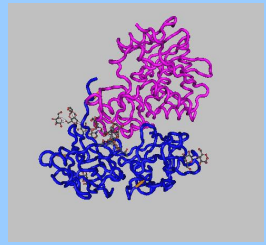


Outsourcing of vaccine development and production :

Development of a cost-effective and portable manufacturing process for the production of a ricin vaccine (*RiVax*).

Christopher J. Dale

Vice President Technology

Cambrex Biopharma.

Introductory statements

Today's presentation and discussion will contain forward-looking statements including statements regarding future operating performance, financial or revenue growth targets, or client project pipelines. These statements may be identified by words such as "expects", "anticipates", "intends", "estimates", "believes" or similar expressions.

These statements are based on Cambrex's current plans and expectations and involve risks and uncertainties that could cause actual outcomes and results to materially differ from those set forth in the forward-looking statements. For discussion of these and other risks and uncertainties, see the "Forward-Looking Statements" and "Risk Factors That May Affect Future Results" sections in the Annual Report Form 10-K for the period ending December 31, 2004.

The Company undertakes no obligation to update forward-looking statements.
For further information, please refer to Cambrex's reports and filings with the Securities and Exchange Commission.

- Introduction : Ricin as a bioterrorist threat
- Biodefense countermeasures : Fundamentals
- Vaccine development and production
- Case study : *RiVax*

Assessment of ricin as a bioterrorist threat

- Category B Biothreat
- 1 million tons of castor beans production worldwide (*Ricinus communis*)
- Potential to yield up to 50,000 tons pure ricin (5% of total)
- High toxicity - behind botulinum toxin in potency
  - Cytotoxic
  - Easy to purify
  - Stable and difficult to inactivate
- Toxic by inhalation, oral ingestion, and systemic exposure
- Ricin is a Type 2 ribosome inactivating protein
  - Specific for mammalian ribosomes
- Additional toxicity is vascular leak syndrome (VLS)
  - Binding of ricin to endothelial tissue: accumulation of fluid in interstitial spaces
- Virtually all mammalian cells are sensitive to ricin; all mammals are susceptible to ricin

Category A

Anthrax (*Bacillus anthracis)*

Botulism (*Clostridium botulinum* toxin)

Plague (*Yersinia pestis*)

Smallpox (*Variola major*)

Tularemia (*Francisella tularensis*)

VHF (Marburg, Ebola, Lassa)

Category B

Brucellosis

Q Fever

Ricin poisoning

Viral encephalitis

Food safety threats (*Vibrio cholerae, Cryptosporidium parvum)*

- There is **no** specific treatment
- Action is rapid and irreversible
- Lethal doses extrapolated from literature

  Aerosol: 3-5 µg/kg (airway damage, irritation to lungs, pulmonary edema and lesions)

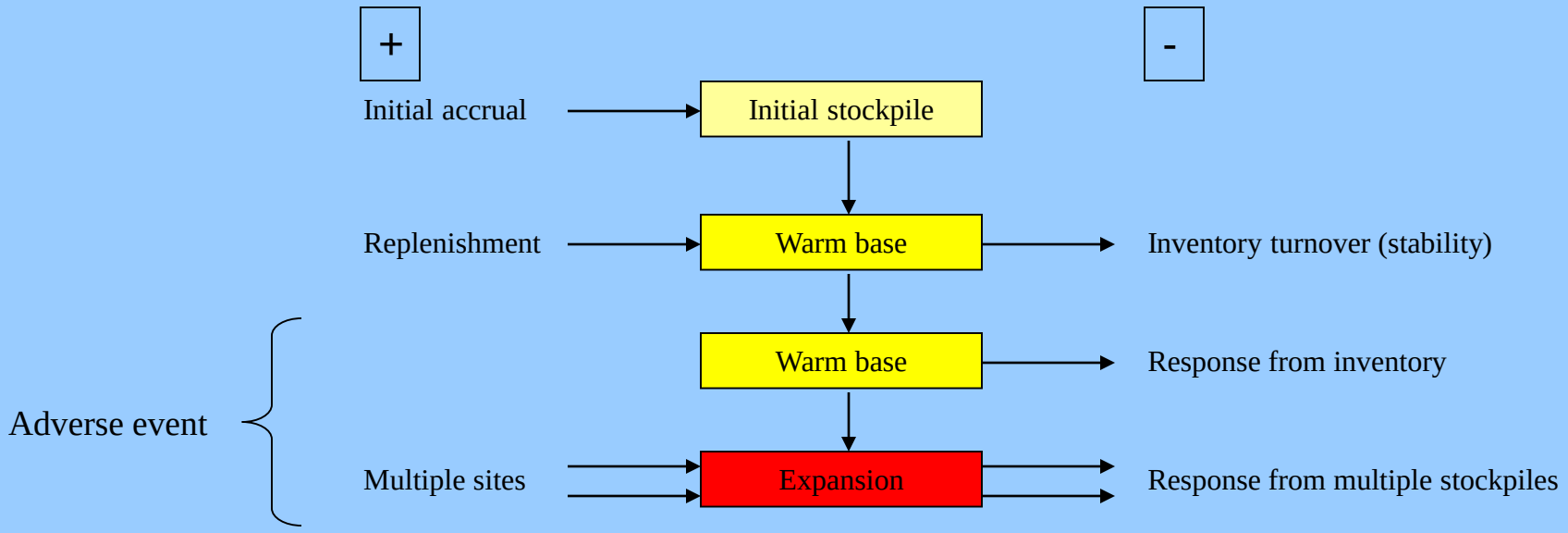
  Injected : 5 µg/kg (internal bleeding leading to central organ failure)

