New enzymes for DNA technologies

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Comparative analysis of RCGY sites methylation in the genomes of Raji and U-937 malignant and normal lung fibroblast cell lines

Novel method of Epigenetic NGS sequencing for cancer research

SUMMARY

DNA methylation in human genome is important for the cells specialization and functioning. An abnormal methylation of the regulation regions of some genes may cause the genes silencing and this phenomenon is often detected in cancer cells. Determination of differences of the genome-wide methylation in normal and tumor cells is useful for understanding the carcinogenesis process and for development of new methods of epigenetic diagnostics.

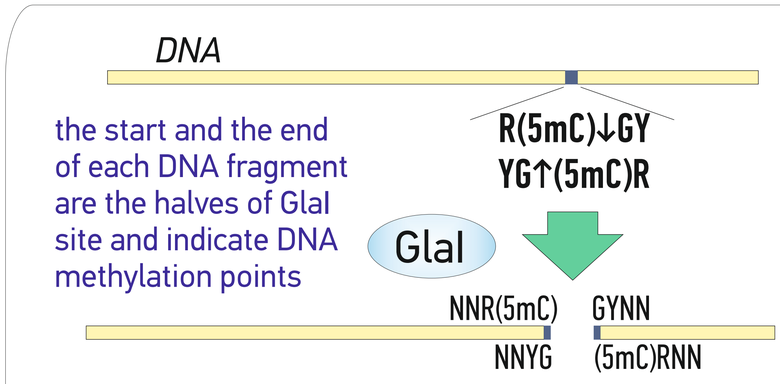
CONCLUSIONS

The analysis showed a significant difference in DNA methylation of three studied human cell lines including CpG islands, some groups of DNA repeats and putative regulatory regions of genes. Thus, we have shown the applicability of the epigenomic sequencing method of search for cancer markers. The suggested approach is reliable and simple if compared to bisulfite sequencing.

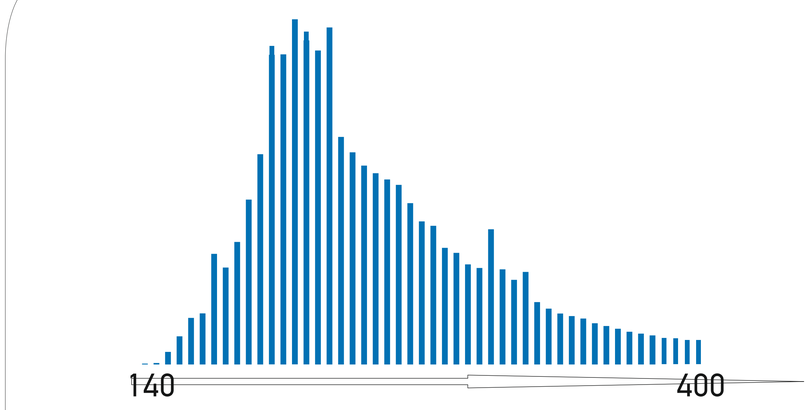
PERSPECTIVES

Taking into account the variability of different types of cancers, more genomes must be included in the analysis to accumulate the genome methylation data acquired by the proposed method. This will allow us to find epigenetic markers specific for certain types of disease. Such database containing epigenetic profiles of tumor and normal tissues allows a rapid development of diagnostic panels based on GLAD-PCR assay [please visit CEAB 09 poster session]

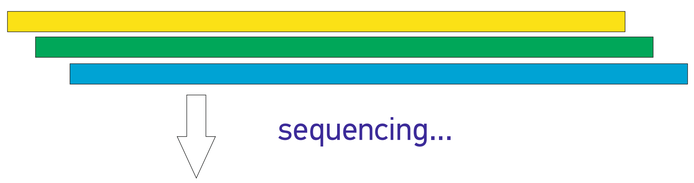
APPROACH



GlaI - DNA fragmentation enzyme produces blunt ends



140 - 400 bp DNA fragments selection

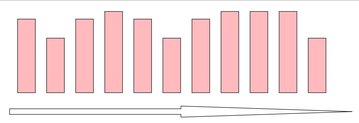


sequencing...

CTGTAGTCTCCAG <....> TCCAGGTGTTTCC**CG**

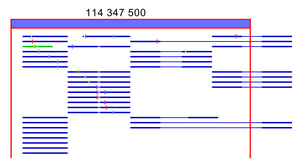
underlined are ends of methylated RCGY sites hydrolized by GlaI

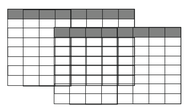
Illumina paired-end 75 bp sequencing



filtering...

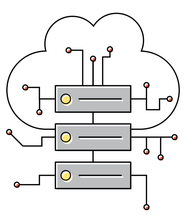
mapping to Human genome reference...





postfiltering, statistics...

Bioinformatics procedures



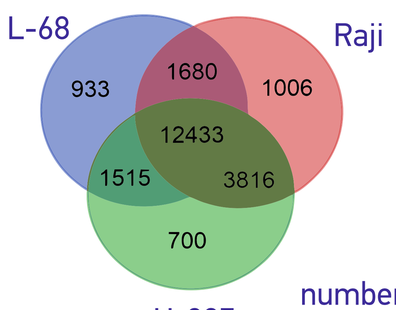
methylation database

online methylation browser

epimarkers searching

rapid epigenetic diagnostics development

Final result and perspectives



numbers of common and unique genes with methylated putative regulatory regions in the genomes studied as test cell lines

AUTHORS

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REFERENCES

Abdurashitov MA, Tomilov VN, Gonchar DA, Kuznetsov VV, Degtyarev SK. Mapping of R(5mC)GY sites in the genome of human malignant cell line Raji. Biol Med (Aligarh). 2015;7:BM-135-15

Online methylation genome browser (beta version) http://mbrowser.sibenzyme.com

GLAD-PCR assay materials http://sibenzyme.com/glad_pcr_assay.php

Patent of Russian Federation RU 2586502 C1