# Improving quantification of rSIV.F/HN vectors for cystic fibrosis gene therapy using droplet digital PCR

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We are developing viral and non-viral vectors for the treatment of a range of acquired and inherited respiratory disorders. We have previously demonstrated that a minimal, self-inactivating, replication-defective simian immunodeficiency virus (rSIV) vector, pseudotyped with the F and HN glycoproteins from Sendai virus (the murine form of human parainfluenza virus I) has high tropism for multiple cell types found within both the conducting airway epithelium and the gas exchanging alveolar regions of the mouse, sheep and human lung. To facilitate clinical development, we routinely produce a large number (multiple Lots/week; target >1e10 TU) of highly purified (anion-exchange/TFF), high-concentration (target >1e9 TU/mL) research-grade preparations of rSIV.F/HN vectors. Our animal-free process is centred on a rocking (WAVE, GE) bioreactor upstream production workflow that utilises suspension-adapted HEK293T cells. We typically use two titre assays to assess vector Lots and to normalise vector dose between studies: (i) a viral particle assay (reported as VP/mL) is derived from the concentration of viral RNA found within vector Lots and is evaluated using real-time qRT-PCR, (ii) a transducing assay (reported as TU/mL) is derived from the concentration of vector-derived DNA within cells transduced with dilutions of viral Lots and is evaluated using real-time qPCR. While both approaches are widely used, and accepted by multiple competent authorities, they can suffer from significant inter- and intra- assay variation. Furthermore, they are dependent on either standard curves of *in vitro* transcribed RNA or linearised DNA molecules, both of which (for optimal assay performance) need to be prepared in a matrix that mimics the ultimate analytes. Interestingly, droplet digital PCR (ddPCR) methods offer the opportunity to quantify nucleic acid targets using the robust three-primer chemistry utilised in the real-time qPCR approach without the need to prepare, qualify and maintain absolute standard curves. After defining optimal primer, target and amplification conditions, the

detection range and lower limit of quantification (LLOQ) of our ddPCR assay was determined using a plasmid construct containing one copy of the *WPRE* target sequence. Under our assay conditions, we found the relationship between the amount of template and number of copies of the target gene was linear between 41 and 2897 copies (linear regression $r^2$ 0.998, $p<0.001$) while the LLOQ was determined to be 31 copies. We subsequently compared the use of qPCR and ddPCR approaches as the final quantification step in our TU/mL assay. A dilution series of a laboratory reference rSIV.F/HN vector Lot was used to transduce suspension-adapted Gibco™ Viral Production Cells. Seventy two hours post-transduction, cellular DNA was extracted using our standard column-based approach (Qiagen 96 well DNeasy kit) and quantified for levels of both WPRE (rSIV.F/HN vector target) and CFTR (single-copy cellular refence gene) using both qPCR and ddPCR. The qPCR method indicated the LV product contained 7.29e8 TU/mL with a relative standard deviation (RSD) of 59% whereas the ddPCR method produced a more robust and repeatable value of 2.28e8 TU/mL (21% RSD). We are now extending the use of ddPCR to our VP/mL assay where the use of transcribed RNA standard for our qPCR approach is particularly time-consuming. Additionally, ddPCR appears to be more robust to variations in matrix composition allowing in-process samples to be analysed without extensive sample preparation – a potentially significant saving in time, effort and cost.