

Accelerating Drug Discovery at Totus Medicines: DNA-Encoded Library Screening with firefly®

Luke D Monroe¹, Richard E Trager², Joselyn S Del Cid¹, Ryan S. Renslow², Melissa L. Netwal³

¹Platform Screening - Totus Medicines, Emeryville, California, US, ²Data and Computational Chemistry - Totus Medicines, Emeryville, California, US, ³SPT Labtech, Melbourne, Cambridgeshire, UK

Overview

- DNA-encoded libraries (DELs) have emerged as a transformative technology within drug discovery. The exploration of large libraries of small molecule ligands as a collective rather than an individual has springboarded DELs to the forefront of high throughput screening.
- Building upon traditional DEL methods, Totus Medicines has developed a platform capable of identifying covalent small molecule ligands via high-throughput cell-based DEL screening. This poster outlines how Totus Medicines is streamlining hit identification, optimization, and technology development, to accelerate the discovery of novel therapeutic candidates using SPT Labtech's firefly automated liquid handling platform.
- We present the metrics that have been collected over one year of screening using Totus Medicine's proprietary covalent DELs to run over 2000 unique selections against a large number of targets, spanning diverse therapeutic areas.

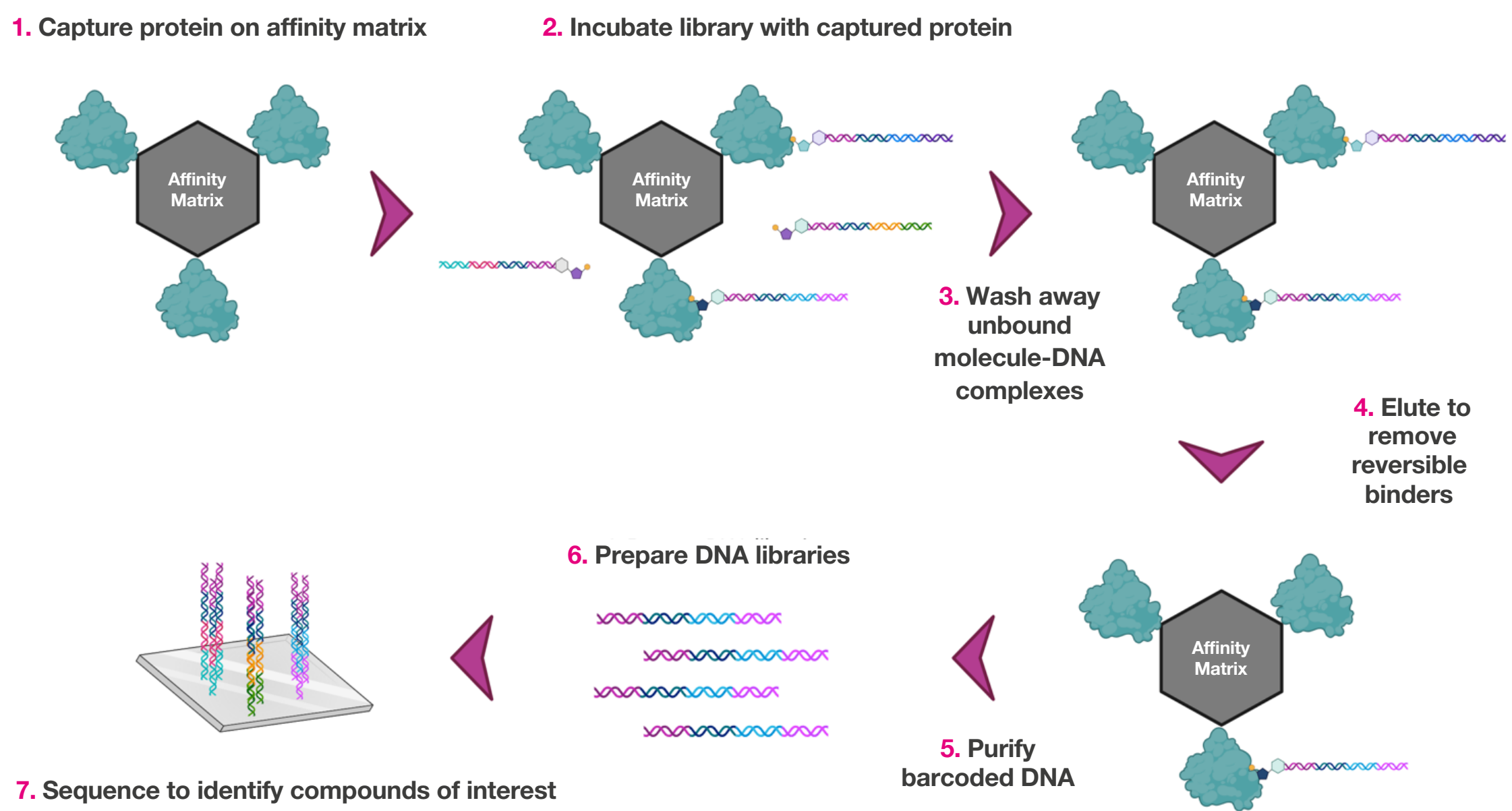
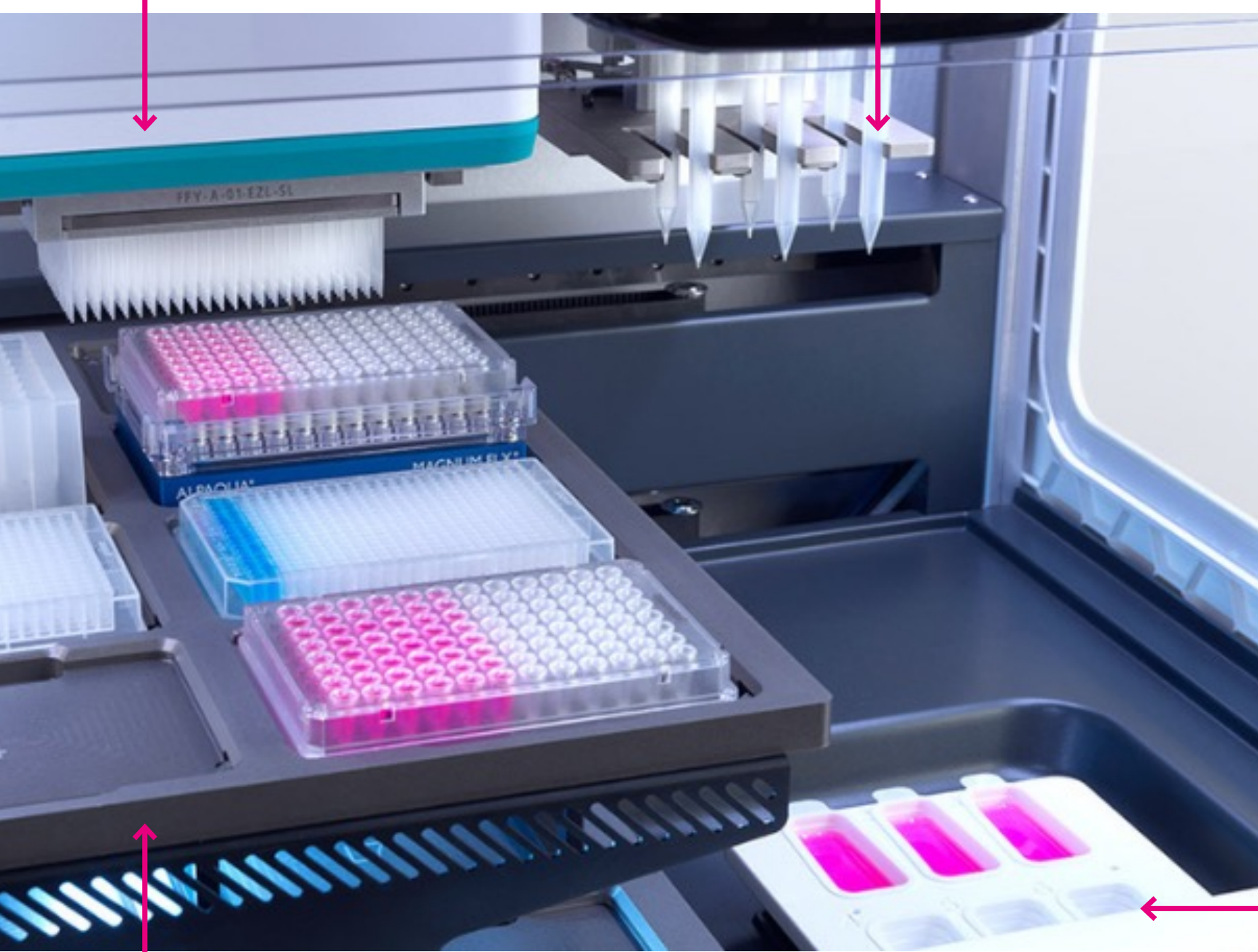


