FivePrime™
Protein Medicines for Life

Development and Integration of a Fully Automated High-Throughput Platform for Cell Line Selection and Cell Culture Development

John Cesarek, BioProcess International Conference
October 12th, 2012 - Providence, RI

fiveprime.com

Five Prime Therapeutics

- Fully Integrated Biologics Discovery and Development Company

- Unique and productive Discovery Platform for identifying innovative protein therapeutics
  - » Custom, fully automated platforms for high-throughput protein production, high-content imaging, ELISA, and gene expression-based screening

- Full suite of preclinical capabilities
  - » Pharmacology, Toxicology, Bioanalytical Development, Translational Science
  - » Large vivarium with capacity for ~9000 animals

- Biologics production cell line and process development
  - » All capabilities from DNA to 100L pilot scale
  - » GMP manufacturing is outsourced
  - » Track record of successful tech transfers

- Small but experienced and successful clinical development group
  - » Lead FivePrime program is in Phase 1b

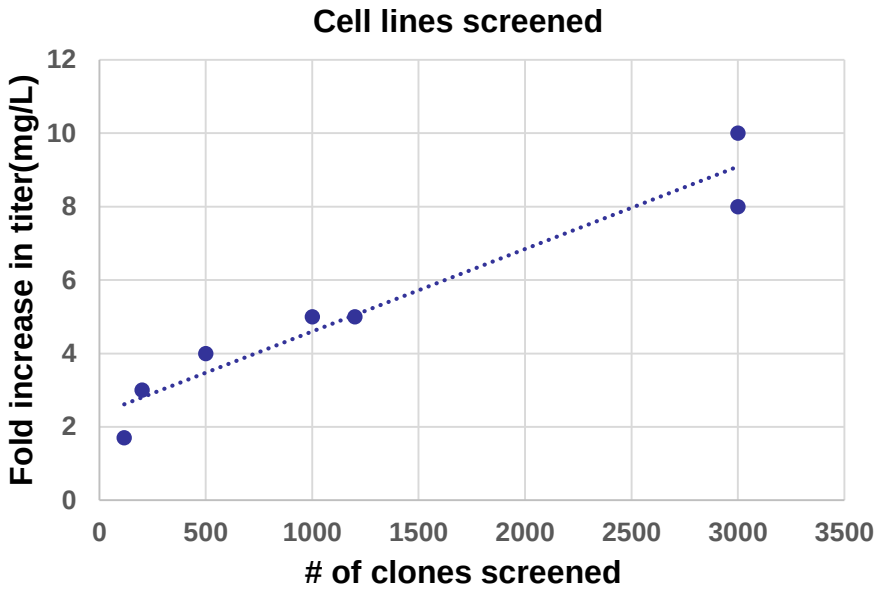
Presentation Outline

- Five Prime Therapeutics' approach to cell line development
- Development and implementation of high-throughput automation in cell line development
  - New automation platform
  - New process workflows
  - Automated data analysis
- Future development

Strategies to Obtain Clonal Cell Lines

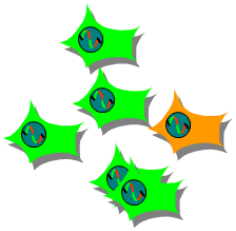
- **Commonly used strategies**
  - ClonePix
  - FACS based single cell deposition
  - Limited dilution

- **Automation aided limited dilution**
  - Numbers game - the more the better, but fold of increase plateaued beyond ~5000
  - Systems like CHO-GS do not require screening of a large number of clones to obtain high producing cell lines

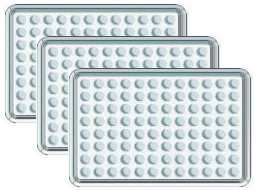


Five Prime's Cell Line Development Workflow

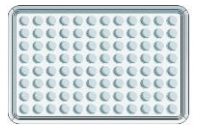
Transfection & Selection



Stable pool

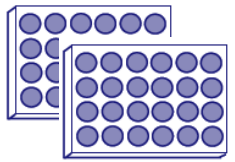


Limited dilution
96-well plates

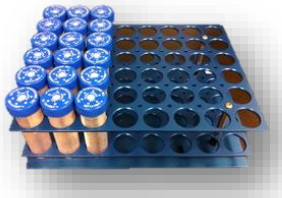


Titer assessment

- 1 molecule
- 3-4 FTEs



Titer assessment
24-well Plates



Suspension adaptation and expansion
Spin Tube

Clone characterization, media and feed optimization

Bioreactor Evaluation

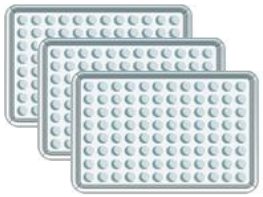
Need for High-Throughput Automation

- Tight project timelines
  - » Process multiple candidate cell lines in parallel
- Limited staffing
  - » Reduce time-intensive and laborious processes
- Quality
  - » Ensure high quality cell lines

