**Extended Materials and Methods**

**M9 buffer, 1000 mL**

* 6 g Na2HPO4
* 3 g KH2PO4
* 5 g NaCl
* 0.25 g MgSO4∙7H2O
* Add deionized water to 1000 mL
* Autoclave

**Potassiumphosphate buffer**[**pH**](http://www.jove.com/science-education/5030/making-solutions-in-the-laboratory)**6.0, 1000 mL**

* 136 g KH2PO4
* Add deionized water to 900 mL
* Adjust pH to 6.0 with 5M KOH
* Add deionized water to 1000 mL
* Autoclave

**Trace metal solution**

* 1.86 g Na2EDTA
* 0.69 g FeSO4 ∙7H2O
* 0.20 g MnCl2 ∙ 4H2O
* 0.29 g ZnSO4 ∙7H2O
* 0.016 g CuSO4
* 1000 mL deionized water
* Autoclave and store in the dark.

**S-basal medium, 1000 mL**

* 5.9 g NaCl
* 50 mL of 1M potassium phosphate, pH 6.0
* 1000 mL deionized water
* Autoclave
* Let the solution cool and then add 1 mL of 5mg/ mL cholesterol (dissolved in Et-OH).

**Potassium citrate 1M, 1000 mL**

* 268.8 g tripotassium citrate
* 26.3 citric acid monohydrate
* Add 900 mL deionized water
* Adjust pH to 6.0 with 5M KOH
* Add deionized water to 1000 mL
* Autoclave

**S-complete medium, 1000 mL**

* 977 mL S-basal
* 10 mL 1M potassium citrate pH 6 (sterile)
* 10 mL Trace metals solution (sterile)
* 3 ml 1M CaCl2 (sterile)
* 3 mL 1M MgSO4 (sterile)
* Add the following components prior to first use of solution:
* 1 mL 5 mg/mL Cholesterol (dissolved in EtOH)

**NGM agar**

* 3.0g NaCl
* 2.5g Pepton (from casein, pancreatic digest)
* 17g Agar
* Add deionized water to 975 mL and a stirring bar
* Autoclave
* After [autoclaving](http://www.jove.com/science-education/5030/making-solutions-in-the-laboratory) cool down to 55°C and add the following components:
	+ 0.5 mL of 1M CaCl2 (sterile)
	+ 1 mL of 5mg/ mL Cholesterol in ethanol
	+ 1 mL of 1M MgSO4 (sterile)
	+ 25 mL Potassiumphosphate buffer, pH 6.0 (sterile)

**TB, 1000 mL**

* 12g Bacto Tryptone
* 24 g Yeast Extract
* 4 mL Glycerol
* Add 900 mL deionized water
* Autoclave
* After autoclaving cool down to 55°C and add the following component:
	+ add 100 mL of 0.17M KH2PO4/0.72M K2HPO4

**0.6 mM Fluorodeoxyuridine (FUDR, sigma cat# F0503), 1000 mL**

* 100 mg FUDR
* Dissolve in 670 mL sterile S-complete, make 10 mL or 45 mL aliquots.
* Store at -20 °C.

**100 mg/ mL Carbenicillin, 10 mL**

* 1 g Carbenicillin
* 10 mL sterile deionized water
* Sterile filtrate and aliquot
* Store at -20 °C.
* Use at a final concentration of 50 μg/ mL

**250ug/ mL Amphotericin B, 4 mL**

* 1mg Amphotericin B
* 4 mL Et-OH
* Store at -20 °C
* Use at a final concentration of 0.1 μg / mL