SELF-PRESSURISED RAPID FREEZING (SPRF)

AN EASY-TO-USE AND LOW-COST ALTERNATIVE CRYO-FIXATION METHOD FOR NEMATODES

Myriam Claeys
DEPARTMENT OF BIOLOGY
NEMATOLOGY RESEARCH UNIT – PROF. WIM BERT

Vladimir Yushin
LAB OF EMBRYOLOGY
NATIONAL SCIENTIFIC CENTER OF MARINE BIOLOGY

Hendrik Claeys
photographs, design
The capillary copper tube used for SPRF fixation: 16 mm long, 350 \( \mu \text{m} \) inner diam., 640 outer diam.
The capillary copper tube inserted into a disposable pipette tip mounted on a 0-20 μl micropipette.
The open end of the capillary copper tube inserted into the specimen suspension and the nematodes are drawn into the tube.
The suction is halted when nematodes emerge from the pipette tip on the opposite side of the copper tube.
Close the copper tube filled with nematodes by squeezing the tube with pliers at both ends for 1-1.5 mm.
The sealed copper tube with the nematodes inside.
The homemade evacuation chamber (Claeys et al., 2017) can be easily replaced by an ordinary desiccator (e.g. Kartell Labware, Italy) fitted with a valve to connect with the rotary vacuum pump (e.g. RV Oil Sealed Rotary Vane Vacuum Pump. Edwards, Sussex, UK).

Inside:
a Styrofoam dish of 12 cm long, 7 cm wide, 5 cm deep and 0.5 cm thick.
After evacuation, the liquid nitrogen is transformed into the nitrogen slush (a semi-solid form of nitrogen).
After aerating and opening the chamber, the sealed copper tube is plunged horizontally into the slush nitrogen.
• After at least 15 s transport the copper tube from the Styrofoam tray in the desiccator to another Styrofoam tray filled with liquid nitrogen. Transportation must be made in container filled with liquid nitrogen.

• A central segment of the copper tube (5 mm long) may be cut out in liquid nitrogen using a pre-cooled tube punch device (Leica Microsystems).

Instead of the tube device, cutting pliers (available in a standard hardware shop) with operating at liquid nitrogen can also be used.
The copper segments containing specimens are transferred in liquid nitrogen into a cryo-vial that contains substitution liquid. The cryo-vials are closed and placed into the freeze substitution apparatus (e.g. AFS, Leica Microsystems).
REMARKS

- SPRF has not been tested on marine nematodes.
- SPRF may be used for other non-marine aquatic plants and animals of small sizes.