Test For Replication Competent Retrovirus

Report Date: 10/14/2015
Report Type: Validation Certificate
Type of Test: Marker Rescue Assay
Part #: 93-0270C2
Cell Line Tested: PathHunter® CHO-K1 CALCRL-RAMP2 β-Arrestin Cell Line
Permissive Host: CHO-K1
Markers Tested: Hygromycin, Puromycin, and G418

Introduction

DiscoverX introduces genetic material into a number of specifically engineered mammalian cells using replication incompetent retroviruses derived from Moloney Murine Leukemia Virus. This document describes the background of the engineered cell lines and reports on the subsequent validation tests done to indicate the cell lines to be free of replication competent retrovirus.

Background of the Cell line

In order to create productive virus a second generation packaging cell line was employed (*http://www.stanford.edu/group/nolan/retroviral_systems/phx.html*). This packaging cell line has been validated to be free of helper virus and to not produce replication competent retrovirus in the absence of exogenously added viral components. The *gag-pol* and *env* proteins are encoded by two different constructs and there is no sequence overlap between the *env* fragment and the LTRs. Moreover, different promoters are employed for the *gag-pol* and *env* constructs to minimize inter-recombination potential.

Cell line testing procedure (Marker Rescue Assay)

The cell line of interest (test cells) were thawed and seeded into one well of a 6-well dish in complete medium. Target cells (permissive host) were thawed and seeded into one well of a separate 6-well dish in complete medium. Once test cells reached confluency (~2 days), the medium in the target cell plate was removed and replaced with medium from the test cells. Target cells were then placed under antibiotic selection (as indicated in the table on page 2) in the presence of polybrene (an agent that facilitates retroviral infection) for 10 days. After selection, surviving cells were stained with a crystal violet solution for 30 minutes, and then washed and scored for live cells. Since crystal violet only stains live cells, positive (+) blue cells indicate the presence of helper virus in the supernatant from test cells. No blue cells indicate absence of any helper virus in the test cell supernatant.

Retroviral Inserts and Marker Selection:

	Overexpressed Targets	Marker
Target 1	CALCRL-PK	G418
Target 2	β-Arrestin2-EA	Hygromycin
Target 3	RAMP2-Stop	Puromycin

Antibiotic Marker	Antibiotic Concentration
G418	800 µg/mL
Hygromycin	300 µg/mL
Puromycin	2.5 µg/mL

Results

Supernatant Host	Selection	# of colonies
Test cells: PathHunter® CALCRL-RAMP2	G418	0
Test cells: PathHunter® CALCRL-RAMP2	Hygromycin	N/A
Test cells: PathHunter® CALCRL-RAMP2	Puromycin	N/A
- Control: CHO-K1	G418	0
- Control: CHO-K1	Hygromycin	N/A
- Control: CHO-K1	Puromycin	N/A
+ Control: G418	G418	>300
+ Control: Hygromycin	Hygromycin	N/A
+ Control: Puromycin	Puromycin	N/A

Hygromycin Positive control: Stable cell line expressing a hygromycin resistant marker
G418 Positive control: Stable cell line expressing a G418 resistant marker
Puromycin Positive control: Stable cell line expressing a puromycin resistant marker

Conclusions

The absence of any detectable transfer of resistance from the host line to the permissive cell line indicates that this cell line is free of any detectable replication competent retrovirus.