  Oral : 1-10 mg/kg (nausea, vomiting, cramps, diarrhea and intestinal bleeding)
- Animals and cells can be protected with antibodies
- Animal studies: short therapeutic window for passive immunization before ricin enters cells
- Specific anti-ricin antibodies are involved in protection
- **Active immunization is only way to protect against exposure**

Biodefense countermeasures : Fundamentals

- Stockpile of countermeasures
- Portability
- Risk management
- Cost of goods (COG)

Model for stockpile



- Inventory of final drug product or bulk drug substance
- Location and strategy for inventory management
- Size of repository (risk)
- Local repositories to serve large urban areas
- Radius of response : Localised response

Scenarios for inventory design and management

- Prioritisation of end-users ?
- First responders
- Vulnerable section of population

Portability of manufacturing processes

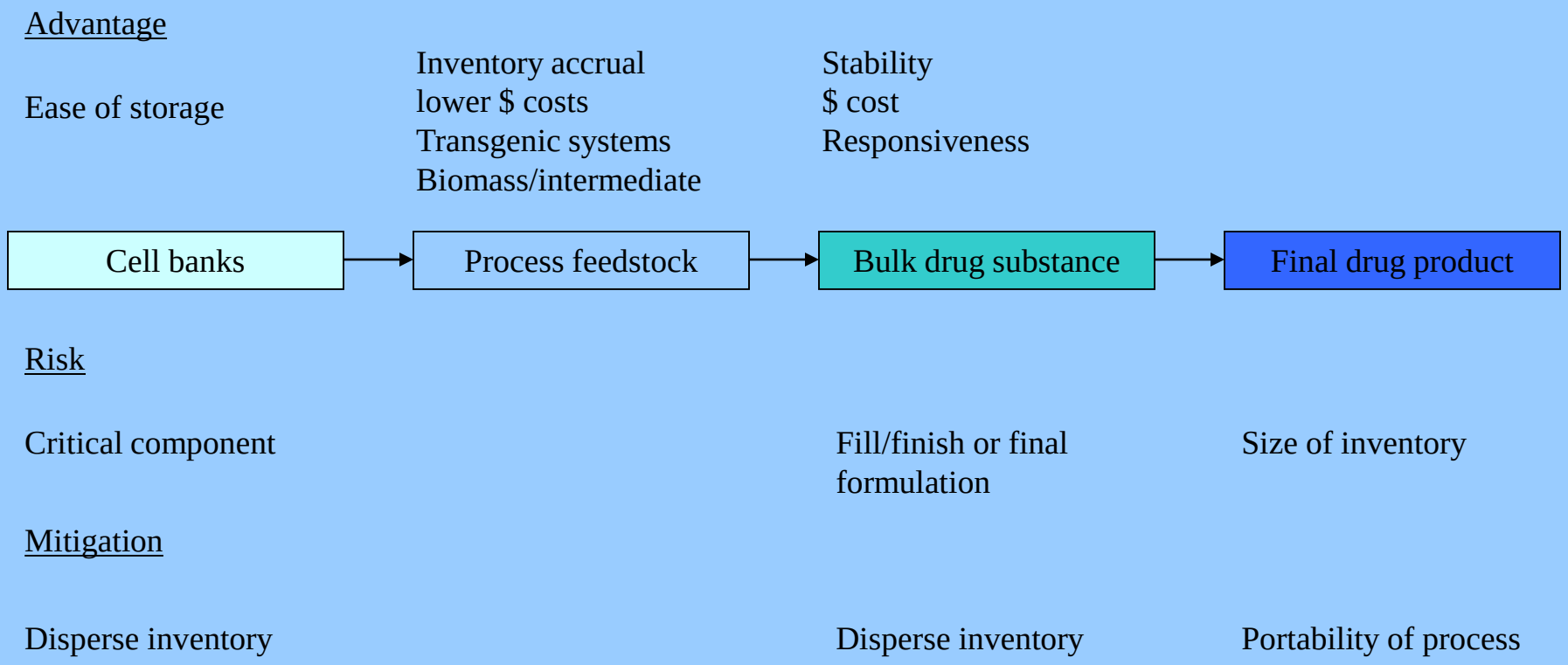
Portable manufacturing processes to enable rapid implementation at multiple sites

- Key elements of portability = Scale of operations and technology
- Manufacturing assets : Relative abundance of manufacturing capacity

Scale	Abundance
10 000L =	Low
1000L =	Medium
100L =	High

- Process intensification to exploit low volume assets
- Utilisation of conventional bioprocess equipment/unit operations
- Diversity of expression systems can confer process advantages (for example use of transgenic milk expression)

