Shipping Refrigerated Embryos

Introduction

The ability to transport unfrozen embryos over long distances has been recognised for many years, although it has not become common practise and most laboratories have preferred to transport frozen embryos at LN_2 temperature. Recently, there has been a resurgence of interest in transporting embryos in the absence of LN_2 which has been focused on simplifying the exchange of mutant mouse strains. Of particular relevance to the mouse community is the ability to transport unfrozen embryos that had previously been cryopreserved. This allows laboratories that don't have access to LN_2 or are not familiar with handling frozen embryos to take advantage of the vast numbers of mouse strains held in archives around the world without resorting to live animal transportation (Takeo et al., 2009; Takeo et al., 2010).

Two important observations underpin the success of the protocol described in the following text. Firstly, a period of *in vitro* culture before transportation promotes embryo viability and secondly, the temperature gradient used to cool the embryos prior to transportation is important for a successful outcome.

A. Thawing embryos

1. Thaw the embryos following an appropriate protocol.

2. Transfer embryos into a 200µl drop of KSOM plus amino acids, overlayed with silicone fluid or mineral oil, and culture for 2-3hrs in

a CO_2 incubator at 37°C.

B. Preparation of embryos for shipment

1. Make 3 x 150µl M2 drops in a culture dish.

2. After the embryos have been cultured in the incubator wash them through the 3 drops of M2.

3. Fill 0.5ml microfuge tube to the top with 0.6ml M2 medium at room temperature, then load 30-40 embryos into each microfuge tube and seal with parafilm (Fig. 1).

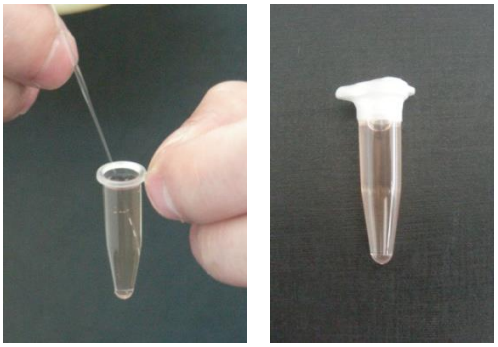

Fig. 1

C. Preparing the refrigerated package

1. Place the tube containing the embryos into a biotube which is supplied within the cold transportation kit (Fig. 2).

Fig. 2

2. Place the biotube into the aluminium lined box (room temperature) (Fig. 3a), then place two gel cool packs (room temperature) into the lined box, so they surround the biotube (Fig. 3b).

