
Facilitating Preclinical Development and Tech Transfer through Informatics

David Bienvenue, Ph.D.
Stefan Ponko, Ph.D.

VLST Corporation



Virulence Factors as a Novel Route to Therapeutics

- Some viral proteins modulate/suppress host immune system
- Facilitate viral infection and influence severity of disease
- Can be homologous or unrelated to host genes
- Targets of viral proteins validated as treatment methods for autoimmune/inflammatory illness

Drug Development Strategy

Viral
virulence
factors

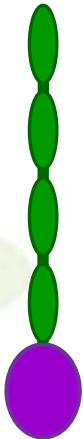
Identify
cellular
targets

Define biologic
consequences
of interaction

Develop therapeutics
mimicking
virulence factors

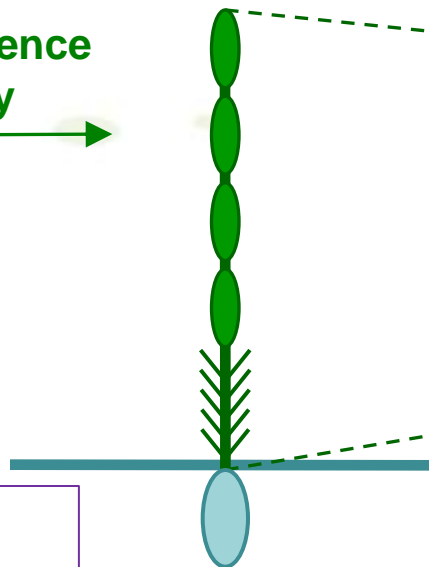
Identification of Secreted Viral TNF Receptor Key First Step in the Development of Enbrel[®]

Shope fibroma
virus-T2

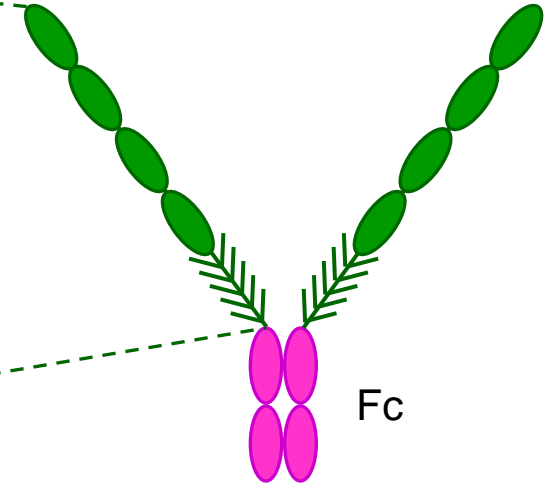


38 % sequence
identity

TNFR2
(p75)



Enbrel[®]
(Entanercept)