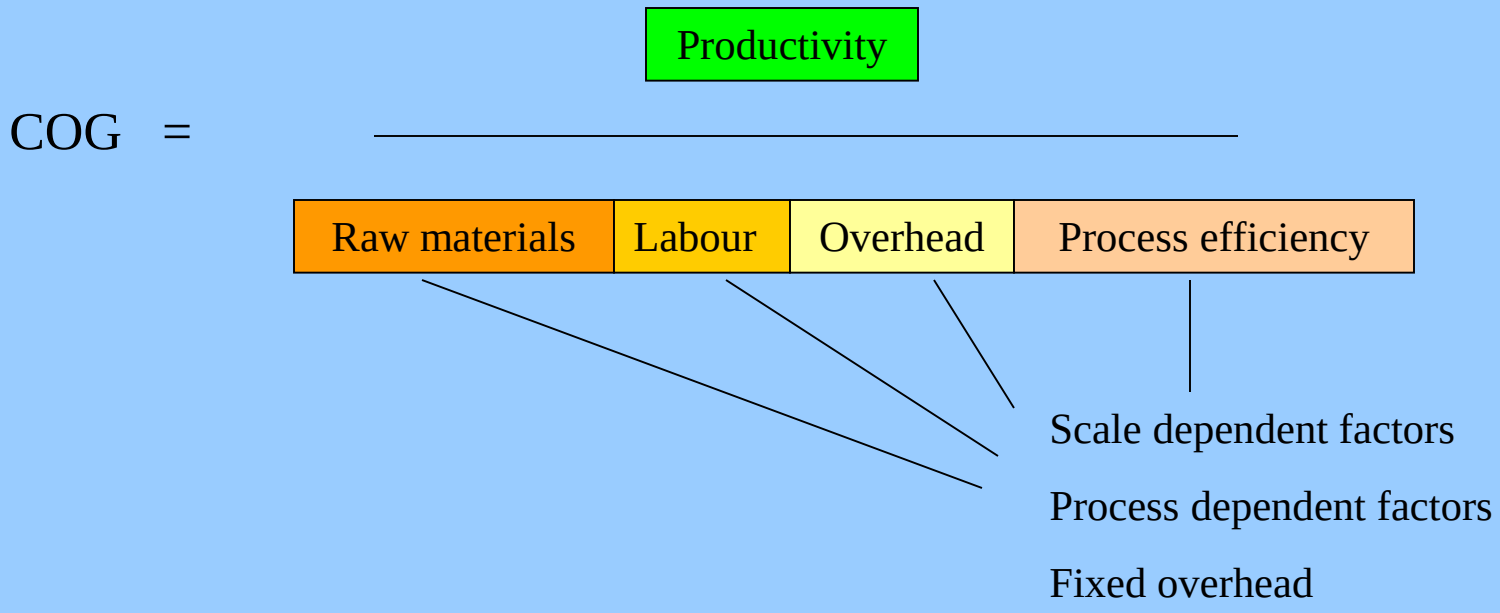
Diversity and risk management



COG structure

Why COG is important in biodefense manufacturing processes :

- Establish and maintain stockpile of products that may never be used
- Focus of threat may change
- If stockpile replenishment rate is low due to high stability ~ low annual demand ?
- Stockpile may be subject to global political change and priorities



RiVax

- Commercial approach to Category B countermeasures
- Target antigen
- Process development

Consortium approach for vaccine development

- "Small biotech" is not well equipped to carry on all the varied activities for vaccine development
  - Lack manufacturing capacity and capability
  - Infrastructure and facilities for containment and animal models
- Large Biotech/Pharma do not accept risky commercial projects, such as the development of biodefense vaccines with ill-defined markets
- Early development and feasibility in academic research: transfer to the development stages
- Lack of definition of the procurement process, as Project Bioshield is for the purchase of "established" countermeasures
- Support for early development through grant funding (SBIR, challenge grants, NIH, DoD, Foundations, etc)

