Oregon National Primate

Research Center

ONPRC

Molecular Virology Support Core

Mail Code L584 - 505 N.W. 185th Avenue, Beaverton, OR 97006 – Lab Tel: 503-629-4042, Web: goo.gl/3kyal

Custom AAV Vector Production Request Form

Please e-mail to completed form to gomes@ohsu.edu and kahl@ohsu.edu

Request Date:

Contact Information

Principal Investigator:

Email Address:

Phone Number:

Laboratory Contact:

Email Address:

Phone Number:

Institution:

Department Name and Code:

Mail code:

Shipping Address:

Project Alias Number (for internal users):

FAID (for internal users):

Name of Fiscal Authority:

FedEx Account Number (for external users):

AAV Vector Information

1. **AAV serotype capsid desired (e.g., AAV1, AAV2, etc.):**

2. **Please indicate AAV vector production scale needed (see general notes for description):**

   ☐ Standard Medium-Scale Prep ☐ Large-Scale Prep

3. **Name of ITR transgene plasmid and concentration (recommended concentration 1 µg/µl). We request that you send us at least 100 µg (for medium scale) or 200 µg (for large scale) endotoxin-free purified plasmid:**

4. **Description of transgene cassette (e.g., Enhanced Green Fluorescent Protein gene driven by CMV promoter):**

5. **Does your transgene cassette contain one of the following regulatory elements?**

CMV promoter	☐Yes ☐No	**CAG promoter**	☐Yes ☐No
SV40 polyA	☐Yes ☐No	**BgH polyA**	☐Yes ☐No

6. **Does your transgene cassette encode oncogenes, select agents, toxins, apoptotic genes, etc. that may be more hazardous than usual?**

   ☐Yes ☐No

7. **What will the AAV vector be used for? (e.g. research area, disease application, target tissue, cells, model organism, etc.)**

8. **Was your transgene ITR plasmid purified endotoxin-free? (we recommend using Qiagen endotoxin-free plasmid purification kits)**

   ☐Yes ☐No

9. **Have you confirmed both AAV 5' and 3' ITRs and other important elements in your transgene vector? If yes, please send us a gel image confirming ITRs.**

   ☐Yes ☐No

10. **Do you have a map and complete sequence for your transgene ITR plasmid vector? If yes, please provide a txt copy of your vector sequence an electronic map indicating restriction sites used for analysis.**

☐Yes ☐No

11. **Does your transgene ITR plasmid encode a conventional single-stranded (two ITRs) or a double-stranded/self-complementary (one ITR) AAV genome?**

    ☐Single-Stranded ☐Double-Stranded/Self-Complementary

12. **Do you have special aliquot size needs? (100 µl is the standard aliquot size unless indicated below; total stock volumes at medium production scale may range from ~500 µl to ~1.5 ml depending on the serotype).**

13. **What date do you need the AAV vector stock by?**

14. **Optional notes and comments:**

General Notes to Users

(1) Institutional Biosafety Committee (IBC) Approval and Biosafety Guidelines

a. According to OHSU guidelines, for each particular project involving recombinant viral vectors, the investigator requires approval from the IBC (for *in vitro* use) and the IACUC (for *in vivo* use) before obtaining AAV vector stocks from the Molecular Virology Support Core (MVSC). For links to IBC, IACUC and up-to-date RDRQ Core Viral Vector Services submission forms go to http://www.ohsu.edu/xd/research/about/integrity (internal users) or contact your local institutional committees (external users). The MVSC has an IBC master protocol in place that describes the underlying AAV vector technology and that can be referred to in your submission (RDRQ questions 3 to 7). If needed, we can assist you in navigating the specific IBC protocol submission process for viral vectors and for training in safe viral vector handling. **Please make sure to forward us your IBC approval letter when granted.**

b. Our recombinant AAV vectors are all helper virus-free, so replication-competent virus (RCV) testing is not required. The minimum Biosafety Level (BSL) or Animal Biosafety Level (ABSL) for work with rAAV is 1. The final BSL/ABSL level may be higher depending on the particular transgene cassette and vector configuration, as well as the cell type or *in vivo* model system used. These are general guidelines, and only the IBC and IACUC can issue project approvals and determine the specific BSL/ABSL for your work. See also http://www.ohsu.edu/xd/research/about/integrity/ibc/upload/Vector-table-01152013.pdf

(2) AAV Insert Size and ITR Integrity

a. The natural AAV genome size is about 4.7 kb. All viral sequences (except for 2 x 145 bp inverted terminal repeats [ITRs]) are deleted in AAV vectors. That provides a transgene capacity of about 4.3 kb. Even though slightly larger transgene cassettes can sometimes still be packaged efficiently, there is a risk of incomplete or non-packaging (i.e. no or low vector titers) with oversized genomes. Therefore, it is recommended to not exceed the AAV packaging capacity. The MVSC can attempt to produce oversize vectors, but success is not guaranteed. Please contact us to discuss if this is a concern.

