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## An optimised protocol to generate high titre lentiviral vectors by extended HEK293T culture following transient transfection and suspension culture

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HIV-1 based lentiviral viruses are considered powerful and versatile gene therapy vectors to deliver therapeutic genes to patients with hereditary or acquired diseases. These vectors can efficiently transduce a variety of cell types when dividing or nondividing to provide permanent delivery and long-term gene expression. Demand for scalable manufacturing protocols able to generate enough high titre vector for widespread use of this technology is increasing. Current methods for LV production either use transient transfection of producer cell lines or production from isolated clonal producer cells engineered to generate vector permanently. Cells can be grown at scale either in 2D relying on culturing producer cells in multi-tray flasks or in roller bottles or cells can be adapted to grow in 3D suspensions in large batch fermenters. Transient transfection is, however, ideal to rapidly generate vector to test for efficacy. In this study, we compared cell survival and LV titres using three different transfection reagents: polyethyleneimine, Fugene® 6 and Genejuice®. LV was produced routinely at titres of 10<sup>9</sup> TU/ml by traditional monolayer conditions for up to 10 days instead of 72 hours using Genejuice® with little drop in titre due to low cellular toxicity. Genejuice® also enabled rapid conversion of HEK293T from 2D to 3D suspension cultures generating titres of 10<sup>a</sup> TU/ml in glass bottles. We propose, these simple changes in vector production enables the generation of larger volumes of high titre vector in a cost-effective manner.