**Response to Editorial and Reviewers’ comments**

The authors would like to thank the Reviewers for his/her comments to their work and they revised the manuscript JoVE52885R1 “Generation of Induced Pluripotent Stem Cells from Frozen Buffy Coats using non-integrating Episomal Plasmids” according to Editorial and Reviewers’ comments. The authors uploaded the revised manuscript with modifications highlighted in red and in “track changes” function.

The text of the manuscript was updated with new comments as indicated below.

**Editorial comments**

**Question 1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.**

**Answer 1.** The authors carefully proofread the manuscript and the errors/inaccurancies of the main text have been corrected.  
  
**Question 2.**

**2.2.2 - How long are cells incubated? Overnight?**

**3.1 - How are the cells collected?**

**3.3 - Incubate the cells in what? A new 12-well plate?**

**Answer 2.** The authors added the additional details required in the following steps: 2.2.2, 3.1 and 3.3.

**Question 3. JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.**

**Answer 3.** The authors added DOIs in the reference list, when DOIs are available.

**Reviewer 1#**

**Question 1.1. Freezing medium (final) composition in sections 1.1.4 and 1.2.9 is different. Please revise and adjust if needed.**

**Answer 1.1.** The authors thank the reviewer for having noticed this mistake and corrected the error in step 1.1.4.  
  
**Question 1.2. Please specify cell count or dilution for freezing in steps 1.2.9-10.**

**Answer 1.2.** The authors revised the text in step 1.2.9 in order to clarify the cell dilution in the freezing medium.

**Question 1.3. Describe if plates are coated prior to cell plating (they might not be) in step 2.1.6**

**Answer 1.3.** The authors clarified that plates are not coated, as suggested by the reviewer.

**Question 1.4. Section 3: Before going into further detail, please describe detachment protocol applied prior to cell collection (also applies to 6.1). Also include plating density or splitting ratio.**

**Answer 1.4.** The authors added more details in section 3 (and step 6.1) in order to address the comment of the reviewer. Specifically, the authors clarified that PBMNCs grow in suspension culture and no detachment protocol was applied, but PBMNCs were collected using a pipette with 1-ml tip.

**Question 1.5. Section 4: It is unclear if you maintain/split cells for a number of days (fixed) or until you reach a critical number of cells. Explain in the text.**

**Answer 1.5.** The authors provided details in the text in Section 4, in order to better explain those steps.

**Question 1.6. Extended explanation on manual colony passaging might be required in step 7.3.**

**Answer 1.6.** The authors explained more in detail the manual colony passaging in step 7.2-7.3.

**Question 1.7.** **Splitting ratio is not specified in step 7.7. Please add**

**Answer 1.7.** The authors added the splitting ration in step 7.7, as indicated by the reviewer.

**Question 1.8.** **Consider adding details on feeder free culture of iPSCs in step 8.6.1.**

**Answer 1.8.** The authors indicated the catalog number of the commercial medium for iPSC feeder-free culture in the Table of Materials and Reagents and the manufacturer reported in details all the procedure that we followed to culture feeder-free iPSC.

**Reviewer 2#**

**Question 2.1. Peripheral blood mononuclear cells (PBMCs) consist of chiefly of lymphocytes and monocytes. These cells, usually considered primary and end cells, in absence of specific mitogens don't proliferate. The medium used for PBMCs amplification should be quoted or, if directly designed by authors, justified for its composition.**

**Answer 2.1.** As stated by the reviewer, PBMNCs are not able to proliferate without specific stimuli. For this reason, the authors used a defined medium previously described in literature to promote and sustain the proliferation of PBMNCs, as indicated in the reference list.

**Reviewer 3#**

**Question 3.1. Please cite Haase et al. Cell Stem Cell. 2009 Oct 2;5(4):434-41. First paper describing cord blood-derived hiPSC induction.**

**Answer 3.1.** The authors thank the reviewer for having noticed this missing reference and added this work in the reference list.

