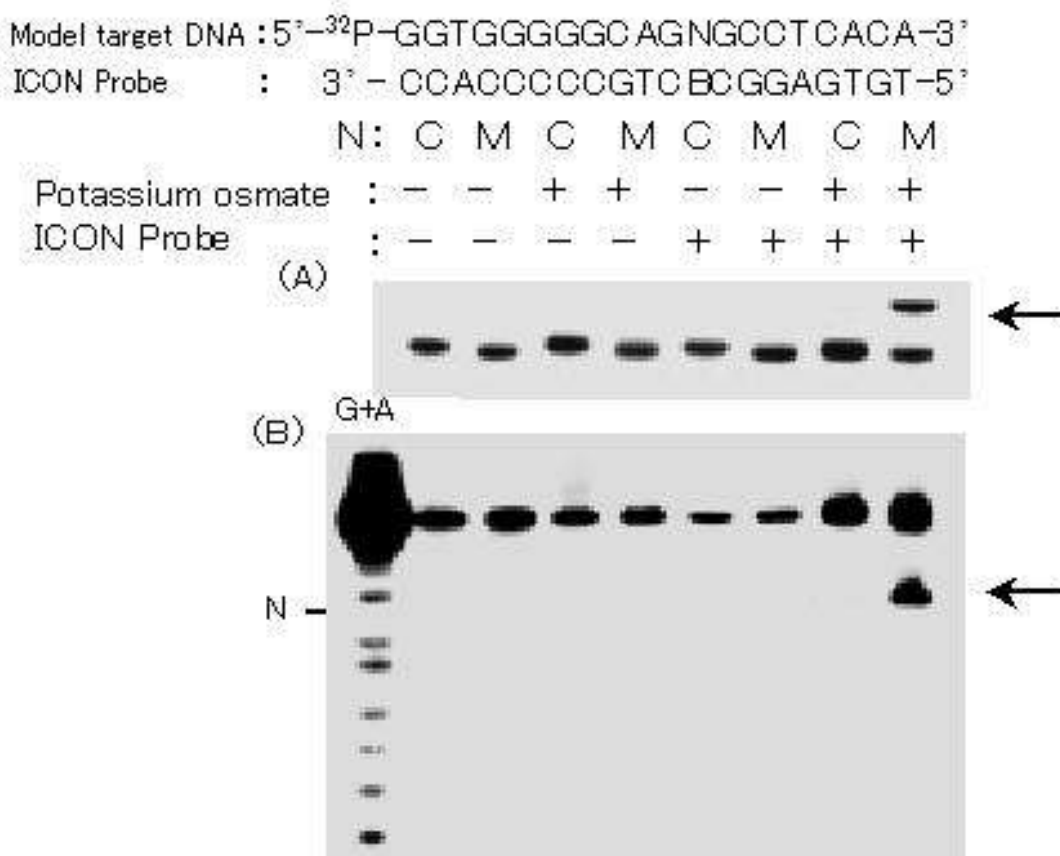


Example Experiment Results

【Test 1】

The ICON Probe forms an osmium complex only with the targeted methyl cytosine. • N in the model target DNA is either cytosine (C) or methyl cytosine (M)

• B in the ICON Probe is the modified nucleotide that forms the complex with the target methyl cytosine.

