Utilising a fully integrated automation platform to enable high-throughput arrayed CRISPR editing for novel target identification

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- The current strategy for target identification and validation uses an approach of **manual**, **semi-automated** CRISPR editing workflows.
- This approach is **personnel**, **time**, and **resource intensive** and lacks **scalability** to test more **targets**, **donors** and **replicates**.
- Translating screening methods to **fully integrated**, **automated** systems allows us to tackle **larger**, more **complex** future screens.
- Here we demonstrate a proof of concept **RNP-electroporation based editing** approach on our high-throughput screening platform.

Why Automate CRISPR Screens?

Robustness & Reproducibility

Repeated processes on identical hardware leads to improved inter-/intra-plate & screen variability

Throughput & Scale

Execution of fully optimised workflows increases screening efficiency, scale & productivity

Lower FTE Requirement

Current CRISPR screen execution requires 2-3 FTE, automated screens require a single user

PC Remote Access

System access and intervention during unsocial hours, monitoring by camera systems

Improved Data Flows & Integrity

Barcode scanners and automated data outputs allow improved data & sample management

Dedicated Support Teams

Collaborative working in Matrix teams links engineers, automation specialists & project leads

Cellular Automated Screening Platform (CASPer)

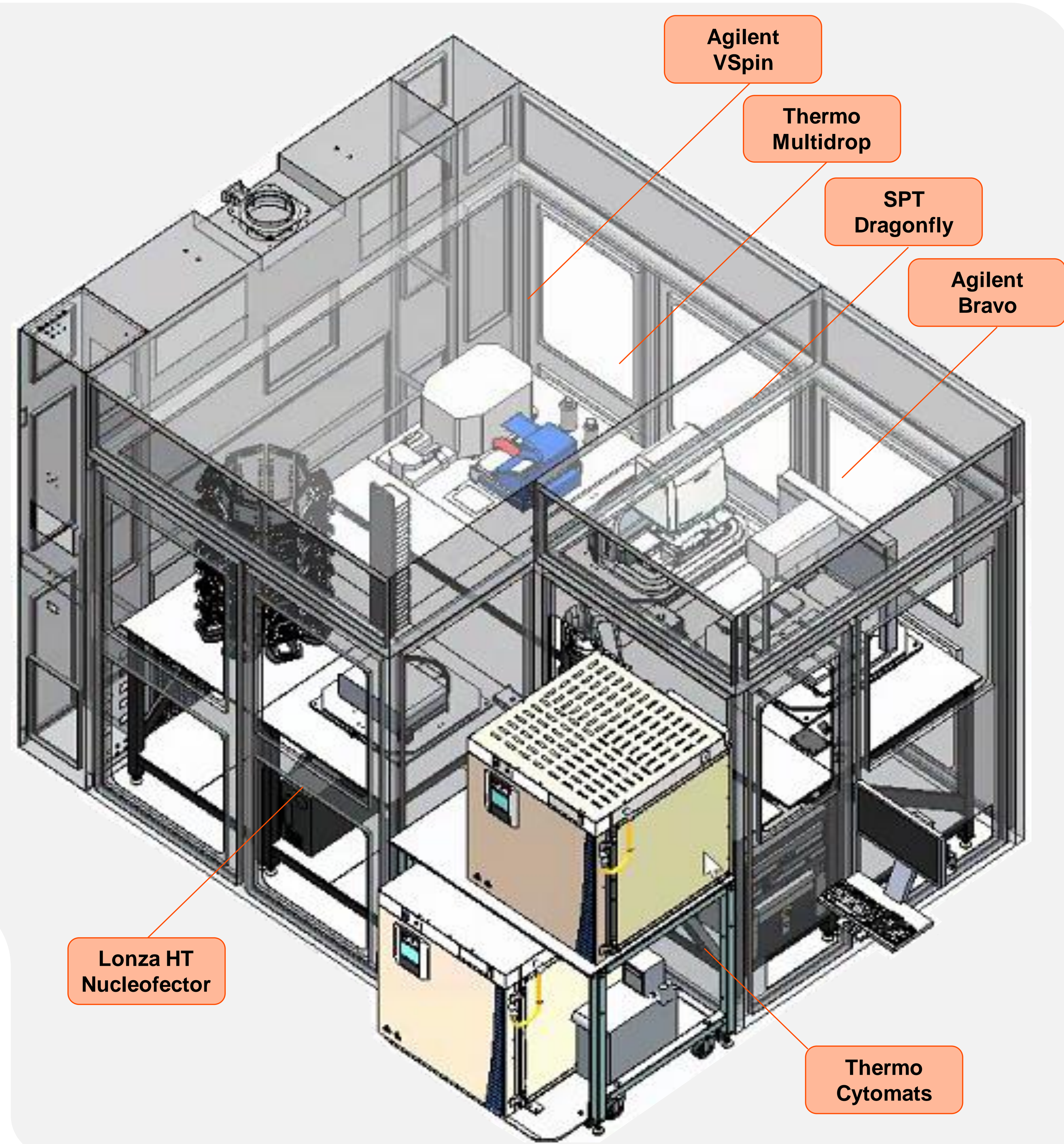
- Fully integrated automation with a focus on CRISPR editing human primary immune cells (e.g. CD4⁺ T cells) & difficult to edit cells, which are more amenable to electroporation based-editing with the Lonza 384-well HT Nucleofector System.
- Enclosed in a CAS recirculating HEPA filtered Class II Biosafety enclosure to ensure sterility and protection to both user and samples.
- CASPer is controlled via Thermo Scientific Momentum workflow scheduling software and hardware is accessed by a F7 robotic arm.
- Semi-automated CRISPR editing workflows have a throughput of 4x 384-well nucleofections a day, requiring 2-3 FTE. Operation of CASPer requires a single FTE and allows sequential batch editing.
- Optimised schedule control reduces nucleofection buffer exposure in CD4⁺ T cells, improving phenotypic and viability variation seen in manual screening (9 min vs 12-15 min exposure, respectively).

