

A Rapid and Inexpensive Fungal Production Platform for Producing Therapeutics and Vaccines

***S. Allgaier, R. Taylor, Y. Brudnaya, D.
Jacobson, E. Cambareri, D. Higgins,
and W. D. Stuart***

Neugenesis Corp, Burlingame, CA





NeuBIOS™ Solves Mission Critical, Major Vaccine and mAb Production Problems

- Proprietary fungal expression platform for producing protein biologics including monoclonal antibodies and vaccines
- Utilizes the filamentous fungus *Neurospora crassa*
- Extremely fast and cost-effective production platform
- Strong patent protection





Neugenes Patent and License Portfolio

Vaccine Production

- Methods and Compositions for Combinatorial-Based Production of Multivalent Recombinant Antigens

Monoclonal Antibody Expression

- Heterologous Dimeric Proteins Produced in Heterokaryons

Gene Diversification Technology

- Single Gene Diversification
- Methods for Diversification of Heterologous DNA Sequences in Living Cells

Combinatorial Biology

- Methods and Composition for Combinatorial Based Discovery of New Multimeric Molecules
- Combinatorial Metabolic Libraries

Expression and Screening

- Neurospora Expression System
- Neurospora Hosts for the Production of Recombinant Proteins and Methods for Producing Same
- Light-regulated Promoters for Production of Heterologous Proteins in Filamentous Fungi
- Production of Heterologous Peptides in Neurospora crassa
- Exploitation of the Cellulase Enzyme Complex of Neurospora
- Method for Peptide Display on the Surface of Conidiospores



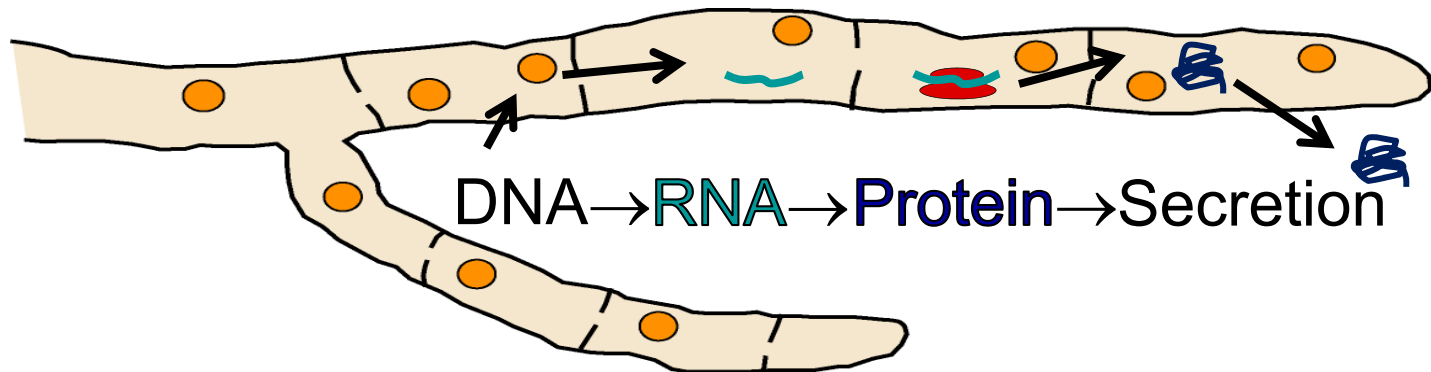
Benefits of Fungal Systems

- Host cell growth is extremely fast
- Media requirements are very simple and low-cost
- Protein secretion rates are among the highest of all organisms
 - Recombinant protein yields are known to exceed 30 g/L
- Fungi are eukaryotic
 - Unlike bacterial systems, protein refolding is rarely an issue
 - Glycosylation is more mammalian-like
- Fungi are used to produce industrial enzymes, small molecules, and biologics
 - NovoNordisk's Levemir® (insulin)
 - Merck's Gardasil® HPV and Recombivax® HBV vaccines
 - Agennix' therapeutic talactoferrin.



UNCLASSIFIED NeuBIOS™ – Neurospora Biological Operating System

- A “Plug and Play” platform for low cost production of protein biologics
 - Hardware = The Fungal Cell The genome + biochemical pathways
 - Software = Expression Cassettes Integrated into the native genome
 - Input = Heterologous Genes



- Strains grown in optimized bioreactors make up the *NeuBIOS™* production factory



