

## Lipid extraction from *Bacillus subtilis*

### Sampling Table

Sample name (date):

Sample No.	Example	No. 1	No. 2	No. 3
Cell suspension (ml)	0.5			
H <sub>2</sub> O from material (ml) — (W)	0.5			
2-Propanol (ml)	5	5	5	5
<u>Extraction</u> Methanol	5	5	5	5
Chloroform (1)	5	5	5	5
H <sub>2</sub> O =4.0-(W)	3.5			
<u>Phase separation</u> CHCl <sub>3</sub>	5	5	5	5
H <sub>2</sub> O	5	5	5	5
Washing with 0.9% KCl	10	10	10	10
Empty vessel (g)				
Total lipid extracts (mg)				

## Day 1

Grow *B. subtilis* in a 100 ml LB medium. Collect cells in a 50-ml screw-capped glass tube by centrifugation. Wash the cells with 30 ml of chilled 1% NaCl. Suspend washed cells in 0.5 ml chilled 1% NaCl and keep on ice. (Frozen cell pellets are thawed in running tap water until the suspension is almost thawed. Then, samples are suspended in 1 ml chilled 1% NaCl and kept on ice.)

During the above procedures, keep 6 ml of 2-propanol in a hot water bath set at 90°C.

To the 1 ml cell suspension, flash 5 ml of hot 2-propanol, using a 10-ml silicon-rubber-capped glass pipette, and immediately transfer the mixture into the hot water bath set at 90 °C.

↓ 5 min, 90°C

Chill on ice.

Add methanol, chloroform, and water as calculated from the **Sampling Table**. The solution must be homogenous.

↓

Add 5 ml chloroform and 5 ml H<sub>2</sub>O, and mix vigorously using a vortex mixer.

↓ 3000 rpm x 15 min (r.t.)

Recover the lower (chloroform) layer into a fresh 30-ml screw-capped glass tube, and then wash with an equal volume of 0.9% KCl.

↓ 3000 rpm x 15 min (r.t.)

Recover the lower (chloroform) layer\*<sup>1</sup> into a 20 ml weighed pear-shaped flask, and evaporate the solvent on a rotary evaporator\*<sup>2</sup>.

\*<sup>1</sup>Special attention should be paid not to recover any material floating in the intermediate phase.

\*<sup>2</sup>If you see water droplets on the bottom of flask, add a few ml of ethanol and evaporate again.

↓

Dry up the residual solvent in a vacuum desiccator, and determine the lipid dry weight.

↓

Add chloroform to make a 10 mg/ml lipid solution while placing the flask on ice

to prevent solvent evaporation.



Transfer the lipid solution into a 1-ml screw-capped sample tube\*<sup>3</sup>.

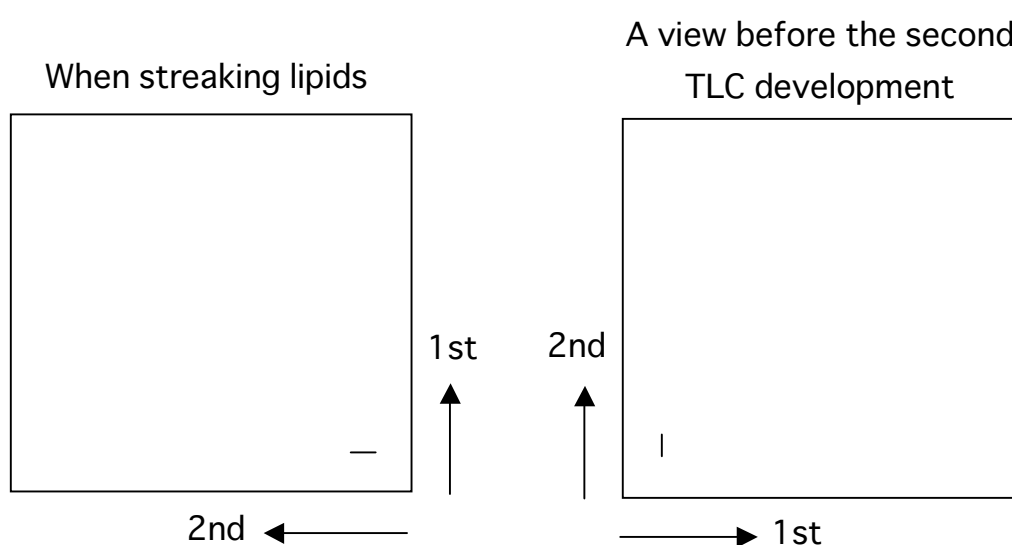
\*<sup>3</sup>Because the cap of the tube is so small, special attention should be paid to ensure that the cap is screwed on tightly. Each cap should then be sealed with parafilm and stored at -30°C. If you store the sample for more than one week, the antioxidant BHT should be added at a final concentration of 10 µg/ml.

Correspondence: Ikuo Nishida, [nishida@molbiol.saitama-u.ac.jp](mailto:nishida@molbiol.saitama-u.ac.jp)

## Day 2

### Separation of lipids by two-dimensional silica gel thin-layer chromatography

Using a 100- $\mu$ l glass micro syringe, streak 100  $\mu$ l of the lipid extract (1 mg lipid equivalent) on a 1-cm line marked in the 2-cm-from-right and 2-cm-from-bottom position of a TLC plate (20 x 20 cm, Silica gel 60, Merck 1.05721.) as shown below.



Estimated weight of lipid classes in 1 mg total lipid extracts:

Pigments	0.59 mg
Glycolipids	0.2 mg
Neutral phospholipids	0.12 mg
Acidic lipids	0.06 mg

#### 1st dimension :

Acetone/Benzene/Methanol/water = 8:3:2:1 (by volume)



Air-dry the plate (you may use warm air from a hair dryer).



#### 2nd dimension :

Chloroform/Methanol/Acetic acid/Water = 170:25:25:4 (by volume)



Dry the plate using warm air from a hair dryer.



Spray 0.01% Purimurin/80% acetone solution over the plate.

Dry the plate using warm air from a hair dryer.



Mark lipid spots under long-wave UV light (360 nm), using a soft pencil (HB).

The fluorescence image must be taken on a digital camera.



Scrape off each spot on medicine paper, and carefully transfer all silica gel powder into a 15-ml screw-capped glass tube<sup>\*4</sup>.

<sup>\*4</sup> Special attention should be paid not to use any tubes with cracks on the mouth or the screw mound.



Add 10 µl each of a 15:0 standard/toluene solution, using a fresh glass capillary pipette.



Add 10 µl of a BHT/toluene solution, using a fresh glass capillary pipette.

(If 14:0 is expected in the lipid samples, BHT must not be added, because 14:0-Me and BHT show the same retention time on a gas chromatograph.)



Add 3 ml each of HCL/methanol solution. Screw on the tube and mix vigorously with a vortex mixer.

↓ 80 °C for 3 h on an aluminum heating block.

### **Day 3**

Chill all tubes on ice.



Extract FA methyl esters with 3 ml distilled hexane.



Evaporate hexane, using a vacuum centrifugation evaporator. Dissolve dried lipid residues in ~5 drops of hexane from a Pasteur pipette and subject to gas chromatograph analyses.

(The hexane solution should be transferred to a 1-ml sample tube for storage or a glass tube for an automatic sample injector.)

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