Application Note: AN-LECF-RETROV-0408

Concentration of Retrovirus Using Thermo Scientific Sorvall® WX Ultra Centrifuge and Thermo Scientific SureSpin™ 630 Swinging Bucket Titanium Rotor

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Key Words

- Ultracentrifugation
- Retrovirus Concentration
- Swinging Bucket Rotor

Introduction

Protein function can be determined by using gain- or loss- of function experiments including the induction of exogenous gene expression in multiple cell lines and *in vivo* models. The utilization of viral transduction to induce exogenous gene expression is becoming an increasingly common technology. This technology is more efficient than transfection as you can selectively target cell populations by using multiple virus types (ie, retrovirus, adenovirus, lentivirus), gene expression is increased in difficult to transfect cell types, and gene expression in infected cells is permanent (Cepko and Pear, 1996). Multiple methods can be used to conduct viral transduction including incubation of target cell populations with dilute or concentrated viral supernatant. Furthermore, concentrated virus can be used for the rapid induction of genetic expression *in vivo* which can eliminate the need for costly and time-consuming transgenic mouse models (Gaiano et al., 1999). The concentration of virus can be completed by ultracentrifugation of viral supernatant harvested from transfected packaging cell lines.

The procedure described below outlines the process for the production and concentration of retrovirus using Thermo Scientific Sorvall WX Ultra Centrifuge and Thermo Scientific SureSpin 630 Swinging Bucket Titanium Rotor. By incubating cortical progenitors with concentrated retrovirus produced by this method, we were able to selectively target gene expression (identified by green fluorescent protein, GFP) to dividing neural progenitors, a primary cell line that has difficulty with exogenous gene expression.

Procedure

Production of dilute virus

- Transfect GP2-293 derived packaging cell line (Clontech) at 90% confluency with 5µg each of pCLE-IRES2-eGFP (Anjen Chenn, Northwestern University, Chicago, IL) and pVSV-G (Clontech) using Lipofectamine™ and PLUS™ Reagent (Invitrogen)
- Approximately 18hrs post-transfection, replace the culture media with fresh media according to manufacturer's instructions
- Place transfected GP2-293 cells at 32°C and 5% CO_2 for virus production
- Harvest virus by removing viral supernatant from confluent monolayer of transfected cells 5 to 6 times at 12 hr intervals and placing into conical tube
- Centrifuge conical tube in Thermo Scientific lowspeed centrifuge at 200 x g for 5 mins at room temperature
- Syringe tube filter viral supernatant through a 45µm filter into new conical
- Freeze each filtered harvest at -80°C

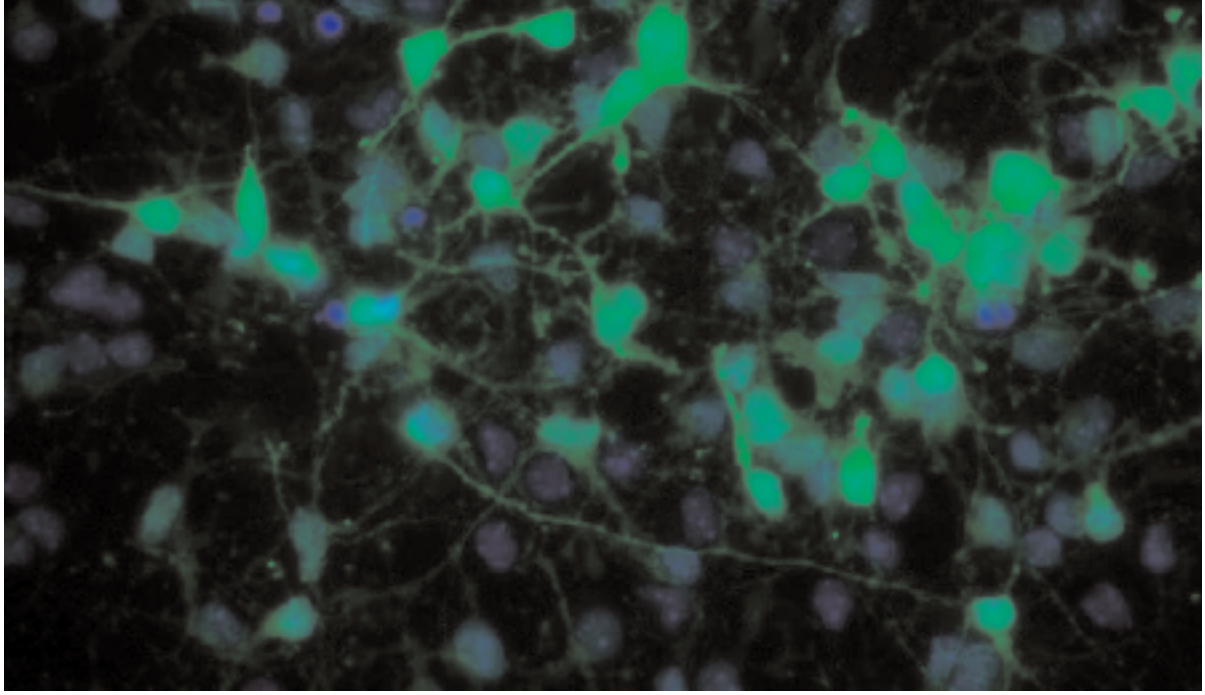

FIGURE 1. Retrovirally infected neural progenitor cells identified by GFP expression. Blue, nuclei, Green, Green Fluorescent Protein.

Concentration of dilute virus

- Thaw viral harvests at room temperature
- Remove viral harvest(s) into PA Thin-Walled Tubes (36 mL, cat #: 03141)
- Place balanced tubes into Thermo Scientific SureSpin 630 (36 mL) Swinging Bucket Titanium Rotor
- Centrifuge tubes in WX Ultra Centrifuge at 112,700 x g (25,000rpm) at 4°C for 1.5 hrs
- Remove supernatant from tubes, leaving viral pellet at the bottom
- Resuspend viral pellet in 40 µL sterile PBS overnight at 4°C and aliquot into small volume working stocks in microcentrifuge tubes
- Store aliquots at -80°C

Discussion

The procedure above describes a method to produce concentrated retrovirus using the WX Ultra Centrifuge and SureSpin 630 Swinging Bucket Rotor. Using concentrated retrovirus we were able to selectively infect dividing neural progenitor cells as detected by GFP expression in small volume without disturbing the culture conditions of the cells (Figure 1, Noles and Chenn, 2007). The above procedure can also be used to produce concentrated retrovirus to induce rapid exogenous gene expression in multiple *in vivo* models (ie, mouse and chick) without the need to create expensive and time consuming transgenic mouse models.

References

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