Methylation analyses

Epigenetic modifications are retrieved both in neoplastic and non-neoplastic disorders. Detection of DNA methylation at individual loci and with promoter-focused studies has been done in formalin-fixed paraffin-embedded archival tissue specimens also as clinical pathology tests. Bisulfite conversion methods have been the most widely used in FFPE samples, because they allow the rapid identification of methylated cytosines in any sequence context **MS-MLPA** requires only small quantities of short fragments, which makes it very suitable for diagnostic application using FFPE DNA

Bisulfite treatment

Genomic DNA amplification

mCGmCGTCTATGmCGAGGCmCGG

↓

mCGmCGTUTATGmCGAGGUmCGG

↓

CGCGTTTATGCGAGGTCGG

GCGCAAATACGCTCCAGCC

Bisulfite treatment

PCR amplification

When a CpG sequence is unmethylated

CGCGTCTATGCGAGGCCGG

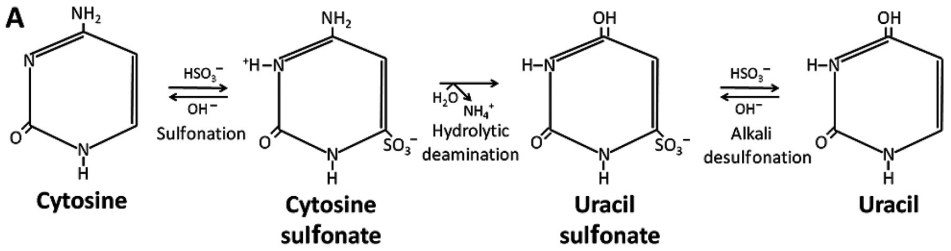
↓