Figure 1. General screening workflow for covalent DNA-encoded libraries. (1) Recombinant protein of interest is bound onto an affinity matrix then (2) incubated with a library ranging from thousands to billions of small molecules bound to barcoded DNA. The unbound (3) library members are then removed through several washing steps following an elution step (4) to remove reversibly bound molecules. The barcoded DNA is then amplified and prepared for sequencing (5-7).

firefly and firefly+ enable streamlined DEL screening

Dual Core Pipetting technology
enables seamless transition between 96- and 384-well screening workflows

Non-contact positive displacement dispensing
3 or 6 independent syringes
Liquid class agnostic
4 mL – 200 nL



Reservoir thermal module (-10°C – 99°C) and reservoirs with limited dead volume

- Standard: 10 mL capacity, 240 µL dv
- LDV: 1.5 mL capacity, 75 µL dv
- New High-Volume: 40 mL capacity, 400 µL dv

16 deck positions across two moving upper and lower decks

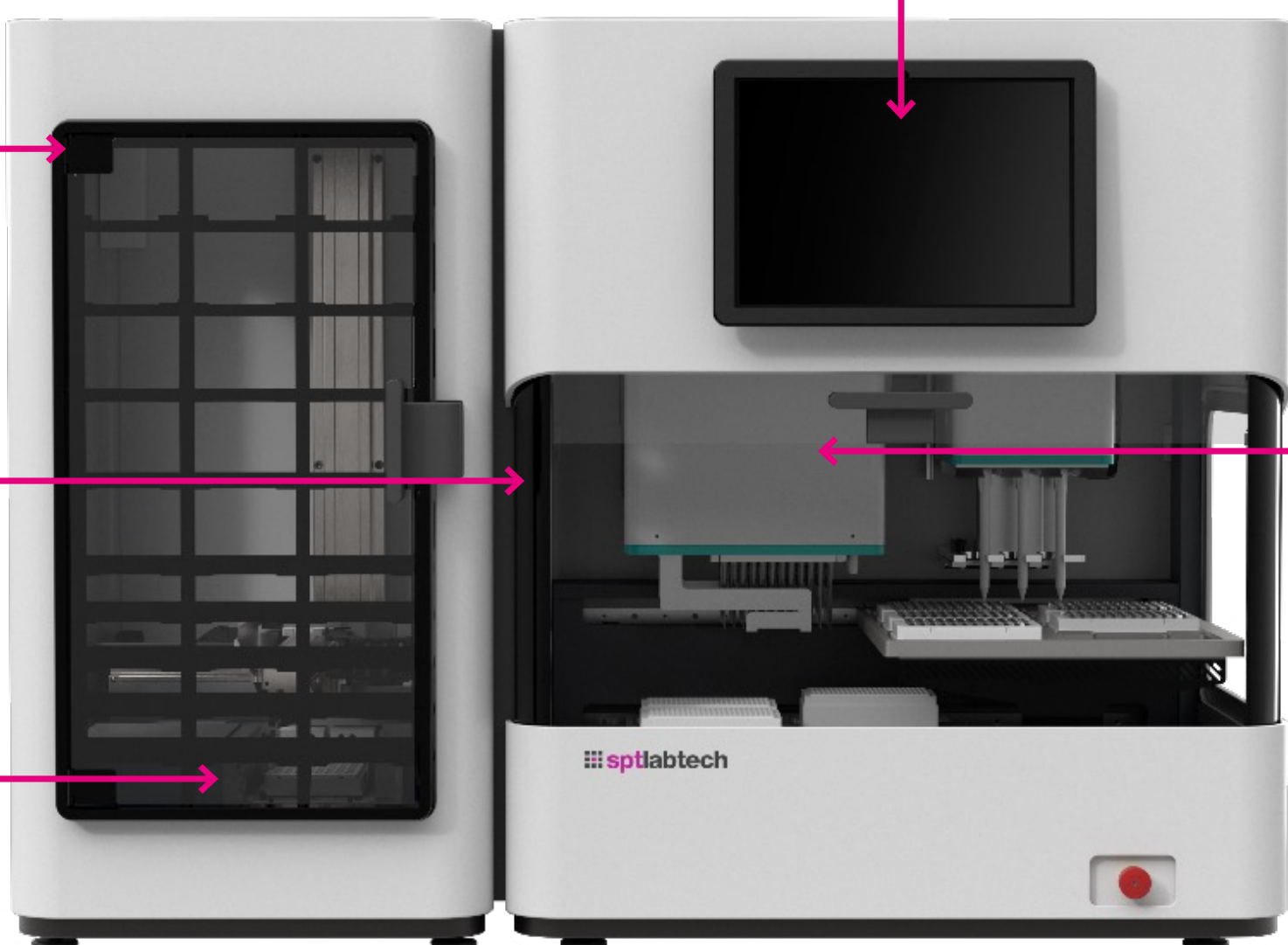
Additional functionality (not shown)
Integrated robust gripper for placing labware on and off the process modules
Integrated bioshake and plate thermal module, -10°C – 99°C

Touch Screen Display for intuitive UI powered by first-in-class software enabling workflow design without compromising flexibility

