A simple and efficient vitrification procedure for cryopreservation of mouse embryos in straws

Based on a method originally published by Nakagata et al (1997)

A. Preparation for vitrification

1. Fill the sandwich box with crushed ice and place the Vitrification Cooling Plate (VCP) on top (Figure 1). The temperature of the VCP will reach to 0°C within 10 seconds.

Figure 1.

2. To prepare the straws, push the cotton/polyvinyl alcohol plug down the straw, from position A to position B, using a metal rod with a stop (Figure 2).

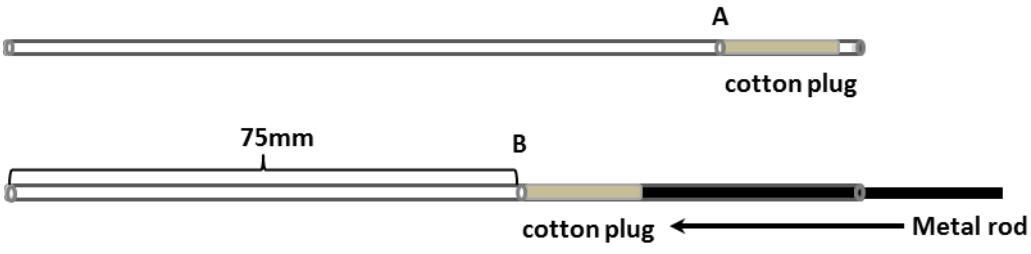


Figure 2.

3. Place the straws on a Perspex support. Using a permanent marker pen, make two calibration marks using the guidelines on the plate (Figure 3). The calibration marks should be placed at 17mm and 31mm intervals from the end of straw (opposite the PVA plug end).

4. Label the straws with the appropriate sample code and place them on the VCP.

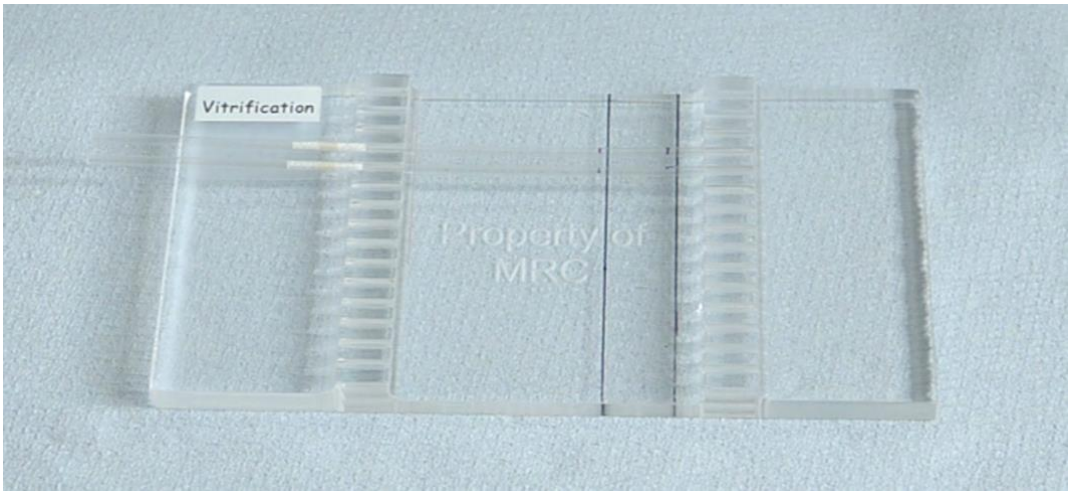


Figure 3.

5. To prepare the dishes, label three 60mm culture dishes (DMSO, DAP213, Sucrose) and place the DAP213 and Sucrose dish onto the VCP (Figure 4). These solutions can be purchased from Cosmo Bio Co., Ltd (www.cosmobio.co.jp)

6. Pipette 45µl drops of DAP213 and 0.25M Sucrose into their corresponding dishes, the number of drops will be the same as the number of straws.

