



Expression of a Vaccine Antigen Candidate Protein using the *Pseudomonas fluorescens*- Based Pfenex Expression Technology™

Bruce Carpick, Ph.D.

Director, Biochemistry Platform

Analytical Research & Development, North America

Sanofi Pasteur

Toronto, Canada

Outline

- Challenges for vaccine protein antigen development
- Background & challenges of the vaccine target
- *Pfenex* Expression Technology™
- High-throughput screening of small-scale expression strains/cultures
- Characterization of small-scale extracts
- Preclinical assessment of antigens using a mouse immunogenicity (neutralization of infectivity) model
- Scale-up expression and preliminary assessment of selected strains
- Next steps



Challenges for vaccine protein antigen development

- Purification and/or scale-up of the native target antigen is often not feasible
- Predictive animal models are complex and may not be relevant to the (human) target population
- In vitro correlates of protection/efficacy may not be available prior to clinical efficacy studies
- Promising antigen candidates are often cell membrane, cell surface, or viral capsid proteins that may be insoluble in traditional recombinant expression systems (e.g. *E. coli*)
 - Target antigen solubilization processes can be costly, time-consuming, and poorly scalable
 - There may be no useful “reference” for the conformation of the refolded antigen



Background and challenges of the target antigen

■ Bacterial membrane protein

- Major constituent/antigen of the outer membrane
- Multiple serotypes relevant to human disease
 - Human serotypes are poorly infective in the established animal protection model
- No high-resolution structure available for the native protein
 - However, the native protein can be purified at lab scale, permitting some biochemical/biophysical analyses

■ Antigen expression strategy: recombinant expression in *E. coli*

- High expression levels, but ~100% in inclusion bodies
- Scaled-up extraction, purification, and refolding processes developed
- Current process results in a highly purified antigen that elicits a neutralizing Ab response in mice

We would like to leverage our process and preclinical experience with the *E. coli*-expressed antigen to investigate an alternative approach

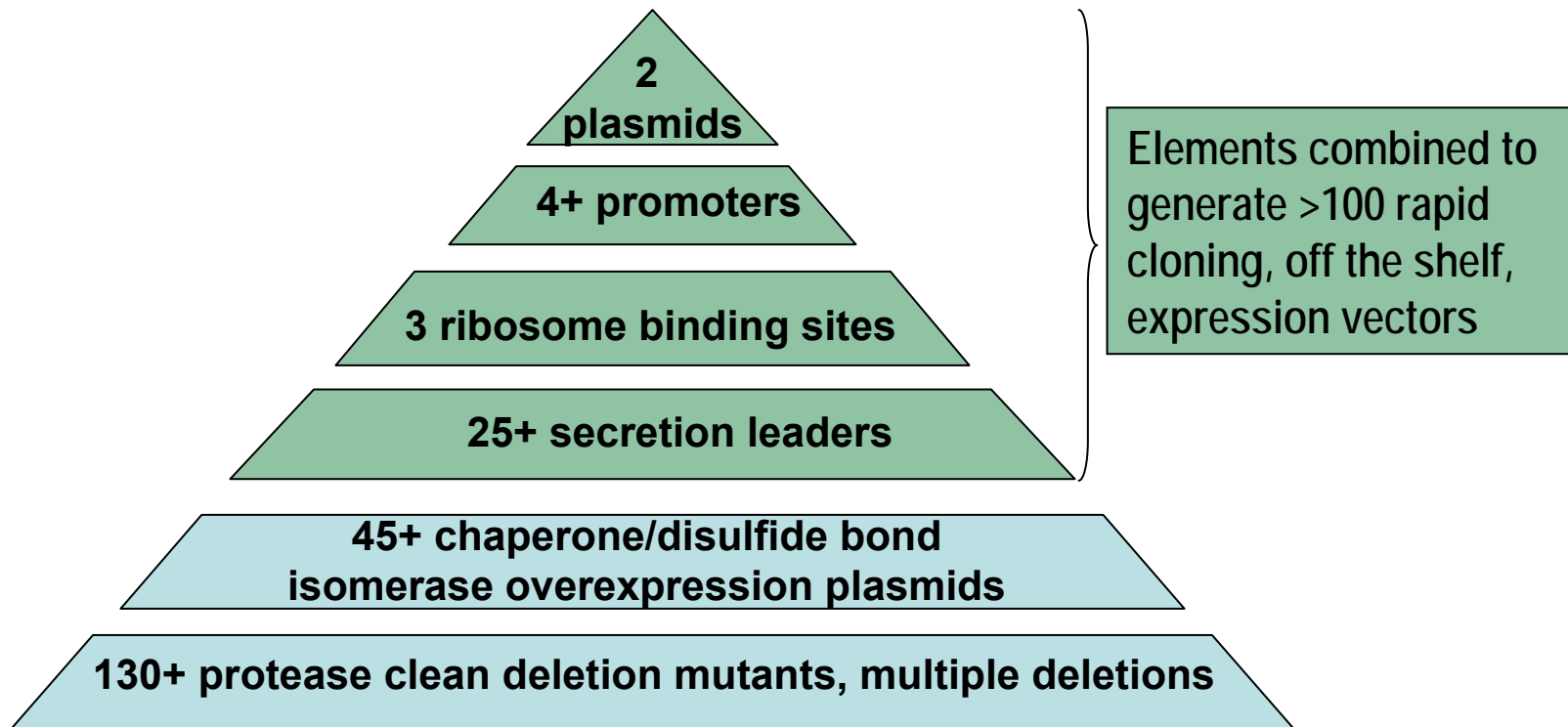
Pfēnex Expression Technology™

- ***P. fluorescens*: Gram negative, non-pathogenic organism**
 - Genome sequenced, leveraged to engineer host strains and expression plasmids
- **Develop effective high throughput growth and assay methods**
 - 96-well growth and assay methods
- **Rapid, effective, scaled down fermentation development**
 - Rapid path to test material