Host Strains - Modified to Reduce Protease Activity and Improve Secretion

<i>N. crassa</i> Strain ▶	N264-79	N307-11	N364	N365	N367	N368	N371	N375	N376	N377	N378	N379	N381	N382
Morphology ▶	wt	wt	wt	wt	<i>col-4</i>	<i>col-4</i>	wt	wt	wt	<i>col-4</i>	<i>col-4</i>	wt	<i>col-4</i>	<i>col-4</i>
Gene KO ▼														
<i>apr-2</i>			Red	Purple	Green	Cyan	Blue	Purple	Pink				Light Green	
<i>apr-3</i>		Pink	Red	Purple	Green	Cyan	Blue	Purple	Pink			Blue		
<i>apr-4</i>	Not yet available													
<i>apr-6</i>								Purple	Pink	Yellow	Green	Blue		
<i>apr-7</i>	Brown	Pink	Red	Purple	Green	Cyan	Blue	Purple	Pink	Yellow	Green	Blue	Light Green	Orange
<i>apr-8</i>														
<i>apr-9</i>				Purple			Blue		Pink				Light Green	
<i>apr-10</i>			Red											
<i>apr-11</i>	Not yet available													
<i>apr-12</i>														
<i>apr-13</i>						Cyan					Green			
<i>mpr-3</i>	Not yet available													
<i>mpr-4</i>	Brown	Pink	Red	Purple	Green	Cyan	Blue	Purple	Pink	Yellow	Green	Blue	Light Green	Orange
<i>pep4</i>	Available from FGSC May 2009, but to be made in-house													
<i>spr-3</i>							Blue		Pink	Yellow	Green	Blue		
<i>spr-4</i>					Green					Yellow				Orange
<i>spr-5</i>			Red	Purple	Green	Cyan	Blue	Purple	Pink		Green		Light Green	
<i>spr-6</i>	Brown	Pink	Red	Purple	Green	Cyan	Blue	Purple	Pink	Yellow	Green	Blue	Light Green	Orange
<i>spr-7</i>	Brown	Pink	Red	Purple	Green	Cyan	Blue	Purple	Pink	Yellow	Green	Blue	Light Green	Orange
<i>spr-8</i>	Available from FGSC May 2009, but to be made in-house													
<i>cre-1 (regulatory gene)</i>	Brown		Red	Purple	Green	Cyan		Purple				Blue		
<i>gla-1 (enzyme)</i>	Brown	Pink	Red	Purple	Green	Cyan	Blue	Purple	Pink	Yellow	Green	Blue	Light Green	Orange

Colored box indicates gene has been KO'd in *N crassa* strain

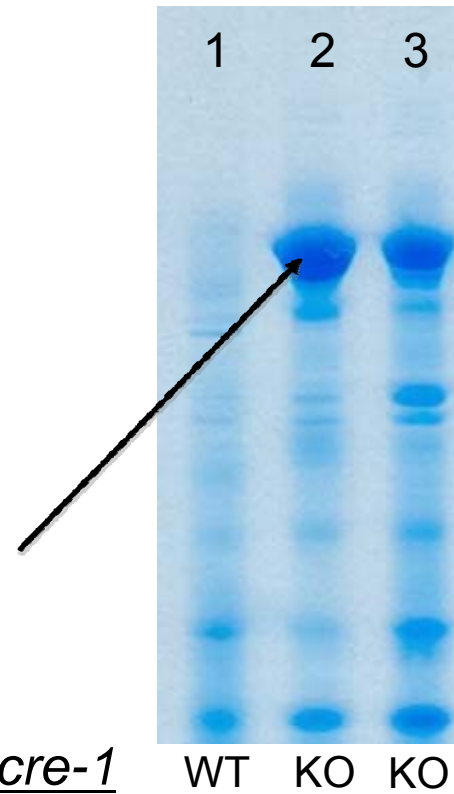


Host Strain Improvement – Regulatory Gene KO

- Knocking out *cre-1* improves overall protein secretion 10-fold

SDS-PAGE comparing total secreted protein from strains \pm *cre-1* repressor gene

