Single cell analysis of lentiviral integration to support ex-vivo gene modified cell therapy development

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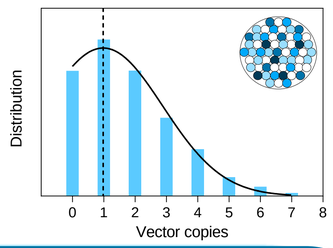
BACKGROUND

Ex-vivo gene-modified cell therapies are increasingly being developed for the treatment of monogenic diseases and cancers. Product safety and consistency of manufacturing are key to the success of the cell therapy field. The generation of genetically-modified cells often entails the use of lentiviral or retroviral vectors, which randomly integrate into the host genome posing a risk for insertional mutagenesis, while product variability is one of the main burdens to successful product generation.

CHALLENGE

Analytical characterisation is fundamental to monitor product variability and safety, but current analytical methods are often inadequate or laborious and only partially aid the evaluation of product's attributes. In particular, population analysis is the standard approach, yet this does not provides insights on the underlying cell-to-cell variability.



PROPOSED SOLUTION

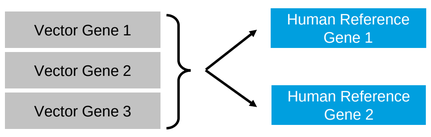
We developed a novel analytical workflow to analyse vector copy number at a single cell level and demonstrated that this is representative of the population of transduced cells. The readout of this assay also provides label-free, PCR-based transduction efficiency, overcoming the challenge of measuring this specification when the standard flow cytometry approach is not applicable.

METHOD

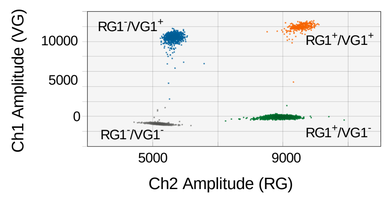
- Lentiviral vector expressing ZsGreen used to transduce CD3$^+$ T cells purified from healthy donors.
- Single cell isolation and processing performed on the Fluidigm C1 system.
- Droplet digital PCR (ddPCR) used as readout.
- An in-house analysis framework based on Bayesian statistics to convert measured data to integer copy number predictions.

1. Multiplex ddPCR can be used on gDNA to measure population Vector Copy Number (pVCN)

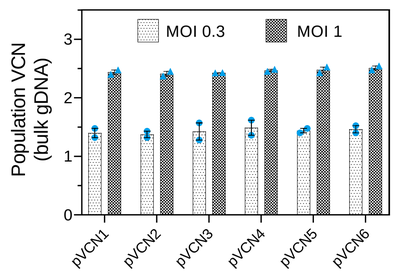
Multiplex TaqMan ddPCR reactions



Exemplar 2D ddPCR amplitude plot



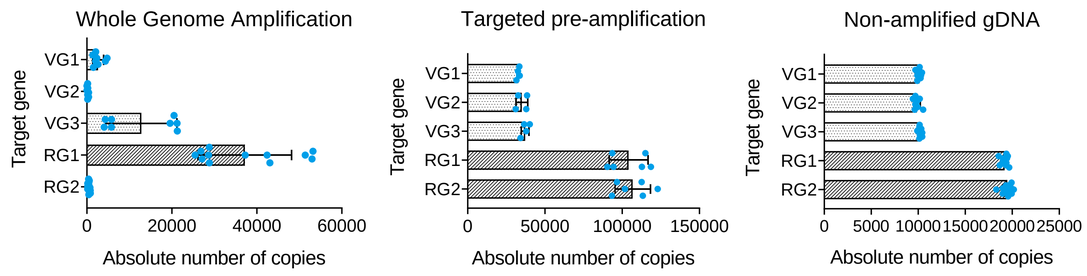
pVCN measured on two samples transduced at differing MOI levels



- All six combinations of multiplex ddPCR reactions provide consistent vector copy number results.
- pVCN analysis by ddPCR is precise and robust on gDNA from transduced cells.

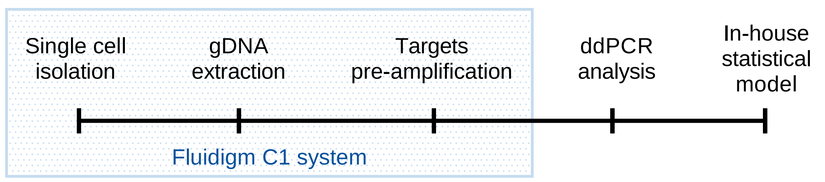
2. A targeted approach is required for linear pre-amplification of the target gene sequences

Representative graphs comparing pre-amplification strategies on gDNA as analysed by ddPCR



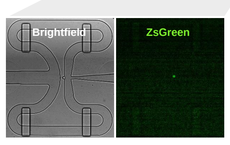
- Three commercial whole genome amplification (WGA) kits and two pre-amplification master mixes tested.
- High variability in targets' amplification observed in all WGA kits.
- Uniform amplification of targets observed with a targeted approach, comparable to non-amplified gDNA quantification.

