OpenPlant Fund Application Form

Title of Project
Cell-free diagnostics for the surveillance of livestock viruses

Primary contact for the team
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Team
Our team 'OpenDiagnostics' (www.open-diagnostics.org) is an interdisciplinary group of graduate students who have been investigating the potential value and impact of cell-free paper based diagnostics across the developing world, since carrying out a Development iTeams project on the technology in late 2016. Having already built partnerships in South Africa and Kenya, the team is now looking to start prototyping diagnostics. They are grateful for ongoing support from both the Synthetic Biology SRI at the University of Cambridge, and the Centre for Global Equality.

Team member	**Contribution**
Laura Mitchell - lsm35@cam.ac.uk Department of Chemistry, University of Cambridge	Laura will use her undergraduate degree in Biochemistry to help mentor and work together with the OpenDiagnostics summer intern student, Punika to replicate the cell-free Zika sensor published by Pardee et al., in the liquid phase.
Raghd Rostom - rr415@cam.ac.uk Wellcome Trust Sanger Institute & University of Cambridge	Raghd will apply her expertise in Bioinformatics and Virus biology to carry out *in silico* design of RNA amplification circuits and toehold switches. She will further help characterise the functionality of these designs in cell-free once the Biomakespace opens.
Emily Groves – egroves113@aol.com Department of Medicine, University of Cambridge	Emily will apply her biological training and research expertise to help construct novel cell-free viral sensing circuits in the Biomakespace.
Andre Zylstra - az308@cam.ac.uk Babraham Institute and University of Cambridge	Andre will be managing Cambridge University Synthetic Biology Society project work from September 2017. He will help with the design of RNA amplification circuits and toehold switches, and help prototype these in the lab.

Summer Intern student – **Punika Ratchachittapong** – pr934@cam.ac.uk, University of Cambridge

Summary

Cell-free paper based diagnostics like those developed by Pardee et al. for the detection of Zika and Ebola viruses, are low-cost, point-of-use and highly stable, and are therefore poised to revolutionise diagnostic testing in resource-limited settings across the Global South.

Livestock viruses such as Foot and Mouth Disease (FMD), Newcastle Disease (ND), and Bovine Respiratory Syncytial Virus (BRSV) are an emerging threat to global food security, farmers' livelihoods, and national economies. In many countries, livestock diseases are endemic; meaning they are impossible to eradicate due to uncontrollable transmission between domestic and wild animal populations. Implementation of disease surveillance programmes is the most effective way of controlling disease-related losses, since rapid detection of disease enables timely action to be taken, preventing further spread of the disease. The greatest barrier to the implementation of disease surveillance programmes in low-middle income countries (LMICs) is the lack of affordable, point-of-use diagnostics.

In the proposed project, we outline how we plan to firstly replicate the work of Pardee et al. 2016 in detecting a fragment of the Zika virus genome with an RNA-based toehold switch-lacZ gene circuit, in a cell-free system. Secondly, we will adapt this circuit to detect a viral fragment from a livestock virus – the specific virus will be selected upon consultation with disease experts at the University of Cambridge and the Pirbright Institute. Adaptation of the circuit will involve *in silico* design of RNA amplification circuits and novel toehold switches, which activate in the presence of amplified viral trigger RNA, to express LacZ, an enzyme which produces a visible colour change.

Proposal

Our top-level goal is to build a liquid phase cell-free diagnostic test for livestock viruses, based on the Pardee et al. 2016 publication "Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components". In order to reach this goal, we have broken our project down into four manageable parts.

Part A: Replication of the cell-free Zika-virus sensor described in Pardee et al., 2016.

Aims:

- Establish whether our stock of MYtxtl cell-free reaction kits are still functional after >6 months storage.
- Replicate the work of Pardee et al. in the MYtxtl system, as an alternative to the NEB system used in their paper.

Logistics:

- This work will be carried out July-August in the Ajioka lab, by our summer intern Punika, under Laura's supervision.

Methods:

- Express GFP in the MYtxtl cell-free system using the p70a-eGFP plasmid provided in the kit.
- Express LacZ in the MYtxtl system, by cloning LacZ into the p70a-eGFP plasmid. Observe and measure a visible colour change upon addition of Chlorophenol Red-β-D-galactopyranoside substrate.
- Clone the toehold switch (ZIKV_Sensor_27B_LacZ, from Addgene plasmid #75006) into the p70a plasmid, to ensure promoter compatibility with the MYtxtl system.
- Clone the trigger RNA (ZIKV_Trigger_27B, Addgene plasmid #75008) into the p70a plasmid, to ensure compatibility with MYtxtl.
- Observe and measure a visible colour change upon addition of Chlorophenol Red-β-D-galactopyranoside substrate to the cell-free system which expresses both the toehold switch and trigger RNA.

Part B: *In silico* design of novel toehold switches

Aims:

- Identify which of three viruses in our shortlist (FMD, ND and BRSV) to prototype a cell-free biosensor for.
- Generate a panel of RNA toehold switch sequences, which are designed to express the colour-change enzyme LacZ when activated by a trigger sequence.
- Devise a scoring system to choose which of the toehold switches should be experimentally tested in the cell-free system.

Logistics:

- Selection of the virus will involve discussions with livestock disease experts from the University of Cambridge Veterinary School, and the Pirbright Institute.
- In silico design of RNA toehold switches can be carried out remotely, as a joint effort between Raghd, Andre and Laura
- We realise this is a non-trivial task and have allocated August-September to complete this.

