FV vector is produced when the media is not changed the morning after transfection (Avg±SD, triplicate).

[0017] FIG. **12** depicts a graph showing FV Titer generated by transfection with PEI with and without media change the day post-transfection, with and without treatment of the plastic with Poly-L-Lysine (PLL). The data show that more FV vector is produced when the media is not changed the morning after transfection, using PLL-coated plates. FV-GFP (Avg±SD, triplicate).

[0018] FIG. **13** depicts a graph showing evaluation of the pattern of FV-CD18 virus production when no media was changed after transfection. Infectious titers were measured on Raw 264.7 cells (Avg±SD, triplicate). The data show that virus titers are the highest at 66 hours post-transfection.

[0019] FIG. **14** depicts a graph showing evaluation of the elution profile of FV-GFP using a salt gradient from 150 mM to 1 M NaCl after binding to a POROS-Heparin column. Data show that infectious virus eluted between 190 and 587 mM NaCl (Conductivity of 22-52 mS/cm). Based on this, the salt concentration for step elution on the AKTAReady was set at 600 mM NaCl.

[0020] FIG. **15** shows that 705 mL of FV-CD18 vector can be effectively loaded onto a 7.9 mL POROS-Heparin (at 267 cm/h with a 2.3 min residence time) without breakthrough, and eluted using 600 mM NaCl buffer with a recovery of infectious virus of 75%. The graph (top) shows the loading volume (Red Line) versus recovery of infectious FV-CD18 virus as measured on Raw 264.7 cells (Blue Curve). Chromatogram (bottom).

[0021] FIGS. **16**A-**16**C depict a graph and related data showing the concentration (20-fold) of POROS-Heparin purified FV-CD18 vector using TFF. FIG. **16**A depicts a graph showing data demonstrating stable pressures without evidence of membrane fouling, using an average Trans-Membrane Pressure (TMP) of 2 psi. FIG. **16**B depicts the figure legend for FIG. **16**A. FIG. **16**C depicts the results at a flux rate of 50 LMH.

[0022] FIG. **17** depicts photographs showing ultracentrifugation (19,000 RPM, 11° C., 2 hours) and pelleting of POROS-Heparin purified and Tangential Flow Filtration (TFF)-concentrated FV-CD18 vector using capped Optiseal BellTop Polyallomer tubes (Beckman Coulter; top pictures), blunt needle for supernate removal (bottom-left), and an extended long-range pipette tip (bottom-right) for re-suspension of the pellet and retrieval of the vector product.

[0023] FIG. **18** depicts a graph showing the effect of benzonase treatment on recovery of FV-GFP vector. Benzonase was added at the media change step post-transfection at concentrations ranging from 0 to 200 U/mL in the presence of 10 mM $MgCl_2$. The data show that FV production is not negatively affected by benzonase up to 200 U/mL for 16 hours and that benzonase can be safely used to reduce residual plasmid during production or after harvest (Avg±SD, triplicate).

[0024] FIG. **19** depicts a graph showing the effect of benzonase on the recovery of FV-GFP. Benzonase was added at 50 U/mL to cells post-transfection in the presence of 10 mM $MgCl_2$ and incubated for 16 or 40 hours (Avg±SD, triplicate). The data demonstrates that longer incubation with benzonase does not negatively impact FV titer and that benzonase treatment can be extended to 40 hours if needed.

[0025] FIG. **20** depicts a graph showing that 40 million 293T cells per T225 (or 1.8×10^5 cells/cm^2), from a range of 20 to 50 million cells per T225, is the optimal density at the time of transfection for production of FV-GFP (Avg±SD, triplicate).

[0026] FIG. **21** depicts a graph showing the evaluation of amount of FV-CD18 in tissue culture flasks supplemented with 10% or 20% fresh DMEM media with FBS at 48 hours post-transfection (Avg±SD, triplicate) to evaluate if fragility of the adherent cell layer and titers could be improved. The data show that addition of fresh media did not impact the cell layer or titer and that fragility of the cell layer is not due to nutrient depletion.

[0027] FIG. **22** depicts a graph showing that after Tangential-Flow Filtration (TFF), FV-CD18 can be filtered through a 0.8 micron filter without loss of titer, and through a 0.45 and 0.22 micron filter with minimal loss of titer showing a 94% and 84% recovery of infectious particles, respectively.

[0028] FIG. **23** depicts a graph showing a stability study of FV-GFP non-purified vector supernate shows that FV is stable for at least 3 days at 4° C. supporting purification to take place over several days with minimal loss of infectious titer. Titered in triplicate (±SE).

[0029] FIG. **24** depicts a graph showing that incubation of POROS-Heparin purified FV-GFP with NaCl at ambient temperature for 2 hours shows a loss of infectious titer with increasing NaCl concentration. This supports the notion that upon elution from the chromatography column with approximately 600 mM NaCl, product needs to be diluted to approximately 150 mM NaCl as soon as possible to preserve titer.

[0030] FIG. **25** depicts a graph showing that titer of FV vector to different concentrations of NaCl for 15 minutes, followed by dilution to 150 mM NaCl prior to storage (corrected for the dilution factor). Titer is significantly reduced by higher molarity of NaCl concentration-dependent (Avg±SD, triplicate). These data supports that FV eluted from POROS-Heparin with 600 mM NaCl should be diluted as soon as possible to isotonic conditions.

[0031] FIG. **26** depicts a graph showing that incubation of FV-GFP at pH 6.9 to 10.1 for 0-24 hours shows loss of infectious titer above pH 8.

[0032] FIG. **27** depicts a graph showing the results of experiments in which POROS-Heparin purified FV-GFP stored frozen at −80° C. in the presence of 5% DMSO was thawed, maintained at ambient temperature for 1, 2 or 3 hours, and re-frozen (Avg±SD, triplicate). Data show no loss of titer indicating that final vector product can be thawed, pooled and aliquoted without significant loss of titer.

[0033] FIG. **28** depicts a graph showing that storage of FV samples at −80° C. in the presence of 300 mM NaCl or higher and 5% DMSO rendered FV completely and irreversibly non-infectious. Percentage GFP of HT1080 cells transduced with samples derived from a POROS-Heparin chromatography run. Cells were transduced with samples fresh or after frozen storage in presence of 5% DMSO. This illustrates that FV vector cannot be stored frozen in the presence of DMSO unless in an isotonic media at 150 mM NaCl.

[0034] FIGS. **29**A-**29**F depict the optimization of FV vector transfection to maximize FV vector titers. HEK293T cells were transfected under various experimental conditions with FV vector plasmids. FV vector supernatants were harvested three days posttransfection and titer (IU/ml) was estimated by infecting HT1080 cells (FV-GFP) or RAW264.7 cells (FV-hCD18). FIG. **29**A depicts FV-GFP