Reagents and Procedures for Pseudomonas Culturing

1 M $MgSO_4$
Dissolve 24.6 g $MgSO_4 \cdot 7H_2O$ in 100 ml DI water.
Autoclave for 20 min.

1 M Tris·Cl
In a volumetric flask, dissolve 121 g/l Tris base; do not bring to volume yet. Adjust to desired pH with concentrated HCl. Mix and bring to volume.

1 M $CaCl_2$
Dissolve 11.10 g $CaCl_2$ in 100 ml DI water.
Autoclave for 20 min.

Trace element solution (Kragelund and Nybroe 1994)
20 mg $CoCl_2 \cdot 6H_2O$
30 mg H_3BO_3
10 mg $ZnSO_4 \cdot 7H_2O$
1 mg $CuCl_2 \cdot 2H_2O$
2 mg $NiCl_2 \cdot 6H_2O$
3 mg $NaMoO_4 \cdot 2H_2O$
10 mg $FeSO_4 \cdot 7H_2O$
2.6 mg $MnSO_4 \cdot H_2O$
1000 ml ultrapure water
Autoclave for 20 min.

Glycerol solution
65 ml glycerol
10 ml 1 M $MgSO_4$
2.5 ml 1 M Tris·Cl, pH 8.0
22.5 ml DI water
Autoclave for 20 min.

Kanamycin stock solution (10 mg/ml)
100 mg kanamycin
10 ml ultrapure water
Dissolve in sterile 15 ml centrifuge tube and sterile filter into another 15 ml tube.

Rifampin stock solution (20 mg/ml)
Add 7.5 ml methanol to a new vial of 150 mg rifampicin
Pipet 1 ml aliquots into sterile 1.5 ml centrifuge tubes and freeze at -20ºC

Kings B Medium

Proteose Peptone	20 g	10 g	2 g
Glycerol	10 g	5 g	1 g
K_2HPO_4	1.5 g	0.75 g	0.15 g
Agar (solid media only)	15 g	7.5 g	1.5 g
DI water	1 liter	500 ml	100 ml
Adjust to pH 7.2 Autoclave 20 min Add sterile 1 M $MgSO_4$			
1 M $MgSO_4 \cdot 7H_2O$	6.09 ml	3.05 ml	0.609 ml

Immediately before use:
For wild-type *P. fluorescens* CY091, add rifampin to a final concentration of 100 µg/ml
For *P. fluorescens* mutant J-1, add 100 µg/ml rifampin and 50 µg/ml kanamycin

Glutamine Glucose Minimal Medium (Worm et al 2000)

Glutamine	0.5 g	0.25 g	0.05 g
Glucose	0.5 g	0.25 g	0.05 g
K_2HPO_4	7.0 g	3.5 g	0.7 g
KH_2PO_4	2.0 g	1.0 g	0.2 g
DI water	1 liter	500 ml	100 ml
Autoclave 20 min Add sterile supplements as follows:			
1 M $MgSO_4 \cdot 7H_2O$	831 µl	416 µl	83 µl
1 M $CaCl_2$	350 µl	175 µl	35 µl
Trace element solution	1000 µl	500 µl	100 µl

Immediately before use:
For wild-type *P. fluorescens* CY091, add rifampin to a final concentration of 100 µg/ml
For *P. fluorescens* mutant J-1, add 100 µg/ml rifampin and 50 µg/ml kanamycin

Glutamine Glucose Casein Minimal Medium (Worm et al 2000)

Glutamine	0.05 g	0.025 g	0.005 g
Glucose	0.5 g	0.25 g	0.05 g
K_2HPO_4	7.0 g	3.5 g	0.7 g
KH_2PO_4	2.0 g	1.0 g	0.2 g
caseinate Na-salt	2.0 g	1.0 g	0.2 g
DI water	1 liter	500 ml	100 ml
Autoclave 20 min Add sterile supplements as follows:			
1 M $MgSO_4 \cdot 7H_2O$	831 µl	416 µl	83 µl
1 M $CaCl_2$	350 µl	175 µl	35 µl
Trace element solution	1000 µl	500 µl	100 µl

Immediately before use:
For wild-type *P. fluorescens* CY091, add rifampin to a final concentration of 100 µg/ml
For *P. fluorescens* mutant J-1, add 100 µg/ml rifampin and 50 µg/ml kanamycin

Allison Lab Protocol, Pseudomonas Culturing, 4/2008, Steve Allison

Pseudomonas agar F plates
Follow instructions on container to make the appropriate amount of media in an Erlenmeyer flask.
Autoclave media and let cool until it's comfortable to touch the flask.
Add the appropriate amounts of antibiotics and swirl gently to mix.

- 100 µg/ml rifampin final concentration (wild type and J-1 mutants)
- 50 µg/ml kanamycin final concentration (J-1 mutants only)

Pour plates and leave lids slightly open while media cools.

Growing bacteria in liquid culture overnight from plates
From a single colony, inoculate 25 ml of Kings B Medium (with appropriate antibiotics) in a 125 ml Erlenmeyer. Cover with a sterile beaker and incubate overnight at 28ºC with shaking at 200 rpm.

Freezing cultures for long-term storage
Tranfer 1 ml aliquots of culture to 1.5 ml centrifuge tubes and spin down at 6000 rpm for 4 min.

Resuspend cells in 1 ml fresh Kings B Medium without antibiotics.

Pipet 500 µl aliquots of the culture into labeled 1.2 ml freezer vials containing 500 µl glycerol solution.

Freeze at -20ºC, then at -80ºC.