SibEnzyme®

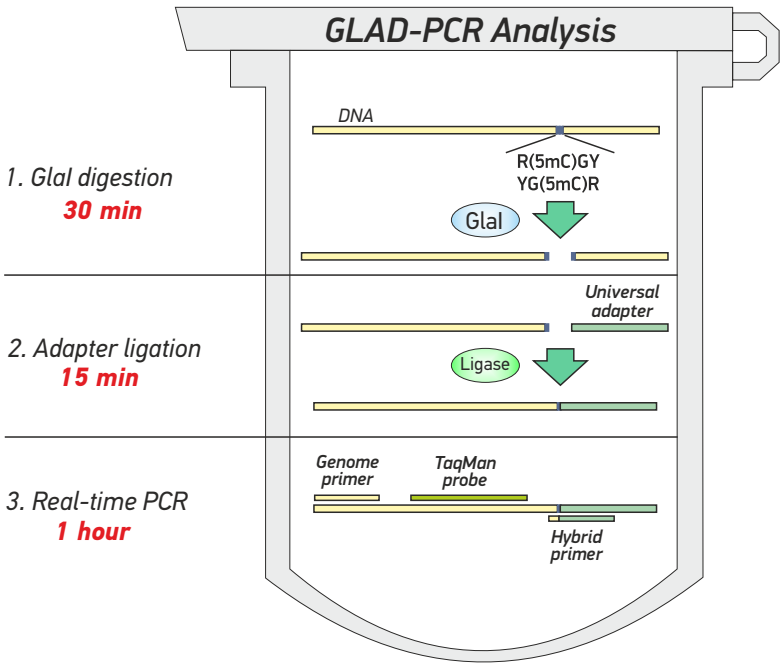
GLAD PCR assay – a new method of DNA methylation analysis for cancer detection

GlaI hydrolysis and Ligation Adapter Dependent PCR (GLAD PCR) is the novel method to determine R(5mC)GY sites produced by methylation with DNMT3A and DNMT3B. GLAD PCR analysis is performed in one tube and includes 3 steps: DNA hydrolysis with site-specific methyl-directed DNA endonuclease GlaI, universal adapter ligation and 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. GLAD PCR analysis has been used to study an aberrant methylation of RCGY sites in regulatory regions of RARB and CEBPD tumor suppressor genes in malignant cell lines. GLAD PCR analysis allows detecting several copies of methylated DNA and may be used in routine laboratory and clinical practice.

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 Pu(5mC)GPy.

Method



Single tube assay

Studied DNA

Malignant cell lines	Control		
Raji – Burkitt's lymphoma	**L-68** – fibroblast cell line	**G** – human peripheral blood DNA	**Mouse** – A/He mouse DNA, negative control

Studied genes

CEBPD	-350	GGAGCGAGGA GGTTCCAAGC CCACAAACAG GAAGAAGAGA AGGCCCTGGA GTGCTGGCAG AGGGAGTGTC ATTCCCAGCA GCGCAGCGCA GGCCGGCCCG CCTCGCTCCT CCAAGGTTCG GGTGTTTGTC CTTCTTCTCT TCCGGGACCT CACGACCGTC TCCCTCACAG TAAGGGTCGT CGCGTCGCGT CCGGCCGGGC
RARB	28	TTCAGAGGCA GGAGGGTCTA TTCTTTGCCA AAGGGGGGAC CAGAATTCCC CCATGCGAGC TGTTTGAGGA CTGGGATGCC GAGAACGCGA GCGATCCGAG AAGTCTCCGT CCTCCCAGAT AAGAAACGGT TTCCCCCCTG GTCTTAAGGG GGTACGCTCG ACAAACTCCT GACCCTACGG CTCTTGCGCT CGCTAGGCTC

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It is well known that an abnormal methylation of certain DNA regions, mostly promoter and first exon of tumor suppressor genes, is a serious sign of cell malignancy. Differences in methylation of CpG island of these regions has been observed for many cancers and earlier we have developed BlsI- and GlaI-PCR assays to discriminate malignant cell lines based on different pattern of CpG islands methylation.

Recently developed epigenetic methods of cancer diagnostics allow to detect the disease at early stages. However, these epigenetic test-systems are based on a method of bisulfite conversion, which is quite complicated and often results in false-positive/negative data. That is why such tests are not widely used.

We have applied GLAD PCR assay to determine an aberrant methylation of selected RCGY sites in regulatory regions of RARB and CEBPD tumor suppressor genes. Results of GLAD PCR assays are fully consistent with the data obtained for these regions by bisulfite sequencing.

