

Small RNA library preparation for teleosts at IMBBC-HCMR

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1. Total RNA extraction

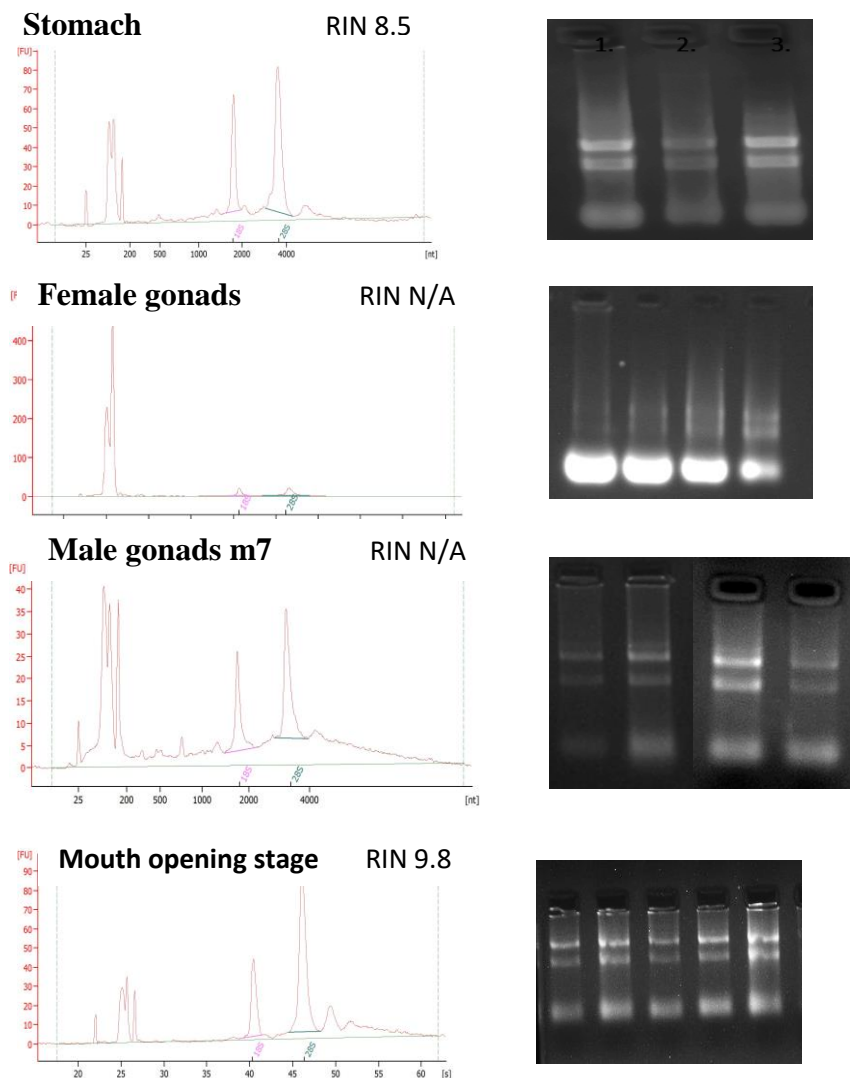
Total RNA extraction is carried out applying NucleoSpin miRNA extraction Kit (Machery Nagel, Cat. No. 740971).

Amount of tissue for RNA extraction is kept below 30mg. For tissues with high DNA content such as spleen even less tissue material is taken. For total RNA extraction, samples in RNAlater or flash frozen are homogenized using liquid nitrogen and passed five times through a 21g syringe as recommended by NucleoSpin.

RNA quality is evaluated by 1% agarose gel as well as by DNAgilent RNA picoChip run. The RIN number is assessed.

! Note in some tissues 5s RNA content may be high, consequently the RIN number may not be assessed or satisfying.

