Isotopic Labeling of Cyanobacteria and DNA Analysis

Bonnie Poulos

Abstract

Protocol for labeling bacteria with N15. The bacterial DNA was made “heavy” so that community viral and host bacterial DNA could be distinguished from each other after sorting by using density gradient centrifugation.

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Materials

Quant-it™ PicoGreen® dsDNA Assay Kit P7589 by Life Technologies

AMMONIUM CHLORIDE (15N, 99%) NLM-467-1 by Cambridge Isotope Laboratories, Inc.

Protocol

Preparation of Growth Media

Step 1.
Prepare Pro99, SN, SNAX or Amp1 medium according to directions, but use 15N ammonium chloride which will provide the heavy isotope. 15NH4Cl is available from Cambridge Isotope Laboratories, Inc. (#NLM-467-1).

REAGENTS
AMMONIUM CHLORIDE (15N, 99%) NLM-467-1 by Cambridge Isotope Laboratories, Inc.

Preparation of Growth Media

Step 2.
Grow cyanobacteria in the medium with heavy nitrogen and transfer at least 3 times before use.

ANNOTATIONS
Bonnie Poulos 11 Jan 2016
Want to ensure that the majority of bacterial DNA has incorporated the heavy 15N and 3 transfers will usually accomplish the maximum incorporation.

Isolation of Hosts Cell DNA

Step 3.
Harvest the bacteria grown in heavy nitrogen.

Isolation of Hosts Cell DNA

Step 4.
Extract bacterial DNA using standard methods.

Isolation of Hosts Cell DNA

Step 5.

Quantify the DNA using Quant-iT Pico Green.

REAGENTS

Quant-it™ PicoGreen® dsDNA Assay Kit P7589 by Life Technologies

Isolation of Hosts Cell DNA

Step 6.

Use at least 10 µg of DNA for density gradient centrifugation.

Isolation of Hosts Cell DNA

Step 7.

For density gradient centrifugation, a Beckman VTi 65 vertical rotor was used with 13x48 mm OptiSeal polyallomer tubes (4.9 ml capacity).

Isolation of Hosts Cell DNA

Step 8.

Mix the DNA with TE buffer (10mM Tris, 1mM EDTA, pH7.6) to a final volume of 0.9 ml.

Isolation of Hosts Cell DNA

Step 9.

Mix the DNA with 4ml of CsCl prepared in TE to a density of p1.8 (measure the density of the final solutions, they should be p1.7).

Isolation of Hosts Cell DNA

Step 10.

Dispense 4.9 ml of the DNA sample in CsCl into the OptiSeal tube and plug with the black caps.

Isolation of Hosts Cell DNA

Step 11.

Load the tubes into the rotor and put caps on all carriers.

Isolation of Hosts Cell DNA

Step 12.

Centrifuge at 44,000 rpm (=184,678.5 g) in a Beckman L70 or L80 ultracentrifuge for 48 hr at 18°C.

DURATION

48:00:00

NOTES

James Thornton Jr 28 Jul 2015

Note: Do not centrifuge at lower temperatures as CsCl may precipitate out.

Isolation of Hosts Cell DNA

Step 13.

Collect 0.2-0.25 ml fractions.

Isolation of Hosts Cell DNA

Step 14.

Calculate amount of DNA in each fraction using Quant-iT Pico Green (perform in duplicate) and measure the density of each fraction.

Isolation of Hosts Cell DNA

Step 15.

Determine the density of the fractions with DNA (plot ng of DNA versus CsCl density).