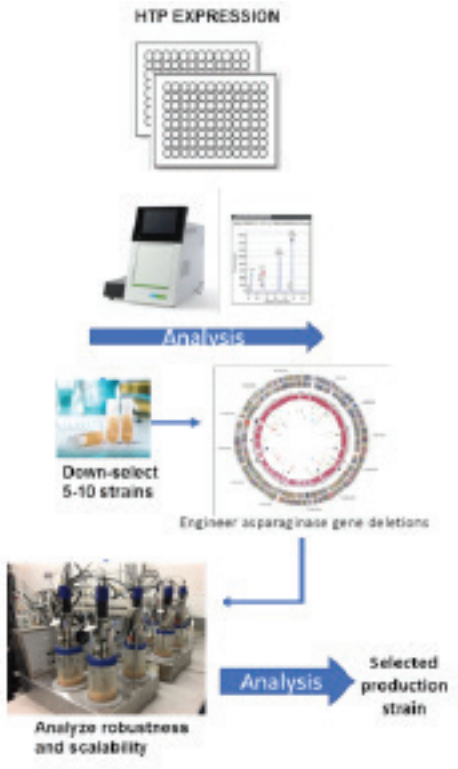
PRODUCT SPECIFIC STRAIN ENGINEERING

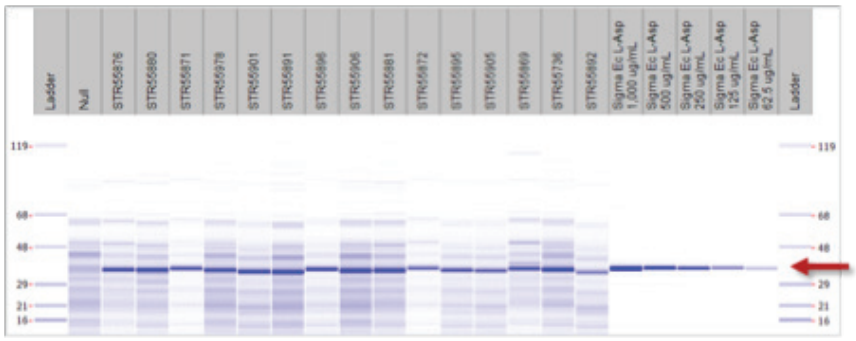
P. fluorescens is readily amenable to genetic manipulation, allowing for rapid generation of genomic modifications that may be required to produce a specific product. To produce recombinant E. chrysanthemi asparaginase (crisantaspase) in Pseudomonas fluorescens, it was necessary to remove any potential for contamination with native asparaginase. Therefore, native asparaginase encoding genes needed to be deleted from the genome. This genomic modification was easily inserted into our standard strain engineering work flow.

A variety of expression strategies and P. fluorescens host strains were screened to identify strains producing high titers of active asparaginase at the 0.5mL culture, 96-well scale. Certain strains were selected for further analysis under a variety of growth and induction conditions at the 1L scale.

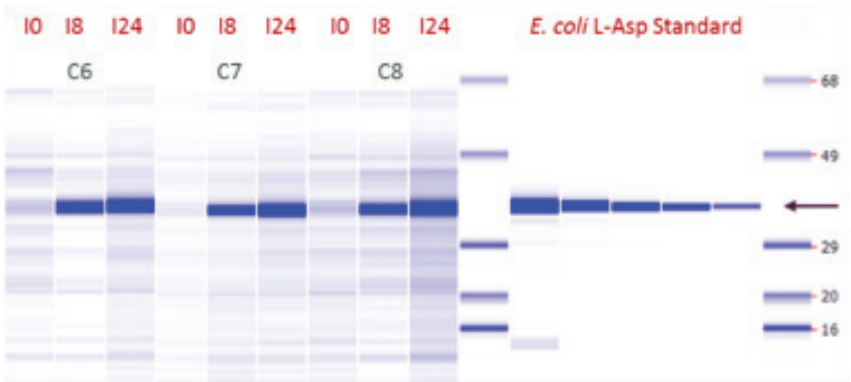
Two native asparaginase encoding genes were precisely deleted from the genomes of the selected host strains within a matter of weeks, and the modified down-selected strains were assessed in 1L fermentations. A production strain was subsequently selected based on product titer, quality and strain robustness.



HTP Strain Screening: Top 15 expression strains show high levels of crisantaspase SDS-CGE analysis of 0.5mL culture samples compared to *E. coli* L-asparaginase standard



Down-selected Strain Assessment: High levels of crisantaspase produced under a variety of induction conditions Titer assessed by SDS-CGE at 0, 8 or 24 hours post induction



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