CATAPULT
Cell and Gene Therapy

Overcoming the quality control barrier in ATMP development: high throughput analytics

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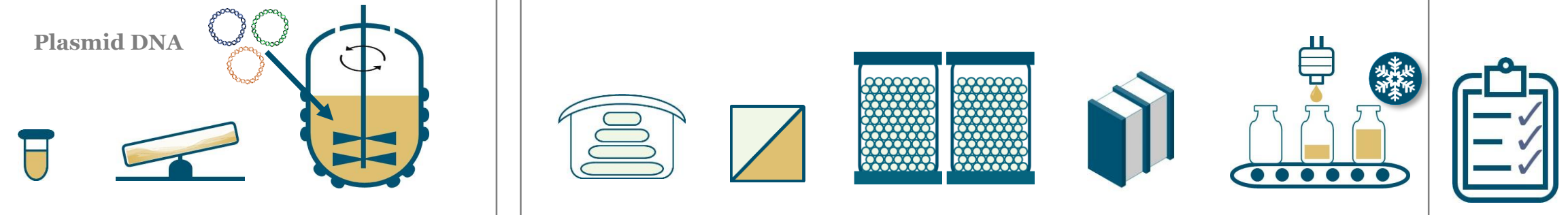
Damian Marshall

Director – New Technologies

Gene therapy product release

Up stream processing

Down stream processing



Titre and Potency	Identity - Physicochemical	Impurities	Safety
• Physical titre • Infectious titre • Infectivity • Transgene function • Stability	• Transgene sequence • Vector proteins • Vector integrity • pH (EP 2.2.3) • Osmolarity	• Residual HCP, HC-DNA, plasmid DNA • Large T antigen protein / DNA • Benzonase • Aggregation • Empty vector	• Sterility (EP 2.6.1) • Mycoplasma (EP 2.6.7) • Endotoxin (EP 2.6.14) • Adventitious viruses • Replication competent viruses

Product release

Industry challenges:

- Large number of batches for release
- Complexity of analytical assays
- Requirement for rapid release

Total yield TU (from 200L)	Target cells	MOI	Cell Number	Transductions
$1.0x10^{12}$	T Cells	5	$3.0x10^{9}$	67
$1.0x10^{12}$	$CD34^{+}$	100	$1.0x10^{8}$	100

Gene Therapy	Condition	Serotype	~ total dose	Doses per 1000 L
RPE65	Retinal dystrophy	AAV2	$2.5x10^{12}$	2400
Factor IX	Hemophilia B	AAV5	$1.4x10^{15}$	4
SMA 1	Muscular atrophy	AAV9	$1.0x10^{15}$	6

July 16, 2019

Kite Announces Plans to Bolster Industry-Leading Cell Therapy Manufacturing Capabilities With New Viral Vector Facility

SANTA MONICA, Calif.--(BUSINESS WIRE)--Jul. 16, 2019-- Kite, a Gilead Company (Nasdaq: GILD), today announced plans for a new 67,000-square-foot facility in Oceanside, California, dedicated to the development and manufacturing of viral vectors, a critical starting

Bluebird ramps up lentiviral vector production with Durham Facility

By Maggie Lynch

10-Apr-2019 - Last updated on 10-Apr-2019 at 13:06 GMT

In an indication of where the growth in the pharma industry is developing, Bluebird Bio is the latest to complete a new viral vector manufacturing facility to produce the investigational gene and cell therapies it is working on.

The biotech is in the process of qualifying the 125,000-square-foot lentiviral vector facility in

Novartis prepped for 'unprecedented' Zolgensma demand

by Dan Stanton

Thursday, April 25, 2019 4:37 am

With over one million square-feet of manufacturing space, Novartis says it is prepared for the imminent approval of AveXis' SMA gene therapy Zolgensma.

Speaking during its Q1 2019 results, Novartis said it is set for the imminent arrival of gene therapy Zolgensma (onasemnogene abeparvovec), added to the firm's pipeline through the acquisition of AveXis. The one-time therapy targeting spinal muscular atrophy (SMA) Type

High production throughput needed

The centre provides access to the expertise, skills, facilities and equipment as the stepping stone needed for organisations to develop new technologies and systems for large scale manufacturing.

- Quality control
- Qualified persons
- Operating policies
- Warehouse management
- Development assistance

Predicted Advanced Medicinal Product Output:
528 batches per year per module
5,280 batches per year for facility

Predicted QC sample output:
>8,000 samples per year per module
>105,000 samples per year for facility

Engineering maintenance:
>3,000 Key equipment pieces for building function

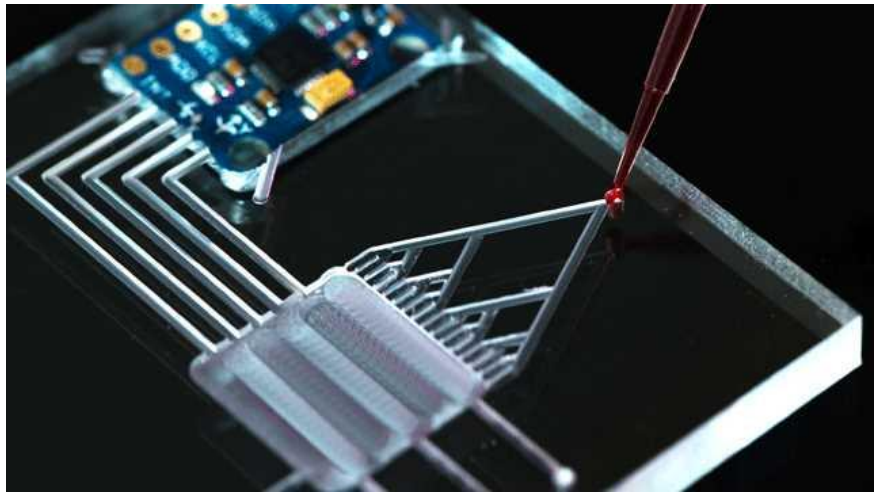
Warehouse:
>1.0M Material picks per year for facility

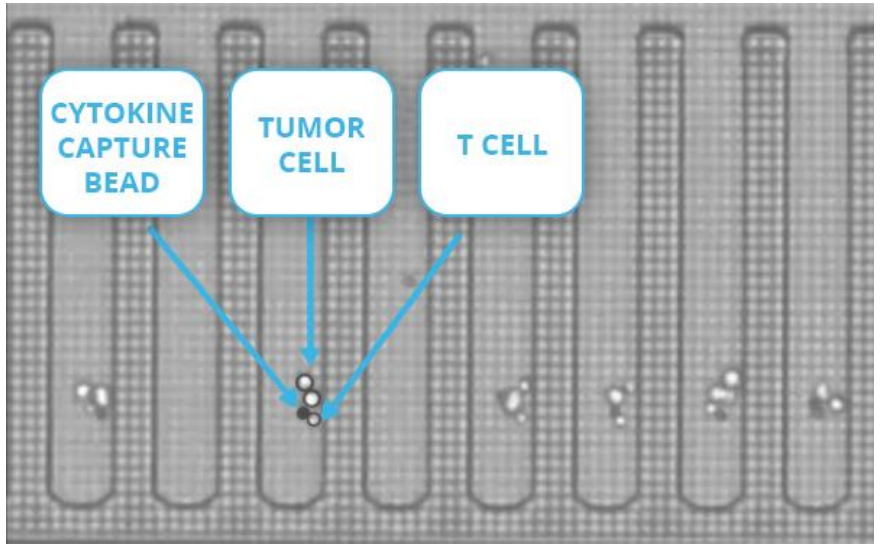
QC lab automation

- Automation can increase facility throughput and make QC faster, more agile, more compliant, and more efficient.
- Automation technologies already exist that could be used to streamline cell and gene therapy product release
- Up to 80% of QC laboratory tasks could be automatable
- automation can also ensure better quality and compliance by reducing manual errors and variability, as well as allowing faster and effective resolution of problems.

Integrating new technologies

- Technologies for cell characterisation are advancing faster than ever before
- This presents an opportunity for technology integration to change the way product release is performed
- These include technologies for rapid analysis:
  - Rapid potency testing
  - Rapid viral characterisation
  - Rapid sterility
- Opportunities to incorporate lab-on-a-chip technologies
  - Sample miniaturisation
  - Multiparametric analysis
- High content technologies (single cell technologies)





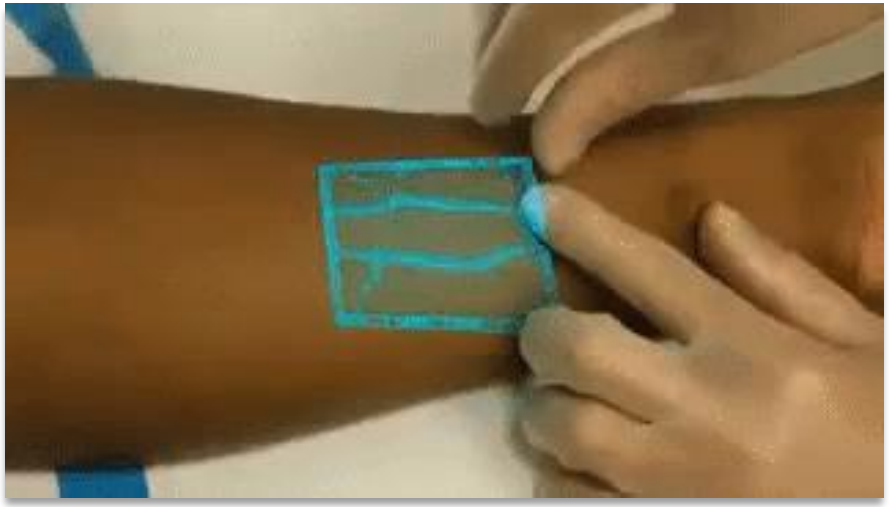
Transformative approaches to QC

- Using state of the art technologies to support product release

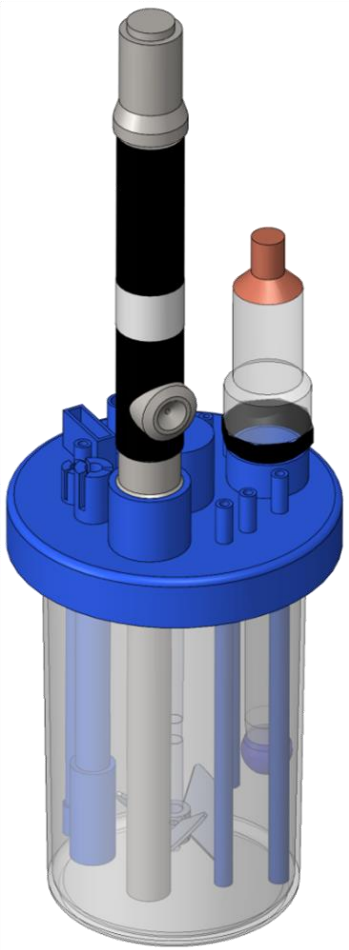
- AR is increasingly being applied in the healthcare sector
  - AccuVein for visualising vasculature
  - brain tumour mapping
  - surgical training

