4. DNA and RNA extractions and Gel documentation

4a. DNA extraction from cultures grown in NMS agar plates

- Prepare a small PCR tubes in a rack and labelled them properly, if you have more than one culture for DNA extraction.
- Add 5 µL of 0.1 M NaOH in each tubes.
- Pick a sterile micropipette tip or sterile toothpick and gently scoop scrap a culture from agar plates.
- Dissolve and mix the culture in NaOH. Incubate in thermocycler for DNA extraction.
- Choose the program DNA extraction in thermocycler, which 95°C for 10 minutes followed by 4°C cooling (forever). Also make sure that the volume of reaction mixture is selected as 5 µL in thermocycler.
- Use the extract as template for PCR reactions.

4b. DNA extraction from E.coli TOP 10 Transformant

- Prepare a PCR tubes with proper labelling and add 5 µL of 0.1 M NaOH in each tubes.
- Pick a single colony (which is positive transformation) and dissolve in 15 µL DNase- free water.
- Take 5 µL culture dissolved in DNase-free water and mix with 5 µL 0.1 M NaOH and incubate under same conditions as in 4a.
- Use the extract as template for PCR reactions.
- Store the remaining 10 µL culture for other use.

4c. DNA extraction from liquid NMS cultures

- Prepare a PCR tubes with proper labelling and add 5 µL of 0.1 M NaOH in each tubes.
- Take 5 µL culture directly from the side arm flask and mix in PCR tube that contain NaOH. Follow the DNA extraction as detailed in 4a.
- Use the extract as template for PCR reactions.

4d. RNA extraction from liquid NMS cultures

- Need two days to complete the extraction.
- Try to finish first day extraction as soon as the sample is collected and followed by extraction incubate at -80°C overnight.
- Complete the second day extractions as soon as possible or before two weeks from the date of overnight incubate at -80°C.

(i) Key points to remember:

- Prepare the 50 mL falcon tubes before sample collection and properly labelled.
- Add 1 mL of stop solution in collection tubes and keep it ready.
- Prepare stop solution fresh and store in 4°C before use (don't use old stock)
- Make sure that all tubes are chilled and placed in ice all the time.
- Add samples one by one keep it aside. Finish your Napthalene assay and sample preparation for metal analysis (which are sensitive parameters too).
- Also, make sure that the other reagent required for the RNA extraction is chilled and readily available to use.

(ii) Reagents preparation:

1. Stop solution - 5% equilibrated phenol in ethanol: take 5 ml of Water Saturated Phenol (Ambion, cat# AM9710) add 45 ml of ethanol (200 proof). Store at 4°C. Use within 1-2 weeks.

2. CTAB solution: dissolve 14g of NaCl in 80 ml H_2O, add 10g of CTAB (Cetyltrimethylammonium Bromide, also known as Hexa-decyl-trimethyl-ammonium Bromide) and adjust volume to 100 ml. Add 100µl DEPC (Diethylpyrocarbonate), incubate overnight and autoclave. Store at Room Temperature

3. Phosphate buffer: To prepare 0.2M sodium phosphate buffer (pH 7.6) dissolve 15.5 g $Na_2HPO_4x2H_2O$ and 2 g of $NaH_2PO_4xH_2O$ in 800 ml of ddH_2O. Adjust volume to 1L, add 1 ml DEPC, and incubate overnight and autoclave. Store at Room Temperature

(iii) Day-I extraction:

- Prepare 2 ml screw-cap tubes (1 tube for one cell culture sample, or 10-14 tubes for one sediment sample)
- Add 0.5 g of 0.1 mm zirconia-silica beads (Biospec products)
- Add 35 µL of SDS 20% and 35 µL of Lauryl Sarkosine 20%
- Add 750 µL of chilled phenol:chloroform:isoamylic alcohol (25:24:1) (this can be added after adding samples).
- Collect biomass pellets from 10 mL culture samples by centrifugation at 5000 rpm for 10-20 min at 4°C. Use 50 mL falcon tube and appropriate rotors.
- Discard the supernatant and add 0.75 ml (for cell culture sample pellet) or 4-5 ml (for sediment sample pellet) of extraction buffer (10% CTAB in 2.4 M NaCl and 0.2 M phosphate buffer pH 7.6, 1:3*)

- mix well to resuspend the pellets into buffer and transfer 750 -800 µL of the mixture to 2 ml screw-cap tubes that is prepared above (should be 1 tube for cell culture sample pellet and 1014 tubes for sediment sample)
- Homogenise in a mini-beater (Biospec products) for 1 min (Note: set 6 for 60 sec)
- Centrifuged at 14,000rpm for 5 min at 4°C
- Take the upper aqueous phase, transfer into a clean 2 ml tube
- Add 750 µL of chilled chloroform:isoamylic alcohol (24:1) (Note: store it in 4°C)
- Centrifuge at 14,000rpm for 5 min at 4°C.
- Prepare 1.5 ml tubes for the next steps
- Transfer the upper aqueous phase (~700 µL) into new 1.5 ml tube and add
    - 0.5 M MgCl2(final concentration 2.5 mM) ~ 3.5 µL (for 700 µL of sample)
    - 0.1 volume of 3 M sodium acetate ~ 70 µL (for 700 µL of sample)
    - 0.7 volume of iso-propanol ~ 490 µL (for 700 µL of sample)
- Incubate the tubes overnight at -80°C.

(iv) Day-II extraction:

- Centrifuge the tubes at 14,000 RPM (18,000 g) for 45 min at 4°C,
- Remove supernatant and add 500 µl of 75% ethanol
- Centrifuge at 18,000 g for 5 min at 4°C,
- Remove supernatant and centrifuge at 18,000 g for 5 min at 4°C
- Remove any liquid residue from the tubes by pipetting and dry samples for 15 min at RT
- Follow the subsequent steps from Qiagen extraction kit to complete the extraction
- Store the samples at -20°C for RT-qPCR.

PCR Mixture preparation

	15 μL rxn	**50 μL rxn**
DNase-free H_2O	~ 6 μL	~20 μL
Forward primer (10 μM)	0.75 μL	2.5 μL
Reverse primer (10 μM)	0.75 μL	2.5 μL
DNA template	0.3 μL	0.3 μL
Polymerase mix	7.5 μL	25 μL

Note: For 15 μL, add <0.3 μL NaOH mix as DNA template (adding more NaOH may interfere with PCR reaction).

Notes: * For transformed *E. Coli*; Can save intact cells left over for growth in liquid, then plasmid extraction