USDA ARS National Animal Germplasm Program

Rainbow Trout Milt Collection, Transportation, Processing and Cryopreservation Protocol

Semen collection and transportation:

Collect trout milt by stripping the males and capture the sample in a zip lock sandwich bag or disposable plastic container.

Fill the container with oxygen gas (preferred) or air and placed in a 5 ºC cooler. Samples can be held in this environment for up to 24 hours prior to use or cryopreservation and can be transported via overnight courier in an insulated container.

Assess the sperm motility with phase contrast microscopy (400 x) by placing a drop (25 to 50 µL of swimming media (please see recipe section), depending on the sperm concentration, on a microscope slide on the microscope stage.

Dip a glass Pasteur pipette into the milt and quickly mix the sample (< 5 microliters) into the swimming media.

Rate the sperm motility with a motility score of 0 to 5, with 0 being no motile cells, and 5 representing large, vigorous wave motion, immediately and without a coverslip.

Quality samples are then diluted 1:3 (v:v; milt to cryopreservation medium) with 12 ºC cryopreservation medium and placed into a 5 ºC cold room or refrigerator.

Load the samples into 0.5 mL straws and freeze using a programmable freezer (e.g. Cryo Bio System Mini Digitcool UJ400, IMV Corporation, Minneapolis, MN) with the following curve: 5 ºC to -70 ºC at 30 ºC per minute and then plunge the samples into liquid nitrogen for storage.

Thaw samples for 1 min in a 12 ºC water bath and analyze motility as described previously.

Artificial Insemination:

Combine a single thawed straw, 90 mL of eggs, and 3 mL of 12 ºC Lahnsteiner Activation Solution in a clean container and gently mix for one minute. Sanitation and incubation are performed according to Purdy et al., 2016.

Recipes (from Coson et al.):

Swimming medium

NaCl	125 mM
CaCl2	0.1 mM
Tris-HCl	30 mM
pH 8.0 to 8.5	

Cryopreservation medium

Glucose	300 mM
DMSO	10% by volume
Egg Yolk	13.30% by volume
pH 8.0 to 8.5	

Lahnsteiner Activation Solution

Sodium bicarbonate	60 mM
Tris (MW 121)	50 mM
pH 9.0	

References:

From: Coson, J. et al. 1999. In: The Male Gamete. Ed: C. Gagnon

Purdy, P.H., Barbosa, E.A., Praamsma, C.J., Schisler, G.J. 2016. Modification of trout sperm membranes associated with activation and cryopreservation. Implications for fertilizing potential. Cryobiology. 73: 73-79. https://doi.org/10.1016/j.cryobiol.2016.05.008.

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