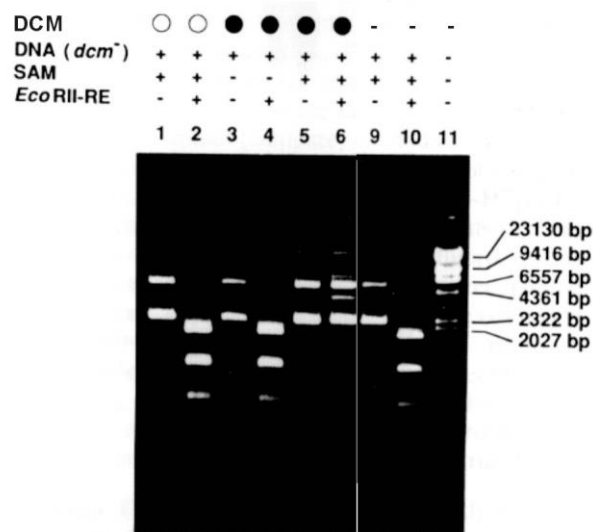


# THE EFFECT OF DNA METHYLATION ON RESTRICTION ENDONUCLEASE CLEAVAGE

**Terms to be familiar with before you start to analyze the figure**

*plasmid \* restriction endonucleases \* agarose gel electrophoresis \* ethidium bromide staining*

**The figure**



In the experiment described below **DNA cytosine methyltransferase** (DCM), an enzyme that catalyzes the methylation of cytosines in DNA using S-adenosyl methionine (SAM) as methyl donor, was tested. Reaction mixtures containing unmethylated plasmid DNA, untreated DCM (●), or DCM heated at 100°C for 10 min (○), SAM and the restriction endonuclease *Eco*RII (recognition site CCA/TGG) were set up, as indicated in the figure. Mixtures were incubated and electrophoresed through an agarose gel containing ethidium bromide (sample 11: *Hind*III-cleaved λ DNA).

Study the figure and answer the questions!

1. Why does the control plasmid (sample 1) give two bands?
2. Why did they use heated DCM?
3. What was the effect of SAM?
4. What is the consequence of cytosine methylation on *Eco*RII cleavage?
5. What enzyme activity is mimicked by DCM?

## The source of the figure

Hanck,T., Schmidt,S., Fritz,H-J. (1993): Sequence-specific and mechanism-based crosslinking of Dcm DNA cytosine-C<sup>5</sup> methyltransferase of *E.coli* K-12 to synthetic oligonucleotides containing 5-fluoro-2'-deoxycytidine. *Nucleic Acids Research* 21, 303-309.

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