

Project Title: Cell-free proteins synthesis as a resource for generating plant proteins

Report Title: Six-month progress report

Summary

Cell-free protein synthesis (CFPS) has emerged as a powerful technique for on-demand, in vitro protein production which reduces labour and increases experimental throughput. However, cell-free systems can be laborious and expensive to set up and there is a shortage of publicly available data comparing different CFPS systems, particularly regarding plant proteins which can be difficult to express. In this project, we developed a resource for researchers to prototype their experimental plans without setting up the system themselves. We compared an in-house generated *E. coli* S30 crude lysate system with a commercial wheat germ platform to quantify their ability to synthesize transcription factors and other plant proteins. This will provide data that can inform which CFPS platform is best suited to a researcher's needs. We held multiple workshops in Norwich and Cambridge to communicate these results and engaged with 20 participants and crowdsourced DNA assembly to 9 workshop participants who tested a gene useful their own research.

Report and outcomes

Outcome #1: CFPS expression vectors pJL1 and pF3 WG (BYDV) Flexi® will be modified to be compatible with Type IIS-based restriction cloning (Golden Gate, MoClo, Loop, etc). Standardized backbones will be available through Addgene (if possible).

Completed: Expression vectors were successfully assembled and used for cell-free expression. Three backbone vectors are available: pEPQD0KN0024 and pEPQD0KN0025 for the *E. coli* system and pEPQD0CB0026 for the wheat germ system. Due to availability, the pEU vector (Addgene #53738) was used in place of the pF3 plasmid. Four C-terminal tags were also cloned into Level 0 vectors (pEPQD0CM0027- pEPQD0CM0030) for detection of expressed proteins.

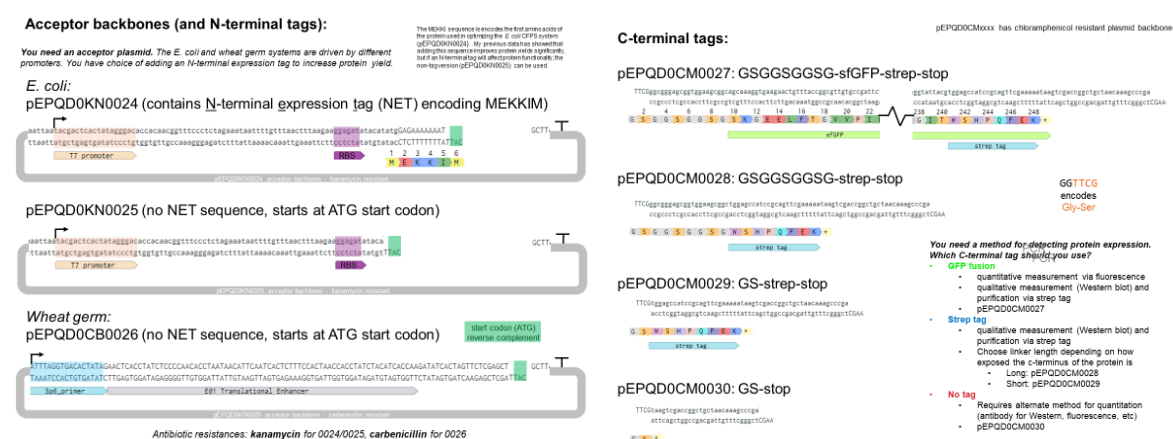


Figure 1. Representation of plasmids generated for this project

In progress: Plasmids will be uploaded to Addgene upon publication of protein expression results.

Outcome #2: Comparison of cell-free protein synthesis of ~12 transcription factors from wheat (with and without fusion to GFP) using both *E. coli* S30 and wheat germ (Promega) kits. (Susan Duncan, EI)

Completed: In coordination with Susan Duncan, we expanded the scope of the project to test 42 transcription factors (TFs) in cell-free expression. 39 were successfully synthesized by Twist Bioscience into an *E. coli* CFPS-compatible vector. 37 were tested in the *E. coli* CFPS system; **11 of 37** successfully expressed. pSD0KN06 was purified by Strep-beads and it appears there is sufficient protein for the DAP-seq protocol.

In progress: DNA assembly for wheat germ plasmids is ongoing; we expect to compare all 39 proteins in the coming months.

