BECKMAN COULTER
Life Sciences

Cell Line Development – Hit Picking

Summary

- Hit picking is a frequent need in many workflows, including cell line development.
- Challenges include:
  - Proper hit/well identification
  - Reformatting to hit plate
  - Combining samples from multiple plates
  - Error-free data tracking
- Biomek's Span-8 system coupled with DART data tracking software enables data-driven transfers for hit picking while ensuring data integrity

Automation is often thought of only as a solution when moving into high-throughput applications or when repeatedly running fixed workflows with no variability. However, there are many workflows that vary from run to run that would benefit from automation due to factors such as complexity, high error rates, and ensuring data integrity. A common and seemingly simple example of this workflow is hit picking.

The basic concept of hit picking is simple – 1) identify hits based on assay data and 2) consolidate these hits into a new labware in an identifiable way. However, there are numerous areas where things can go wrong when performing this task at the bench. First, analyzer data must mapped back to the correct source wells and the wells containing hits must be identified. These wells must then be transferred in a consistent order to ensure no hits are mislocated. This process is even more challenging when consolidating hits from multiple source plates and/or working with small volume or deep well plates where the presence or absence of liquid is difficult to determine visually. Despite all of these potential sources of error, there is little in the way of safeguards when performing this task manually.

Here we will describe how hit picking was automated as part of a cell line development workflow. This lengthy and complex workflow had multiple hit picking steps. After plating cells by limiting dilution, the ~33% of wells that received a single cell were identified by imaging on a CloneSelect Imager (Molecular Devices), (Figure 1A, green wells). Roughly 50% of these monoclonal wells showed growth after three weeks, and these randomly distributed "primary hit" wells (Figure 1B, pink outlines) required sampling for protein titer.

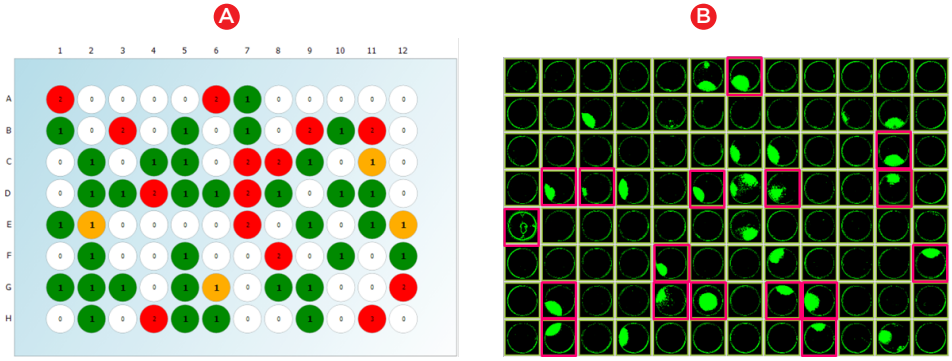


Figure 1. Hit identification. A) Following limiting dilution, wells with a single cell were identified by imaging on a CloneSelect Imager (green/yellow). B) Cells were grown for three weeks and wells with colony growth were identified by imaging. Monoclonal wells with growth ("primary hits") that were assayed for protein titer are indicated by pink squares.

We utilized the Span-8 pod of a Biomek i7 (Figure 2A) to rapidly sample the media from the primary hit wells across four 96-well plates. The transfers were directly driven by the imported confluence data and the spanning capability enabled the simultaneous sampling of multiple spaced wells per column to accelerate the process (Figure 2B). In addition, the fine level of pipetting control allowed slow aspirate speeds and optimal tip location to avoid disrupting the loosely adherent cells. The Span-8 pod dispensed replicate wells for each primary hit well into a single black 384-well plate – a challenging labware to execute a reformatting process with manually. This plate was then analyzed on an Octet HTX (Pall Forte Bio) to determine IgG titers.

