Automation of a Secondary Drug Screen for ALS Using iPSC-Derived Human Neurons

Paul Guyett[1], Melissa Martowicz[2], Laura Simdon[2], Michael Hendrickson[1] | 1. BrainXell, Inc., Madison, WI, USA; 2. Gilson, Inc., Middleton, WI, USA

Overview

- Automation of serial dilutions and dosing on PIPETMAX provides a more accurate and less labor-intensive alternative to manual pipetting for rescreening
- PIPETMAX control software is flexible and provides user-defined options at run time
- The automated rescreening workflow validated that BX-1000 and BX-1200 restore NFL expression

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by the death of motor neurons. A hallmark of ALS is low expression of neurofilament light chain (NFL) in motor neurons. A high-throughput phenotypic assay using patient-derived induced pluripotent stem cells (iPSCs) was used to screen over 6,000 compounds. Eighty compounds restored expression of NFL in reporter cell lines. These compounds were rescreened and validated by measuring reactivation of NFL expression in non-reporter lines by ELISAs. Given the error prone and time-consuming nature of secondary screening in 384-well plates, the process was automated on the Gilson PIPETMAX. The method prepared 8-point, 10-fold serial dilutions in triplicate across a 384-well plate followed by dosing of neurons. The automated screen was validated with a side-by-side comparison to manual pipetting. Two compounds, BX-1000 and BX-1200, were validated by the rescreening assay. Automation on PIPETMAX reduced hands-on time, eliminated human error, and had higher precision compared to manual pipetting.

Materials and Methods

Neuron Differentiation from Human iPSCs

Motor Neuron differentiation from human iPSCs was based on protocols described previously (Du et al. 2015. Nat Commun. 6:6626). Briefly, human iPSCs were treated with small molecules for 1 week to induce neuroepithelial progenitors (NEPs). The NEPs were split and treated with additional patterning molecules for another week to generate subtype-specific neuronal progenitors. These progenitors were expanded with a combination of small molecules and frozen in cell freezing medium. To accelerate maturation after thawing and seeding, neurons were cultured in medium supplemented with BrainFast Maturation Supplement for another 1-2 weeks.

Reporter Cell Lines

Reporter cell lines were created as described in “ALS Drug Discovery via High-Throughput Phenotypic Screening Using iPSC-Derived Human Motor Neurons” (available here: www.brainxell.com/motor-neurons) Briefly, CRISPER technology was used to integrate an Nluc reporter into the neurofilament-light chain exon 4.

Plate Coating and Washing

Poly-D-lysine (PDL) was manually added to 384-well plates. PIPETMAX aspirated the PDL and washed the plates three times with sterile distilled water.

Rescreen and Validation of Hit Compounds

Eighty hit compounds from the HTS were rescreened in 384-well plates to determine efficacy and EC50. The process was automated on a Gilson PIPETMAX. The program prompts the user to select which column of a 384-well plate will be diluted during the run. PIPETMAX then prepares triplicate 8-point, 10-fold serial dilutions across a 384-well plate. Upon completion of the dilutions, a prompt is sent to place the neurons on the bed of the PIPETMAX. The neurons were dosed with a user-defined volume of the serial dilutions. The PIPETMAX protocol was validated with a side-by-side comparison to the standard manual procedure.

Expression of the reporter gene was measured 4 days post-dosing. Nluc activity was detected using the Nano-Glo Luciferase Assay kit (Promega), and luminescence was measured on a plate reader (Tecan)

Compound Screening with iPSC-derived Human Motor Neurons Culture Workflow Using Gilson PIPETMAX

Figure 1. Gilson PIPETMAX and Consumables PIPETMAX is an automated pipetting platform ideal for biological workflows in 96- and 384-well formats.

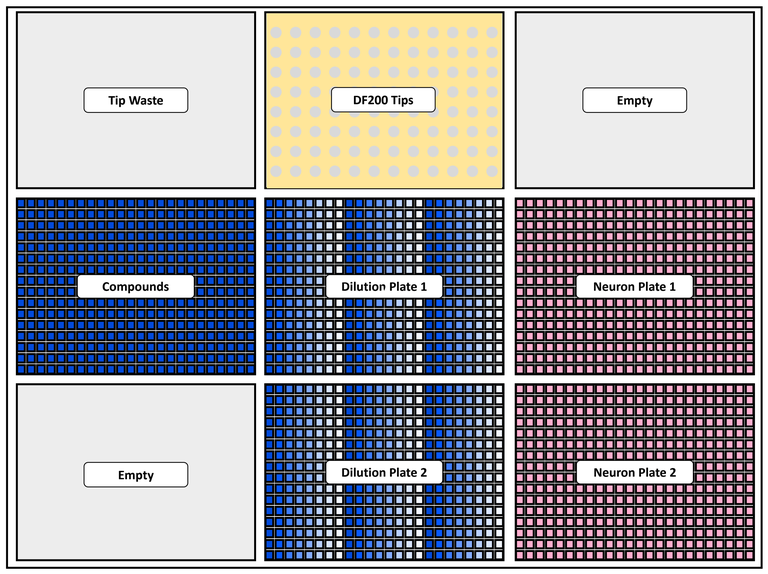


Figure 2. PIPETMAX Bed Layout for Serial Dilutions and Dosing

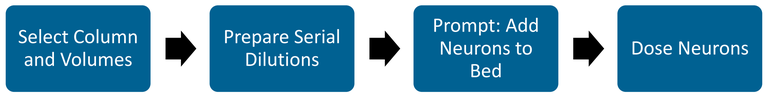


Figure 3. PIPETMAX Workflow for Serial Dilutions and Dosing Each run prepares an eight-point, 10-fold serial in the dilution plate of one user-defined column of the “Compounds” plate. Upon completion of the serial dilution the user is prompted to place the neurons on the bed. An option to run a second set of dilutions is available.

