Single cell analysis of lentiviral transduction to support ex-vivo gene-modified cell therapies.

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Industry's need of novel analytical assays
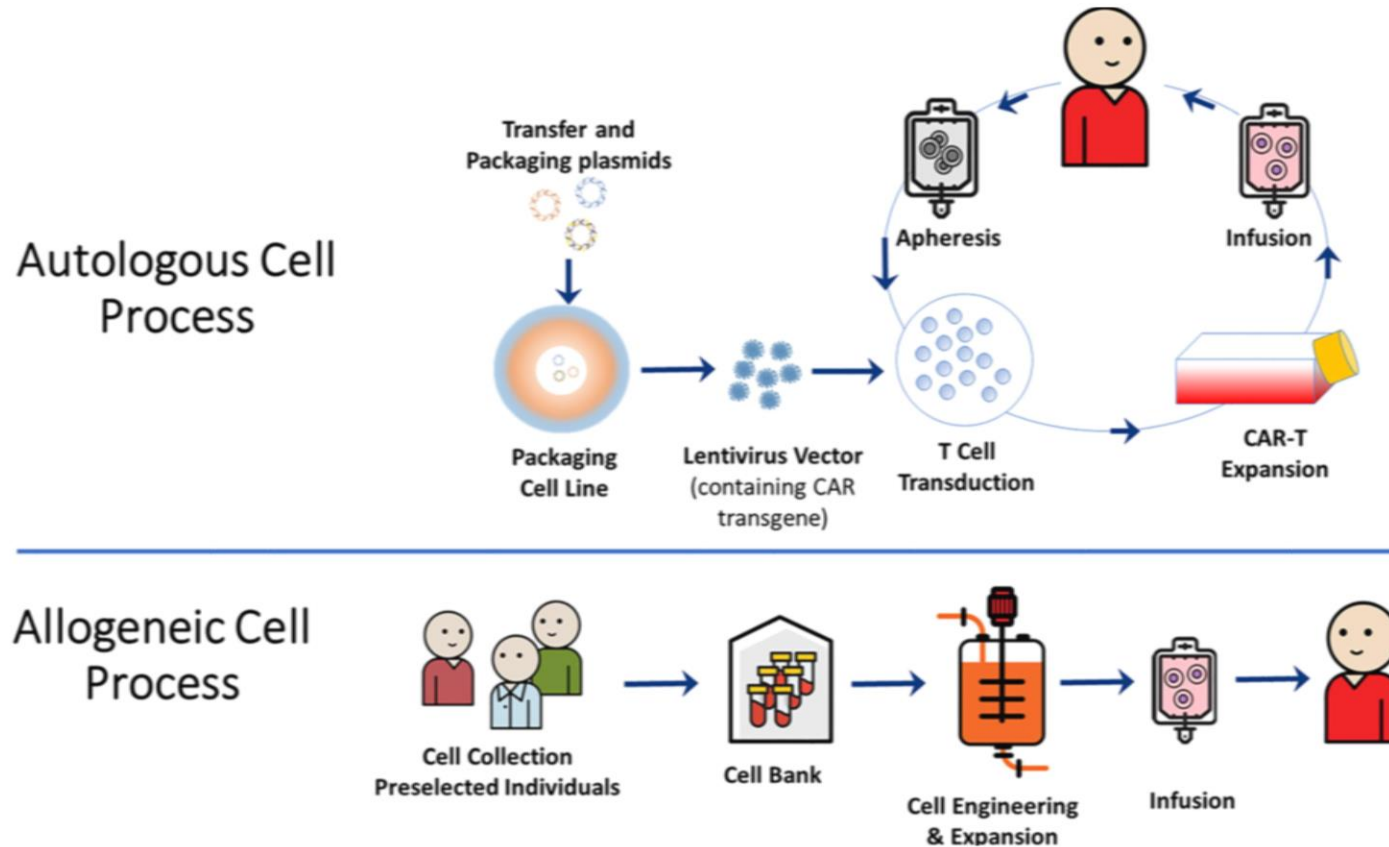
Background

Address an unmet need of measuring critical quality attributes for cell therapy products using fast and reliable solutions suitable for in-process control during manufacturing.

