**Project Title:**

“Visualising genetic circuits in space and time with paper-based cell-free translation”

**Report Title:**

Developing *E.coli* cell-free transcription-translation reaction suitable for outreach and education purposes

**Summary**

The project’s ultimate aim is to develop paper-based cell-free transcription translation (TXTL) platform that is suitable for visualising activity of genetic circuits. Implementation of this aim is dependent on three tasks: establishing a protocol for production of robust paper-based TXTL system, identifying TXTL-activity reporters and prototyping gene circuits on the interconnected paper-based TXTL layout using chosen reporters. Currently, we have managed to find a robust protocol for production of highly active TXTL system in solution using sfGFP as a reporter. Numerous trials of TXTL-reaction on paper have largely failed and we are currently troubleshooting this. As an alternative strategy to paper-based TXTL we found that freeze-dried TXTL solution mix shows a significant activity upon its reconstitution and thus can a viable option to paper-based cell-free reaction setup. We propose using lyophilised solution-based TXTL setup for further outreach and educational activities.

**Report and outcomes**

The project started with accommodating already known protocols for production of highly active TXTL E.coli mixtures. For this I have employed two protocols: a traditional approach with cells being opened by French press (Emulsiflex) and high-speed centrifuge and a cheaper and more streamlined protocol using Sonication and cooled table-top centrifuge. Independent of the protocol for E.coli lysate preparation activity has been comparable and close to the activity of a commercial mixture (Promega T7 high yield S30) (Fig1A and B). The protocols for both approaches have been posted in details to https://www.protocols.io/researchers/zakir-tnimov. The procedures have also been reported on a workshop in Cambridge Biomakespace as well as during a master class at James Haseloff Lab. The later showed the ease and robustness of obtaining TXTL active mixtures in house.

Next, according to implementation schedule, I have tried to recapitulate conditions for obtaining paper-based TXTL largely following James Collins laboratory publications. While cell-free reactions reportedly showed high activity in the solution, paper-based option could only give signal very close to the background. The latter could only be visualised with highly expensive instrumentation (Typhoon Trio or Plate reader) (Fig1C and D) and thus could not be used in any outreach low-cost settings. In a process of finding proper conditions, I have varied amount of the lysate being loaded on the paper, type of paper and blocking of paper with BSA but none of the trials gave any reasonable activity. Therefore, I thought to find a viable alternative that would be suitable for outreach as, ultimately, goal of the current project is to built an educational kit for teaching synthetic biology. Thus instead of using lyophilised TXTL on the paper (Fig1E), instead I freeze-dried TXTL in the tube and tested its activity after reconstitution with mQ water. As oppose to the lyophilised paper-based TXTL, lyophilised solution-based TXTL showed activity very close to the intact lysate. Therefore I suggest using this type of a TXTL-reaction in future outreach activities.

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**Figure 1. A.** Comparison of In-house prepared *E.coli* BL21 (DE3) extract to extract from Promega . **B.** Optimisation of preparation of *E.coli* extract by sonication. S30 extract from *E.coli* BL21 cells have been prepared using conventional protocol using Frenchpress and centrifuged at 30000 g, while S20 lysates have been prepared by Sonicator with indicated amount of energy acquired by the sample and centrifuged in table-top centrifuge at 20000g. **C.** Synthesis of sfGFP in Paper-based TXTL. **D.** Comparison of TXTL that proceed in solution and on paper. Same reaction mix has either been run in the tube and then applied onto the paper or freeze-dried and reconstituted the paper. **E.** Assessment of how freeze-drying and following reconstitution of the TXTL mixture affects its performance. As a control same but not lyophilised lysate and a Master Mix was used in preparing TXTL.

**Changes to team**

None

**Expenditure**

|  |  |
| --- | --- |
| **Item** | **Spent, £** |
| Oligonucleotides | 14.30 |
| Whatman Grade 42 Quantitative Filter Paper Ashless (Ash 0.007%), circle, 42.5 mm | 9.47 |
| Disposable Biopsy Punch, 2.0mm diameter | 153.00 |
| Corning Low Volume 384 Well Black with Clear Flat Bottom NBS Microplate 10 /bag w/out Lid Nonsterile | 310.34 |
| Shipment | 7 |
| TREHALOSE FOR BIOCHEMISTRY | 56.08 |
|  | Total spent 550.19 |

**Would you like to claim the £1,000 follow-on fund?**

*Yes, we would like to claim the £1,000 follow-on fund to be used as described below.*

*I already have agreed with ‘Pilgrim’ international summer school (Russian Federation) to run two-day workshop on Synthetic Biology with using in-house prepared freeze-dried TXTL over 13-14 of August. In addition to this I plan to arrange one or two workshops on preparing TXTL in Cambridge Biomakespace and also plan to travel to Norwich to give a presentation in Biomakers club.*

**Follow on Plans**

Given the success of using lyophilised TXTL in the tube I plan to prepare a large batch (100ml) of S30 extract to be further used in outreach activities by me and by larger Synthetic Biology communities in Cambridge (e.g. Biomakespace). This will necessitates baying components for the master mix with the budget shown below the text (thus far I have used reagent from my Jason Chin lab and MRC LMB communal consumables such as microbiology media). I plan to prepare a set of vectors containing fluorescent protein reporters besides sfGFP as well as proteins and RNAs that can be employed in a simple genetic circuit (Cas9, its catalytically inactive variant, sgRNA). The latter will be used in Synthetic Biology workshops (in *‘Pilgrim’* for instance) to where I plan to travel in mid-August this year.

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| --- | --- |
| Component | Price, £ |
| 19 Amino acids (1g each) | 275.26 |
| Potassium L-glutamate (100g) | 40.47 |
| Ammonium L-glutamate (100g) | 68.50 |
| Hemimagnesium L-Glutamate (250g) | 32.95 |
| Potassium oxalate (50g) | 23.32 |
| Ribonucleotide triphosphates (>250mg) | 285 |
| Spermidine (1g) | 16.53 |
| Putrescine (5g) | 19 |
| Phosphoenolpyruvate (250mg) | 91 |
| Whatman filter paper | 32 |
| chlorophenol red-b-D-galactopyranoside (100mg) | 64 |
| 4-Nitrophenyl N,N’-diacetyl-b-D-chitobioside (5mg) | 190 |
| Total | 1138 |

Genetic construction

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| --- | --- |
| Component | Price, £ |
| Oligonucleotides | 100 |
| PCR reagents (Q5 polymerase 500U) | 224 |
| Gibson Assembly Master Mix | 121.6 |
| Total | 445.6 |

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| --- | --- |
| Component | Price, £ |
| Traveling to Norwich (train and bus), 2 persons | 60 |
| Traveling to ‘Pilgrim’ bio-school in Russia (fight, ground travel and a stay), 1 person | 600 |
| Total | 660 |