ADDGENE
ACQUIRE - DISTRIBUTE - DISCOVER

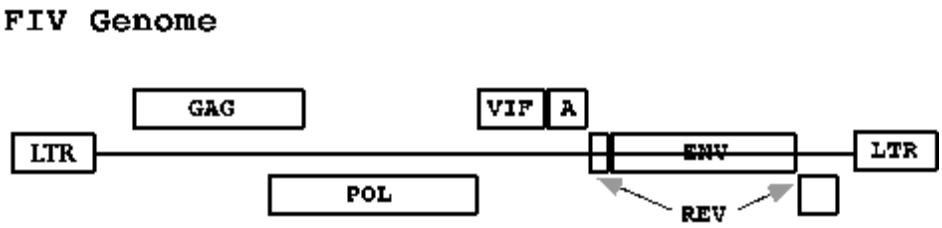
One Kendall Square, Bldg 600, 3rd Flr, Cambridge, MA 02139
Phone (617)225-9000, Fax (617)225-9004, Email:info@addgene.org

Felix Protocols

From: Nolan Lab (http://www.stanford.edu/group/nolan/index.html)
Note: A Retroviral Tutorial is also available at the Nolan Lab's website

Felix lentivirus vectors

The FELIX vector system is derived from Feline Immunodeficiency Virus (FIV). FIV is a member of the lentivirus subfamily of retroviruses, and causes an immunodeficiency syndrome in cats which closely resembles that caused by HIV in humans. The genome of FIV is shown below:



The FELIX vector system consists of three plasmids - structural, envelope, and transfer vector - which are cotransfected into 293T cells to produce virus. The structural construct makes Gag-Pol and Rev (some variants also make Vif and A). Any envelope construct can be used for pseudotyping - VSV-G tends to give the highest efficiency and broadest host range. The transfer vector contains the viral LTRs and packaging signal. Any expression cassette (promoter and gene of interest) can be cloned into the vector. Inserts can be up to 8.5kB.

FIV Biology

As with other retroviruses the FIV genome is a tightly compacted genome that makes multiple structural and regulatory proteins that allow its replication. The most important proteins are listed here:

- The Gag polyprotein is proteolytically processed into the structural components of the virion. The Gag-Pol polyprotein is made by translational frameshifting at the Gag to Pol junction. Pol is proteolytically processed to yield the protease, reverse transcriptase, integrase and dUTPase.
- Vif, as in the primate lentiviruses, is believed to control virion release from lymphocytes.
- A, like the primate lentiviral Tat, is a transcriptional activator of the LTR; however, A is only a weak transactivator and does not seem to bind to any ordered secondary structure in the

LTR.

- Rev mediates the nucleocytoplasmic export of intron-containing viral mRNAs by binding to the RRE (located in the 3' end of Envelope).
- The FIV envelope mediates binding and entry into lymphocytes and macrophages, among others, and is believed to use CXCR4 as its primary receptor.

Some applications of FELIX vectors are listed below:

- Transduction in vitro of primary, non-dividing cells (e.g. CD34+ stem cells, neurons, dendritic cells) to study their biology.
- In vivo gene transfer for gene therapy to non-dividing cells such as hepatocytes and CD34+ stem cells. 3) cDNA library screens in primary cells

FELIX Virus Production: $CaPO_4$ Transfection

1) Split 293T cells out the day before transfection. 2-2.5 X 10^6 cells/6cm plate generally works well. At the time of transfection cells should be 70-85% confluent in 3mL of DMEM w/10%FCS.

2) Add 2ul of 50mM Choloroquine to plates prior to transfection.

3) To a 15mL tube, add 5ug structural vector (e.g. CPRDEnv), 3ug transfer vector (e.g. pFLX-5CL), and 2ug envelope vector (e.g. pCI-VSVG).

4) Add 61ul of 2M $CaCl_2$ (Malinkrodt only) - try to wash the DNA down the tube.

5) Add 430ul sterile ddH_2O - must be room temperature.

6) Spin liquid to bottom of tube.

7) Add 500ul of 2XHBS and bubble 2-10sec in the water/CaCl/DNA mix. For information on making 2XHBS see the Phoenix protocol page.

8) Add the mixture dropwise to the plated cells.

9) Incubate cells at 37 degrees for 8hrs.

10) Change media to fresh DMEM w/10% FCS.

11) Change media again in 22hours.

