

Cryopreservation of Mouse Lines

Each freezing technique involves a number of stages, outlined below. The default method will be sperm freezing. If quality control results are not satisfactory, embryos will be frozen. The choice of stage will depend on a variety of factors including strain background.

SPERM FREEZING WITH QC

- sacrifice of males for sperm collection and freezing
- superovulation of females and oocyte collection, followed by IVF
- embryo transfer to pseudopregnant females
- sacrifice of E14.5 pregnant females to assess embryo quality.

2-CELL EMBRYOS (IVF)

- superovulation of females and oocyte collection
- sacrifice of males for sperm collection for IVF
- freezing 2-cell embryos
- embryo thawing and transfer to pseudopregnant females
- sacrifice of E14.5 pregnant females to assess embryo quality.

MORULA FREEZING

- superovulation of females
- morula collection and freezing
- embryo thawing and transfer to pseudopregnant females
- sacrifice of E14.5 pregnant females to assess embryo quality.

The overriding consideration is to minimize pain and distress to the animals. The following guidelines will be adhered to:

1. Superovulation and collection of oocytes or morulae: Three or five days prior to oocyte or morula collection 3-5 week-old female mice will be injected IP with 5 units of Pregnant Mare's Serum (PMS). 46 hours later mice will be injected with 5U Human Chorionic Gonadotropin (HCG). For oocytes, females will be sacrificed by cervical dislocation the following morning. For morula mice will be mated individually with a single male mice and checked for vaginal plugs the following morning. They will be sacrificed by cervical dislocation at E2.5. Oviducts will be removed and oocytes or embryos will be flushed from them. Oocytes will be used for IVF. Healthy looking morula will be frozen using the slow freezing technique.

2. Sperm Collection and IVF: 10 days before sperm collection, male mice will be stimulated by mating with females for five days, the females will then be removed from the cages. After a further five days, the males be sacrificed by cervical dislocation to remove the epididymis for sperm collection. For sperm freezing, sperm will be frozen in straws in the vapour phase of LN2. For IVF, fresh or thawed sperm will be incubated with the oocytes for four hours, following which the oocytes will be cultured

overnight. Oocytes which have undergone division to the two cell stage, will be transferred to the oviducts of pseudopregnant females.

3. Embryo Transfer: Female ICR mice will be mated with vasectomized male mice (2 females/male). The following morning female mice will be checked for presence of vaginal plugs. Females that have not been plugged will be set aside and used in future experiments of this nature. Plugged females will be anesthetized by an IP injection of ketamine/xylazine. Once anaesthetized their backs will be wiped with alcohol and a single incision will be made in the lower back region, on one side of the spinal cord. The oviducts will be exposed, and the ovarian bursae torn. About 15 embryos will then be introduced into the infundibulum with the aid of a mouth-controlled glass pipette. The oviducts will then be returned into the abdomen of the mouse, and the incisions closed with surgical clips. Females will be kept in warmed cages until they regain consciousness, after which they will be returned to the mouse rooms. At E14.5 females will be sacrificed by cervical dislocation to assess embryo quality. A small tissue sample will be taken from the embryo for PCR analysis.