Capability Snapshot

384 HT-Electroporation

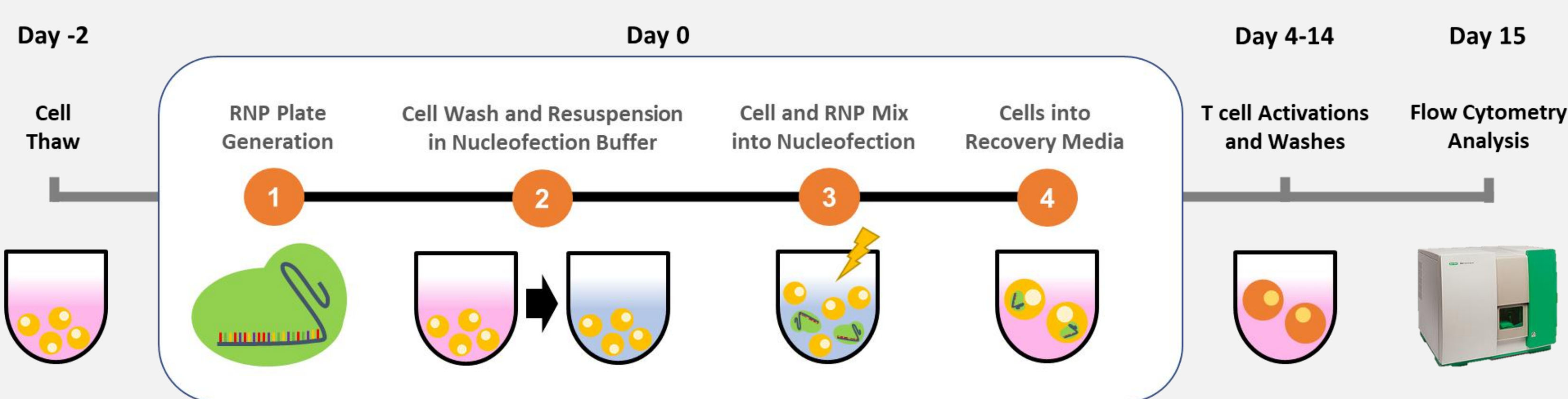
Lentiviral Transduction

Long-term Primary Cell Editing & Culture



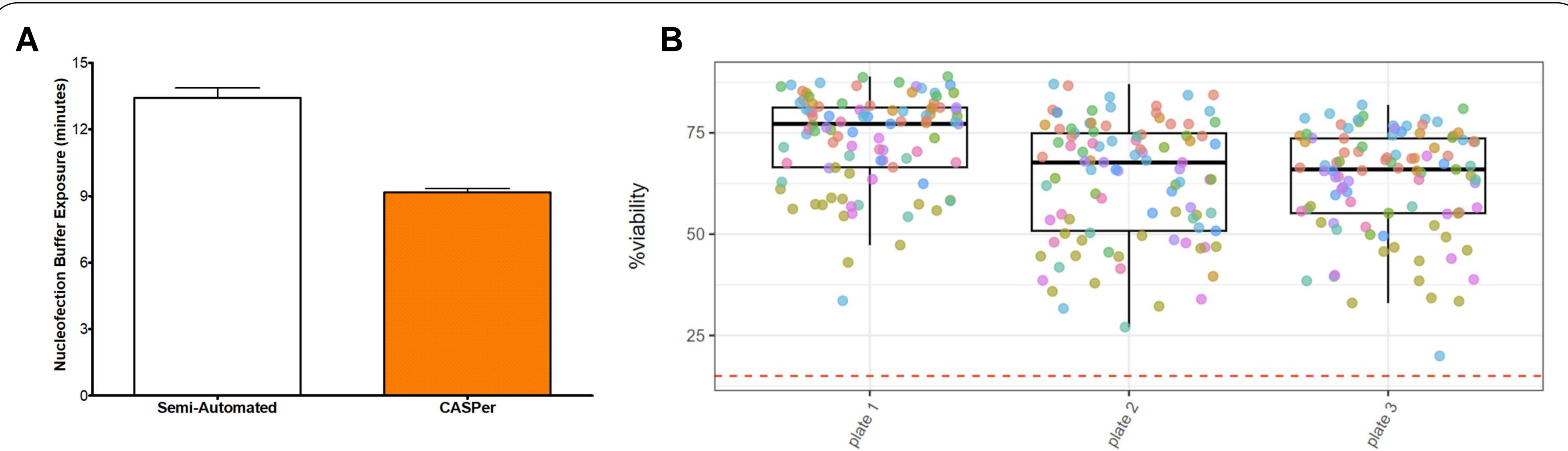
Proof of Concept Aim and Design

Perform a RNP-electroporation based CRISPR edit (Day 0) against 10 gene targets using CASPer, to expand a specific sub-population of CD4+ T cells against the total CD4+ T cell population (n=3).



