Homemade Glycogen (Molecular Biology Grade) for DNA/RNA Precipitation

Hanli Fan; 10/17/06

NOTE: Glycogen is a convenient substitute for tRNA or seeDNA as a carrier for nucleic acid precipitation. Although Molecular Biology grade glycogen can be purchased from a number of vendors, it is rather expensive (e.g., about $100/20-40mg). The following protocol describes a simple and inexpensive approach to preparing a large amount of glycogen, which is suitable for most Molecular Biology applications, such as DNA and RNA precipitations.

I. Reagents and Stock Solutions

Chloroform (*Sigma, catalogue #C-2432*)
Ethanol, 100%
D-Glycogen (Beef Liver) (*Fisher, catalogue #BP676-5*)
Isoamyl alcohol (*Sigma, catalogue #I-9392*)

II. Experimental Protocol

1. Add 5 grams of glycogen to 30 ml of DD-H_2O and stir until the glycogen is fully dissolved all dissolve into solution. This will take about 1-2 hours.
2. Aliquot the glycogen solution into 2 ml eppendorf tubes, approximately 1 ml per tube. Add an equal volume of PC-8 to the glycogen solution and vortex thoroughly. NOTE: After vortexing, the phenol phase should be the lower phase.
3. Centrifuge at 14,000 rpm in the microcentrifuge for 10 minutes at 4^{o}C. Transfer the upper aqueous phase (containing glycogen) into new Eppendorf tubes. Discard the lower phenol phase.
4. Add an equal volume of cold (4^{o}C) of chloroform: isoamyl alcohol (50:1 vol: vol) into the glycogen phase and vortex thoroughly. NOTE: After vortexing, the chloroform phase should be the lower phase, and the glycogen should be in the upper aqueous phase.
5. Centrifuge at 14,000 rpm in the microcentrifuge for 10 minutes at 4^{o}C. Transfer the upper phase glycogen phase into new Eppendorf tubes. Discard the lower chloroform phase.
6. Add an equal volume of room temperature absolute (100%) ethanol into the glycogen phase, and mix well by inverting 4-5 times.
7. Centrifuge at 14,000 rpm in the microcentrifuge for 10 minutes at 4^{o}C. Discard the supernatant.
8. Dry the precipitate in the Speedvac for 8 hours or overnight at 30-40ºC with the vacuum on until the glycogen is completely dried.
9. Pool the pellets and weigh the pooled pellet.
10. Dissolve the pellet in DD-H_2O at a concentration of 20mg/ml. Getting the glycogen into solution may require frequent shaking at room temperature for about 1-2 hours.
11. Aliquot and store at –70ºC.