Isotopic Labeling of Cyanobacteria and DNA Analysis
Version 2

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Preparation of Growth Medium

1. Prepare Pro99, SN, SNAX or Amp1 medium according to directions, but use ^{15}N ammonium chloride which will provide the heavy isotope. $^{15}NH_4Cl$ is available from Cambridge Isotope Laboratories, Inc. (#NLM-467-1).
2. Grow cyanobacteria in the medium with heavy nitrogen and transfer at least 3 times before use.

Isolation of Host Cell DNA

1. Harvest the bacteria grown in heavy nitrogen.
2. Extract bacterial DNA using standard methods.
3. Quantify the DNA using Quant-iT Pico Green (Invitrogen #P7589).
4. Use at least 10 µg of DNA for density gradient centrifugation.
5. For density gradient centrifugation, a Beckman VTi 65 vertical rotor was used with 13x48 mm OptiSeal polyallomer tubes (4.9 ml capacity).
6. Mix the DNA with TE buffer (10mM Tris, 1mM EDTA, pH7.6) to a final volume of 0.9 ml.
7. 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).
8. Dispense 4.9 ml of the DNA sample in CsCl into the OptiSeal tube and plug with the black caps.
9. Load the tubes into the rotor and put caps on all carriers.
10. Centrifuge at 44,000 rpm (=184,678.5 g) in a Beckman L70 or L80 ultracentrifuge for 48 hr at 18°C (note: do not centrifuge at lower temperatures as CsCl may precipitate out).
11. Collect 0.2-0.25 ml fractions
12. Calculate amount of DNA in each fraction using Quant-iT Pico Green (perform in dulicate) and measure the density of each fraction.
13. Determine the density of the fractions with DNA (plot ng of DNA versus CsCl density).