

### **Recombinant viral vector request form**

Email completed form to [albert.blanchart-aguado@ki.se](mailto:albert.blanchart-aguado@ki.se)

For further information or questions regarding price list Please call (+46) 8 524 87 387 before submitting request

#### **What to provide to the core:**

1. Obtain the necessary **Material Transfer Agreement (MTA)** if needed. If the customer provides a plasmid purchased from a company or a non-profit organization (i.e. AddGene), it is the customer's obligation to contact the depository entity (laboratory or company which produced the plasmid), to find out if some MTA agreement must be fulfilled, in order for the VirusTech core facility to work with those plasmids.
2. Download the **L-anmälan** (Permits and notifications for Genetic Modified Microorganisms work) documents in Swedish and/or in English from the core facility's web page (<http://ki.se/en/cmb/virustech-core-facility>). Read the Guidelines and Instructions to fill the document.
3. This **order form** properly filled.
4. Your Lentiviral or AAV expression vector (plasmid) at the right concentrations:  $\geq 150 \mu\text{g}$  of transgene AAV plasmid and  $\geq 300 \mu\text{g}$  of the LV expression vector at a concentration of at least  $0.30 \mu\text{g}/\mu\text{l}$  (preferably  $\geq 1 \mu\text{g}/\mu\text{l}$ ). The DNA should have been purified using an endotoxin-free protocol (e.g. Endo-free maxi/mega/Giga plasmids purification kits or equivalent).
5. High Quality DNA: Plasmid DNA should be checked for purity and have an A260/280 ratio no lower than 1.8 (*the actual value of this ratio must be provided to the core*).
6. Additional **SmaI/SrfI digestion analysis** on the AAV transgene plasmids should be performed and the results should be also submitted to the core. *Please attach a gel image to this form.*
7. As indicated in the guidelines from our web site we recommend transforming and growing your LV and AAV plasmids in recombination deficient cells such STBL#, NEB stable or SURE at 30°C for no more than 16 hours.
8. Vector map and sequence file.
9. We encourage you to submit when applicable a picture of in vitro cultured cells transfected with your transgene plasmids (containing reporter genes).

Billing information. Order number: \_\_\_\_\_ (To be filled by the core)

Principal Investigator (PI):	Requesting Investigator:
Order Date:	Contact Phone:
<b>For KI users only. ZZ code:</b>	Contact E-mail:
<b>For External users. PO number or Reference number:</b>	
Shipping address:	Billing address:

**Order details:** (Please copy-paste the following table and submit as many as needed for each construct)

Lentiviral Orders:

Construct name (please specify all sequences inserted):		
Origin/AddGene number (if applicable):		
Name (tag) on aliquots:		
<b>Plasmid Information:</b>		
Insert (bp):		
Plasmid (bp):		
Volume ( $\mu$ l):		
Concentration ( $\mu$ g/ $\mu$ l):		
A260/280:		
<b>Transfer plasmid and packaging:</b>		
Transfer plasmid type	Pseudotype Needed	Packaging system
<input type="checkbox"/> 2 <sup>nd</sup> Generation <input type="checkbox"/> 3 <sup>rd</sup> Generation <input type="checkbox"/> 3 <sup>rd</sup> Generation SIN 3' LTR <input type="checkbox"/> 3 <sup>rd</sup> Generation SIN CMV 5' LTR	<input type="checkbox"/> VSV-G	<input type="checkbox"/> 2nd Generation (p.MD2.G/psPAX2) <input type="checkbox"/> 3rd Generation (p.MD2.G/ pRSV-Rev/ pMDLg-pRRE)
<b>Additional information:</b>		
Titration:	Titration Only:	Others:
<input type="checkbox"/> Transduction + FACS <input type="checkbox"/> Transduction + qPCR <input type="checkbox"/> RT-qPCR	<input type="checkbox"/> Transduction + FACS <input type="checkbox"/> Transduction + qPCR <input type="checkbox"/> RT-qPCR	
<b>Product delivery</b>		
<input type="checkbox"/> Non-concentrate supernatant	<input type="checkbox"/> Concentrated small volumes	<input type="checkbox"/> Concentrated large volumes
Number and size of aliquots:		

(If more than one construct is submitted, copy this table and paste below as needed)

Attach a map of the transfer plasmid:

(If more than one construct is submitted, copy this table and paste below as needed)

Gamma-Retrovirus Orders:

Construct name (please specify all sequences inserted):		
Origin/AddGene number (if applicable):		
Name (tag) on aliquots:		
<b>Plasmid Information:</b>		
Insert (bp):		
Plasmid (bp):		
Volume ( $\mu$ l):		
Concentration ( $\mu$ g/ $\mu$ l):		
A260/280:		
<b>Transfer plasmid and packaging:</b>		
Pseudotype Needed		
<input type="checkbox"/> Ecotropic pCL-Eco <input type="checkbox"/> Amphitropic (pUMVC + pMD2.G –VSV.G env-)		
<b>Additional information:</b>		
Titration:	Titration Only:	Others:
<input type="checkbox"/> Transduction + FACS <input type="checkbox"/> Transduction + qPCR <input type="checkbox"/> RT-qPCR	<input type="checkbox"/> Transduction + FACS <input type="checkbox"/> Transduction + qPCR <input type="checkbox"/> RT-qPCR	
<b>Product delivery</b>		
<input type="checkbox"/> Non-concentrate supernatant	<input type="checkbox"/> Concentrated small volumes	<input type="checkbox"/> Concentrated large volumes
Number and size of aliquots:		

(If more than one construct is submitted, copy this table and paste below as needed)

Attach a map of the transfer plasmid:

AAV Orders:

Construct name (please specify all sequences inserted):		
Origin/AddGene number (if applicable):		
Name (tag) on aliquots:		
<b>Plasmid Information:</b>		
Insert (bp):		
Plasmid (bp):		
Volume (µl):		
Concentration (µg/µl):		
A260/280:		
<b>Transfer plasmid and packaging:</b>		
Pseudotype Needed		
<input type="checkbox"/> rAAV2/1	<input type="checkbox"/> rAAV2/5	
<input type="checkbox"/> rAAV2/6	<input type="checkbox"/> rAAV2/2	
<input type="checkbox"/> rAAV2/8.ape		
<b>Additional information:</b>		
Titration:	Titration Only:	Others:
<input type="checkbox"/> Transduction + FACS	<input type="checkbox"/> Transduction + FACS	
<input type="checkbox"/> Transduction + qPCR	<input type="checkbox"/> Transduction + qPCR	
<input type="checkbox"/> RT-qPCR	<input type="checkbox"/> RT-qPCR	
<b>Product delivery</b>		
<input type="checkbox"/> Crude AAV production	<input type="checkbox"/> Concentrated and Purified (Iodixanol and Anion Exchange)	
Number and size of aliquots:		

(If more than one construct is submitted, copy this table and paste below as needed)

Attach a picture of restriction digest confirming plasmid integrity:

(If more than one construct is submitted, copy this table and paste below as needed)

Attach a map of the transfer plasmid:

(If more than one construct is submitted, copy this table and paste below as needed)