Fig. 3a Aluminium lined box

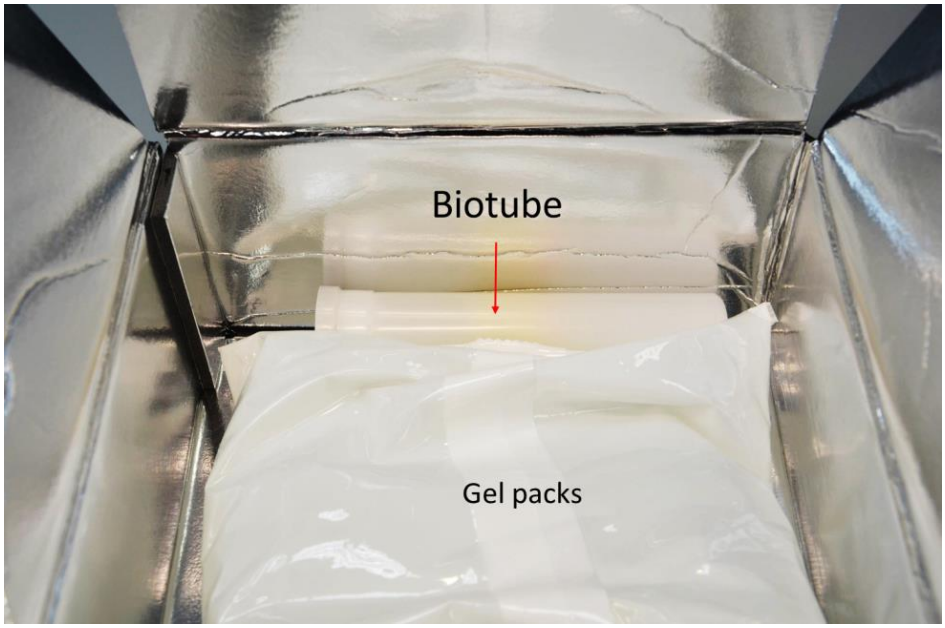

Fig. 3b Inside of the aluminium lined box

3. Seal the aluminium box with sellotape.

4. Place the aluminium box into the polystyrene container following the assembly instructions. Then seal the polystyrene container with packing tape (Fig. 4). This thermal control unit will maintain a temperature of 4-8°C for up to 72hrs (Fig. 5). The embryos will be viable for at least 72hrs under these conditions.

