

Assessing the Relationship Between Functionality and Proteome of Cell-Free Protein Systems from *Yersinia Pestis*

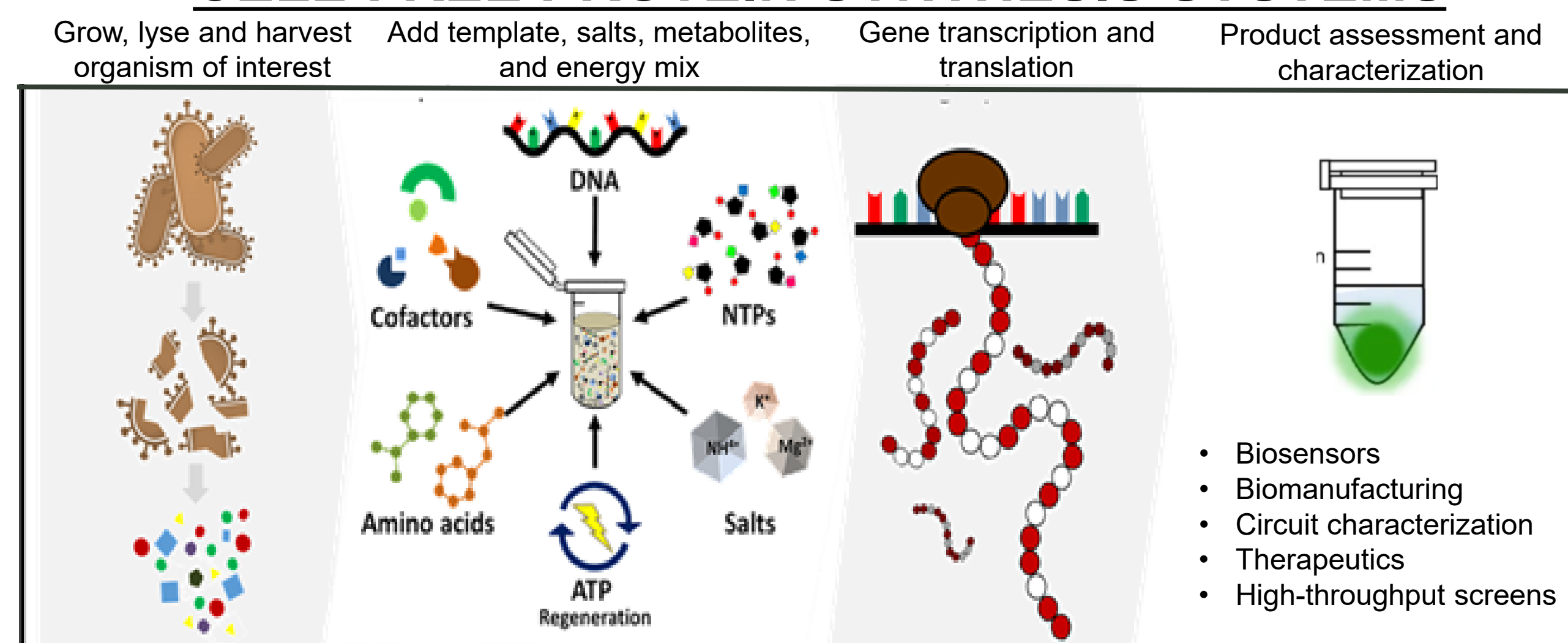
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ABSTRACT

Cellular lysates capable of transcription and translation have become valuable tools for prototyping and evaluating genetic circuits, screening engineered functional parts, and producing biological components. Despite the increasing use of lysates, analysis of the composition of proteins in these lysates as well as their contribution to overall productivity has yet to be fully explored. The ability to characterize a lysate as productive vs. non-productive without a major investment in resources would be a useful capability for biotechnological pursuits. Here we report the overall productivity, as measured by expression of sfGFP (superfolder Green Fluorescent Protein), of several lysates derived from attenuated strains of *Yersinia pestis* grown at 21°C, 26°C, and 37°C. Lysates from all temperatures were generally capable of *in vitro* transcription and translation but certain strains and temperatures were shown to produce higher amounts of desired protein. To explore this relationship, we conducted a full proteomic analysis of several strains of *Yersinia pestis* grown at varying temperatures identify correlative relationships of the amount of protein produced, to the presence of specific proteins within the lysates. We also assessed the functionality of expressed protein to determine if certain lysates produced more functional proteins than others. Finally, we compared our *Y. pestis* lysates to standard systems made from *E. coli* from both commercial sources and those made in-house following accepted protocols to determine if *Y. pestis* lysates offer any advantage over traditional systems. Together these data demonstrate that *Y. pestis* lysate productivity is dependent on strain as well as growth temperature which effect the proteomic profile of the organism. The ability to generate these functional cell-free lysates from organisms other than *E. coli* provides an additional tool to deliver solutions to the warfighter via several avenues including the discovery of enhanced functionalities for biotechnological applications or by mitigating the risks associated with pathogen and emerging threat assessments.

