Algal culture harvest and RNA extraction for RNA-Seq

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Abstract

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Protocol

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

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

Step 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.

Step 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

Step 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

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

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

Step 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

Step 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