Ambient hotel for additional plates and tips

Integrated robotic arm for seamless transfers

On-deck thermalcycler for end-to-end selection workflows



New air displacement pipetting head increases pipetting flexibility

- High volume tips
- Cherry picking

Compact footprint
(W x D x H)
1080mm x 560mm x 780mm

Manual screening vs screening on firefly

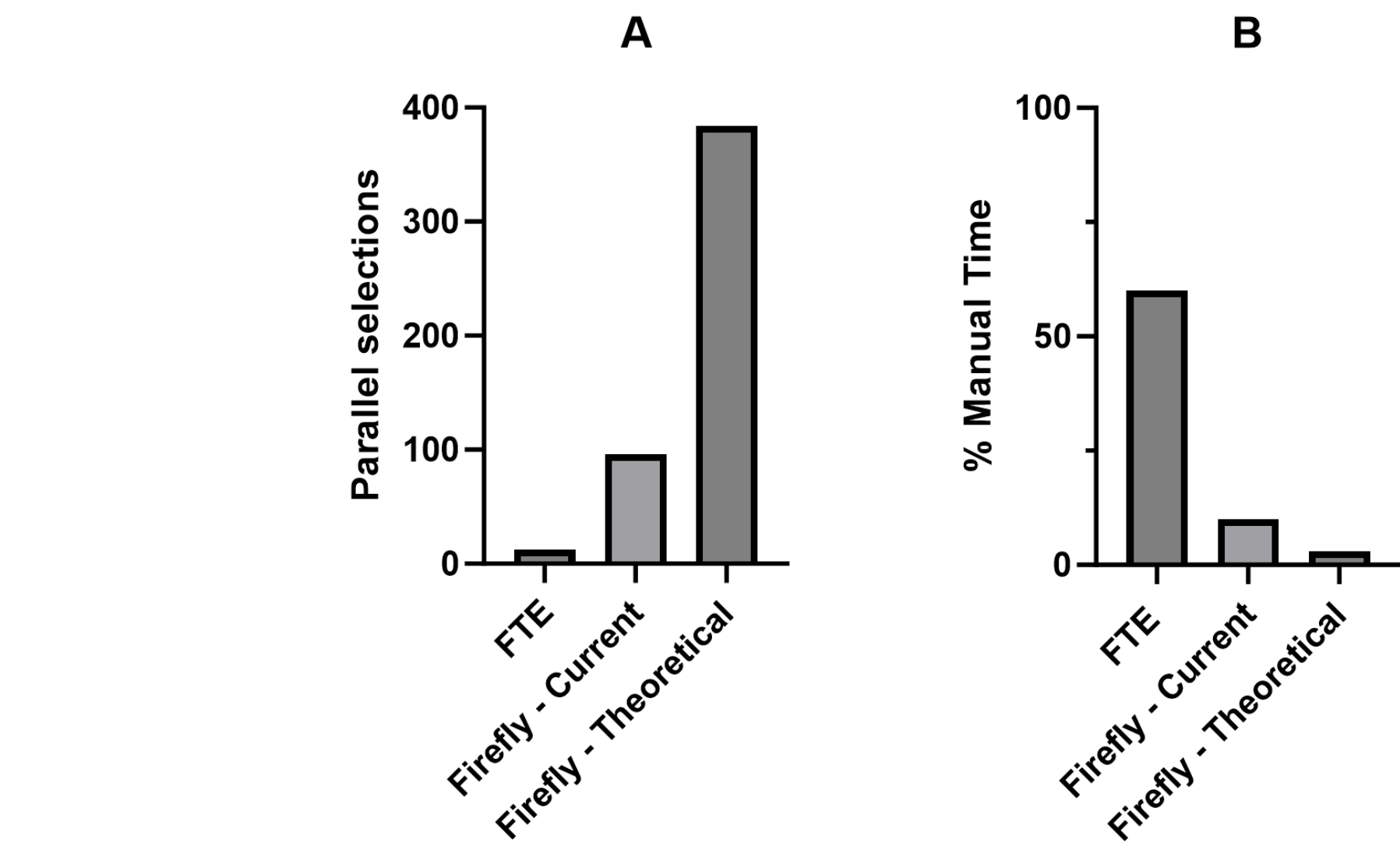


Figure 2. firefly vastly increases sample throughput for screening workflows (A) Totus' current firefly SOPs bring selections up to 96 samples per run, with future development increasing throughput to 384-well plates. (B) Manual intervention required by individual personnel is dramatically reduced by automation of DEL screening with firefly. User inventions on Totus' current firefly SOPs account for less than 10% of the total experimental time. Expansion to firefly+ is expected to reduce user interface time even further.

