

**Docket #:** S95-004

# **Rapid, Stable, High titre production of Recombinant Retrovirus**

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This method of rapid production of high titre retrovirus for large scale production involves a retrovirus construct with an Epstein Barr Virus origin of replication and EBNA protein.

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The nuclear replication and retention functions of the Epstein Barr virus have been used to maintain retroviral vectors episomally with in human-based retroviral packaging cell lines. These hybrid EBV/retroviral vectors are capable of producing helper-free recombinant retrovirus as soon as 48 hours, and for at least 30 days, after transfection into 293T-based ecotropic and/or amphotropic retroviral packaging cells. Viral titers greater than  $10^7$  CFU/ml were obtained after puromycin selection of transfected retrovirus packaging cell lines. This episomal approach to retroviral production circumvents limitations inherent in transient and chromosomally-stable retroviral producer systems thereby affording reproducibly rapid, large scale, high titer retrovirus production.

## **Innovators**

- Todd Kinsella
- Garry Nolan

## **Licensing Contact**

**Michael Bellas**

Licensing Associate

[Email](#)