Standard operating procedure for cryo-freezing tissue samples

Updated 17th March, 2016 by Amanda Chamberlain

Sampling tissue and cryo-freezing

Tissues must be cryo-frozen as soon as possible after an animal's death to preserve RNA. Therefore if large numbers of tissues are to be sampled a large number of people are required. They should be assembled into the following teams / roles

- Dissection team - Consisting of veterinarians, in the case of large animals there should be up to three, they will be responsible for the euthanasia of the animals, identification and dissection of tissues.
- Tissue tracker – One person ticking off which samples have been dissected and collected by tissue handlers.
- Tissue handling team – Two to three people collecting samples from dissection team, labelling correctly and transferring to tissue processing area and placing on ice for tissue processing team.
- Tissue processing team – The number of people in this team will depend on the number of samples being collected. The processing area should be set up close to dissection area but out of the way. They are responsible for taking collected tissues, removing any connective tissues, dissecting the organ/sample into appropriately sized pieces, placing in correctly labelled tubes and freezing in liquid nitrogen.

One animal should be euthanized and then dissected at a time.

Warnings

This procedure uses the following **hazardous chemicals**:

- Liquid Nitrogen
- Dry ice

Both liquid nitrogen and dry ice displace oxygen and are therefore asphyxiants. Both can cause cold burns. All staff using these chemicals are required to undergo cryogenics training. Both should be used in a well ventilated area and personal must not travel with either in enclosed spaces, such as cars or lifts. Cryogenic PPE should be used when handling these chemicals. See AgriBio Operations Manual section 3 Cryogenics for standard operating procedures.

This procedure has the following **manual handling risks**:

- Cutting from sharp knives. Cut resistant gloves must be worn on the non-cutting hand.
- Injury from large animals. Only farm staff trained in animal handling will handle animals.

Requirements

- Pre-labelled (animal, tissue) 5ml cryogenic tubes (x3 per animal/tissue) – use cryogenic labels and also label lids with a marker
- Pre-labelled (animal, tissue) 50ml cryogenic tubes (x1 per animal/tissue) – use cryogenic labels and also label lid with a marker
- Labels (animal, tissue) for zip lock bags (x1 per animal/tissue)
- 20cm x 20cm zip lock bags (x1 per animal/tissue)
- Paper or plastic plates (x1 per animal/tissue)
- Sharpened butchers knives (x1 per cutting team member)

- Cut resistant gloves (x1 per cutting team member)
- Ethanol spray bottles x 4
- Markers
- Waste bucket (x2) and bags (lots)
- Liquid nitrogen canister large enough to hold all samples filled with liquid nitrogen
- Second liquid nitrogen canister filled with liquid nitrogen for topping up.
- Cryogloves, cryoapron and face shield
- Gloves
- Kim wipes / Paper towels

Protocol

Dissection team:

1. Restrain animal and give an intravenous injection of 10% Zylazil (to be done by a veterinarian), adequate to cause moderate sedation. Release animal from their restraints into a holding area.
2. Once the animal has lay down euthanize by lethal injection (to be done by a veterinarian) using Pentabarb (sodium pentobarbitone 200mg/mL). Administer intravenously at dose rates greater than 100mg/kg. The veterinarian will then identify when the animal is expired.
3. Dissect the animal, identify and surgically remove each tissue type, either the whole organ or a 10cm x 10cm piece where the organ is large.

Tissue tracker and handlers:

4. Place organ/sample directly in a labelled zip lock bag.
5. Record tissue collection and transfer to tissue processing area and place on ice.

Tissue processing team:

6. Take the sample/organ from the ice, collect 3x labelled 5ml tubes and 1x labelled 50ml tubes.
7. Remove sample from zip lock bag and place on clean paper plate.
8. Using clean butchers knife, and wearing cut resistant glove on the none cutting hand, remove any connective tissue from the organ/sample.
9. Cut three 1cm x 1cm samples and place each one in one of the 5ml tubes.
10. Place remainder of organ/sample into 50ml tube.
11. Make sure all tubes are correctly sealed (not under or overtightened). If liquid nitrogen gets into the vial during storage, they can explode. Ensure lids labelled with a marker, in case labels come off.
12. Using appropriate PPE, place 5ml and 50ml tubes into liquid nitrogen canister.
13. Dispose of paper plate and clean down bench and knife with ethanol.
14. Repeat for other samples.
15. Once all the samples are processed, top up liquid nitrogen canisters with liquid nitrogen.
16. Transport samples to laboratory and remove tubes from liquid nitrogen in well ventilated area using appropriate PPE. Place tubes on dry ice for 10 minutes to evaporate remaining liquid nitrogen and then store samples at –80°C.

Cryogenic grinding using the Geno Grinder 2010

Warnings

This procedure uses the following **hazardous chemicals**:

- Liquid Nitrogen
- Dry ice

Both liquid nitrogen and dry ice displace oxygen and are therefore asphyxiants. Both can cause cold burns. All staff using these chemicals are required to undergo cryogenics training. Both should be used in a well ventilated area and personal should not travel with either in enclosed spaces, such as cars or lifts. Cryogenic PPE should be used when handling these chemicals. See AgriBio Operations Manual section 3 Cryogenics for standard operating procedures.

Requirements

- Thick-walled polystyrene container with dry ice
- Empty thick-walled polystyrene container for liquid nitrogen
- Small dewar containing liquid nitrogen
- 5ml Geno Grinder tubes with beads
- Cryo-Blocks that will hold 5ml Geno Grinder tubes
- Tweezers
- Plastic funnel

Protocol

1. Remove samples from storage and place in dry ice to transport to Geno Grinder work area.
2. Place grinding tubes into Cryo-Blocks and place 3 medium sized beads into each tube.
3. Pour enough liquid nitrogen into the polystyrene container to cool the Cryo-Blocks.
4. Place the Cryo-Blocks and tubes into the container with liquid nitrogen.
5. Transfer each sample (1cm x 1cm cube) from its storage tube into a grinding tube that has now been cooled by the liquid nitrogen.
6. Take the Cryo-Blocks out of liquid nitrogen using cryogenic gloves. Clamp the Cryo-Blocks with tubes into the Geno Grinder. Place plastic spacers on top of the tubes if necessary to ensure that the tubes are held securely. Ensure that weight is distributed equally between the two sides of the clamp.
7. Close the Geno Grinder cover.
8. Grind at 1500rpm for 3minutes.
9. After grinding, immerse tubes in liquid nitrogen, check if sample completely ground, if not return to grinder and grind at 1500rpm for 1 minute.
10. Immerse a funnel and a pair of tweezers in liquid nitrogen.
11. Take a tube from the cryo-block and place on dry ice. Using the cold tweezers, remove the beads and any tissue that is not ground.
12. Tip the ground tissue into its corresponding 5ml sample tube using the cold funnel.
13. Place the sample tube into liquid nitrogen.
14. Rinse the tweezers and funnel in deionised water and place back into liquid nitrogen.
15. Repeat steps 12-15 for all samples

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16. Transfer samples to dry ice if proceeding immediately with extractions. If not proceeding transport on dry ice and store at -80ºC.