Total RNA extraction from *Sparus aurata* tissues

The total RNA extraction was performed with NucleoZOL reagent. We followed the protocol that that isolates small (<200nt) and large RNA (>200nt) in one fraction.

1. Homogenization

A suitable amount of each tissue (Table 1) was weighed while still frozen and homogenized in liquid nitrogen. The powder was added to 1ml NucleoZOL/100mg tissue. For extra homogenization samples were passed through a 0.9 mm needle (20 gauge) fitted to a syringe

2. Precipitate contaminants

400 ul RNase-free water/ml of NucleoZOL were added to the lysate, which was mixed vigorously for 15 sec and incubated 5-15 min at RT. Then the mix was centrifuged for 15 min at 12.000 x g.

3. Precipitate total RNA

The supernatant was transferred to a new tube and 1ml of 100% isopropanol/ml supernatant was added.

4. Wash total RNA

500ul of 75% ethanol were added to the pellet. The samples were centrifuged for 3 min at 8.000 x g and the supernatant was discarded with a pipette. The washing step was repeated 2 more times, and the pellet was left to dry until no ethanol residual was left.

5. Reconstitute RNA

30ul of RNase-free water was added to reconstitute the total RNA. The RNA was dissolved by flicking and then kept on ice (or at -20o C for later use).

Table 1: The amount of tissue and reagents which were used for total RNA extraction

Sex	Tissue type	mg of tissue	Amount of NucleoZOL (ul)	H_2O (contaminants precipitation) (ul)	100% Isopropanol (RNA precipitation) (ul)	75% EtOH (Wash) (ul)	H_2O (RNA dissolving) (ul)
Male	Liver, Gills, Distal intestine,	30-40	500	200	700	250	30
	Brain	50-60	600	240	840	300	30
	H.kidney	15	300	120	420	100	30
	Gonad	5-10	200	80	280	100	30
	Muscle	100	1000	400	1400	500	30
Female	Liver, Gills, Distal intestine,	30-50	500	200	700	250	30
	Brain	40/60	500/600	200/240	700/840	250/300	30
	H.kidney	15	300	120	420	150	30
	Gonad	50	500	200	700	250	30
	Muscle	70-80	800	320	1120	400	30

For all muscle tissues the total amount of supernatant (at precipitation step) was equally divided in 2 new tubes in which the extraction was completed using the half of the amount of isopropanol, EtOH and H_2O (Table 1) in each tube. Finally, the dissolved RNA was joined in one tube ending in total volume of RNA solution of 30ul.

Each sample was measured in nanodrop spectrophotometer. Two ul (2 ul) of total RNA were loaded in agaroze gel (1.5%) and visualized by staining with ethidium bromide under UV light. Finally a sample of tissues was electrophoresed on the 2100 Bioanalyzer system using the RNA 6000 pico assay.