A Novel Flow Cytometry Method for Rapid Assessment of Lentiviral Detection

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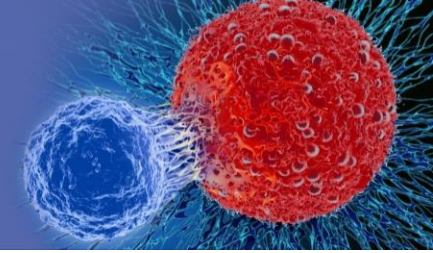
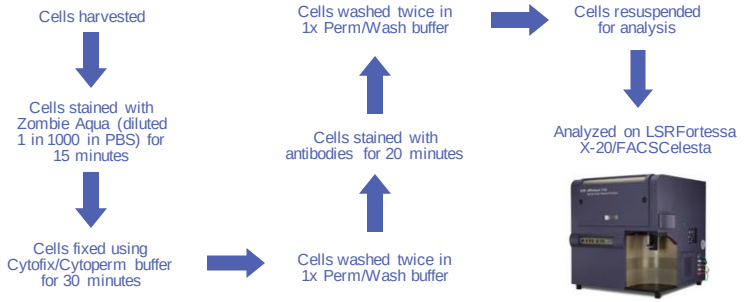


Introduction

- Lentiviral vectors are commonly used in gene and cell therapies for cancer treatment.[1]
- They facilitate transduction of T-cells with genes encoding engineered chimeric antigen receptors and T-cell receptors (TCRs).[1]
- To assess lentiviral vector batch-to-batch variability, whether it is in process development, manufacturing, or product characterization, a range of analytical methods are required.[2]
- Current analytical tools to assess vector titer, by measuring the transduction of peripheral blood lymphocytes (PBLs), are low throughput and slow to deliver data, taking several days to complete.
- Here, we describe a novel flow cytometry method, called "Intracellular Vector Component Staining" (IVeCS), that offers fast, high-throughput evaluation of in-process vector titers.

Figure 1. IVeCS Workflow



Cytofix/Cytoperm (BD, Franklin Lakes, NJ); IVeCS, Intracellular Vector Component Staining; LSRFortessa X-20/FACSCelesta (BD, Franklin Lakes, NJ); PermWash (BD, Franklin Lakes, NJ); PBS, phosphate buffered saline; Zombie Aqua (BioLegend, San Diego, CA).

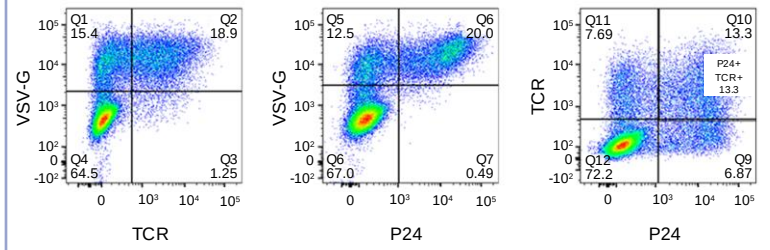
Methods

- An IVeCS method was developed to evaluate vector transfection levels in human embryonic kidney 293T (HEK293T) cells (**Figure 1**).
- A range of antibodies were assessed to determine their suitability to bind key vector components within the cells.
- One antibody was optimized for each of the 3 essential proteins, vesicular stomatitis virus G protein (VSV-G), gag-pol (P24), and TCR, and then used to measure levels of expression by the HEK293T cells during vector production, in parallel with harvesting of the vector material.
- These data were compared with a conventional method of measuring vector titer involving the time-consuming transduction of PBLs.
  - Linear regression analysis was used to determine if there was a correlation between expression of the 3 proteins tested and the titer of the vector harvest.
- Our new method to evaluate protein vector components can be completed within 3 hours of the vector material harvest.
- The proteins can be checked at any stage post transfection of the vector manufacturing process.

Results

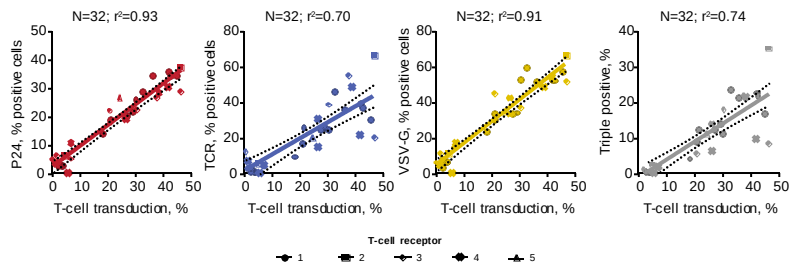
- We successfully optimized antibodies recognizing 3 key proteins in the same flow panel (**Figure 2**).
- There was a strong correlation between the biological titers produced by the conventional method of transducing PBLs and VSV-G and P24 expression (**Figure 3**; r^2>0.9).
  - Correlation between TCR and T-cell transduction efficiency was more variable than the other two components measured, potentially due to the dependency of TCR antibody used on the TCR being tested.
  - Level of triple positive cells was more variable than P24 or VSV-G staining and aligns more closely to the level of TCR staining.
- IVeCS method was optimized for vectors coding for multiple TCRs (**Figure 4**).

Figure 2. Relationship Between 3 Key Proteins Marking Co-Transfection



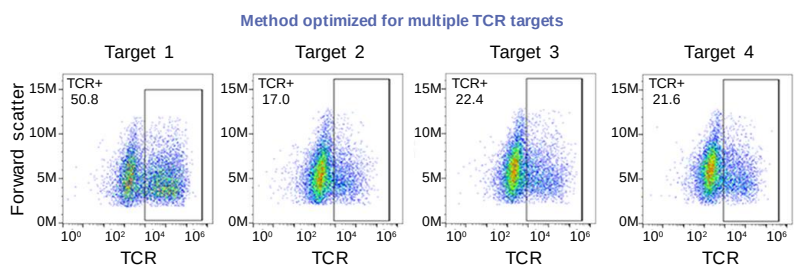
P24, gag-pol; TCR, T-cell receptor; VSV-G, vesicular stomatitis virus G protein.

Figure 3. Correlation Between P24, TCR, and VSV-G Expression and Biological Titer



Linear regression protein analysis. Dashed lines are 95% confidence intervals of best-fit line. Data from TCRs 1–5 were collected via separate experiments. P24, gag-pol; TCR, T-cell receptor; VSV-G, vesicular stomatitis virus G.

Figure 4. HEK293T Cells Stained for TCR Protein Expression



HEK293T, human embryonic kidney 293T; M, million; TCR, T-cell receptor.

Conclusions

- We developed a flow cytometry method, called IVeCS, to allow rapid assessment of vector protein levels expressed within HEK293T cells post-transfection.
- We have shown strong correlation between lentiviral vector protein expression and PBL titer.
- This new method can be used as a predictive tool when comparing vector production runs under the same conditions.
- Being able to monitor protein expression of key vector components during vector manufacture has led to process improvements.

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References
1. Transfiguracion J, et al. *Mol Ther Methods Clin Dev*. 2020;18:803. 2. Murphy ME, et al. *Mol Ther Methods Clin Dev*. 2016;3:16005.

Abbreviations Used in Text
HEK, human embryonic kidney; IVeCS, Intracellular Vector Component Staining; PBL, peripheral blood lymphocyte; TCR, T-cell receptor; VSV-G, vesicular stomatitis virus G protein.

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