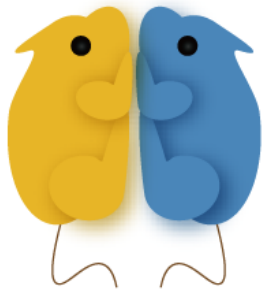


User's Guide

Genetically Modified ES-Mouse/Virus Production
Service by IRCN ES-Mouse/Virus Core Facility



E/VER

ES-Mouse/Virus Engineering and Resource

The IRCN ES-Mouse/Virus Core strategically advances the IRCN scientific mission with state of the art methods of common need.

August 2019

Note: This document is subject to change without notice. The current version of this document is available for download at: <https://core.ircn.jp/en/es-virus-core/>

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1. Services

	Price	
	IRCN Users	External users
Knock-in (with selection markers) ES-mouse	Discount price (Please inquire)	600,000 JPY
Knock-in (without selection markers) ES-mouse		900,000 JPY
Knockout ES-mouse (Knockout ES cell derived ES-mouse)		600,000 JPY
AAV (Medium-scale preparation)		180,000 JPY
AAV (Small-scale preparation)		60,000 JPY (3 or less samples/order)
		50,000 JPY (4 or more samples/order)
Others by request	Please inquire	

ES-mouse: Fully ES-cell-derived mouse. There is **no requirement for the crossing of chimeric mice** to produce mice that carry the KI gene in all cells of the body.

AAV:

Medium-scale preparation with the guarantee of titer, $>1 \times 10^{12}$ vg.

Small-scale preparation without the guarantee of titer, the usual titer is 2.5×10^{11} vg.

2. Outline

User

EVER

Obtain approval of recombinant DNA experiments.

Preparation of DNA Constructs (Targeting vectors etc.).

Obtain approval to use recombinant mouse/viruses.

Pre-order inquiries

Consultation

Estimated price

Order

Submit materials

Progress information

Delivery of products

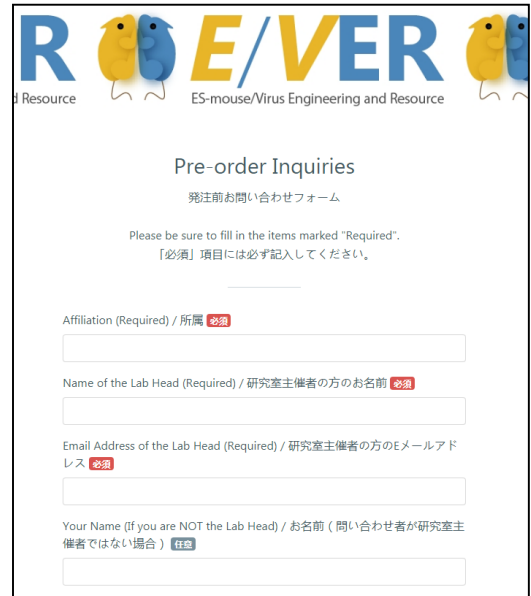
Design the genome-editing tools

Obtain approval for recombinant DNA experiments.

Obtain approval to make recombinant mice.

3. Procedures Before Ordering

Users are strongly encouraged to **contact the E/VER staff as early in a project as possible.** Please fill out and submit a “Pre-order Inquiries Form” (<https://form.run/@ever-pre-order>) before beginning a project. The website can be used from either a PC or mobile phone.



Pre-order inquiries form

3-1. E/VER

After receiving the pre-order inquiries form,

- **E/VER** will propose the optimum plan to make the mouse/virus, as a result, the user will be able to avoid mistakes (e.g., in design of targeting vector). **E/VER does not charge for advice or consultation.**
- **E/VER** will ask users for the information necessary for review and approval by the safety committee of recombinant DNA experiments, and will immediately submit any additional experiment application documents. **E/VER will not accept formal orders until the recombinant DNA experiment application documents are approved, and will not start making mice until the animal experiments application is approved.**
- **E/VER** will present an estimated cost to the client.

