iGEM2013 – Microbiology – BMB – SDU	
Title: Northen blotting **SOP number:** SOP0028_v01 **Version number:** 01	**Date issued:** 2013.08.26 **Review date:** 2013.08.26 **Written by: PRA**

1. Purpose

To run RNA on a gel, blot it onto a membrane and scanning the results

2. Area of application

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Northen blotting apparatus		●	
Polyacryl-amide gel chamber		●	
Pippettes p20, p100		●	
		●	
		●	
		●	
		●	

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

Name	Components	Supplier / Cat. #	Room (hallway	Safety

			storage)	considerations
Purified RNA				
Polyacryl-amide gel				
Nothern blot laoding dye				
TBE buffer				

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. Running the gel

1. Prerun the gel at 300V for 20 min before loading the RNA
2. Load 5-50 mg total RNA to the well with dissolved in Northen blot loading dye
    1. Make sure that urea has been removed from the wells before loading
3. Run the gel for 2-4h

2. Blotting the membrane

1. Cut 6 pieces of Whatman 3MM paper and 1 piece of Zeta-probe membrane in appropriate size for the gel.
2. Wet 2 pieces of Whatman paper in 1 x TBE and place them on the top electrode (cathode)
3. Place a dry piece of Whatman paper on the gel and cut away the gel not

covered by the Whatman paper.

4. Wet it carefully in 1 x TBE and place on top of the sandwich
5. Wet the membrane in 1 x TBE and place on top of the gel
6. Wet the remaining Whatman papers in 1 x TBE and place them on top of the membrane.
7. Carefully remove air bubbles from the sandwich by rolling a glass or plastic pipette over the sandwich
8. Assembly the blotting device and turn it around. Run at 400mA for 1h.
9. Check that the blue color from the loading dye has been transferred from the gel to the membrane to check if the blot has run for long enough.
10. Disassemble the sandwich and air dry the membrane on a piece of paper
11. Cross-link the RNA to the membrane facing up in the UV oven (1200 on display, 254nm)

3. Hybridizing the membrane

1. Pre-hybridize the membrane at 42°C for 10 min in 10 mL hybridization buffer in hybridization oven .
2. Add 1-10 uL probe (add to buffer, not directly to membrane)
3. Hybridize for 0.5 hr - ON

4. Washing and scanning the membrane

1. 2-3 x 10 min with 2 x SSC and 0.1% SDS at 42°C
2. Air dry the membrane and wrap in Vita-wrap.
3. Place membrane in a cassette
4. Scan cassette

9. Waste handling

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

10. Time consumption

- 24 hours
- 4 hours 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	01	The SOP has been approved

12. Appendices