# Assessing functionality of cell-free protein synthesis systems from attenuated pathogenic organisms

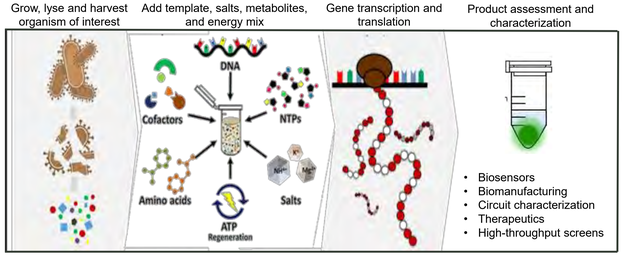
Nathan D. McDonald[1], Katherine Rhea[1], Aleksandr Miklos[1]
[1]U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD



## ABSTRACT

Cellular lysates capable of transcription and translation have become valuable tools for prototyping and running genetic circuits, screening engineered functional parts, and producing biological components. Despite the increasing use of lysates, the diversity of organisms used to produce lysates trails behind the diversity of organisms studied and manipulated for biotechnological pursuits. Here we report that lysates derived from several attenuated strains of *Yersinia pestis* are functional and when driven by the housekeeping σ70 or the T7 bacteriophage promoters can produce GFP at levels comparable to similar systems derived from *Escherichia coli*. We also show that *Y. pestis* specific regulatory promoters are functional in the *Y. pestis* derived lysates but not in *E.coli*. Due to the unique natural lifestyle of *Y. pestis*, lysates were produced from *Y. pestis* grown at 21°C, 26°C, and 37°C. Lysates from all temperatures were generally capable of *in vitro* transcription and translation. To assess the utility of these *Y. pestis* lysates, we further demonstrate they can express a native virulence marker, the F1 capsule-like protein, and non-native proteins of interest. Finally, we developed functional cellular lysates from attenuated strains of *Bacillus anthracis* and *Francisella tularensis* which both rely on native constitutive promoters for GFP expression. Together this data demonstrates that, without genetic enhancements, cell-free extracts from these non-*E. coli* chassis organisms are functional and for *Y. pestis* the productivity of the lysates is dependent on strain as well as growth temperature. The ability to generate these functional cell-free lysates from organisms other than *E. coli* provides an additional tool to discover enhanced functionalities for biotechnological applications and to mitigate risks associated with pathogen and emerging threat assessments.

## CELL-FREE PROTEIN SYNTHESIS SYSTEMS




## *YERSINIA PESTIS* CELL-FREE LYSATES

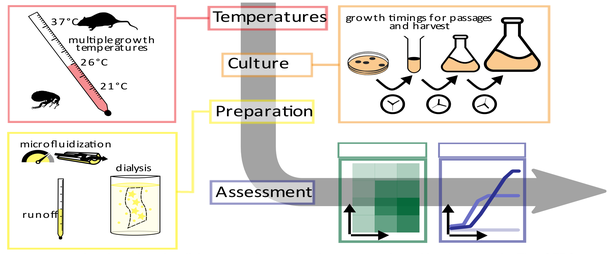



**Figure 2: Generating functional cell-free protein synthesis systems from *Yersinia pestis*.** The CFPS systems from attenuated *Y. pestis* CO92⁻ were optimized through a variety of conditions. The growth and culture temperatures and timing were chosen based on the lifecycle of *Y. pestis* along with maximizing protein content. The extracted lysates were further processed for enhanced functionality. Each of the lysates were characterized and optimized in CFPS reactions.

## CHARACTERIZING *YERSINIA PESTIS* CFPS

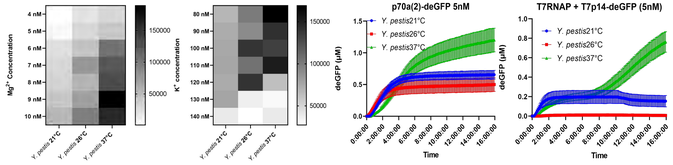
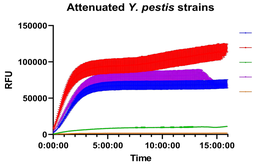



**Figure 3: Optimizing and assessing CFPS systems from *Y. pestis*.** The lysates from attenuated *Y. pestis* CO92⁻ were optimized for magnesium and potassium ion concentrations which are known factors influencing CFPS reactions. Once each lysate was optimized for salt concentrations, the productivity of each lysate was assessed with a reporter under the control of a housekeeping σ70 promoter or a bacteriophage T7 RNA polymerase dependent promoter. In both cases, the CFPS systems from a 37 °C lysate was the most productive.




| Strain | Plasmids | Attenuation |
|---|---|---|
| D-12 | pCD1/pMT1 | pCD1- |
| Kim6 | pCD1/pMT1 | Lcr- |
| EV76 | pCD1/pMT1 | Pgm- |
| CO92 | pCD1/pMT1 | Pgm- |
| N-41 | pPCP/pCD1/pMT1 | Pgm- |

**Figure 4: Evaluating lysates generated from multiple attenuated strains of *Y. pestis*.** Having demonstrated that CFPS systems from *Y. pestis* are functional, we set out to assess productivity differences across attenuated strains. Lysates were generated from five strains of attenuated *Y. pestis* grown at 21 °C and evaluated with the σ70 controlled reporter. We found that productivity is strain dependent with some strains having little or no functionality.

