

**AAV Reference Material Working Group  
Bid Submission Form**

**Method of vector purification, RFP 2.0**

Method of vector formulation, RFP 3.0

Item for Submission

A proposal for purification of one lot of AAV2CMVGFP vector with a final yield of  $\geq 1 \times 10^{15}$  virus genomes. Any method of purification for AAV vectors that is able to meet the yield requirement with a total recovery rate of 30-60% of input vector genomes will be considered, methods based on the column chromatography are highly preferable. Both affinity and ion exchange columns will be acceptable.

General Requirements for Bidding

The purification process must be described in detail. Particularly, the following critical information should be provided: reagents needed such as - enzymes, buffers and other chemicals, plastic ware (pipets, tubing and other accessories, bottles/bags and other containers, filtering units, etc), down stream processing equipment such as those for filtration and concentration, chromatography materials and equipment such as resins, column and a work station and operating temperature of each step. QC test, methods and QA standard for cleaning and sanitizing the equipment used for purification of the AAV reference material need to be reported. This report should also include the detection of lipopolysaccharides. Conditions and procedures for storage and shipment of crude virus lysates and purified virus before vialing should also be described. The bidder should include a description of the facility, plus the qualifications of the individuals who will do the work. There should also be information about similar work that has been accomplished in this facility by these personnel.

Documentation Requirements

Provide information as stated above. Batch records of purification runs or summary information of purified vector lots purified by the proposed method should be included to demonstrate performance of the purification process (e.g. percentage of virus genome recovery, sizes of vector lots purified, ratios of virus genomes to infectious particles, plasmid, vial and cellular DNA contamination, cellular, viral and transgene protein contamination, etc).

**Please complete the following fields:**

***Contact Information – RFP 2.0***

Contact Individual:	<i>Richard O. Snyder</i>
Institution:	<i>University of Florida</i>
Address:	<i>PO Box 100266, Gainesville, Fl 32610-0266</i>
Phone Number:	<i>352-392-8459</i>
Email Address:	<i>rsnyder@gtc.ufl.edu</i>

Please indicate if your institution is also submitting proposals for the other activities:

- AAV reference material production
- Donation of Cell Bank
- Tissue culture reagents
- Tissue culture plastic wares and equipment
- Formulation and storage condition for the reference AAV vector
- Adenovirus Helper Virus or rHSV Helper virus purification
- Plasmid purification
- Down stream processing equipment and materials (filtration, concentration, etc)
- Column chromatography materials (resins, columns, tubing, bags, containers and other accessories)
- Chromatography work station
- Donation of other supplies/services
- Vialing, storage and distribution of AAV reference material

*Please attach:*

Documentation mentioned above

Submit this completed form and all attached information for receipt **by September 1, 2003** to the address below. Electronic submissions are encouraged. Final decisions will be communicated by or about September 15, 2003. Please note that all information submitted will be publicly available. Please do not mark any information confidential, as we cannot honor that request. Please include an estimated cost and market value for goods and services donated.

**Williamsburg BioProcessing Foundation**  
**Attn: AAV Reference Material Working Group**  
**1206 Laskin Road, Suite 201**  
**Virginia Beach, VA 23451**  
**(757) 423-8823**  
**Fax: 423-2065**



This proposal is for purification of one batch of AAV2CMVGFP vector with a yield of  $\geq 2 \times 10^{15}$  virus genomes in total using chromatography.

## **Manufacturing Process**

### Summary

A Cell Harvest is thawed, lysed in 0.5% sodium deoxycholate in 20 mM Tris, pH 8.0 and 150mM NaCl, treated with Benzonase<sup>®</sup> and then disrupted by microfluidization. Virions are then purified by STREAMLINE<sup>™</sup> heparin affinity chromatography. Peak fractions are pooled and the NaCl concentration is adjusted to 1M. The pooled fractions undergo Phenyl Sepharose column chromatography, and the rAAV is collected in the flow-through. This flow through is diluted with water and purified and concentrated by Sulfopropyl cation exchange chromatography. Vector is eluted in excipient consisting of 135mM NaCl in phosphate buffered saline. This Purified Bulk is stored frozen.