CELL-FREE PROTEIN SYNTHESIS SYSTEMS



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YERSINIA PESTIS CELL-FREE LYSATES

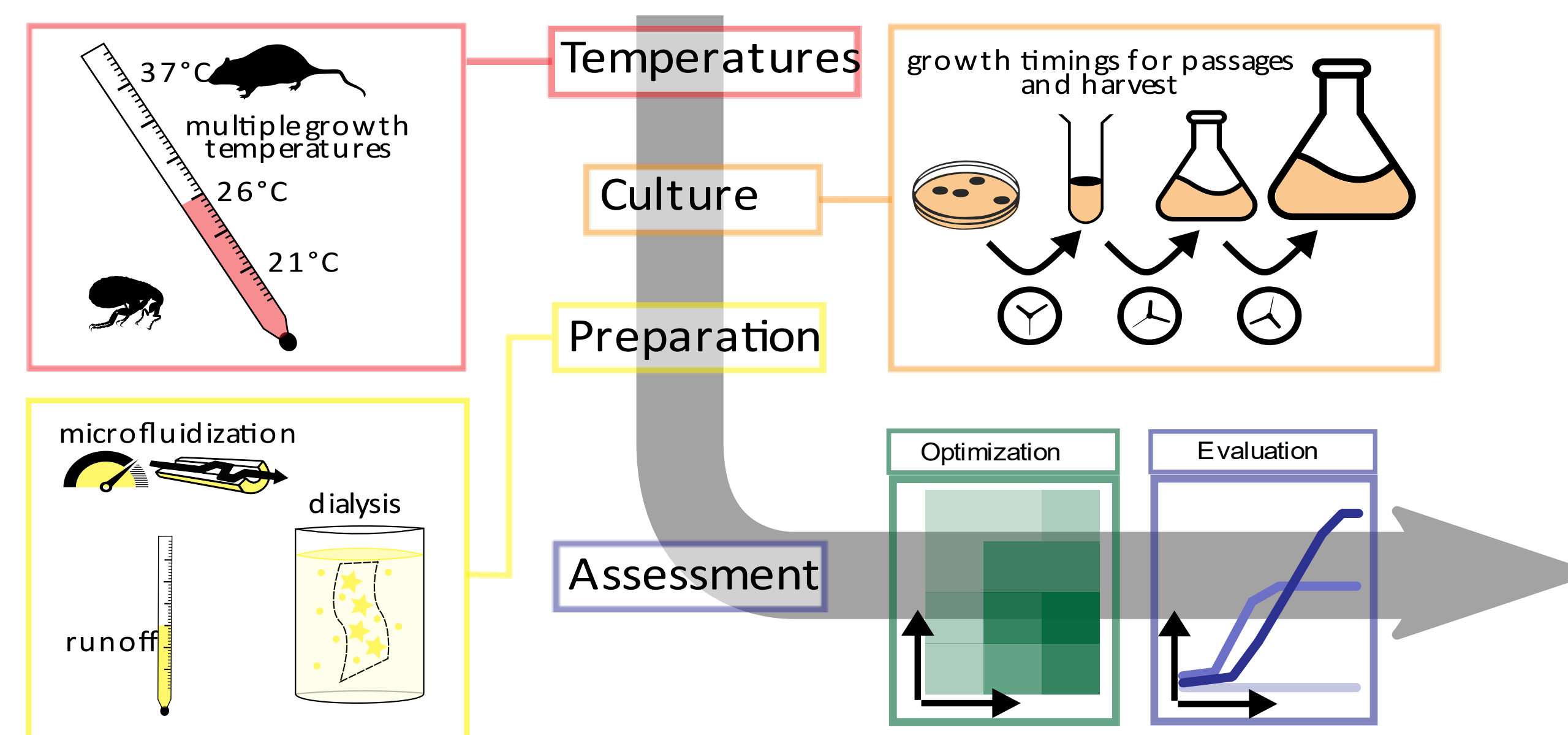


Figure 2: Generating functional cell-free protein synthesis systems from *Yersinia pestis*. The CFPS systems from attenuated *Y. pestis* CO92⁻ were optimized through a variety of conditions. The growth and culture temperatures and timing were chosen based on the lifecycle of *Y. pestis* along with maximizing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