UGUGTUTATGUGAGGUUGG

↓

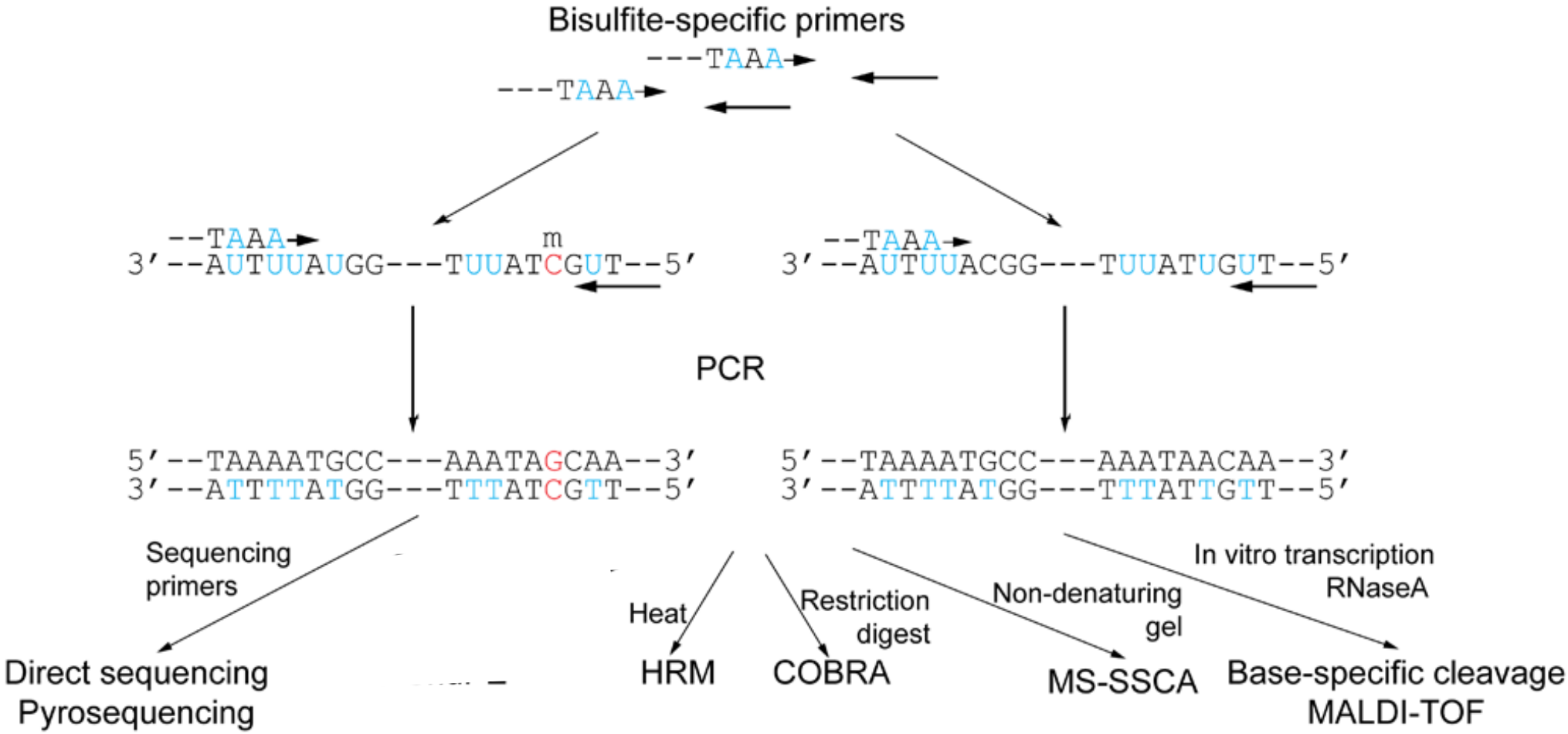
TGTGTTTATGTGAGGTTGG

ACACAAATACACTCCAACC

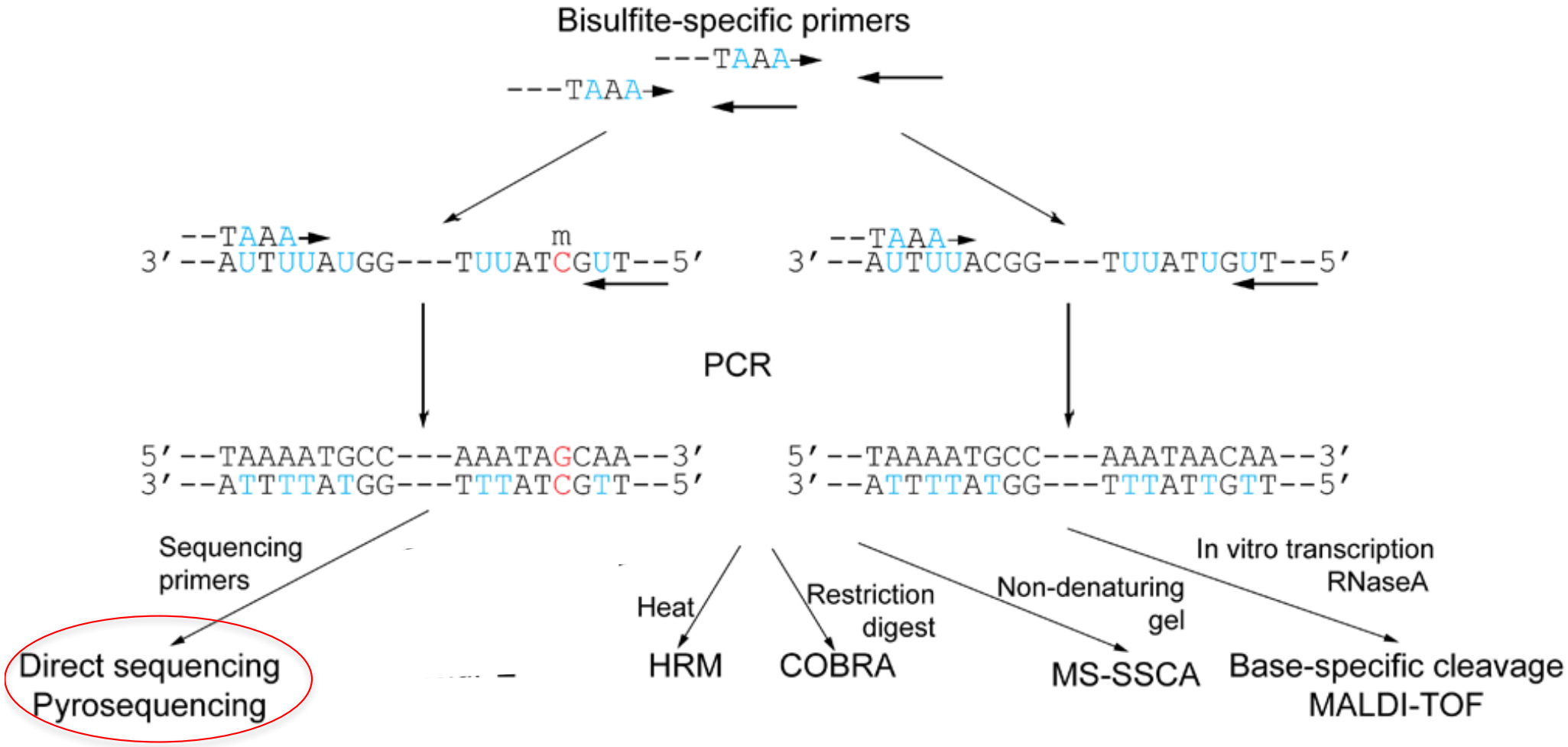


B

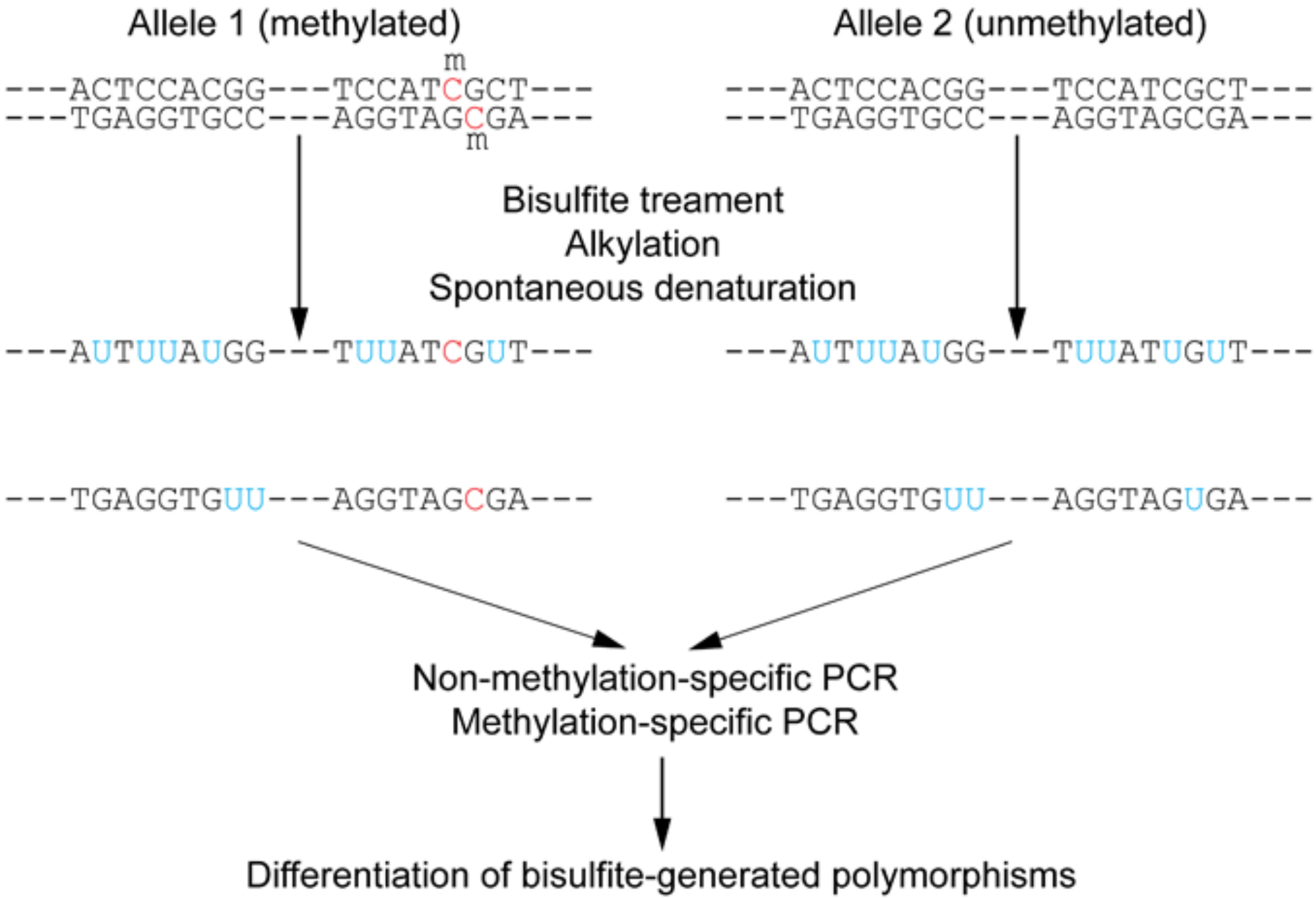
	Original sequence	Sequence after bisulfite treatment
Unmethylated DNA	A-T-**C**-**G**-G-T-C-A-T-**C**-**G**-C-A-T	A-T-**U**-**G**-G-T-U-A-T-**U**-**G**-U-A-T
Methylated DNA	A-T-**C**-**G**-G-T-C-A-T-**C**-**G**-C-A-T	A-T-**C**-**G**-G-T-U-A-T-**C**-**G**-U-A-T

