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Feasibility of Lentiviral Vector Production using a Novel Airlift Bioreactor

Matthew B. Welty[1], Qyunh Mai Trinh[1], Emily L. Hopewell[1], Adam Ostrowski[2], Daniela Bischof[1]
[1]Indiana University, Indianapolis, IN, [2]Cellexus International Ltd., Dundee, UK

ABSTRACT

Background & Aim:

With more cell and gene therapy products reaching the clinic, there is a growing demand for lentiviral vectors. Due to the large number of flasks required for adherent cell lentiviral vector production, manufacturers have turned to bioreactors and suspension adapted cells to reduce space requirements, labor, and costs. Traditional stirred tank bioreactors use mechanical agitation to provide mixing and aeration of the culture volume. This method of agitation can generate large shear forces that can damage the health of the cells thus reducing lentiviral vector yields.

Cellexus has developed an airlift bioreactor system to reduce these shear forces. Instead of mechanical agitation, this bioreactor uses gas sparging to gently mix the culture volume while still maintaining vessel homogeneity and sufficient aeration. In this experiment, a suspension adapted HEK293 cell line was expanded in the CellMaker bioreactor and then transiently transfected to produce lentiviral vector. The vector was then tested for functional titer.

Methods, Results, and Conclusion:

IU293T(S), an HEK293T suspension adapted cell line developed by IU School of Medicine, cells were expanded in shake flasks and finally the Cellexus CellMaker airlift bioreactor. The cultures were also supplemented with Antifoam C and Pluronic™ F-68 to reduce foaming. Samples were periodically collected to generate a growth curve. Once a sufficient number of cells had been generated for a 6L batch, they were transiently transfected using a third-generation plasmid system.

This system included a gag and pol containing plasmid, VSVG envelope plasmid, Rev containing plasmid, and a lentiviral vector plasmid expressing GFP driven by a CMV promoter. PEIpro® transfection reagent and sodium butyrate were used to enhance transfection. 64 hours after transfection the supernatant was collected, clarified, aliquoted, and stored at -80°C.

Aliquots were then thawed and used to obtain p24 values. Adherent HEK293 cells were then transduced with the vector and subsequent GFP expression levels measured by flow cytometry to determine the functional titer.

These results demonstrate the feasibility of using the Cellexus CellMaker bioreactor for lentiviral vector production.

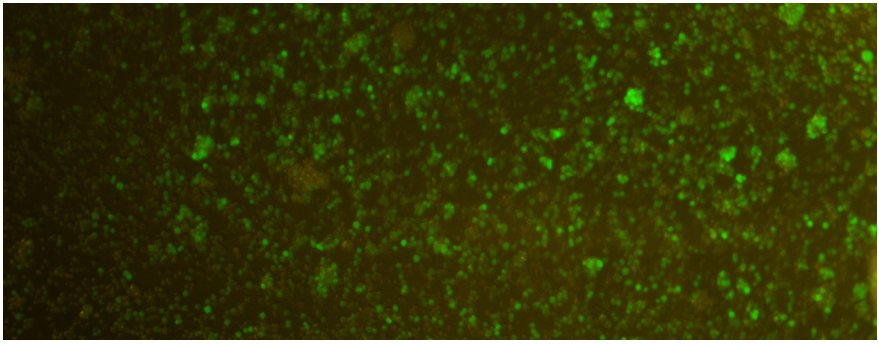

Fig 1: HEK293T cells were transfected with a third-generation lentiviral vector expressing GFP. Prior to harvesting the supernatant, a sample was collected and observed under a fluorescent microscope. 4x.