Analysis of DNA methylation using SNuPE

Single Nucleotide Primer Extension is a powerful method which can be used for the precise analysis of methylation in a certain position. The procedure is shown on the figure. You treat your DNA with bisulphite and then anneal a primer which ends immediately before the site of analysis. Then you perform two primer extension reactions, one with radiolabelled dCTP (ddCTP) and another with dTTP (ddTTP). In this reactions only one nucleotide will be added to the primer. Then you have to denature your reaction, run your products on acrylamide gel and analyse the activity of the labelled primer. This method can also be adapted for the use with fluorescence labelled nucleotides or terminators.

The primers for SNuPE should be defined to bisulphite treated sequence. If the primer contains a CG sequence you have to use (Y=C/T) at the position of C.

SNuPE protocol for ^{32}P labelled nucleotides

1. Treat the DNA with bisulphite as described and amplify the region of interest.
2. Purify the desired fragment with any amplimer purification protocol taking care that no nucleotides remain in the reaction.
3. Prepare two reactions containing the same amount of PCR product, 1xPCR buffer, 1 pM of the primer, 1 U Taq polymerease and 1µCi of ^{32}P dCTP or dTTP in 20 µl final volume.
4. Run the following program: denaturing at 95°C, 1 min; annealing at the Tm of the primer, 2 min; extension at 72°C, 2 min.

5. Transfer the samples on ice and add 4µl of formamide loading buffer to each sample.

6. Denature the samples for 2 min at 95°C and load on preliminary pre-run 10% acrylamide gel containing 1xTBE and 7 M urea.

7. Run the gel at 40-45 mW/cm^2 for 30-40 min (BioRad Protean II system).

8. Dry the gel on a vacuum dryer for 2 h at 80°C.

9. Perform autoradiography for 10 min to 2 h (depending on the signal intensity) at room temperature.

10. Align obtained autoradiogramms to the gel and excise the pieces of the gel corresponding to the bands.

11. Measure the amount of radioactivity incorporated using a scintillation counter.

12. The percentage of methylation can be determined as a ratio of the sample C activity (as c.p.m.) to the total (C+T) incorporation (as c.p.m.).

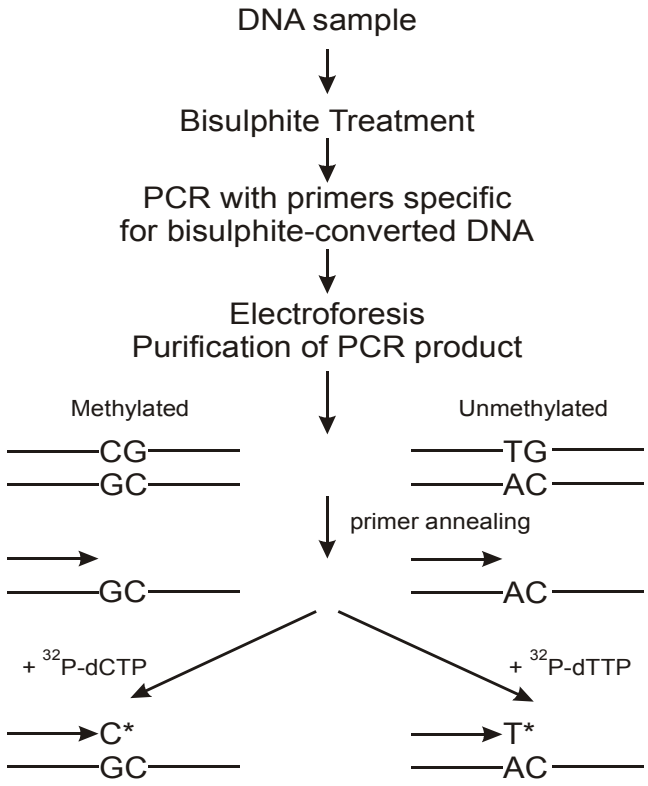

Figure: *SNuPE procedure*