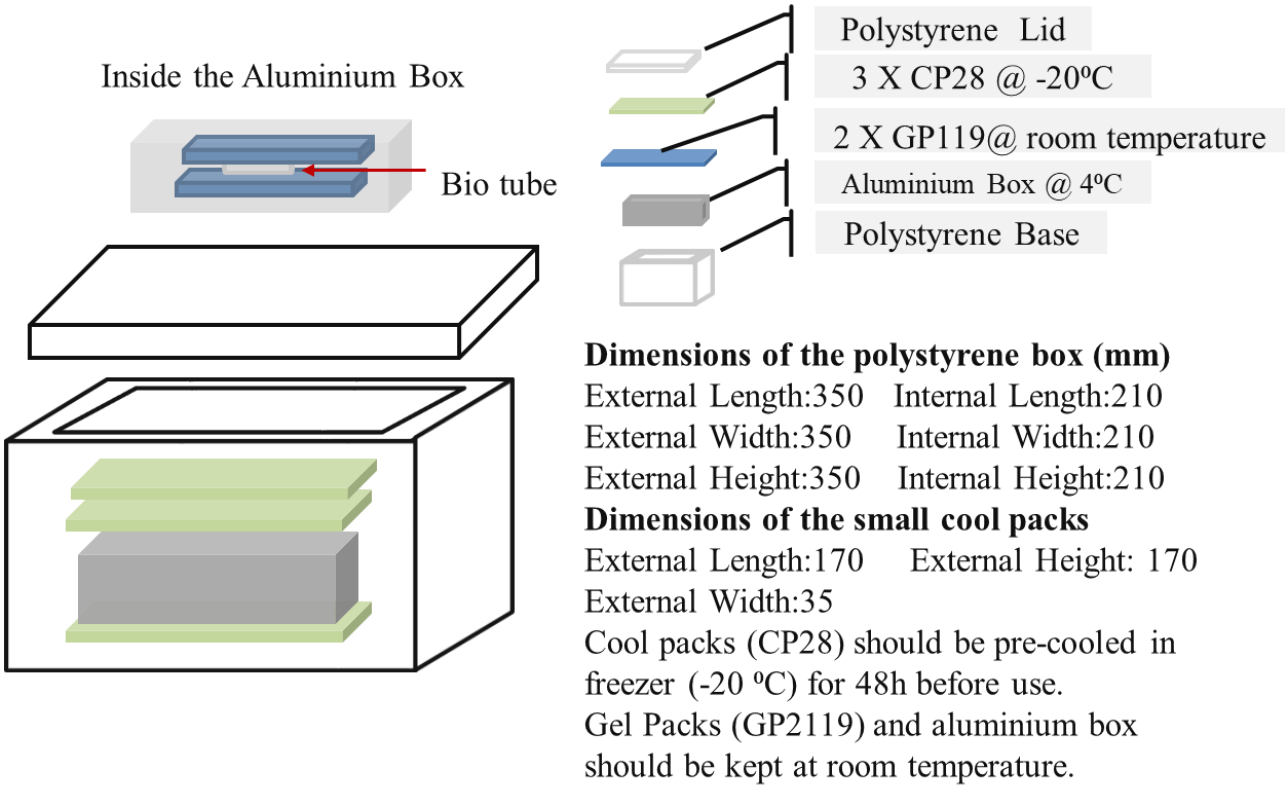

Fig. 4 Cold package assembly instructions

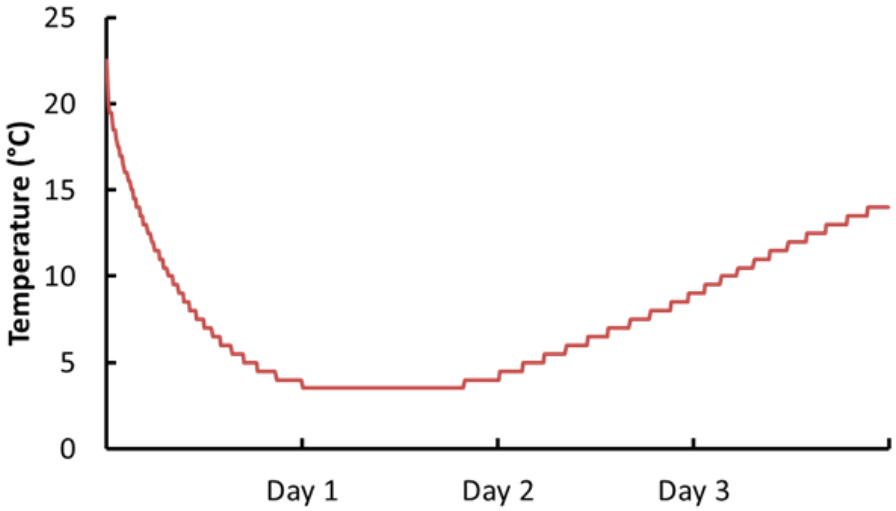

Fig. 5 Temperature profile of the cold package during transportation

5. Send the samples via a regular delivery service.

D. Arrival of the refrigerated package

1. When the cold package arrives, remove biotube from aluminium box, and remove the microfuge tube containing embryos from the biotube.

2. Allow tube to stand vertically at room temperature for 30mins avoiding direct exposure to light.

3. Open the microfuge tube containing the embryos and gently re-suspend the embryos.

4. Aspirate the entire M2 medium from the tube using 1000µl pipette, and then transfer the solution to the centre of a 60mm culture dish.

5. Locate the embryos and transfer them to a 200µl drop of fresh M2 medium.

6. The embryos are now ready for use and may be transferred into recipient females or cultured in KSOM plus amino acids, overlayed with silicone fluid or mineral oil, until required.

E. Experimental results

Table 1 *In vitro* development of frozen/thawed 2-cell C57BL/6NTac embryos after being held for 24, 48 and 72hours in M2 medium at 4-8 °C. The data represent the total number of embryos tested over 3 replicate experiments.

Duration held at 8°C	No. embryos tested	No. developed into blastocysts	Mean blastocyst development rate (%)	SEM
0	100	85	87.33	5.98
24hrs	94	79	84.35	2.65
48hrs	93	77	85.42	4.81
72hrs	95	58	66.99	20.05

Table 2 *In vivo* development of frozen/thawed 2-cell C57BL/6NTac embryos after being held for 24, 48 and 72hours in M2 medium at 4-8 °C. The data represent the total number of embryos tested over 3 replicate experiments.

Duration held at 8°C	No. embryos transferred	No. offspring produced	Mean Birth Rate (%)	SEM
0	108	40	37.04	10.32
24hrs	108	51	47.22	8.50
48hrs	108	36	33.33	13.14
72hrs	108	32	29.63	13.08

F. References

Takeo T., Kaneko T., Haruguchi Y., Fukumoto K., Machida H., Koga M., Nakagawa Y., Takeshita Y., Matsuguma T., Tsuchiyama S., Shimizu N., Hasegawa T., Goto M., Miyachi H., Anzai M., Nakatsukasa E., Nomaru K., and Nakagata N. 2008. Birth of mice from vitrified/warmed 2-cell embryos transported at a cold temperature. *Cryobiology.* 58(2): 196-202

Takeo T., Kondo T., Haruguchi Y., Fukumoto K., Nakagawa Y., Takeshita Y., Nakamuta Y., Tsuchiyama S., Shimizu N., Hasegawa T., Goto M., Miyachi H., Anzai M., Fujikawa R., Nomaru K., Kaneko T., Itagaki Y., and Nakagata N. 2010. Short-term storage and transport at cold temperatures of 2-cell mouse embryos produced by cryopreserved sperm. J Am Assoc Lab Anim Sci. 49(4): 415-419.