CORRELATING PRODUCTIVITY WITH PROTEOMIC PROFILES

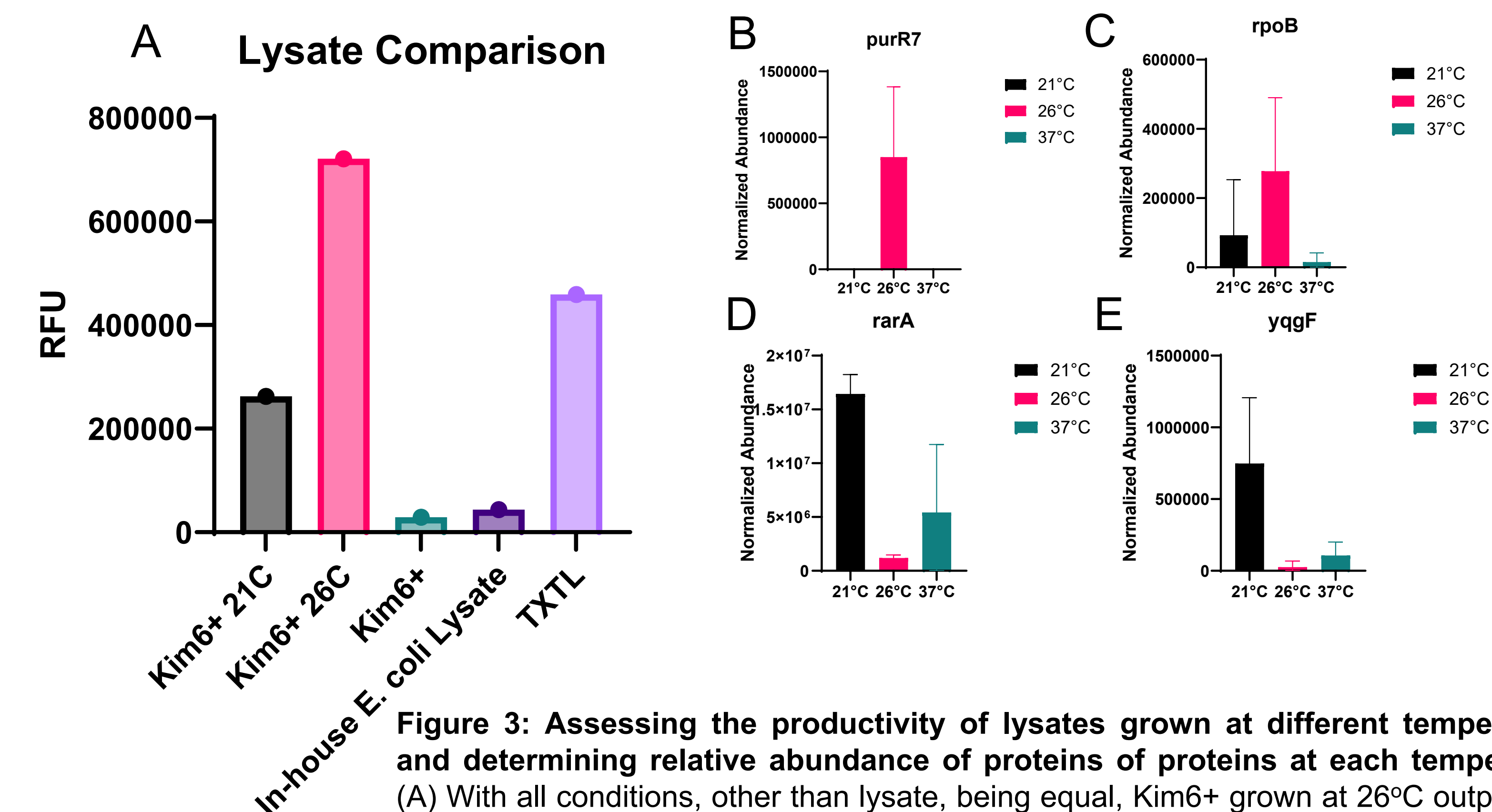


Figure 3: Assessing the productivity of lysates grown at different temperatures and determining relative abundance of proteins of proteins at each temperature. (A) With all conditions, other than lysate, being equal, Kim6+ grown at 26°C outperforms even the commercial kit myTXTL. (B) purR7 is abundant in the 26°C lysate, it is a transcriptional regulator that suppresses the production of hypoxanthine nucleotide (IMP). (C) rpoB is also at increased concentrations in the 26°C lysate and is the beta subunit of bacterial RNA polymerase that contributes to the active center. (D) rarA, a DNA repair protein, responsible for post-replication gaps is significantly decreased in the 26°C lysate. (E) yqgF, a pre-16S rRNA nuclease, is at significantly lower concentrations in the 26°C and 37°C lysates.

