Reviews for "Monitoring hPSC genomic stability in the chromosome 20q region by ddPCR"
Authors: Caroline Becker, Sema Aygar, Laurence Daheron
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Reviewer 1

Has selected to remain anonymous.

Originality, novelty and significance of results:	Good
Technical Quality of Work:	Excellent
Comprehensibility and Presentation of Paper:	Excellent
What is the overall impression:	Excellent

Reviewer Recommendation Term: Revise and resubmit pending minor revisions

Narrative (as sent to corresponding author):
Becker and colleagues present a detailed method for assessing BCL2L1 copy number variation in human pluripotent stem cells. The BCL2L1 locus is one of the most frequently amplified regions in genome edited hPSCs. Although the method has been previously described elsewhere, this protocol provides practical and in depth instructions on how to implement this assay in practice. Additionally, it adds novel points such as the use of a different reference gene that better coincides with BCL2L1 replication timing. This manuscript is potentially very useful for the stem cell and gene editing communities where it is extremely important to monitor genomic stability when generating gene edited cells.

Several minor points need to be addressed before the manuscript can be considered for publication:

1. Please define CNV when this acronym is used for the first time.

2. A short background description on how ddPCR works, especially in the context of CNV detection would be helpful.

3. It would be helpful to number each step so it's easier to follow the protocol.

4. In Droplet Reading and Data Analysis sections, it would be helpful to take screen shots/photos and indicate with arrow or similar tools so that it is easier to follow the instructions.

5. In Data Analysis, it is stated that interpretation is based on 19 hPSC control samples. It is unclear if there is literature reference for this panel or this is based on in-house data.

6. What are the recommendations for borderline abnormal clones? Should these clones be tossed or further testing is recommended and what what test(s)?

7. It is listed in the CNV table that CNV<=2.15 is considered normal with gDNA as input. However, the parental PGP-1 line used in the Notch3 gene editing example has a CNV value of 2.23. Why was this cell line considered to have a normal CNV?

8. The normal CNV cutoff for lysate samples is higher presumably due to the impurities present in the samples. However, have the authors assessed the effect of different lysis buffer compositions on normal CNV cutoffs?

Reviewer 2

Has selected to remain anonymous.

Originality, novelty and significance of results:	Good
Technical Quality of Work:	Good
Comprehensibility and Presentation of Paper:	Excellent
What is the overall impression:	Excellent

Reviewer Recommendation Term: Accept

Narrative (as sent to corresponding author):
This is a very useful protocol for rapid testing of genomic stability in chromosome 20q which can have a profound impact on the functionality of pluripotent stem cell lines (PSCs). It is both timely and comprehensive. It is logically presented with detailed instructions to carry out testing and to analyse the results. It will enable groups to check their cell lines prior to starting experimental workflows and provide valuable information for both decision making and downstream functional assay analysis. The testing is applicable to both maintenance of PSCs and to gene editing. The troubleshooting section is thorough and provides solutions to problems that might arise when applying this technology to samples and data analysis. All in all a great protocol paper to enable this testing to be added to QC workflows.

Author's reply to the reviews:

Resubmitted with updated paragraphs highlighted.

Reviewers' response to the revision:

Reviewer 1

Originality, novelty and significance of results:	Excellent
Technical Quality of Work:	Excellent
Comprehensibility and Presentation of Paper:	Excellent
What is the overall impression:	Excellent

Narrative (as sent to corresponding author):
Accept

THE EDITOR DECIDED TO ACCEPT THE PAPER