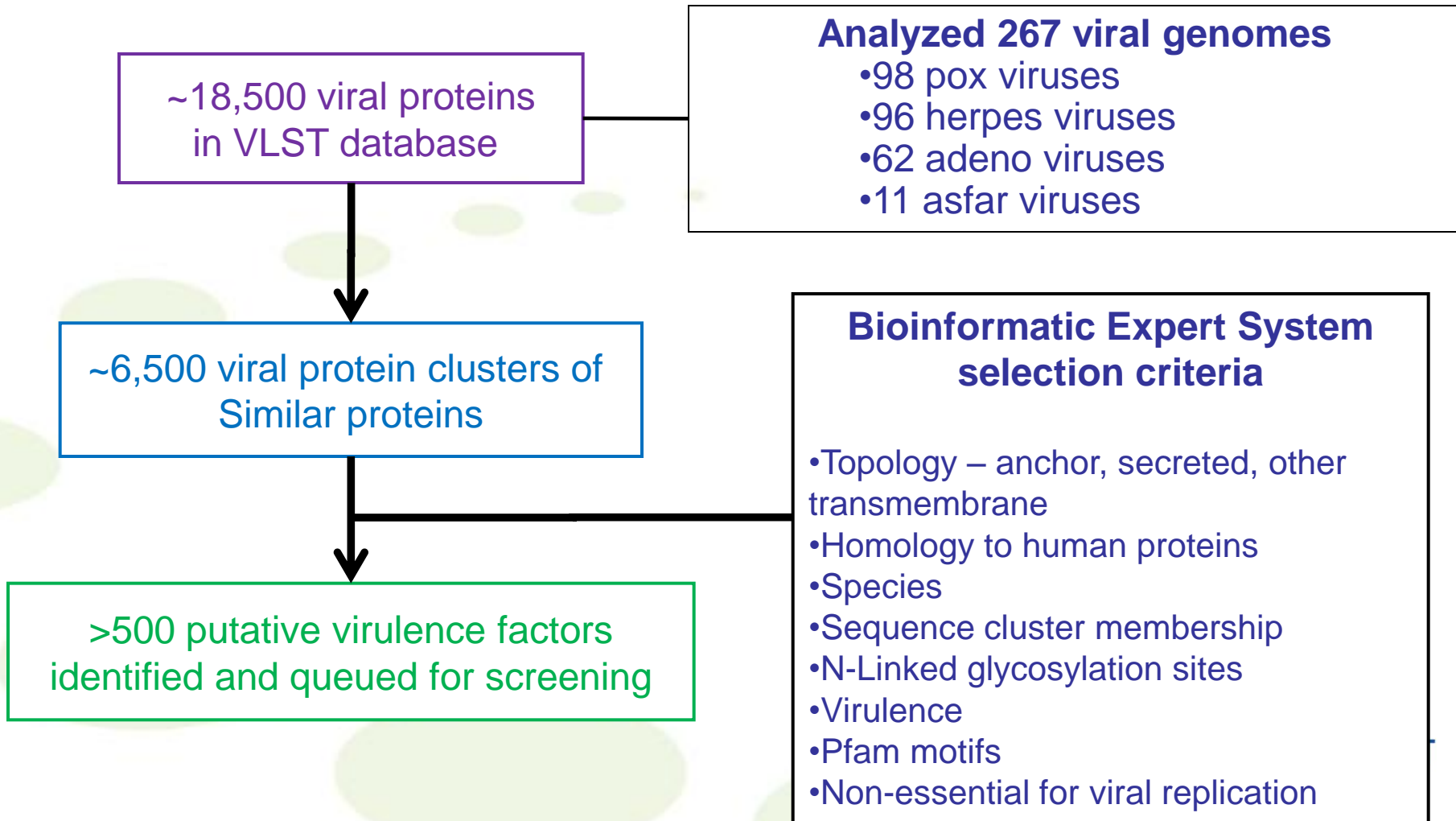


Fc

Smith *et al* (1990) Science 248: 1019
Smith *et al* (1991) BBRC 176: 335

Numerous examples of viruses encoding
chemokine and cytokine binding proteins

Genomic Scale Search for Viral Virulence Factors



Identification of Host Proteins Interacting with Viral Virulence Factors

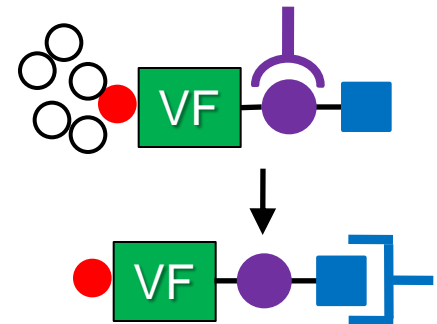
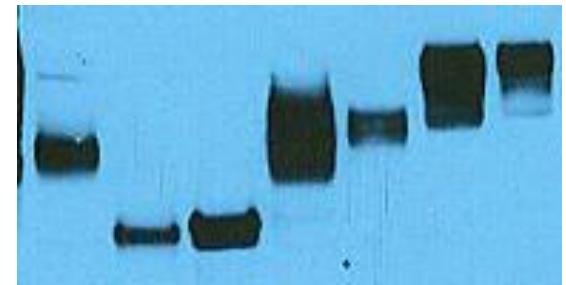
Bioinformatic mining for virulence factors



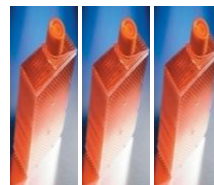
Synthesize viral genes



Transiently express tagged viral proteins



Screen cell lysates & conditioned media



Target identification by LC-LTQ MS



Capture target(s) with tandem affinity tags

Data Overload!

- Huge amounts of data generated during target discovery
 - DNA/protein sequences
 - Different vectors, cell lines
 - Expression/purification methodology
- As protein drugs move through development, track important changes to the construct and/or process
- Using spreadsheets has limitations
 - Version control
 - Access control
 - Prone to human error
 - Limited functionality

Design Goals- Protein Database



VS



Start simple!

