**Editorial comments:**  
  
**• Your manuscript has been modified by your editor, please maintain the current formatting throughout the manuscript. Please use the updated manuscript located in your Editorial Manager account (under “File Inventory”) for all subsequent revisions.**

Thank you for modifying the format, I have kept it consistent with this latest revision.

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

The manuscript has been thoroughly proofread to ensure no spelling or grammatical errors.

**• 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.**

I have gone through to ensure that the DOI is listed for each reference. I have found the DOI number for “Lewis, I. D. et al. Umbilical cord blood cells capable of engrafting in primary, secondary, and tertiary xenogeneic hosts are preserved after ex vivo culture in a noncontact system. Blood. 97 (11), 3441-3449 (2001)” and have manually included it in the references. I am unfortunately unable to find the DOI number for “Vaskova, E. A., Stekleneva, A. E., Medvedev, S. P. & Zakian, S. M. "Epigenetic memory" phenomenon in induced pluripotent stem cells. Acta Naturae. 5 (4), 15-21 (2013).”

**• Please disregard this comment if all of your figures are original. If you are re-using figures from a previous publication, please obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."**

All figures are original.

**• 3.2: Please mention the cell culture medium used.**

**In step 3.2 I have included the culture media used for MEFs.**

**Reviewers' comments:**  
  
**Reviewer #1:**  
  
*Manuscript Summary:*  
  
**The manuscript by Daniel et al. describes a method for viral reprogramming of mouse embryonic fibroblasts using four transcription factors, Gata2, Gfi1b, cFos, and Etv6. Introduction of these 4 factors bypasses the pluripotent intermediate to specify cells with hematopoietic potential. The text is well-written, easy to follow, and includes specifics necessary for a researcher with moderate technical knowledge to conduct the procedures independently. A negative control for placental aggregation is suggested. Potential applications are outlined and expected results are fairly represented. References cited are appropriate. Overall, this will be a great resource to have available on JoVE.**

*Major Concerns:*  
There are no major concerns.

*Minor Concerns:*  
**I offer only minor suggestions, which may or may not be useful for eventual video production.**  
**-4.18, Line 361) Can authors comment on why the colonies need 3-4 weeks in the methylcellulose CFU assay? Typical CFU are counted from other murine sources 10-14 days after initial plating. In fact, the figure 3 legend also references use of 10-14 days.**

I apologize, you are correct. I have edited the protocol to use the correct timing of 10 – 14 days.

**-Line 398. Typo: hematopoetic**

Thank you, I have fixed this to the correct spelling.

**-Line 450. Typo: epigentically**

Thank you, I have fixed this to the correct spelling.

**-Fig 1. At day 4, the MEFs are transferred to a large plate on filters in aggregate. Perhaps a cartoon of a floating filter on a large plate or use of the word "aggregate" would better represent the stepwise written protocol.**

You are correct; this could be made clearer in the figure. To maintain the simplicity of the figure but still reflect that information, I have included “(placental aggregation protocol)” at the CFU assay analyses point to make clear that this assay is done through the placental aggregation protocol.

*Additional Comments to Authors:*  
N/A  
  
**Reviewer #2:**  
  
*Manuscript Summary:*  
  
Outlines the procedures to demonstrate direct conversion from MEF to blood progenitors.  
  
*Major Concerns:*  
  
None.  
  
*Minor Concerns:*  
  
none  
  
*Additional Comments to Authors:*  
  
Excellent paper.  
  
**Reviewer #3:**  
  
*Manuscript Summary:*  
  
The manuscript reported a protocol to induce hemogenic program in mouse embryonic fibroblasts (MEFs) via doxycycline-based overexpression of transcription factors (TFs). And they showed that, based on FACS, about 12.7% of reprogramming MEFs after 35 days were GFP+ and CD45+ which is a pan-hematopoietic marker, indicating high efficiency of this reprogramming technology. While it is very interesting, however, there are some questions for this manuscript.  
  
*Major Concerns:*  
  
**I know this should be the detailed protocol from their Cell Stem Cell paper (2013 August 1). In this abstract, the authors clearly said that "Starting with 18 candidate TFs, and through a process of combinatorial elimination, we obtained a minimal set of factors that would induce the highest percentage of GFP+ cells. We found that Gata2, Gfi1b, and cFos were necessary and the addition of Etv6 provided the optimal induction." However, the protocol never mentioned how did they narrow down the TFs from 18 to 4, this should be the most important part for this protocol, which this details, it will increase much more impact for this protocol, because it will allow other labs to use this technology to screen other factors.**

Thank you for your comment. The process of combinatorial elimination of TFs was done through literature review and subsequent deletion of TFs one by one similar to the approach taken by Yamanaka for finding the pluripotency factors (Takahashi and Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell. 2006 Aug 25;126(4):663-76. Epub 2006 Aug 10.) until the optimal yield of GFP+ colony numbers was identified. The GFP+ colonies are based on a double transgenic system that we used for the screening. Our system involves using a human CD34rtTA mouse crossed with a TetO-H2BGFP mouse (DOX off). We isolated MEFs from these mice, removed residual GFP+ and CD45+ cells by flow and then transduced them with the TF combinations. When the CD34 gene is turned on the GFP fusion protein is expressed. We have included this in the introduction to make it clear how the final set of TFs was determined. The DOX inducible lentiviruses we used for the final reprogramming were incompatible with the double transgenic MEFs. For the JoVE protocol we focused on the reprogramming strategy necessary to replicate our findings with the finalized set of TFs rather than on how the final set was reached, as the 2013 Cell Stem Cell paper covered this in detail.

**The authors did have a strategy for hemogenic induction in MEFs in figure 1, but it would be much better if they could also show the protocol steps in this figure (1.1-4.19).**

Thank you for your comment. In the effort of simplicity we chose to display the generalized reprogramming strategy using TF-inducible vectors, which can use MEFs from any source, not necessarily through our protocol of isolating MEFs from our double transgenic mouse. Including the generation of virus and the placental aggregation would complicate the figure, and warrant multiple additional panels to visualize these techniques. We selected to have the simple reprogramming strategy so that others who want to follow this process can see the generate plate layout.

**The authors mentioned that the reprogramming efficiency is 12.7%, however, Figure 2A/2B suggested a much higher efficiency (based on double positive of GFP and CD45, may be higher than 50%?), unless not a representative image.**

You are correct, the images from figures 2A/2B demonstrate an area of a GFP+ cluster, but they are not indicative of the entire field of transduced cells in the plate. Figure 3, demonstrating a yield of 12.7% GFP+CD45+ cells includes the entire population of cells in the dish, which is not represented by the images in Figure 2.

*Minor Concerns:*  
  
**At page 4, 1.11) Cut the remaining tissue into small pieces using forceps and transfer to a 50 ml conical tube containing 10 ml of trypsin. "forceps" should be "scissors and forceps".**

You are correct, I have fixed this error.

*Additional Comments to Authors:*  
  
N/A  
  
**[Editorial recommendation**: Please keep JoVE’s protocol requirements in mind as you address the above comment - the protocol must contain sufficient details in order to enable users to accurately replicate your technique. We recommend NOT removing any details from the protocol text.]

I would like to thank the editor and the reviewers again for their comments and edits to the manuscript. I hope I have addressed each comment sufficiently. If any concerns remain please let me know and I will address them as soon as possible.