Reproducibility on firefly

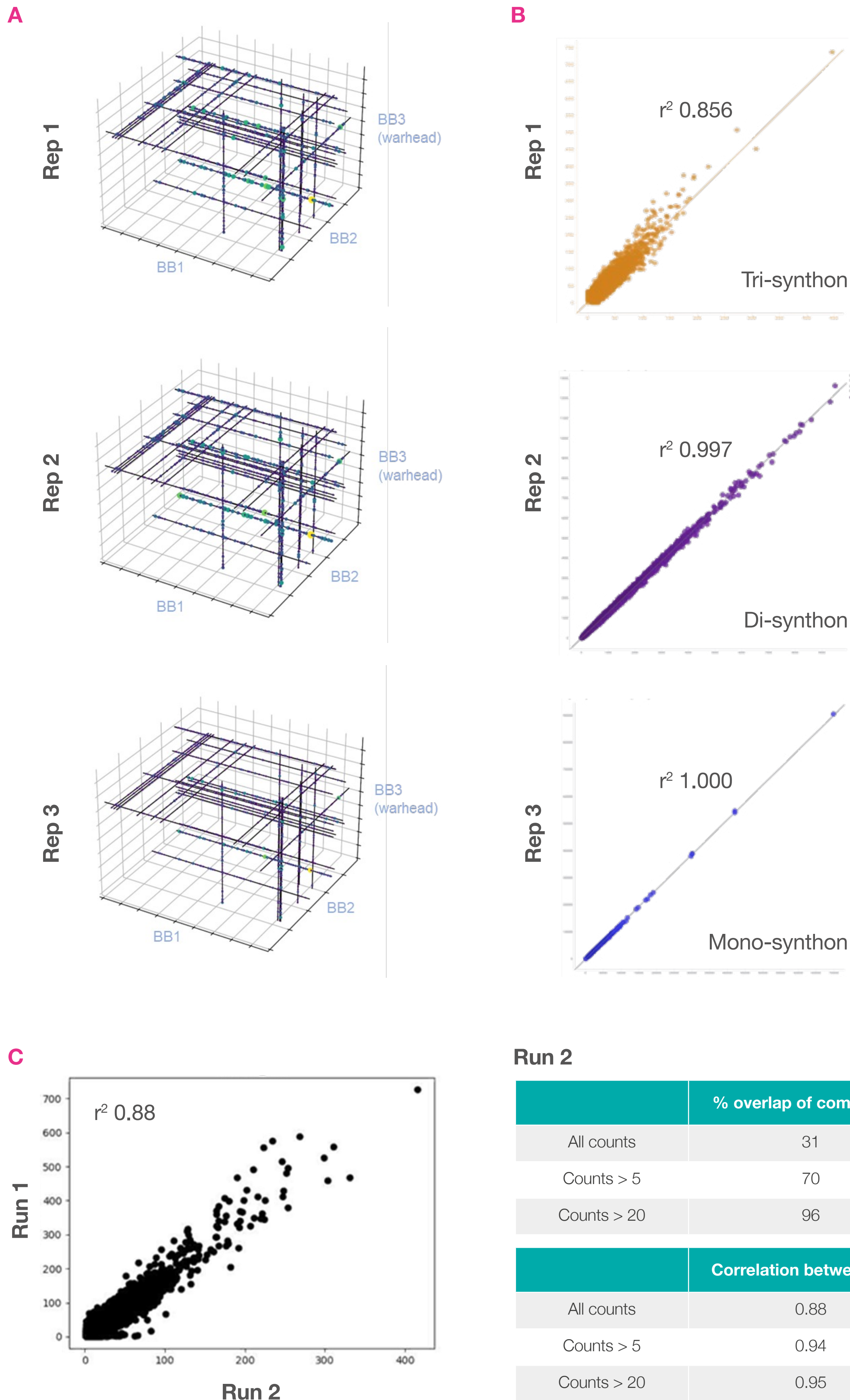


Figure 3. Totus' proprietary DEL screening platform results in identification of multiple hit candidates with high reproducibility across sample replicates. Reproducibility between samples in the same firefly run (intra-run comparison) show high correlation, including similar synthon patterns (A) and conserved structure-activity relationships (SARs) as shown by increasing correlation from tri-, di- and mono-synthon level analyses (B). Reproducibility is also correlated between runs (inter-run comparison) across time (C).

Conclusions

- The transition from manual to automated DEL screening workflows increases throughput, reduces FTE strain and enables identification of reproducible hit candidates.
- firefly has enabled Totus Medicines to complete over 2,000 unique selections to date, using a variety of workflow protocols. The versatility of firefly has enabled their screening platform to continue evolving, enabling drug discovery on traditionally "undruggable" targets.
- firefly+ will further increase Totus' screening capacity.
- firefly's dispense head significantly reduces reagent dead volumes compared to other liquid handlers where SBS reservoirs are required for reagent addition.

