CGAP Freezing Human Tissue in Isopentane

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Abstract

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Protocol

Step 1.

Isopentane	20ml	ThermoFisher (10468030)
Forceps	2	ThermoFisher UK Ltd (15232290)
100mm Petri Dish	1	Corning (430591)
Scalpel	1	Swann-Morton Ltd (0507)
Labelled 15ml falcon tubes	3 (Per Tissue)	Falcon (352097)
Small plastic weighing boats	3 (Per Tissue)	Fisher Scientific (HEA1420AF)

Step 2.
Receive tissue and place on ice.

Step 3.
Place the container of isopentane on dry ice.

Step 4.
Place 1 pair of forceps with the end in the dry ice.

Step 5.
Add lumps of dry ice into the isopentane to achieve the correct temperature -70°C for oesophagus, spleen, lung, liver and other organs (-40°C for striated muscle), monitoring with the -100°C thermometer.

Step 6.
Place labelled 15ml falcon tubes on dry ice.

Step 7.
Using the room temperature forceps, place the first tissue sample to be frozen onto the petri dish.

Step 8.
Cut tissue into chunks for freezing (1 for bulk RNA, 1 for spatial transcriptomics, 1 for fresh dissociation if not taken already).

Step 9.
Place each piece of tissue for freezing into a separate weighing boat

Step 10.
Using the cold pair of forceps, firmly pick up one side of the weighing boat and carefully lower the tissue directly into the isopentane (taking care to ensure the tissue doesn't float out of the weighing boat).

Step 11.
Hold the sample submerged in the isopentane for approximately 10-20 seconds (depending on the size of the sample) until there are no more bubbles produced from the tissue.

Step 12.
Drain as much of the isopentane off the weighing boat as possible.

Step 13.
Using the cold forceps, place each piece of tissue into the correspondingly labelled 15ml falcon tubes and keep on dry ice.

Step 14.
Transfer the labelled 15ml falcon tubes into the -80°C freezer.

Step 15.
Take the Mr Frosty cryo-container out of the dry ice and leave the lid off in a fume cupboard and allow isopentane to evaporate.