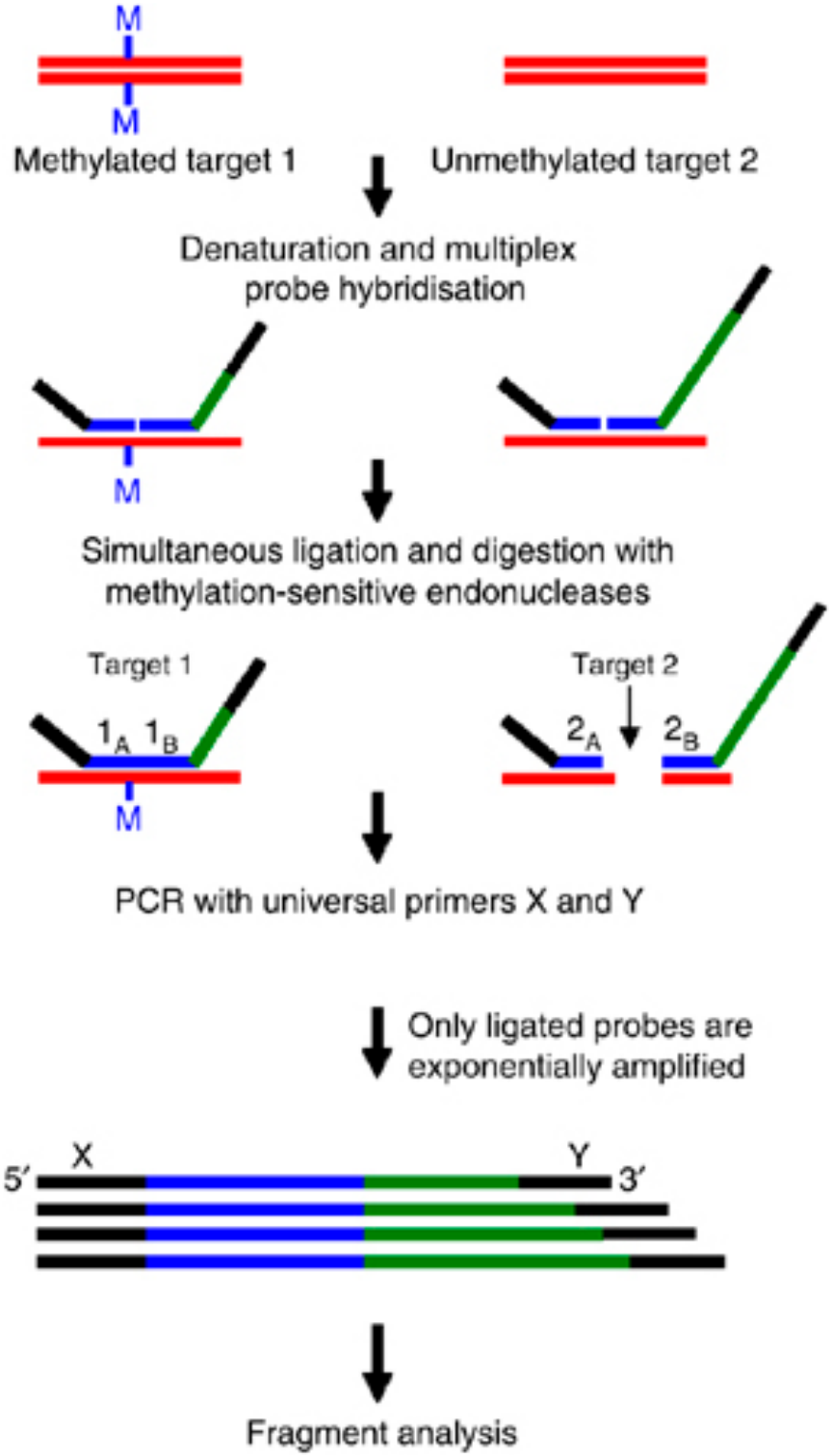


DNA methylation analysis methods not based on methylation-specific PCR. Following bisulfite conversion, the genomic DNA is amplified with PCR that does not discriminate between methylated and non-methylated sequences. The numerous methods available are then used to make the discrimination based on the changes within the amplicon as a result of bisulfite conversion.



Following PCR amplification of the region of interest, Pyrosequencing is used to determine the bisulphite-converted sequence of specific CpG sites in the region. The ratio of C-to-T at individual sites can be determined quantitatively based on the amount of C and T incorporation during the sequence extension.


Allele 1 (methylated)
Allele 2 (unmethylated)
m
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
m
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
Bisulfite treament
Alkylation
Spontaneous denaturation
---AUTUUAUGG---TUUATCGUT---
---AUTUUAUGG---TUUATUGUT---
---TGAGGTGUU---AGGTAGCGA---
---TGAGGTGUU---AGGTAGUGA---
Non-methylation-specific PCR
Methylation-specific PCR
Differentiation of bisulfite-generated polymorphisms



MS-MLPA

Only undigested, therefore methylated sequences, are amplified

The amplified material is then separted by CE.