

Department of Neurobiology

SCHOOL OF MEDICINE

412 Bryan Research building

Dec 28th, 2018

Bing Wu, Ph.D.  
Review Editor  
JoVE

**Re:** Manuscript number: JoVE59241R2

     Corresponding authors: Kantor Boris and Chiba-Falek Ornit  
    Title: “Lentiviral vector platform for efficient delivery of epigenome-editing tools into hiPSC-derived disease models”

Dear Dr. Wu:

Thank you for your interest in our manuscript and for the opportunity to resubmit a revised manuscript for further consideration. We found the editorial comments on our manuscript very helpful and would like to respond as follows:

**Editorial comments:**  
*1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*

**Our reply:** We thank the reviewer for this comment. We carefully proofread the manuscript and ensured that no spelling or grammar issues were present.

*2. JoVE cannot publish manuscripts containing commercial language. This includes company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. Examples of commercial language in your manuscript include Addgene, etc.*

**Our reply:** We thank the reviewer for this comment. In the revised manuscript, we excluded all commercial language and we reference all the products in the Table of Material and Reagents.

*3. Unfortunately, there are a few sections of the manuscript that show significant overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please check the iThenticateReport attached to this email.*

**Our reply:** We thank the reviewer for this comment. We carefully checked the iThenticate Report attached to the email, and sections that showed significant overlap with previously published work have been rewritten using original language.

*4. Step 1.1.1: Please write this step in the imperative tense.*

**Our reply:** We thank the reviewer for this comment. In the revised manuscript, in the **Protocol** section, page 4, lines 193-196, this step has been written in the imperative tense, and read as follows: “Obtain the deactivate (dead) version of SpCas9 (dCas9) via site-directed mutagenesis (data not shown). Replace the clone harboring D10A and H840A mutations in HNH and RuvC catalytic domains of the enzyme with the active Cas9 in pBK30129 by exchanging between AgeI-BamHI fragments, respectively (**Fig. 3**).”

*5. 1.1.1-1.1.3: Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?*

* + 1. **Our reply:** We thank the reviewer for this comment. In the revised manuscript, in the **Protocol** section, page 4, lines 198-213, we included more details to these steps. The revised manuscript read as follows: “1.1.2. Derive DNMT3A catalytic domain from *p*dCas9-DNMT3A-eGFP (See Table of Materials) by amplifying DNMT3A portion BamHI-429/R 5’-GAGCGGATCCCCCTCCCG-3’ BamHI-429/L 5’-CTCTCCACTGCCGGATCCGG-3’ (**Fig. 3**). To amplify the region containing DNMT3A use the following conditions: (1) 95 °C for 60 s, (2) 95 °C for 10 s, (3) 60 °C for 20 s, (4) 68 °C for 60 s. Repeat steps 2-to-4 30 times. Final extension: 68 °C for 3 min, hold 4 °C. 1.1.3. Clone DNMT3A-fragment, digested by BamHI restriction enzyme, into BamHI site of the modified pBK301 vector carrying dCas9. Verify cloning by direct Sanger sequencing. The resulted plasmid harbors dCas9-DNMT3A-p2a-puromycin transgene. The plasmid expresses gRNA scaffold from human U6 promoter (**Fig. 3**).1.1.3 Replace puromycin reporter gene with the GFP to create dCas9-DNMT3A-p2a-GFP. Digest dCas9-DNMT3A-p2a-PURO plasmid with FseI. Purify the vector fragment using gel-purification method. Prepare the insert by digesting pBK201a (plenti-GFP) with FseI. Clone FseI fragment into the vector. The resulted plasmid pBK539 harbors dCas9-DNMT3A-p2a-GFP transgene (**Fig. 3**).”

*6. 1.3.3: Please write this step in the imperative tense.*

**Our reply:** We thank the reviewer for this comment. In the revised manuscript, this step has been included in the step 1.3.2. In the **Protocol** section, page 5, lines 241-248, text read as follows: “To prepare the plasmid mix use the four plasmids as listed (the following mix is sufficient for one 15 cm plate: 37.5 µg of the CRISPR/dCas9-transfer vector (pBK492 (DNMT3A-PURO-NO-gRNA or pBK539, DNMT3A-GFP-NO-gRNA; 25 µg of pBK240 (psPAX2); 12.5 µg pMD2.G; 6.25 µg of pRSV-rev (**Fig. 4A**). Calculate volume of the plasmids based on the concentrations and add the required quantities into 15-ml conical tube. Add 312.5 µL 1 M CaCl2 and bring up to 1.25 mL final volume using sterile dd-H2O. Gently add 1.25 mL of 2x BBS solution while vortexing the mix. Incubate for 30 min at room temperature. Cells are ready for transfection once they are 70 - 80% confluent.”

*7. 1.5.16: Please write this step in the imperative tense.*

**Our reply:** We thank the reviewer for this comment. In the revised manuscript, text has been revised. In the **Protocol** section, page 7, lines 313-316, read as follows: “Wash the tubes with additional 50 µL 1x PBS and mix as before. At this step, the volume of the final suspension is ~120 µL and appears slightly milky. To obtain a clear suspension, proceed with a 60 s centrifugation at 10,000 x g. Transfer the supernatant to a new tube, make 5 µL aliquots, and store them at -80 °C.”

*8. 2.1.1: Please write this step in the imperative tense.*

**Our reply:** We thank the reviewer for this comment. In the revised manuscript, this step has been rewritten in the imperative tense. In the **Protocol** section, page 9, lines 417-420, text read as follows: “Culture hiPSCs under feeder-independent condition in feeder-free ESC-iPSC culture medium (See Table of Materials) onto hESC-qualified basic matrix membrane (BMM)-coated plates (See Table of Materials). Wash confluent colonies with 1 mL DMEM-F12, add 1 mL of dissociation reagent (see Table of Materials), and incubate for 3 min at room temperature.”

*9. 2.2.29: Please write this step in the imperative tense.*

**Our reply:** We thank the reviewer for this comment. In the revised manuscript, this step has been rewritten in the imperative tense. In the **Protocol** section, page 11, lines 524-526, text read as follows: “**d 13 – d 17**: Perform a daily medium change using completed N2B27 medium. Cells are ready for passaging when cultures are 80-90% confluency.”

*10. 2.2.36: Please write this step in the imperative tense.*

**Our reply:** We thank the reviewer for this comment. In the revised manuscript, this step has been rewritten in the imperative tense. In the **Protocol** section, page 12, lines 552, text read as follows: “Passage cells once they reach 80-90% confluency.

*11. After you make all changes, please highlight fewer than 2.75 pages of protocol steps (including spacing) in yellow for filming.*

**Our reply:** In the revised manuscript, 2.75 pages of protocol steps (including spacing) have been highlighted in yellow for filming.

We hope that our revised manuscript thoroughly and satisfactorily addresses all of the concerns raised by the reviewers. We hope that your editorial team will find it now acceptable for *JoVE*.

Sincerely,

*B.KANTOR*

Boris Kantor, Ph.D.

Assistant Professor of Neurobiology

Director, Duke Viral Vector Core Facility

Duke University Medical Center

DUKE UNIVERSITY SCHOOL OF MEDICINE DURHAM, NC 27710  PHONE 919- 681-1068 Fax 919-684-0044

An Affirmative Action / Equal Opportunity Institution