Reviewer's report

Title: A new assay for measuring chromosome instability (CIN) and identification of drugs that elevate CIN in cancer cells

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Reviewer: Daniela Moralli

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Overview
In this study, the authors describe a new assay for measuring chromosome instability, based on FACS analysis of the segregation of a model chromosome (human artificial chromosome), expressing GFP.

The method described does not require lengthy cytological analysis and so can represent an easily quantifiable, faster alternative for the characterization of genotoxic substances. The paper is clear, and the data sound. For these reasons, the present manuscript represents a very interesting and useful advance in the field of chromosome instability studies, and falls well within the remit of BMC Cancer journal. The manuscript should be accepted after minor revisions.

Discretionary revisions
1) The authors state that the EGFP cassette loaded onto the HAC is protected from silencing by a chS4 insulator. Does this mean that all the CHO and HT1080 clones containing the HAC have identical levels of GFP expression, or is there variability? For example, on page 11, 2nd paragraph, the authors write that “Ten BS-resistant clones that expressed the GFP transgene were isolated…”. Were there any GFP negative BS-resistant clones?

This point should be clarified, as the presence of variable GFP levels between clones could hint at epigenetic effect on GFP expression, possibly affecting the FACS readings.
Quantitive RT-PCR data on GFP expression would be useful.

2) The FACS analysis is conducted 2 weeks after the treatment with the aneugens compounds. If a cell gains growth advantages because of gain/loss of an essential chromosome, it would quickly take over the whole population during the 14 days period. The majority of the cells would either contain the HAC or not, depending on the HAC status of the founder cell. Thus, the aneugenic potential of the compound under test could be under/over estimated. Are three replicates sufficient to exclude this scenario? In figure 4, some of the treatments show much larger error bars than others. Could this be due to a founder effect? This point should be clarified and discussed.

3) In the legend to Figure 5 the authors write “Our model assumes that when
mis-segregation occurs during mitosis, one daughter cell will inherit a HAC while
the other daughter cell does not”. What about non-disjunction errors, where one
cells inherits two copies of the chromosome and the other none? How does the
model take this phenomenon into account? This point should be discussed.

3) Figure 2 would be more informative with the addition of a map of the HAC
vector, showing the various markers, and a phase contrast image of the GFP
expressing cells.

Minor essential revisions
1) Nocodazole is misspelled in Table 1, 2 and 3, and Figure 4.
2) Figure 2 legend should clarify which probe has been used for the FISH in
Figure 2B, as some of the endogenous chromosomes appear to be labelled.
2) Figure 3 does not show the A and B.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a
statistician.

**Declaration of competing interests:**

I declare that I have no competing interests