- Focus on immediate needs of company
- Build/add functionality as needed
 - Easier, cheaper, faster to get up and running
 - Less likely to overwhelm users with too many functions
- Initially, you may just need a butter knife...

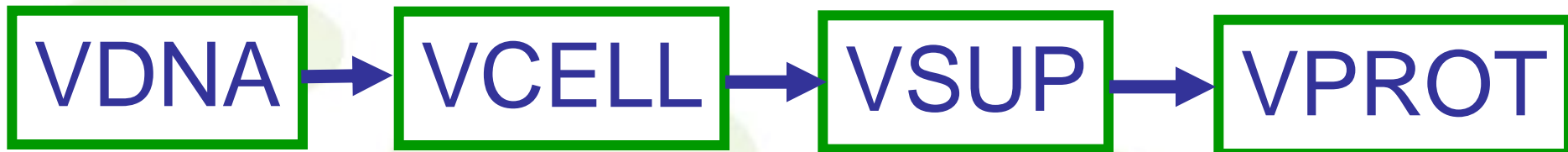
Design Goals- Data Tracking

Decide what information to track and format

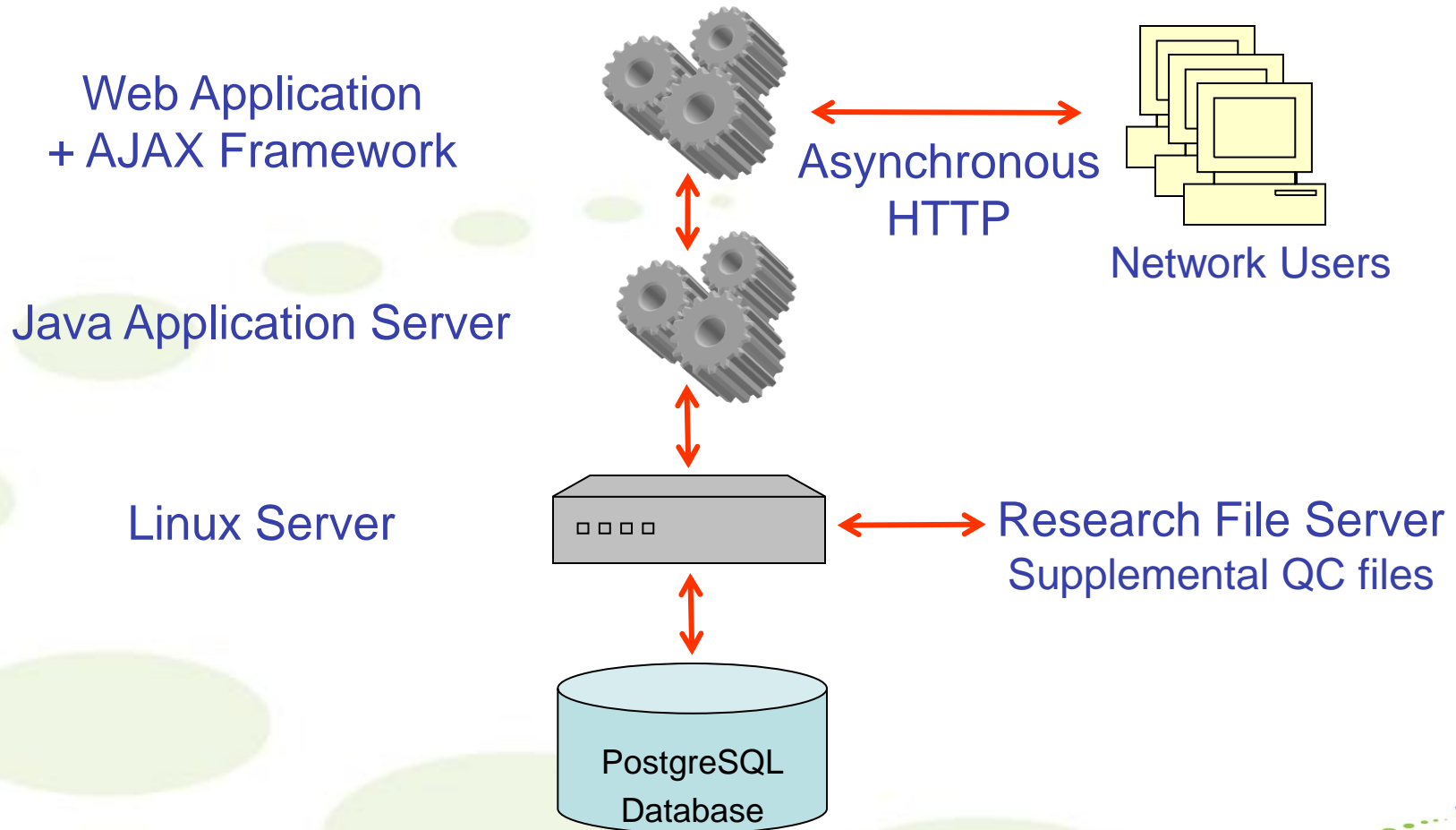
- DNA info → Data Table, Sequence file
- Cell line info → Data tables
- Cell supernatant → Data tables
- Purification details → Data tables
- QC data → Annotated gel/blot, SEC traces

