Recommended Protocol for ^{3}H labeling of DNA

Overview

1. Obtain tritiated SAM: NEB recommends: Perkin Elmer Adenosyl-L methionine, S-[methyl-^{3}H]

   Code: NET155V001MC (or UC)
   15 Ci/mmol (diluted from 78 Ci/mmol), which is about 66 µM.

2. Reaction set-up (add in the following order):

Nuclease-free Water	up to 12 µl
Supplied Methyltransferase Reaction Buffer (10X)	2 µl
Diluted SAM	4 µl
DNA	1 µg
Methyltransferase	4 – 25 units (1 µl)

3. Mix, pipette up and down at least six times

4. Incubate at 37°C for one hour

5. Stop the reaction by heating at 65°C for 20 minutes

Notes

1. The volume of DNA should be 25% or less of the total reaction volume. When using more dilute DNA, increase the reaction volume to 50 µl. Using too much DNA volume in the reaction can cause inhibition by changing the pH or salt concentration of the reaction.

2. The volume of SAM can be increased to 8 µl without inhibition. If more label is required, larger volumes of SAM can be dried in a spin vacuum. The reaction is then set up using the tube containing dried SAM.

3. The incubation time can be increased to 4 hours. Overnight incubations do not give significant increases in methylation.

4. Using a similar protocol, we were able to label 1 µg of Lambda DNA to 3.43 x 10^{6} cpm measured by a standard filter-binding assay. Quenching in the assay was about 40%. (D. Robinson, unpublished observation)