**Question 3.2. Provide some sentences on the characteristics of the episomal plasmids used in this study already in the introduction.**

**Answer 3.2.** Episomal plasmids used in this work are commercial available (Addgene) and the catalog number for each plasmid is reported in the Table of Materials and Reagents. The manufacturer reported in details all the information and characteristics of the episomal plasmids. Specifically, these plasmids were described for the first time in the following paper by Yamanaka’s group as cited in the reference list (Reference 10: Okita, K. et al. A more efficient method to generate integration-free human iPS cells. Nat. Methods. 8 (5), 409-412, doi: 10.1038/nmeth.1591 (2011)). Also, these plasmids were already used to reprogram skin fibroblasts in another work, quoted in the reference list (Reference 15: Kim, C. *et al*. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature.* **494** (7435), 105-110, doi: 10.1038/nature11799 (2013)). Of note, the introduction (133-134) has been modified in order to better clarify that the plasmids used in this work have been already used in previous publications and additional episomal plasmid details are added in the method section (step 4.3).

**Question 3.3. Provide a brief method sections on:**

**3.3.1 Plasmid preparation, isolation, quality assessment;**

**3.3.2 Method to test transfection efficiency via reporter gene constructs;**

**3.3.3. Method to assess cell viability after electroporation;**

**3.3.4. Assay to test and proof loss of the episomal plasmids in the generated hiPSC colonies / clones;**

**3.3.5. Method on how to measures / define reprogramming efficiency and provide expected efficiency data on fresh vs. frozen PMBMCS samples. This is important but currently only briefly mentioned in the discussion**

**Answer 3.3.** Additional information are provided in order to address the following points highlighted by the reviewer:

**3.3.1** Purification and isolation of the four plasmids was performed using the plasmid purification kit (Qiagen) listed in the Table of Materials and Reagents. The manufacturer reported in details all the procedure followed by the authors to isolate and check the quality of the purified plasmids used in this work. Specifically, the authors added this comments in the text at the beginning of Section 4 (lines 280-283).

**3.3.2.** To test the transfection efficiency, the authors added the step 4.8**.**

**3.3.3.** To assess cell viability after electroporation, cells were counted before and after electroporation protocol and cell viability was evaluated based on trypan blue exclusion (see in the text the step 4.6).

**3.3.4.** The authors would thank the reviewer for giving the chance to clarify this point. Indeed, the authors splitted in two different section qRT-PCR for gene expression analysis and qPCR for transgene exclusion, in order to better explain how to test the loss of episomal plamsids (see Sections 8.4-8.5).

**3.3.5.** The authors would thank the reviewer for giving the possibility to clarify this point and also to give the chance to revise and correct a mistake that the authors missed in the previous submission, regarding the reprogramming efficiency (see the correction in the text at line 674). Specifically, the authors measured the reprogramming efficiency as the percentage of average iPSC colony formation, calculated as number of iPSC colonies/total number of electroporated cells as stated in Section 7 (lines 346-347) (Hasegawa K, Zhang P, Wei Z, Pomeroy JE, Lu W, Pera MF. Comparison of reprogramming efficiency between transduction of reprogramming factors, cell-cell fusion, and cytoplast fusion. [Stem Cells.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Comparison+of+Reprogramming+Efficiency+Between+Transduction+of+Reprogramming+Factors%2C+Cell%E2%80%93Cell+Fusion%2C+and+Cytoplast+Fusion) 2010 Aug;28(8):1338-48. doi: 10.1002/stem.466). Of note, the reprogramming efficiency obtained in the present work is comparable to those reported in previously published works (Reference 14: Chou, B. K. et al. Efficient human iPS cell derivation by a non-integrating plasmid from blood cells with unique epigenetic and gene expression signatures. Cell Res. 21 (3), 518-529, doi: 10.1038/cr.2011.12 (2011) and reference 23: Staerk, J. et al. Reprogramming of human peripheral blood cells to induced pluripotent stem cells. Cell. Stem Cell. 7 (1), 20-24, doi: 10.1016/j.stem.2010.06.002 (2010)). In the present work the reprogramming efficiency did not differ from the two frozen starting materials (see the text at line 674-675), but the authors cannot provide efficiency data on fresh blood PBMNCs samples, because only frozen PBMNCs were used in this study.

Also, the authors would thank the reviewer for his/her minor comments and changes in the text have been addressed, according reviewer’s suggestion.