iGEM2013 – Microbiology – BMB – SDU	
Title: RNA purification **SOP number:** SOP0027_v01 **Version number:** 01	**Date issued:** 2013.08.23 **Review date:** 2013.08.23 **Written by: PRA**

1. Purpose

To purify RNA from cell pellet

2. Area of application

E. coli cell pellet

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Heating block		●	
Fume cupboard		●	
Eppendorf centrifuge		●	
		●	
		●	
		●	
		●	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Blue pipette tips		Contact	Micro storage	

(RNase free)		lab-manager		
Green pipette tips (RNase free)		Contact lab-manager	Micro storage	
NaCitrate		Contact lab-manager	Micro storage	
NaAcetate pH 4.5		Contact lab-manager	Micro storage	
EDTA		Contact lab-manager	Micro storage	
SDS		Contact lab-manager	Micro storage	
Phenol		Contact lab-manager	Chemical room	
Chloroform		Contact lab-manager	Chemical room	
8-hydroxyquinoline		Contact lab-manager	Chemical room	
96% ethanol		Contact lab-manager	Chemical room	
70% ethanol		Contact lab-manager	Chemical room	
Phase-lock tubes		5 prime / 2302830		
2mL eppendorf tube (RNase free)		Contact lab-manager		

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0026_v01_Growth for RNA purification - ara inducible plasmid

SOP0027_v01_RNA purification

SOP0028_v01_Nothern blotting

7. Environmental conditions required

8. Procedure

1. Spin the phase-lock tubes at max speed for 2-5 min
2. Add 150 µL solution 2 followed by 300µL chloroform and 700µL phenol
3. Resuspend cell pellet in 150µL cold solution 1

4. Transfer suspension into the phase-tock tube
5. Invert the tubes and transfer to 80°C for 3-4 min
    1. Invert tubes after 1-2 min (make sure to press the lids while inverting; otherwise the tubes will pop open)
    2. Meanwhile, add 1.5mL cold 96% ethanol to an empty 2 mL eppendorf tube, and place on ice
6. Chill the phase-lock tubes on ice
7. Spin at max speed for 3-5 min
8. Transfer aqueous phase in the phase-lock tube to the cold ethanol
    1. Make sure not to transfer any phenol bubbles
9. Incubate at -20°C or -80°C for 1hr or ON
10. Spin at max speed for 0.5-1hr at 4°C
11. Wash pellet 3 times; 150µL 70% cold ethanol and then 96% ethanol twice
    1. The pellet should turn white and more visible
12. Dry the pellet in a maxed fume cupboard
13. Resuspend the pellet in 20-100 µL of H_2O and pipette up and down
    1. evt. freeze it a few times

14. Preparation of **Solution 1**
    1. Mix components with end concentrations as indicated and sterile filtrate
        1. 10mM NaCitrate
        2. 10mM NaAcetate pH 4.5
        3. 2mM EDTA
15. Preparation of **Solution 2**
    1. Mix components with end concentrations as shown and sterile filtrate
        1. 10mM NaAcetate pH 4.5
        2. 2 % SDS
16. Preparation of acidic phenol
    1. Prepare in brown bottle
        1. 100 mL phenol “dissolved” in H_2O with 2cm of aqueous phase
        2. 3.33 mL of NaAcetate pH 4.5
        3. 0.1 g of 8-hydroxyquinoline

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
Chloroform			
Phenol			

10. Time consumption

- 30 min hands-on time

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.08.22 / PRA	01	The SOP has been written
13.08.23 / TJK/AK	01	The SOP has been approved

12. Appendices