**RNA extraction of Chlamydomonas (large scale)**

1. **Trizol (Invitrogen) + RNeasy Midi Kit (Qiagen, modified)**
2. For 1.5x108 pelleted cells (~ 1x 50ml liquid culture @ 3x106 cells/ml).
3. Resuspend cells in 1ml of lysis buffer (Prepare before use).

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| **SDS-EB lysis buffer** | **Stock** | **Vol** |  |
| **50 mM Tris-HCl (pH 8.0)** | 1M | 50ul |  |
| **200 mM NaCl** | 5 M | 40ul |  |
| **20 mM EDTA** | 0.5 M | 40ul |  |
| **Nuclease-free H2O** |  | 620ul |  |
| **2% SDS** | 10% | 200ul |  |
| **Proteinase K (20mg/ml)** |  | 50ul | 1mg/ml |
|  | ***Total*** | 1000ul |  |

1. *Note: Make sure no clumps is visible.*
2. Add 30ml TRIzol reagent. Mix well.
3. *Note: Do not let sample in lysis buffer for too long. This may cause degradation.*
4. Incubate 5mins at RT. (or store at -80°C)
5. Spin down 3 tubes of Maxtract HD (15ml) at 1500g, 1 min.
6. Transfer Trizol solution (10ml into each 15ml Maxtract HD).
7. Add 1/5 volume (2ml) of chloroform.
8. Shake vigorously for 15s. Incubate 5mins at RT.
9. Centrifuge 1500g, 5mins, RT.
10. Pour out the aqueous phase to a 50ml tube.
11. Add 1.5x volume of 100% ethanol and mix well.
12. Set a RNeasy Midi column on a vacuum manifold.
13. Transfer mixture into each tube into RNeasy Midi column.
14. Put the column back onto the collection tube.
15. ***On-column DNase treatment (RNeasy Mini spin column, Qiagen)***
16. Pipette 2ml Buffer RW1 into RNeasy Midi spin column. Centrifuge 3200g for 5mins RT. Discard flow-through.
17. Add 140ul Buffer RDD to 20ul DNase I stock solution (aliquot). Mix gently. DO NOT vortex!
18. Add DNase I incubation mix (160ul) onto the spin column membrane. Incubate at 30°C for 15mins.
19. (Following 3 steps can be done on vacuum manifold)
20. Wash column with 2ml RW1. Centrifuge 3200g for 5mins at RT. Discard flow-through.
21. Pipette 2.5ml Buffer RPE onto RNeasy Mini spin column. Centrifuge 3200g for 2mins at RT. Discard flow-through.
22. Wash with another 2.5ml Buffer RPE. Centrifuge 5000g for 5mins at RT. Discard flow-through.
23. If steps 23-25 were done on a vacuum manifold
	1. After final RPE wash centrifuge spin column at 3200g for 5min to remove all residual ethanol from column filter.
24. Transfer spin column to a 15ml collection tube.
25. Elute with 250ul RNase-free water. Centrifuge 3200g, 5mins.
26. Repeat elution with the same 150ul RNase-free water.
27. QC1: Nanodrop
28. Typical profile should be as shown below, ratio 260/280 ~2, ratio 260/230 >2.

1. QC2: Agilent Bioanalyzer (*Important: Denature sample before loading!*)
2. RNA Plant Assay, typical profile should be as shown below, RIN >8, two distinct peaks, 18S peak shows overlap with smaller peaks (16S), 25S peak should be higher than 18S, no visible smear after 25S (potential gDNA contamination), typical yield = ~200ug

1. QC3: Qubit RNA BR (ng/ul) (Dilute RNA 1 :10 before Qubit QC)
2. Store RNA at -80°C.