Fully Automated Cell Line Development Workflow

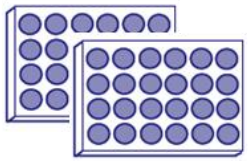
Transfection & Selection

Stable pool



Limited dilution
96-well plates

Titer assessment



Titer assessment
24-well Plates

Suspension adaptation and expansion
Spin Tube

Clone characterization, media and feed optimization

Bioreactor Evaluation

Requirements for Improving Cell Line Development

- **High-throughout process for cell line screening and selection**
  - » Flexible, analytical measurement options (HTRF, ELISA, Well Imaging)
  - » "Cherry Picking" from 96-well to 24-well and 96-DWP
  - » Easy setup, operation, and management to minimize personnel

- **High-throughout process for cell culture development**
  - » Maintain continuous fed-batch productions
  - » Inoculation to spin tubes
  - » Spin tube feeding, sampling, and cell concentration and viability measurement
  - » Easy setup, operation, and management to minimize personnel

- **Link analyzed data of clones throughout process**
  - » Automate data analysis and report generation
  - » Track history of clones in process development

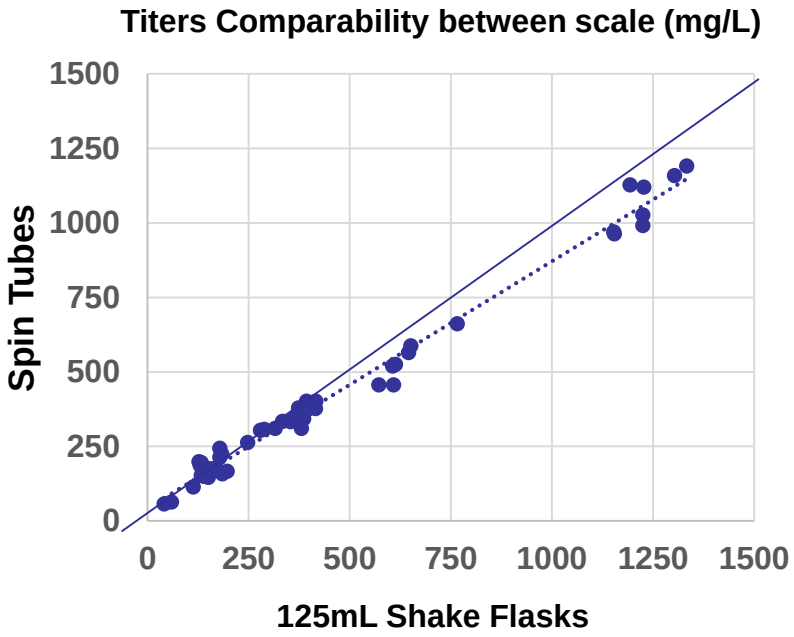
Building vs. Buying a CLD Automation Platform

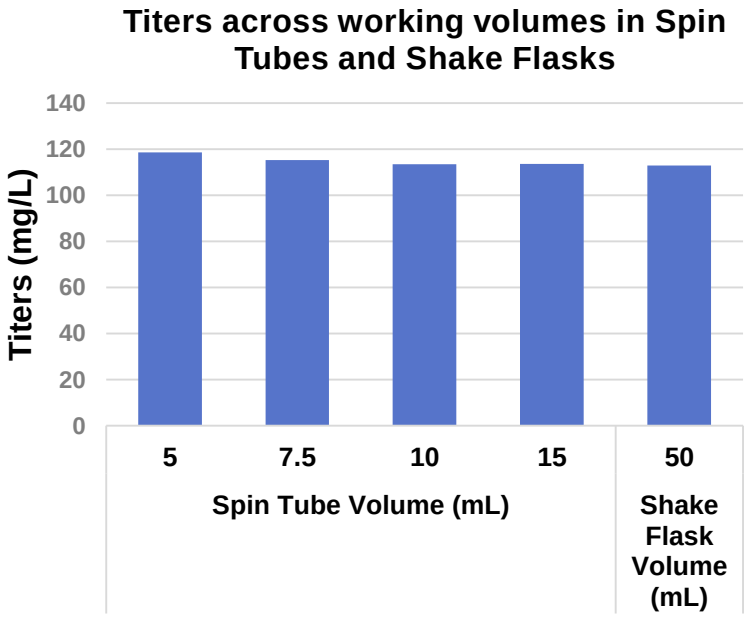
- **Internal Resources – Automation Technologies Group**
  - History of design and implementation of several large, high-throughput platforms (ELISA, protein production, cell-based screening)
  - Engineering solutions
    - Custom hardware
    - Custom automation scheduling and device drivers software tools
    - Data integration
- **Full Control of Process**
  - Easy to adapt to changing processes
  - Less reliant on vendor support (Limits downtime)
- **Limited Company Resources**
  - Small company - Need to make use of what is available
  - Tight timelines
  - Cost

