# Mouse Morula Freezing and Thawing Protocol

**Mutant Mouse Resource and Research Center**

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# Mouse morula freezing

**Supplies:**

- Flushing and holding media (FHM) or comparable
- 1.5 M DMSO in FHM (add 1065 µL DMSO in 8.935 mL FHM)
- 0.5 M sucrose (0.86 g sucrose in 4.5 mL FHM, filtered)
- Sterile CBS straws (0.3 mL) and weights
- Syringe and CBS straw adapter
- Hand or mouth micropipette
- 35mm Petri dishes (Falcon 1008)
- Heat sealer
- Timer
- Crysalys freezer
- Liquid nitrogen ($LN_2$)

**Procedures:**

1. Load each straw with freezing media according to **Figure 1**. There should be approximately 3 times more sucrose than DMSO solution each straw.
2. Select high quality embryos for freezing (up to 35 embryos per straw).
3. Pick up the embryos with a micro pipette tip with as little media as possible. Be careful to keep the embryos close together, to prevent too much media from entering the 1.5 M DMSO column.

   ***Note: Load the micro pipette with a small amount of 1.5 M DMSO prior to picking up the embryos. This will minimize the amount of other media diluting the 1.5 M DMSO column.***
4. Insert the pipette into the straw and gently expel the embryos into the 1.5 M DMSO column. Quickly remove the pipette tip.
5. Seal both ends of each straw and confirm embryos are in the proper column under a microscope.
6. Allow the embryos to equilibrate at room temperature for a minimum of five minutes, but no more than 15 minutes, in the column of 1.5 M DMSO before starting the freezing program.
7. Place the straws into the Crysalys using the morula freezing program.
   a. Morula freezing program: Starting temperature: 22.0°C, cool to –7°C at a rate of -2°C/minute, hold at –7°C for 10 minutes to allow for manual seeding, before proceeding to -40°C at a rate of -0.5°C/minute.

   ***Note: Manual seeding is done by gently touching forceps cooled in $LN_2$ to the very top of the DMSO column.***
8. When the program has reached -40°C, straws should be quickly plunged into $LN_2$.
9. Transfer the straws to their appropriate storage location.


**Figure 1. A straw for cryopreservation of mouse embryos.** Add a large column of 0.5 M sucrose (A), air bubble, a small column of 1.5 M DMSO (B), another air bubble, and a smaller column of 0.5 M sucrose. Do not allow media to touch the filter (C).

## Mouse Morula Thawing:

**Supplies:**

- Flushing and holding media (FHM) or comparable
- Equilibrated (37°C; 5% $CO_2$) KSOM Culture Dish
- Hand or mouth micro pipette
- Syringe and syringe tip adapter for CBS straws
- Scissors
- Beaker with room temperature water (approximately 22°C)
- Kimwipes®
- Timer

**Procedure:**

1. Hold a single straw in air for 15 seconds.
2. Plunge the straw into room temperature water and hold for approximately 10 seconds.
3. As soon as the ice dissipates, pull the straw out of the water and wipe off with a Kimwipe®.
4. Holding the straw horizontally, cut the sealed end, opposite the filter.
5. Direct the cut end into a Petri dish and then cut the opposite end (below the filter) to expel the contents using a syringe with adapter (**Figure 2**).
6. Using a timer, allow embryos to equilibrate for 5 minutes.
7. Collect the embryos and transfer them into another petri dish with fresh FHM or comparable solution. Wash the embryos 2 more times before transferring to equilibrated KSOM dishes.

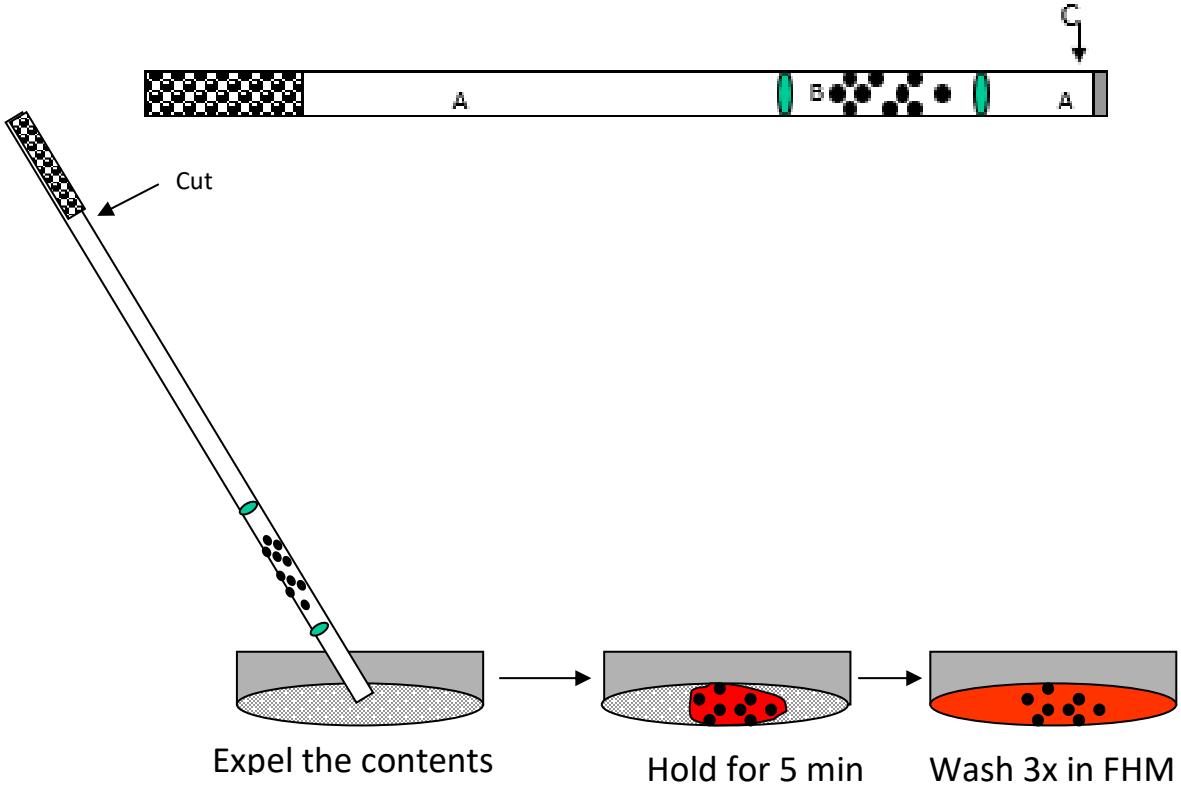


**Figure 2** Thawing mouse embryos: holding the straw horizontally, cut the sealed end opposite the end with the label. Direct the cut end into a Petri dish and then cut the straw again, just below the filter to expel the contents. Allow embryos to equilibrate for 5 minutes. Collect the embryos and transfer into another petri dish with fresh FHM. Wash the embryos 2 more times before further culture.