Current challenges in LV-based cell therapy manufacturing:

- High Cost of Goods, e.g. viral vector for gene delivery
- Integration of lentivirus and retrovirus in host cells
- Ensure safety for patients
- Sample heterogeneity may affect consistency of products

A growing need for advanced analytical characterisation of cell therapy products

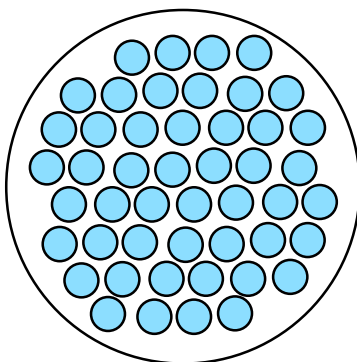## Ex vivo cell therapies are complex processes

Autologous Cell Process

Transfer and Packaging plasmids → Packaging Cell Line → Lentivirus Vector (containing CAR transgene) → T Cell Transduction → Apheresis → Infusion → CAR-T Expansion

Allogeneic Cell Process

Cell Collection Preselected Individuals → Cell Bank → Cell Engineering & Expansion → Infusion
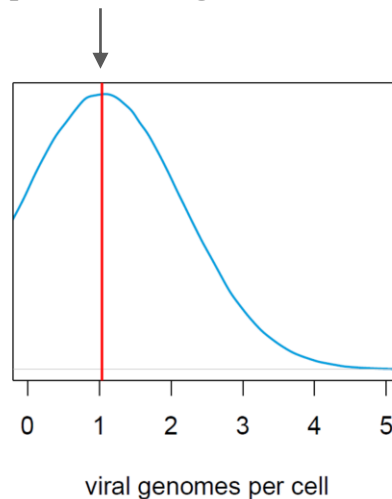
Sources of variability

- Donor material
- Lot variations
- Process robustness
- Manual operations
- Source of reagents
- Lack of controls and reference materials
- Operational variability
- …

Burke et al., Regen. Eng. Transl. Med., 2019

Can current analytical tools measure cell-to-cell variability?

population average

VCN estimation on cell population

viral genomes per cell

VCN estimation on single cells
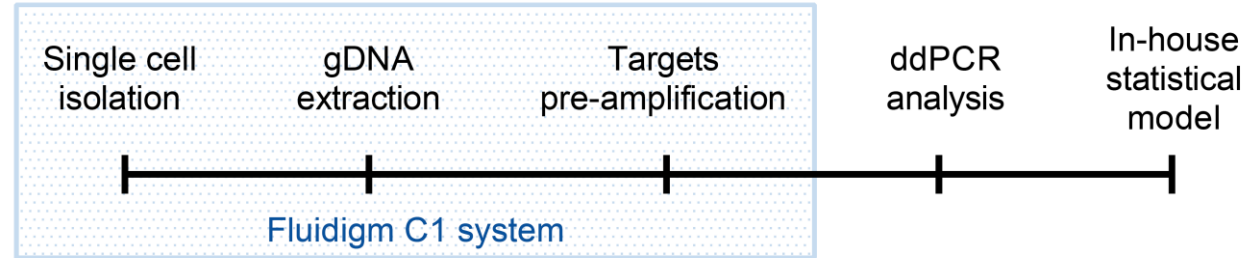
viral genomes per cell
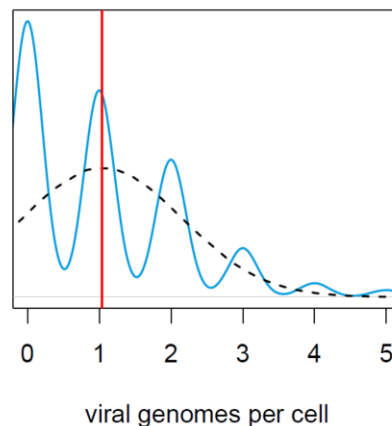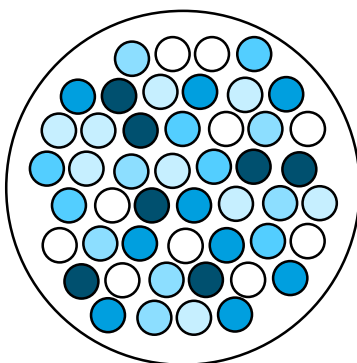
Advantages of single cell analysis

