**Reviewer Report**

## Title: Clinker: visualising fusion genes detected in RNA-seq data

**Version:** **Revision 1** **Date:** 5/24/2018

**Reviewer name:** **Andreas Hoff**

**Reviewer Comments to Author:**

By revising the manuscript, the authors have slightly improved the overall description of the useful fusion visualization tool; Clinker. Most of the points I raised have been satisfactory answered and accounted for. The paper provides an excellent description of the Clinker software and demonstrates its application with an example of the P2RY8-CRLF2 fusion and detection of multiple fusion transcript isoforms in B-ALL. However, I am still missing some supporting data for the functional experiments and conclusions.In response to my previous comment #2, the authors added Sanger sequencing data confirming the presence of three alternative breakpoints of P2RY8-CRLF2 in patient 6. However, I also asked about Sanger sequencing data confirming the cloned vectors containing canonical, alternate and frameshift PCR products before being transduced into BaF3 cells. The authors should have this data, as they in the methods write: "PCR products were cloned into P-GEM-T easy vector (Promega), Sanger sequenced and then subcloned into a retroviral pMSCV-GFP retroviral expression vector". This information would further support the flow cytometry data and provide credibility that we are actually seeing overexpression of CRLF2 as a result of the expression of the canonical and alternate fusion transcripts. Especially since the alternate and frameshift fusions were cloned using the same primers. The data can easily be added as a subpanel to supplementary figure 4.In addition, I am wondering if the authors know what the longer band (~620bp) and RT-PCR product is in supplementary figure S3 and if this is backed up by RNA-seq data and visualization by Clinker?Some minor comments:- Please make sure you refer to each supplementary item in the text. I cannot see a reference to supplementary figure 1. Also the added supplementary figure 3 should be referred to on page 9, line 10.- The reference to supplementary figure 3 on line 29/30 should be changed to supplementary figure 4.- In the figure legend for figure 3 you refer to supplementary tables 1 and 2. However, I can only see one supplementary table.- In the caption of figure S3 your write "DNA gel..". The gel is showing RT-PCR products confirming the presence of fusion transcripts at the RNA level.

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