– 50% of total protein secreted is a specific glucoamylase

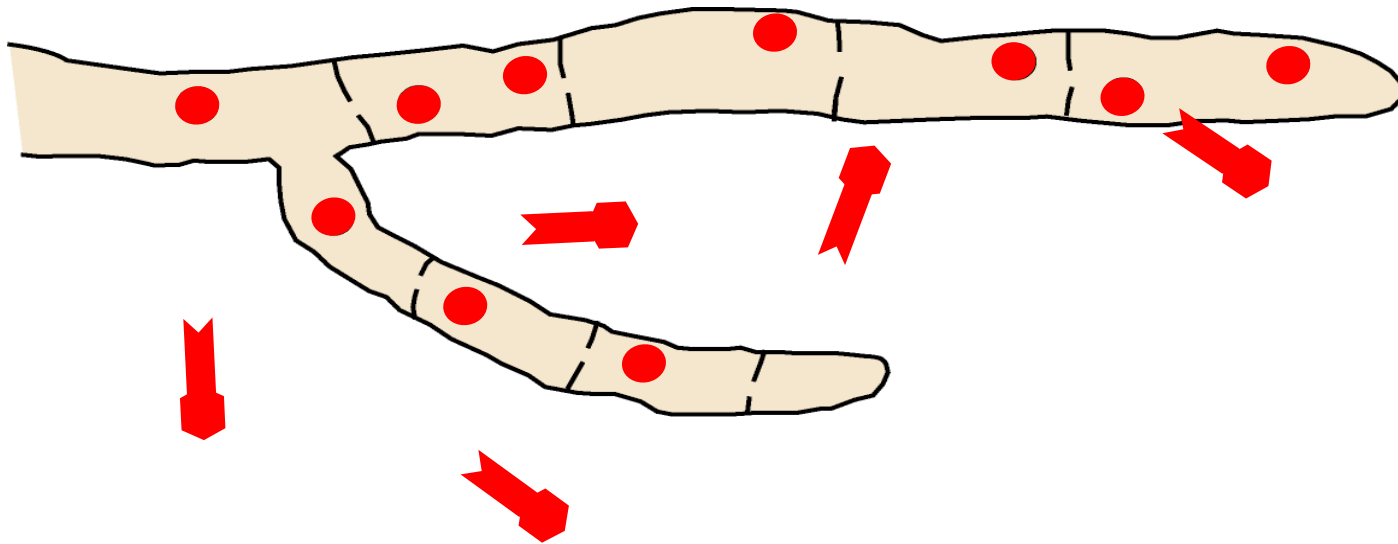


global regulator *cre-1*



Recombinant Vaccine Antigen Production

- *Neurospora* host strain transformed with an expression cassette coding for a recombinant protein

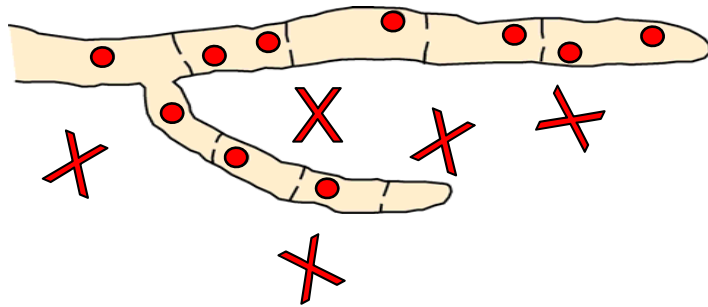




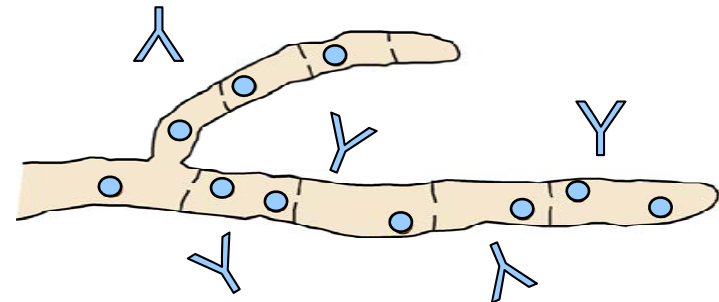
Heterokaryon Production Strain

- Sibling host strains are transformed to produce different proteins

Protein X Producing Strain

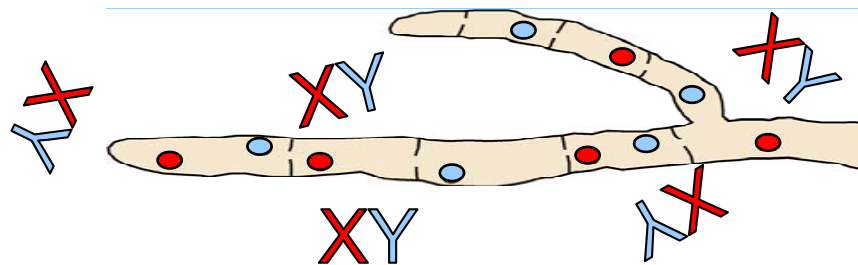


Protein Y Producing Strain



- Then host strains are fused to express both proteins

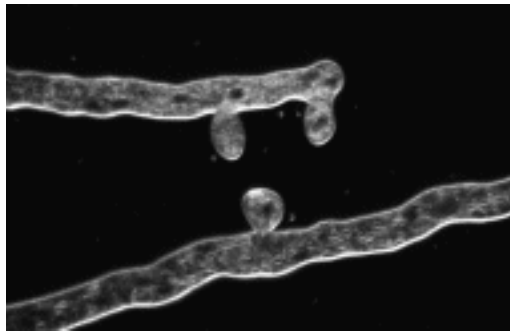
Protein X and Y Producing Strain



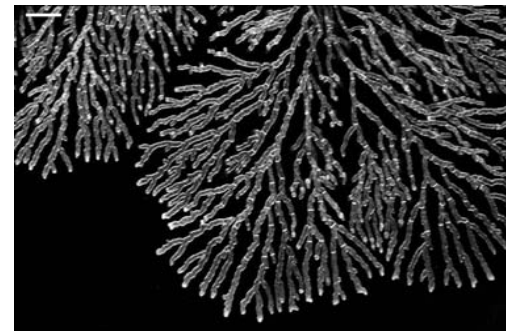


Heterokaryon Technology

- Allows the production of flexible combinations of subunit heteromeric proteins such as monoclonal antibodies
- Can also be applied to produce multivalent viral vaccine mixtures
- The ratio of each set of nuclei in the heterokaryon can be artificially and stably set allowing the empirical “tuning” of the expression levels of the subunits by effectively changing the gene dosage.