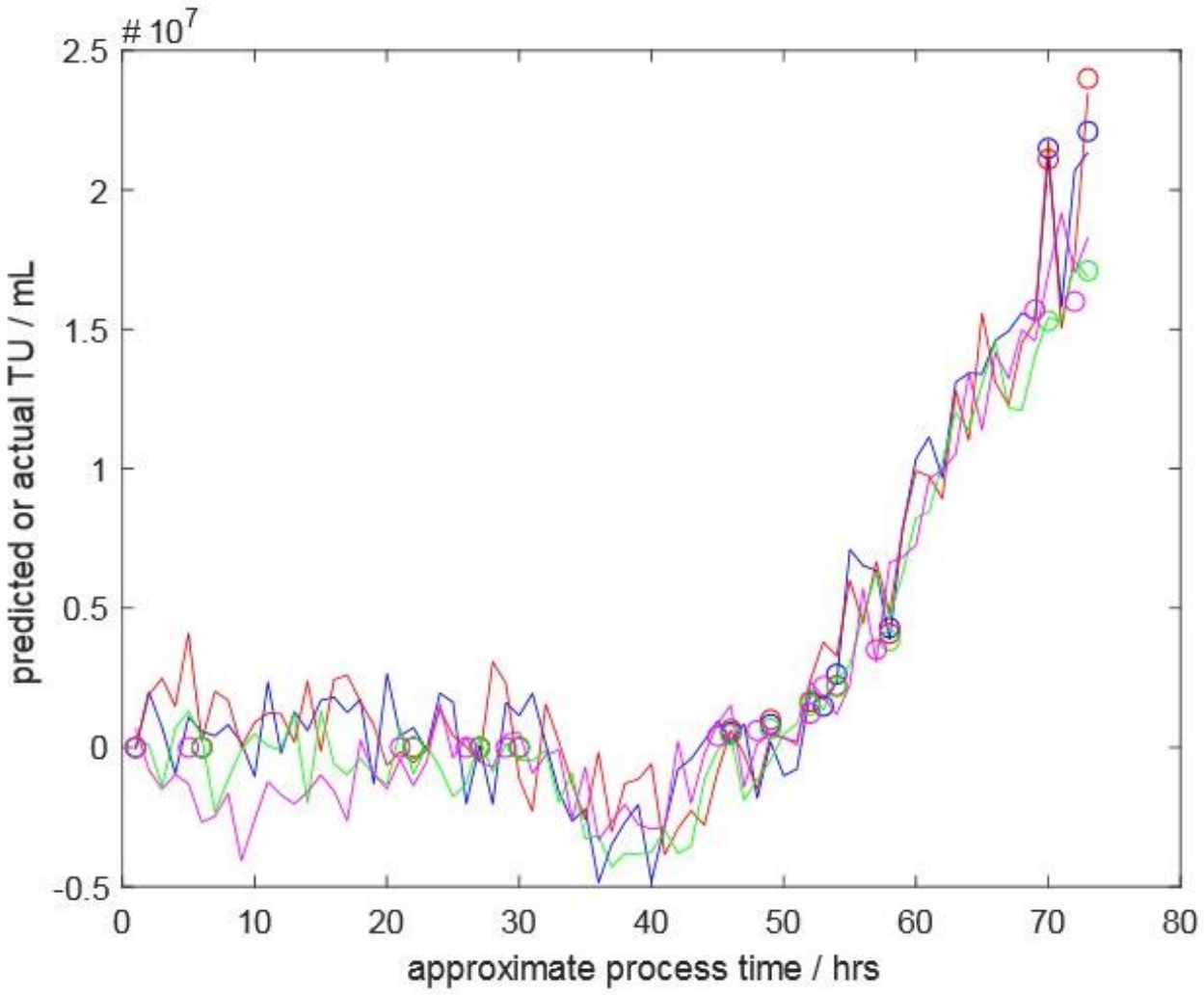
- AR is also being investigated as a new approach to support GMP manufacturing by large pharma

- Are there opportunities for augmented reality in QC?
  - Advanced electronic data recording
  - Lowering skill barriers
  - Increasing operator output



Sensor technology integration





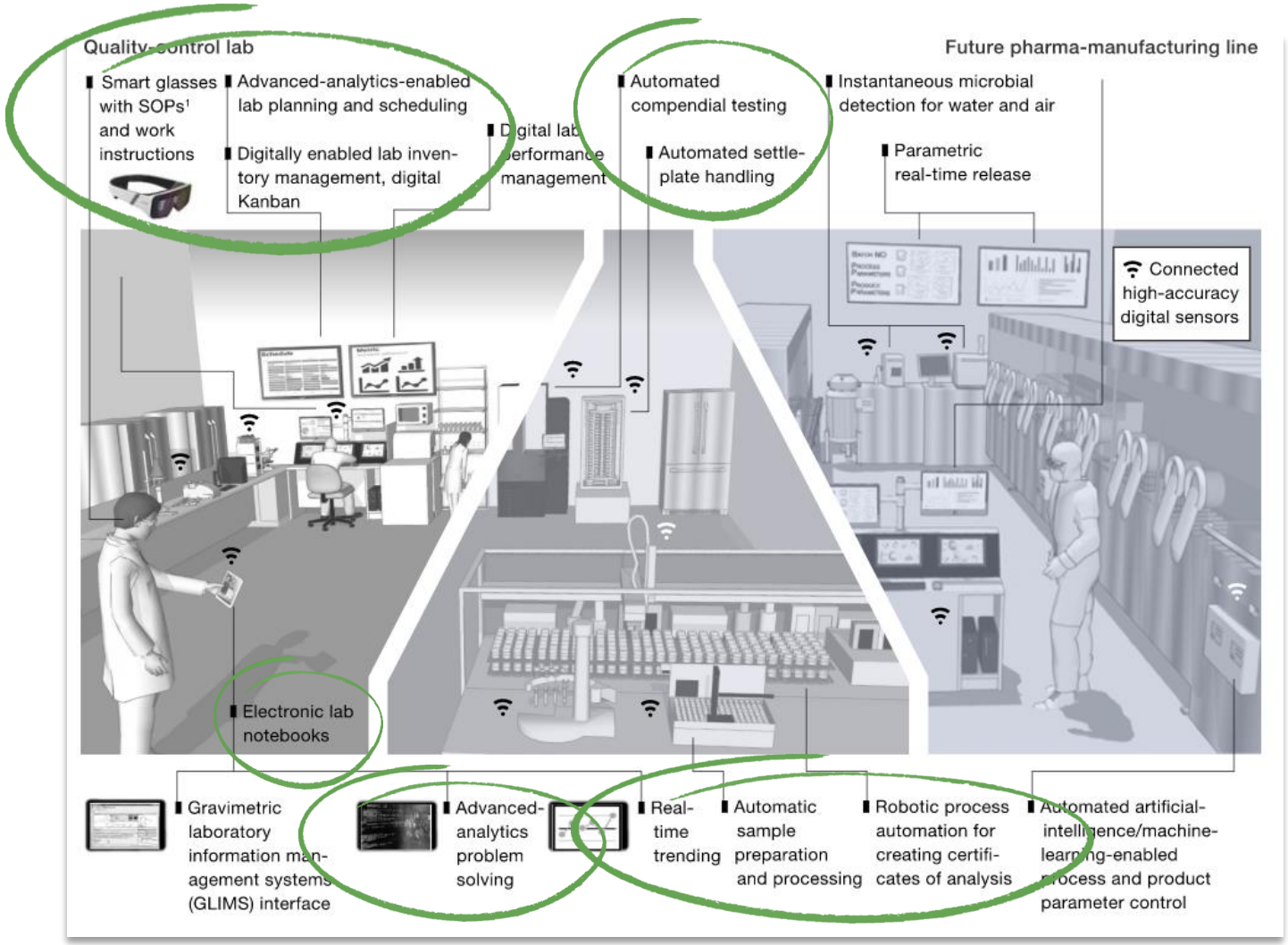
Real Time Release Testing

- RTRT is a framework to ensure the quality, safety and efficacy of the final drug product based on data generated during the process.

