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Cryopreservation of transgenic mouse embryos — an eight years' experience

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Abstract

A total of 70,629 predominantly transgenic mouse embryos prepared from 9,727 pregnant female donors were cryopreserved using a method leading to a high revitalisation rate. Against loss, 125 mutant mouse lines were protected. An average of 7.26 embryos (eight-cell embryos) per pregnant donor was received. To reduce the number of animals required as embryo donors, a special breeding exclusively for cryopreservation was omitted if possible and subsequently the number of animals used for freezing was reduced remarkably. The advantage of this strategy is that (mutant) mouse lines out of current use do not have to be kept in a breeding nucleus.

In parallel, this procedure leads to rederivation and improves the export of mice to other facilities. The cryopreservation of these 125 mutant lines keeps the potential to save approximately 20,000 laboratory mice per year to be bred if they were kept in a breeding stock. This is a major contribution to the "3R" requirements developed by Russel and Burch to reduce the number of laboratory animals.

Keywords: Cryopreservation; Transgenic mouse embryo; Rederivation; Embryo banking; Embryo transfer; Revitalisation; 3R

Abbreviations: LN2, liquid nitrogen; p.c., post coitum; PBS, phosphate buffered saline; SPF, specified pathogen free; VP^+, vaginal plug positive

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Introduction

In the past 30 years, many basic questions of gene regulation and function were addressed and answered using spontaneously or targeted mutants. 20 years ago it became possible to generate stable mutated rodents with the help of transgenic technologies. Consequently, a tool was available to study the role of single genes in more complex organisms. These techniques, however, resulted in a dramatic increase of transgenic animals, mainly mice (four *Medline* cited publications in 1982, about 6,500 in 2002). In the beginning, transgenic overexpressers (Palmiter et al., 1982) were used, followed — with the availability of the embryonic stem-cell technology (Evans and Kaufman, 1981; Martin, 1981) — by targeted mutagenesis or by combinations of both techniques.

Generation and characterisation of transgenic animals require great efforts. The population of those mutant lines is most often very small, yet their scientific benefit is enormous. Following generation and characterisation, transgenic lines must be kept in stock, even if they are out of any experimental use. Otherwise they will be lost and can be recovered only hardly.

Needs to save space (and money) for mouse housing, to rederivate infected mouse lines, and the increasing problems to import or export mice made a search for alternatives to standard breeding procedures mandatory: As an alternative to maintain (mutant) lines in stock and to protect them against an unexpected loss, several techniques were developed preserving early embryonic stages or spermatozoa of (transgenic) rodents, preferentially of mice. The cryopreservation technique for embryos was developed in the early 1970s (Leibo and Mazur, 1971; Whittingham, 1971a, b; Whittingham et al., 1972; Leibo et al., 1974) to preserve murine inbred strains. Several reports describe a long-term storage of cryopreserved embryos without major problems (Whittingham, 1971a, b; Whittingham, 1974; Muhlbock, 1976; Leibo, 1977, 1986; Mobraaten, 1986; Hedrich and Reetz, 1988, 1990; Dulioust et al., 1995). In addition to the protection of mutants against dying out, the idea of animal welfare, e.g. the requirements of Russel and Burch (1959) to reduce the number of laboratory animals used (3R hypothesis), was taken into consideration.

Consequently, we decided to consistently cryopreserve (mutant) mouse lines kept by different research groups in the German Cancer Research Centre (DKFZ) and neighbouring facilities. The aim of this work was to utilise a technique to cryopreserve bulk numbers of mutant mouse lines on the genetic background they were investigated. The aim was not to develop sophisticated techniques to preserve "difficult" lines or to backcross those lines to a certain genetic background within the same procedure. Therefore, animals or embryos, respectively, of different housing units exhibiting different technical and hygienic standards were to be handled.

After the assessment of several freezing protocols (Wood and Farrant, 1980; Mobraaten, 1986; Kasai et al., 1990; Kasai et al., 1992; H. Hedrich, S. Leibo, L. Mobraaten, H. Mossmann and I. Reetz, pers. comms.), the revised two-step method published originally by Leibo (1986) yielded most efficient in our laboratory.