High-Throughput Spin Tube System

- Adaptable to high-throughput process with pierceable septum
- Comparable correlation between spin tubes and shake flask
- Consistent titers across working volumes

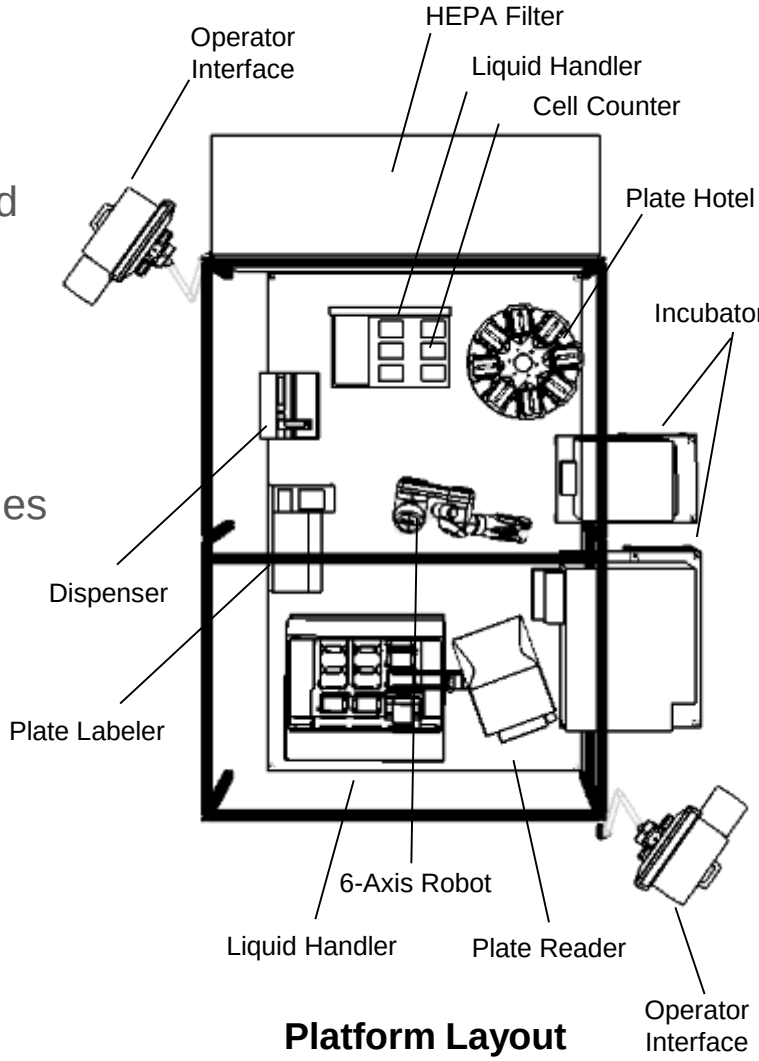
Custom 50-mL Spin Tube
- Vented cap with pre-slit septum
- Integrates with 1mL disposable tips





Cell Line Development Platform

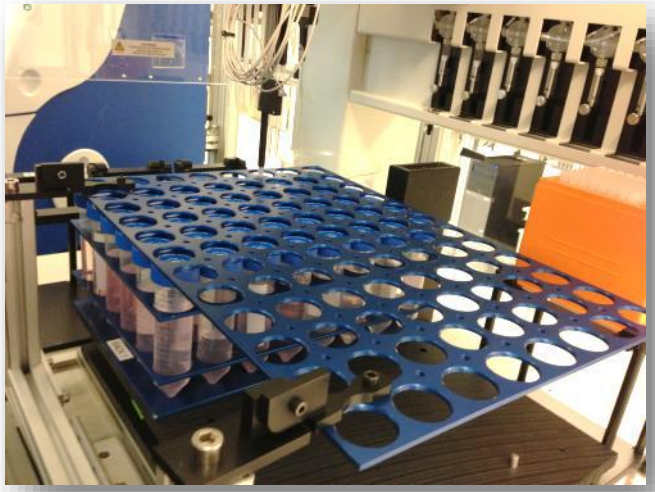
- **Platform Key Features:**
  - Multiple HEPA-filtered enclosure
  - “Smart Automation” for robust processing and error handling
  - 24/7 fully unattended operation
  - High plate capacity (> 220 microtiter plates)
  - Multiple cell plate incubators to isolate cell lines
  - Tube rack and plate inventory tracking
  - Modular design for flexible integration



