

Cost Distribution for Cryopreservation of Mouse Embryos

Introduction

Currently the Center for Comparative Medicine provides mouse embryo cryopreservation services at \$2,000 per line to freeze 300-500 embryos. Embryos are quality sorted at the 8-cell morula stage and only those embryos with eight high quality blastomeres are frozen and retained in the repository. To perform this at this reduced price (compared to commercial firms offering the service), components of the cryopreservation effort are cost shared with the investigator. The purpose of this document is for scientists to understand what their costs will be when they request a line of mice be cryopreserved. The cost sharing for cryopreservation is given in Table 1.

Table 1. Cryopreservation Cost Sharing

Purchase of mice to be superovulated	Scientists
Per diem of mice during superovulation	Scientists
Hormones for superovulation and their administration	Comparative Medicine
Media and disposable plasticware for embryo handling	Comparative Medicine
Technician time for collecting, sorting and cryopreserving embryos	Comparative Medicine
Liquid nitrogen for embryo storage	Comparative Medicine
Database entry and maintenance of embryo storage information	Comparative Medicine

The time it takes to collect morula of adequate quality to complete the cryopreservation of a line of mice is highly variable. We superovulate wildtype mice, preferably C57BL/6J, because the process is reliable and most scientists desire this genetic background for their mice. Transgenic mice are highly variable in their response to hormonal injections, either due to the genetic background or to the influence of the genetic manipulation. The reliability of superovulation is the greatest determinant in the cost of the process to both the scientist and Comparative Medicine. Table 2 below contains common mouse strains and their response to hormonal superovulation (from *Manipulating the Mouse Embryo* 2nd edition, Hogan et al, 1994, CHSP, Cold Spring Harbor, NY).

Table 2. Ovulatory Response of Mice to Hormonal Superovulation

High ovulators	Low ovulators
C57BL/6J	A/J
BALB/cByJ	C3H/H3J
129/SvJ	BALB/cJ
CBA/CaJ	129/J
CBA/H-T6J	129/ReJ
SJL/J	DBA/2J
C58/J	C57/L
BALB/cByJ x C57BL/6F1	BALB/cJ x A/JF1
C57BL/6J x CBA/CaJF1	FVB
C57BL/6J x DBA/2F1	
C57BL/6J x C3H/HeJF1	

Finally, to insure that the cryopreserved embryos are viable and to confirm the genetic integrity of the line we perform a thaw and implantation of embryos. A known number of embryos are thawed and implanted into a pseudopregnant female, the number of implantations is counted and tissues are provided to the scientist for genotyping. The cost sharing associated with this quality control process is shown in the Table 3 below.

Table 3. Cost Sharing of Cryopreservation Quality Control

Purchase of recipient females	Scientists
Per diem of recipient females	Scientists
Purchase of male vasectomized mice	Comparative Medicine
Per diem of vasectomized males	Comparative Medicine
Media and disposable plasticware for embryo handling	Comparative Medicine
Technician time for thawing and implanting embryos	Comparative Medicine
Collection of implantation sites	Comparative Medicine
Data entry in database system	Comparative Medicine
Genotyping of tissues provided	Scientists

We will forgo the quality control portion of the cryopreservation effort at the request of the scientist if cost is a consideration. However, we do recommend this be performed as it validates the entire process that has been performed.