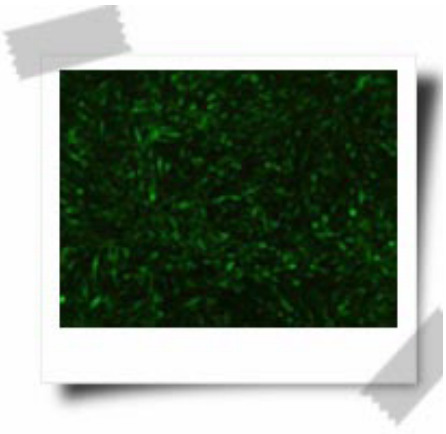
Gryphon™ Retroviral Packaging Cell Lines (Ampho and Eco)

Website: www.allelebiotech.com
Call: 1-800-991-RNAi/858-587-6645 (Pacific Time: 9:00AM~5:00PM)
Email: oligo@allelebiotech.com

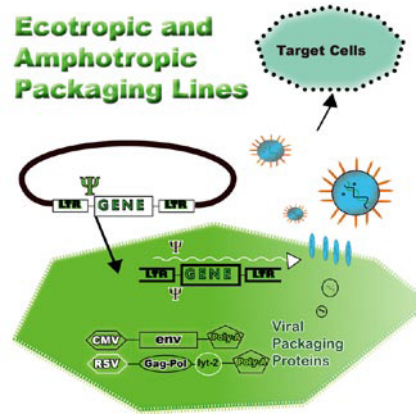
For Technical Support:
Email:vivec@allelebiotech.com

Safety Issues: Retroviral vectors should be handled using NIH BSL - 2 safety guidelines. For more information, please see Biosafety in Microbiological and Biomedical Laboratories <4th edition> which is available on the Web sites of the National Institutes of Health at http://bmbl.od.nih.gov.



Note:

Gryphon™ Ampho and Eco packaging cell lines are second-generation retrovirus producer lines for the generation of helper free amphotropic and ecotropic retroviruses. They are currently used in over 2500 laboratories worldwide for delivery of genes and libraries to cells for biomedical research.



The lines were created by placing into 293T cells (a human embryonic kidney line transformed with adenovirus E1a and carrying a temperature sensitive T antigen co-selected with neomycin) constructs capable of producing gag-pol, and envelope protein for ecotropic and amphotropic viruses. Gag-pol was introduced with hygromycin as the co-selectable marker.The envelope proteins were introduced with diphtheria toxin resistance as the co-selectable marker. An IRES-CD8 surface marker was also introduced downstream of the reading frame of the gag-pol construct to monitor gag-pol production which can be readily monitored by flow cytometry. For both the gag-pol and envelope constructs different non-Moloney promoters were used to minimize recombination potential.

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. That promises even with minimum amount of background knowledge or experience of working with viruses, you can produce infection-ready viruses by easily transfecting and collecting.

Gryphon™ Ampho Cell Line (amphotropic) is to deliver genes to dividing cells of most mammalian species, including human, but not hamster. Gryphon™ Eco Cell Line (eotropic) is to deliver genes to dividing cells of murine or rat. (See Product List below)

Product List

Gryphon™ Ampho Cells Line	ABP-RVC-10001	10^6 cells in 1 vial
Gryphon™ Eco Cells Line	ABP-RVC-10002	10^6 cells in 1 vial

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Protocols

Storage: Store in liquid nitrogen

Content: 10^6 cell/vial

Protocols:

A. Thawing Gryphon™ Cells:

1. Remove the vial containing frozen cells from liquid nitrogen or shipping box. Thaw rapidly at 37°C by holding the vial and gently shaking in the water bath. Take out the vial from the water bath when the frozen cells start to thaw (about 1-2 minutes). The key point is NOT to let the cells thaw completely.

2. Immediately add 1 ml of Growth Medium (High glucose DMEM containing 10% heat inactivated fetal bovine serum, 100 U/ml Penicillin, 100 U/ml Streptomycin, 2 mM L-Glutamine) to the cells and gently transfer them to a 15 ml sterile conical screw cap tube.

3. Add 2 ml of GM and gently mix the cells to allow osmotic equilibration.

4. Add 10 ml of GM, close the tube, invert the tube several times and spin cells at 500 x g for five minutes.

5. Remove the supernatant, resuspend cell pellet in GM, and transfer cells to a 10 cm tissue culture dish.

Note: It is important to freeze multiple vials of each producer cell line after first receiving and expanding them to ensure a ready supply of backup vials to allow for uniform virus production over several years. If the cells are to be carried in selective media, this should not be applied until after the first passage.

B. Growth and passage of Gryphon™ cells:

Gryphon™ cells derived from 293 cells are carried in GM and grown in a 37°C incubator supplied with 5% CO2. To split and passage the cell lines:

1. Gently rinse cultured cells 1x with PBS (without Ca++ or Mg++).

2. Trypsinize (.05% trypsin/0.53 mM EDTA) until the cells easily detach and can be readily pipetted into a single cell suspension.

3. Trypsinization is quenched with GM prior to subculture in fresh medium.

Note: Do not split the cells at densities more dilute than 1:5 in order to maintain the uniformity of the cells in culture and minimize the outgrowth of clonal variants. The cells should not be allowed to grow over-confluent. This leads to the formation of cell clumps in culture which can cause uneven cell distribution after replating and result in less efficient transfection.

C. Passaging Gryphon™ cells:

To achieve optimal cell conditions, passage cells at 1:4 or 1:5 at 70-80% confluent every 2-3 days. Never let cells reach confluence since this will reduce transfection efficiency in the short term. Passage of Gryphon™ cells every few months in Hygromycin (300 ug/ml) and Diptheria Toxin (1ug/ml) containing medium for one week is recommended.

Cells can be analyzed and sorted by fluorescent activated cell scan (FACS) for expression of mouse CD8 (a proxy measure of gag-pol in this cell line) and for surface expression of envelope protein with 83A25 antibody.

D. Freezing Gryphon™ cells

1. Wash, trypsinize, and quench cells as described in B.

2. Centrifuge the cells at 500 x g for 5 min.

3. Remove the media and add 1 ml of freezing solution (Freezing Medium: 90% heat-inactivated fetal calf serum, 10% DMSO) per 106 cells.

4. Transfer to a 2 ml cryogenic vial.

5. Place the freezing vial at -70 °C overnight and transfer to liquid nitrogen on the following day.

Technical Notes

* It is recommended that during initial set up, the user optimize the system by using a retroviral vector expressing an easily detectable marker such as GFP or lacZ. Only when one is satisfied with the transfection conditions and infection rates should one proceed to using vectors with no readily detectable marker.

* The initial plating of the cells may be the most important step in successfully obtaining high retroviral titers. It is extremely important that the cells are not overly clumped and are at the correct density. If they are overconfluent, it may be necessary to split them 1:2 or 1:3 for several passages prior to plating for transfection. In general, the cells should be plated at a density so that they are 95-100% confluent at 24 hours post-transfection.

* The addition of chloroquine to the medium appears to increase retroviral titer by approximately two fold. This effect is presumably due to the lysosomal neutralizing activity of the chloroquine.

Read more notes for viral production in the manual of Gryphon Retroviral Sytem.