Design Goals (Cont.)

- Determine how different types of data relate?
- Assign unique identifier to each new vector, batch, etc.
- Make it possible to backtrack to original data (lab notebook)
- Make it function as a request system for reagents



Protein Tracking Application Architecture



Submit Requests

Transient Expression

Stable Expression

Existing Batch

Request system for batches of protein, sorted by type of request

View Requests

Transient Expression

All Others

Aids in resource management

Add Batch

View Batches

Search Batches

Add Supe

View Supes

Search Supes

“Add” function is password protected

Add Cell Line

View Cell Lines

Search Cell Lines

Anyone can view, search records

Add VDNA

View VDNAs

Search VDNAs

DNA Record

VDNA Number	517	Project*	
Construct*	pBIG.GLuc.HAC	Insert*	GLuc
Vector NTI File Name*	VDNA517pBIG.GLuc.HAC.gb		
Notebook #*	VL052-RM-1	Notebook Page	98
Vector	pBIG	Tag	HAC
Date completed & boxed		QC Initials	
Theoretical Monomeric Mol. Wt.	31666	Extinction Coefficient <small>(M⁻¹ cm⁻¹)</small>	23085
Absorbance <small>.1% (=1 g/l)</small>	0.729	Construct Expression	To Be Expressed
VPID		Comments	
Categories		N-Linked glycosylation site count	0

•Hyperlink to DNA sequence

•Automatically calculated from mature sequence
•Useful information for purification

Mature Sequence
(required if construct is to be expressed)

Supe Record

- Linked back to stable cell line and DNA
- Captures important harvest information

VSup Number			
Cell Line*	78-293 t17 from atcc p=10	transfection VDNA* <i>(only for parentals)</i>	230-409-HAC-7803 229-409-HAC-7667 228-409-HAC-2946 227-409-HAC Sol. Delta 226-409-CFLAG-SolSigma 225-pFUSEmIlgG2Aa-Fc1
Harvest Date	07/10/08	Staff	merrill/tompkins
Production Container	plates	Number of Passages	5
Transfection Method	lipofectamine/plus	ORF	
Tag	HAC	Culture Media	dmem + 10%fbs
Culture Media Volume	1000 mL	Culture Media Volume Conc.	1x
Freezer	Protein Science -80 Freezer	Notebook Reference	VLST-0010
Harvest Cell Density (1e6)	5x10E6	Harvest Viability (%)	65%
		Transfection Request <i>(only for parentals)</i>	Select One
		Insert	G1uc
		Expression Level	4
Sup Quality	1 - Mostly Degraded	Link to QC Data	072808-hac-vsups-582-583-584-585.ppt
MSD Protein Concentration <i>(ng/ul)</i>	14.9	Endotoxin Test Results	Negative

- Link to PowerPoint file with Western blot
- Endotoxin test results

Protein Batch Record

“History” of DNA thru purified batch, linking all records

VPROT230: |VSUP359-VCCELL142-VDNA517

VPROT Number	230	Quarantined	<input type="checkbox"/>
Common Name	Gluc-HAC	Purification 1	QSepharose Fast Flow
Affinity Tag	HAC	Purification 2	GE Streptavidin
Endotoxin Test Results	0.5 Eu/mL	Purification 3	Superdex 200 XK26/60, SEC
		Purification 4	
		Formulation Buffer	PBS
Current Number of Aliquots	8.0	Supes <i>(id-transfection-vdna)</i>	359-stable, pooled with vsup356-517
		Final Purified Protein Mass (ug)	1,911
		Mass of Protein Remaining (ug)	728
		Purification Operator's Initials	JB
		Storage location of purified protein	-20 C
		Link to SDS-PAGE/Western Data	VPROT 0230.ppt
Purified Volume (mL)	2.1		
Purification Operator's	VI-078-JB-3		

