

**Docket #:** S96-139

# **Phi-Nx Helper-free Retrovirus Producer Lines**

The Nolan laboratory has created second-generation retrovirus producer lines, termed Phoenix, for the generation of helper free ecotropic and amphotropic retroviruses. The lines are based on the 293T cell line (a human embryonic kidney line transformed with adenovirus E1a and carrying a temperature sensitive T antigen co-selected with neomycin). The unique feature of this cell line is that it is highly transfectable with either calcium phosphate-mediated transfection or lipid-based transfection protocols--up to 50% or higher of cells can be transiently transfected.

Two cell lines were created, Phoenix-ECO and Phoenix-AMPHO. Both lines were tested for helper virus production and established as being helper-virus free and are fully compatible for library generation for retroviral gene transfer experiments. The titres produced by these lines using standard polybrene-enhanced retroviral infection were approaching or above  $10^7$  per ml for both Phoenix-Eco and Phoenix-Ampho when carrying episomal constructs. When transiently produced virus is made, titres are usually 1/2 to 1/3 that value.

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