Consortium approach for vaccine development

Project structure

•Dor Biopharma awarded grant from NIH

• Design of integrated vaccine development program

• Assembly of specialist teams at multiple sites

• Cambrex to provide process development and GMP manufacturing services

Challenges

Management of multiple teams

Management of diverse disciplines

Multiple locations

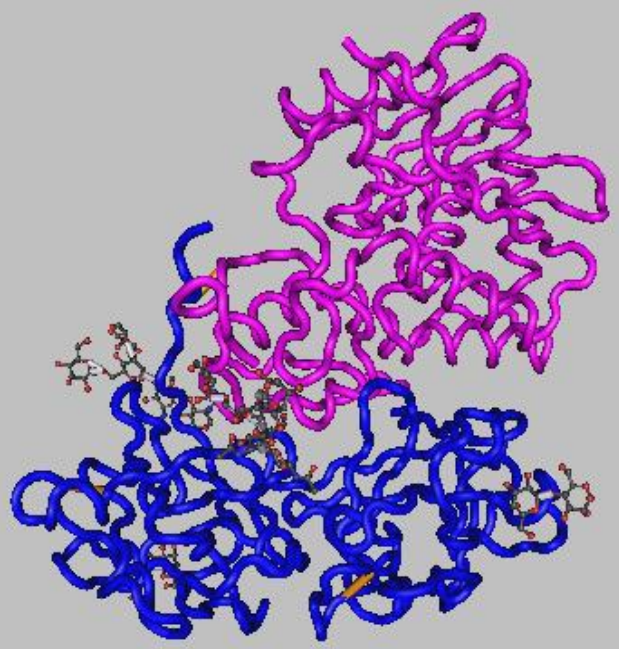
Fast-track timeline

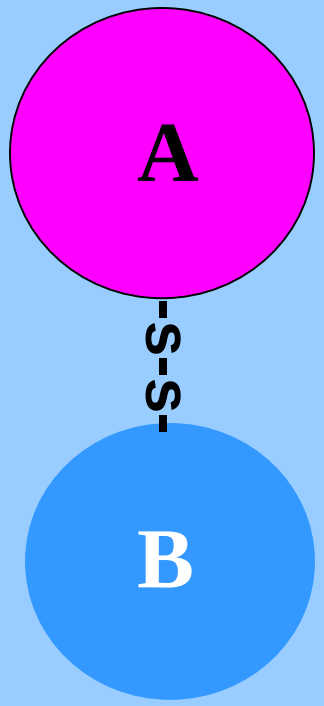
Sustained progress to report to grant awarding body

Ricin vaccine candidates

Vaccine Candidate	Status
Ricin Toxoid (formaldehyde-treated holotoxin)	Difficult process for control of residual toxicity
dgRTA (chemically deglycosylated RTA)	Expensive process
Plant derived or recombinant RTA	Toxicity associated with retention of enzymatic activity
RT-B	Not as immunogenic as RT-A
Recombinant A chain double mutant (***RiVax™,*** DOR Biopharma)	Under development – Phase I
Recombinant A chain deletion (USAMRIID)	Under development

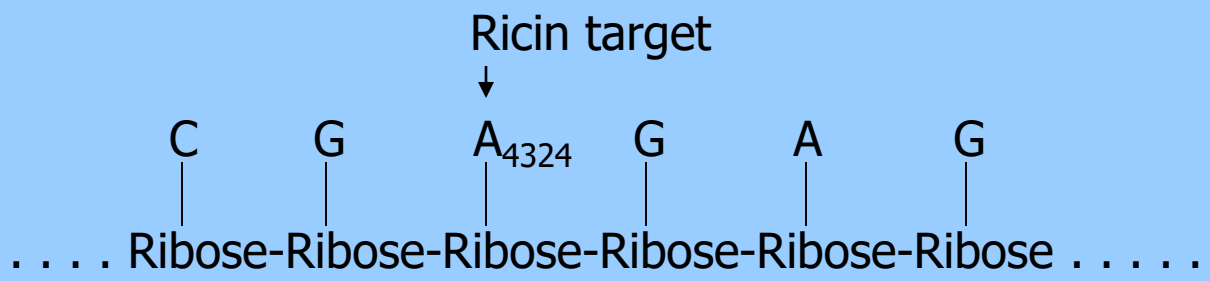
Ricin Structure and Major Enzymatic Activity





RTA, 267 aa, 32K
N-glycosidase, cleaving adenine 4324 in 28S rRNA

RT-B, 262 aa, 32K
Binds to terminal galactose residues



Engineering of Recombinant Ricin A Chain

Key features

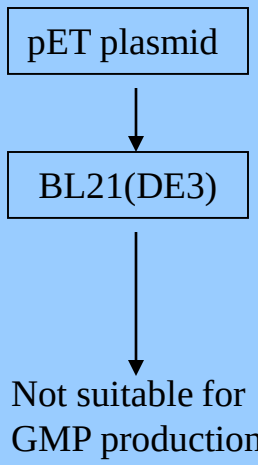
- Site specific amino acid substitutions
- Double mutant (*RiVax*™)
- Valine to Methionine (76), Tyrosine to Alanine (80)
- Elimination of enzymatic site and vascular leak syndrome (VLS)