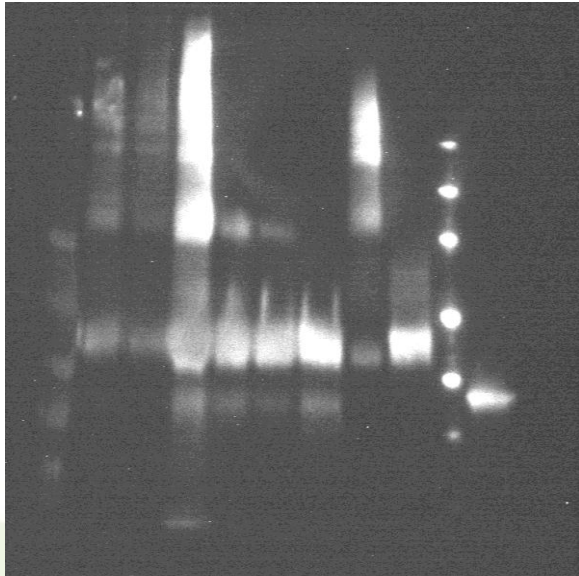
Purification details, including formulation

Yield info and amt. in inventory

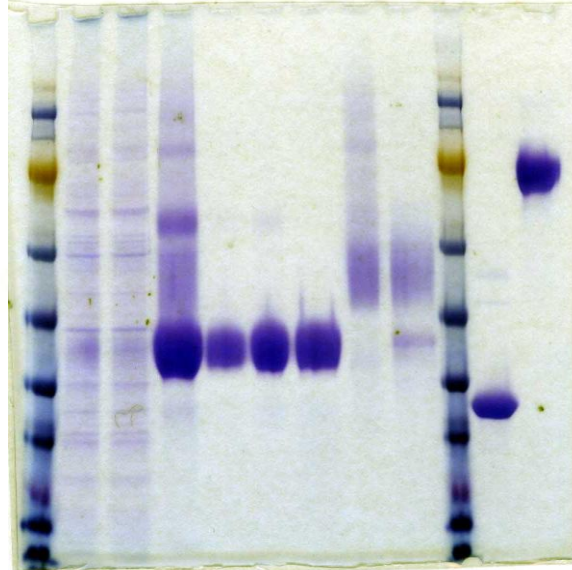
Link to PowerPoint file with QC Data

Protein Batch QC

1 2 3 4 5 6 7 8 9 10 11 12



1 2 3 4 5 6 7 8 9 10 11 12



<u>Lane</u>	<u>Sample</u>
1	MW Marker
2	Supe (NR)
3	AC-Flow Thru (NR)
4	AC-Eluate (NR)
5	SEC Pool
6	VPROT 613(5ug or 1ug, NR)
7	VPROT 613 (5ug or 1ug, Red)
8	VPROT 618 (5ug or 1ug, NR)
9	VPROT 618 (5ug or 1ug, Red)
10	MW SB+2 or Perfect Protein
11	Gluc-HAC (5ug or 1ug, NR)
12	VLST007-Fc (5ug or 1ug, NR)

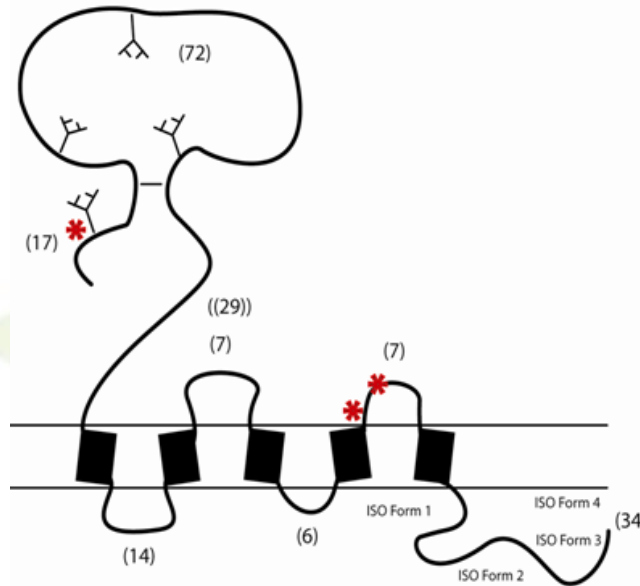
- Standardized format for all research reagents
- Users can make their own assessment of quality
- Minimizes time spent digging through notebooks