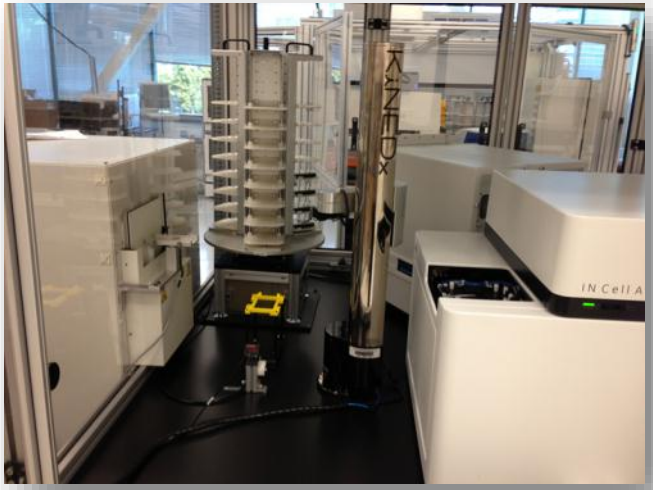
Platform Layout

Cell Line Development Platform

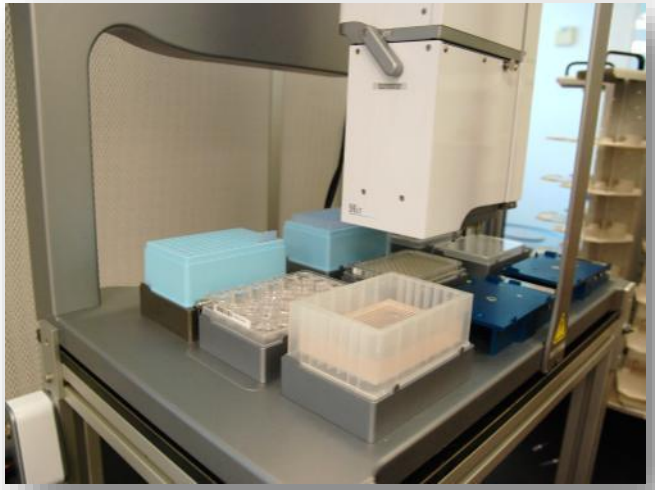
CLD Platform



Custom Spin Tube Rack



Imaging Platform



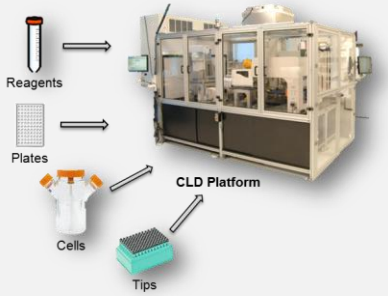
“Cherry Picking” Process

End-User Experience with the CLD Platform

1 Project Setup

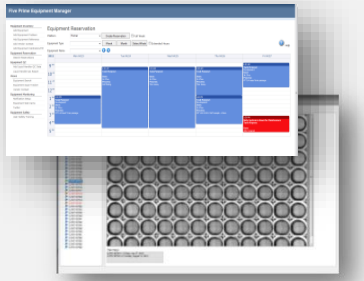
Setup project parameters (Project name, Project Type, Clone ID, # of Plates, etc.)

2 Process Setup



Load cells, consumables, and reagents

3 Monitor



Automated resource scheduling

Monitor cell growth (Image once a week)

4 Decision Making

Prioritize plates to screen based on colony size, growth rate, and changing project parameters

"Smart Automation" - Multiple software tools and integrated informatics to setup automation protocols, track inventory, and aid in error recovery.

