Cryopreservation of embryos : a way to reduce the number of housed animals and the genetic drift.

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Abstract

The **GIGA Mouse facility platform** has recently improved its mouse line cryopreservation technique. The method of embryo cryopreservation by rapid cooling also called **aseptic vitrification** has been selected. Vitrification media, key steps and timing have been optimized and validated.

After a first partial exposition of the embryos to cryoprotective solutions, they are immersed in a vitrifying mixture of penetrating and non-penetrating cryoprotectants for a short time. The straw containing the embryos is immediately sealed before to be plunged in LN2, resulting in a brutal solidification in which crystallization does not have time to occur.

Introduction

Number of new transgenic mouse strains is continuously expanding with, as a consequence, a rapid increase in maintenance cost.

Additionally, as research evolves, some strains are no more immediately needed.

Mouse embryo cryopreservation represents a way of response to all these questions:

i/ drastic reduction of maintenance cost,

ii/ protection of cryopreserved lines are from genetic drift,

iii/ protection again infectious disease.

Results

Various tests are carried out to verify the quality of the cryopreservation procedure. The technique has been validated at GIGA. Vitrification media are prepared by the platform. Each medium batch is controlled by MEA.

In order to be in compliance with the European Union Tissues and Cells Directives 2006/17/EC and 2006/86/EC, our vitrification method is now performed aseptically. **Aseptic vitrification** allows us to avoid any cross-contamination between straws during the storage and all manipulations. To perform aseptic vitrification, the GIGA Mouse Facility and Transgenics Platform have recently acquired an UltraSeal 21™ manual sealer for straw to ensure that the health status of cryopreserved embryos is preserved during the whole cryopreservation procedure: cooling, storage and warming. Regarding strain housed in the conventional facility, the embryo harvesting can be combined with rederivation.

Straws of each strain are stored in two different LN2 containers. Each straw is clearly identified with the client name, strain name, genetic background, allelic status, cryopreservation date, embryo number and stage of development. This information is also recorded in a database, with the number of straw, embryos per straw, storage location (container, canister, visotube)…

Efficiency of the technique: test thaw quality control to live pups

The ultimate goal is the revitalization of transgenic lines. But embryo viability depends not only on the cryopreservation process but also to the genetic background and the presence of a transgene. So embryo survival is monitored for each line: **i)** *in vitro* developmental rates are noted immediately after thawing and after one hour of culture, and **ii)** survival embryos are used for *in vivo* control by performing embryo transfers. For this *in vivo* control, 15-25 two-cell-stage embryos are transferred to post coïtum pseudopregnant fosther mother and the rate of live-born pups is observed. These tests are repeated twice.

An appropriate number of embryos are vitrified in order to obtain between 30 and 50 live-born pups. Optimally, 250 (homozygote strain) or 500 (heterozygous strain) embryos need to be cryopreserved. These effectives have been determined in absence of embryo lethality or a reduced prolificacy related to the transgene. In absence of interference between the transgene and prolificity, we request 5-10 male miceand 25-50 female mice. 5-10 females should be supplied every week or every fortnight.

References

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