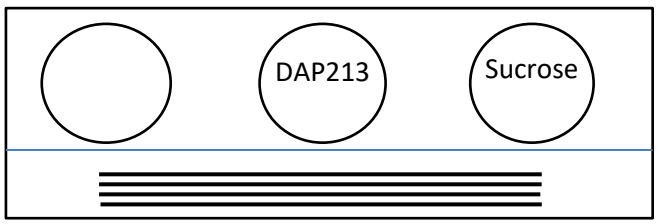
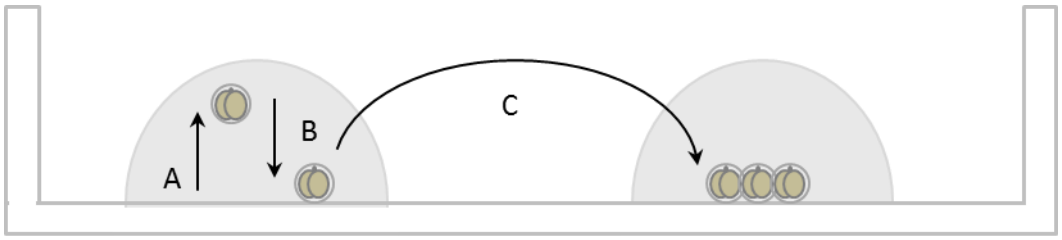


Figure 4.

7. Filter 1M DMSO and place one 80µl wash drop into the 60mm culture dish. Place subsequent 45µl holding drops into the same dish, the number of 45µl drops will be the same as the number of straws.

B. Pre-treatment of embryos in 1M DMSO solution

1. Load an embryo handling pipette with 1M DMSO solution and transfer the embryos from the culture medium into the 80µl wash drop of DMSO. The embryos will float on top of the drop and then sink to the bottom within 1-2mins.
2. Once the embryos have settled, split them equally between the remaining 45µl drops (Figure 5).
3. Once this is done, place the DMSO dish on the VCP for 5mins.



A. Embryos float in the drop
B. Embryos sink to the bottom of dish in 1- 2min
C. Embryos transferred and grouped to other drops

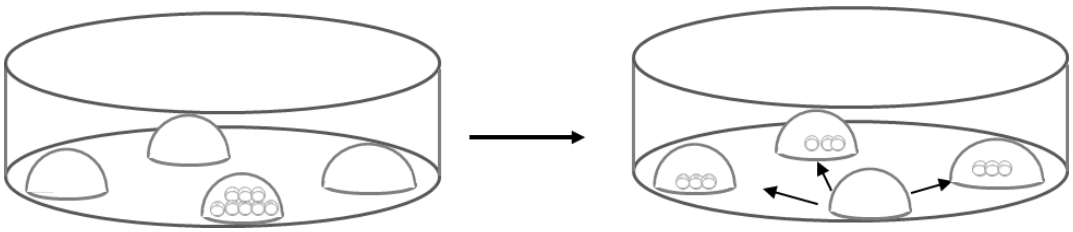


Figure 5.

C. Embryo Exposure to DAP213 solution

1. After 5mins have elapsed, move the DMSO dish to the microscope and use a wedge-shaped 10µl pipette tip to transfer 5µl 1M DMSO containing the embryos to the top of one of the 45µl DAP213 drops, keeping the dish on the VCP and taking care not to touch the drop with the pipette tip (Figure 6).

2. Set a timer for 5 mins after the embryos from the first drop have gone into the DAP213.

3. Repeat the process for any subsequent drops.

(**Tip:** to stop the embryos sticking, pick up 1µL of DMSO first, then the embryos).

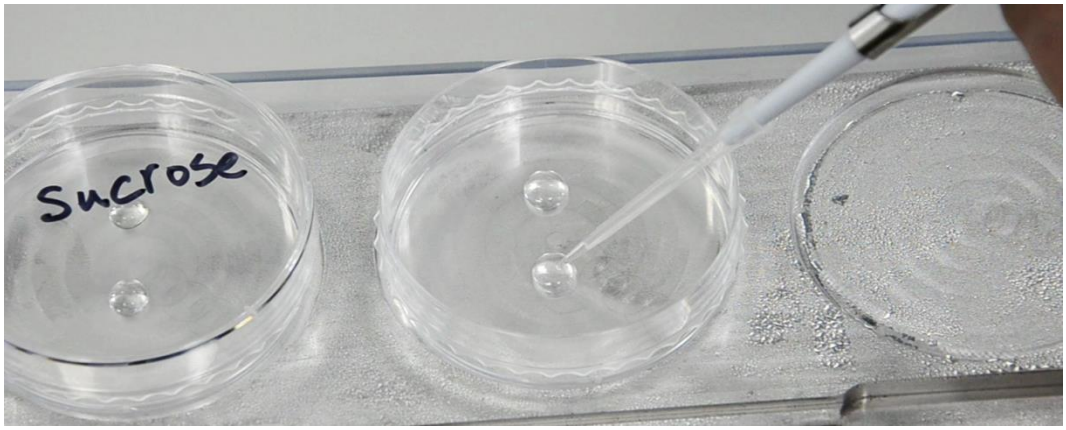


Figure 6.

