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Sommario/riassunto	By means of this ‘Frontiers in Genetics’ research topic, we are celebrating 30 years of the Comet Assay. The first paper on this single-cell gel electrophoresis assay was published in 1984 by O. Ostling and K.J. Johanson (Biochem. Biophys. Res. Commun. Vol.123: 291-298). The comet assay is a versatile and sensitive method for measuring single- and double-strand breaks in DNA. By including lesion-specific enzymes in the assay, its range and sensitivity are greatly increased, but it is important to bear in mind that their specificity is not absolute. The comet assay (with and without inclusion of lesion-specific enzymes) is widely used as a biomarker assay in human population studies - primarily to measure DNA damage, but increasingly also to assess the capacity of cells for DNA repair. Ostling and Johanson (Biochem. Biophys. Res. Commun., 1984) were also the first to report experiments to measure DNA repair, by simply following the decrease of DNA damage over time after challenging cells with ionising radiation. However, this approach is time-consuming and laborious as it requires an extended period of cell culture and is therefore not ideal for biomonitoring studies, which typically require high-throughput

processing of many samples. As an alternative approach, the in vitro comet-based repair assay was developed: a cell extract is incubated with a DNA substrate containing specific lesions, and DNA incisions accumulate. The in vitro comet-based repair assay has been modified and improved over the past decade: it was first devised to measure base excision repair of oxidised purines in lymphocytes (Collins et al., Mutagenesis, 2001), but has since been adapted for other lesions and thus other repair pathways, as well as being applied to tissue samples in addition to cell suspensions.Even after 30 years, the comet assay is still in a growth phase, with many new users each year. Many questions are repeatedly raised, which may seem to have self-evident answers, but clearly, it is necessary to reiterate them for the benefit of the new audience, and sometimes being forced to think again about old topics can shed new light. Different applications of the comet assay are discussed, including: genotoxicity testing, human biomonitoring, DNA repair studies, environmental biomonitoring and clinical studies. Furthermore, we will consider and where possible answer questions, including the ones raised by Raymond Tice at the 8th International Comet Assay Workshop in Perugia (Italy 2009): i) What is the spectrum of DNA damage detected by the various versions of the Comet assay?; ii) What are the limitations associated with each application?; iii) What should be done to standardize the assay for biomonitoring studies?; iv) Can the Comet assay be used to monitor changes in global methylation status?; What are the best cell types to use for detecting genotoxic substances in vitro?; v) Can the assay be fully automated?; and more. So this ‘Frontiers in Genetics’ research topic will be written for the beginner as well as for the experienced users of the Comet Assay.