Fusion



Cell Growth



Recombinant Proteins Produced by Neugenes in *Neurospora*

Proteins

	Secreted	Western Reactive	Bioactive
Viral			
Flu H5N1M1	√	√	√
Flu H1N1M1	√	√	√
Flu H1	√	√	√
Mammalian			
rhM-CSF	√	√	√
Lactoferrin	√	√	ND
HSA	√	√	ND
Chymotrypsin	√	√	√
Relaxin	√	√	ND
DSPA	√	√	√
Plant			
Zeamatin	√	√	√

Monoclonal Antibodies

	Secreted	Western Reactive	Bioactive
IgG1			
*A	√	√	√
B	√	√	√
*C	√	√	√
D	√	√	ND
E	√	√	ND
F	√	√	ND
G	√	√	ND
IgG2			
**H	√	√	√
scAb			
I	√	NA	ND

* Humanized mAbs

** Fully human mAb

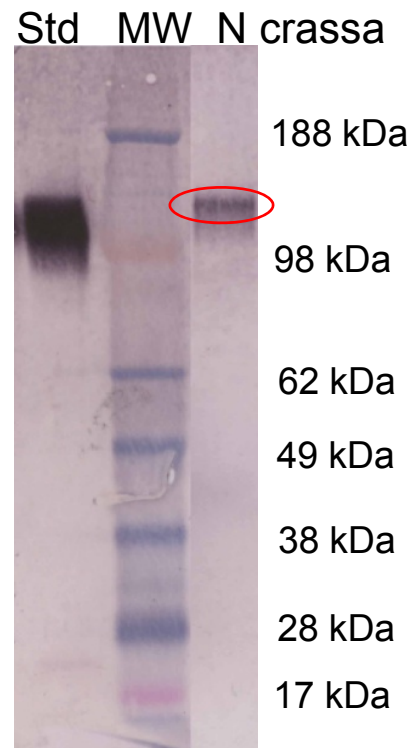


Monoclonal Antibody Produced in *Neurospora* is Intact

Identity/Fragmentation:

- Protein A + CM IEX

Western Blot

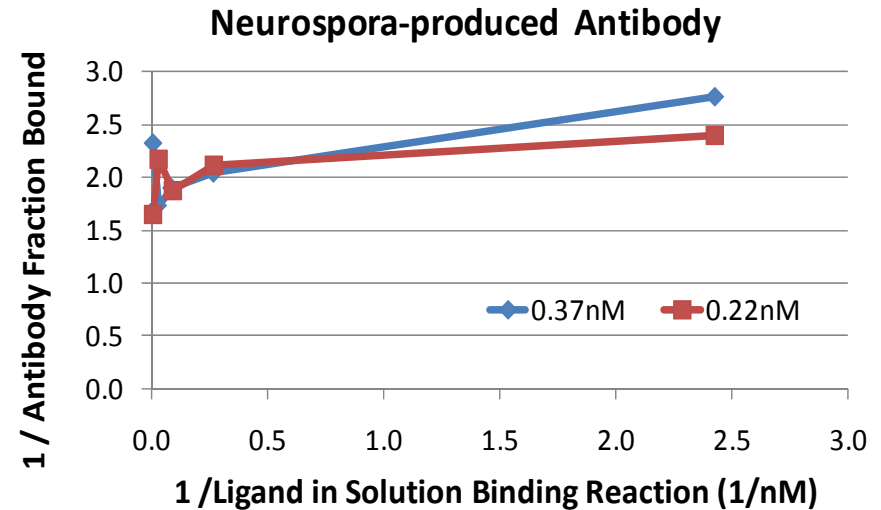
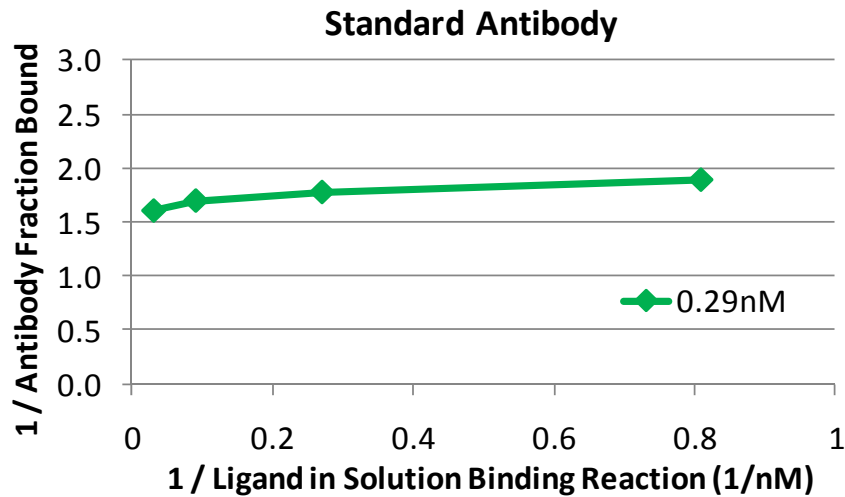