3. Development of the single cell VCN (scVCN) workflow

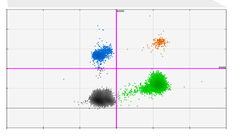


Single cell isolation in a closed automated system

Automated droplet digital PCR analysis

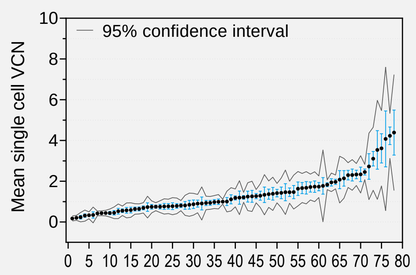


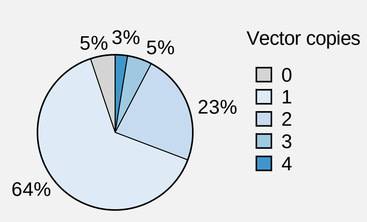
Quality control of cells isolation and number



Absolute quantification of viral and human targets

scVCN analysis on "low" pVCN sample

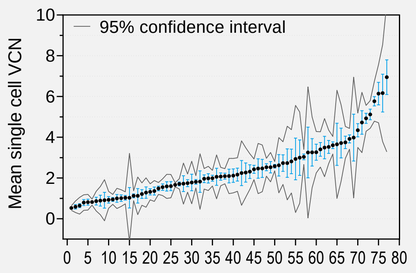


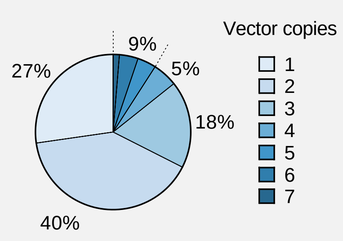


N= 78 cells

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

scVCN analysis on "high" pVCN sample





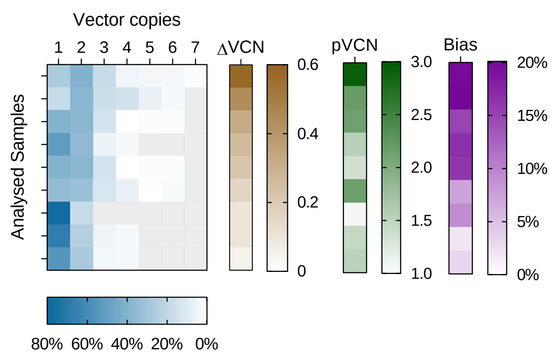
N= 77 cells

Mean of scVCN predictions= 2.35
Bulk gDNA VCN/cell= 2.93 ± 0.12

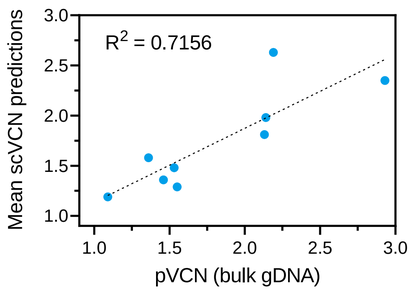
- Single cell VCN analysis can estimate the number of vector units in each analysed single cell.
- Bayesian statistics is applied to convert measured scVCN values by ddPCR into integer values corresponding to vector units per cell.
- Comparison of scVCN mean with the population VCN from gDNA analysis indicates that the assay can be predictive of the cell population specifications.

4. Accuracy of the scVCN assay is verified on a larger sample set

scVCN distribution across nine samples compared to the population VCN and assay bias



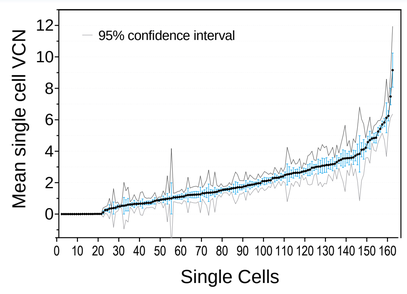
Linear regression comparing scVCN mean with population VCN

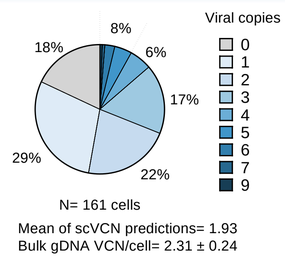


- Comparison of scVCN predictions mean with pVCN is used to estimate the accuracy of the assay.
- The absolute difference between these values ranges from 0 to 0.6 copies, corresponding to an accuracy ≥ 80%.
- Linear regression test shows positive correlation between population and single cell VCN.

5. scVCN assay optimisation for gene-modified cell therapy products

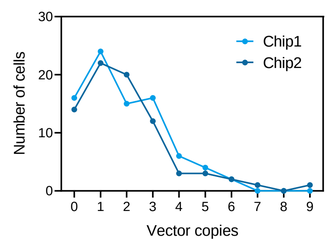
scVCN analysis on a non-FACS sorted sample

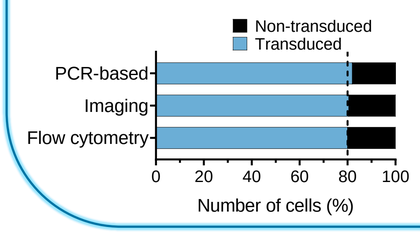




N= 161 cells

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





- The scVCN assay can be applied to non-sorted cell sample used as a proxy for a typical cell therapy product.
- The sample size can be increased by analysing multiple microfluidics chips for the same population. No statistical difference between scVCN distributions is observed here (p = 0.4297, two-tail Wilcoxon test).
- PCR-based transduction efficiency can be measured with this assay and is comparable to conventional flow cytometry or imaging analysis.

Conclusions

- We designed a novel analytical method for advanced analysis of vector copy number and label-free transduction efficiency at a single cell level to improve the characterisation of gene-modified cell therapy products.
- The scVCN assay can improve the control over the safety of gene-modified products by indicating the proportion of single cell with a high number of vector integrations, which are generally associated with higher risk of insertional mutagenesis, before therapy delivery and during follow-up studies.
- We envisage a broad applicability of the method to monitor product's variability due to process, donor-to-donor, and operational variations.
- Although it was developed on a lentiviral-based cell therapy model system, we anticipate this to be amenable to VCN analysis of any transgene from viral or non-viral vectors, by simple modification of the primer sequences.

We work with Innovate UK

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