Recovery of embryos vitrified in EFS (ethylene glycol – ficoll - sucrose) solution

＊ Media ＊

Thawing sol. 1 (TS1): 0.75 M sucrose in PB1 or M2 (37^0C)

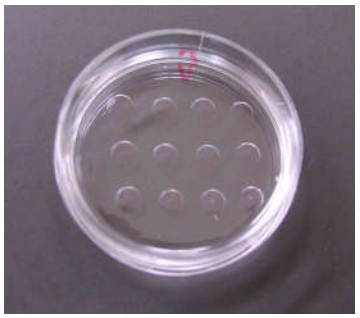
Thawing sol. 2 (TS2): 0.25 M sucrose in PB1 or M2 (room temperature)

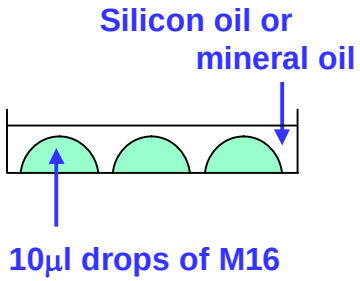
Culture medium : M16 (Sigma) containing 0.4% (w/v) BSA and equilibrated under 5% CO_2 in air at 37^0C

＊ Equipments ＊

Dissecting microscope, timer、glass capillaries with mouthpieces (for handling embryos)、pipettes 1,000 μl, 100 μl, watch glasses or 50 mm petri dishes（e.g., Falcon #1007）, 35 mm petri dishes for embryo culture (e.g., Falcon #1008), forceps, leather cryo gloves, face mask.

Fig. 1

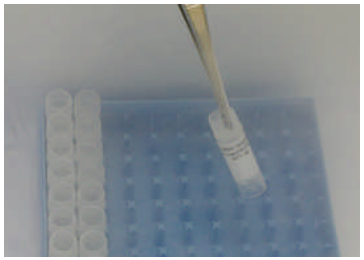




<Fig.1>
Prepare the culture dish with 10μl aliquots of M16.

Warm the TS1 to 37^oC.

Fig. 2



Retrieve a tube from the liquid nitrogen.

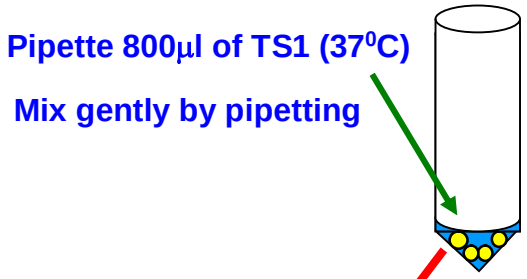
<Fig.2>
Put on the face mask and cryogloves and retrieve a cryotube from the liquid nitrogen container using forceps.

Fig. 3

Discard the liquid nitrogen and wait for 30 s.

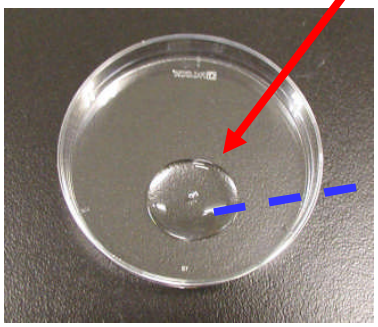
<Fig.3>
Quickly discard the liquid nitrogen in the tube, and let it stand for 30 s at room temperature.

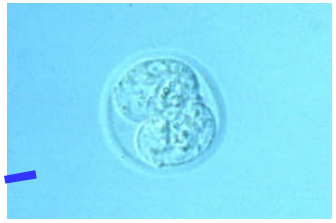
Fig. 4



<Fig.4>
Put 800 μl of TS1 (37°C) into the tube by a single out-pipetting, and then mix the solution gently by pipetting ten times in 25 sec. Please make sure that the solution is evenly dissolved.

Fig. 5





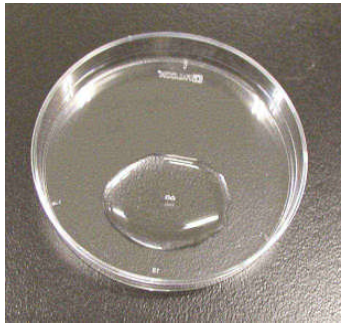
Shortly after recovery in TS-1

<Fig.5>
Using a 1000 μl pipette, transfer the entire volume to a watchglass or 50 mm petri dish. Hereafter, embryos are handled at room temperature until they are placed in M16.

Set the timer for 3 min and start it.

3 min

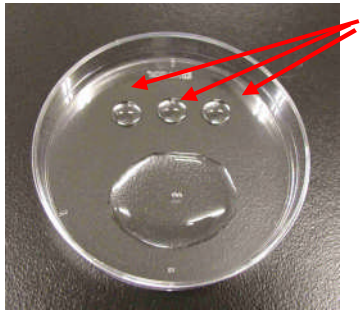
Fig. 6



<Fig.6>
Spread the solution over the surface of the watchglass or petri dish by gentle shaking.