b. Inverted-terminal repeat (ITR) plasmids (a.k.a. cis or vector transgene plasmids) are prone to rearrangements and deletions. This can lead to failed AAV vector productions. Therefore, many ITR-containing plasmids need to be grown in recombination-deficient (rec-) E.coli strains (such as Sure, Stbl2, or Stbl3) as low-copy plasmids at 30°C. Expect significantly reduced yields. We recommend using 2XYT medium and doubling growth volumes. For example, for a Qiagen Mega Prep set up a 2 x 500 ml overnight culture. Use twice the volumes of P1, P2, and P3 buffers for initial re-suspension and cell lysis. Then purify over a single Mega column following standard Qiagen protocol.

c. Before supplying your ITR plasmid to us, validate ITR integrity by restriction digests using enzymes that cut within the ITRs, such as Xma I, Msc I, Ahd I, Pau I or Pvu II.

(3) AAV Production Sizes and Expected Yields

a. Our standard medium production scale is at ~2,000 cm^2 cell culture surface area (13 x T-150 flasks). If your vector is low yielding at regular scale or if you need very large quantities, we also offer a large production scale at ~6,000 cm^2 cell culture area (40 x T-150 flasks). For internal OHSU users, the cost of a standard medium-scale production is $1,169 and $2,262 for a large- scale production.

b. Important note about vector yields: The MVSC makes every effort to optimize and improve production protocols to provide our users with the highest possible yields. For high titer AAV serotypes, such as AAV8 and AAV9, medium-scale yields for standard or stock vectors (such as marker gene vectors) are typically in the range of E+12 to E+13 total viral genomes (vg). For lower titer serotypes, such as AAV1, 5 and 7, vector yields can range from E+11 to E+12 total vg. Note that yields for a particular AAV serotype vector can vary significantly when custom transgene expression cassettes are used, due to inherent differences between cassettes. Due to these variables, yields cannot be guaranteed for custom vector requests.

(4) AAV Titers

a. We titer all virus productions for viral genome (vg) titer either by qPCR (if common regulatory elements, such as CMV promoter, CAG promoter, SV40 polyA, or BgH polyA are present) or by a sequence-independent Quick Titer assay (fluorescent dye-based method). We check purity of our productions by SDS–PAGE gels. Final virus product will be 0.22µm sterile-filtered into an injection-compatible and stability-enhanced buffer (DPBS + 35 mM NaCl + 5% glycerol).

(5) If you require controls for your assays please do not hesitate to ask us. We generally have marker stocks of the most common serotypes (such as AAV1, 5, 7, 8 and 9) with a CMV-driven eGFP reporter gene in our inventory (10 µl, 25 µl, 50 µl, and 100 µl aliquot sizes) for immediate purchase. Other common promoters and marker genes may be available upon request for cloning purposes or vector production.

Shipping and Billing

For ONPRC and OHSU investigators, all materials are supplied either in person or shipped directly using the Inter-Campus Courier to the laboratory address provided by the requesting investigator. An email will be sent prior to shipment to the requesting user confirming the day of shipment. Packages are normally delivered the following day (i.e. 24 h turnaround), and we request that you confirm receipt via email. If you haven't received your package by 2pm the following day, please contact us so we can track your package. Internal investigators receive an invoice at the end of the month with a description of the charges and the project alias number that will be billed.

In order to ship your ITR-containing plasmid to us, please use the following label and fill in the relevant information. Plasmids can be shipped via the Inter-Campus Courier service.

To: Michelle Gomes/Don Siess/Christoph Kahl ONPRC, Molecular Virology Support Core Mail Code L584 Research Building 046 505 NW 185th Avenue Beaverton, OR 97006 Phone: 503-629-4042/ 503-629- 4033/ 503-645-1141	**From: (PLEASE PROVIDE SENDERS NAME, SHIPPING ADDRESS AND PHONE NUMBERS)** **RESPONSIBLE PERSON FOR THIS SHIPMENT**
Emergency Contact Number:	

For external (non-OHSU) users, materials will be shipped and received via FedEx using the provided address and account number. An email will be sent prior to shipment to the requesting user confirming the day of shipment and the tracking information once available. Please inquire for specific routine or custom service requests, and we can generate a quote for you. External billing proceeds through the ONPRC business office. Note that center guidelines require that external users supply us with a purchase order (PO) before initiating a service. After the work is completed, external users will receive an invoice for review and the supplied PO will be billed. Please contact Laurie Schweiker at schweike@ohsu.edu or 503-690-5210.