Discard linear, iterative approach, adopt parallel,
HTP method for microbial strain development

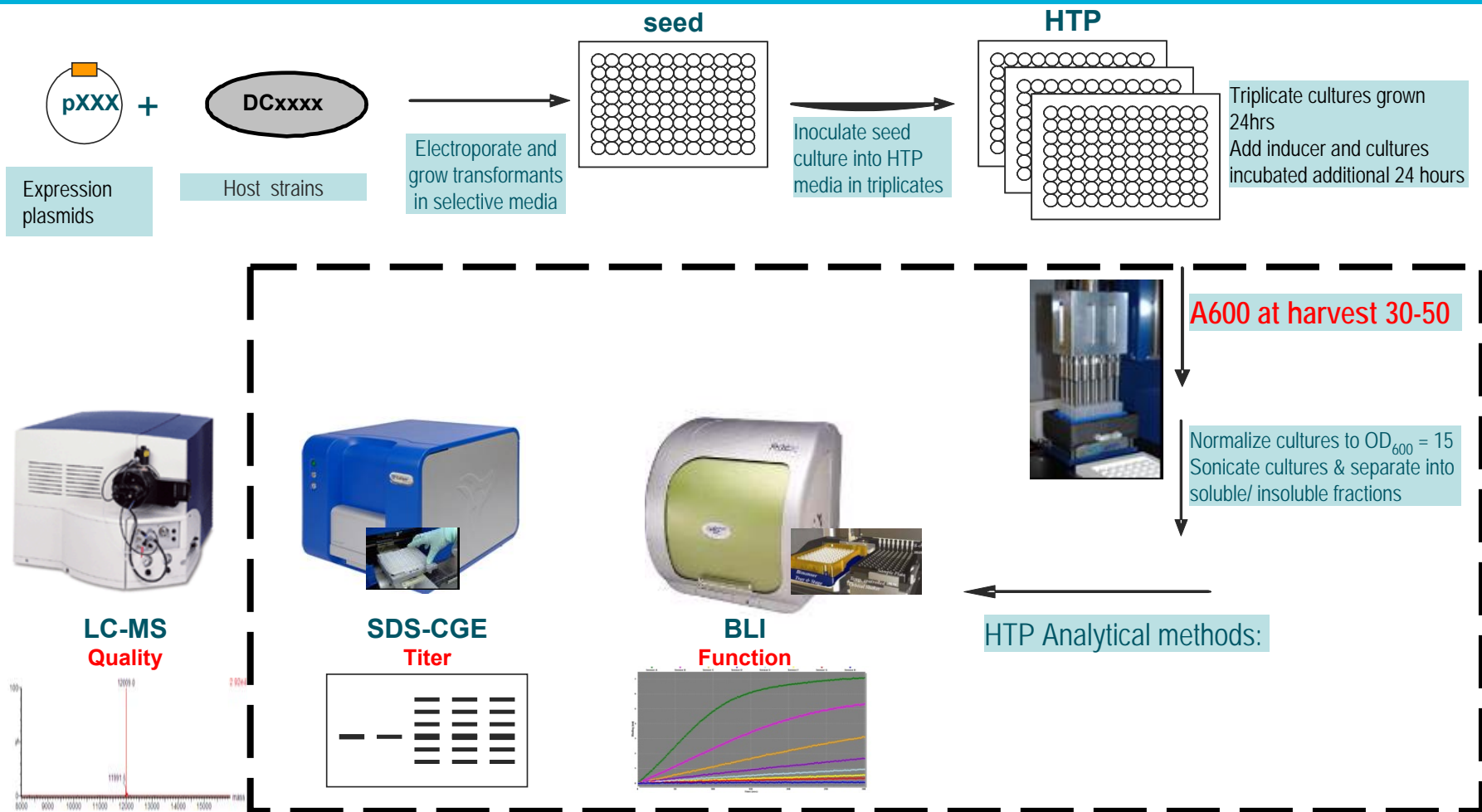
Cell Biology → Expression strain

Pfēnex Toolbox: The Next Generation for Bacterial Strain Engineering



Thousands of unique combinations are harmoniously combined to enable strain engineering for optimal protein production