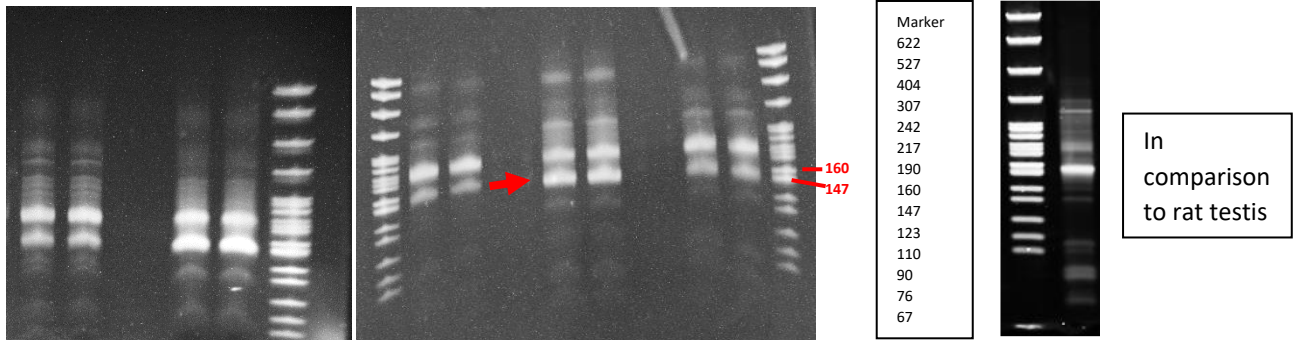
1.1 Typical outcome of total RNA extractions of different tissues:



2. Small RNA library construction

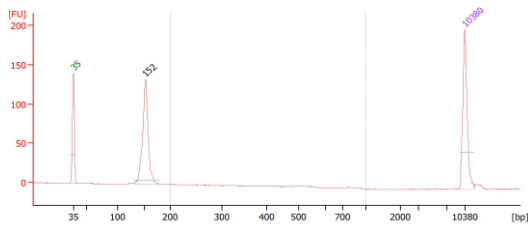
Small RNA library is generated using NEB smallRNA library construction kit. (New England BioLabs Cat. No E7300).

2.1 Typical Gel picture obtained from teleost tissues

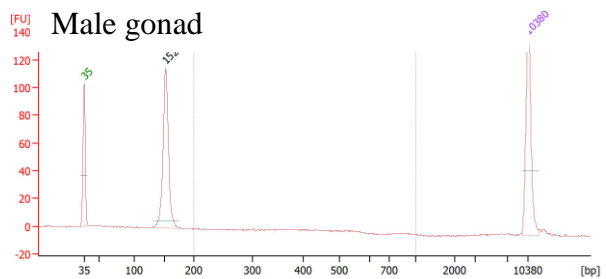


2.2 Evaluation via DNA High Sensitivity Kit

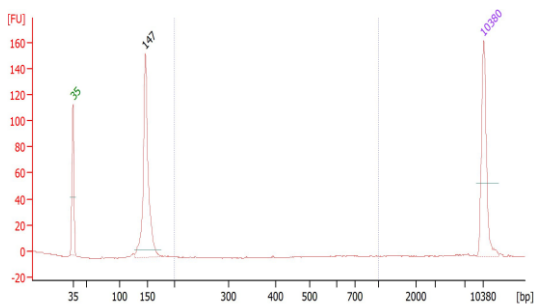
Female gonad



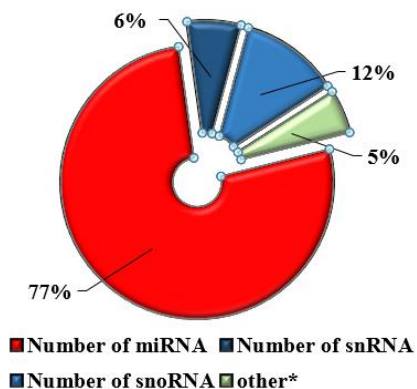
Male gonad



Developmental stages



3. Typical distribution of obtained sncRNAs



*other includes ribosomal gene, tRNA, mt trna and miscRNA