AAV Reference Material Working Group
Bid Submission Form

Method of vector production, RFP 1.0

Addendum

Contact Information – RFP 1.0

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This addendum to the proposal for production of one batch of AAV2CMVGFP vector with a yield of $\geq 2X10^{15}$ virus genomes in total using transient transfection methodology.

Additional data

The Vector Core now transfects approximately 800 cell factories per year (each $1X10^9$ cells) including 500 individual cell factory preparations (average vector yield per preparation = ~1X10e13 vg, see Fig 1) and 300 cell factories used in large preparations (10 cell factories or more combined) of rAAV vectors.

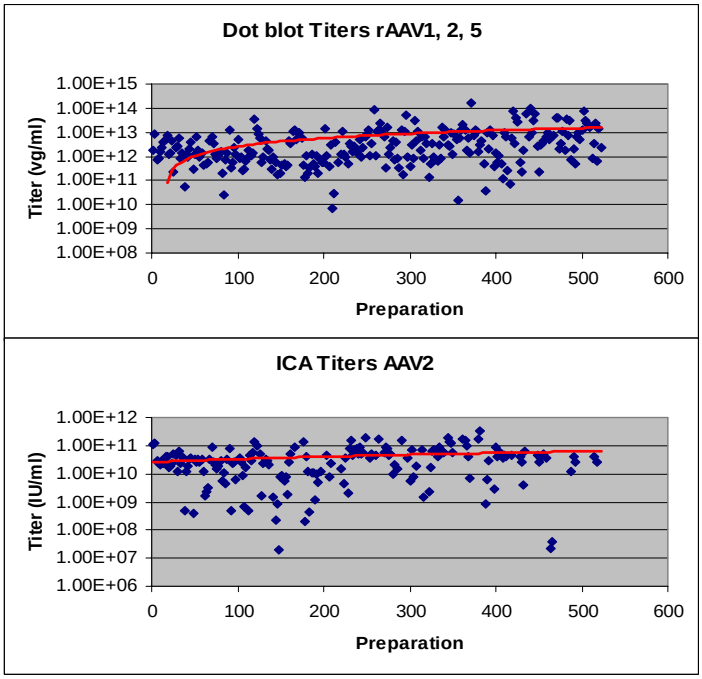

Figure 1. Small-scale rAAV preparations made during yr2002. 67% of all preps were AAV2. The remaining were AAV2 capsid mutants, or AAV1 or AAV5 vectors.

For AAV2 vectors, the particle to infectious titer (P:I) ratio averages 100 with a range of 20-200 following iodixanol gradient centrifugation and heparin chromatography, or a three-column chromatographic purification method (see Fig 1). The ratio of full (dot-blot assay) to empty AAV2 vector capsids (A20 ELISA) is 1-3 when purified by iodixanol gradient centrifugation and heparin chromatography, and 5-10 for the three-column chromatographic purification method. These two purification methods each have yields of 30-50%, so the amount of vector that is available at harvest is approximately 2-3X10e13 vg per cell factory.

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 ^{o}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.

Legal

The University of Florida (UF) Office of Technology Licensing's position is to make the pTR-UF-11 vector available with no claims as to all of the elements included in the vector. The Vector plasmid can be deposited with ATCC and its use will come under MTA from UF. UF has an MTA for non-profit and for-profit organizations that are executed frequently.

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.