Robotically Enabled High Throughput Strain Development

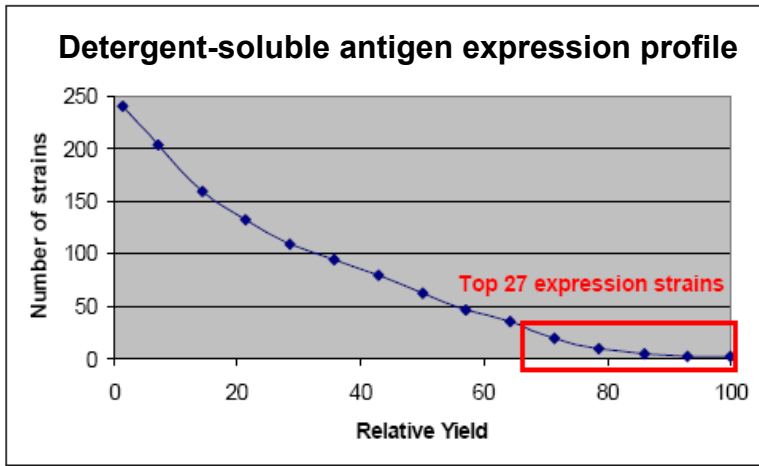


A Thousand Strains Evaluated in < 5 Weeks

HTP screening of plasmids and host strains

- Target gene was cloned into 12 different expression plasmids. Each of these were used to transform 20 different host strains
12 plasmids x 20 host strains = 240 expression strains
- Cultures (0.5-ml) grown in 96-well plates
- Cell pellets lysed in presence of detergent
 - Total, detergent-soluble, and insoluble fractions were analyzed
- Extracts analyzed for
 - Target antigen concentration (SDS-CGE)
 - Reference: In-house purified (*E. coli*) antigen protein standard
 - Identity (Western blot, LC-MS)

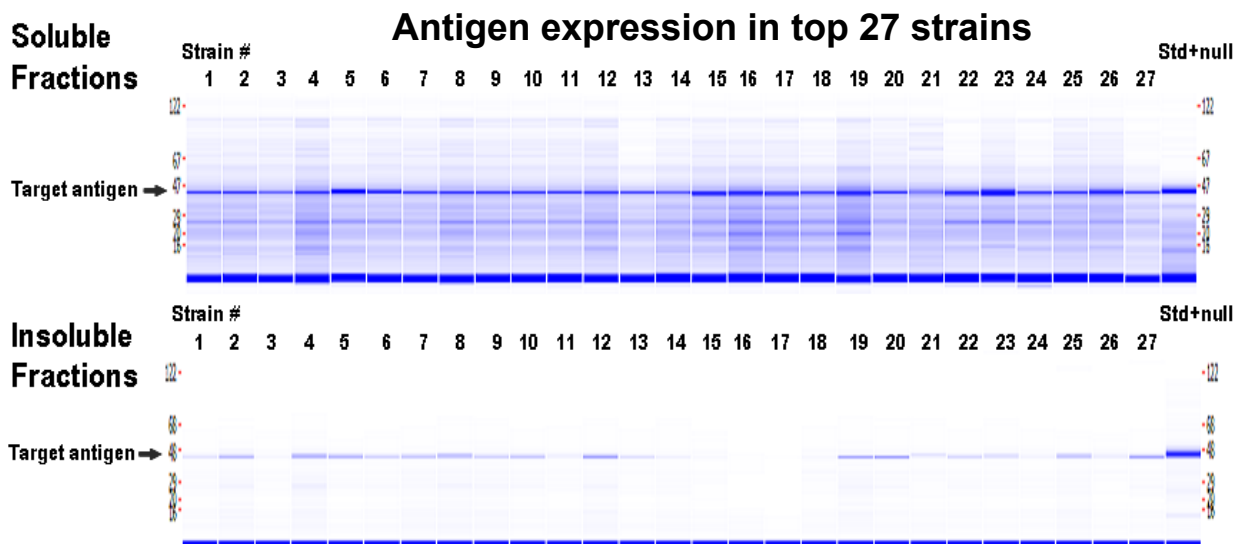
HTP expression screening results



Most strains expressed detergent-soluble antigen

Only a small subset produce very high levels of protein

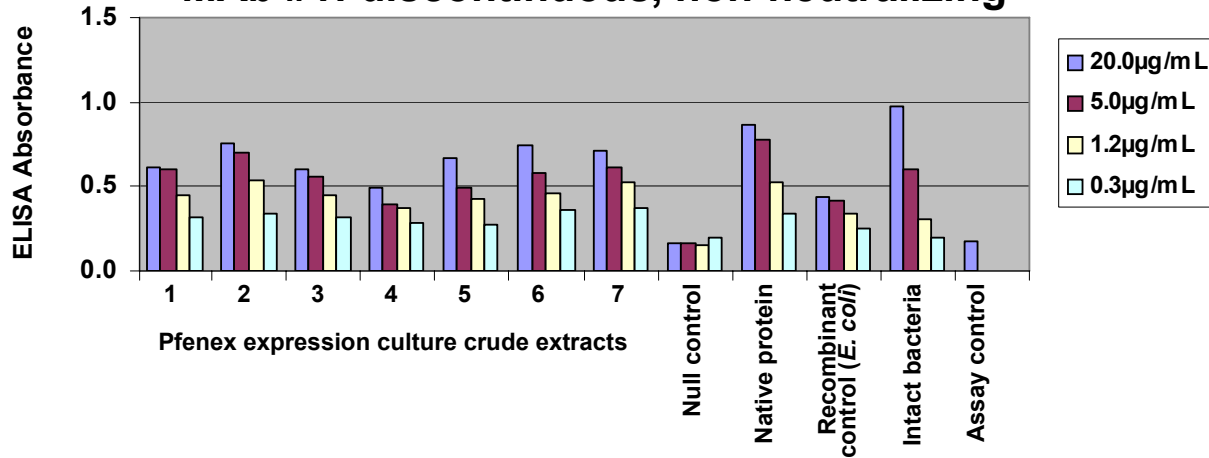
Top 27 strains were selected for further analysis, by SDS-CGE



