October 18, 2018

Vineeta Bajaj, Ph.D.

Review Editor

*JoVE*

1 Alewife Center Suite 200

Cambridge MA 02140

Re: Invited Manuscript JoVE58933.R3

Dear Dr. Bajaj:

Please find attached a third round of revisions to our manuscript, which we have re-titled “**Preparation and Gene-Modification of Nonhuman Primate Hematopoietic Stem and Progenitor Cells**," as you requested. Our point-by-point responses to the editorial comments inserted within the document have been included below. With these further modifications, we hope that this manuscript will be suitable for publication in JoVE.

Sincerely,

Christopher W. Peterson

Staff Scientist, Stem Cell and Gene Therapy Program

Fred Hutchinson Cancer Research Center

Research Assistant Professor, Department of Medicine, Division of Medical Oncology

University of Washington School of Medicine

**Editorial comments:**  
Table 2 can be combined with the materials table. if interested in retaining, please provide xxxx et al. instead of the reference number.

**Response:** Table 2 has been merged with the Table of Materials. Tables 3-4 have been adjusted as Tables 2-3 throughout the manuscript.

Line 2: Need a better word here

**Response:** We have modified the title as requested.

Line 123: Some of the subsection headings can be removed. This will help save the lines and will also reduce the protocol length.

**Response:** We have deleted individual headers to shorten the protocol.

Line 199 and 202: Reworded to reduce the length of the step.

**Response:** Thx. Reads well.

Line 265: Since this is highlighted we need a linking sentence from BM lysis to CD34 enrichment. So maybe some parts of section 1.3

**Response:** We did highlight the section header in line 205 (step 1.3) as a linking sentence in between the hemolysis and the CD34 plating.

Line 296: Please use degree symbol for this

**Response:** The degree symbol has been added.

Line 304: Since this is just a preparation of the flow cytometer, do you want to film this part? Are the settings novel for your protocol or is generally the norm? If it is general setting this can just stay in the protocol as is since it is important for the protocol but no need to film.

**Response:** That is a valid point. The settings are not really unique and among the users well established. What is more important is how the assessment and gating look. We have reassessed the highlighted section and included more relevant parts for filming.

Line 305: This is redundant and can be removed:

**Response:** The language in all substeps of 2.2 has been cleaned up and redundant phrases removed.

Line 308: This is redundant as it is already described in 2.2.1. So, you are basically providing all the button clicks and making the steps crisper as well.

**Response:** Redundant language has been removed in steps 2.2.2-2.2.5 according to instructions.

Line 328: Converted to imperative tense. Please check. Also, please avoid the usage of words like could, should etc.

**Response:** The tense has been changed.

Line 338 and 361: This is not an action step and hence converted to a note. Converted to a note since not in imperative tense and not an action step.

**Response:** Thank you for converting it.

Line 372: Sorting for CFC assays is only performed with cells from the CD34-enriched product (**Table 3**, Sample 10).

**Response:** This is a technical note and not in conflict with the referenced note. Sorting will only be performed from Sample 10. Within this sample, we identify subsets of cells which are sorted individually.

Line 376: Incubate cells for how long, what conditions etc.

**Response:** Information has been added to step 2.4.3.

Line 377: This is redundant.

**Response:** Redundant language has been removed.

Line 393: Does this contain virus as well?

**Response:** No, this is the coating. Reference to step 3. 1 has been added to prevent confusion.

Line 405: Removed substep 3.4 and combined together – to reduce the highlighted sentences to fit 2.75 pages.

**Response:** Thank you.

Line 420: From the flask coated with CH296

**Response:** This has been clarified above. The virus-conditioned medium is produced upfront and frozen at -80°C. We changed the language to prevent confusion.

Line 421: Which flask is the infection performed?

**Response:** References have been added to prevent confusion.

Line 433 and 494: Check the step number.

**Response:** Incorrect references have been changed.

Line 466: If this is highlighted, step 4.4.2 needs highlighting as well to bring out continuity.

**Response:** We have reassessed the highlighted section as requested.

Line 523: Imperative tense.

**Response:** Tense was changed.