- Advanced analytical characterisation of CQAs

- Improved safety profile (e.g. proportion of clones with high copy number)

- In-depth analytical comparability during process development

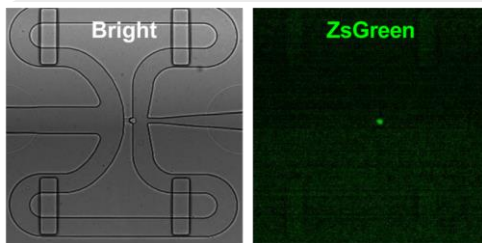- Advanced control over process consistency

*VCN = vector copy number

A Vector Copy Number assay at single cell level

Single cell isolation → gDNA extraction → Targets pre-amplification → ddPCR analysis → In-house statistical model

Fluidigm C1 system

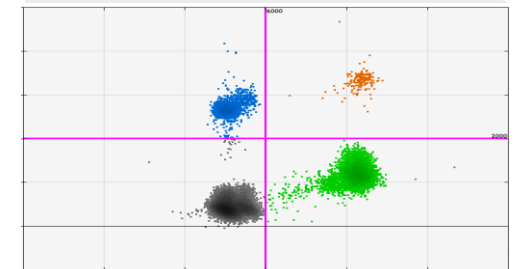**Single cell isolation in closed automated system**

Automated droplet digital PCR analysis

Quality control of cells isolation and number

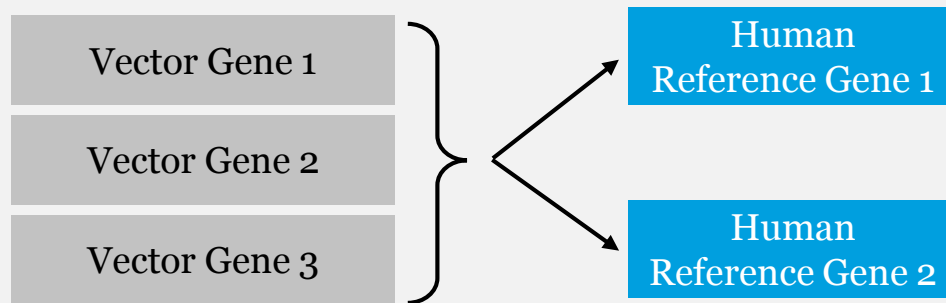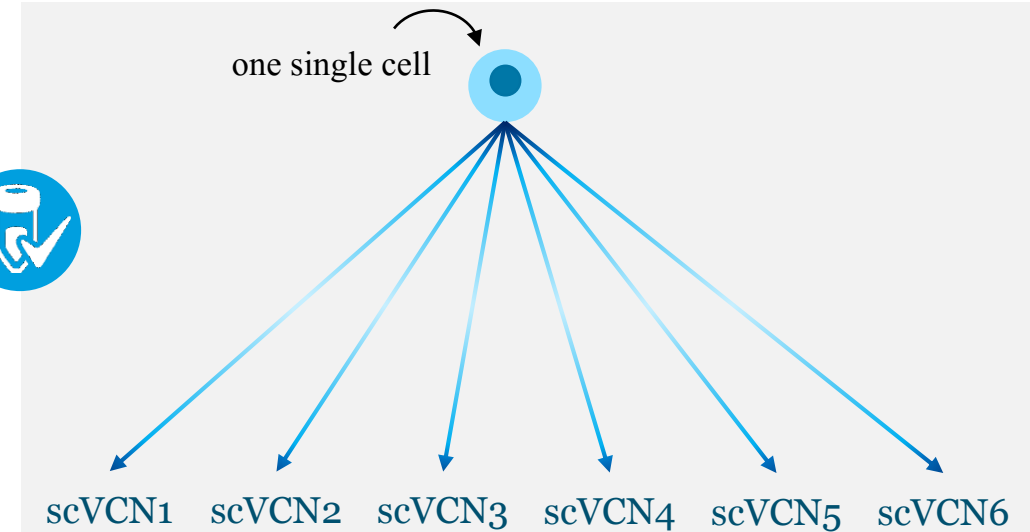*Absolute quantification of viral and human targets*

