Apr 26, 2016

Algal culture harvest and RNA extraction for RNA-Seq

DOI
dx.doi.org/10.17504/protocols.io.ewjbfcn

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Protocol Citation: Alle Lie 2016. Algal culture harvest and RNA extraction for RNA-Seq. **protocols.io https://dx.doi.org/10.17504/protocols.io.ewjbfcn**

Protocol status: Working

Created: April 26, 2016

Last Modified: February 21, 2018

Protocol Integer ID: 2731

Keywords: rna extraction for rna, rna extraction, algal culture harvest, rna, seq, extraction

Troubleshooting

1 Use sterile techniques to harvest 800 ml of culture, and divide into 4 250 ml centrifuge bottles with 200 ml each.

2 Spin the centrifuge bottles at 5000 g for 10 min.

3 Carefully decant the supernatant. Use 0.5 ml of RNALater to resuspend the pellet and transfer it to a microcentrifuge tube. Use another 0.25 ml of RNALater to wash the bottle and obtain any remaining cells. Transfer this 0.25 ml into the same microcentrifuge tube.

4 Depending on whether you want pseudo-replicates, you can keep the 4 harvested pellet separate or mixed.
Store the harvested cells at or below -20oC until ready for RNA extraction

5 Before RNA extraction, the RNALater needs to be removed.
Thaw samples if they are frozen. Centrifuge samples at > 10 k g for 10 min at 4oC.
RNALater sometimes affects the buoyancy of the cells, and make them a bit harder to pellet. If you find that the cells are not pelleting, spin harder for a longer time.
Decant supernatent with RNALater

6 Extract RNA using a RNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions

7 Remove any DNA from the extracted samples using DNase (Sigma), following manufacturer's instructions

8 Clean up the total RNA using a RNA Clean & Concentrator kit (Zymo), following the manufacturer's instructions
Quantify the RNA using any preferred method, e.g. Qubit fluorometer

9 Store the RNA at -80oC
It may be ideal to aliquot some RNA out so that you don't need to thaw the entire RNA sample if you need some for any reason