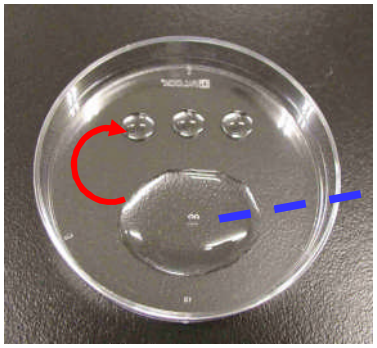
Dispersion of the solution makes it easier to spot the embryos.

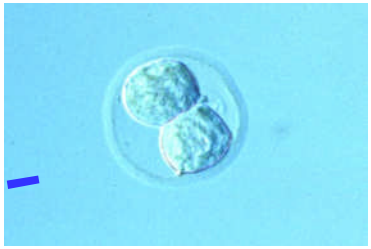
Fig. 7



<Fig.7>
Pipette three 50 μl drops of TS2 onto the surface of the dish.

Fig. 8





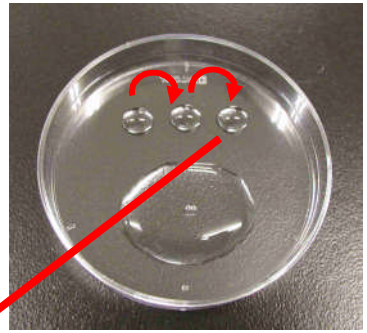
3 min after recovery in TS-1

<Fig.8>
After 3 min of warming (Fig.5), retrieve the embryos using a sterile mouth-pipette, and transfer them from the drop of TS1 to a drop of TS2.

Set the timer for 3 min and start it.

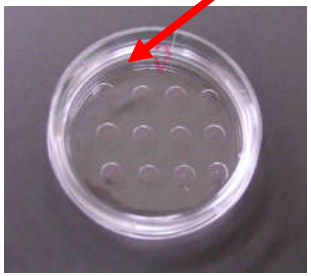
3 min.

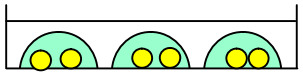
Fig. 9



<Fig.9>
Transfer the embryos to the second drop of TS2.
Then, transfer them to the third drop of TS2.

Fig. 10





Culture in M16 at 37^{0}C.

<Fig.10>
After 3 min (Fig.8), transfer the surviving embryos to 10 μl drops of M16.
Incubated embryos under 5% CO_2 in air at 37^{o}C until transfer to the oviducts.

1) Preparation of BSA-free PB1

		PB1 (BSA-free)	
	M.W.	mM	mg/100ml
NaCl	58.4	136.98	800.0
KCl	74.6	2.68	20.0
KH2PO4	136.1	1.47	20.0
Na2HPO4,12H2O	358.14	8.04	288.1
MgCl2,6H2O	203.3	0.49	10.0
Glucose	178.6	5.56	100.0
Na pyruvate	110	0.33	3.6
CaCl2,2H2O	147	0.9	13.2
Penicillin G			6.0 (approx)

Sterilize the solution by filtration.

2) Preparation of TS1 (0.75 M sucrose solution)

2-1. From PB1

Dissolve 7.7 g of sucrose in PB1 (BSA free) and bring the total volume to 30 ml.
Mix by gentle shaking until sucrose is completely dissolved.
Add 90 mg of BSA onto the surface the solution and leave it stand until completely dissolved.
Sterilize the solution by filtration.
Aliquot and store in a refrigerator.

2-2. From M2

Dissolve 7.7 g of sucrose in M2 and bring the total volume to 30 ml.
Mix by gentle shaking until sucrose is completely dissolved.
Sterilize the solution by filtration.
Aliquot and store in a refrigerator.

3) Preparation of TS2 (0.25 M sucrose solution)

Dilute TS1 with x2 volume of PB1 or M2, as appropriate.
Aliquot and store in a refrigerator.

Please note the followings:

1) The temperature of the thawing media and the duration of embryo exposure to them are critically important for the survival of embryos. Please compare the morphology of your embryos with that of embryos in the photos shown above. You may extend the period for the first thawing medium (TS1) until the embryos recover a shrunken morphology.

2) The embryos just after thawing are very sensitive to physical damages. Please handle them as gently as possible.

3) Please first thaw the C57BL/6 wild type embryos and make sure that the embryos finally look normal in the M16 medium. If you find any trouble with this step, please contact us.

4) Embryos should be transferred into day 0.5 (on the day of viginal plug) oviducts on the day of thawing. Ideally, 16-20 embryos are to be transferred per female so that they may get pregnant efficiently.

If I can be of any further assistance, please do not hesitate to contact me.

Thank you!

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