RNA Stable Isotope Probing Experimental Set Up

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Abstract

This is a protocol for carrying out RNA Stable Isotope Probing experiments using seawater or vent fluids to examine microbial bicarbonate uptake. It was developed at Axial Seamount to examine autotrophy in deep-sea hydrothermal vent fluids. We have used modified versions in seawater, cold crustal environments, and other hydrothermal vents as well.

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Before start

This protocol assumes you have already collected your fluid, eg seawater, vent fluids, etc.

Protocol

Step 1.
To an evacuated, acid-washed and sterile, stoppered 500 mL pyrex bottle, add 8.83 mL of 12C or 13C DIC (600 mM solution, sterile, in serum vials) using a 10 mL syringe with a filter on it. Do the 12C first. Final concentration = 10mM. This concentration will depend on your experimental design.

Step 2.
Using a peristaltic pump, fill each bottle to 530mL (premarked) using 60-100 mL/minute. Fill 12C first, then 13C. Change needle in between. Change tubing between isotopes. If bottles look like they are not filling it might be due to gas build up. Release pressure with a needle.

Step 3.
Add 1 to 1.5mL of 10% HCl to each bottle with a 1 mL syringe, checking pH repeatedly until you reach desired pH (e.g. pH < 6.5).

Step 4.
Add 20 mL of hydrogen gas to each bottle using small hydrogen bottle of 100% hydrogen with a 60mL syringe with a stopcock.

Step 5.
Incubate bottles on their sides for appropriate period of time at chosen temperatures (e.g. 30 °C for 36 hrs, 55 °C for 18-24 hrs, and 80 °C for 9-18 hrs).

Step 6.
To end the experiment, place a needle attached to peristaltic pump tubing and also a vent needle in the stopper, turn upside down in a ring stand and filter fluid through a 0.22 uM Sterivex at 60 mL/min. Use 12C tubing for controls, 13C tubing for experiment; watch which isotope as well. Run pump

backward.

Step 7.
When all fluid has been filtered, fill Sterivex with filter-sterilized RNAlater, cap with Medex caps, and store at 4ºC for 24 hours and then move to -80ºC.

Step 8.
To clean tubing between experiments, use peristaltic pump and a beaker to pass DI/MilliQ, 10% HCL, MilliQ, 70% EtOH, and then MilliQ through tubing, then empty and dry.

Warnings

hydrogen gas is used in this protocol to over-pressurize bottles. use caution.