Process Workflow – Cell Line Screening and Selection

Cells

Cell Dispensing

Cell Dispenser

Cell Plates

Cell Incubation

Incubator

Plate Imaging

Imaging Platform

Colony Identification

CLD Platform

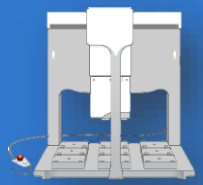
Assay Plate

Titer Measurement

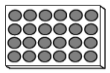
Plate Reader

Cell Plates

Clone Transfer



Liquid Handler



24-well and 96-DWP

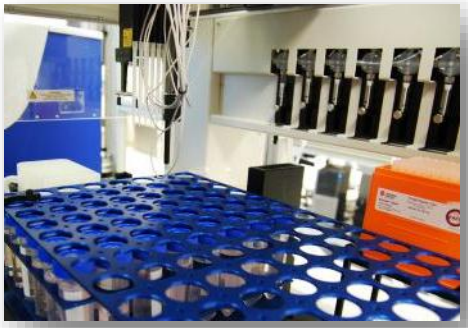
FivePrime

Process Workflow – Cell Culture Development

Shaking Incubators

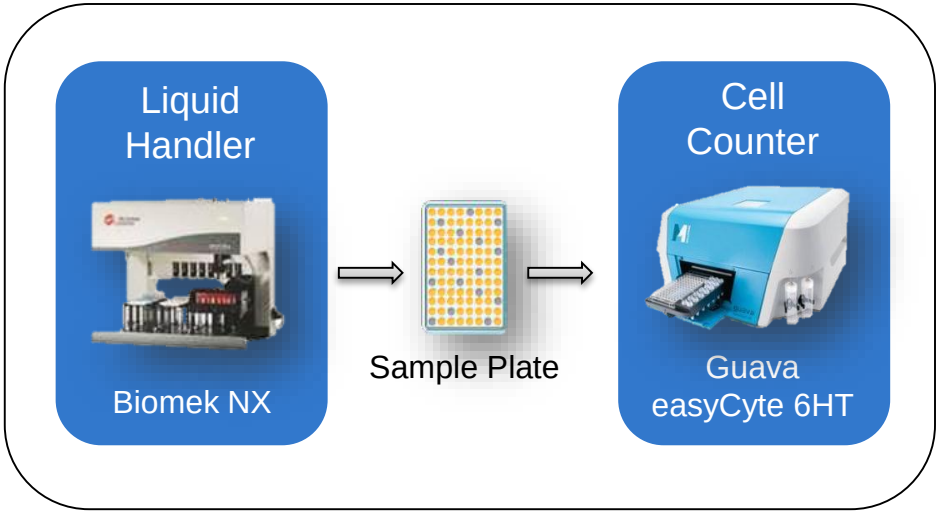
48/96-Well Spin Tube Rack

CLD Platform



Biomek NX with Custom Deck

- Fully automated seed train maintenance and fed-batch production
  - » Tube inoculation
  - » Cell splitting and passaging
  - » Cell sampling (cell count and viability)
  - » Feeding



Automated Data Capture, Storage, Analysis & Retrieval

- Real time data analysis and reporting tool
- Prioritization and ranking of clones
- Plate data and image archiving to Cell Line Development Database (SQL Server)
- Multiple project tracking

