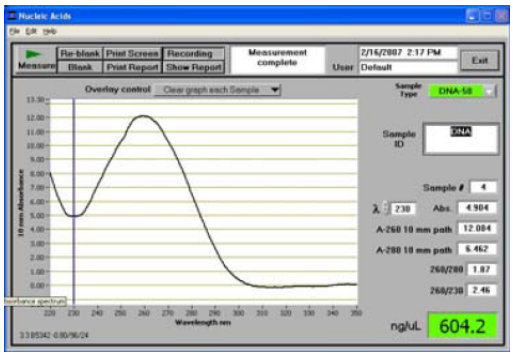
RNA extraction of Chlamydomonas (large scale)

1. **Trizol (Invitrogen) + RNeasy Midi Kit (Qiagen, modified)**
2. For $1.5x10^8$ pelleted cells (~ 1x 50ml liquid culture @ $3x10^6$ cells/ml).
3. Resuspend cells in 1ml of lysis buffer (Prepare before use).

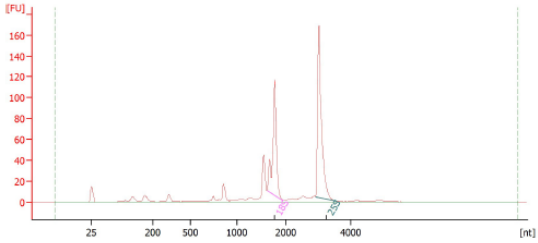
SDS-EB lysis buffer	**Stock**	**Vol**	
50 mM Tris-HCl (pH 8.0)	1M	50ul	
200 mM NaCl	5 M	40ul	
20 mM EDTA	0.5 M	40ul	
Nuclease-free H_2O		620ul	
2% SDS	10%	200ul	
Proteinase K (20mg/ml)		50ul	1mg/ml
	Total	1000ul	

4. *Note: Make sure no clumps is visible.*
5. Add 30ml TRIzol reagent. Mix well.
6. *Note: Do not let sample in lysis buffer for too long. This may cause degradation.*
7. Incubate 5mins at RT. (or store at -80°C)
8. Spin down 3 tubes of Maxtract HD (15ml) at 1500g, 1 min.
9. Transfer Trizol solution (10ml into each 15ml Maxtract HD).
10. Add 1/5 volume (2ml) of chloroform.
11. Shake vigorously for 15s. Incubate 5mins at RT.
12. Centrifuge 1500g, 5mins, RT.
13. Pour out the aqueous phase to a 50ml tube.
14. Add 1.5x volume of 100% ethanol and mix well.
15. Set a RNeasy Midi column on a vacuum manifold.
16. Transfer mixture into each tube into RNeasy Midi column.
17. Put the column back onto the collection tube.
18. ***On-column DNase treatment (RNeasy Mini spin column, Qiagen)***
19. Pipette 2ml Buffer RW1 into RNeasy Midi spin column. Centrifuge 3200g for 5mins RT. Discard flow-through.
20. Add 140ul Buffer RDD to 20ul DNase I stock solution (aliquot). Mix gently. DO NOT vortex!
21. Add DNase I incubation mix (160ul) onto the spin column membrane. Incubate at 30°C for 15mins.
22. (Following 3 steps can be done on vacuum manifold)
23. Wash column with 2ml RW1. Centrifuge 3200g for 5mins at RT. Discard flow-through.

24. Pipette 2.5ml Buffer RPE onto RNeasy Mini spin column. Centrifuge 3200g for 2mins at RT. Discard flow-through.
25. Wash with another 2.5ml Buffer RPE. Centrifuge 5000g for 5mins at RT. Discard flow-through.
26. If steps 23-25 were done on a vacuum manifold
    a. After final RPE wash centrifuge spin column at 3200g for 5min to remove all residual ethanol from column filter.
27. Transfer spin column to a 15ml collection tube.
28. Elute with 250ul RNase-free water. Centrifuge 3200g, 5mins.
29. Repeat elution with the same 150ul RNase-free water.
30. QC1: Nanodrop
31. Typical profile should be as shown below, ratio 260/280 ~2, ratio 260/230 >2.



32. QC2: Agilent Bioanalyzer (*Important: Denature sample before loading!*)
33. 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



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