SDS-CGE results confirmed by W. blot, LC-MS, SDS-PAGE

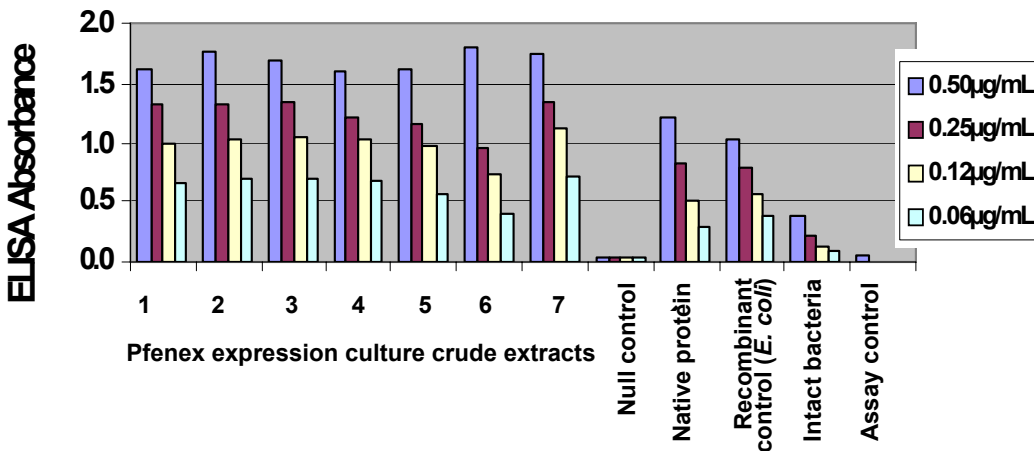
Epitope analysis of selected crude antigen detergent-soluble extracts by antigen ELISA

MAb #1: discontinuous, non-neutralizing

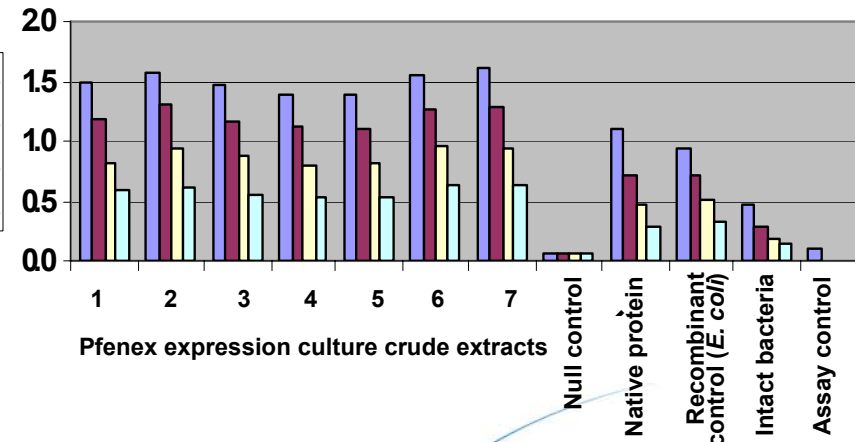


Antigen ELISA: Antigen capture using specific polyclonal antisera, followed by detection by antigen-specific monoclonal Ab

MAb #2: continuous, neutralizing



MAb #3: continuous, neutralizing



Preclinical assessment of crude antigens expression culture detergent-soluble extracts

23 clones selected for screening in mouse immunogenicity model

Controls:

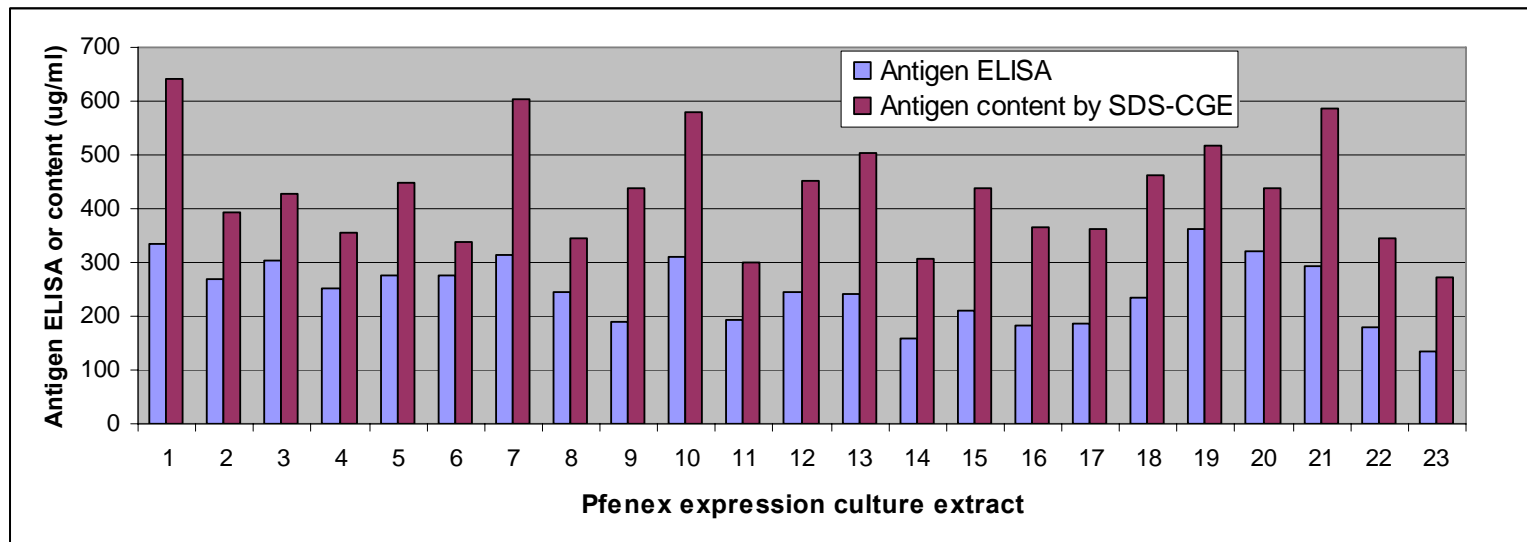
- Null expression control
- Purified recombinant antigen protein (*E. coli*-expressed)

