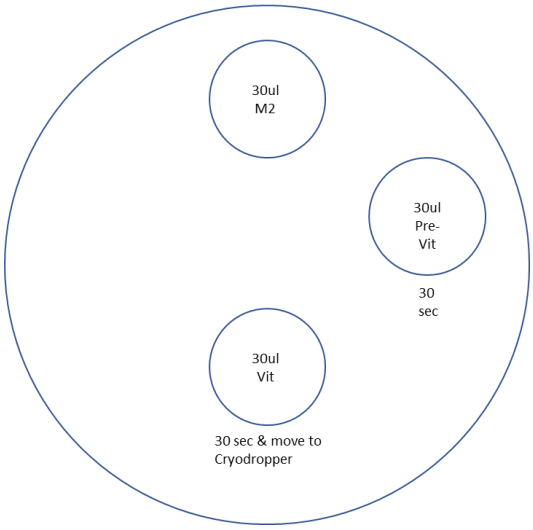
Protocol for Cryodropper Vitrification of Mouse Embryos

Vitrification of embryos

1. Embryos are kept in M2 at 37°C until cryopreservation.
2. Make vitrification and pre-vitrification media.
3. Set up portable LN_2 vapor phase freezer. [We use a Styrofoam cooler and float.]
4. Label each Cryodropper. [We color code or label just the bulb portion.]
5. Preload 100µl 0.5M Sucrose M2 in the Cryodropper bulb. Place the 100µl drop on a petri dish and pipette into the Cryodropper. Hold the Cryodropper by the open end and gently flick the media into the bulb portion. Gently squeeze the bulb to remove any media remaining in the embryo loading area and wipe the liquid off with a Kimwipe. Preload <5µl vitrification media into the center of the straw area by gently positioning a drop using the same method as before or with a gel loading tip. Store open end up in an Eppendorf rack.
6. On a cover of a 35mm dish, a drop of M2 (20-30µL per drop), a drop of pre-vitrification solution, and a drop of vitrification solution is loaded.
7. Label and precool the cryogenic storage vial. [We use a 4ml cryovial and label as needed. 4 Cryodroppers will fit per vial.]
8. Embryos are first transferred to the drop of M2 in the 35mm dish.
9. Prefill an embryo pipette with a small amount of the pre-vitrification solution. Under the dissecting microscope, transfer embryos from the M2 drop to the drop of pre-vitrification solution.
10. Incubate 30 seconds.
11. Prefill the embryo pipette with a small amount of vitrification solution. Transfer embryos from the pre-vitrification solution drop to the vitrification solution drop.
12. Incubate 30 seconds.
13. Collect the embryos and load them into the vitrification solution preloaded in the Cryodropper. [It helps to focus the microscope on the embryos in the pipette.]
14. Seal Cryodropper at the end with a heat sealer. [Gently test to be sure the device is sealed, if not, seal again.]
15. Plunge Cryodropper into LN_2 until media in bulb freezes (@10 seconds). Store on raft in LN_2 vapor until all samples are processed. Transfer to vials.
16. The vial is then transferred to a LN_2 dewar and stored in the vapor phase.