- This typically includes the measurement of CQA's during the process in combination with real-time monitoring of process parameters

- RTRT can provide a higher assurance of product quality –
  - Real-time control of process
  - Enhanced process understanding
  - Operational flexibility
  - Framework for continuous manufacturing
  - Support of continual improvement

Summary



Data connectivity, advanced analytics, robotics and automation have the potential to revolutionise ATMP product release

Digitization, automation, and online testing: The future of pharma quality control. McKinsey &Company 2019

INTRODUCTION TO ANTHA

Lab automation as the key to realising an integrated and flexible digital strategy

Markus Gershater, PhD
CSO Synthace
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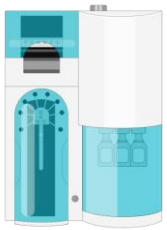
TRACKING LAB PROCESSES
AUTOMATED DATA STRUCTURING AND CONTEXT

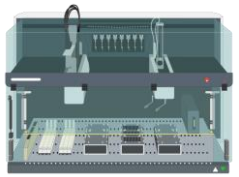
Expansion and Transduction

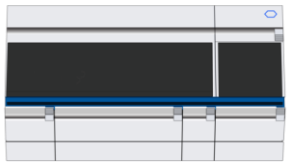
Clarification / Buffer Exchange

Purification / UFDF

Sterile Filtration / formulation & fill finish







Analytical Development / QC

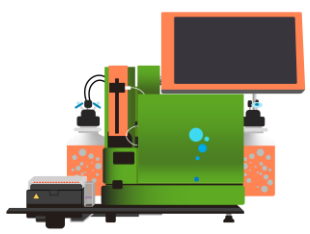
TRACKING LAB PROCESSES
AUTOMATED DATA STRUCTURING AND CONTEXT

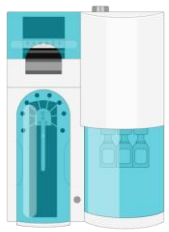
