iGEM2013 – Microbiology – BMB – SDU	
Title: Growth for RNA purification - ara inducible plasmid **SOP number:** SOP0026_v01 **Version number:** 01	**Date issued:** 2013.08.23 **Review date:** 2013.08.23 **Written by: PRA**

1. Purpose

To grow cells with plasmids containing arabinose inducible promoter for RNA purification

2. Area of application

E. coli with arabinose inducible promoter

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Erlenmeyer flasks	Hall with glass ware	•	
Centrifuge	Lab	•	
p1000, 100, 20		•	
Spectrophotometer	Growth room	•	
Liquid nitrogen container		•	
		•	
		•	

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

Name	Components	Supplier / Cat. #	Room (hallway	Safety

			storage)	considerations
Blue pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
15 mL Falcon tube		Contact lab-manager	Micro storage	
Cuvettes		Contact lab-manager	Micro storage	
Liquid nitrogen				
Fort. LB media		The new Anne-Mette	Autoclave room	
20% arabinose		Contact lab-manager	Chemical room	
20% glucose		Contact lab-manager	Chemical room	

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. Prepare the appropriate amount of Erlenmeyer flask with 10mL LB for each sample to be taken from the culture + 5mL (E.g. 25mL when 2 samples is to be taken)
2. Incubate bacteria in 37°C incubator.
3. Make sure a centrifuge for 15mL Falcon tubes is at 4°C, when samples (step 5-6) are taken.
4. Grow cells to OD_{600}=0.7
5. Transfer 10mL culture to a 15mL Falcon tube and induce the rest of the culture with arabinose (0.2% end concentration)

    1. Speed freeze the 10mL in liquid nitrogen
    2. Keep on ice
6. Repeat the procedure without further addition of arabinose for the rest of the samples
    1. Optionally: at any time you can stop induction of Para
        1. Spin the culture at 3500rpm for 5min at 4°C
        2. Discard the supernatant and resuspend in (same volume as culture spun (step 6.1)) LB with 0.2% glucose
        3. Let the resuspended culture grow at 37°C and take samples the same way as described in step 6
7. Centrifuge at 4°C for 7 min at 7000 rpm
8. Continue to RNA purification

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
LB media from culture		**Biowaste - container in fume cubard in growth room**	

10. Time consumption

- Total-time 5 hours.
- Hands-on-time 1.5 hours.

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	01	The SOP has been approved

12. Appendices