

Automated extraction of PCR-grade gDNA from *Ciona intestinalis* tissue

This protocol describes genomic DNA extraction from *Ciona intestinalis* specimens and it is an adaptation of a similar protocol developed for marine plants and vertebrates. It is suitable for large-scale approaches, such as in population genetics, by means of an automated liquid handler, and therefore is particularly useful when downstream applications are also automated. The present protocol is for 96-well plates, thus reducing processing time to approx. 50 min

1. A small (1 cm²) piece of muscle tissue is dissected from the oral syphon of anesthetized or 70% EtOH-stored ascidians, by using sterile scissor/blade, and is placed in a 1.5 ml vial on ice.
2. Tissue samples are transferred to 96-well plate and then lysed at 56°C (ca. 4 hrs) with 245 µl of SDS-containing T1 buffer (specially developed for blood and tissues, it contains chaotropic salts, denaturing agents and detergents) and 5 µl of Proteinase K stock (see Solutions) on shaker, according to the manufacturer's procedures.
3. Sample plates are centrifuged at 2000 rpm x 2 min and the supernatant is transferred to a new 96-well plate that is placed again in the liquid handler..
4. The gDNA-containing supernatant is purified on a liquid handler dual bridge by mixing it with 250 µl of binding buffer and 250 µl of 96-100% EtOH for optimal binding to silica membrane and loaded on binding plate that contains 96 columns.
5. gDNA is removed from silica membranes by washing with two different buffers (see manufacturer's instructions) and then eluted in 100 µl of H₂O or TE buffer. It is now ready-to-use for downstream applications.
6. After gDNA is gel controlled (electrophoresis in a 1% agarose gel in 0.5X TBE and ethidium bromide), PCR reactions are prepared in automation with 0.5/1.0 µl of DNA, 0.2/0.5 [pmol/µl] of each oligo and Taq pol. [0.075u/µl] in 20 µl total volume.

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Ciona: fresh or alcohol preserved adults.

Apparatus: Gripper tool; Vacuum manifold; Macherey Nagel Nucleospin 96; Biomek FX (Beckman Coulter Inc.) equipped with ORCA Robotic arm, PCR machine and Plate Carousel, electrophoretic apparatus.

Solutions: Proteinase K stock: (prepare fresh just before use) weight 75 mg proteinase K and resuspend them in 2.6 ml; Proteinase K buffer PB (from the manufacturer's kit).

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

Borra M., Mauriello E., Campili F., Biffali E. 2007. High throughput genomic DNA purification, PCR and fragments analysis reaction setup on microalgae using Macherey-Nagel Nucleospin 96 Plant Kit* and Beckman Coulter's Biomek® FX* Laboratory Automation Workstation equipped with ORCA® robotic arm. MGE Newsletter N° 30 Month April 2007
Macherey-Nagel. Genomic DNA from Tissue. User Manual. July 2009/Rev.05
