^{13}C Incubation Protocol

In field:
- collect subsamples for incubations from each layer in "microbial pit"
  place subsamples into 18% sterile saltwater in falcon tubes (prepped anaerobic with DTT and aerobic without)
- place sample into cooler inside of cooler filled with ice to keep cold but not frozen or touching ice

In lab:
- bring vortexed anaerobic sample into COY to set up anaerobic incubations
- set up aerobic incubations on bench
  2mL vortexed sediment slurry with 2mL 25% ^{13}C incubation media (CO_2, acetate, glucose)
- incubate ~60 days on bench (aerobic) or in COY (anaerobic)

Sample incubation headspace:
- remove headspace for $^{13}CO_2$ and $^{13}CH_4$ analysis
  Flush clean exetainer with N_2 gas in COY. Pull ~15mL headspace gas out of incubation tube, then put all headspace into flushed exetainer
- remove ~7mL from headspace exetainer, put into a new flushed, labeled exetainer

Solution Recipes

288mL Saltwater media w/ 0.07M CO_2

Autoclave:

distilled H_2O	95.135mL
30% saltwater	172.973mL
1M TrisHCl pH 7.5	8.649mL

After autoclave:

Add (Each solution is filter sterilized before adding):

Minimal Salts Soln	3.459mL
0.5M KPO_4 buffer (pH 7.5)	0.562mL
Thiamine & biotin Soln	0.259mL

Then add:

$NaHCO_3$ Soln	7.35mL

Anaerobic (+ 0.05% DTT)

Transfer 144mL of 288mL CO_2 saltwater media to sterile bottle and add:

Dithiothreitol	0.072g

144mL Saltwater media w/ 0.07M glucose

Autoclave:

distilled H_2O	47.568mL

30% saltwater	86.486mL
1M TrisHCl pH 7.5	4.324mL

After autoclave:
Add (Each solution is filter sterilized before adding):

Minimal Salts Soln	1.730mL
0.5M KPO_4 buffer (pH 7.5)	0.281mL
Thiamine & biotin Soln	0.130mL

Then add:

Glucose Soln	3.676mL

+ 0.05% DTT
Transfer 72mL of 144mL glucose saltwater media to sterile bottle and add:

Dithiothreitol	0.036g

144mL Saltwater media w/ 0.07M acetate
Autoclave:

distilled H_2O	47.568mL
30% saltwater	86.486mL
1M TrisHCl pH 7.5	4.324mL

After autoclave:
Add (Each solution is filter sterilized before adding):

Minimal Salts Soln	1.730mL
0.5M KPO_4 buffer (pH 7.5)	0.281mL
Thiamine & biotin Soln	0.130mL

Then add:

Acetate Soln	3.676mL

+ 0.05% DTT
Transfer 72mL of 144mL acetate saltwater media to sterile bottle and add:

Dithiothreitol	0.036g

9mL Minimal Salts Solution (store @ 4C)

MQ Water	8mL
1M NH_4Cl	0.2g
0.5M $CaCl_2$	0.25g
Trace elements	0.75mL

Bring final volume to 9mL

Trace elements

MQ H_2O	100mL
$MnCl_2 \cdot 4\ H_2O$	36mg
$ZnSO_4 \cdot 7\ H_2O$	44mg
$FeSO_4 \cdot 7\ H_2O$	230mg
$CuSO_4 \cdot 5\ H_2O$	5mg

10.8mL Thiamine & Biotin soln

Thiamine (1mg/mL)	9.6mL
Biotin (1mg/ml)	1.2mL

0.5M KPO_4 buffer (pH 7.5)

50mL:

MQ H_2O	35mL
1M K_2HPO_4	3.632g
1M KH_2PO_4	0.565g

bring to 40mL with MQ H_2O, pH to 7.5, bring to 50mL

1000mL 30% saltwater solution

Modified from: Halohandbook, Version 7.2, March 2009 - Compiled and edited by Dr Mike Dyall-Smith

MQ H_2O	850mL
NaCl	240g
$MgCl_2 \cdot 6\ H_2O$	30g
$MgSO_4 \cdot 7\ H_2O$	35g
KCl	7g
1M TrisHCl pH7.5	20mL

Dissolve and bring to final volume of 1000mL

2400mL 18% Saltwater from 30% SW Stock

30% Saltwater	1440mL
MQ H_2O	960mL

Filter sterilize

+ 0.05% DTT

Transfer 600mL of 18% saltwater to sterile bottle and add:

Dithiothreitol	0.3g

Carbon Source Solution

Carbon Source	**25% ^{13}C**
CO_2 Volume	**7.35mL**
H_2O	7.35mL
^{13}C	0.441g
^{12}C	1.270g
Glucose Volume	**3.68mL**
H_2O	3.68mL
^{13}C	0.470g
^{12}C	1.362g
Acetate Volume	**3.68mL**
H_2O	3.68mL
^{13}C	0.215g
^{12}C	0.620g