RiVax : Cambrex scope of work

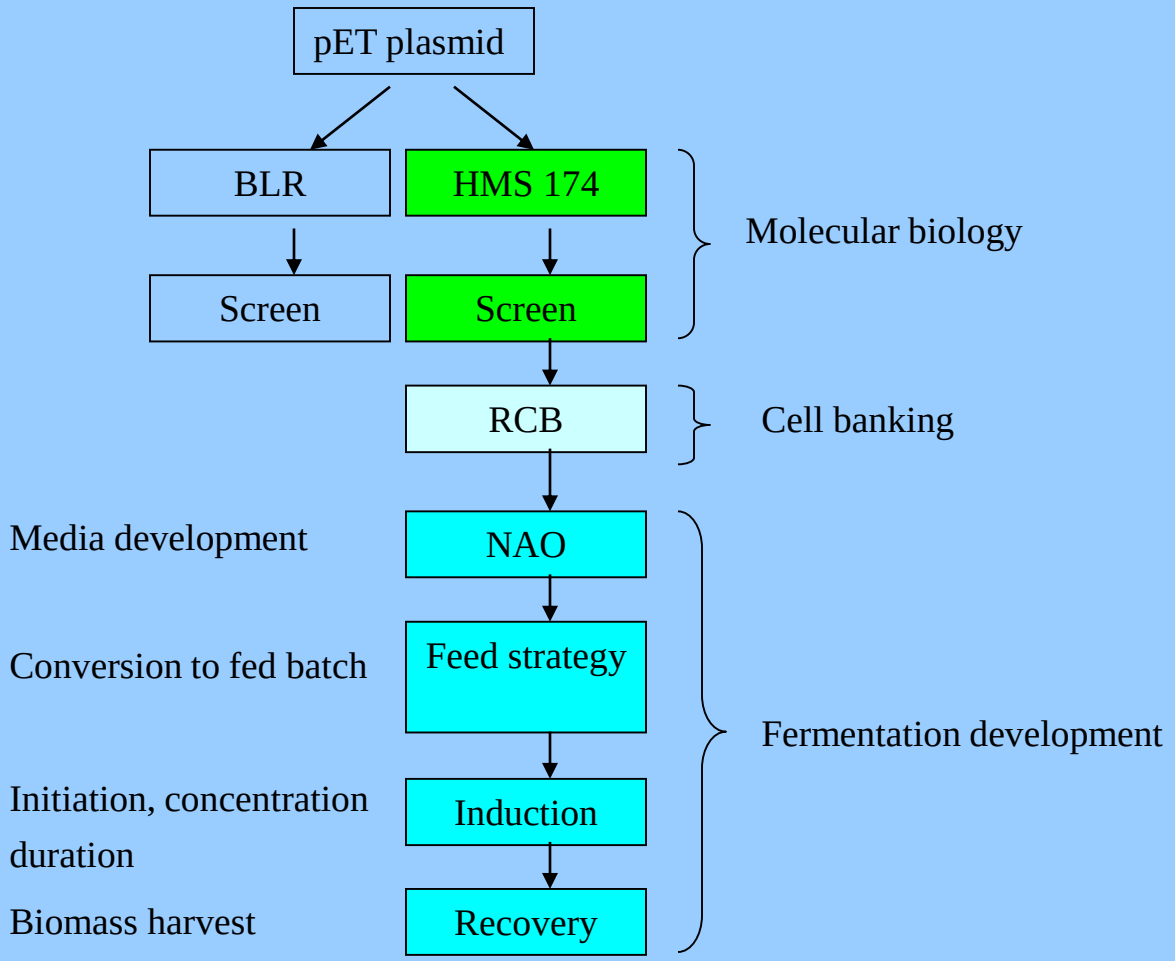
- Re-engineer plasmid/host system (*E.coli*)
- Target soluble expression
- Eliminate all animal derived components
- Fed-batch fermentation for high cell density
- Develop efficient extraction methodologies
- Develop robust purification regime with minimal # unit operations
- Interim formulation

RiVax : Molecular biology and fermentation development

Academic process



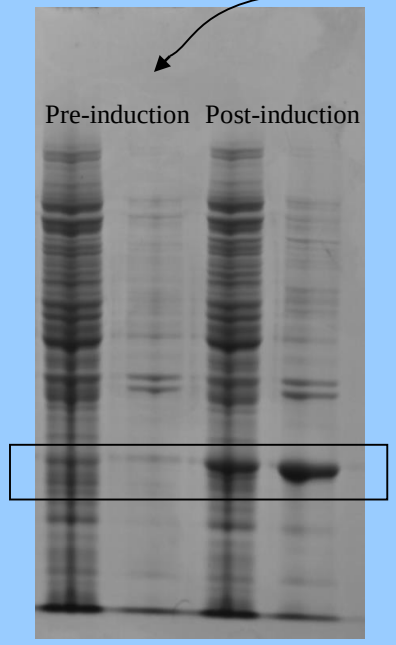
New scope of work



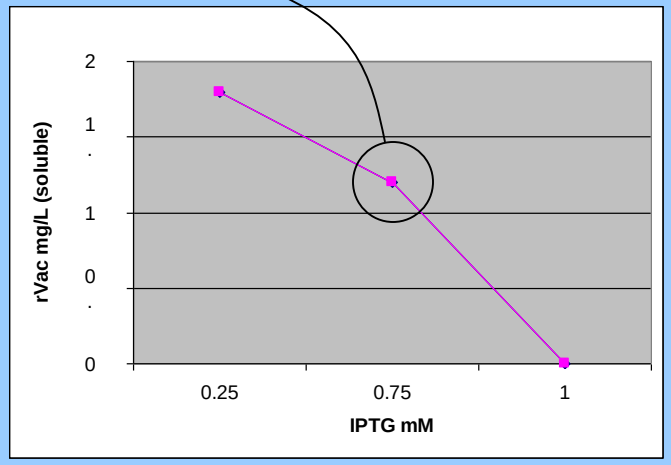
Fermentation : Localization of expression

- Localization of expression (soluble vs IB)
- Soluble expression to circumvent refold, minimize # unit operations
- Strategies for directing product localization

[IPTG] : Solubility and expression level / Bias to soluble intracellular expression



