

Brigham and Women's Hospital Transgenic Mouse Facility  
Arlene H. Sharpe, M.D., Ph.D., Director  
77 Avenue Louis Pasteur, NRB-837  
Boston, MA 02115  
TEL: (617) 432-6569 FAX: (617) 432-6570

## In vitro Gene Targeting Application

### I. GENERAL INFORMATION

Date: \_\_\_\_\_

Name of Principal Investigator: \_\_\_\_\_

Institution: \_\_\_\_\_

Department: \_\_\_\_\_

Address: \_\_\_\_\_

\_\_\_\_\_

Tele: \_\_\_\_\_ Email: \_\_\_\_\_

Investigator interacting with facility: \_\_\_\_\_

Institution: \_\_\_\_\_

Department: \_\_\_\_\_

Address: \_\_\_\_\_

\_\_\_\_\_

Tele: \_\_\_\_\_ Email: \_\_\_\_\_

### II. FUNDING SOURCE

BWH Investigators: Provide BWH Cost Center Number \_\_\_\_\_

Non-BWH Investigators: Provide Purchase Order Number  
**and attach a copy of the purchase order to this application** \_\_\_\_\_

Please provide the following information for monthly invoicing:

Accounts Payable Contact Person (at *your* institution): \_\_\_\_\_

Address: \_\_\_\_\_

\_\_\_\_\_

Telephone: \_\_\_\_\_ Fax: \_\_\_\_\_

Email: \_\_\_\_\_

*Note: Project will not be initiated until cost center number or purchase order form is received.*

**III. SCIENTIFIC RATIONALE**

Briefly describe the specific aim of the study, and the rationale for generating this knockout strain.

**IV. ANIMAL PROTOCOLS**

Appropriate protocol number(s), obtained from the Harvard Medical Area Standing Committee for Animals

---

---

**V. DNA CONSTRUCT**

Attach a schematic diagram of the DNA construct; include restriction map, location and size of exons and introns, and drug resistance genes used (e.g. neomycin, TK, hygromycin, puromycin).

## **In vitro gene targeting application (continued)**

We require 75  $\mu$ g of DNA for the electroporation. What is the concentration of your DNA solution?

Indicate how the concentration was determined.

How was the construct linearized?

## **VI. EMBRYONIC STEM CELLS**

What type of ES cells should be targeted (e.g. J1 cells from 129 mice, Bruce 4 cells from BL/6 mice)?

What selection should be applied after transfection (e.g. G418 to select for neomycin resistance, ganciclovir to select against TK)?