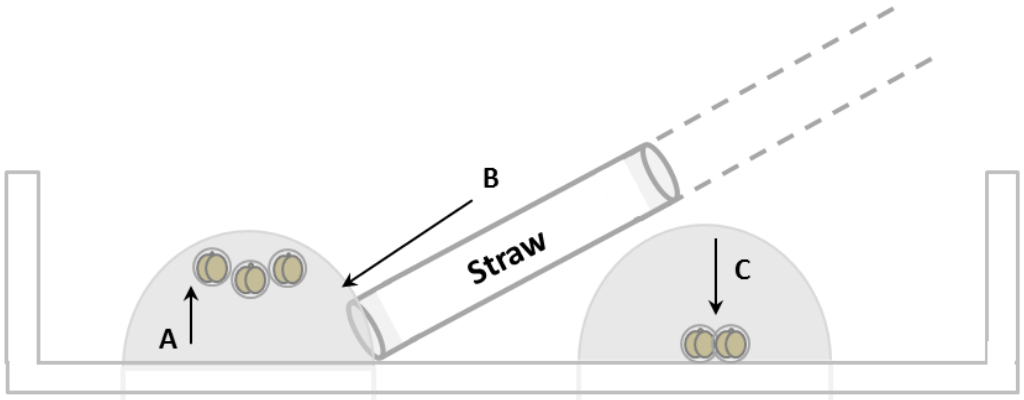
4. After 1 minute, when the embryos have partially equilibrated in the DAP213 drop, start to load the embryos into the pre-cooled straws (Figure 7):

   a) Attach a 1ml syringe to the labelled end of the straw and aspirate 0.25M Sucrose solution, until the meniscus reaches mark 1.

   b) Then aspirate air, to move the sucrose meniscus to mark 2.

   c) Then aspirate the entire drop of DAP213 containing embryos into the straw.

   d) Aspirate air until the sucrose meniscus is drawn half way along the cotton/PVA plug.



A. Leave the embryos in the DAP213 drop for 1min to partially equilibrated;
B. Loading the straw: to avoid embryos attaching to the outside of wall of the straw, place the straw towards the bottom of the drop;
C. Do not leave the embryos in the drop for more than 3min as they may start to sink to the bottom of the drop, which will cause embryo lost during loading.

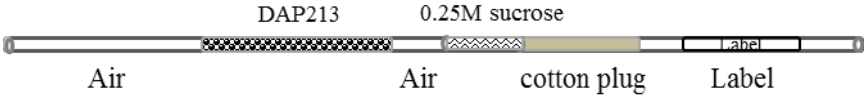


Figure 7.

5. Place the straws back to VCP and seal the unlabelled end of the straw.

6. Once the 5min equilibration time is up, plunge the straws into LN_2, and transfer the straws to a secure LN_2 location.

D. Warming Vitrified Embryos

1. Place a tube of 0.25M sucrose warming solution (1ml) in incubator for at least 5min before warming.

2. Add 0.9ml 0.25M sucrose (pre-warmed to 37°C) into a 60mm culture dish, and place the dish on a 37°C heated stage.

3. Remove the required straw from the storage tanks and place in a flask of LN_2.

4. Using forceps take the straw from the LN_2 and place it in a 37°C water bath (Figure 8). Once the DAP213 fraction has melted, remove the straw immediately from the water bath.

Figure 8.

5. Wipe the straw with a tissue, then holding the straw firmly and horizontally, cut through middle of the cotton/PVA plug.

6. Cut off the heat sealed end of the straw, then holding the straw vertically over the drop of 0.9ml 0.25M sucrose, use a metal rod to push down the remaining cotton/PVA plug, expelling the contents of the straw into the drop. Do not allow the tip of straw to touch the drop. Set a countdown timer for 3mins.

7. Gently rotate the dish to mix the solution.

8. After 3mins, collect the embryos from the solution, and transfer them into a drop M2 and assess for damage before moving them to a second drop of M2.

9. After the final wash the embryos are ready to be transferred into the recipient female.

10. If you are not ready to set up the embryos transfers the embryos should be moved to a KSOM^{+AA} drop, under oil, and held in the incubator until required.

E. References

Nakao, K., Nakagata, N., and Katsuki, M. 1997. Simple and efficient vitrification procedure for cryopreservation of mouse embryos. Experimental animals / Japanese Association for Laboratory Animal Science 46:231-234.

Nagy A., G.M., Vintersten K., Behringer R. 2003. Manipulating the Mouse Embryo - A laboratory manual (3th Edition). Cold Spring Harbor Laboratory press, New York.