Leukapheresis

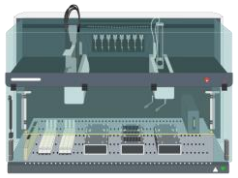
Activation / Modification

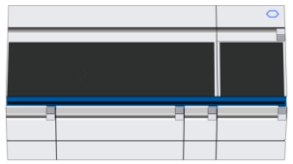
Expansion

Harvest / Cell Washing and Formulation



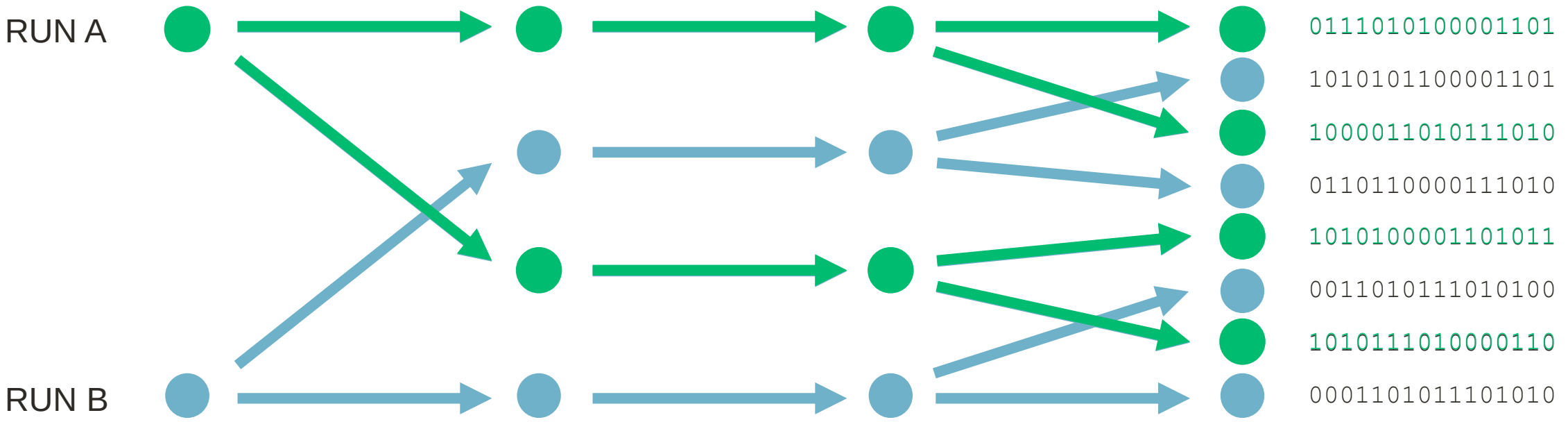




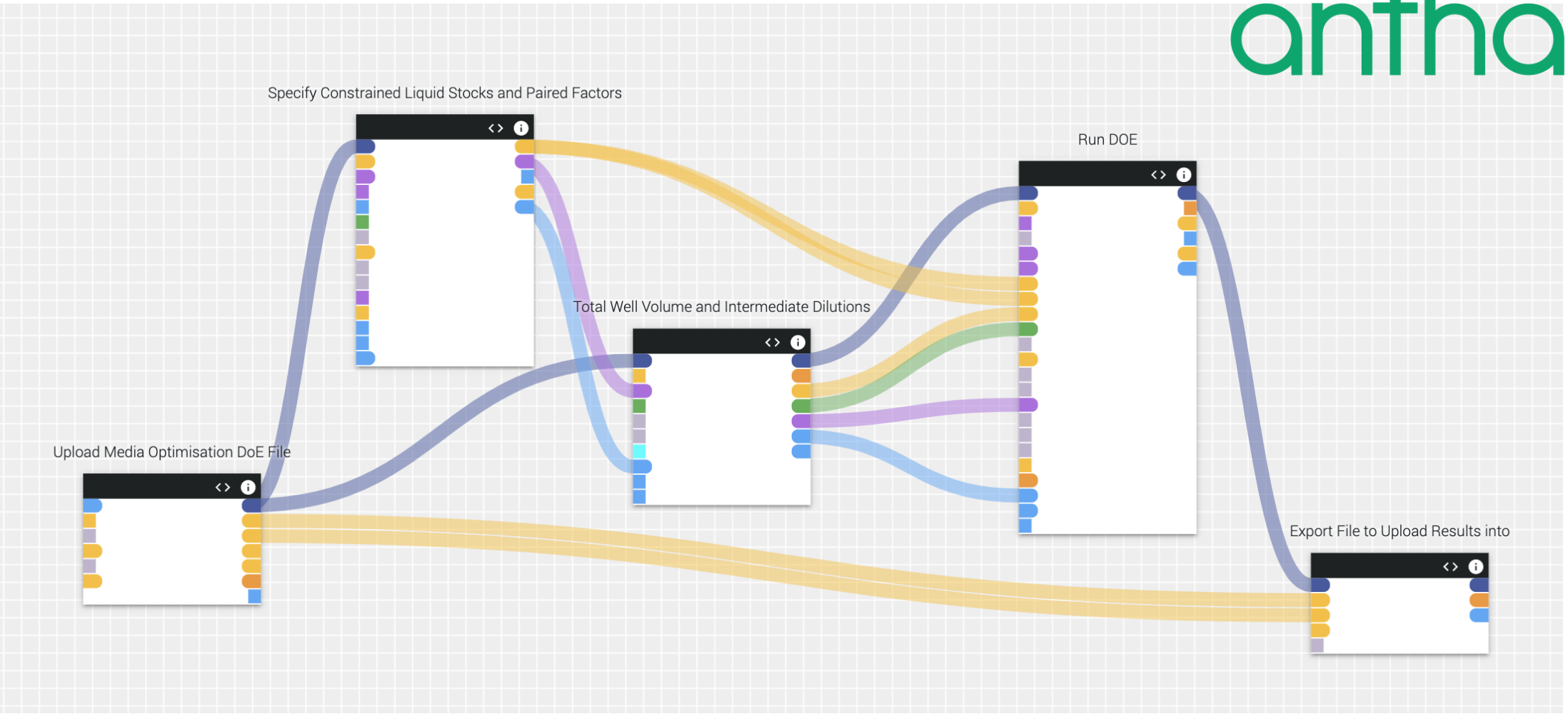


Analytical Development / QC

TRACKING LAB PROCESSES
AUTOMATED DATA STRUCTURING AND CONTEXT



BUILDING BLOCKS OF AUTOMATION

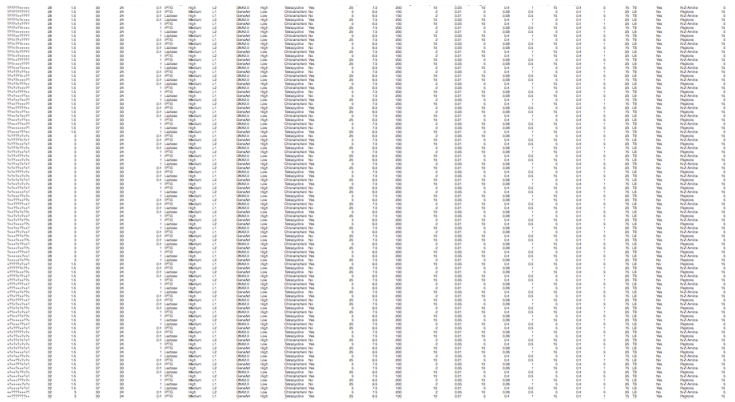


AUTOMATED OPTIMISATION

High throughput DoE needed to rapidly explore the design space of each protocol

OPTIMISED: AUTOMATING DOE

Experimental Design File

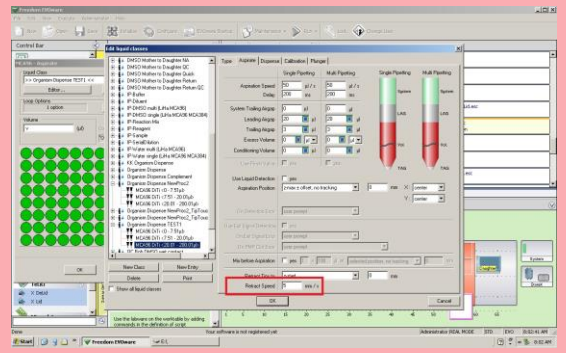


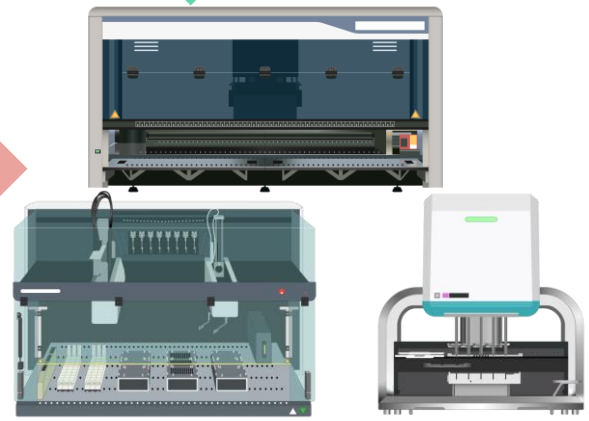
Automated planning and programming

antha

Planning

Hours of programming





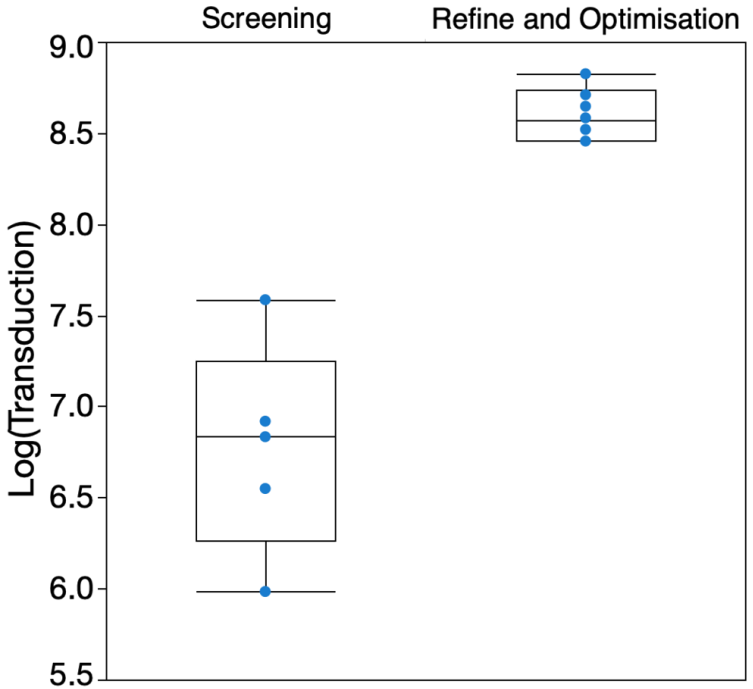
OPTIMISED: AUTOMATING DOE

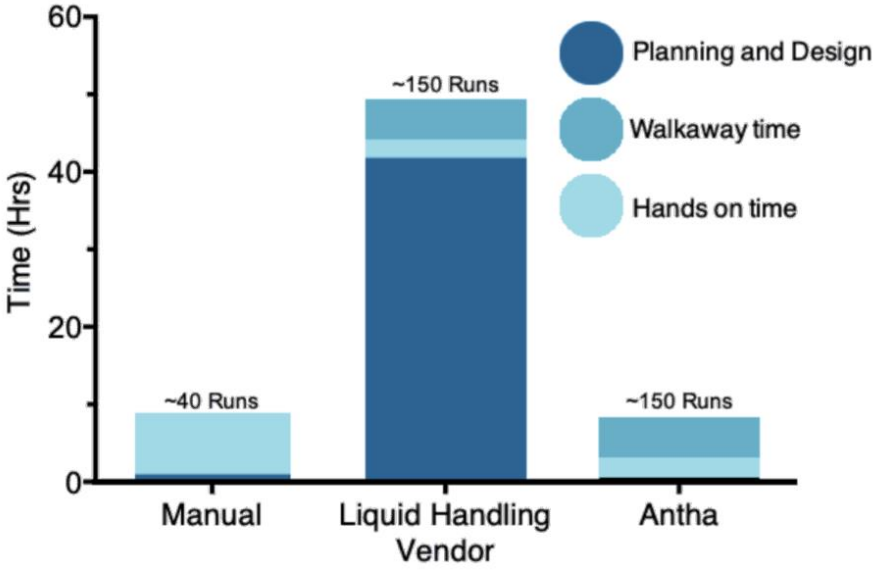
Watch video

OPTIMISED:
SCIENTIFIC AND OPERATIONAL BENEFITS

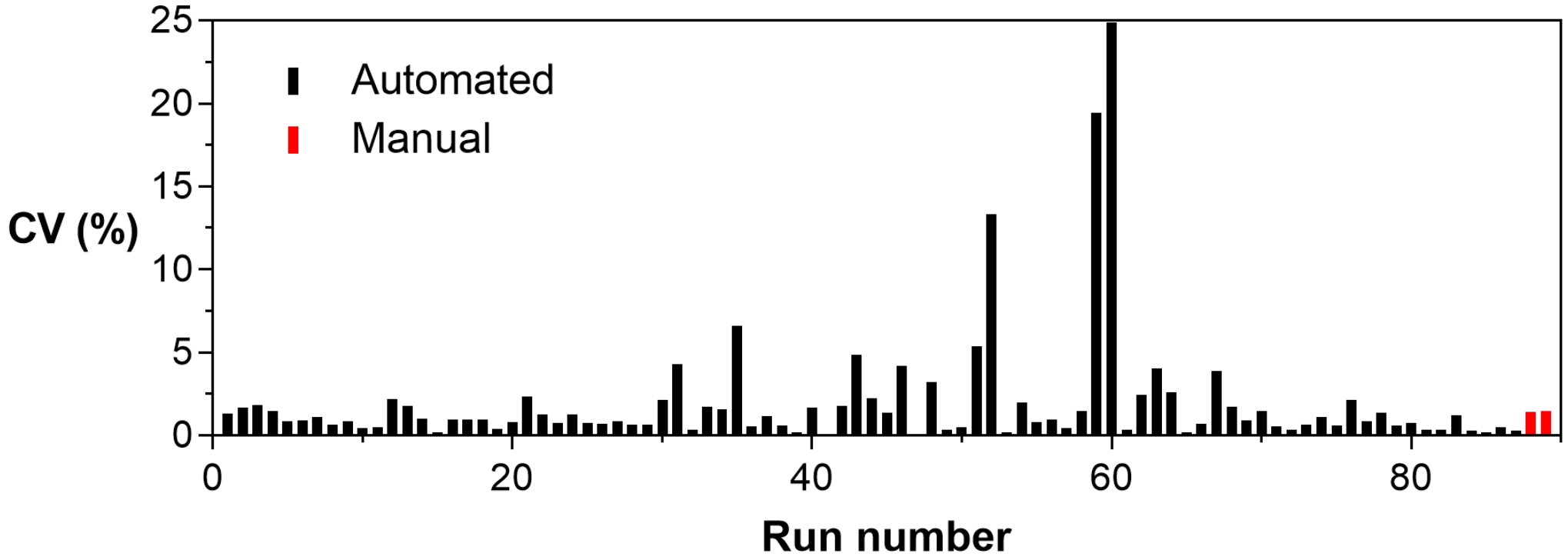
Antha optimization of transfection gave 3-10 fold increase in viral titre, whilst providing 83% time and 32% resource savings.

Oxford Biomedica



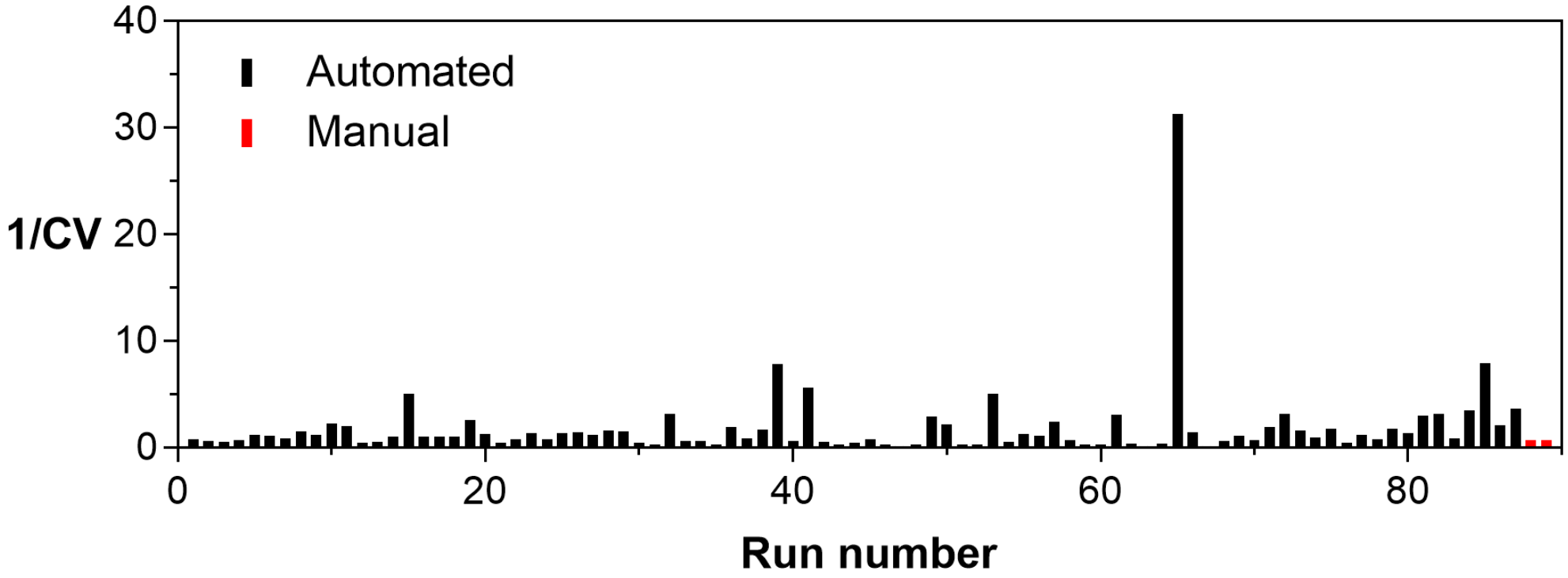


OPTIMISED:
DOE FOR AUTOMATION DEVELOPMENT



Automatically generated array of liquid handling strategies for qPCR, tested over 4 replicates

