***Prosthecochloris aestuarii* 2K Liquid Growth Medium:**

Prosthecochloris aestuarii strain 2K was obtained from the culture collection of Dr. Thijs Aartsma at the University of Leiden before his retirement. The original *P. aestuarii* culture was first isolated by Vladimir Gorlenko in 1970. The strain 2K is different enough from the type strain (DSM 271^T) that it should be classified as a distinct species; however, since strain 2K has never been separated into a pure culture, it has not been renamed.

Solutions:

A. 10x Basic Salts (makes 1 L, can be refrigerated):
   1. KH_2PO_4: 10 g
   2. NH_4Cl: 10 g
   3. $MgCl_2 \cdot 6H_2O$: 50 g
   4. NaCl: 200 g
   5. $CaCl_2$: 0.9 g (mix in first, or else it won't dissolve)
   6. Trace Elements Solution (See Solution G): 10 mL
   7. Fill to 1 L mark with d.d.H_2O and then autoclave.

B. Iron Sulfate Solution (makes 0.1 L, can be refrigerated)
   1. $FeSO_4 \cdot 7H_2O$: 0.05 g
   2. 0.4 M HCl: 100 mL

C. **Fresh** $Na_2S \cdot 9H_2O$ solution (0.4 g per 100 mL d.d.H_2O; pass through a 0.2-µm filter into a sterile flask)

D. **Fresh** $NaHCO_3$ solution (8 g per 100 mL d.d.H_2O; pass through a 0.2-µm filter into a sterile flask)

E. Ethanol (95% v/v)

F. Vitamin B_{12} Solution (2 mg per 1 mL d.d.H_2O; pass through a 0.2-µm filter into a sterile bottle). Can be refrigerated.

G. Trace Elements Solution "PA" (makes 1 L, can be refrigerated):
   1. $FeCl_3 \cdot 6H_2O$: 4 g
   2. H_3BO_3: 0.14 g
   3. $ZnSO_4 \cdot 7H_2O$: 0.44 g
   4. $Co(NO_3)_2 \cdot 6H_2O$: 0.25 g
   5. $CuSO_4 \cdot 5H_2O$: 0.02 g
   6. $MnSO_4 \cdot 4H_2O$: 0.02 g
   7. Fill to 1 L mark with d.d.H_2O. Pass through a 0.2-µm filter into a sterile bottle.

To mix medium:

1. Into a bottle of sufficient volume, mix using the scheme below. If using carboys (for volumes of 10 L or more), the media in its entirety may be directly mixed into the carboy:

	0.5 L Media	1 L Media	2 L Media	10 L Media	12 L Media	15 L Media
Solution A	50 mL	100 mL	200 mL	1.0 L	1.2 L	1.5 L
Water	395 mL	790 mL	1.58 L	7.90 L	9.48 L	11.85 L

Autoclave on a 30 minute liquid cycle. Allow to cool to room temperature, then add in order:

Solution B	2.5 mL	5 mL	10 mL	50 mL	60 mL	75 mL
Solution C	25 mL	50 mL	100 mL	500 mL	600 mL	750 mL
Solution D	25 mL	50 mL	100 mL	500 mL	600 mL	750 mL
Solution E	2.5 mL	5 mL	10 mL	50 mL	60 mL	75 mL
Solution F	13.5 µL	27 µL	52 µL	270 µL	325 µL	400 µL

2. Adjust pH to 7.0 with concentrated H_2SO_4 or Na_2CO_3. The pH should be measured by aseptically removing small aliquots and testing on litmus paper.
3. For smaller cultures, aliquot media into bottles or capped culture tubes. To exclude oxygen, fill the bottles or tubes to near the lip (leaving enough space for the inoculation volume).
4. Inoculate 1-2% (v/v) using established culture. Grow at 30° C under incandescent lights for 2-4 days. The strain may produce white sulfur globules that sink to the bottom of culture; this is normal.

Compiled July 21, 2014 by Gregory Orf, Blankenship Lab, Washington University in St. Louis