Data analysis – from ddPCR to vector copies

Six unique combinations of target genes

Multiplex ddPCR analysis

Vector Gene 1
Vector Gene 2
Vector Gene 3

Human Reference Gene 1
Human Reference Gene 2

$$scVCN = 2 \times \frac{Vector\ gene\ copies}{hRefGene\ copies}$$

one single cell
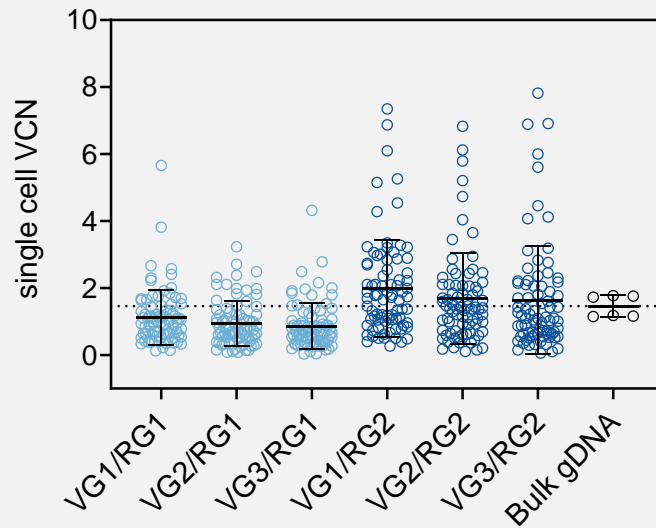
scVCN1 scVCN2 scVCN3 scVCN4 scVCN5 scVCN6

Probability model to compute maximum likelihood
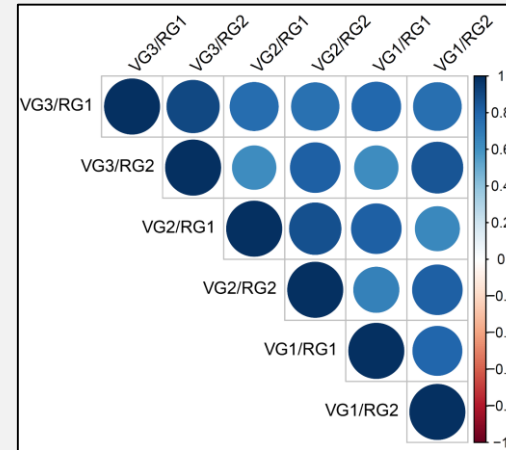
Integer vector copy in the single cell

The back end of the data analysis framework
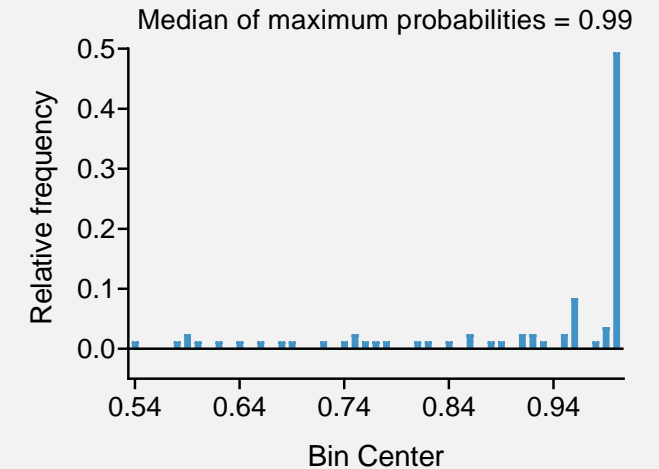
1. Analyse raw data

Raw data distribution and presence of outliers (technical artefacts, e.g. targets dropouts).