CHARACTERIZING YERSINIA PESTIS LYSATES

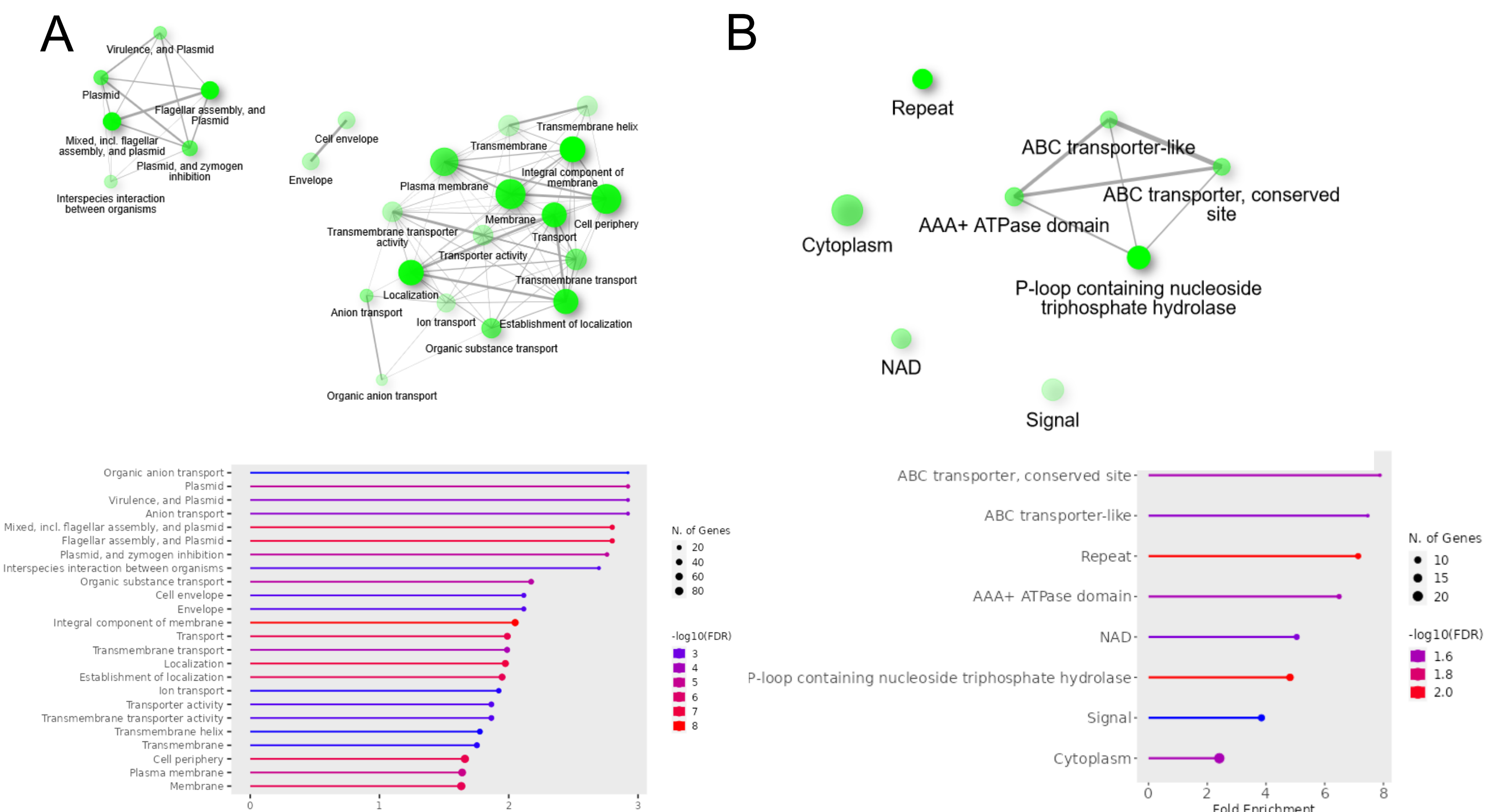


Figure 4: Evaluating lysates generated from multiple attenuated strains of *Y. pestis*. The overall pathway composition and associated genes for each strain are significantly different. CO92⁻ (A) shows and increased amount of membrane associated proteins as well as transporter proteins whereas (B) Kim6+ has in increased amount of cytoplasmic, signal and metabolic proteins.

CHARACTERIZING YERSINIA PESTIS LYSATES

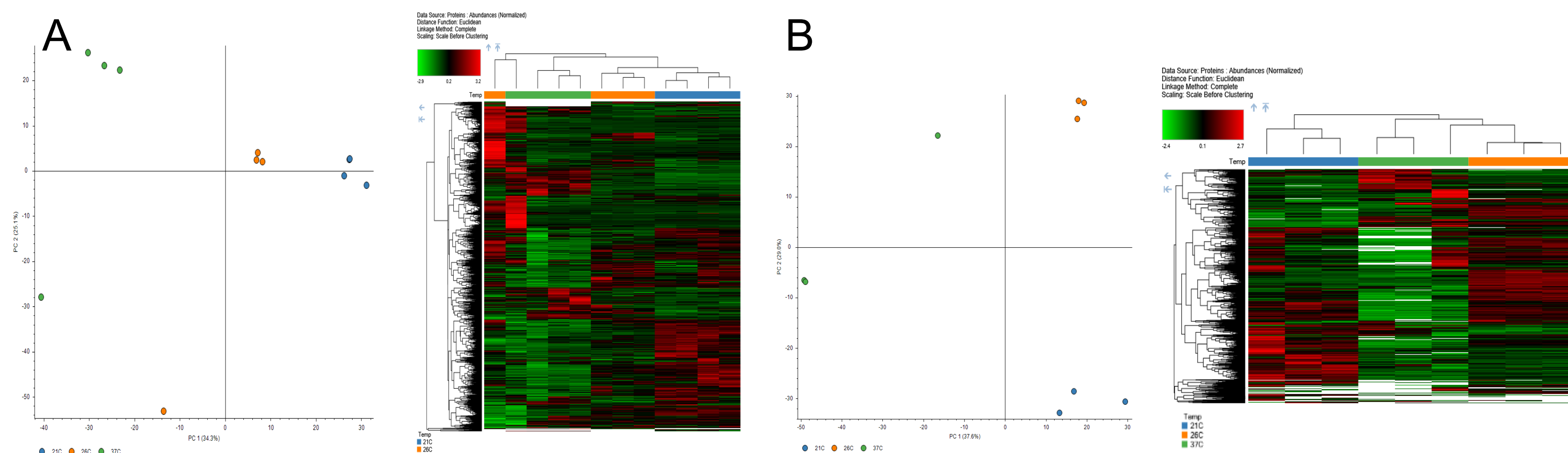


Figure 5: Determining Proteomic profile of different strains of *Y. pestis* lysates grown at different temperatures. Lysates from attenuated *Y. pestis* CO92⁻ (A) and Kim6+ (B) were generated at three distinct growth temperatures (21°C, 26°C and 37°C) in biological triplicates. These lysates were processed and run using Mass Spectrometry and then analyzed to determine relative abundances of normalized proteins. The scatter plots show distinct protein clustering that appears to be dependent on growth temperature and unique patterns for each *Y. pestis* strain. The heatmaps of the growth temperatures and separate strains also appear to show clustering based on growth temperature while also showcasing unique compositions for each strain of *Y. pestis* grown.

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Conclusions

- Functionality of lysates is strain and growth temperature dependent.
- Growth temperature and strain of organism greatly impacts the proteomic profile of the lysate.
- Up/down regulation of transcriptional promoters, methyl transferases and DNA repair mechanisms is growth temperature specific.
- These DNA/RNA regulatory proteins may potentially effect protein producing capabilities of the lysates.

Future work

- Further characterize the relationship between protein composition and functionality of lysates..
- Optimize energy buffer components based on proteomic profile of lysates to increase productivity of specialized products.

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