Quantitative ELISA performed on each extract

- More accurate readout for specific antigen content

Readouts:

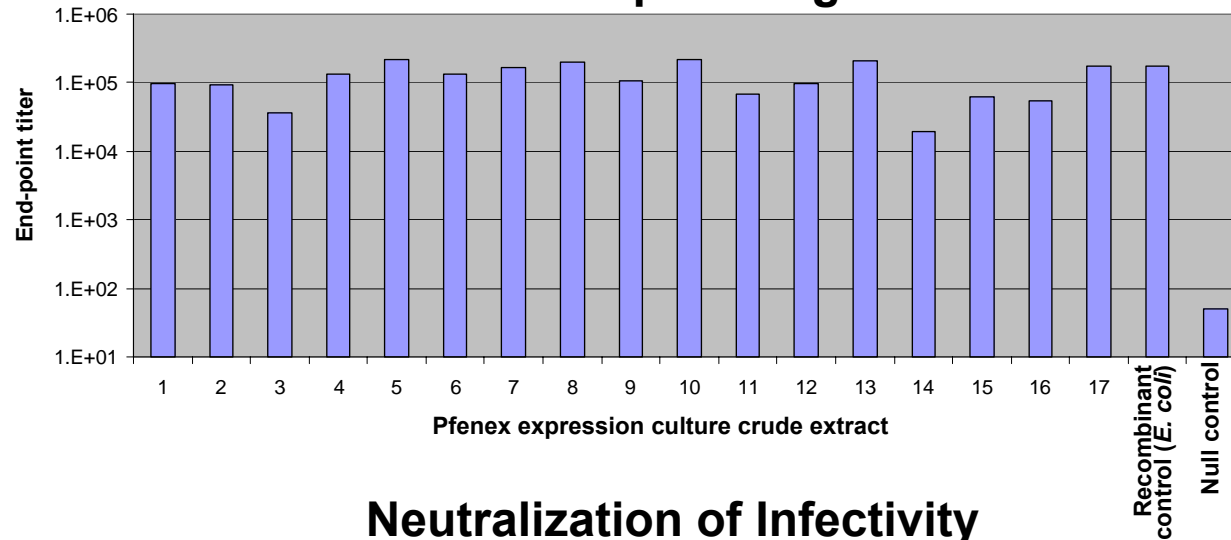
- Specific IgG titer
- Neutralization of infectivity titer