Monoclonal Antibody Produced in Neurospora Binds to Ligand

Activity:

Ligand Binding by Competitive ELISA





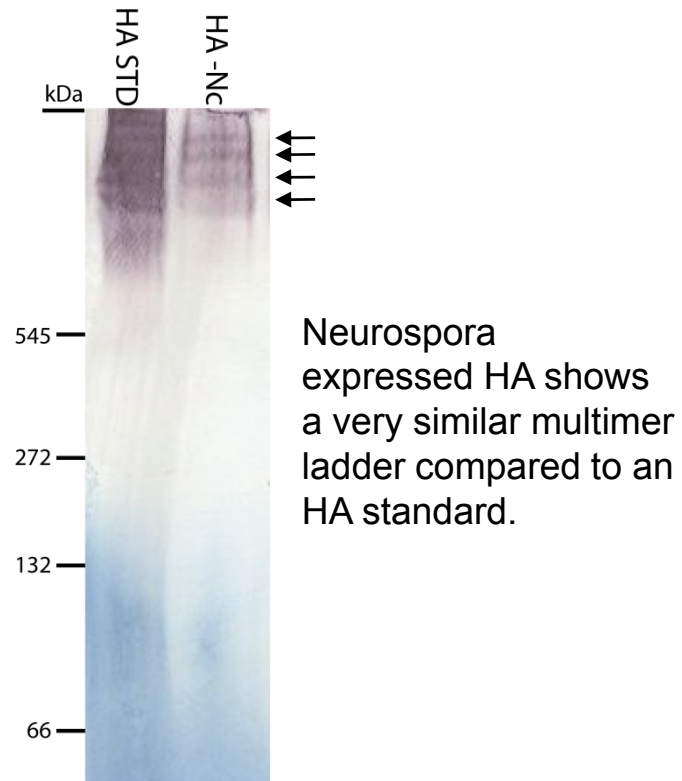
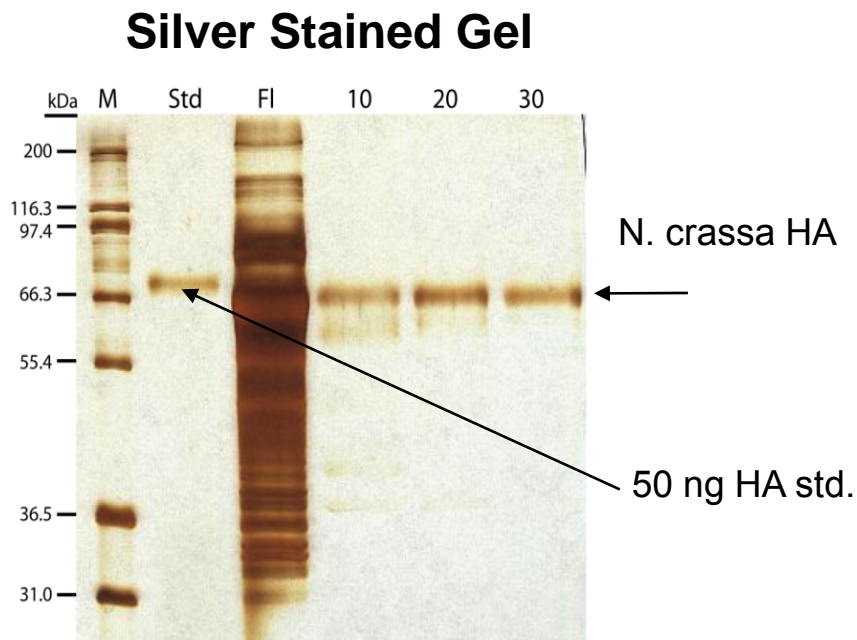
Flu HA Produced in *N. crassa* is Intact and Binds to Specific Antibody

Fragmentation:

- Single-step < 10%

Identity/Folding:

Blue-Native Gel Western Blot



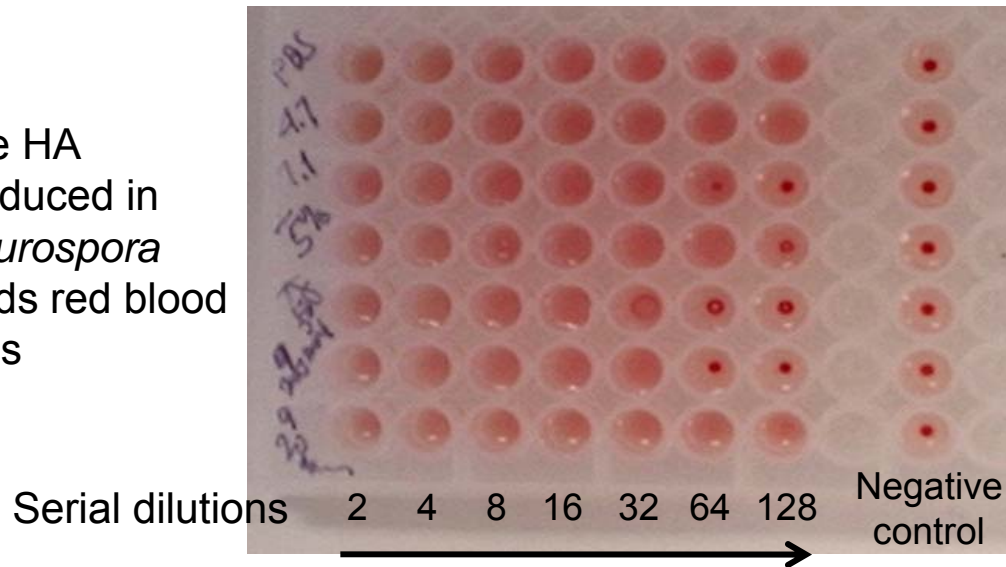


Influenza HA Produced in *N. crassa* is Bioactive

Activity:

Hemagglutination Activity Assay

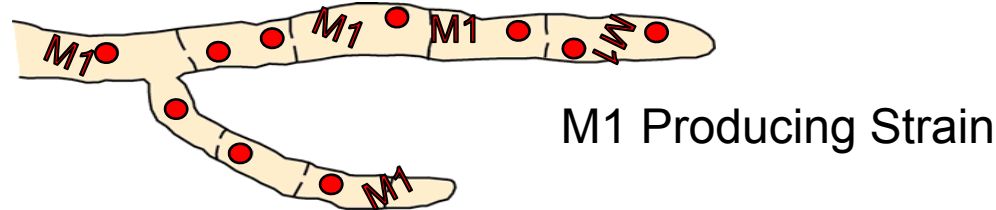
The HA produced in *Neurospora* binds red blood cells



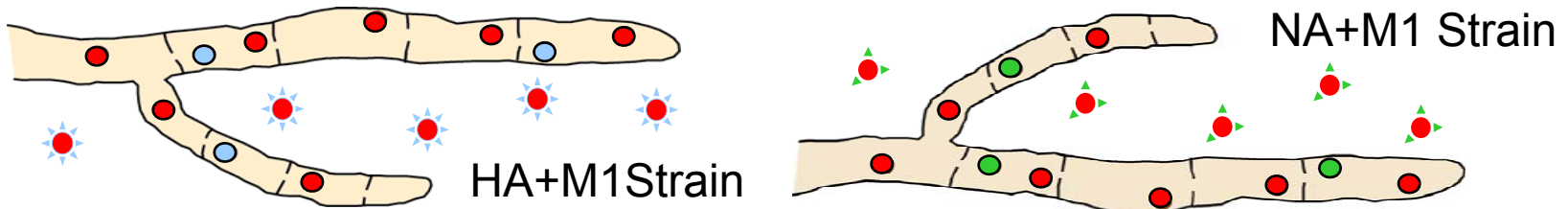


Heterokaryon Technology to Produce Influenza VLPs

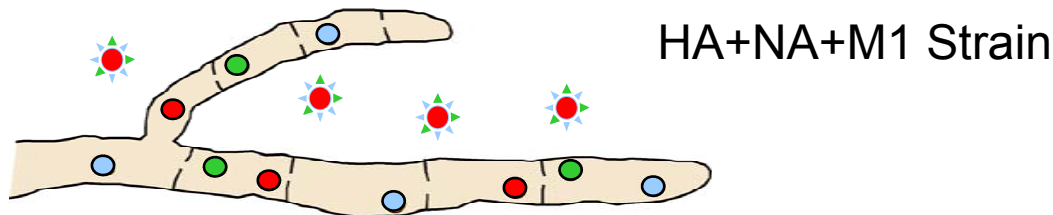
- Generate M1 producing host strain



- Transform M1 strain separately with HA and NA constructs



- Fuse HA+M1 and NA+M1 strains to generate HA+NA+M1 heterokaryon producing VLPs





NeuBIOS Platform is More Efficient for Flu Vaccine Production

Production Method	HA mg/L (Final Yield)	Vaccine Doses/L	L to generate 10 MM Doses
Eggs	7	470	21,277
Mammalian Cell Culture*	2.7	180	55,000
NeuBIOS**	100	6,667	1,500

Numbers based on production of monovalent bulk and 15 ug HA dose.

