkingsniveau MLI

5.2 Activities with a genetically modified micro-organism whose host is listed in List A1 in Appendix 2 (E.coli die jullie gebruiken staat hierin) and whose vector is either listed in List A2 in Appendix 2 or meets certain criteria, as follows:

i. the size of the vector is known;

ii. a vector map or description is available, including all the vector's composite parts and their relative positions;

iii. the functions and origins of the composite parts are known;

iv. the oris present in the vector are known;

v. the composite parts are not members of the group of insertions listed in Appendix 2, List A3; (vertaling beschrijving; schadelijke genproducten zoals toxines etc.) if they are, then those parts should be regarded as donor sequences;

vi. the vector contains no viral sequences from viruses hosted by higher eukaryotes, which might enable the vector to act as a viral vector; if it does, then those sequences should be regarded as donor sequences.

Activities in which characterised donor sequences are used:

i. The sequence contains no genetic information that codes for a harmful gene product. Classification: ML-I.