SibEnzyme®

New enzymes for new DNA technologies

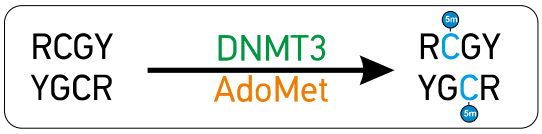
GLAD PCR assay – a new method of DNA methylation analysis for epigenetics

A new method of GLAD PCR assay has been developed to determine minimal quantities of methylated sites in presence of excess of unmethylated DNA. This is typical for DNA from clinical samples of blood and tissues. Method allows to determine methylation of RCGY site of interest in human and mammalian genomes.

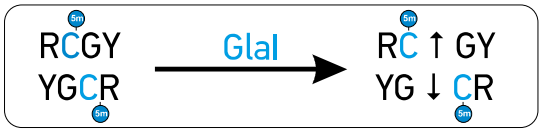
Method includes:
- GlaI hydrolysis of studied DNA,
- the universal adapter ligation
- and subsequent real-time PCR with Taqman probe. One primer is designed for DNA region of interest, structure of another primer is based on an adapter sequence.

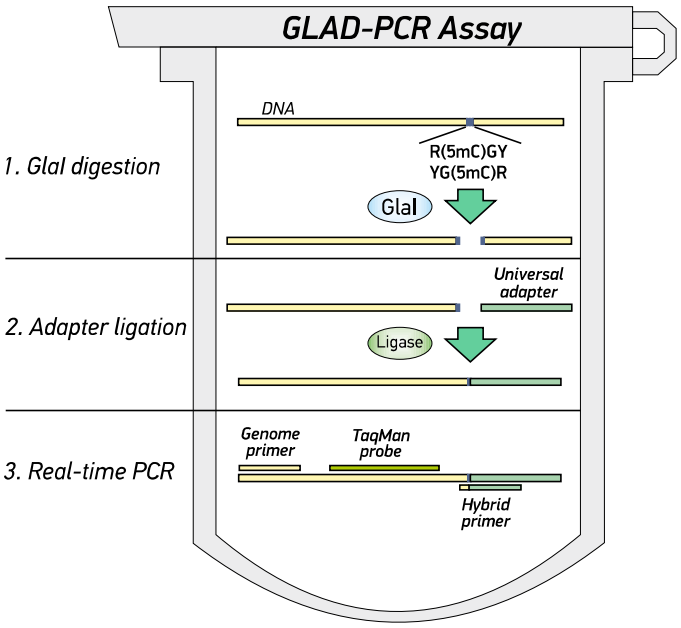
Method is performed in one tube, takes about 2-3 hours and determines even several copies of R(5mC)GY site of interest.

De novo DNA methylation in mammals is performed by DNMT3A and DNMT3B DNA methyltransferases.



Recently we have discovered and characterized a new DNA-endonuclease GlaI. GlaI belongs to the novel type of site-specific methyl-directed DNA-endonucleases which hydrolyze only methylated DNA. GlaI recognizes DNA sequence R(5mC)GY.





Today an abnormal methylation of certain DNA regions, mostly promoter and first exon, of a number of genes was shown at initial stages of aging deceases such as cancer, cardiovascular decease, diabetes and other deceases involving epigenetic changes.
Thus GLAD PCR assay may be used for development of new epigenetic PCR diagnostics.

In comparison with other epigenetic methods GLAD-PCR has strong advantages:
- Simple – 3 easy steps
- Requires only real time PCR-machine
- Quick - only 2-3 hours
- Sensitive – detects even several copies of selected R(5mC)GY site

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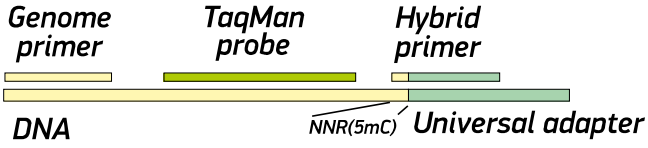
GLAD-PCR Assay Kit

A new kit is designed for 200 GLAD-PCR reactions in two 96-wells PCR plate.
All reagents are included except primers and TaqMan probe.



Reaction requires:

- sample DNA
- genome primer and TaqMan probe designed for R(5mC)GY site of interest
- hybrid primer (includes constant part complimentary to the universal adapter and tetranucleotide part complimentary to DNA at the point of GlaI hydrolysis). Hybrid primers may be ordered on the request.

More information and order at:

sibenzyme.com/info7819.php

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