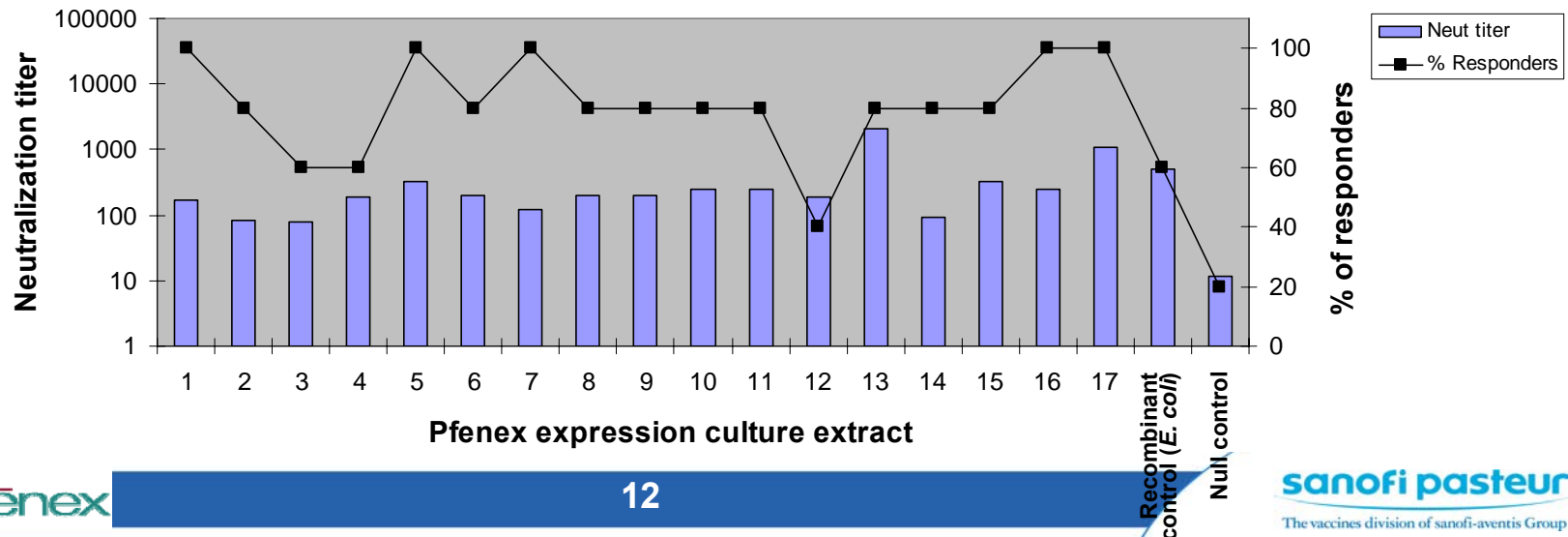


Immunogenicity of antigen expression culture extracts

Total Specific IgG



Neutralization of Infectivity





Conclusions from HTP screening

- **27 (of 240) strains had high levels of detergent-soluble antigen expression**
- **The expressed proteins are antigenically similar to the purified *E. coli* recombinant & purified native proteins**
 - Quaternary structures may be different
- **The crude detergent-soluble extracts are highly immunogenic, compared to null control**
 - Several strains had high neutralization titers in mice
 - Data interpretation is complicated by presence of impurities in the extracts
- **Four strains were selected to proceed to the scale-up (1-L) fermentation culture screening stage**

Characteristics:

 - High expression in detergent-solubilized fraction
 - Good immunogenicity profiles: total specific IgG, neutralization of infectivity titers, and % responders
 - No toxicity within immunized mice groups

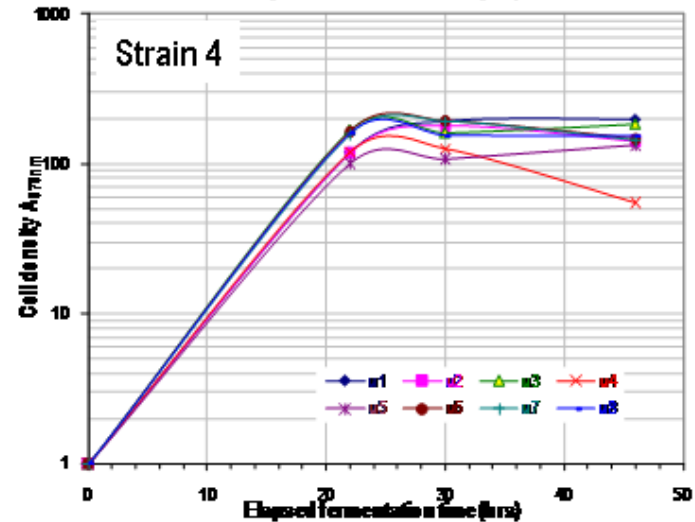
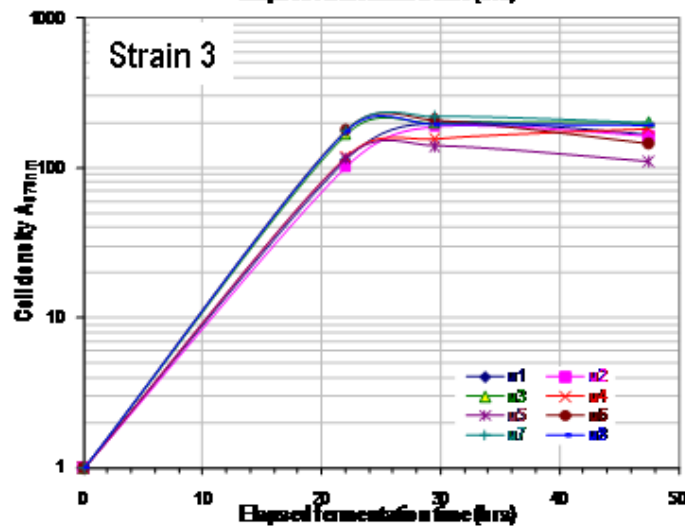
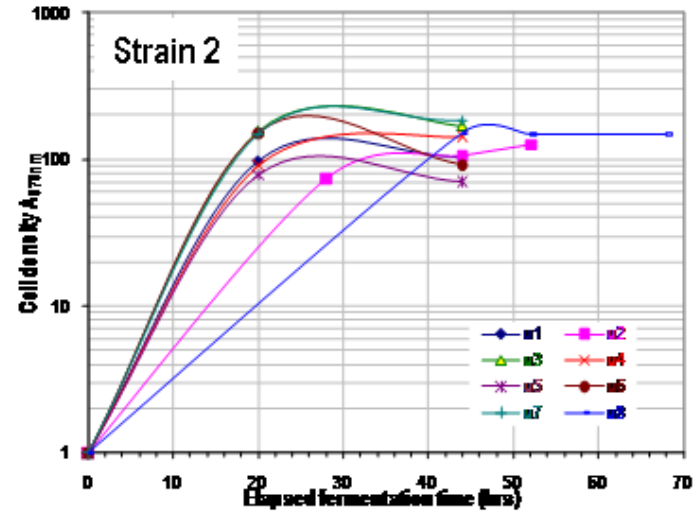
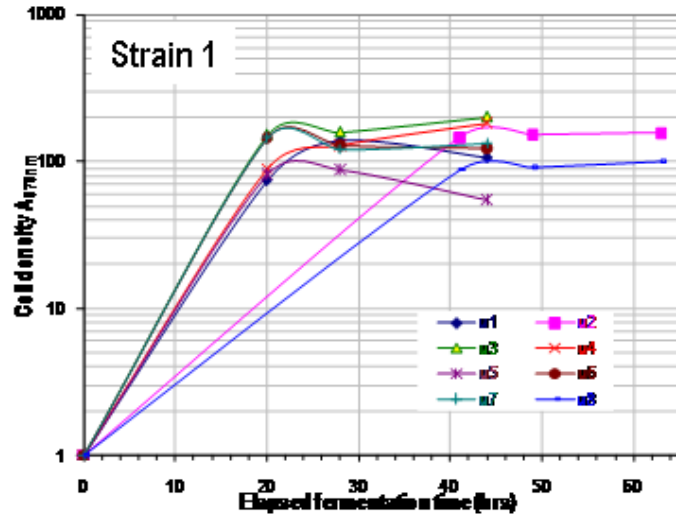
Fermentation Work Flow