OPTIMISED:
DOE FOR AUTOMATION DEVELOPMENT



Automatically generated array of liquid handling strategies for qPCR, tested over 4 replicates

AUTOMATED ANALYTICS

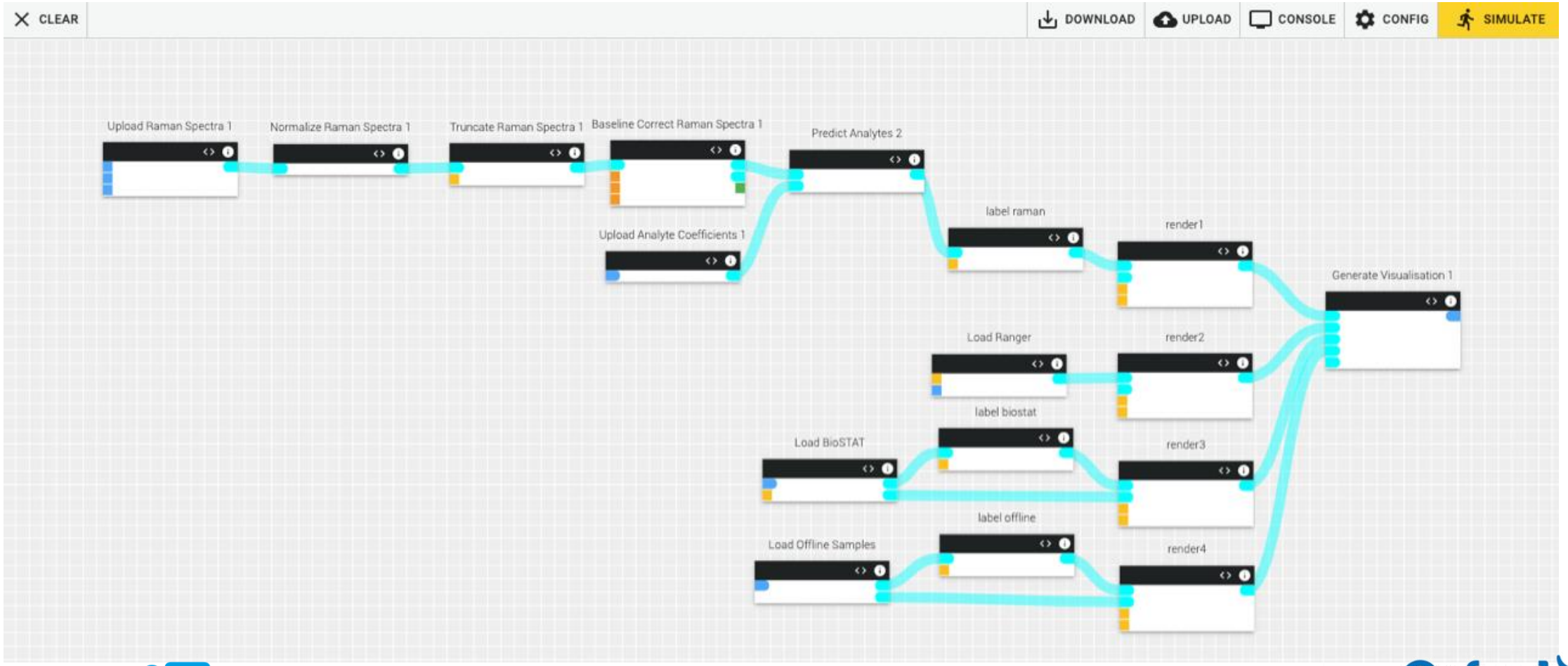
Robust protocols are automated flexibly to adapt any workflow without extensive reprogramming

Watch video

AUTOMATED DATA STRUCTURING

Automated integration of bioreactor, analytical and sample data

CONNECTED:
CASE STUDY: IN-LINE RAMAN



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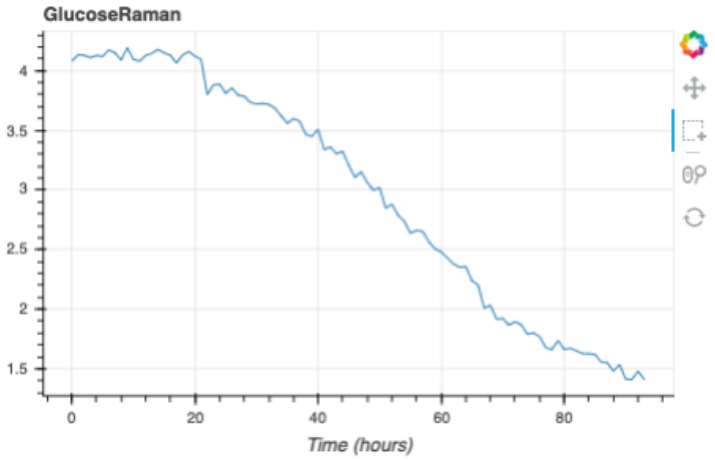
Oxford Biomedica

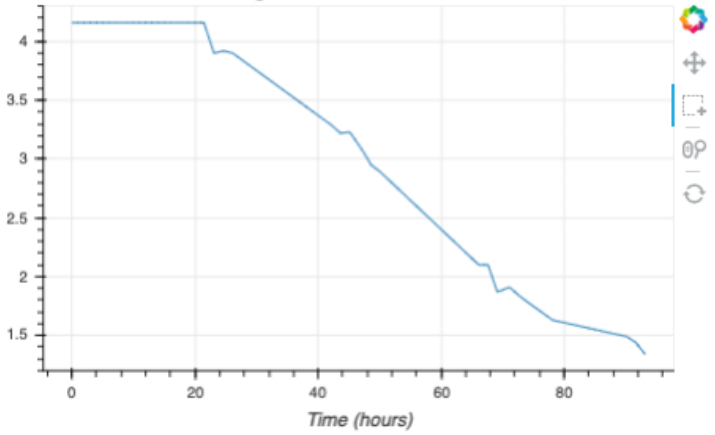
CONNECTED:
CASE STUDY: IN-LINE RAMAN

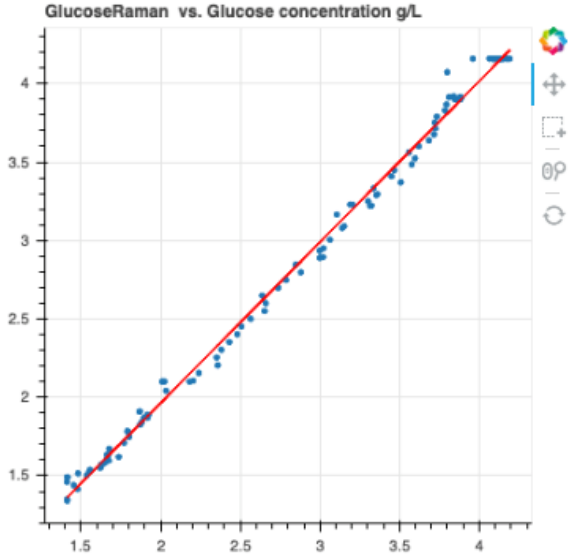
reactor17

GlucoseRaman



Glucose concentration g/L





#	Statistic	Sensor 1	Sensor 2
0	count	114.00	114.00
1	mean	2.91	2.89
2	std	0.97	1.00
3	min	1.41	1.34
4	25%	1.88	1.87
5	50%	3.04	2.98
6	75%	3.83	3.90
7	max	4.19	4.16

#	Linear Regression Fit	Values
0	Regression Coefficient	1.0258
1	Intercept	-0.0877

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Oxford Biomedica

CONCLUSIONS

Automation of lab and data processes

Rapid, comprehensive optimisation of automated analytics

Run automated analytics flexibly

Automatically structure data

Thanks: CATAPULT Cell and Gene Therapy, Oxford Biomedica

CATAPULT
Cell and Gene Therapy

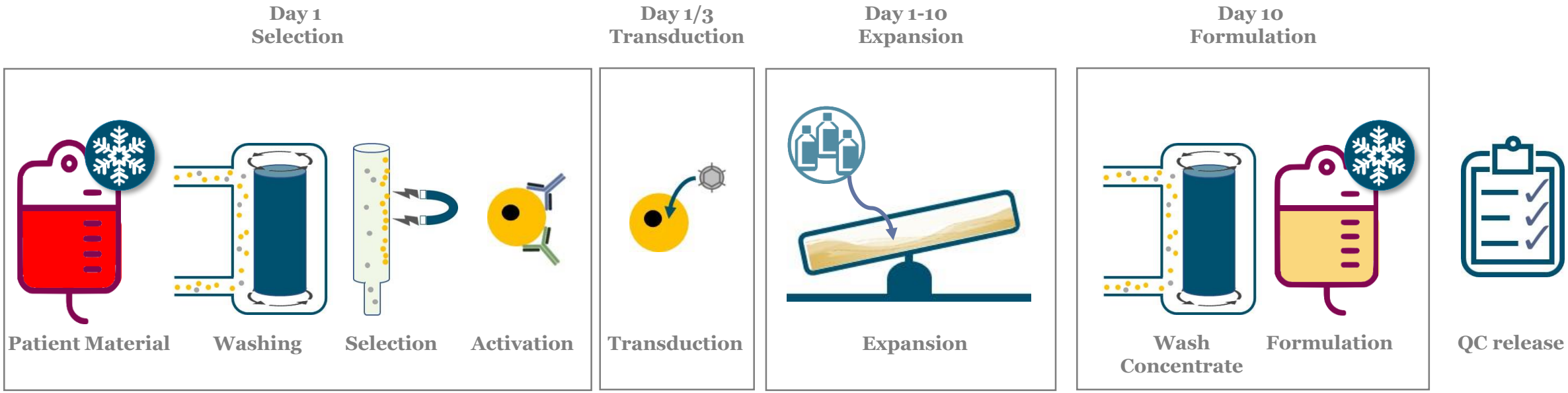
Rapid Analytics

Shortening time for complex product release assays

Juan Miguel Sánchez-Nieto

Analytical Development Scientist

Challenge: reduce time between product formulation and patient administration



Identity	Impurities	Safety	Potency
• Transduction efficiency • Immunophenotype • Appearance	• Percentage non-$CD3^+$ cells • Large T antigen protein/DNA	• Genome viral copy number • Sterility (EP 2.6.1) • Mycoplasma (EP 2.6.7) • Endotoxin (EP 2.6.14) • Replication competent viruses	• Viable cell count • CAR/TCR expression • Cell killing activity • Cytokine stimulation