Method:

- In silico design of RNA toehold switches will involve A) creating design specifications for de novo generation of RNA sequences, B) Generation of RNA sequences with the NUPACK software package, C) Filtering of sequences with in-frame stop codons, and poor predicted thermal stabilities, D) Identification of sequences with minimal predicted cross-talk (i.e. high specificity to the trigger RNA).

Part C: Construction and characterisation of a Nucleic Acid Sequence Based Amplification (NASBA) circuit.

Aims:

- All RNA-based diagnostic tests will likely require an amplification step to achieve clinically relevant sensitivities. Pardee et al. report the successful use of an isothermal amplification step which we would aim to replicate using either the same or similar technology.
- Testing and characterising isothermal amplification reactions. Particularly important to assay will be the capacity and time taken to amplify clinically relevant quantities of RNA to robustly and reproducibly detectable thresholds.
- Gather information on primer sequence constraints for optimal performance. To be integrated into the *in silico* design process.

Logistics:

- Selection of the appropriate isothermal amplification technology will consider factors such as price, reaction efficiency, ease of use, and long-term reagent stability under anticipated usage and storage conditions.
- Testing to be carried out at Biomakespace with work contributions from Andre, Laura, Raghd & Emily
- Testing can be potentially be carried out in parallel with work on Parts A and B. Expected to take place August - November.

Methods:

- Most amplification systems rely on DNA templates and primers. Initial characterisation of isothermal technologies would use dilution series of suitable DNA template sequences and complementary primers. The endpoint DNA concentration would be quantified using a NanoDrop™ spectrophotometer, or similar instrument.
- Later testing would attempt to incorporate a reverse transcriptase enzyme, enabling use of RNA input, and RNA polymerase to enable a full NASBA circuit.
- Finally, testing using primers directed against the virus of interest would enable incorporation into the full toehold sensor detection workflow using the amplification reaction products as input to the toehold sensor cell-free reaction.

Part D: Characterisation of *in silico* designed toehold switches in the cell-free system

Aims:

- To identify which of our designed toehold switches successfully generate a LacZ mediated colour change, in the presence of the trigger RNA.

Logistics:

- The majority of our consumables budget will be spent on this part of the project.
- Laura, Raghd, Andre and Emily will carry out this part of the project at the Biomakespace, October-December, during evenings and weekends.
- We hope students from the Cambridge University Synthetic Biology Society will be excited about joining our project at this stage.
- We have accounted for the purchase of 40 toehold switches, but it is probable that we might not need to characterise so many if a functioning switch is identified in a preliminary round of screening.

Method:

- Order the top scoring toehold switches and cognate trigger sequences, identified during *in silico* design, as geneblocks, and clone these into MYtxtl-compatible plasmids.
- For each toehold switch, measure the colour change that occurs with the addition of trigger RNA at concentrations of 0nM and 3000 nM. Colour change observed for experiments with 0 nM trigger RNA are indicative of signal leakage.
- Measurements will be the change in absorbance at 570 nm over time, using a plate reader.
- The toehold switches that generate the largest fold increase in absorbance, over the shortest time, will be selected for characterisation of sensitivity to trigger RNA concentration.

Possible Project Extension

Lastly, if the we are successful in completing all of the above parts, we will move onto attempting to freeze-dry cell-free virus-sensing circuits onto paper substrates. We have identified freeze-drying equipment that is available in the Departments of Chemistry, Haematology, and Metallurgy. To optimise the freeze-drying process, we will need to find the optimal “recipe”; comprising temperature, pressure and time settings.

Benefits and outcomes

This project fits with the cell-free synthetic biology theme, and with the growing interest in Cambridge into the development of cell-free genetic circuits for sensing applications. The significant contributions from this project will be: demonstration of the first functioning cell-free virus-sensing circuit in Cambridge, and the synthesis and characterisation of novel toehold switches. All experiments will be fully documented.

With hands-on experience building circuits, the OpenDiagnostics team will be better prepared for the second aspect of their mission – to develop training resources for people around the world to learn how to build their own cell-free genetic circuits and biosensors.

Sponsor for the research and cost centre

Dr. James Ajioka – ja131@cam.ac.uk
Department of Pathology, University of Cambridge

I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).

Budget

OpenPlant Fund Budget

Item	**Cost/£**	**Comment**
Consumables for biological prototyping[1]	3730	40x custom toehold switch sequences (£30 each) = £1200 40x cognate RNA trigger sequences (£25 each) = £1000 Pardee et al. toehold switch and trigger (Addgene) = £100 Transformable E.coli = £170 Restriction and ligation enzymes = £200 Miniprep DNA extraction kit (250 reactions) = £200 DNA sequencing costs = £200 Colour change substrate for LacZ (Chlorophenol Red-β-D-galactopyranoside) = £25 Miscellaneous consumables e.g. primers, agar, plates, antibiotics, plasticware etc. = £335
Biomakespace membership	180	4 people for 3 months at ~£15/month/pp
Travel to Pirbright Institute, Surrey	90	To discuss project with livestock disease experts. To cover 200 mile return journey by car (45p/mile).
Total cost	**4000**	

[1]We already have 288 reactions worth of MYtxtl cell-free reaction mixture.

Additional funding available to support the project:

Grant/Prize	**Value/£**	**Comment**
Small grants scheme for SRIs	2000	This will cover part of the maintenance and consumables costs for Punika's internship.
Parmee Prize	2000	We anticipate applying for Santander matched funding to support an engineer or biology intern.
Cambridge University Entrepreneurs £1k Prize	1000	This will cover the remainder of maintenance costs for Punika's summer internship.
London Healthtech Challenge	10000	
Total	**15000**	**Of which £10000 is available to progress the project long term.**