- 4 strains
- 8 variable induction conditions each
- 8-unit 1-L multiplex bioreactors
- Gram scale

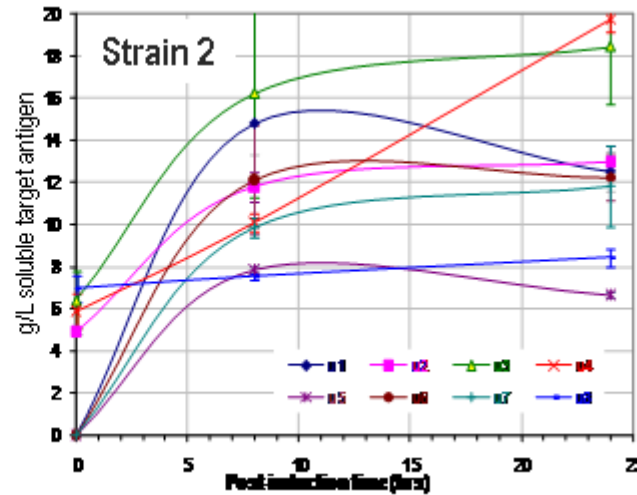
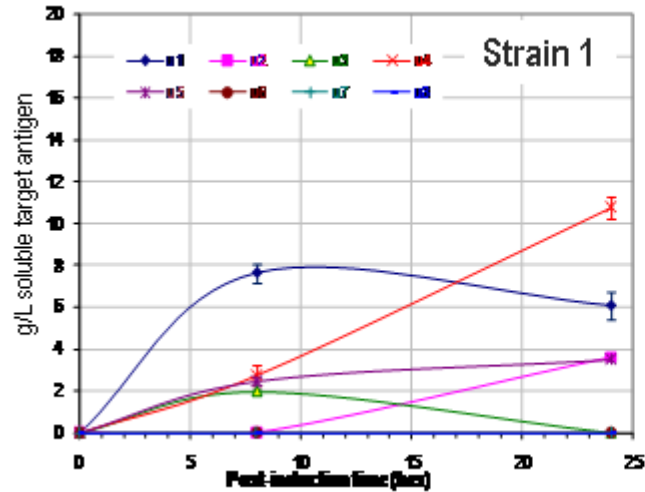


- Multiple time-point samples taken for expression analysis
- Cultures harvested to generate lysates/extracts

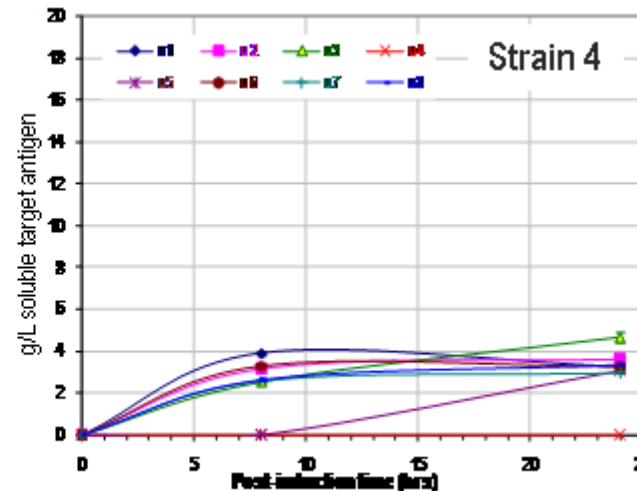
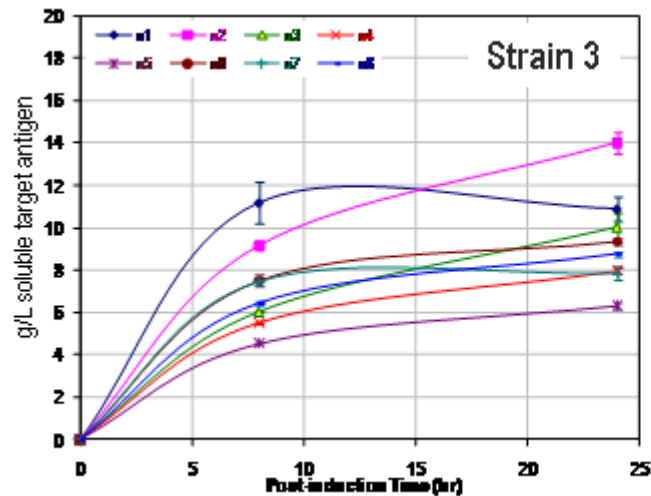
1-L fermentation culture growth curves