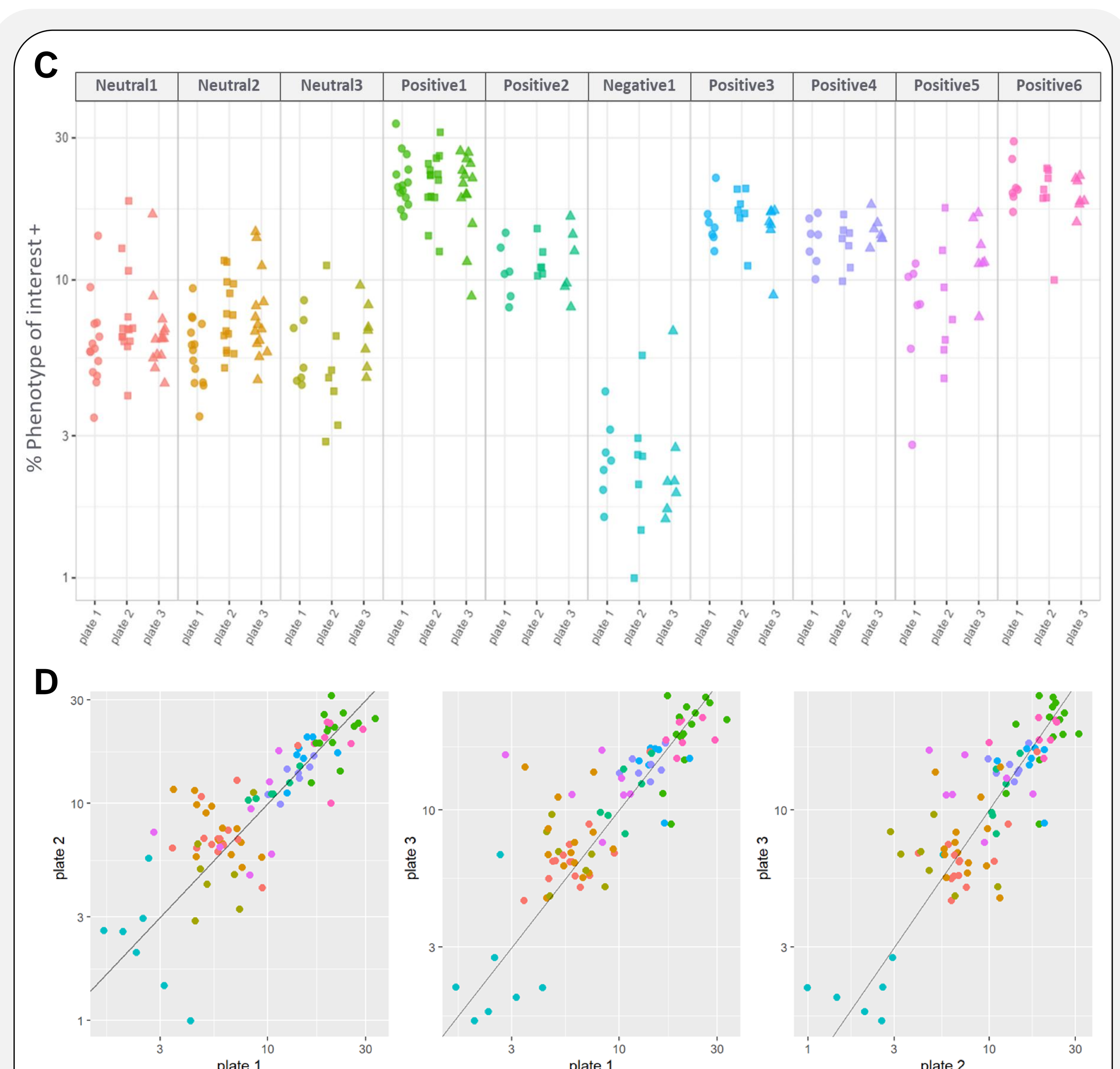
Cell thaw consists of 5 pooled donors. 5x10⁵ cells in P3 buffer with 67.5pmol sgRNA, 40pmol Cas9 per well, nucleofection programme EH115. T cell activation with TransAct in a 1:750 dilution. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

Automated Editing of CD4+ Primary T Cells



A. Time CD4+ cells spent in undiluted P3 nucleofection buffer during an automated CASPer edit compared to a representative semi-automated offline edit (n=3). Overall time is reduced with improved consistency between plate replicates. **B.** Viability box plot of edited T cells across 3 sequential edits. Average viability was >70% across all plates, an improvement against a typical semi-automated edit.

- Successful automated CRISPR reproducibly alters our T cell sub-population of interest, with **improved timings** and post-editing **viability** compared to offline workflows.
- Further parallel validations with a greater number of targets and replicates are ongoing.



C. The effect of 10 targeted CRISPR KOs over 3 sequential edits on our % phenotype of interest in a total cell population. Comparison of positive and negative controls relative to the neutral controls demonstrate successful directional CRISPR editing; data presented on a log scale. **D.** Correlation analysis of our % phenotype of interest shows linearity and reproducibility between plates.

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