# 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.

*1. Media*

1.1. M2 Medium
1.2. KSOM (e.g., Millipore/Chemicon: MR-020P-5F)
1.3. MEM Amino Acids Solution (50X) (e.g., Life Technologies: 11130036)
1.4. MEM Non-essential Amino Acid Solution (100X) (e.g., Sigma: M7145-100ML)

*2. Equipment*

2.1. PCR tube with attached flat cap polypropylene 0.5ml Brand (Fisher Scientific)
2.2. Parafilm
2.3. Dissecting microscope
2.4. Laboratory timers
2.5. Code 570 Biotransporter boxes (Air Sea Containers)
2.6. Ice packs (Fisher Scientific, ICE-910-020W)
2.7. ThermoCafé by Thermos 0.5 Litre Flask (Argos, 927/0518)
2.8. Maxim Integrated Products iButton Thermochron F5 (Digi-Key Corporation, DS1921G-F5#-ND)
2.9. 7.0ml Bijou or similar container

*3. Protocol steps*

3.1. Thaw the embryos following an appropriate protocol
3.2. Transfer embryos into a 200µl drop of KSOM, plus amino acids and culture for 2-3hrs in a $CO_2$ incubator at 37°C.
3.3. Make 3 X 150µl M2 drops in a culture dish.
3.4. After the embryos have been cultured in the incubator wash them through the 3 drops of M2.
3.5. Fill 0.5ml microfuge tube with 0.6ml M2 medium at room temperature, then load 30-40 embryos into each microfuge tube and sealed with parafilm (Fig 1).

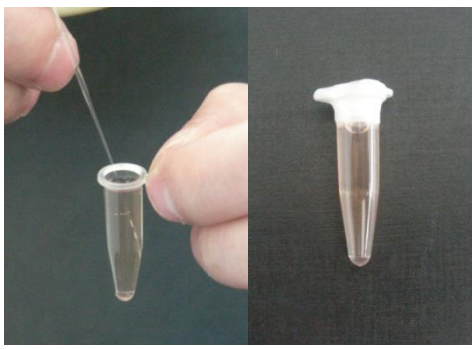


Fig. 1

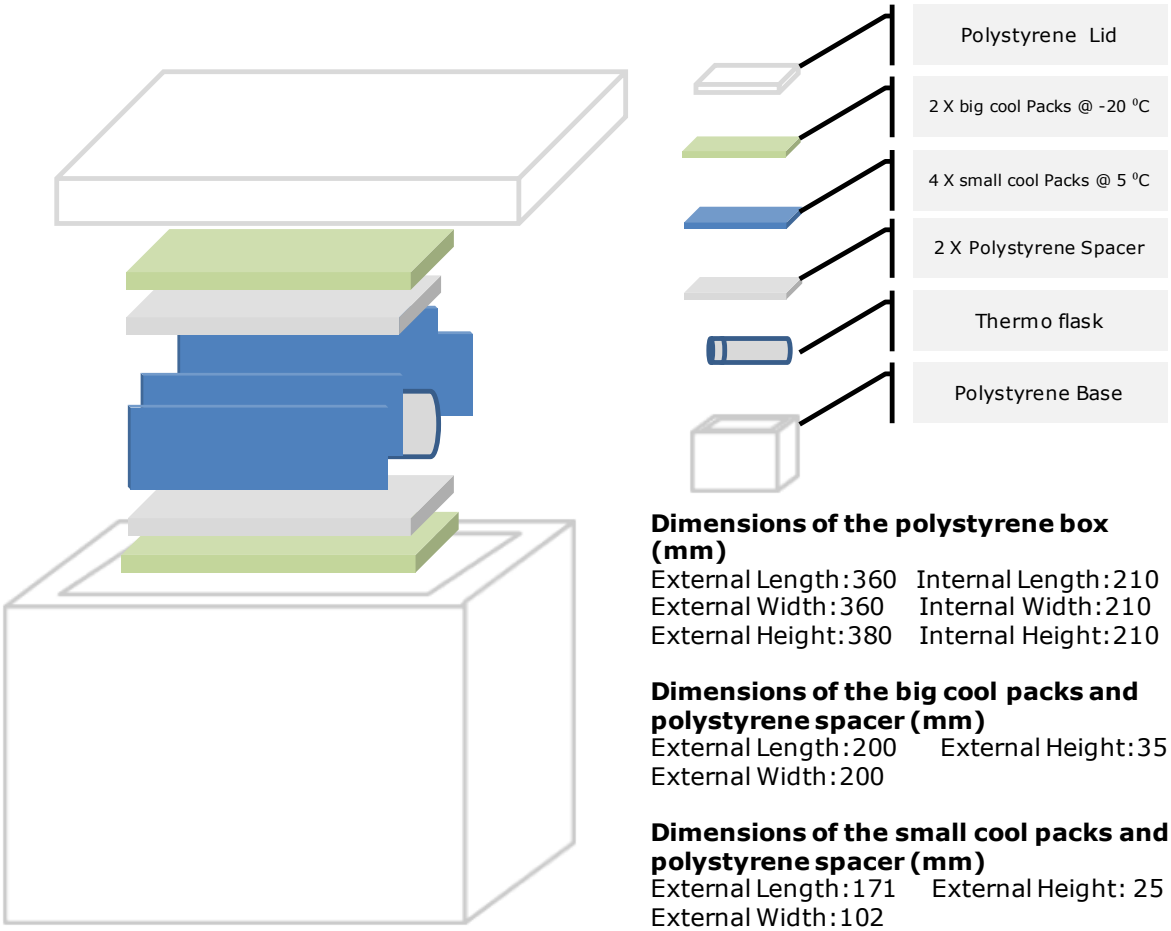
3.6. Place the tube containing the embryos into a 7ml Bijou (Fig 2).