In process testing is performed on Purified Bulk. One or more Purified Bulks which meet set specifications are combined, sterile filtered, and then vialled. Final bulk is dispensed into 1.2ml cryovials and stored at  $-80^{\circ}\text{C}$ .

### Methods

**Cell Lysis:** The Harvest ( $\sim 1 \times 10^{10}$  cells) is thawed and 2X DOC Lysis buffer is aseptically transferred to the Harvest bioprocess container (BPC) bringing the final deoxycholate concentration to 0.5%. Benzonase (50 U/mL final concentration) in the presence of  $\text{MgCl}_2$  and Tris/NaCl buffer is added to the Harvest BPC. After incubation with intermittent mixing for 60 minutes at  $37 \pm 2^{\circ}\text{C}$ , the benzonase treated Harvest is further disrupted by passing it once through a microfluidizer. The Crude Lysate is collected in a small bioprocess container and aseptically attached to the ATKA FPLC to immediately begin chromatographic purification.

**Purification:** The lysate is applied to a STREAMLINE heparin column, which captures the AAV type 2 vector particles. The column is washed with PBS and the vector is then eluted with a bump using PBS/NaCl. The vector containing fractions (the HE peak) are collected in another bioprocess container and stored at  $2 - 8^{\circ}\text{C}$ .

The salt concentration of the HE Peak is increased from 350 mM to 1.1 M by the addition of 4 M NaCl into the BPC. The BPC is then attached to the FPLC and the vector preparation is loaded on a Phenyl Sepharose Column. The column is monitored by UV absorbance and the vector elutes in the flow through. Vector is collected in a BPC.

Water for Injection is added to the PS Flow Through to decrease the salt concentration to approximately 140 mM NaCl. This material is then passed through a SP HP (Sulfopropyl cation exchange) Column and the Purified Bulk is eluted with a PBS/NaCl bump.

All process equipment undergoes cleaning and sanitization using NaOH according to standard operating procedures.

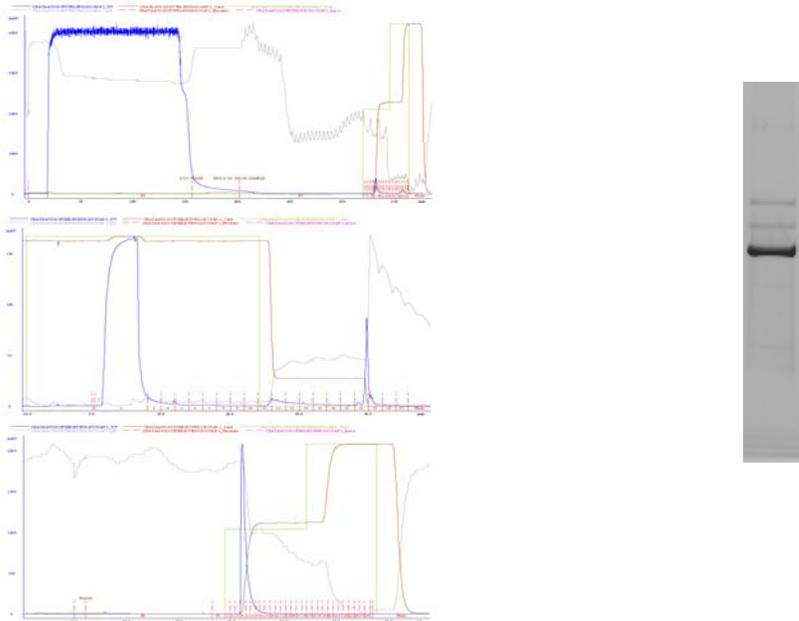
***In-process testing:*** The Purified Bulk is then sampled for Bioburden, Vector Genome Titer, and Vector Identity and Purity testing. The Purified Bulk is then sterile filtered using a 0.2µm filter and stored frozen at -70 to -90°C.