Preclinical Development Application of Informatics

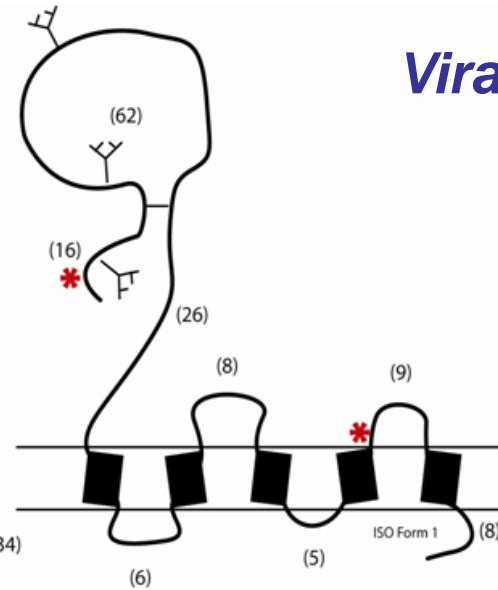
- Beyond tracking information on research reagents, is system useful for process development/tech transfer activities?
- **Case study:** Development, scale-up and tech transfer of CD47-Fc

Case study: CD47-Fc

Human **CD47**



Viral **CD47**



- Viral CD47 interrupts CD47-signalling by forming heterodimer
- Make soluble Fc-fusion protein of hCD47

Case study: CD47-Fc

- Extracellular domain fused to IgG1 Fc, expressed and purified as dimer
- Multiple constructs evaluated during program
- 7 N-linked glycosylation sites
 - Assume process may impact glycoform heterogeneity
- Initial process development performed at VLST, tech transfer of process and reagents to CMO

Case Study: Tracking Construct Variations

- **~115 different vectors** were evaluated
 - Murine, human and viral constructs
 - Different expression systems
 - Different affinity tags
 - Different truncated versions of binding domain
- Multiple stable cell lines and clones
- Multiple purified batches

- Protein Database used to track of all these variations

Case Study: Tracking Process Variations

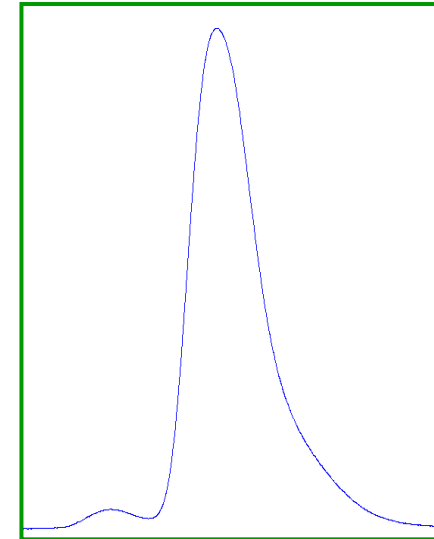
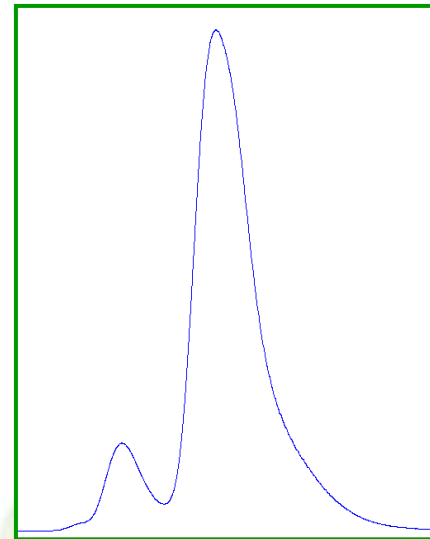
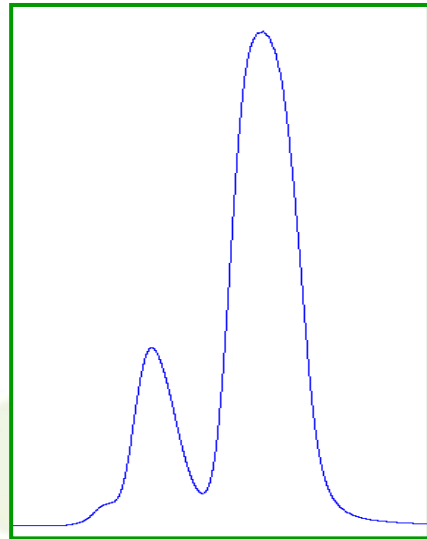
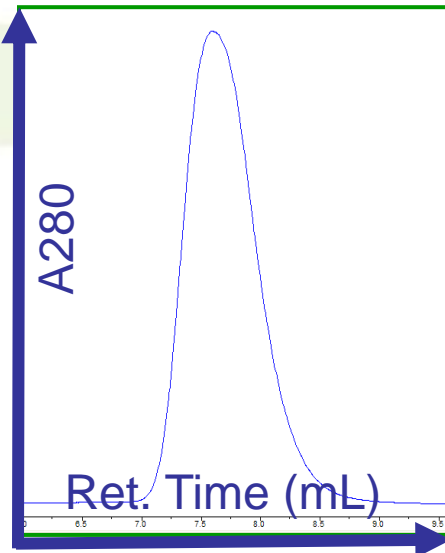
- Aggregation observed after CEX on analytical SEC
- Different process conditions evaluated, tracked