A

B

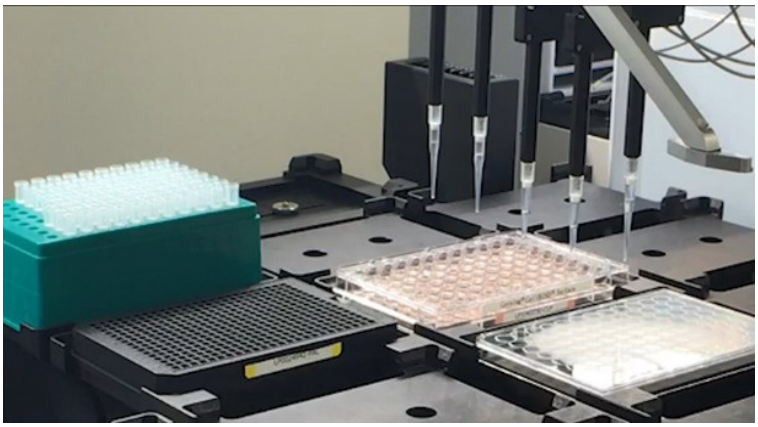
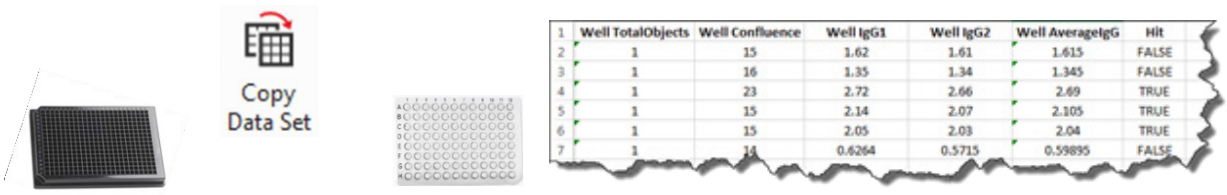


Figure 2. Automated hit picking. A) Biomek i7 with HEPA filters and integrated devices utilized for cell line development. B) Media from the ~15% of wells with colony growth from a single cell were sampled using the Span-8 pipettors and transferred to a black 384-well Tilted Well Plate for analysis on the Octet HTX (Pall Forte Bio).

As challenging as the physical aspects of hit picking are, ensuring data integrity during a reformatting process is just as essential. In this example, data that is generated on the 384-well assay plate must be correctly attributed to the source wells to drive a second hit picking step. During liquid transfers, Biomek can automatically transfer the source well information (e.g. location) to the assay plate. This allows the complementary DART data software to automatically copy the assay data from the 384-well plate back to the source plates and wells (Figure 3A), thereby preventing data loss or copy/paste errors. Not only does DART provide an easily-accessible database for all screen data (Figure 3B), but the data can be used to drive the second hit picking step of transferring high-expressing cells into larger well plates for expansion ("secondary hits"). The source of the clone (such as the bar code and well) or the clone tracking ID can also be moved as the cells are moved so that the final plate will have traceable information back to the cell's origin.



	Well TotalObjects	Well Confluence	Well IgG1	Well IgG2	Well AverageIgG	Hit
2	1	15	1.62	1.61	1.615	FALSE
3	1	16	1.35	1.34	1.345	FALSE
4	1	23	2.72	2.66	2.69	TRUE
5	1	15	2.14	2.07	2.105	TRUE
6	1	15	2.05	2.03	2.04	TRUE
7	1	14	0.6264	0.5715	0.59895	FALSE

Figure 3. Data integrity during reformatting. A) DART software ensures data integrity by automating the transfer of data from the reformatted assay plate back to the original source wells. B) The centralized data can be accessed through reports (shown), web browsers, and used by the automation for additional data-driven hit picking steps.

While just a small part of a larger cell line development workflow, the hit picking steps would likely be the most error prone when manipulating both samples and data. We have shown how automating both the physical transfers and data handling aspects of these reformatting steps enable a rapid and error-free hit picking process.

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