1-L fermentation culture productivity: detergent-soluble antigen expression

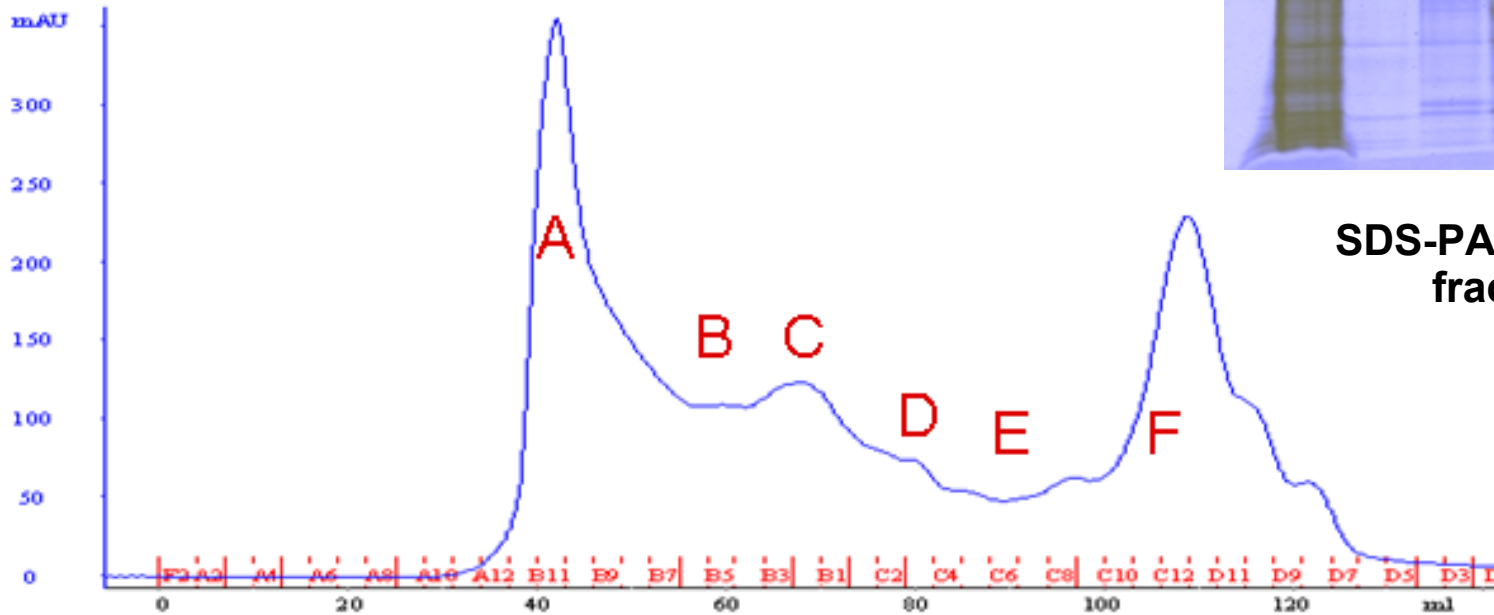


Highest expression (20 g/L)

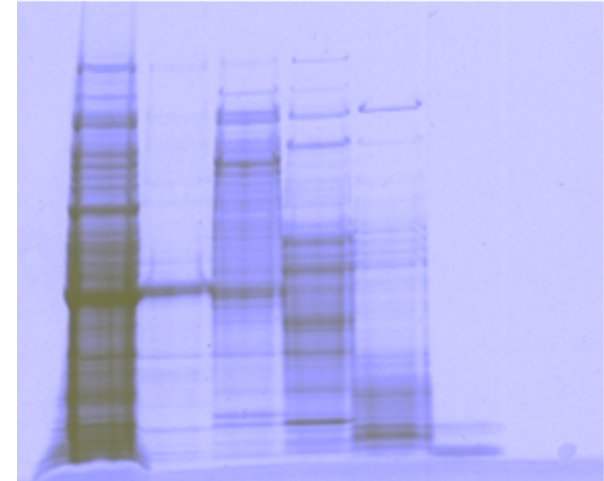


Preliminary purification of scale-up culture detergent extract

Preparative size-exclusion chromatography (SEC)



Total A B C D E F



SDS-PAGE of SEC fractions



Next steps

- **Development of a lab-scale purification process**
- **Optimization of extraction/solubilization conditions**
- **Characterization of highly purified antigen protein**
 - Biochemical/biophysical
 - Immunochemical
- **Immunogenicity testing in mouse model**



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