## UTILIZING *Y. PESTIS* CFPS TO MAKE THE F1 CAPSULE-LIKE PROTEIN

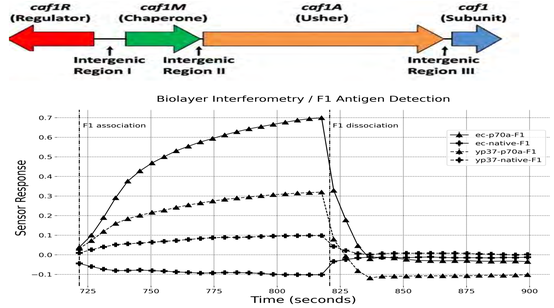



**Figure 5: Producing the F1 capsule protein in *Y. pestis* CFPS.** A hallmark feature of *Y. pestis* is the production of the proteinaceous capsule comprised of the F1 protein. A) Genetic organization of the F1 capsule in *Y. pestis*. B) A construct comprised of the *caf1* gene under the control of the native promoter (intergenic region I) or σ70 was expressed in attenuated *Y. pestis* or *E. coli* lysates. The production of F1 was assessed with an F1 specific Bio-layer interferometry antibody binding assay. The both the native and σ70-promoter successfully expressed *caf1* in the attenuated *Y. pestis* lysate.

## GENERATING AND CHARACTERIZING A *FRANCISELLA TULARENSIS* CFPS

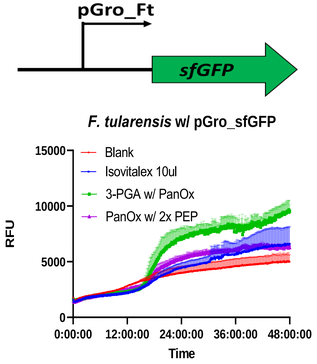



**Figure 6: Generating functional cell-free protein synthesis systems from *Francisella tularensis*.** The growth, timing, and nutritional requirements were optimized for attenuated *F. tularensis* to generate lysates. A variety of expression constructs were tested and not functional in the CFPS (not shown). The promoter controlling constitutive expression of *groEL in F. tularensis* was to express *sfGFP* and this was found to function in the CFPS reactions. After establishing a functional reporter system, a variety of cell-free energy mixes and reactions were evaluated with the *F. tularensis* lysate. We found that by including two sources of energy in the mix; 3-PGA along with PanOx, yielded the greatest amount of GFP production. In addition, the overall length of the CFPS reactions were extended to 48 h where it was observed that GFP production continued to increase.

## GENERATING AND CHARACTERIZING A *BACILLUS ANTHRACIS* CFPS

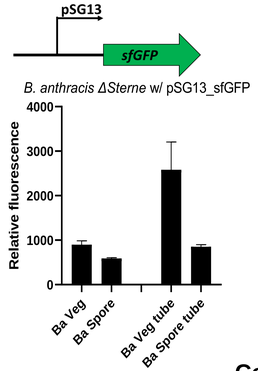



**Figure 7: Generating functional cell-free protein synthesis systems from *Bacillus anthracis ΔSterne*.** The growth, timing, and nutritional requirements were optimized for attenuated Bacillus anthracis *ΔSterne* to generate lysates from both vegetative and spore material. A B. anthracis constitutive promoter, pSG13 was used to control the reporter GFP construct and evaluated in the lysates generate from B. anthracis *ΔsterneΔ*. Endpoint fluorescent measurements from CFPS reactions run in both a 96-well plate as well as Eppendorf tubes found that lysates made from both the vegetative and spore material could express GFP. In both cases the vegetative material was more productive, as expected, and the reactions performed better in tubes compared to the plate reactions. This expression is on par with what has been observed from previously described Bacillus CFPS systems.

## Conclusions

- Demonstrated that functional cell-free protein synthesis systems can be generated from multiple attenuated strains of *Y. pestis*
- Functionality of lysates is strain and growth temperature dependent
- *Y. pestis* lysates are capable of driving gene expression from native promoters.
- *Francisella tularensis* CFPS systems require unique energy buffer composition and extended reaction times. This system functions from a constitutive *F. tularensis* specific promoter.
- *Bacillus anthracis ΔSterne* lysates are functional from a native promoter in CFPS reactions when derived from vegetative cells.

## Future work

- Develop additional threat relevant lysates for cell-free protein synthesis using attenuated *Burkholderia pseudomallei* Bp82.
- Fully characterize the current functional lysate panel using proteomics analysis to determine relevant protein composition and identify any potential novelties.

**Acknowledgements:** We would like to thank DTRA-JSTO for providing funding for this effort. The views expressed in this poster are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.

**DEVCOM CBC @ DTRA CBD S&T Conference**
Scan the QR Code to view all of CBC's
2022 DTRA CBD S&T Conference materials
https://cbc.devcom.army.mil/cbdst-conference/