2. Quality controls

Pairwise correlations between each combination shows **robustness** of the assay.
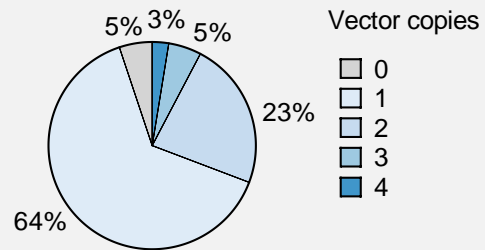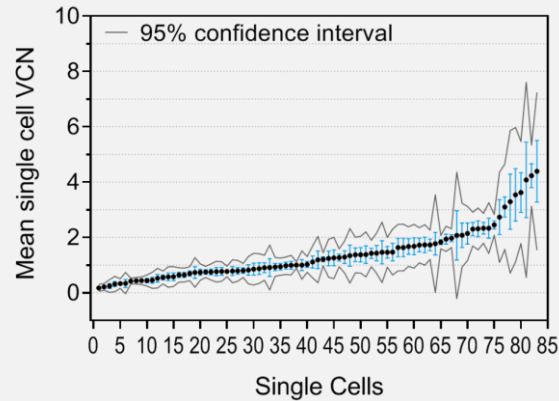
3. Statistical analysis

The in-house statistical tool converts measured scVCN into predicted viral units, assigning to each conversion a **maximum likelihood**.

*VCN = vector copy number

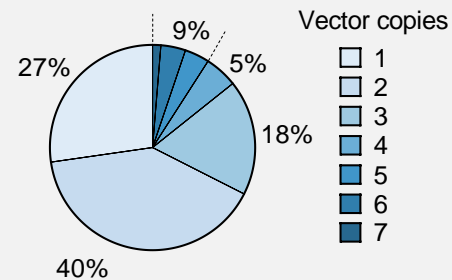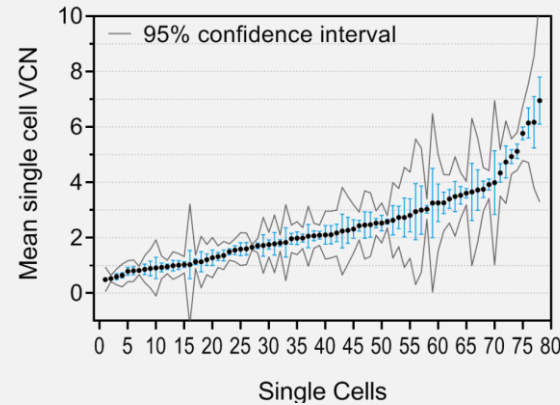# The scVCN assay predicts different distribution ranges

Low bulk VCN

N= 78 cells

Mean of scVCN predictions= 1.36
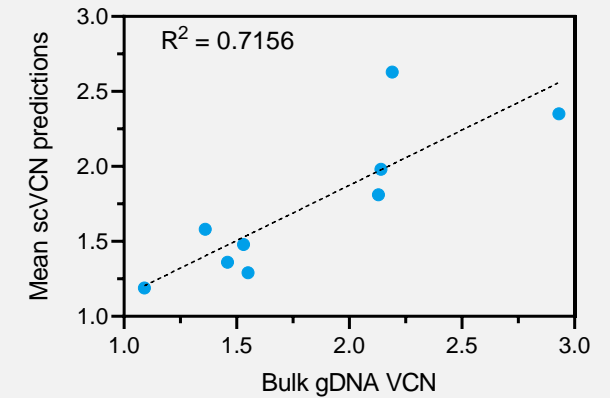Bulk gDNA VCN/cell= 1.46 ± 0.32

