Viral Particle Request Form
Viral Vector Core Facility
The Miami Project to Cure Paralysis

All information *must* be completed. Use a separate form for each type of viral particle to be produced and email to: VVC@med.miami.edu

All information will remain confidential.

Section I: Requestor Information

Date	
Requestor's Name	
Requestor's Email Address	
Requestor's Phone Number	
Principal Investigator	
Billing Account Number	

Section II: IBC Information

You *must* have IBC approval to produce and/or use viral particles.

IBC Protocol Number	
Principal Investigator	

1. Is this your first time ordering this calendar year? Select Yes ◯ No ◯

1a: If you answered "Yes", include your IBC protocol letter of approval, a copy of the protocol submitted to the IBC, and any amendments relevant to the virus to be produced.

1b: If you answered "No", does the current request fall under the same protocol? Select Yes ◯ No ◯

If you answered "Yes", the IBC documents are not required.

If No, include any relevant IBC protocol and approval letter for the new request.

Leave the following blank: the VVC will complete them.

I. Total charges: **$**

II. Prep information:

III. Special VVC notes:

Section III: General Information About Viral Particles to be Produced

1. Type of viral particles to produce (check one)? Lentivirus ◯ Adeno-associated virus (AAV) ◯

2. Will you transduce *in vitro* or *in vivo* (check one)?

3. What cell type(s) are you going to transduce? *In vitro* ◯ *In vivo* ◯ Both ◯

4. What biological question(s) will you address using these viral particles?

5. **We *cannot* produce viral particles that exceed a biohazard safety level 2 (BL2) rating.**
Describe all dangers that may be uniquely presented by production and/or use of your viral particles. Will expression of the transgene present a biohazard (e.g., encodes an oncogene, toxin, *etc*.)?

Section IV: Purity and Charges

Standard preparation includes FPLC purification for AAV particles, and ultracentrifugation for lentiviral particles. Other options are available at additional cost.

6a. **Lentiviral particles.** Standard preparations are typically ~450 µL of >2.0 x 10^{11} viral particles/mL* in 1X PBS/1%BSA. Half-size preps are also available. Aliquots are 20 µL in screw-cap vials. What size and how many preps do you want?

☐ Total number of full-size preps

☐ Total number of half-size preps (Titer same as full-size preps)

*Lentiviral concentrations are determined by ELISA for the virus p24 protein. Typical preps yield >1.0 x 10^{7} pg/mL of p24, corresponding to >1.0 x 10^{11} viral particles/mL. However, the actual transduction depends on the cell-type and other conditions, and thus the Transducing Units (TU) should be determined empirically. See LentiWeb.com for further information.

6b. **AAV particles.** AAV particles are FPLC-purified and typically yield ~200 µL of >1.0 x 10^{13} viral particles/mL (AAV-8) or >1.0 x 10^{12} viral particles/mL (AAV-2) in 1X HBSS (based on qPCR). Indicate how many preps you want and the serotype. Serotypes available: AAV1, 2, 5, 6, 8, 9, AAVretro, AAVPHP.eB and AAVPHP.S

☐ Total number of standard preps

☐ Total number of half preps (Titer same as full-size preps)

Choose serotype:		

7. Provide any additional viral particle production instructions below (e.g., higher concentration):

Section V: Information About Your Transfer Plasmid

You must provide a *high-quality, endotoxin-free maxi-prep* of your transfer plasmid that is free of genomic DNA. We make the packaging plasmids. Alternatively, we can perform the plasmid preparation for you as a separate service.

8a. Into which backbone transfer plasmid is your transgene cloned?

☐ pLenti-MP2 (lentivirus) ☐ pRRLsinPPT.CMV.MCS.Wpre (lentivirus)

☐ pAAV-MCS (AAV)

☐ Other (please describe)

8b. From whom did you obtain the backbone plasmid?

☐ Viral Vector Core

☐ Other (please specify)

9. What gene(s) of interest is inserted into the vector?

10. What is the length of your insert?

Note: The maximum insert size is ~4 kbp for many lentiviral constructs (e.g., pLenti-MP2 and pRRLsinPPT.CMV.MCS.Wpre), and ~3 kbp for many AAV (e.g., pAAV-MCS).

11. Do you have a tag or antibody for the construct? If "Yes", describe below. ☐ Yes ☐ No

12a. With which maxi-prep kit did you prepare the plasmid (vendor, kit name, and catalog #)?

12b. What are the O.D. readings and concentration?

260 nm		**260 nm / 280 nm**	
280 nm		**Concentration**	

Note: the plasmid concentration can be *no less* than 0.5 mg/mL.

12c. What is the total amount of plasmid being supplied?

For lentiviral particles, a standard preparation requires 100 µg of transfer plasmid.
For AAV particles, a standard preparation requires 250 µg of transfer plasmid.

12d. Did you sequence your transgene subcloned into the transfer vector?

☐ Yes (fully) ☐ Yes (partially) ☐ No (why not? Please spend $8.00 and get it sequenced)

13. Insert the following below:
(1) a gel image below showing **(a)** 0.5 µg of undigested plasmid, and **(b)** 0.5 µg of digested plasmid (cut to yield at least 2 bands), both from the final maxi-prep DNA being delivered for packaging.

Gel image

(2) a map of the transfer plasmid (GenBank-formatted annotated sequence for all Miami Project VVC transfer plasmids are available from the Viral Vector Core).