3-2. The User

Before (/After) submitting the pre-order inquiries form,

- **The User** must obtain approval for recombinant DNA experiments, and submit the copies of approved documents to E/VER. **E/VER will not accept formal orders until the recombinant DNA experiment application documents are approved,**

After submitting the pre-order inquiries form,

- **The User** prepare the DNA materials (such as a Targeting vector) according to the requirements of E/VER.
- **The User** must obtain approval for the use of recombinant mice/viruses, and submit copies of approved documents to E/VER. **E/VER will not deliver mice/viruses until receiving the approved copies.**
- **The User will** optimize the screening PCR conditions (Option for making a knock-in mouse).

4. Ordering

The user checks the following items for formal ordering.

- ✓ Submit copies of the approved recombinant DNA experiment application documents to E/VER.
- ✓ Apply for the use of recombinant mice/viruses.
- ✓ Preparation of the DNA materials.
- ✓ Optimization of the screening conditions (Option)
- ✓ Approval of fee
(There shall be no discount or refund of the determined expenses.)
- ✓ Approval of Authorship Policy (See Page 10)

After completing the above, fill out and submit an “Order Form” (<https://form.run/@ever-order>).

Then provide the DNA material according to contact from E/VER (See Page 8).



The image shows a screenshot of the E/VER Order Form. At the top, there is a logo for E/VER (ES-mouse/Virus Engineering and Resource) featuring two stylized mice. Below the logo, the text "Order Form" and "注文書" is displayed. The form includes instructions in English and Japanese, stating that users must first send a "Pre-order Inquiries Form" if they haven't done so. It also notes that required items must be filled in. The form contains three input fields: "Affiliation (Required) / 所属 必須", "Name of the Lab Head (Required) / 研究室主催者の方のお名前 必須", and "Email Address of the Lab Head (Required) / 研究室主催者の方のメールアドレス 必須". At the bottom, there is a field for "Your Name (if you are NOT the Lab Head) / お名前 (問い合わせ者が研究室主"

5. DNA submission

Quality: Prepare high-quality plasmid DNA suspended in water for use in transfection. We recommend the Promega PureYield Plasmid Midiprep System or similar for **endotoxin free** plasmid isolation.

Quantity (Targeting vector for knock-in): More than 3 micrograms. Adjust the concentration to 300, 600, or 900 (ng/ μ L). Higher concentrations may improve targeting efficiency. However, please do not concentrate the DNA by isopropanol precipitation.

Quantity (Virus vector for virus production):

For small-scale packaging (Every 1 sample):

pAAV vector: total >7ug, Conc. >1ug/uL

Rep/Cap vector: total >25ug, Conc. >1ug/uL

For medium-scale packaging (Every 1 sample):

pAAV vector: total >15ug, Conc. >1ug/uL

Rep/Cap vector: total >50ug, Conc. >1ug/uL

If you need to concentrate the DNA, use ethanol instead of isopropanol.

6. Sharing Progress Information

A **project ID** (such as GE0001 or AAV0001) is given to each project and notified to the user. Then, E/VER shares the progress information sheet (Google spreadsheet) created for each laboratory with the user. The user can check project progress at any time.

7. Delivery

E/VER will deliver the mouse/virus after receiving a copy of the animal experiment approval document.

ES-mouse: It will be delivered one week after birth. All produced mice are delivered and not maintained by E/VER. On the other hand, the original targeted ES cells are stored by E/VER, but we will also hand over the ES cells at your request. However, the efficiency of mouse production by re-injection using delivered ES cells is not guaranteed.

AAV: All produced viruses are delivered and not stored by E/VER.

8. Payment of Fees

For The University of Tokyo and Organization of the country or local government and similar institutions, the fee is postpaid, and **in the case of companies, the fee is prepaid based on a Collaborative Research Agreement**. In the case of a Collaborative Research Agreement, we will separately bill Research Support Expenditure of 10% to 30%. IRCN Financial Planning Team (Email address: finance.ircn@gs.mail.u-tokyo.ac.jp) is responsible for billing.

9. Authorship Policy

In principle, core staff will not seek authorship. Only in cases where additional effort was required to produce results beyond the standard core mission will their co-authorship be considered. This may include, for example, the development and successful implementation of technologies or procedures that go significantly beyond published work, the production of multiple or special mouse lines intended as common resources for multi-lab research teams (including intra-IRCN lab collaborations), or the mentoring of approved visiting IRCN researchers in learning the core's technologies. Undetermined cases will be resolved on the advice of the SC to the Director.