Research Article

High-quality RNA extraction from the sea urchin Paracentrotus lividus embryos

Nadia Ruocco1,2,3, Susan Costantini4, Valerio Zupo5, Giovanna Romano6, Adrianna Ianora6, Angelo Fontana3*, Maria Costantini1*

1 Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, Napoli, Italy, 2 Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant’Angelo, Via Cinthia, Napoli, Italy, 3 Bio-Organic Chemistry Unit, Institute of Biomolecular Chemistry-CNR, Via Campi Flegrei 34, Pozzuoli, Naples, Italy, 4 CROM, Istituto Nazionale Tumori “Fondazione G. Pascale”, IRCCS, Napoli, Italy, 5 Center of Villa Dohrn Ischia-Benthic Ecology, Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, P.ta S. Pietro, Ischia, Naples, Italy, 6 Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, Napoli, Italy

* marinacostantini@szn.it (MC); afontana@icb.cnr.it (AF)

Abstract

The sea urchin Paracentrotus lividus (Lamarck, 1816) is a keystone herbivore in the Mediterranean Sea due to its ability to transform macroalgal-dominated communities into barren areas characterized by increased cover of bare substrates and encrusting coralline algae, reduced biodiversity and altered ecosystem functions. P. lividus is also an excellent animal model for toxicology, physiology and biology investigations having been used for more than a century as a model for embryological studies with synchronously developing embryos which are easy to manipulate and analyze for morphological aberrations. Despite its importance for the scientific community, the complete genome is still not fully annotated. To date, only a few molecular tools are available and a few Next Generation Sequencing (NGS) studies have been performed. Here we aimed at setting-up an RNA extraction method to obtain high quality and sufficient quantity of RNA for NGS from P. lividus embryos at the pluteus stage. We compared five different RNA extraction protocols from four different pools of plutei (500, 1000, 2500 and 5000 embryos): TRIzol®, and four widely-used Silica Membrane kits, GenElute™ Mammalian Total RNA Miniprep Kit, RNAqueous® Micro Kit, RNeasy® Micro Kit and Aurum™ Total RNA Mini Kit. The quantity of RNA isolated was evaluated using NanoDrop. The quality, considering the purity, was measured as A260/A280 and A260/230 ratios. The integrity was measured by RNA Integrity Number (RIN). Our results demonstrated that the most efficient procedures were GenElute, RNeasy and Aurum, producing a sufficient quantity of RNA for NGS. The Bioanalyzer profiles and RIN values revealed that the most efficient methods guaranteeing for RNA integrity were RNeasy and Aurum combined with an initial preservation in RNAlater. This research represents the first attempt to standardize a method for high-quality RNA extraction from sea urchin embryos at the pluteus stage, providing a new resource for this established model marine organism.