Editorial comments:

1. Please keep manuscript formatted per JoVE guidelines (see attached manuscript)–letter (8.5" x 11") paper size, 1-inch margins, 12 pt Calibri font throughout, all text aligned to the left margin, single spacing within paragraphs, and spaces between all paragraphs and protocol steps/substeps.

> Response: To the best of our knowledge, this draft now follows the JoVE formatting guidelines.

2. Protocol: This may be a general procedure, but we need specific examples for ease of scripting/filming and for readers to follow along with the protocol. Please include specific DNA sequences, mice strains, etc., that you used for, e.g., Dnmt1.

> Response: We have now included a supplementary data file that contains an annotated murine *Dnmt1* sequence as an example of steps 1.1-1.3 and 1.5.1. It highlights regions of intron 1 that should be avoided for insertion of the landing pad based on bioinformatic analysis of the region. Further, it shows a possible sgRNA to use for landing pad insertion, as well as PCR primers for assessing insertion. Additionally, we have added Supplementary Figure 1 to aid visualization of steps 1.3 and 1.5.1, as described further in our response to item 3 below. Regarding the mouse strain, we have added our recommendation of using embryos from B6C3F1/J mice within the protocol (steps 1.4 and 2.4), as well as listed this strain in the Table of Materials.

3. 1.3/1.5.1: Note that these steps cannot be filmed without more details.

> Response: We have added Supplementary Figures 1A-B to demonstrate step 1.3. Supplementary Figure 1B shows a specific example of a DNA template designed for the *Dnmt1* gene. We have removed step 1.5.1 from the film, as detailed PCR primer design is beyond the scope of this protocol. However, we have added Supplementary Figure 1C to demonstrate primer design for the *Dnmt1* example.

4. 1.4/1.6/2.4: Please provide a reference for materials prep and microinjection.

> Response: Thank you for pointing this out. We have added references to steps 1.4/2.4[1,2] and 1.6[3-5]. These references include detailed instructions on how to prepare materials for and conduct microinjections.

5. Please remove 'Figure 1'/'Figure 2'/etc. from the Figures themselves.

> Response: The figure titles have been removed from the figures themselves.

Bibliography

1 Harms, D. W. *et al.* Mouse Genome Editing Using the CRISPR/Cas System. *Curr Protoc Hum Genet.* **83** 15.17.11-27, (2014).

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3 Ohtsuka, M. *et al.* Improvement of pronuclear injection-based targeted transgenesis (PITT) by iCre mRNA-mediated site-specific recombination. *Transgenic Res.* **22** (4), 873-875, (2013).

4 Cho, A., Haruyama, N. & Kulkarni, A. B. Generation of Transgenic Mice. *Curr Protoc Cell Biol.* **Chapter** Unit-19.11, (2009).

5 Pu, X. A., Young, A. P. & Kubisch, H. M. Production of Transgenic Mice by Pronuclear Microinjection. *Methods Mol Biol.* **1874** 17-41, (2019).