Preparation of total RNA

1) Harvest $1x10^5$ to 10^8 cells. Wash 1x w/PBS and freeze in liquid N_2 and store @ -70°C

2) Resuspend each pellet in 1.5ml lysis buffer (300µl 5x Lysis, 75µl 200mM VR, 1.125ml H_2O

3) Incubate on ice 5minutes and spin 20min at 8k at 4°C.

4) Add equal volume of 2x proteinaseK buffer and 30µl of 20mg/ml ProtK. (Final conc. 0.2mg/ml)

5) Incubate 30 minutes at 42°C

6) Extract with 1-2 volumes of Phenol $CHCl_3$. Spin 10min at 8k at 4°C.

7) Repeat step 6 if interface is viscous.

8) Extract with 3mls of $CHCl_3$ (Chloroform)

9) Add 2µl of glycogen and 2.5volumes (~7.5ml) of EtOH. PPT at -70°C or on dry ice.

10) Spin 10min at 8k at 4°C in swinging bucket Sorvall.

11) Wash w/70% EtOH and dry

12) Resuspend in appropriate volume of 1xTE (50-500µl)

13) Run on a 1% EtBr gel. (28s @ 4.8kb, 18s @ 1.9kb, 5s @ 160bp)

5x Lysis	**[Final]**	**2xPK buffer**
14ml 5M NaCl	140mM	20ml 1M Tris pH7.5 (10mM)
750µl 1M $MgCl_2$	1.5mM	5ml 0.5M EDTA
5ml 1M Tris pH 8.6	10mM	6ml 5M NaCl
2.5ml NP40	0.5%	10ml 20% SDS
78ml H_2O		59ml H_2O