*MMC information from the Novartis website.

** NeuBIOS™ calculations use a conservative yield estimate for the system

NeuBIOS:

- High HA yield/ liter results in
- more vaccine doses/ liter, which translates to
- smaller production volumes needed to manufacture large numbers of vaccine doses
- = cost savings



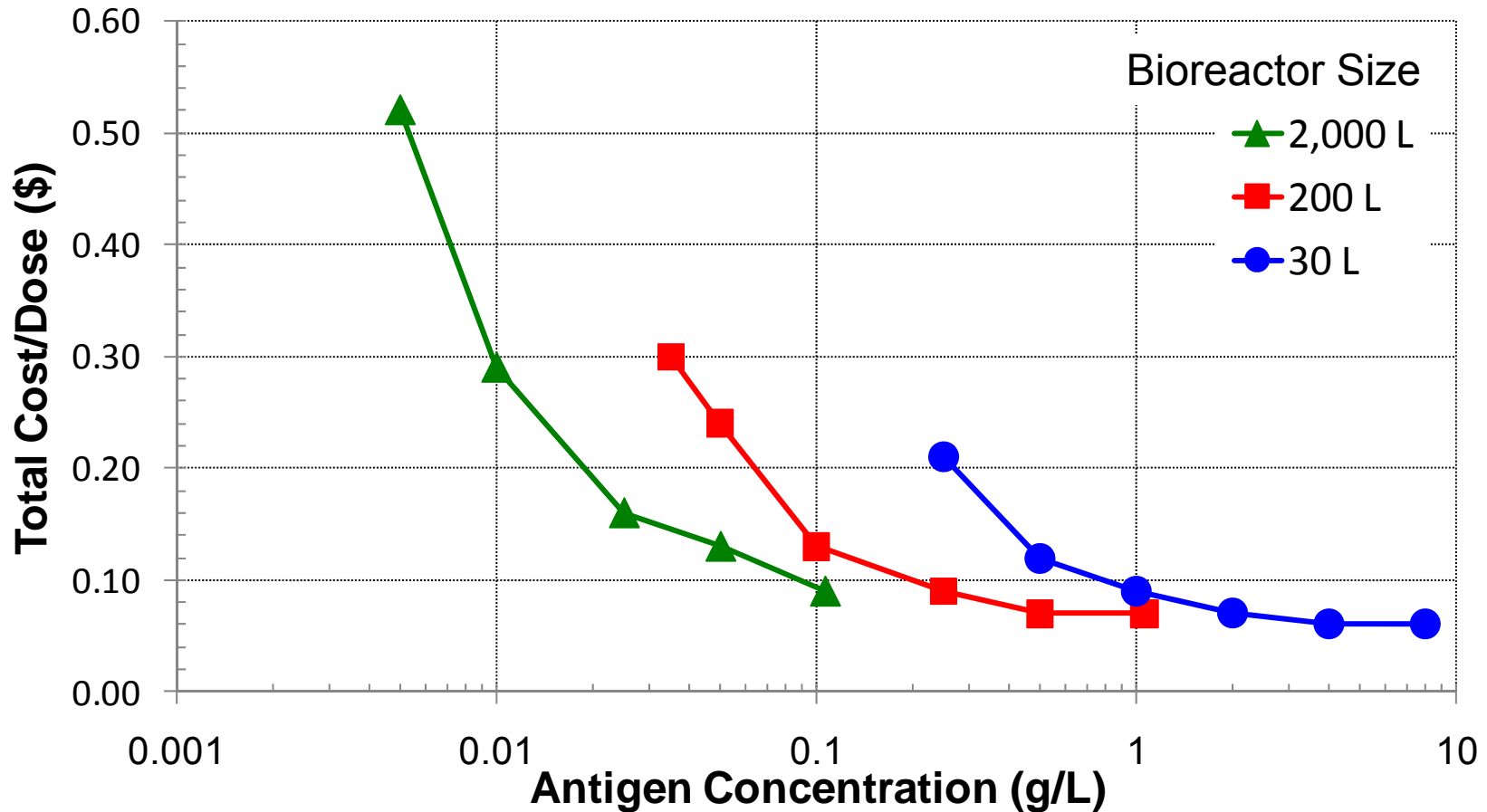
NeuBIOS™ for Influenza Vaccine Production

Advantages

- The system does not use eggs for vaccine production
 - Removes risk that avian influenza could compromise production supply
- Virus growth is not required
 - Removes risk to vaccine workers
 - Yield unaffected by poor growing virus strains
- Can be used to generate tailored multivalent vaccines
 - Mix and match antigens such as VLPs
- Vaccine against new variants can be produced in weeks rather than the current 6-9 months