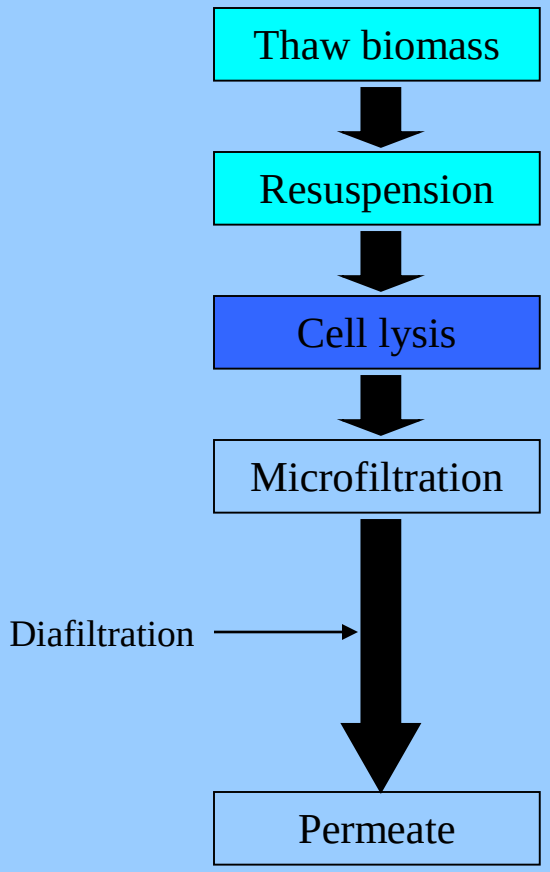
RiVax



S = Soluble fraction

I = Insoluble fraction

RiVax : Recovery



	Conditions	Function
Thaw biomass	2-8ºC for 48-60 hours	
Resuspension	25mM sodium phosphate pH 6.5	Lysis buffer
Cell lysis	10 000 psig , T < 20ºC	Release of intracellular soluble product
Microfiltration	0.2µm Hollow fiber	Clarification : Removal of particulate material
Diafiltration	25mM sodium phosphate pH 6.5, 3-4 DV	Increased efficiency of *RiVax* recovery
Permeate		

Development of a capture chromatography unit operation

Key drivers
- Selectivity
- Capacity
- Recovery

Functionality	Chromatography resin	Results
AEX	Q Sepharose FF	Ability to bind *RiVax* but poor selectivity relative to HCP
HIC	Phenyl Sepharose 6 FF (High Sub)	No binding of *RiVax* but capture of HCP (possible flow-through unit operation)
CEX	CM Sepharose FF SP Sepharose FF SP 550C Poros HS50	Low capacity ~ 0.2mg/mL Low capacity ~ 0.6mg/mL Capacity 3mg/mL but poor recovery (56%) Capacity 9mg/mL, recovery 75-88%

Development of a capture chromatography unit operation

Poros HS50 and SP 550C selected as potential capture chromatography resins

Dynamic binding capacity decreased at low linear flow rates

Increasing linear flow rate firstly to 100cm/h then to 500cm/h greatly improved dynamic binding capacity.

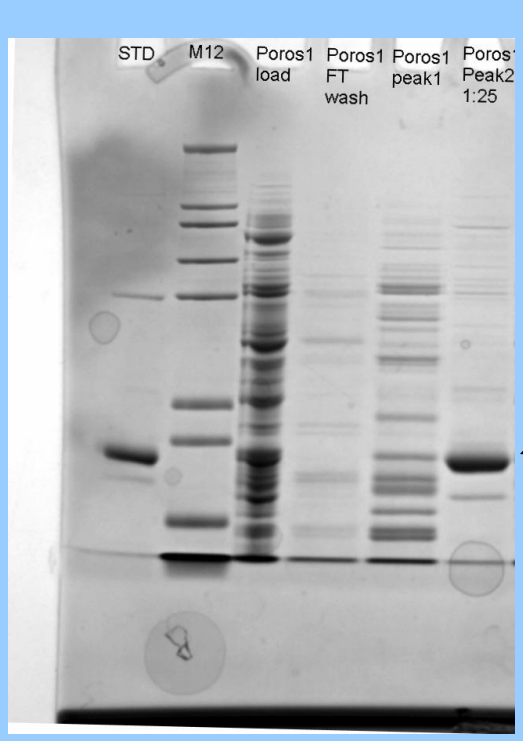
Pressure flow characteristics of SP 550C unsuitable for larger scale columns at high linear flow rates (at 100cm/h).