VPROT 0230		WALLACE, WENHAST		Project*	
VPROT Number	0230	Quarantined	<input type="checkbox"/>		
Common Name	GLu-FHC	Purification 1	<input type="checkbox"/>		
Affinity Tag	HAC	Purification 2	<input type="checkbox"/>		
Endotoxin Test Results	0.00E+00	Purification 3	<input type="checkbox"/>		
Concentration Assay Used	A280 SEC 0.1M 0.75M	Purification 4	<input type="checkbox"/>		
Aliquot Volume (mL)	0.1	Formulation Buffer	PEB		
Original Number of Aliquots	210	Supes (at production vol)	0.00E+00		
Current Number of Aliquots	80	Final Purified Protein Mass (ug)	0.91		
Mass per Aliquot (ug)	0.10	Mass of Protein Remaining (ug)	0.78		
Final Purified Concentration (ug/mL)	9100	Purification Operator's Initials	JA		
Purified Volume (mL)	0.1	Storage location of purified protein	02C		
Purification Operator's Notebook Number	VL076-B-3	Link to SOC-PAGE/Western Data	VPROT 0230.ppt		
Storage Entry Date	06/27/01				

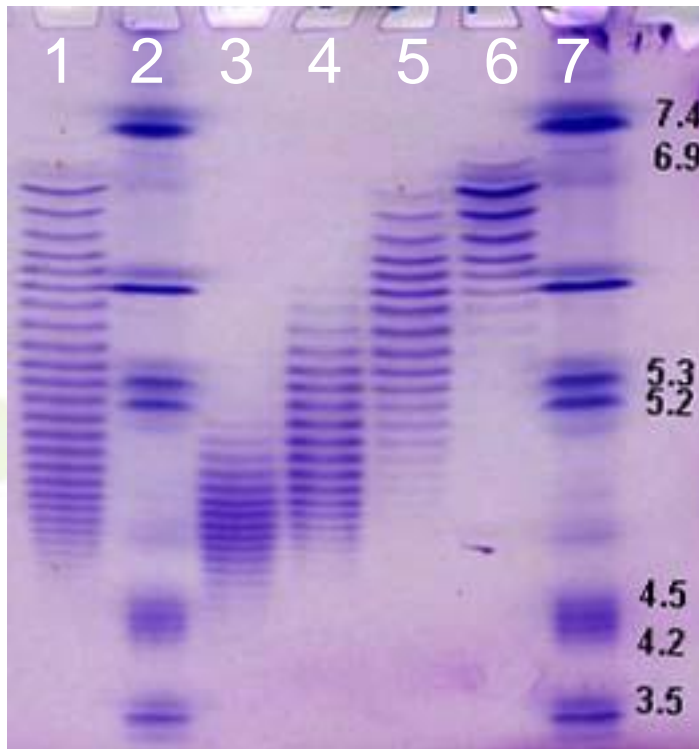
VPROT 0230		WALLACE, WENHAST		Project*	
VPROT Number	0230	Quarantined	<input type="checkbox"/>		
Common Name	GLu-FHC	Purification 1	<input type="checkbox"/>		
Affinity Tag	HAC	Purification 2	<input type="checkbox"/>		
Endotoxin Test Results	0.00E+00	Purification 3	<input type="checkbox"/>		
Concentration Assay Used	A280 SEC 0.1M 0.75M	Purification 4	<input type="checkbox"/>		
Aliquot Volume (mL)	0.1	Formulation Buffer	PEB		
Original Number of Aliquots	210	Supes (at production vol)	0.00E+00		
Current Number of Aliquots	80	Final Purified Protein Mass (ug)	0.91		
Mass per Aliquot (ug)	0.10	Mass of Protein Remaining (ug)	0.78		
Final Purified Concentration (ug/mL)	9100	Purification Operator's Initials	JA		
Purified Volume (mL)	0.1	Storage location of purified protein	02C		
Purification Operator's Notebook Number	VL076-B-3	Link to SOC-PAGE/Western Data	VPROT 0230.ppt		
Storage Entry Date	06/27/01				

