

## REAL-TIME PROTEIN SYNTHESIS MONITORING DEVICE

- EASY TO USE
- PRECISE
- PROTOTYPE FOR A  
DIAGNOSTICS DEVICE

### TEAM

**Maxim Rossmann (primary contact)**, University of Cambridge CIMR, Department of Haematology, [mr579@cam.ac.uk](mailto:mr579@cam.ac.uk). Maxim will contribute to the project with his knowledge of biophysics and will work on the development of the assay and device design. Maxim has experience in project management and also has worked on the development of commercial diagnostic tests.



**Shengjiang Tan**, University of Cambridge CIMR, [st299@cam.ac.uk](mailto:st299@cam.ac.uk). Shengjiang will contribute to the project with his knowledge of cell biology and expertise in the ribosomopathies. He will work on the assay design and refinement.



**Norberto Escudero**, University

of Cambridge CIMR,  
[ne272@cam.ac.uk](mailto:ne272@cam.ac.uk)

Norberto has expertise in molecular biology and genetic and will be working on the assay development and optimization.



**Christine Hilcenko**, University of Cambridge CIMR, Department of Haematology, [ch@mrc-lmb.cam.ac.uk](mailto:ch@mrc-lmb.cam.ac.uk). Christine is an experienced protein chemist and will work on the assay development. She will contribute by providing high quality proteins and ribosomes.



**Christie Nel**, Scaturio Ltd., Cambridge, [christie.nel@gmail.com](mailto:christie.nel@gmail.com). Software engineer experienced in development of LabView applications and instruments based on platform. Christie will support the development of electronics and software.



## **SUMMARY**

Our lab works on understanding how ribosomes are put together from their component parts and how this process is regulated by the cell. In this project we aim to design a tool for monitoring protein synthesis (translation) *in vitro*. We aim to design a simple device that will allow for the tracking of translation dynamics, which can be used for the discovery of novel translation-inhibiting or activation molecules and ribosome profiling for biophysical or structural studies. The device could also be used for teaching purposes and will serve as a prototype for a diagnostic device for the preliminary/quick detection of ribosomopathies,

*i.e.* inherited disorders characterized by the disruption in the ribosome assembly pathway and low protein synthesis capacity - an emerging class of human developmental and cancer predisposition disorders.

## **PROPOSAL**

### **i) Problem**

Ribosomes and protein synthesis in general are subjects of intensive research. But measuring ribosomal activity is cumbersome and requires expensive reagents and equipment. A robust and low-cost system is needed [**Ref. 1-2**] to perform basic experiments for characterisation of ribosomes and investigation of translation cycle effectors.

### **ii) Biological system**

To develop a prototype we will use commercial in vitro translation system PURExpress kit (NEB) as a characterized ribosome source. A liquid sample containing DNA or mRNA encoding for the eGFP protein will be mixed with the kits components, which will result in the formation of fluorescent protein. Fluorescence intensity will be monitored in real-time and used to quantify the fraction of active ribosomes. Normalised translational activity will be used to draw conclusions about the ribosome number and performance.

### **iii) Design goals for the hardware**

We aim to perform reaction and analysis using commercially available disposable 10  $\mu$ L slides Countess (Invitrogen) (**Fig. 1-2**), which ensure precise reaction volume control. Upon mixing the reagents fluorescent eGFP will be produced and the intensity of the fluorescent light will be measured in real-time by a RGB colour sensor with I2C interface. A UV LED will be used as the source of incident light. We will decide whether to use any additional optical filters or multiple channels during the trials involving the model and reference samples. The data logging and primary processing will be done on the PC using the colour sensor manufacturers software provided with the demo boards. The device will be assembled in a 3D printed enclosure (**Fig. 3**), controlled by Arduino and operated via a touch screen provided in the starter kit.

### **iv) how you plan to implement the project**

We will start the project with the development of the detector unit. Using standard fluorescent solutions we will determine the optimal distance between the RGB detector and sample and the UV lamp and learn how to read the output. In the next step we will work on the optimal reaction condition using standard plate reader and then translate knowledge onto the prototype. Once we have a robust and consistent output we will engineer the enclosure and let it print using 3D printing service at the Addenbrookes Hospital.