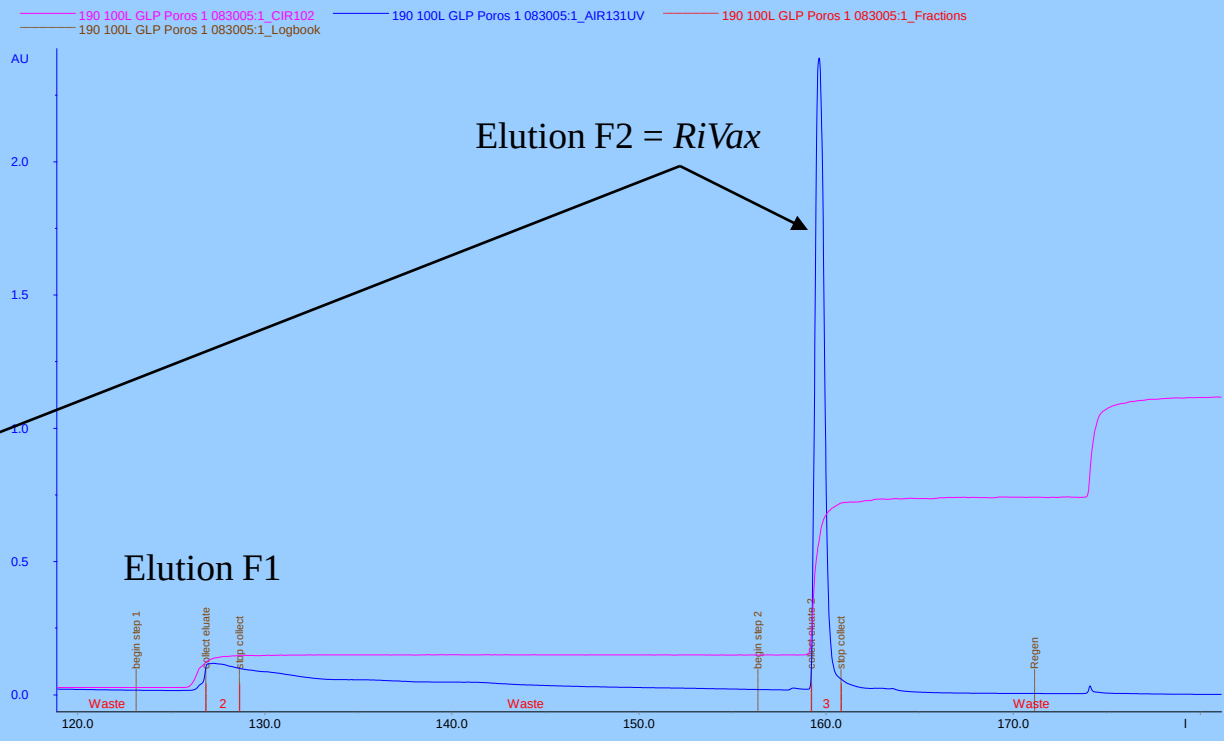
Poros HS50 selected as capture chromatography resin

LFR (cm/h)	Capacity (mg/mL)	Recovery (%)	Purity (%)
500	9	85	85

• Development of specifications for conductivity of load material and establishment of step elution conditions.

Poros HS50





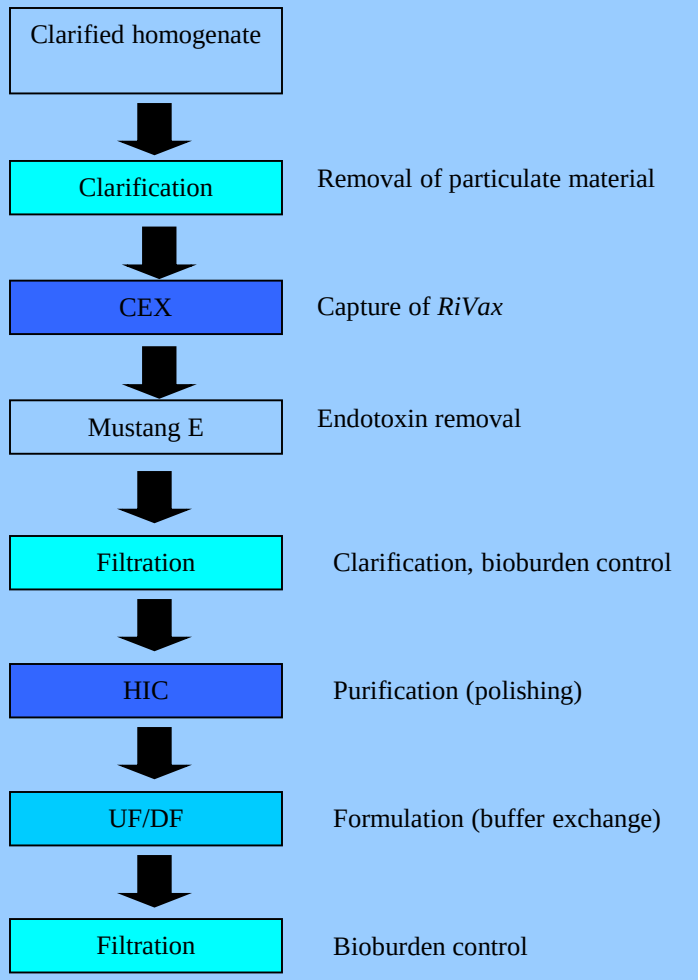
Poros HS50 elution profile and SDS PAGE analysis of fractions

