

Internal Notes:

Processed by:\_\_\_\_\_\_\_\_\_\_\_

Assigned on:\_\_\_\_\_\_\_\_\_\_\_\_

Final Titer: \_\_\_\_\_\_\_\_\_\_\_\_\_

Shipped/picked up on:\_\_\_\_\_\_\_\_\_\_\_\_\_

**Gene Transfer, Targeting and Therapeutics Facility**

**Recombinant viral vector request form**

Email completed form to GT3@salk.edu

To obtain a quote for services - Please call 1-858-453-4100 x1891 before submitting request

**What to provide to the core:**

**1**. 300ug (preferably >1ug/ul) of your lentiviral or rAAV plasmid. If large scale rAAV production is requested, 500ug of rAAV plasmid is required. DNA should have been purified using an endotoxin-free protocol (e.g. Endo-free maxi/mega/giga plasmid purification kits or equivalent). We do not accept miniprep purified DNA.

**2.** Plasmid DNA should be checked for purity and have an A260/280 of >1.8.

**3.** AAV ITR plasmids should be checked for recombination by digestion with SmaI or XmaI. Each ITR contains two SmaI/XmaI sites - digestion will cut out your insert. Excessive amounts of linearized full-length plasmid indicate recombination has occurred.

**4.** Lentiviral and retroviral transfer plasmids should be digested with appropriate restriction enzymes to confirm correct insert size.

**5.** Please provide a gel image of your SmaI or XmaI digested AAV2 ITR plasmid, or your lentiviral transfer plasmid with the insert excised.

**6.** To avoid recombination we recommend transforming and growing your lentiviral and rAAV transfer plasmids in recombination deficient cells such as STBL3 @ 30°C IN 2XYT broth for no more that 16 hrs with shaking.

**7.** Vector map and Sequence file - we encourage you to submit your plasmids to our vector library. We will be happy to work with you on establishing MTA agreements for your reagents.

**8.** Information about your gene of interest or insert and any special requirements for handling e.g. toxicity, oncogenic, pro-apoptotic, etc.

## Billing Information. Order#:

|  |  |  |
| --- | --- | --- |
| **Principal Investigator (PI):** | **Requesting Investigator / Lab contact:** | |
| **Order Date:** | **Contact Phone:** | |
| **Salk Users. Fund number:** | **Contact E-mail:** | |
| **External Users. PO Number**  **(credit cards are NOT accepted):** | |  |
| **Billing address:** | **Shipping address:** | |