12) Collect supernatant at 48hrs post-transfection. (NB: supernatants can be collected as early as 35 hours post-transfection and as late as 72 hours and will still contain good titre - so you can get 2 or 3 virus collections per transfection, peek is somewhere between 48 and 60 hours post. Also, moving plates to 32 degrees ~8hrs prior to collection can increase yields.)

13) Clear the supernatant by spinning in a 15mL tube at 1500RPM for 5 minutes and/or by filtering through a .45um filter. (if you are filtering you will lose some titre, this loss can be minimized by pre-wetting the filter with serum or serum-containing media and by using low-protein binding filters).

14) OPTIONAL: Virus can be concentrated by centrifugation at >40000XG for 1.5-2hrs. I use 50mL tubes in a fixed angle Beckman JA.20 or JA25.50 at 20000 RPM. Conventionally you concentrate in an ultracentifuge (SW41 or SW28 rotor) at 50000XG for 1.5hrs. The pellet should be resuspened in TNE (Tris-NaCl-EDTA).

15) Target cells are infected by adding virus-containing supernatant or purified, concentrated virus diluted in media in the presence of polybrene (1000X stock is 5mg/mL) or protamine sulfate (1000X = 8mg/mL). For best results cells should be infected in multi-well plates and then spun at 2500RPM for 90 minutes at 30degress immediately following addition of virus. Spin infection significantly enhances infectivity.

16) Place cells containing virus at 32 degrees for 6-36hrs - usual overnight works well.

17) Change infected cells to fresh media.

18) Analyze protein expression >48hr post-infection.

FELIX Virus Production: FuGENE 6 Transfection

NB: FuGENE 6 can be obtained from Boehringer-Mannheim and has proven to be the best lipid reagent for transfection of 293T cells in our hands.

1) Split 293T cells out the day before transfection. 2-2.5 X 10^6 cells/6cm plate generally works well. At the time of transfection cells should be ~50% confluent in 3mL of DMEM w/10%FCS.

2) In a sterile 1.5mL microfuge tube add 266ul of serum-free DMEM. Add 27ul FuGENE 6 directly to media without allowing it to touch the sides of the tube.

3) Incubate the FuGENE/media mixture for 5 minutes at room temperature.

4) To a second sterile 1.5mL tube add 5ug structural vector (e.g. CPR/\Env), 3ug transfer vector (e.g. pFLX-5CL), and 2ug envelope vector (e.g. pCI-VSVG).

5) Dropwise add diluted FuGENE from step 3 to the tube containing the DNA from step 4.

6) Tap the tube to mix the contents.

7) Incubate the FuGENE/DNA mix for 15minutes at room temperature.

8) Add the mixture dropwise to the plated cells. Swirl to distribute.

9) Incubate cells at 37 degrees for 16-30hours.

10) OPTIONAL: Change media to fresh DMEM w/10% FCS.

11) Change media at 30hours post-transfection.

12) Collect supernatant at 48hrs post-transfection. (NB: supernatants can be collected as early as 35 hours post-transfection and as late as 72 hours and will still contain good titre - so you can get 2 or 3 virus collections per transfection, peak is somewhere between 48 and 60 hours post. Also, moving plates to 32degrees ~8hrs prior to collection can increase yield.)

13) Clear the supernatant by spinning in a 15mL tube at 1500RPM for 5 minutes and/or by filtering through a .45um filter. (if you are filtering you will lose some titre, this loss can be minimized by pre-wetting the filter with serum or serum-containing media and by using low-protein binding filters).

14) OPTIONAL: Virus can be concentrated by centrifugation at >40000XG for 1.5-2hrs. I use 50mL tubes in a fixed angle Beckman JA.20 or JA25.50 at 20000 RPM. Conventionally you concentrate in an ultracentifuge (SW41 or SW28 rotor) at 50000XG for 1.5hrs. The pellet should be resuspened in TNE (Tris-NaCl-EDTA).

15) Target cells are infected by adding virus-containing supernatant or purified, concentrated virus diluted in media in the presence of polybrene (1000X stock is 5mg/mL) or protamine sulfate (1000X = 8mg/mL). For best results cells should be infected in multi-well plates and then spun at 2500RPM for 90 minutes at 30degress immediately following addition of virus. Spin infection significantly enhances infectivity.

16) Place cells containing virus at 32 degrees for 6-36hrs - usual overnight works well.

17) Change infected cells to fresh media.

18) Analyze protein expression >48hr post-infection. NB: This protocol can be scaled up for larger plates by multiplying ALL reagent and dilution and DNA volumes by the difference in surface area between the desired plate/flask and a 6cM plate.

Last modified: 5/4/05