### **v) proposed outcomes and benefits**

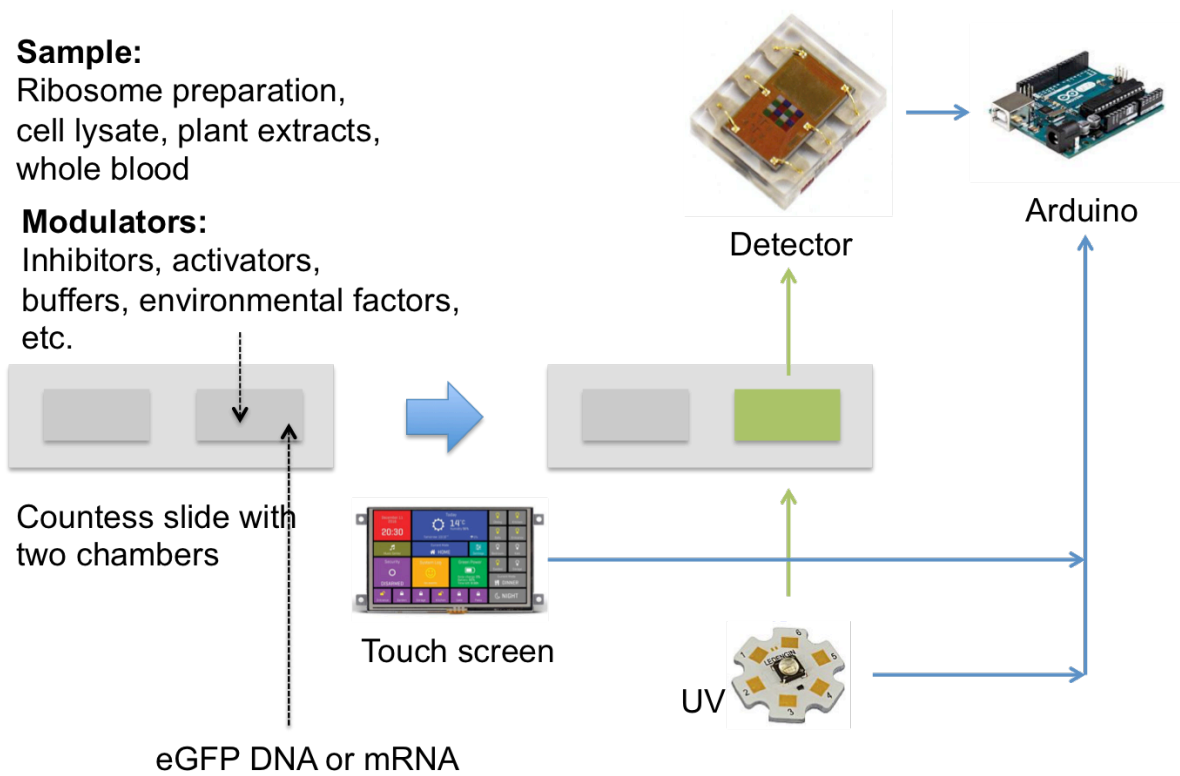
In the first place we plan to use the developed device for the analysis of the ribosome preparations. The device will also be used to measure translation inhibition and activation in microbiology, cell- and plant biology, drug discovery projects targeting ribosomes and could serve as a prototype for a simple diagnostic device to detect ribosomopathies,

such as for example SBDS (Shwachman-Diamond Syndrome) [Ref.3].

