Embryo sampling for total RNA extraction

Overview:

This protocol describes a method used to sample gilthead seabream embryos for isolation of small and large RNA with NucleoZOL (MACHEREY-NAGEL)

Consumables:

- Watchmaker forceps
- 2 ml Eppendorf tubes
- Dissection microscope
- Glass Petri dish 80 x 15 mm
- Dry ice or liquid nitrogen
- PBS 1X
- NucleoZOL (MACHEREY-NAGEL)
- nanodrop spectrophotometer
- Agarose gel electrophoresis system
- 2100 Bioanalyzer system

1. Gilthead seabream eggs were transferred in a Petri dish, and the developmental stage was identified under the microscope. The desired number of eggs (~100) was then transferred in a 1.5 ml eppendorf tubes and flash frozen in dry ice or liquid nitrogen. Eggs were stored at -80 ^{o}C until total RNA extraction.

2. Before total RNA extraction the eggs were defrosted on ice. The desired amount of eggs was collected and the PBS was removed (30-60 eggs, depending on the availability, with 500ul NucleoZOL).

3. Eggs were disrupted with a frozen pestle and mortar in liquid nitrogen.

4. NucleoZOL was added and total RNA extraction was performed according to the manufacturer's protocol (Isolation of total RNA). All samples were eluted in 30ul RNase-Free water.

5. Each sample was measured in nanodrop spectrophotometer. In addition 2µl of total RNA were loaded in agaroze gel (1.5%) and visualized by staining with ethidium bromide under UV light. Also 1µl of the total RNAs were electrophorised by 2100 Bioanalyzer system using the RNA 6000 pico assay.