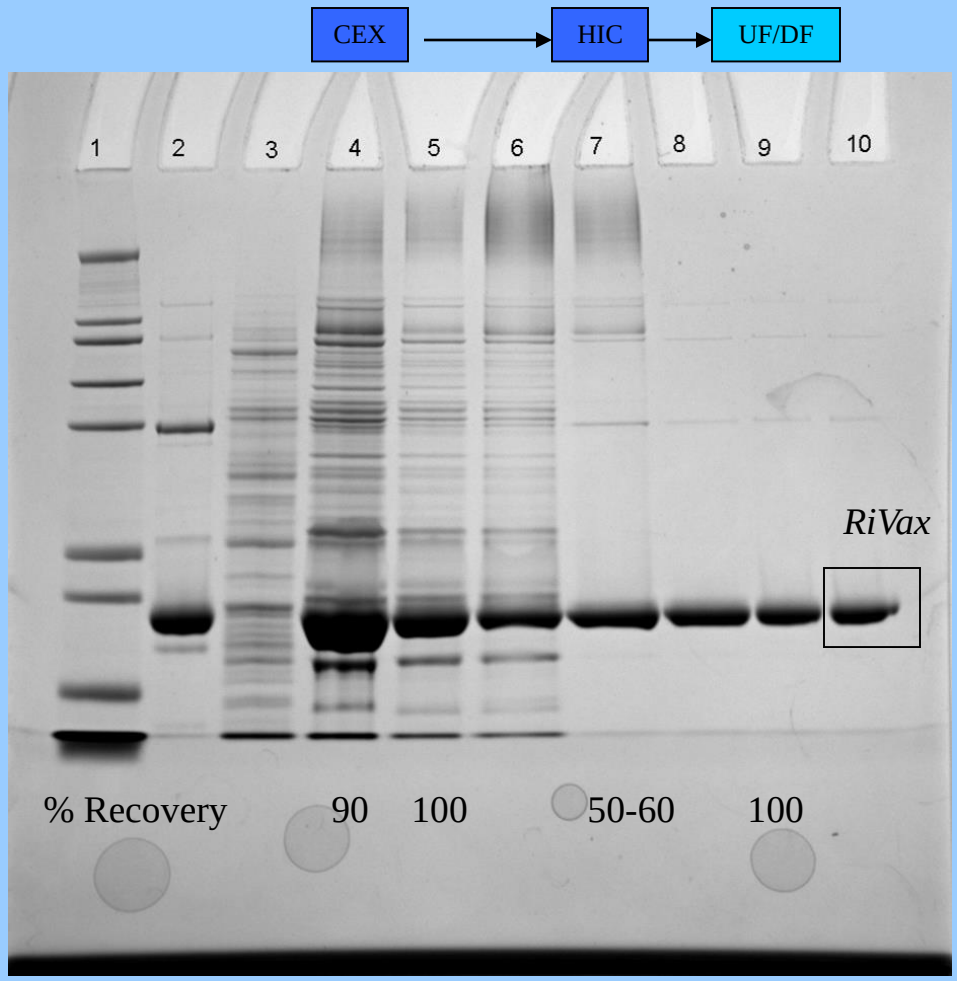
Development of a purification chromatography unit operation

- HIC displays high binding of HCP components
- Butyl-S Sepharose 6 FF selected as purification resin
- Specifications for conductivity to facilitate binding of *RiVax* to the resin (min/max)
- Dynamic binding capacity (*RiVax*) ~ 22mg/mL
- Elution of *RiVax* within conductivity range 142-104mS/cm (HCP major components elution at < 83mS/cm)
- Selection of start/stop fraction collection to avoid minor HCP components at leading edge of *RiVax* elution fraction.

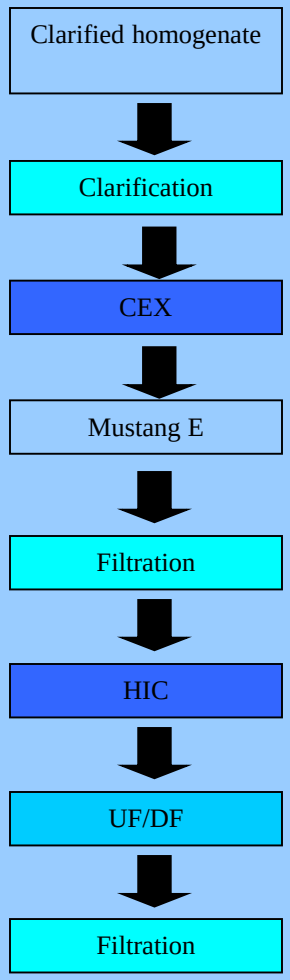
LFR (cm/h)	Capacity (mg/mL)	Recovery (%)	Purity (%)
500	22	81	>95

RiVax : Purification





RiVax : Endotoxin removal



		EU/mL
Clarified homogenate		
Clarification	Removal of particulate material	2.8×10^6
CEX	Capture of *RiVax*	$0.7\text{-}1.7 \times 10^6$
Mustang E	Endotoxin removal	200
Filtration	Clarification, bioburden control	
HIC	Purification (polishing)	16
UF/DF	Formulation (buffer exchange)	48
Filtration	Bioburden control	< 16

HF TFF primary clearance

Mustang E filter provides additional assurance in cases of high endotoxin input

RiVax manufacturing process : Summary of productivity, yield and cost effectiveness

Expression = 1.5 -2.0 g/L in fermentation (100L scale of operations)
Compound yield = 50-60% in extraction – purification

Quality attributes
Purity = >95%
Endotoxin = <10 EU/mg
Residual DNA = <15 pg/mg

$ indicators (full analysis pending approval to release $ data)
Cycle time (Fermentation – purification) = 7 days
Material requirements : Low
Longevity of multi-use components : Medium
Process risk : Low

Portability : High

- Conventional raw materials and equipment
- 4 x UO impact product yield
- 5 x UO impact product quality attributes
- Stable BDS

Conclusions

- Limited number of organisations are developing biodefense products (vaccines, therapeutics).
- Consortium approach is an effective mechanism for commercialisation
- Cost of goods critical to product economics
- Need for rational design to maximise productivity, yield and portability
- A portable (100L scale) and cost-effective process for the manufacture of *RiVax* has been successfully developed
- *RiVax* : A vaccine against a ricin bioterrorist threat is now under clinical evaluation

Acknowledgements

Dor Biopharma (Robert Brey)

Cambrex Corporation