Fig. 2

3.7. Insert two small cold packs and a thermo iButton into a thermos flask, and then insert the Bijou containing the embryos and close the cap (Fig.3).


Fig. 3

3.8. Place the thermos flask into polystyrene box following the assembly instructions below (Fig. 4). This arrangement will keep the embryos at 4-8C for 72hrs (Fig. 5). The embryos will maintain their developmental potential ability during this holding period.




**Dimensions of the polystyrene box (mm)**
External Length:360 Internal Length:210
External Width:360 Internal Width:210
External Height:380 Internal Height:210

**Dimensions of the big cool packs and polystyrene spacer (mm)**
External Length:200 External Height:35
External Width:200

**Dimensions of the small cool packs and polystyrene spacer (mm)**
External Length:171 External Height: 25
External Width:102

Fig. 4

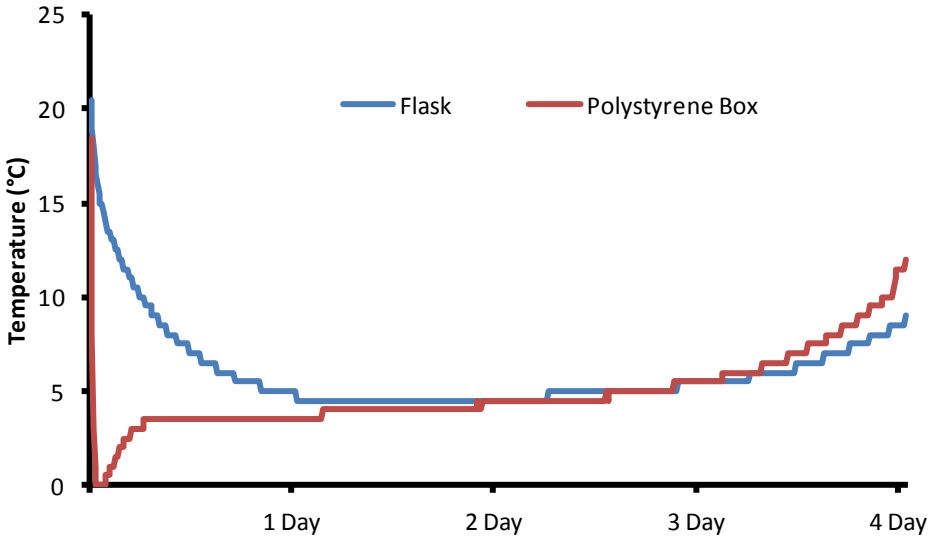



Fig. 5

3.9. Send the samples via a regular delivery services.

3.10. When the cold package arrived, remove the Bijou from the thermos flask.

3.11. Allow the Bijou to stand vertically at room temperature for 30mins avoiding directly exposure to light.

3.12. Open the Bijou and remove the microfuge tube containing the embryos and open it.

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

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

3.15. The embryos are now ready for use and may be transferred into recipient females or in KSOM, plus amino acids until required.

**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 |

## Reference

1. 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

2. 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.