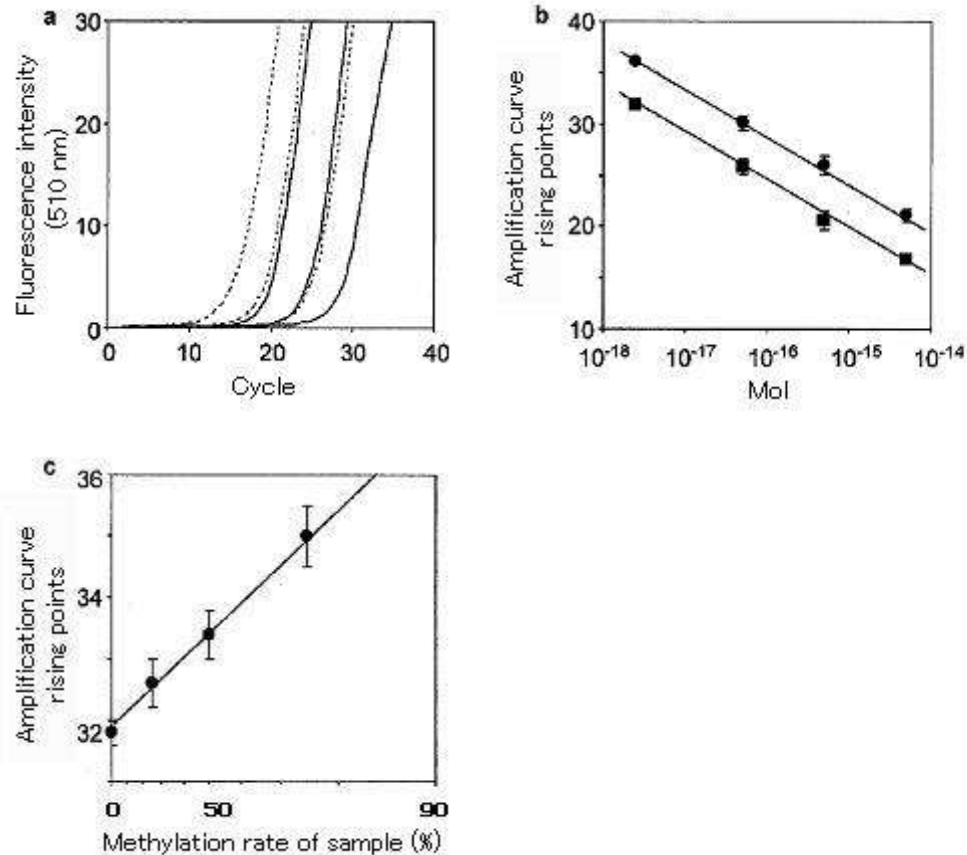


(A) PAGE was conducted on a sample taken from the osmium complex formation reaction under the presence of the ICON Probe on DNA labeled at the 5' terminal with ³²P. The crosslink bodies between the strands generated as a consequence of the formation of the osmium complex can be confirmed as bands of low mobility on PAGE (arrow).

(B) Thermal alkali processing was conducted on a sample after reaction, followed by PAGE. The band of high mobility on electrophoresis can be confirmed (arrow) so the occurrence of crosslinking due to the target DNA and the ICON Probe forming a complex at the methyl cytosine can be confirmed. Severance at the methyl cytosine site can be understood by comparison with the guanine/adenine base-severed G+A lane on the furthest left.

【Test 2】

Sequence selective quantification of methylated DNA using an ICON Probe



(a) Amplification inhibition of crosslink complex between ICON probe and DNA strand due to quantitative PCR

In comparison to non-methyl cytosines (dotted lines), with methyl cytosines (solid lines), the number of cycles required to start amplification is delayed due to the formation of crosslink bodies between the ICON Probe and the DNA strand.

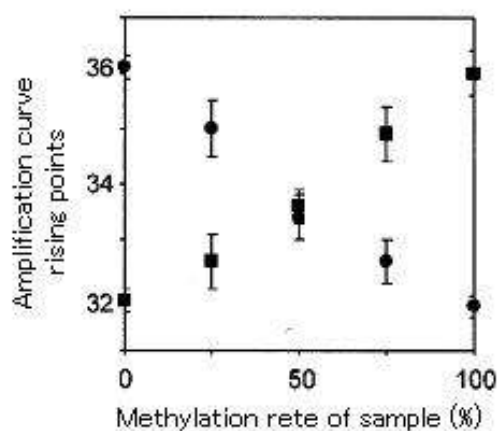
(b) Calibration curve of sample volume created from the curve in (a)

The number of cycles required for initiation of amplification (vertical axis: using the local maximal value of the secondary differential of the curve) differs linearly in accordance with the volume of the initial methylated DNA.

(c) Calibration curve for determination of the methylated ratio

The number of cycles required for initiation of amplification (vertical axis: using the local maximal value of the secondary differential of the curve) differs linearly in accordance with the proportion of target cytosine methylation. This method allows determination of the methylated ratio within the sample.

There is no interference on other methylation regions existing simultaneously in the



quantification of methylation

Two fictitious types of DNA including sequences with methylation sites in 2 locations (C1, C2) (C1: methylated C2: non-methylated and C1: non-methylated C2: methylated) are mixed in fixed proportions and reacted. The horizontal axis shows the methylation rate of C1 in the mixed system.

■ shows the probe for C1, and ● shows the results of measurement using the probe for C2.

Even if the proportions of methylation of cytosines in 2 different locations are changed, there is no impact on the quantification of methylation of the target cytosine.

【Test 4】

Measurement of the quantity of methylation in genome samples taken from various mouse tissues

We measured methylation rates using genome samples (all 20 ng) taken from 4 types of tissues and ICON Probe for two CpG sequences assumed to be mouse tissue-specific methylation regions.

Methylation levels specific to each different tissue were confirmed at these two methylation sites. (the coverexplanatory note)

Figures show the methylation rate (%)

Figures in brackets show the standard deviation obtained from 5 independent experiments

【reference book】

Reference literature for the experiments above: Tanaka, K. et al.:J. Am. Chem. Soc., 129: 14511-14517, 2007