Outcome #3: Publication of a computational model incorporating a publicly available web interface for comparing and predicting cost of plant protein CFPS (Nicolas Larus-Stone, UC).

Completed: Preliminary data for protein expression of wheat transcription factors (Objective 2) and other proteins (Objective 4) is 80% complete.

In progress: Unfortunately, with the delays in collecting expression data, Nicholas Larus-Stone has completed his master's thesis and is unavailable to continue development a computational model. In response, we are planning a short paper summarizing these results to be published in an open-access journal.

Outcome #4: Two hands-on workshops (at Norwich and Cambridge) will allow attendees to run and quantify a protein with the option of providing a DNA template of their own choosing.

Completed: Three workshops were completed with **9 participants** who cloned and expressed proteins of interest to their research (8 Norwich, 1 Cambridge) along with **11 observer participants** who expressed GFP as part of the workshop (2 Norwich, 9 Cambridge).

Workshop dates:

July 3, 2018 - Norwich, Earlham Institute G52

July 5, 2018 - Norwich, Earlham Institute G52

July 19, 2018 - Cambridge Department of Plant Science Teaching Lab

August 2, 2018 - Norwich, Earlham Institute G52

In progress: There are two additional participants who experienced delays in DNA assembly but have now completed their plasmids. There will be a final joint workshop next week to express their proteins. While no further workshops are planned, additional reagents are available for future collaborations on an ad hoc basis

Summary table (selected results; two participants have yet to characterize their proteins)

Protein	<i>E. coli</i> PANOx-SP	TNT® Coupled Wheat Germ Extract System (Promega)
RNA-induced silencing complex protein from <i>Arabidopsis thaliana</i>	very low	very low
transcription factor from <i>Triticum aestivum</i> (wheat)	good	not tested
signalling kinases from <i>Arabidopsis thaliana</i>	1 good	good
	2 none	low expression
meiotic protein from <i>Arabidopsis arenosa</i>	1 low expression	good
	2 none	very low
hydrolases from <i>Bacteroides thetaiotaomicron</i> (a gut bacterium)	1 good	not tested
	2 good	not tested
	3 good	not tested
	4 good	not tested

Supplementary photos:



Figure 2. Participants of the Norwich workshops

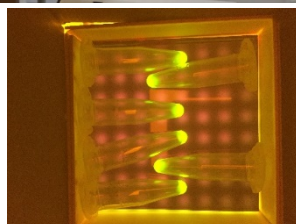


Figure 3. Participants in the Cambridge workshops. In bottom left is GFP expressed by workshop participants using the *E. coli* system

Changes to team

The team has not changed in composition; however, Nicholas Larus-Stone has graduated and we are exploring alternate methods to publish and promote our results.

Expenditure

<i>Consumables</i>	<i>£394</i>
<i>Chemicals/Reagents</i>	<i>£2,846</i>
<i>Shipping of reagents to Cambridge</i>	<i>£60</i>
<i>Travel for workshops</i>	<i>£56</i>
TOTAL	£3,356

Note that I need to reimburse the Patron lab for use of reagents for Western blots ~£250

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.

Follow on Plans

- 1. Complete DNA assembly for wheat transcription factors into vectors for expression using the Promega wheat germ cell-free protein synthesis kit. Additionally, we will try expressing these in *N benthamiana*. Thus, we could compare three systems for expression in parallel. Reagents and consumables for DNA assembly and protein purification will costs ~£200. This will be in coordination with Susan Duncan's follow-up proposal in her OpenPlant fund award. **Aug 2018 to Dec 2018***
- 2. Optimize wheat germ kit for expressing P450 enzymes from the O'Connor group. This will require purchasing two more reagents (~£50) and likely more of the Wheat Germ master mix (£100-450) **Aug 2018-Nov 2018***
- 3. Try expressing a panel of plant chaperones in the *E. coli* system to improve its ability to express and fold transcription factors or NLR immune proteins (cost to synthesize/assemble DNA ~£400) **Nov 2018-March 2019***
- 4. Explore possibility of generating lysate from BY2 tobacco cells in which vacuoles are removed from the protoplasts (unknown cost, need to obtain access to a nitrogen mini-bomb for lysis). This aim does not have a timeline or funding allocated at this time.*