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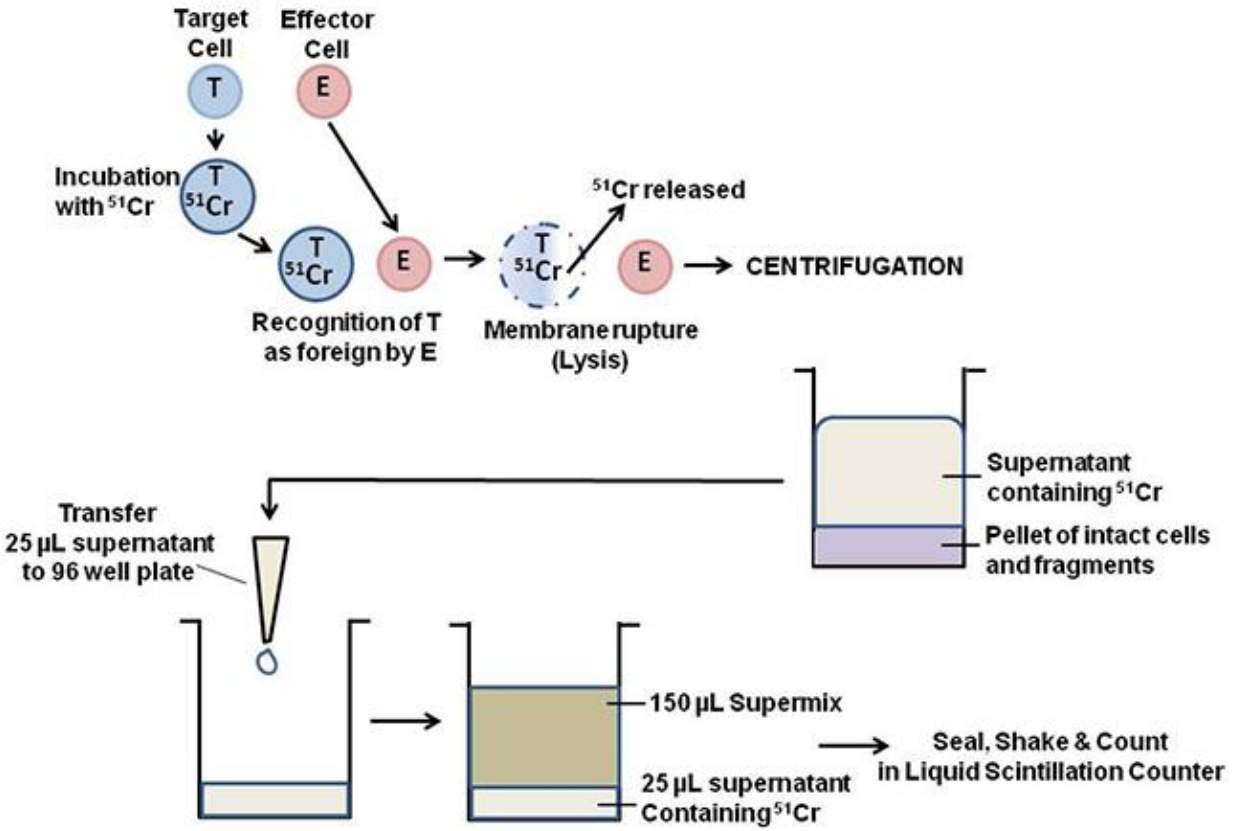
Potency assays for immunotherapies

Current methods to evaluate T-cell potency

Chromium release

- Gold standard
- Limitations:
  - Time – leakage
  - Safety – use of radioactive material
  - Cell requirements – high effector to target ratios | physiological relevance



Example protocol. Image: Perkin Elmer

Alternatives to Cr51 release assay

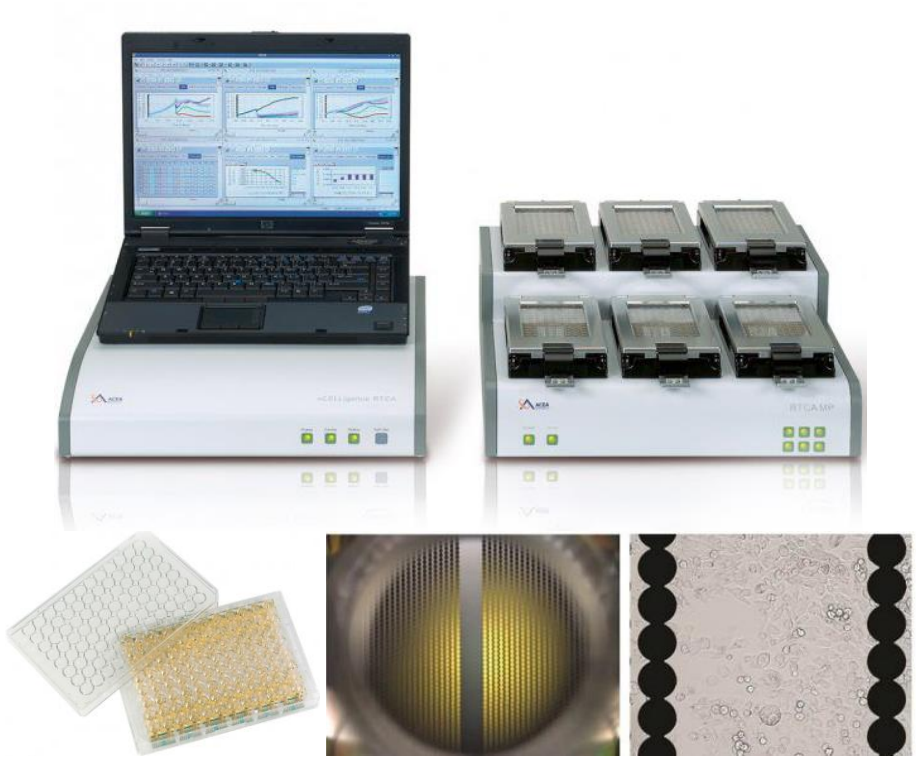
Assay	Measure	Readout
CytoTox-96	LDH	Absorbance
Cell Titer-Glo	ATP	Luminescence
Calcein-AM	Dye release	Fluorescence
Delfia EuTDA	BATDA release	Fluorescence
Flow cytometry	Cytokine/cell death	Fluorescence

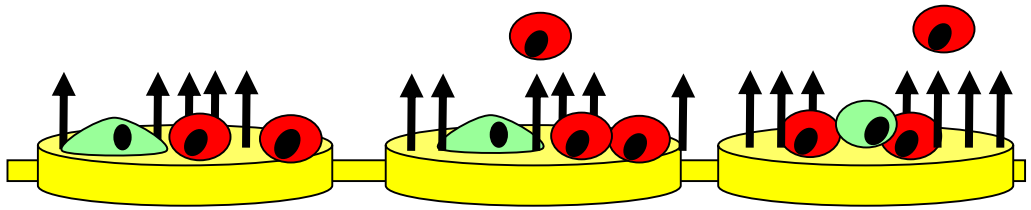
Solution: impedance – based potency assay

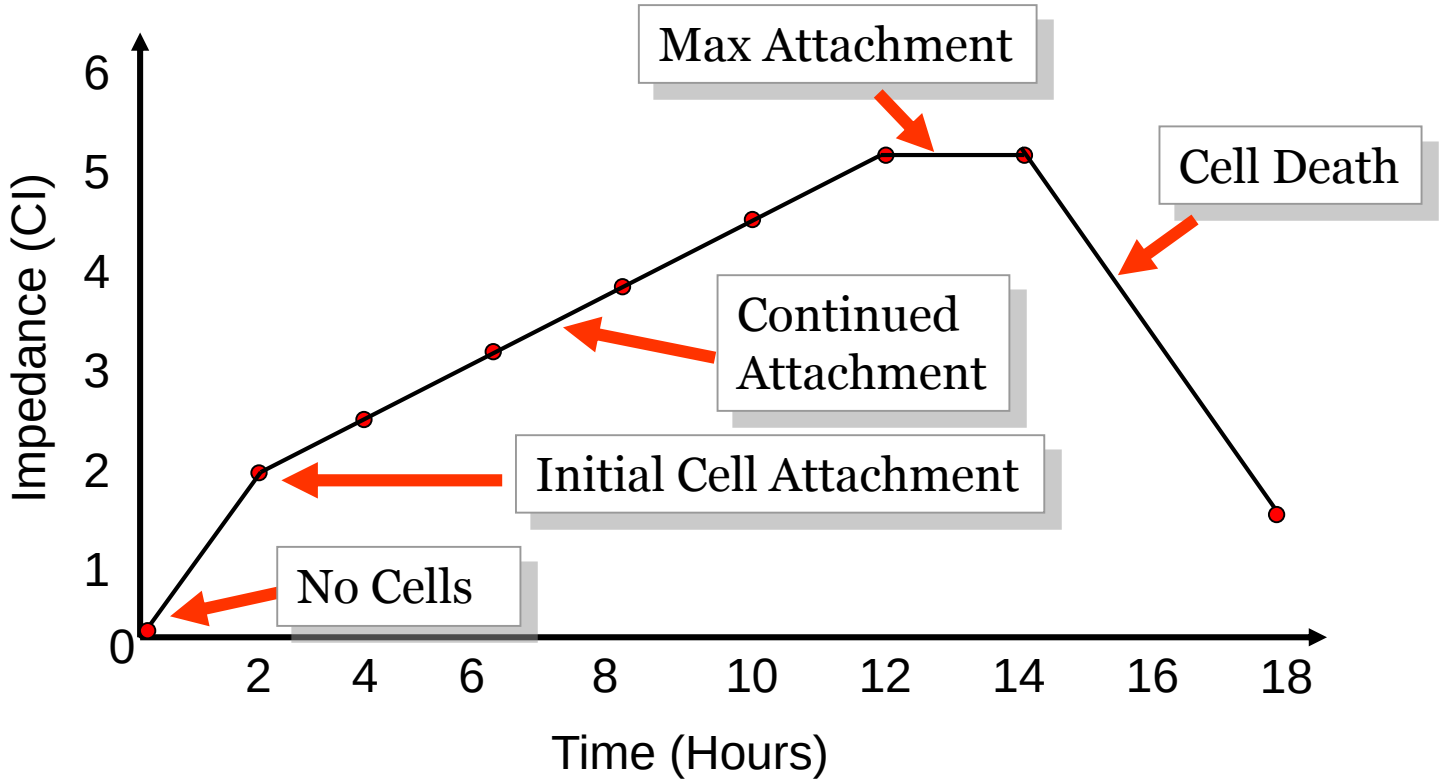
Real Time Cell Analysis system:

