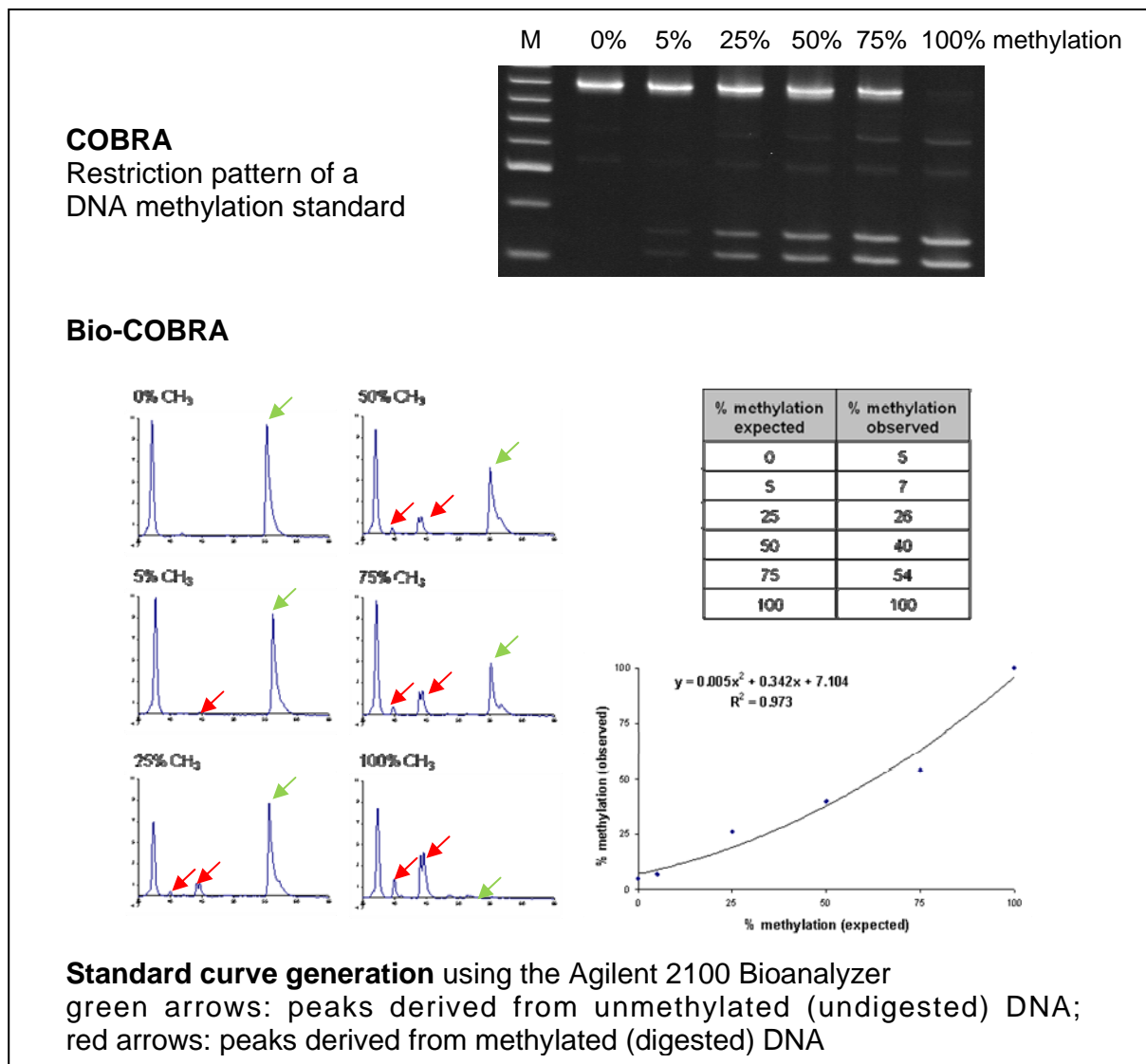


## Bio-COBRA

DNA hypermethylation in promoter CpG islands is a hallmark of cancer. Therefore, it is of great interest to not only qualitatively, but also to quantitatively measure DNA hypermethylation. The coupling of **Combined Bisulfite Restriction Analysis (COBRA)** with an electrophoresis step in microfluidics chips provides a rapid, accurate and cost-efficient quantification of methylation patterns in DNA samples (Brena *et al.*, Nat. Protoc. 2006).

The principle of Bio-COBRA is as follows: Bisulfite treatment of genomic DNA converts unmethylated cytosine residues to uracil, whereas methylated cytosine residues remain unchanged. Then, the sequence of interest is amplified by PCR, followed by a restriction digest with a restriction enzyme that contains a CpG dinucleotide in its restriction site. Because of bisulfite treatment-induced changes of the DNA sequence, the enzyme cleaves the PCR fragment only if it was originally methylated. Subsequent electrophoretic separation of DNA fragments on acrylamide gels gives semiquantitative information on DNA methylation. By using the Agilent 2100 Bioanalyzer accurate quantification of methylation is achieved, and calibrations of Bio-COBRA results to a standard curve give exact DNA methylation percentages.



Brena, R.M., Auer, H., Kornacker, K., and Plass, C. (2006). Quantification of DNA methylation in electrofluidics chips (Bio-COBRA). Nat. Protoc. 1, 52-58.