Cost per Dose – The System is Very Inexpensive

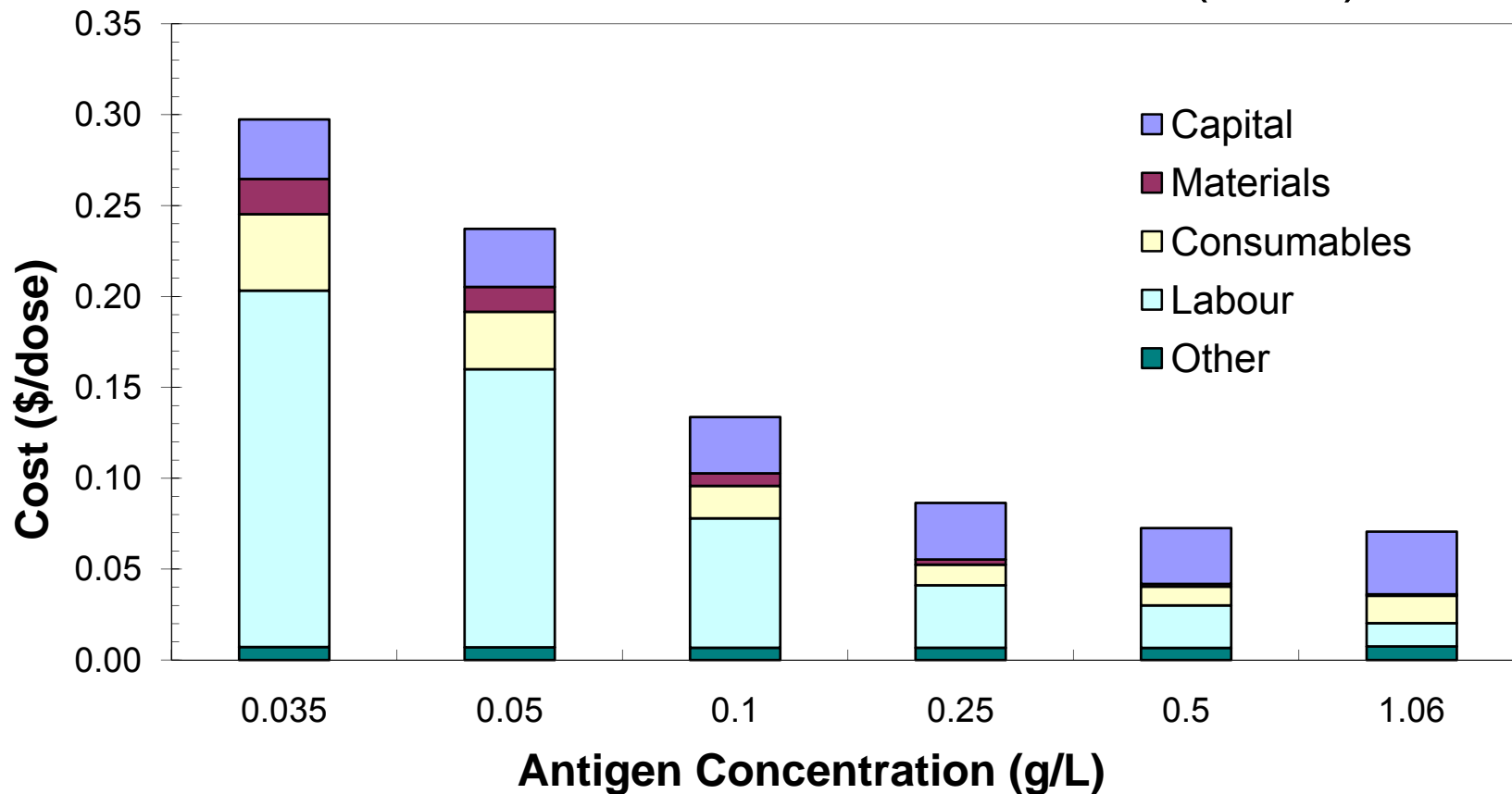


Projections from BioPharm Services, costs based on 50 ug dose



Non-capital Costs Fall Rapidly with Increased Antigen Concentrations

Cost Breakdown vs Concentration (200L)



Projections from BioPharm Services, costs based on 50 ug dose



NeuBIOS™ Platform Summary

- Non-pathogenic host organism
 - Animal-free system
 - Ability to store production strains
- High production yield potential
 - Uses minimal media and simplified protein recovery
- Scalable and flexible
- Reduced capital requirements
 - Bioreactors <1000 liters
- Production efficiency translates to significant cost savings



Acknowledgements

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Edward Cambareri

Kevin Eng

Paul Escarpe

WJ Feng

Judith Greengard

Binh Le Haynes

Debbie Higgins

David Jacobson

Steve Karp

Kathryn Koprivnikar

Hong Lam

Jung Lee

Calvin Settachatgul

Andy Simmons

Steve Stoufer

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