***Formulation:*** One or more Purified Bulks are thawed and mixed. The Final Bulk is then filtered using a 0.2µm filter, and filled.

***Filling.*** 5000 vials of 0.5ml @ 1X10e12vg/ml will be filled under a separate RFP. The vials are then frozen at -70 to -90°C and stored.

Frozen final product vials are submitted to the Reference Standard Working Group for final product testing, stability testing, and characterization.

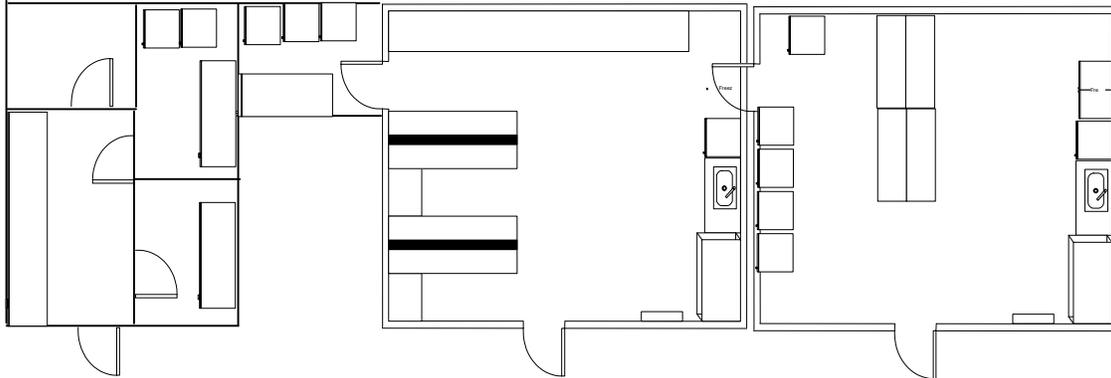
Example



**FPLC purification of rAAV.** The equivalent of ten Nunc Cell Factories (~1x10e10 cells) of rAAV-UF11 was microfluidized in the presence of deoxycholate and immediately loaded on the first of three FPLC columns. The first column is a Streamline (Pharmacia) heparin column. The second column is Phenyl sepharose and the third column is SP sepharose. Shown are tracings for A<sub>280</sub>, conductivity and fraction numbers. The final product is shown on the silver stained gel.

## Facility

The University of Florida Vector Core Laboratory facilities include two laboratories of 660 sq. ft each, and a laboratory of 330 sq. ft (floorplan shown below). In addition, the Vector Core shares an equipped darkroom, cold room, equipment room, phosphoimager, dishwashing facilities, and a microscope room with three other faculty members.



Major equipment located in the laboratory includes: Beckman centrifuges: (2) J6-HC, (2) J2-HS, (3) LE-80 ultras, LE-90 ultra with appropriate rotors; Microfluidics 110S Microfluidizer; Biosafety cabinets: Forma 4-foot A2/B3, (2) Baker 6-foot, Baker 4-foot, (3) NuAire 4-foot, NuAire 6-foot; (2) Biometra OV-5 hybridization ovens; (2) Forma -86°C Ultra low freezers; (3) Fisher Isotemp refridgerators; (3) Sears -20°C Freezers; (16) Forma CO<sub>2</sub> Tissue Culture Incubators; Barnstead Water purification system; PE PCR machine; Spectrophotometer; UVP UV Transilluminator; FPLC systems: (3) Pharmacia AKTA FPLC with dedicated computers and BioRad FPLC system all with fraction collectors; Bacterial shakers: New Brunswick and Amerex; Microscopes: Nikon light microscope, Meiji light microscope, Zeiss Axiovert 25 with Fluorescence, Zeiss Axiovert 135 with Fluorescence, and Nikon Optiphot-2; (6) Eppendorf microfuges; (3) electronic balances; Conductivity meter; pH meter.