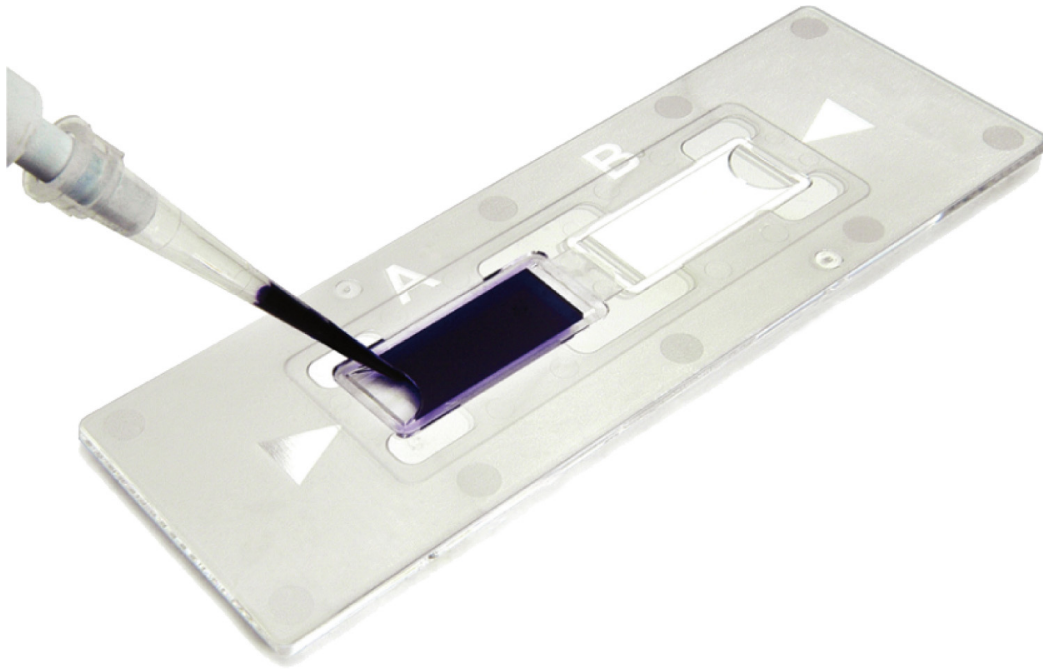
**Budget:**

LED UV5TZ-390-30:	50
RGB Sensor TCS3472 I2C	55
3D printing costs	200
PURExpress Kit E6800S	190
Reagents	100
Expression plasmid	90
Disposable <i>Countess</i> slides	30
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<b>Total</b>	<b>715</b>

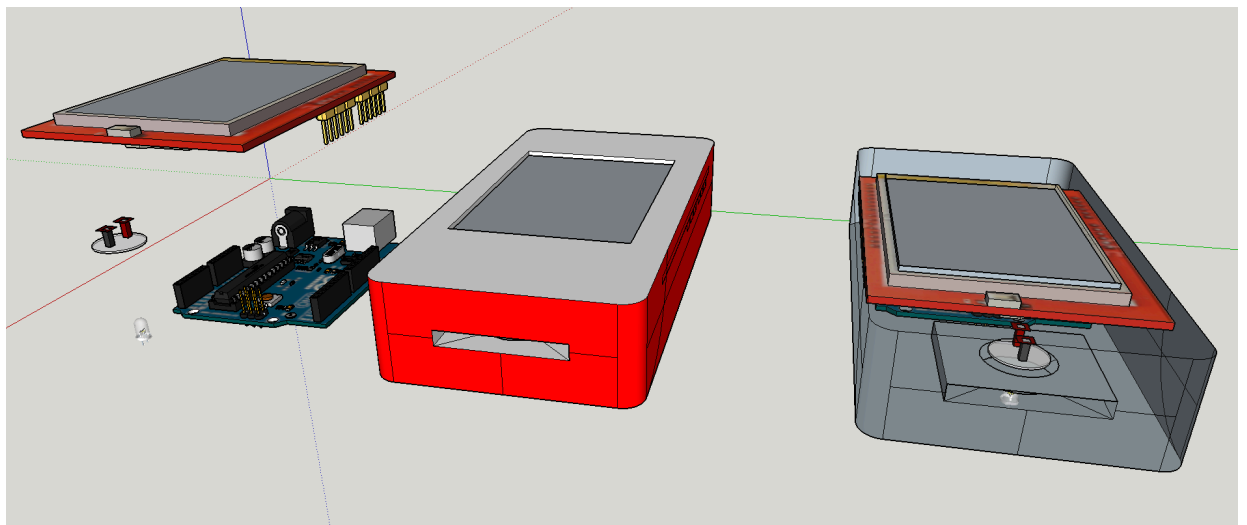
**Fig 1.** RiboReader - general design.



**Fig 2.** Countess disposable 10uL slide chamber



**Fig 3.** RiboReader.



### References:

- (1) Rosenblum G., 2012
- (2) Capece CM, 2015
- (3) Wong CC., 2011