SOUTHERN RESEARCH

Legendary Discoveries. Leading Innovation.

Acoustic Droplet Ejection – New Liquid Handling Technology, New Challenges in Automation and Implementation into the Work Flow

Lynn Rasmussen, Clinton Maddox, Sara McKellip, E. Lucile White

Abstract No. MP86

Abstract

The introduction of Acoustic Droplet Ejection (ADE) liquid transfer technology has created a paradigm shift in compound handling for High Throughput Screening. Benefits of this technology include accurate, low volume transfers that eliminate the need for intermediate dilutions, conservation of compound and production of higher quality data. In addition non-contact transfers eliminate the use of pipette tips, saving hundreds of thousands of dollars in consumables. The benefits of using ADE technology are obvious and compelling. What is not obvious at first is how the introduction of this technology into the compound handling work flow can impact other steps in the process. We describe the evolution of the work flow in the Southern Research Institute High Throughput Screening Center when a Labcyte Echo 550 became part of the drug preparation process. Mixing and compound precipitation, evaporation control for nanoliter volumes transferred to assay plates, water absorption by source plates and automation of the process were all challenges addressed as the Echo ADE technology was integrated into work flow at Southern Research Institute.

Compound Libraries

Compound libraries used for High Throughput Screening (HTS) are designed to represent chemical space defined by certain criteria such as the Lipinski "Rule of Five". What this means for the HTS lab is that every compound in the library is different and has the potential to behave in a unique way. In reality the majority of the compounds within a library are well behaved in that they are readily soluble in DMSO at concentrations of 10-100mM and that they stay in solution in spite of freeze thaw cycles and the absorption of water by the DMSO solvent. That still leaves a small group of compounds within the library that behave badly, primarily by precipitating during routine handling of the compound plates during the screening process. When compounds are used for screens, the stock solution is assumed to be homogenous. This will not be the case for compounds that have precipitated, even if they go back into solution before use.

Homogenous Solutions

To insure that stock solutions of compounds used for setting up HTS screening campaigns are homogeneous they are routinely "mixed" prior to use. For contact type dispensers using pipette tips, this is normally accomplished by aspirating and dispensing within the well a number of times, prior to transfer of the compound to the assay plate. For Acoustic Drop Ejection liquid handling instruments, there is no way to "mix" prior to transfer so other options were evaluated. The Covaris L8 (Figure 1), which is also based on acoustic technology, was selected as the platform for mixing/re-solubilizing compound source plates prior to transfer by the Echo 550. The L8 uses a line transducer which is compatible with SBS footprint microtiter plates of any well density. This allows even 1536-well compound plates to be mixed, which is very difficult with any other technology. One limitation of the L8 is that the high-energy acoustic mixing, while very effective, causes splatter within the well. Plates need to be well sealed prior to mixing on the L8 and need to be centrifuged afterward to ensure that all of the compound solution is in the bottom of the well. The process of feeding sealed plates to the L8 has been automated, but centrifugation and seal removal are done by hand. Based on our screening throughput this is not the rate-limiting step in the process.

Homogenous Solutions, continued

Figure 1 Non-contact mixing is accomplished with the Covaris L8 Acoustic mixer. It can accommodate any microtiter plate as long as it is SBS standard footprint.

Automation

What is frequently rate-limiting in the work flow is the transfer of compounds from source plates to assay plates. To address this bottleneck, plate handling for the Echo 550 has been automated. A Peak Robotics KiNEDx articulated arm (Fig 2-A) controlled by PAA Overlord software is the basis of the automation. To provide sufficient plate capacity a 10-position stacker carousel (Fig. 2–B) is used on the system. A lid station is also included to handle delidding and relidding of both source and assay plates (Figure 2-C).

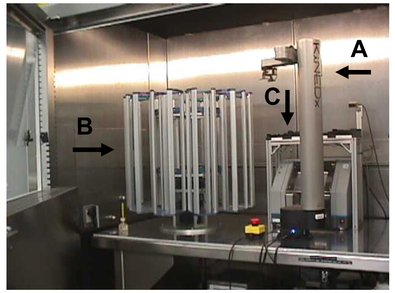

Figure 2 System components include the Peak Robotics KiNEDx arm (A), a 10 position stacker carousel (B) and the delidding station (C).

Evaporation and Water Absorption

Due to the extremely low volume of compound transferred to the assay plates (five to twenty five nanoliters) it was obvious that evaporation was going to be a problem for overnight runs. The other obvious problem was the absorption of water by the compound source plates if they were left unsealed for extended times, especially with the high relative humidity common in Birmingham, Alabama. To address both of these problems, MicroClime lids from Labcyte were used. These lids are a sandwich construction with an absorbent core (Figure 3). The core is saturated with DMSO and when used on assay plates, prevents evaporation of 5nl drops for at least 24 hours. When used on compound source plates, the DMSO-containing lids absorb water from the atmosphere before it can get to the compound solution. This minimizes the amount of water absorbed by the compounds themselves, while they are on the system for extended runs. The only difficulty encountered with the use of MicroClime lids was on the compound source plates. These plates were sealed using adhesive foil seals from Beckman Coulter. We have found these to be the most effective seals for sealing plates containing compounds in 100% DMSO. When the seals are removed, following mixing on the L8 and centrifugation, a very small amount of adhesive can remain on the source plate. At times, there is enough adhesive to cause the MicroClime lid to stick to the plate. At this point the weight of the plate, especially for 1536-well ones, can be insufficient to hold the plate down, resulting in improper lid removal. To resolve this problem, the lid station was modified to include a vacuum base for holding the plate securely while the lid was removed.(Figure 4).

Figure 3
Labcyte MicroClime lids prevent evaporation of DMSO from the destination plates and prevent hydration of the compound stock plates during extended automated runs.

Figure 4
The updated delidding station outfitted with a solenoid activated vacuum clamp to hold the plate during delidding.

Conclusions

The ADE technology employed by the Echo 550 brought substantial improvements to our compound handling process. Compound waste was eliminated; accuracy, precision and reproducibility improved. Additionally, the elimination of pipette tips reduced cost. But because this was a radical change in liquid handling technology and not simply a refinement of an existing technology, much of the rest of the process had to be modified as well. This took time, money, ingenuity and persistence. It is important to understand up front that bringing new technology into an established work flow will not be a simple substitution for existing technologies and peripheral issues will arise during the process. It is also important to establish a collaborative relationship with the Automation and Integration groups, whether internal or out-sourced, to address issues as they arise. In the end we worked with Let's Go Robotics, Labcyte and Covaris to put together an integrated system that would meet our needs. The system as built (Figure 5) is housed in a Class II Biosafety cabinet.

Figure 5 The system as built and installed in the Southern Research Institute HTS Center. The system is housed in a Baker BioProtect II Biosafety Cabinet to maintain sterility or provide biological containment as required by the screen.

Acknowledgements

This project was funded by Southern Research Institute. Thanks to Let's Go Robotics, Covaris. and Labcyte for working together on the integration. Special thanks to Nicholas Pratte and Chinapong Songchan from LGR for overcoming many obstacles during the installation of the system.

2000 Ninth Avenue South ● Birmingham, AL 35205 ● www.SouthernResearch.org ● BusDev@SouthernResearch.org ● (888) 322-1166 ● 001 (205) 581-2830