- Non-invasive system – electrical impedance
- Label free
- High throughput – 6x 96-well plates
- Flexible
- Limitation:
  - Optimisation required for each target cell line

How does the impedance-based potency assay work?



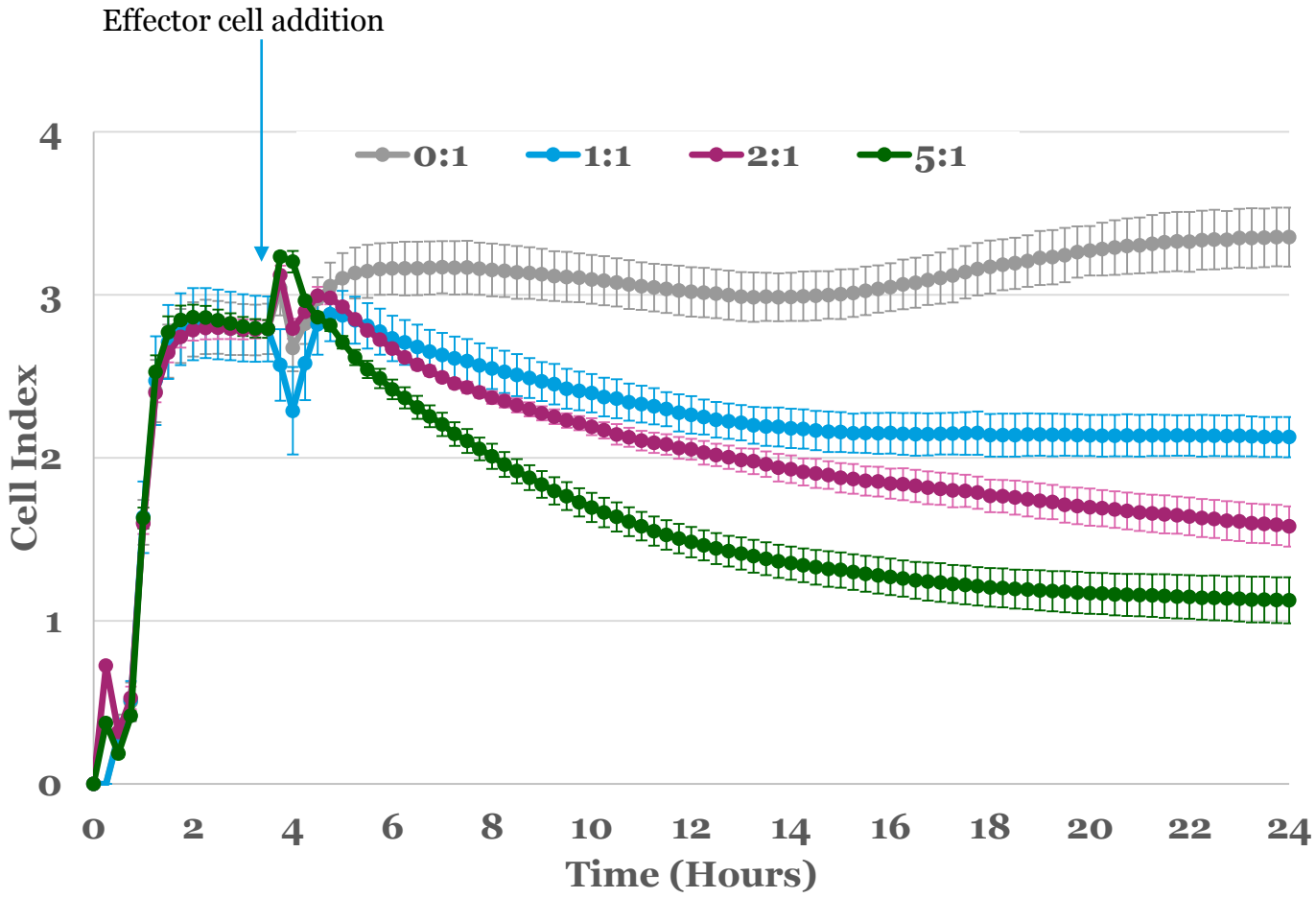




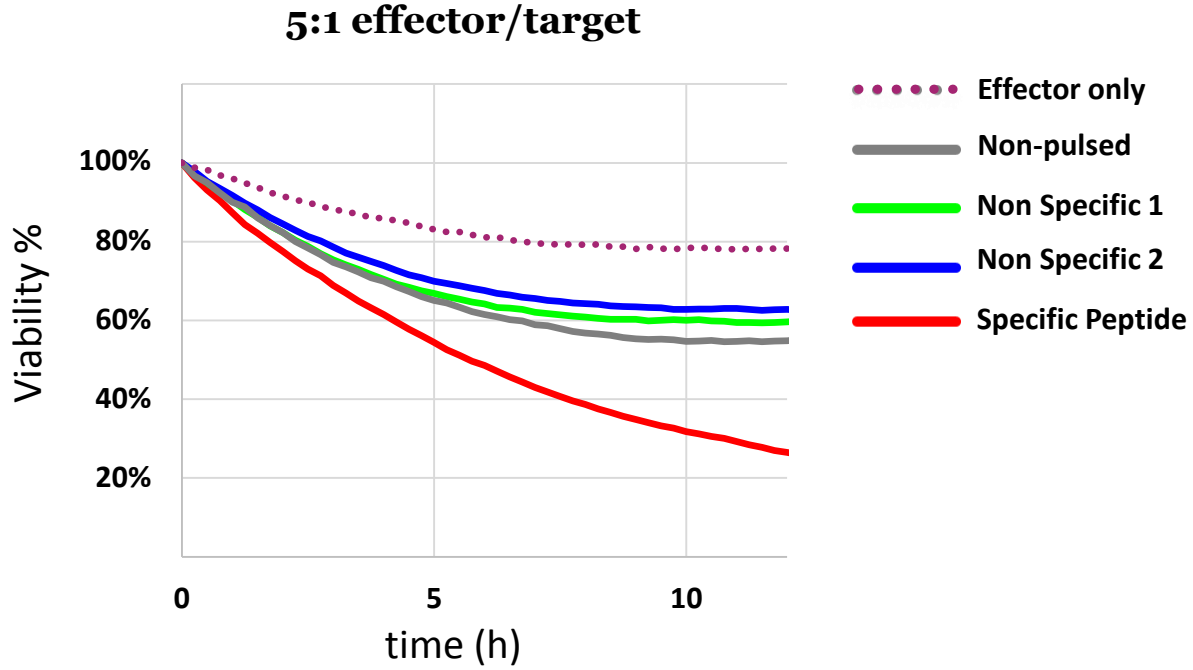
Optimisation of Effector:Target cell ratios for a TCR therapy

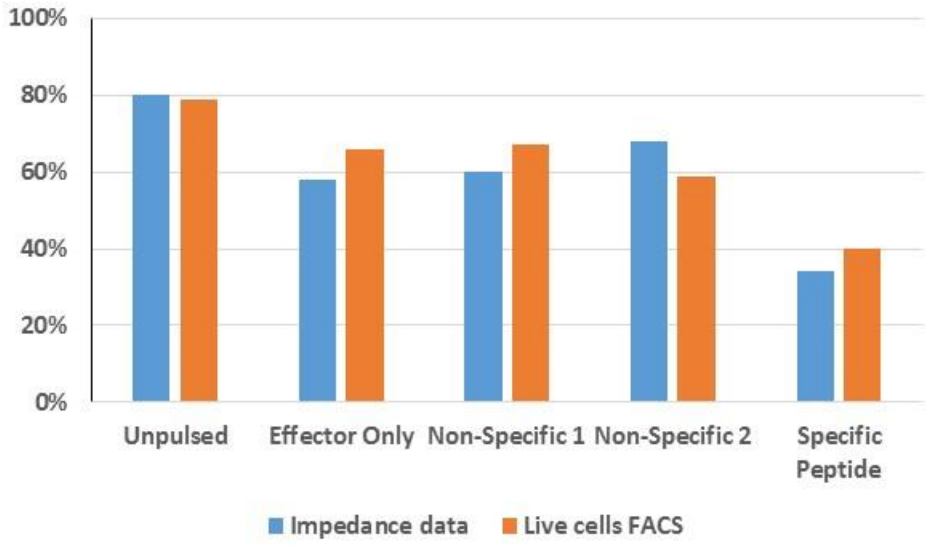
Assay outline:

1. Target cells are pulsed for 2 hours with peptide prior to plating
2. Target cells are plated and allowed to attach for 4 hours – impedance readings are initiated
3. Cells are washed prior to killing assay
4. Transduced T cells are added
5. Killing response is measured every 15 minutes for up to 24 hours

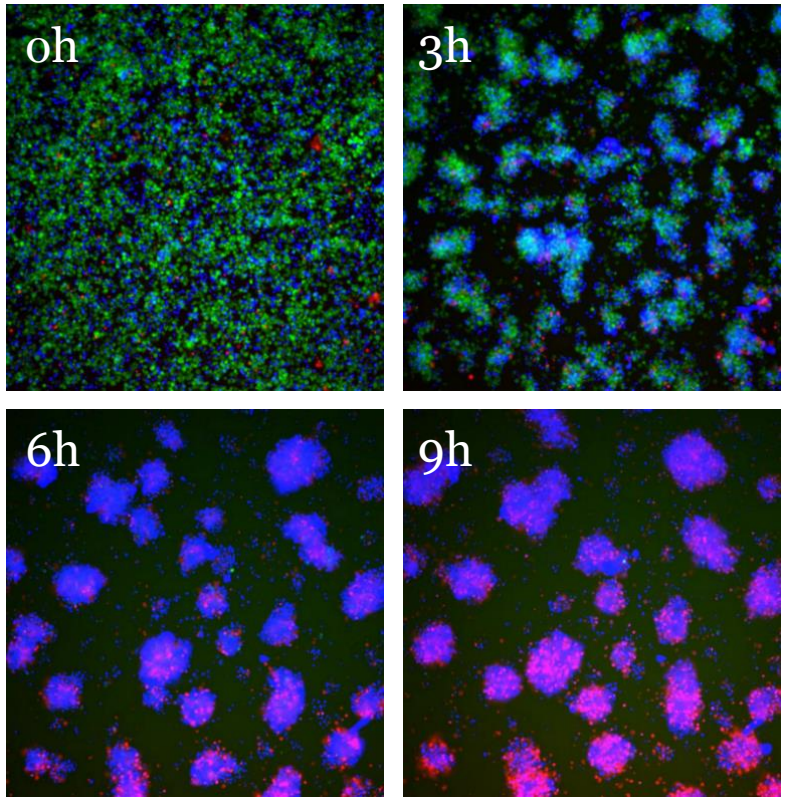


Comparability between impedance and flow cytometry – TCR therapy

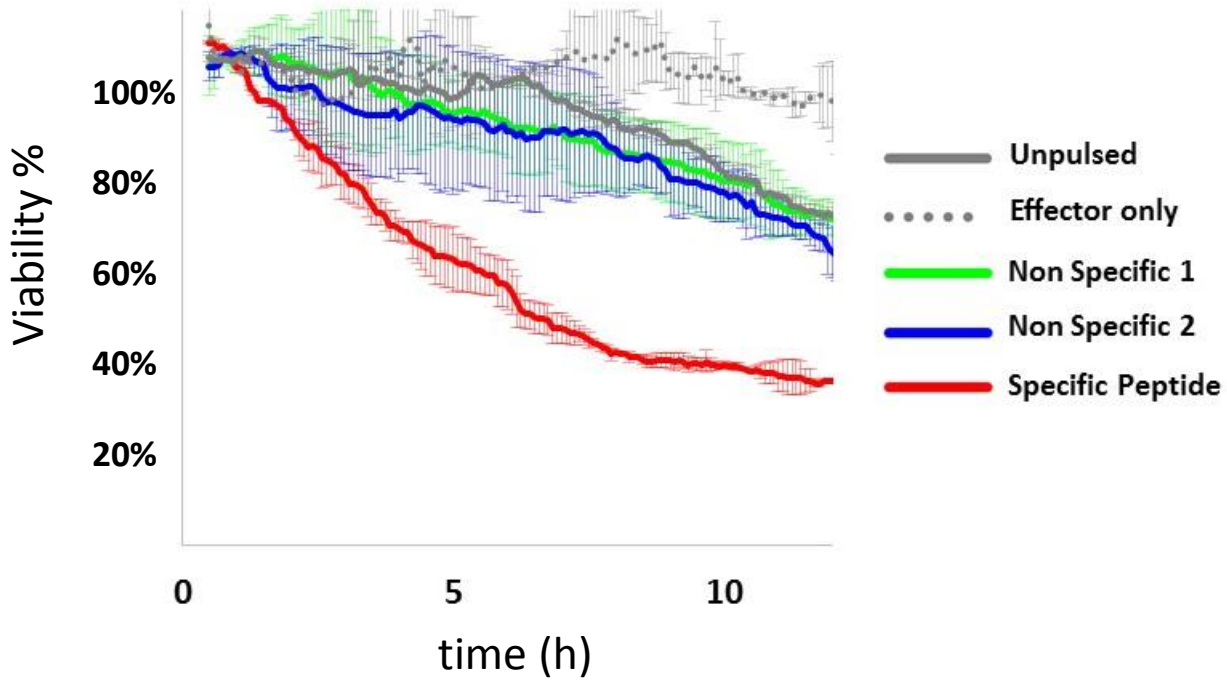




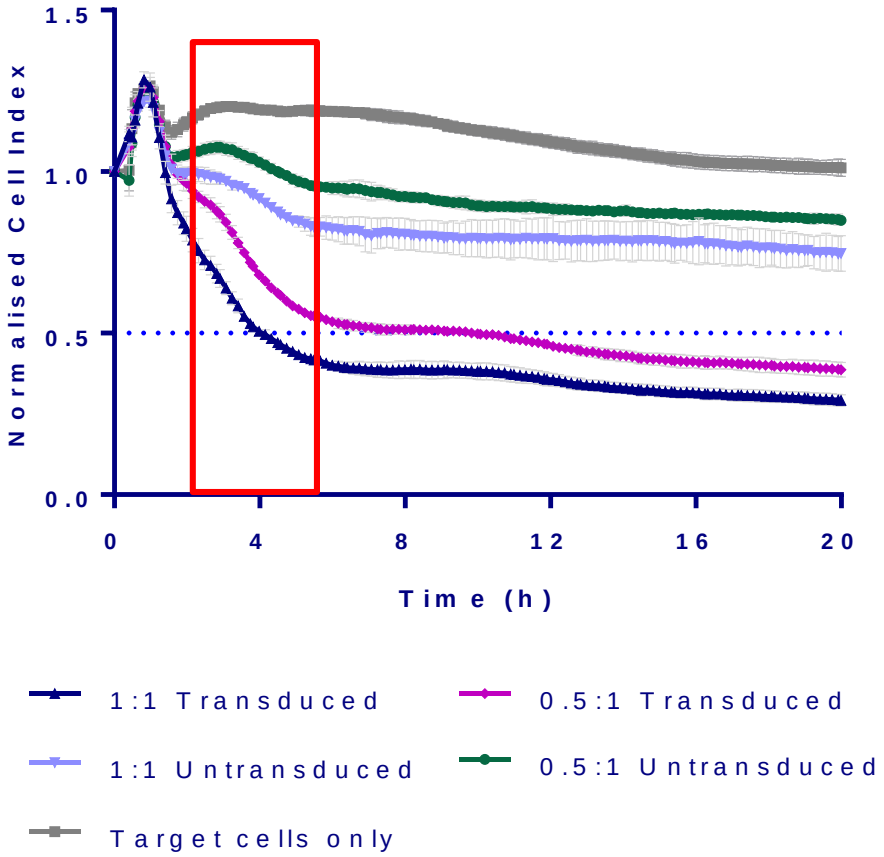
Correlation with impedance and quantitative image analysis – TCR therapy

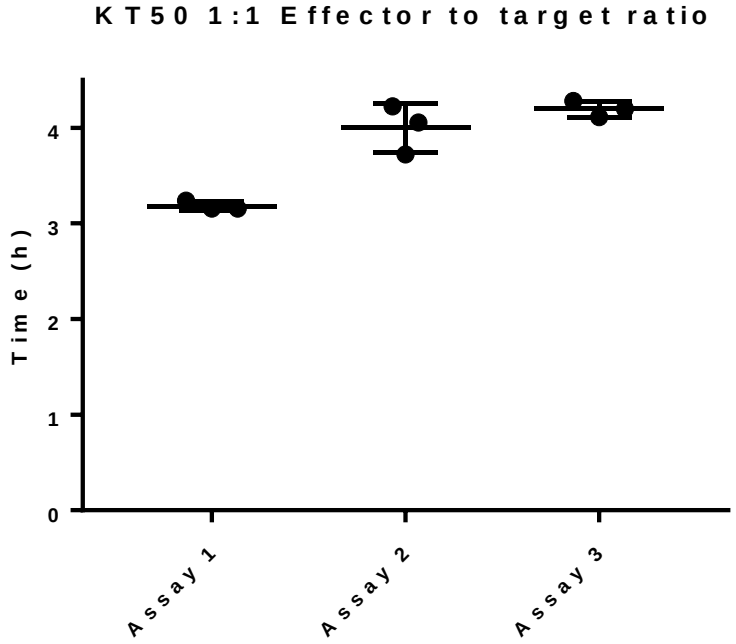


Target cells | **Effector Cells** | **Dead cells**



Real time detection of product's potency within 4h - CAR-T cell based therapy





Summary

- TCR and CAR-T immunotherapy potency can be reliably measured using impedance spectroscopy
- We have shown specificity of the assay independently of the therapy used
- Assay readout correlates with FACS analysis and image analysis
- The impedance assay is label free and provides **kinetic data** of cell killing
  - KT50
- This assay provides a **fast** and **high-throughput** alternative to current methodologies

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We work with Innovate UK