## PROJECT INFORMATION (please submit separate form for each construct)

**Top of Form**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **CONSTRUCT NAME:** | | | | |
| **PLASMID INFORMATION:** | | | | |
| **Insert (bp):** | **Plasmid (bp):** | **Volume (ul):** | **Concentration (ug/ul):** | **A260/A280:** |
| **INFORMATION NEEDED FOR LENTIVIRAL ORDERS**   |  |  | | --- | --- | | **TRANSFER PLASMID TYPE**  **2nd gen**  **3rd gen SIN HIV-1 5’LTR**  **3rd gen SIN RSV 5’LTR**  **3rd gen SIN CMV 5’LTR**  **EIAV**  **OTHER (PLEASE DESCRIBE):** | **PSEUDOTYPE NEEDED**  **VSV.G**  **Rabies.G**  **ASLV EnvA**  **ASLV EnvB**  **FuG-B2**  **TITRATION ONLY:**  **Transduction + QPCR**  **FACS** | | | | | |
| **INFORMATION NEEDED FOR RETROVIRAL ORDERS:**   |  |  | | --- | --- | | **TRANSFER PLASMID TYPE**  **MMLV TRANSFER PLASMID**  **MCSV TRANSFER PLASMID**  **OTHER (PLEASE DESCRIBE):** | **PSEUDOTYPE NEEDED**  **VSV.G**  **ECOTROPIC**  **AMPHOTROPIC** | | | | | |
| **INFORMATION NEEDED FOR AAV ORDERS:**    **Crude AAV production (without purification). Number of plates requested:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**  **Small scale production – Iodixanol purification. Final Volume Requested:\*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ul**  **Large scale production – CsCl purification. Final Volume Requested:\*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ul**   |  |  | | --- | --- | | **SEROTYPE NEEDED**  **rAAV2/1**  **rAAV2/2**  **rAAV2/3**  **rAAV2/4**  **rAAV2/5** | **rAAV2/6**  **rAAV2/LK03**  **rAAV2/8**  **rAAV2/LK19**  **rAAV2/9** **rAAV2/retro**  **rAAV2/rh10 TITRATION ONLY:**  **rAAV2/DJ**  **QPCR** | | | | | |
| \* - Both production protocols give 2-3mL final product, which can be further concentrated to as little as 100ul, which will increase the concentration/ul, but not the total number of particles. Please indicate the final volume you want your preparation to be concentrated to **prior** to titration. | | | | |
| **INFORMATION NEEDED FOR ADENOVIRAL ORDERS:**  **E1 deleted Ad5 amplified from Pac1 digested DNA**  **E1 deleted Ad5 amplified from viral stock**  **OTHER (PLEASE DESCRIBE)** | | | | |
| **INFORMATION NEEDED FOR G-DELETED RABIES ORDERS:**  **ENVELOPE NEEDED**  **RABIES SADB19.G**  **ENVA.G**  **VSV.G**    **Construct:**  **Recovery from Plasmid (please provide plasmid info above)**  **From GT3 stocks:**  G-deleted Rabies-eGFP G-deleted Rabies mCherry-Myc  G-deleted Rabies-mCherry G-deleted Rabies FLPo-dsRedXpress  G-deleted Rabies-ChR2-mCherry G-deleted Rabies AlstR-GFP  G-deleted Rabies GCaMP3-dsRedXpress G-deleted Rabies GcAMP3  G-deleted Rabies eGFP-Er(T2)CreEr(T2) G-deleted Rabies BFP  G-deleted Rabies eGFP-rtTA G-deleted Rabies dsRedXpress | | | | |
| **Attach picture of restriction digest confirming plasmid integrity:** | | | | |

**Bottom of Form**

**Project Description:**

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| --- |
| **Give a brief overview of the project and its aims:** |
| **Describe any potential or expected toxicity or additional biohazardous concerns, in particular expression/knockdown of oncogenes or tumor suppressor genes, and expression of inserts that may induce apoptosis:** |
| **Attach a map for the supplied transfer plasmid:** |

TERMS AND CONDITIONS OF SALE AND LIMITED USE AGREEMENT BETWEEN THE SALK INSTITUTE FOR BIOLOGICAL STUDIES (“SALK”) AND RECIPIENT OF BIOLOGICAL MATERIALS

Biological materials to which this Limited Use Agreement applies:

**Lentiviral vectors, Retroviral vectors, Adeno-associated viral vectors and Adenoviral vectors, Herpes Simplex viral vectors, Rabies viral vectors and Vesicular Stomatitis viral vectors generated by the Salk Institute Gene Transfer, Targeting and Therapeutics Core Facility (GT3).**

- and any progeny or unmodified derivatives thereof and any related information or material supplied in connection therewith by Salk (the "Biological Materials"). Salk retains ownership of Biological Materials, including any Biological Materials contained or incorporated in modifications. Ownership of modifications and derivatives of Biological Materials will be determined in good faith by the parties hereto depending upon the parties' relative contributions to the creation of said modifications and derivatives.

We are pleased to provide the Biological Materials, from the GT3 Core Facilityof Salk, subject to terms contained herein.

1. Your institution and your investigator WILL:
   1. Use the Materials only for academic research.
   2. Use them safely and in compliance with all laws, regulations, and NIH guidelines.
   3. Be responsible for any injury or damages that your use may cause.
   4. Acknowledge Salk’s investigator as the source of the Materials in publications.
   5. Return or destroy the Materials when no longer needed or on Salk’s request.
   6. Determine with Salk in good faith the ownership of modifications and derivatives.
   7. Pay Salk for actual shipping costs or provide Federal Express account number.
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