Computer: Three Dell 8100 (Pentium 4) computers with two laser printers located in offices. In the laboratory space: a Dell Dimension computer (Pentium II) with laser printer, a Gateway computer (Pentium I), a Compaq Computer (Pentium III), a flat bed scanner.

Other: The Department of Molecular Genetics and Microbiology and the Powell Gene Therapy Center provide all secretarial and fiscal services that are needed for projects. A machine shop and electronic shop are located in the Health Center and are available on a fee-for-service basis. Photographic services are also available on a fee basis. The University of Florida has core facilities (ICBR) for oligonucleotide synthesis, peptide synthesis, and monoclonal antibody generation. Protein microsequencing and electron microscopy are available on a fee-for-service basis.

### **Personnel**

Richard O. Snyder, Ph.D. is the Director of the Vector Core and Human Applications Laboratory. Mark Potter, the Assistant Director of the Vector Core, heads a group of 13 technicians involved in producing and purifying research-grade vector. In addition, a The Human Applications Laboratory (cGMP manufacturing facility) operates in a separate building with a separate staff of 15 people. A Quality Control group (5 people) and a Quality Assurance Unit (3 people) work closely with the Vector Core and Human Applications Laboratory.

For the Vector Core, General training sessions are held on a periodic basis for personnel working in Manufacturing, QC, and QA. Individuals are presented with protocols covering a particular procedure before the training session. The individual receives hands-on training under guidance of a supervisor. When individuals can complete the task under supervision, they are allowed to complete the tasks on their own and in teams. Personnel have been trained in aseptic technique and specific procedures, and undergo yearly refresher training. Additionally, dedicated facilities maintenance operators have been trained and oversee the regular cleaning and maintenance of the facility and equipment. The Assistant Director of Quality Control coordinates environmental and personnel monitoring and testing of raw materials, in-process and product samples. The Director of Quality Assurance has independent oversight of manufacturing and oversees Quality Control. The Director of QA is responsible for release of raw materials and final product, validation support, document development and review, training, change control, auditing, document control, and conducting deviation and failure investigations.

### **Shipping**

At harvest, the culture media is discarded, cells are washed with PBS, and harvested using PBS containing 5mM EDTA. The collected cells are centrifuged at 1000g for 10 minutes, resuspended in 60 ml Lysis Solution (150 mM NaCl, 50 mM Tris pH 8.4) or other buffer that will be compatible with purification, combined, and stored at -20°C until purified. Alternatively, cell pellets can be combined, centrifuged, supernatant removed, and cell pellets frozen at -20°C without buffer. Routinely in the Vector Core, harvests are stored for 2-4 weeks at -20°C or -80°C prior to purification. Harvests have been stored as long as 8 months prior to purification and had similar yields.

When the harvest is shipped for purification, the stability of the frozen cell harvest should be maintained when shipped on dry ice. Shipping temperature could be monitored using a TempTale. This device tracks and stores temperature on a small recorder that is packed in the shipping container. The data would be retrieved using the software and interface to download and read the device. Mock shipments could be performed and the recipient could send the TempTale back to UF to be read by the software. Alternatively, a protocol could be developed to expose the container to various temperatures for various length of time.

### **Value**

Approximate value of the donation: \$80,000 (this includes labor, equipment maintenance, and facilities costs, and excludes all other reagent, and testing costs).

Legal

For distribution of the viral reference standard stock made using the pTR-UF-11 vector plasmid, the University of Florida would require the signature of a form stating 1) that the University of Florida would be held harmless (no liability) by users of the reference standard, 2) that UF is not responsible for infringement of elements in the vector, 3) that the recipient shall not distribute the vector to any other institution, and 4) that it shall be used only as a reference standard.

Potential delay

If the contract is awarded to UF, there is a potential delay in producing the cell harvest because the Vector Core is relocating in July/August of 2004. Practically, if the contract is awarded to UF, the secondary RFPs for reagent donations (Media, serum, plasmid DNA, plasticware, etc) will need to be drafted and awarded prior to transfection. So this relocation may not be an issue.