High bulk VCN

N= 77 cells

Mean of scVCN predictions= 2.35
Bulk gDNA VCN/cell= 2.93 ± 0.12
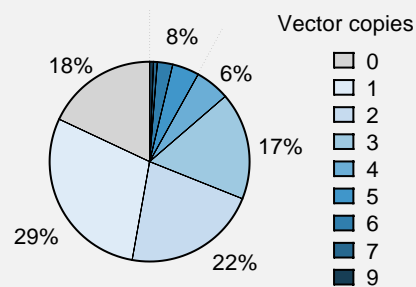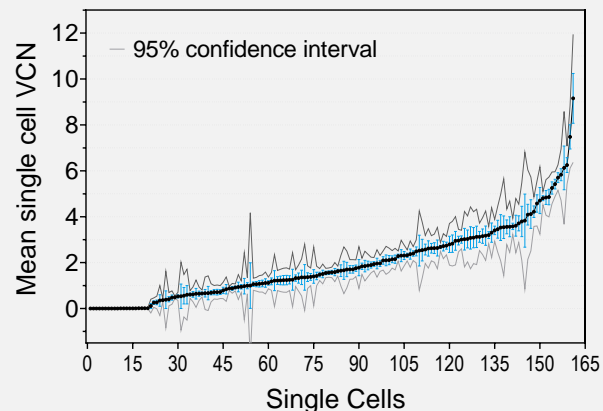
Correlation with bulk population

$R^2 = 0.7156$

- Accurate prediction compared to bulk gDNA VCN

- Difference from population analysis lower than 20%

*VCN = vector copy number

# An in-depth analytical characterisation of gene-modified cell therapy products

Vector Copies Distribution

Mean single cell VCN vs Single Cells, with 95% confidence interval.

Pie chart — Vector copies:
- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 9

8%, 6%, 17%, 22%, 29%, 18%

N= 161 single cells

Mean of scVCN predictions= 1.93
Bulk gDNA VCN/cell= 2.31 ± 0.24

Reproducibility across sampling

Number of cells vs Vector copies, Chip1 and Chip2.
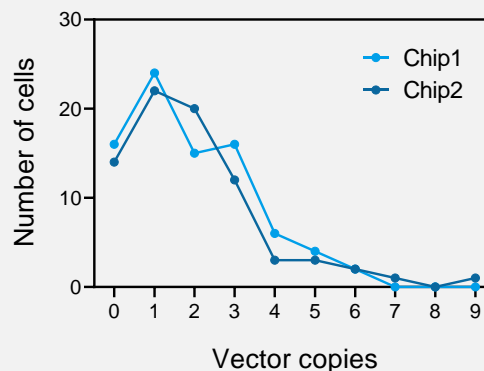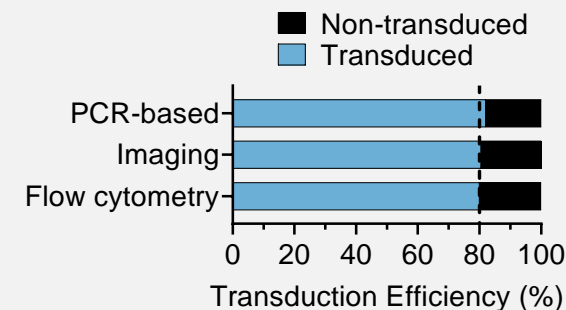
Population size can be scaled out by increasing the number of microfluidic chips.

Single cell distribution is consistent across two microfluidic chips.

Label-free Transduction Efficiency

Transduction Efficiency (%): PCR-based, Imaging, Flow cytometry. Non-transduced / Transduced.

Rapid analysis of % transduced cells using a digital PCR-based approach at a single cell level.

*VCN = vector copy number

Take home message

Key points

- One assay for two critical quality attributes (VCN and transduction efficiency).

- Information on heterogeneity of sample/process.

- Possibility to scale out to increase analysis throughput.

- Useful as process diagnostic tool to monitor process consistency and inform on cost of goods.

- Advanced tool to monitor safety of the product (e.g. % of clones with >5 vector integrations, as per FDA recommendation).

- Flexible and customizable to analyse any transgene in any cell type.

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