VPROT 0230		WALLACE, WENHAST		Project*	
VPROT Number	0230	Quarantined	<input type="checkbox"/>		
Common Name	GLu-FHC	Purification 1	<input type="checkbox"/>		
Affinity Tag	HAC	Purification 2	<input type="checkbox"/>		
Endotoxin Test Results	0.00E+00	Purification 3	<input type="checkbox"/>		
Concentration Assay Used	A280 SEC 0.1M 0.75M	Purification 4	<input type="checkbox"/>		
Aliquot Volume (mL)	0.1	Formulation Buffer	PEB		
Original Number of Aliquots	210	Supes (at production vol)	0.00E+00		
Current Number of Aliquots	80	Final Purified Protein Mass (ug)	0.91		
Mass per Aliquot (ug)	0.10	Mass of Protein Remaining (ug)	0.78		
Final Purified Concentration (ug/mL)	9100	Purification Operator's Initials	JA		
Purified Volume (mL)	0.1	Storage location of purified protein	02C		
Purification Operator's Notebook Number	VL076-B-3	Link to SOC-PAGE/Western Data	VPROT 0230.ppt		
Storage Entry Date	06/27/01				

VPROT 0230		WALLACE, WENHAST		Project*	
VPROT Number	0230	Quarantined	<input type="checkbox"/>		
Common Name	GLu-FHC	Purification 1	<input type="checkbox"/>		
Affinity Tag	HAC	Purification 2	<input type="checkbox"/>		
Endotoxin Test Results	0.00E+00	Purification 3	<input type="checkbox"/>		
Concentration Assay Used	A280 SEC 0.1M 0.75M	Purification 4	<input type="checkbox"/>		
Aliquot Volume (mL)	0.1	Formulation Buffer	PEB		
Original Number of Aliquots	210	Supes (at production vol)	0.00E+00		
Current Number of Aliquots	80	Final Purified Protein Mass (ug)	0.91		
Mass per Aliquot (ug)	0.10	Mass of Protein Remaining (ug)	0.78		
Final Purified Concentration (ug/mL)	9100	Purification Operator's Initials	JA		
Purified Volume (mL)	0.1	Storage location of purified protein	02C		
Purification Operator's Notebook Number	VL076-B-3	Link to SOC-PAGE/Western Data	VPROT 0230.ppt		
Storage Entry Date	06/27/01				



Case Study: Examining Impact of Glycosylation via IEF-PAGE



<u>Lane</u>	<u>Sample</u>
1	VPROT 287
2	Serva pI marker
3	VPROT 365
4	VPROT 366
5	VPROT 367
6	VPROT 368
7	Serva pI marker

- Facilitated tracking data generated by other groups (immunologists, analytical CRO's)

Case Study: Tech Transfer to CMO

- Research reference standard sent to CMO given ID#
- Samples of protein generated by CMO, transferred to VLST, were also entered in database
- Facilitated tracking data generated at VLST and CMO

Facilitating Preclinical Development and Tech Transfer through Informatics

Conclusions

- Phased approach was successful
- Utilized by majority of lab staff
- Facilitates interdepartmental work
- Continue to add/enhance functions as necessary
- Wiki page for instructions for use
- Disadvantages?
 - Time required for data entry
 - Transparency is not always good!

Acknowledgements

Bioinformatics and Proteomics

- **Stefan Ponko, Ph.D.**
- Ajamete Kaykas, Ph.D.

Protein Sciences Group

- Jeff Bartron
- Chris Tompkins
- Laura Hajny
- Patrick Mosher
- Ryan Kelly
- Ryan Merrill



Senior Management

Martin Simonetti, CEO

Paul Carter, Ph.D., CSO