INCELL 2000

Cell Growth

Automation Scheduler

CLD Platform

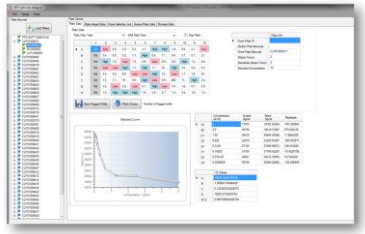
Guava easyCyte 6HT

Cell Count/ Viability

Cell Line Development Database

Envision Plate Reader

Clone Titer

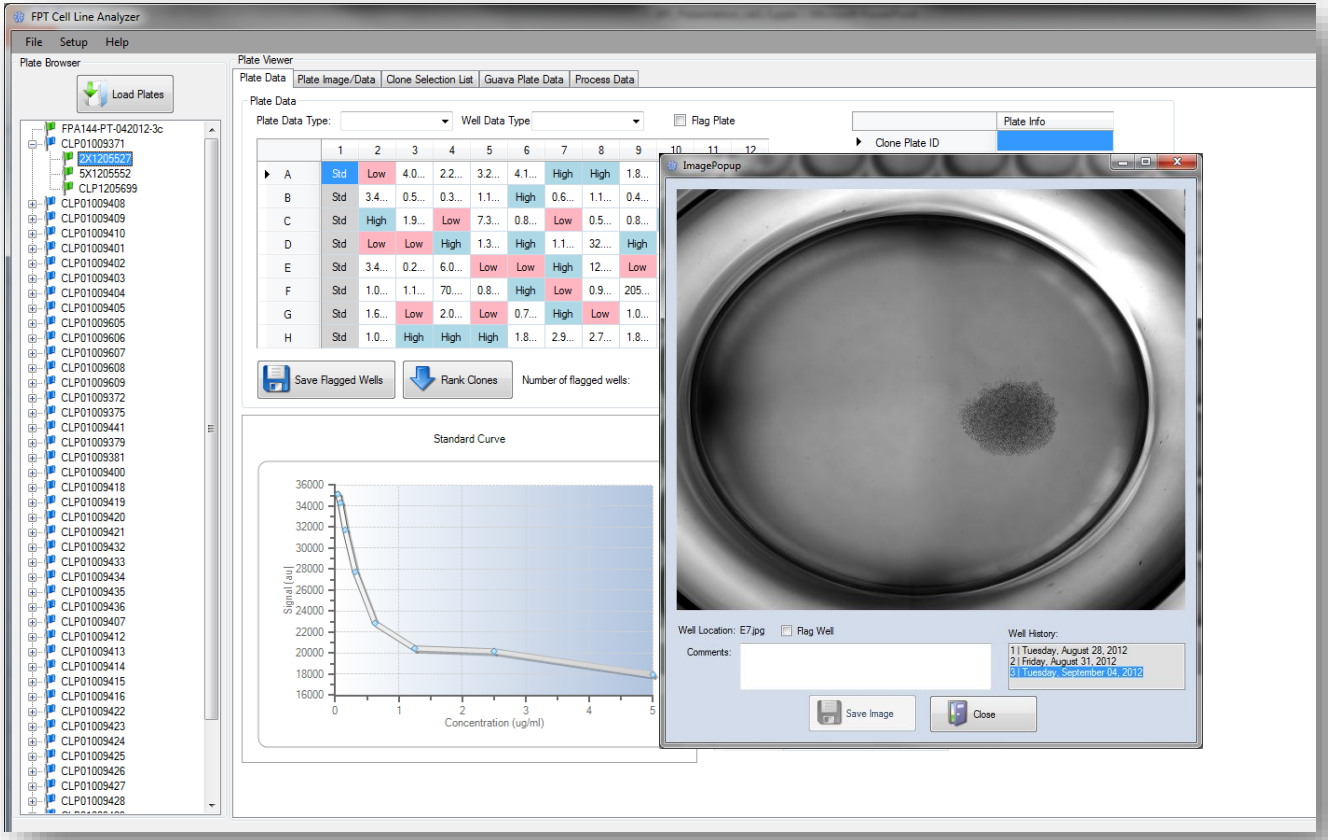


Data Analyzer

FivePrime

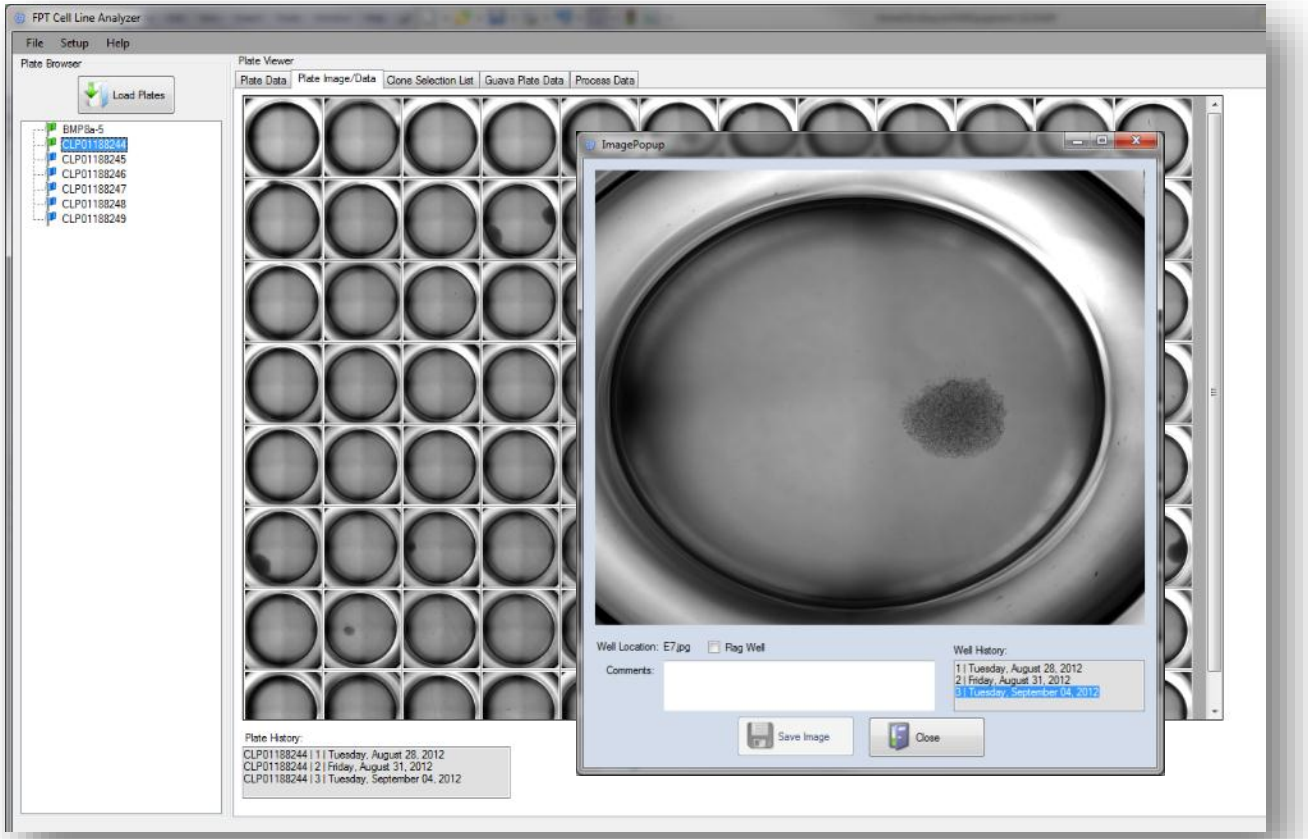
Automated Data Analysis – Clone Titer

- **Clone Titer Analysis**
