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Recommendations to enhance reproducibility and reliability in comet assay

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Comet assay is a very sophisticated technique use to assess plethora of DNA damages at the level of individual cells and can be applied in a range of fields from human and environmental biomonitoring, routine genotoxicity assessment, DNA repair studies, and clinical studies to molecular epidemiology. Due to its demonstrated advantages and applications it is increasingly acceptable by regulatory authorities as a part of battery of assays used for regulatory submissions in genetic toxicology. Yet the major drawback of this technique is the unreliability in making reproducible data, due to miscellaneous conditions used in different laboratories and due to lack of understanding of the critical steps. Comet assay was performed according to the protocol described by Raymond Tice, with some modifications. Some critical steps that affect the final outcome of the assay were optimized during thisstudy. As the concentration of low melting agarose greatly influenced the comet formation and assay reproducibility, melting of agarose in a boiling water bath was found to be better than melting using a microwave oven. Optimal agarose solidification time was 30 min at 4°C to attain adequately solidified agarose layers which reduce the fragility. Optimal lysis time was 2 h at 4°C. Alkaline unwinding time was increased up to 30 min for adequate denaturation of DNA. The best way to calculate the required voltage to get a voltage drop of 1 V/cm, was to use the spreadsheet developed by Gunnar Brunborg. Electrophoresis for 45 min was found to be sufficient to obtain a considerable DNA migration. However, a small factor, that one would not consider, significantly matters the final outcome and the assay reproducibility.

Biography

Baragamaarachchi R Y is a first year PhD student who graduated with a first class in BSc Genetics from University of Bangalore. She obtained MSc in Molecular Life Sciences in 2014 from University of Colombo, Sri Lanka. Her research interests lie in Molecular Biology, Immunology, Medicinal plants, Genetics and Microbiology. She has served as a resource person at workshops based on Immunological techniques and awarded at 2nd International Conference on Frontiers in Molecular Life Sciences held in Sri Lanka (2014), for outstanding poster presentation.

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