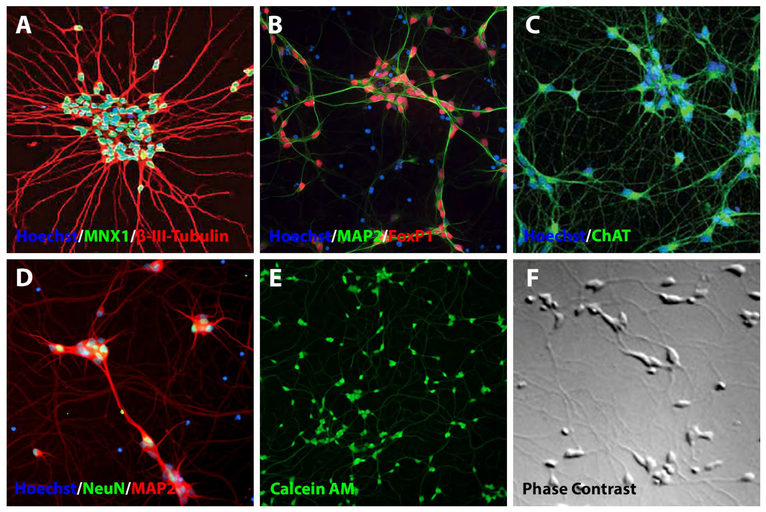
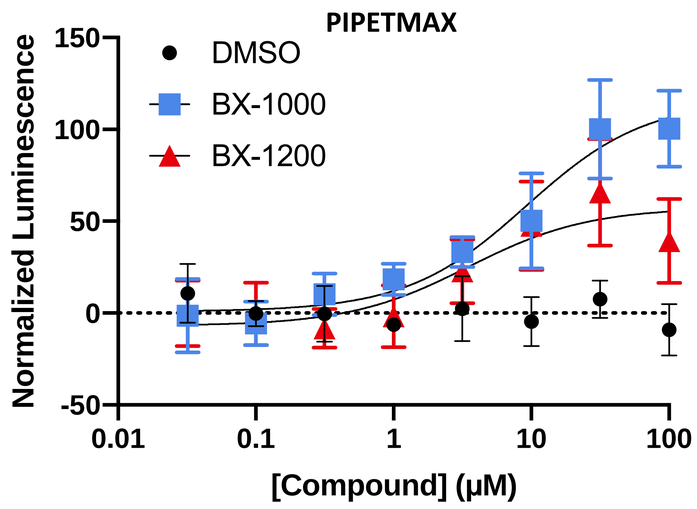


Figure 4. Expression of Motor Neuron Markers
(A-C) Neurons express markers associated with spinal motor neuron identity, including MNX1, FoxP1, and ChAT. *(D)* Approximately 60%–65% of motor neurons are positive for the mature neuronal marker NeuN. *(E-F)* Extensive neurite outgrowth, which begins within a day of plating, is shown by calcein AM staining and phase microscopy.

Variable	Value
Column of Stock in Source Plate to Dilute	1-24
Volume of stock compound (initial dilution)	20
Serial Dilution Volume	20
Mix cycles during serial dilution	1
Volume of dilutions to add to neurons	5
Mix volume during serial dilution	10
Mix cycles during dosing	1
Mix volume during dosing	10

Figure 5. User-defined Variables add Flexibility to PIPETMAX Protocols Source wells, volumes, mix cycles, mix volumes, and dosing volume are user-defined variables.



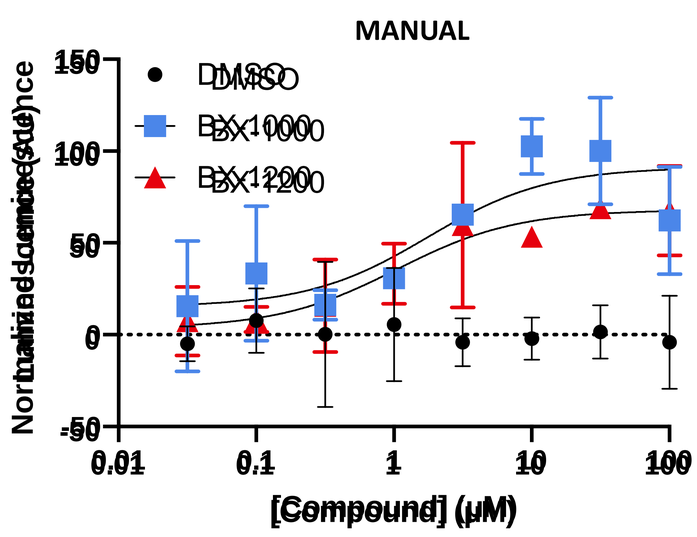


Figure 6. PIPETMAX vs. Manual Pipetting Serial dilutions and dosing were performed by PIPETMAX (upper panel) or by manual pipetting (lower panel). The error bars are one standard deviation of biological triplicates.

Conclusions

- Automation of serial dilutions in 384-well plates flexible and accurate
- Eighty compounds were rescreened and 2 were validated as hits
- Smaller sample volumes = money saved
- Reduced waste and increased accuracy

Contact

Gilson’s PIPETMAX: mmartowicz@gilson.com
BrainXell’s neurons and supplements: mhendrickson@brainxell.com