In comparison with other epigenetic methods GLAD-PCR has strong advantages:

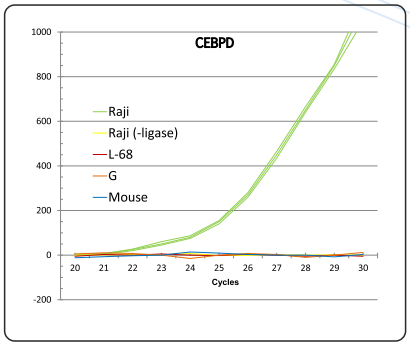
- Simple – 3 easy steps in one tube
- Requires only real time PCR-machine
- Quick - only 4-6 hours
- Sensitive – detects several copies of selected R(5mC)GY site in a presence of excess of corresponding RCGY site

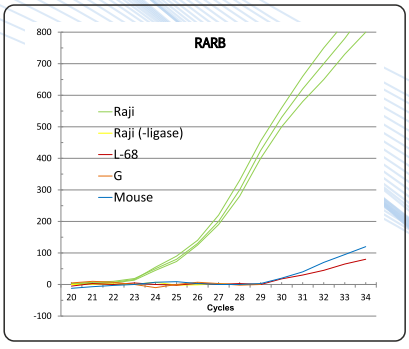
GLAD PCR assay may be used for detection of minimal quantities of methylated DNA in a presence of excess of unmethylated DNA in clinical samples of blood and tissues.

Fig. 1. GLAD PCR analysis of DNA methylation in regulatory region of **CEBPD** tumor suppressor gene. RCGY site is in positions -270..-267 from transcription start.

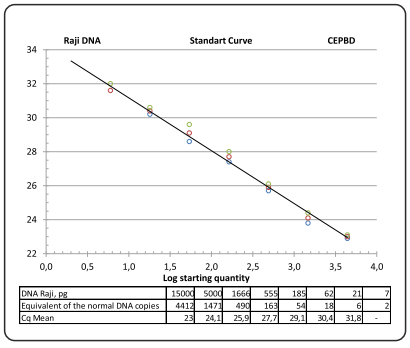
Fig. 2. GLAD PCR analysis of DNA methylation in regulatory region of RARB tumor suppressor gene. RCGY site is in positions 112..115 from transcription start.

a — Amplification chart of GLAD PCR assay of 15 ng DNA per reaction using Bio-Rad CFX96. We accept Raji DNA methylation to be 100%.

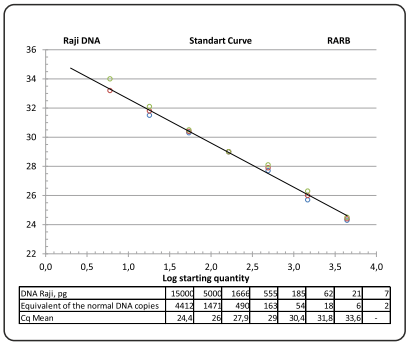




b — Sensitivity determination of GLAD PCR.



DNA Raji, pg	15000	5000	1666	555	185	62	21	7
Equivalent of the normal DNA copies	4412	1471	490	163	54	18	6	2
Cq Mean	23	24,1	25,9	27,7	29,1	30,4	31,8	-



DNA Raji, pg	15000	5000	1666	555	185	62	21	7
Equivalent of the normal DNA copies	4412	1471	490	163	54	18	6	2
Cq Mean	24,4	26	27,9	29	30,4	31,8	33,6	-

A new method of GLAD PCR assay has been developed to study DNA methylation. Method allows to determine methylation of RCGY site of interest in human and mammalian genomes in excess of corresponding unmethylated sites.

Method includes GlaI hydrolysis of studied DNA, the universal adapter ligation and subsequent real-time PCR. Method is performed in one tube, takes about four-six hours and determines even several copies of R(5mC)GY site of interest.

GLAD PCR assay has been applied to study an aberrant methylation of selected RCGY sites in regulatory region of RARB and CEBPD tumor suppressor genes. All studied RCGY sites are highly methylated in Raji cells. GLAD PCR assay may be used in epigenetic diagnostics especially in early cancer detection.

Patent RU 2525710 C1

See details on md.sibenzyme.com

MD-DNA endonuclease GlaI

BlsI- and GlaI-PCR Assay

Epigenetic typing of human cancer cell lines