  - 4-Parameter curve fit
  - Clone prioritization and ranking
  - Well data linked to well images



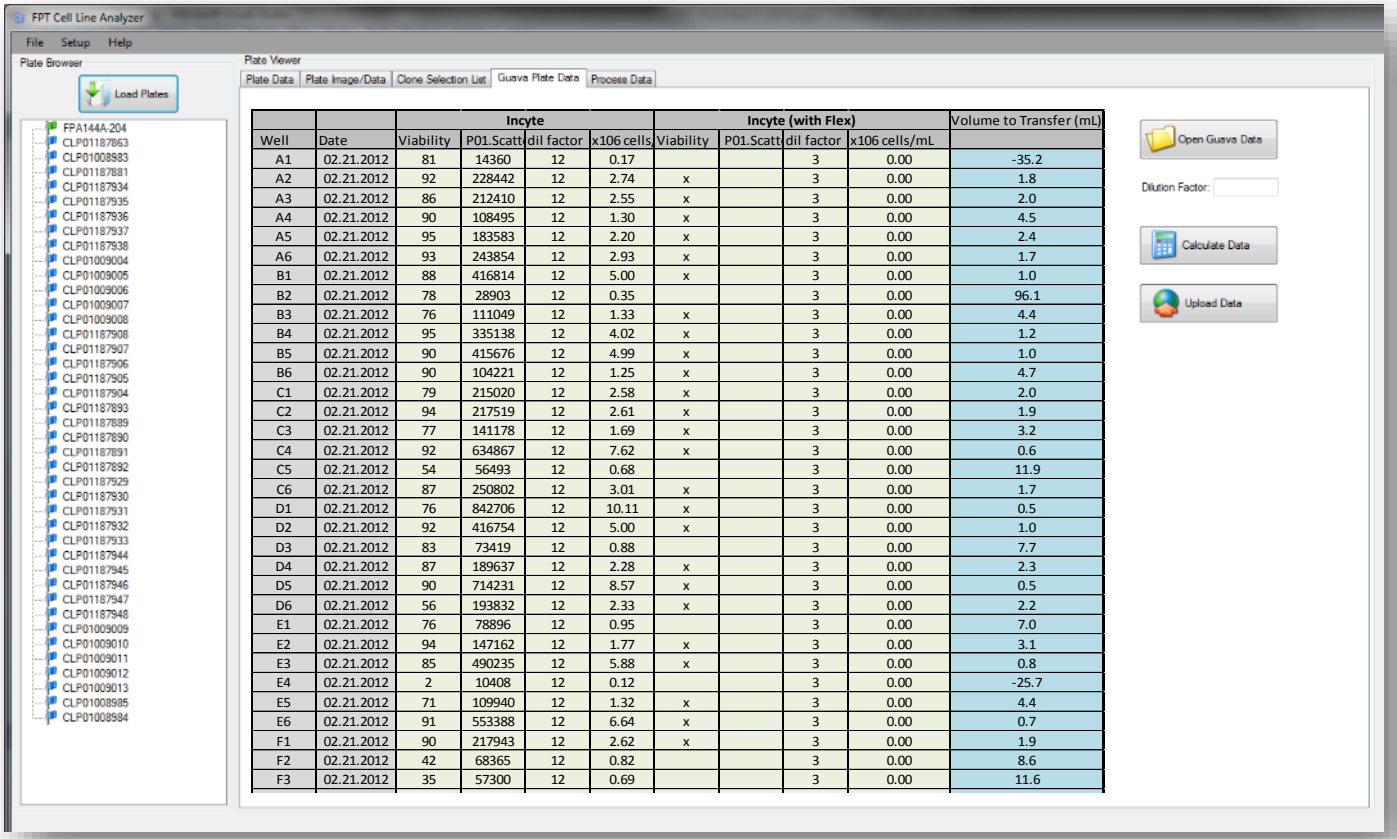
Automated Data Analysis – Clone Images

- **Plate Overview of Well Images:**
  - 96 and 24-well plates
  - Plate/well contamination flagging
  - Cell growth tracking (% Confluence) and monoclonality detection
  - Plate/well image history



