Improving Mammalian Cell Line Development Process Through Innovative Automation Approaches

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Biotechnology drugs prescribed by medical specialists are growing twice as fast as traditional prescription drugs.
From a single program in 1996 to over 60 programs today, Pfizer continues to increase its Biologics Portfolio.
Pfizer’s Marketed Biotherapeutics

Genotropin

Rebif® 22 mcg/0.5 mL
(interferon beta-1a)

Fragmin®
(dalteparin sodium injection)

SOMAVERT®
(pegvisomant for injection)

MACUGEN™
PEGAPTANIB SODIUM INJECTION

$1.5 Billion Estimated 2006 Sales
Global Biologics … positioned to receive biologics from all Pfizer Discovery campuses

Adapted from: Melcer, R., STL Post Dispatch, 08 Jan 2004
Why Automation for Cell Line Development?

- The development of cell lines suitable for commercial manufacture has long been a challenge for the biopharmaceutical industry.
- The techniques for introduction of recombinant genes into cells, as well as the method for the selection of candidate cell lines, are random processes.
- Heterogeneity resulting from random integration events requires that many individual cell lines be screened to identify the rare integration events that give rise to stable and high producers.
- Conventional approaches to identify high producers are labor-intensive, costly, and inefficient due to practical limitations.
- Manual data entry, analysis and archiving are error prone. Often data is stored in a variety of different formats and multiple locations including lab notebooks, spreadsheets and lab computers.
Cell Line Development Process

Transfections

96-W plates

6W/24W plates

Cloning

T-Flasks

Shakes

Fed-Shakes

Cell Bank

Bioreactors

Cell Line Stability
Development of an Automation System for Cell Line Screen and Selection

**Wish list:**

- High-throughput ELISA screening of cell lines for IgG quantitation
- High-throughput image screening of antibody-producing cell lines
- High-throughput “cherry-picking” of cells from multiple source plates
- The system needs to be fully-integrated, capable of reconfiguration and is easily re-adapted to new devices
Fully-integrated System for Static Cell Culture

- Walk-away, around-the-clock operations with error handling capability
- Single-run capacity of >100 microtiter plates
- Integrated data processing
- Enclosed class-100 environment controlled by six built-in HEPA filters
- Price < $750K
System Capability: Cell Line Screening and Selection

- Cell plate
- Assay plate
- Medium Trans.
- ELISA assay
- Software interface (Developed in-house)
- "Cherry-Picking"
- Auto-imaging

Software interface (Developed in-house)
Hardware

Key System Components:

- CRS robot table (2.00 m X 2.44 m)
- CRS F3 arm with six degrees of freedom and a 900 mm reach
- CRS Plate Carousel
- CRS Plate De-lidder and barcode reader
- Automated Nikon microscope
- Tecan Spectrafluor Reader®
- TECAN Genesis®
- Liconic StoreX® Incubators (37°C and 4°C)
Two Software interfaces developed in-house

- Software interface to generate taskfile using the parameters defined by the researchers based on ELISA readout

- Interface software generates a Work List of all the samples to be “Cherry-Picked” based on the outcomes of the imaging analysis
ELISA screening to identify high-producing cell lines
- Process up to 4000 cell lines in 3 hours
- Standard deviation of 2.4%

Auto-focused microscope to follow the Taskfile for cell imaging
- Bright-field
- Fluorescent
- 40 minutes/96-well plate

TECAN liquid handler to follow worklist for “Cherry-picking” of candidate cell lines
- Up to 120 cell lines/hour
Manual vs. Automation on cell line performance

- ELISA by Human (µg/mL)
- ELISA by Robot (µg/mL)

Manual vs. automation on ELISA assay

y = 1.0679x
R² = 0.987
How long it took us......

- **Vendor Evaluations: February, 2004**
  - Up to 4 different manufacturers were evaluated
  - Thermo-CRS selected

- **System design completed: October, 2004**
  - System configurations: May, 2004
  - System software: October, 2004

- **Manufacture Site Test completed: December, 2004**

- **System delivered and installed: May, 2005**

- **Site Acceptance Test completed: June, 2005**

- **System performance validation completed: March, 2006**
  - Performance validation of individual components
  - Functional validation of the integrated system
Lessons Learned from Our 1st Customized Integrated System

◆ Software
  • Complex system required experienced operator with extensive automation experience. Extensive training on use of the system was required.
  • Dedicated operators needed for proper operation and error handling.
  • Vendors lack of in-depth knowledge of 3rd party devices caused project delays
  • Lack of local vendor support prevented swift problem solving.
  • Relied heavily on internal IT experts for trouble shooting.

◆ Hardware
  • Complex system required lengthy installation and validation
  • Too many units to integrate in the configuration resulted in longer timelines to align cell culture process.
  • As a result, automation benefit was not fully realized, hence reduced the return on investment.
Conclusions

◆ Automation is the solution to meet the demand for higher throughput, higher process accuracy, higher productivity, and higher flexibility.

◆ Automation does not necessarily work faster than people, it does, perform “around the clock” and “walk-away” operations.
  - Automation does increase overall throughput and accuracy.

◆ There is no existing fully-integrated system that can handle all of the steps in a cell line process
  - Modular systems provide the greatest flexibility

◆ Flexibility, robustness, and easy of use are essential elements for the successful integration of an automation system. Expert application support of assay automation scripting is also important.

◆ Handling the widest range of methods and conditions, labware, and reagents is highly desired.
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