Vitrification Layout

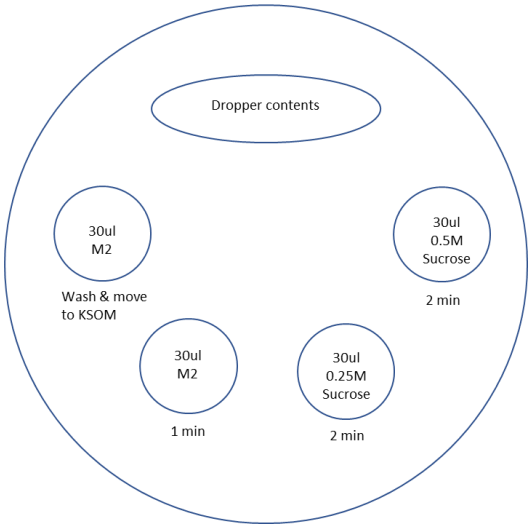


Protocol for Cryodropper Recovery of Mouse Embryos

Thawing of vitrified embryos

1. Prepare thaw dish for each cryodropper as follows: a 60mm lid with space across the top (for 0.1 mL of 0.5 M sucrose in M2), and then 20-30µl drops of each clockwise: 0.5M sucrose in M2, 0.25M sucrose in M2, and M2 (2 drops). Prepare incubation dish as follows: 60mm dish with 100µl KSOM elongated drop through center, 30µl KSOM drop on either side for washing embryos prior to culture. Cover with paraffin and equilibrate at 37°C with 5% CO_2 for at least 30 min.
2. The cryodropper containing vial is removed from the storage vessel and kept in LN_2 vapor until ready for thaw.
3. The cryodropper is immersed in a water bath at 37˚C until the media in the dropper is thawed (@10 sec).
4. The tip of the cryodropper is cut off and the embryos are deposited onto the culture dish. The 0.5M sucrose M2 in the Cryodropper bulb is gently flicked down to the straw and expelled from the cryodropper into the embryo drop, minimizing bubbles. The embryos are immediately collected from the drop using an embryo pipette prefilled with 0.5M sucrose in M2.
5. The embryos are transferred to a new drop of 0.5M sucrose in M2 followed by an incubation of 2 min.
6. Using an embryo pipette prefilled with 0.25M sucrose in M2, the embryos are transferred quickly to the drop of 0.25M sucrose in M2 and are incubated for another 2 min.
7. Using an embryo pipette prefilled with M2, the embryos are transferred quickly to the first drop of M2 and are incubated for 1 min.
8. The embryos are then washed through the last drop of M2, through the 2 drops of KSOM, and are then transferred to the long drop of KSOM under oil.
9. Embryos are cultured at 37°C with 5% CO_2 (for 2 days for late morula or blastocyst stage).

Recovery Layout



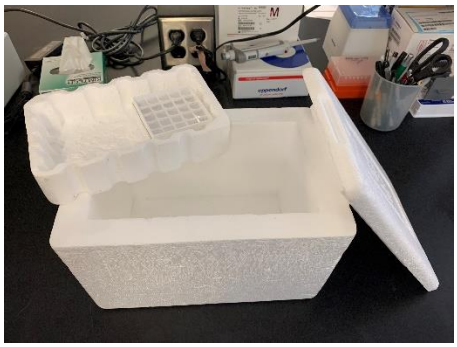
References:

Adapted by B. Stone from Wai Hung Tsang and King L. Chow *BioTechniques Protocol Guide 2010* (p. 55) doi 10.2144/000113258.

Cryodropper Video
https://youtu.be/J3iSHdoJi2E

Equipment:

Cryodropper for Embryo Vitrification (black line: ParaTechs 80010)
Cryovial (4 ml) or other suitable LN_2 storage option (USA Scientific 1440-9100)
CO_2 incubator at 37˚C
Slide warmer or 37˚C incubator
Water bath @ 37˚C (500ml beaker of water in a 37˚C incubator works well)
Pipettors and tips; ex: 1ml, 200ul, 20ul, 2ul
Eppendorf tubes, racks
Portable LN_2 vapor phase freezer with Styrofoam raft (see photo)
Fine tip Sharpie for labeling
Kimwipes
Tissue culture dishes; 35mm, 60mm
Embryo handling pipettes and mouth pipettor
Stereomicroscope with adjustable light source
Timer
Impulse heat sealer (American International Electric AIE-105T)
Forceps
Scissors
LN_2 vapor phase storage dewar
0.22µm filter units for sterilization (ex: Millipore SCGVUORE and SLGP033RS)
Refrigerator/freezer for media storage



Media and Reagents Required:

M2 medium (Millipore MR-015-D)
Ficol PM70 (Sigma-Aldrich F2878)
Sucrose (Sigma-Aldrich S1888)
Ethylene Glycol (Sigma-Aldrich 102466)
Dimethyl sulfoxide (DMSO)(Sigma-Aldrich D2650)
0.5 M sucrose
FS
Pre-VS
VS
0.25M sucrose
Paraffin Oil (Sigma-Aldrich 18512)
KSOM^{AA} medium (Millipore MR-121-D)

Recipes					
Medium	Component	Amount	Total Volume	%	Notes
FS	Ficol PM70	3.0g	10 ml		in PBS (pH 7.4), filter sterilize
	Sucrose	1.7g			
Pre-VS	Ethylene glycol	10ul	100 ul	10%	prepare fresh
	DMSO	10ul		10%	
	M2	80ul		80%	
VS	Ethylene glycol	15ul	100 ul	15%	prepare fresh
	DMSO	15ul		15%	
	FS	60ul		60%	
	M2	10ul		10%	
0.5M sucrose	sucrose	34.0g	100ml		prepare in M2, filter sterilize, store in 1.5ml aliquots at -20˚C
	M2	100ml			
0.25M sucrose	sucrose	1.7g	10ml		prepare in M2, filter sterilize, store in 100ul aliquots at -20˚C
	M2	10ml			