Automated Data Analysis – Cell Count and Viability

- **Spin-Tube Data Handling**
  - Automated calculation of cell count and viability
  - Worklist generation for inoculation, feeding, and passaging
  - Spin tube and tube rack tracking



		Incyte				Incyte (with Flex)				Volume to Transfer (mL)
Well	Date	Viability	P01.Scatt	dil factor	x106 cells	Viability	P01.Scatt	dil factor	x106 cells/mL	
A1	02.21.2012	81	14360	12	0.17			3	0.00	-35.2
A2	02.21.2012	92	228442	12	2.74	x		3	0.00	1.8
A3	02.21.2012	86	212410	12	2.55	x		3	0.00	2.0
A4	02.21.2012	90	108495	12	1.30	x		3	0.00	4.5
A5	02.21.2012	95	183583	12	2.20	x		3	0.00	2.4
A6	02.21.2012	93	243854	12	2.93	x		3	0.00	1.7
B1	02.21.2012	88	416814	12	5.00	x		3	0.00	1.0
B2	02.21.2012	78	28903	12	0.35			3	0.00	96.1
B3	02.21.2012	76	111049	12	1.33	x		3	0.00	4.4
B4	02.21.2012	95	335138	12	4.02	x		3	0.00	1.2
B5	02.21.2012	90	415676	12	4.99	x		3	0.00	1.0
B6	02.21.2012	90	104221	12	1.25	x		3	0.00	4.7
C1	02.21.2012	79	215020	12	2.58	x		3	0.00	2.0
C2	02.21.2012	94	217519	12	2.61	x		3	0.00	1.9
C3	02.21.2012	77	141178	12	1.69	x		3	0.00	3.2
C4	02.21.2012	92	634867	12	7.62	x		3	0.00	0.6
C5	02.21.2012	54	56493	12	0.68			3	0.00	11.9
C6	02.21.2012	87	250802	12	3.01	x		3	0.00	1.7
D1	02.21.2012	76	842706	12	10.11	x		3	0.00	0.5
D2	02.21.2012	92	416754	12	5.00	x		3	0.00	1.0
D3	02.21.2012	83	73419	12	0.88			3	0.00	7.7
D4	02.21.2012	87	189637	12	2.28	x		3	0.00	2.3
D5	02.21.2012	90	714231	12	8.57	x		3	0.00	0.5
D6	02.21.2012	56	193832	12	2.33	x		3	0.00	2.2
E1	02.21.2012	76	78896	12	0.95			3	0.00	7.0
E2	02.21.2012	94	147162	12	1.77	x		3	0.00	3.1
E3	02.21.2012	85	490235	12	5.88	x		3	0.00	0.8
E4	02.21.2012	2	10408	12	0.12			3	0.00	-25.7
E5	02.21.2012	71	109940	12	1.32	x		3	0.00	4.4
E6	02.21.2012	91	553388	12	6.64	x		3	0.00	0.7
F1	02.21.2012	90	217943	12	2.62	x		3	0.00	1.9
F2	02.21.2012	42	68365	12	0.82			3	0.00	8.6
F3	02.21.2012	35	57300	12	0.69			3	0.00	11.6

Summary

- Five Prime's Automation Technology group has successfully designed and implemented a high-throughput platform for cell line selection and cell culture development
- Highly customizable platform allows for changing project parameters
- "Smart Automation" allows for a robust, full walk-away system to reduce the number of FTE's
- High quality process delivers low plate contamination and reduced human errors
- Spin tube system enables a large number of cell lines to be screened by fed-batch culture
- Fully automated data analysis and record keeping provides key data for process decision making

Future Development

- Incorporate data analysis and reporting for downstream process development
  - » Link clone data throughout process development
- Develop cell culture development process in 96-DWP
- Integrate cell line development data with electronic lab notebook

Acknowledgements

- **Process Development**
  - Jin Zhou
  - Marion Glenn
  - Aileen Zhou
- **Automation Technologies**
  - Nallakkan Arvindan
  - Justin Jager
- **Optimum Processing Inc.**
  - Peter Florez