

## Answering reviewers

The manuscript titled, “The preparation and identification of Gpm6a/ReelinGFPCreERT2 construct,” (manuscript # 28961) by Shi H. et al. describes how two constructs were cloned using BAC with the use of restriction enzymes and homologous recombination in bacteria. Although the significance of generating these constructs is great, the presentation of the cloning work was rudimentary. Figures were centered on basic cloning/screening gel data which, for most researchers, would not be presented for publication. It would be much better to focus on the pros and cons of the authors’ approach in generating these constructs and showed with data that their methods are superior. Specific comments are in the following:

1. Should elaborate on description about hepatic mesothelial cells and hepatic stellate cells in terms of why it is important to generate mouse lines to trace these cells.

**Answer:** Excessive extracellular matrix (ECM) of the liver results in cirrhosis, the end stage liver disease of high mortality for which efficacious medical treatments are not currently available except for liver transplantation. Hepatic stellate cells (HSCs) activation is considered a major mechanism in the formation of fibrosis and cirrhosis. However fundamental questions concerning cell fate regulation of HSCs remain largely underexplored. Hepatic mesothelial cells are the potential precursors for HSCs in the development of liver and can transdifferentiate into myofibroblast cells in mouse liver fibrosis. So it is important to generate mouse lines to trace hepatic mesothelial cells and HSCs.

2. Is it a common practice to use Gpm6a and Reelin as markers? What are other markers people have used? Why are Gpm6a and Reelin better?

**Answer:** In the normal adult liver, quiescent HSCs are characterized by desmin expressing, lipid drops storage, and extensive dendrite-like processes. Upon injury, HSCs are activated, lose lipid drops and transform into a myofibroblastic phenotype expressing smooth muscle actin (SMA) and excessive extracellular matrix proteins (ECM). Desmin, SMA and ECM proteins are the common markers of HSCs, but it is not specific. The  $Wt1^{CreERT2}$  mice are useful to trace hepatic mesothelial cells, but the labeling efficiency and specificity is low. Specific genes have been identified in hepatic mesothelial cells and HSCs by microarray. Glycoprotein M6a (Gpm6a) has been identified as a specific surface marker of hepatic mesothelial cell. Reelin is extracellular matrix glycoprotein, which is a specific HSCs marker and has similar amounts in resting and activated HSCs.

3. What is the common practice in generating these Gpm6a and Reelin constructs?

**Answer:** The common practice in generating Gpm6a/Reelin<sup>GFPCreERT2</sup> construct is to insert GFPCreERT2 fragment into a vector containing Gpm6a/Reelin promoter, which is conventional restriction enzyme/ligation cloning of recombinant DNA. For

conventional vector, its advantage is easy to handle, but it has low expression of the reporter genes and low specificity because DNA size is about 3-5kb containing only promoter. For bacterial artificial chromosome (BAC) vector, its advantages include relatively high efficiency and relatively high specificity, but BAC DNA is fragile and can break easily because BAC DNA is about 200kb containing all regulatory sequences upstream and downstream of the encoding sequence. We choose BAC vector to prepare the construct of Gpm6a<sup>GFPCreERT2</sup> and Reelin<sup>GFPCreERT2</sup>.

**4.** Typo: should be Table I rather than Figure 1.

**Answer:** Yes, I have substituted Table 1 for Figure 1 in the manuscript by red marker.

**5.** Fig 4 and 5 are not referenced in the text.

**Answer:** Yes, I have referenced Fig 4 and 5 in the text by red maker.

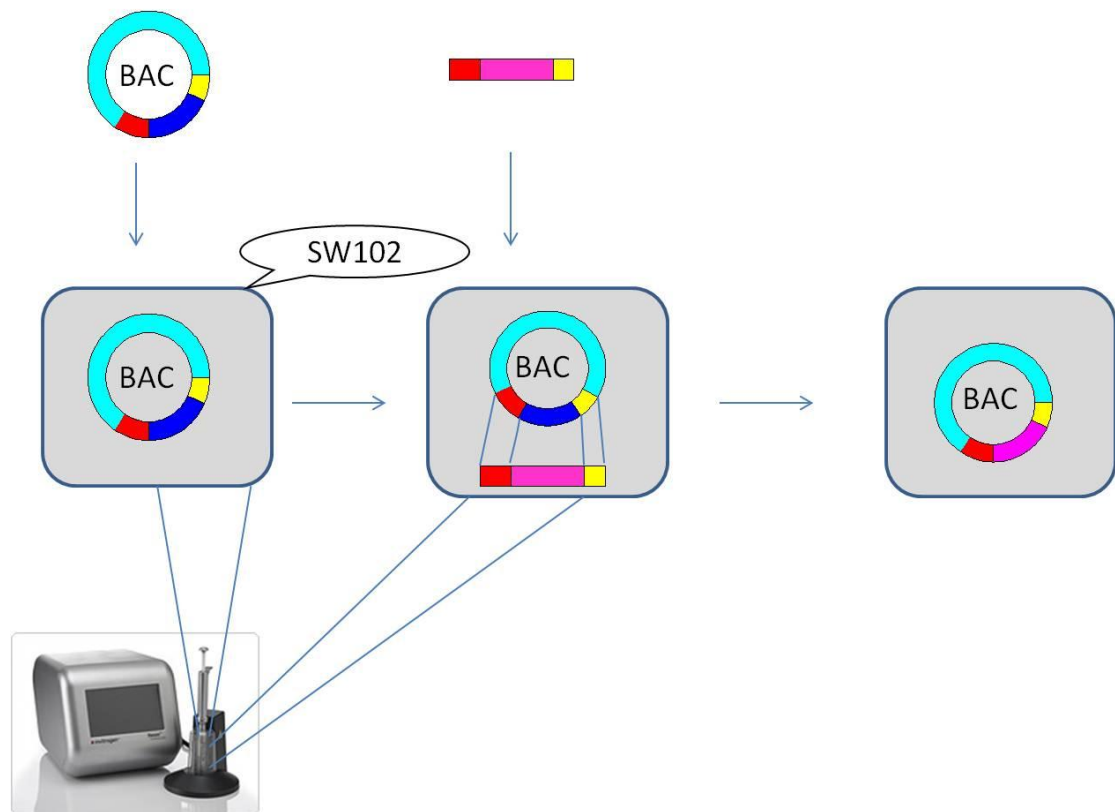
**6.** How does this cloning approach different from Gibson assembly? Need to compare and contrast.

**Answer:**

	Gibson assembly	bacterial artificial chromosome (BAC) cloning
Vector	conventional vector	bacterial artificial chromosome (BAC) vector
Vector shape	linear	circle and close
Vector size	3-5kb	200kb
Vector sequence	only promoter	all regulatory sequences upstream and downstream of the encoding sequence
Vector	Easy to handle	fragile and easy to break
Gene expression efficiency	low	relatively high
Gene expression specificity	low	relatively high
DNA assembly method	homologous recombination	homologous recombination
Restriction digestion	no	no
DNA inserts	more than 10	only one

**7.** Need a flowchart to illustrate cloning strategy.

**Answer:**



8. A. and B. are missing in Fig. 1. Or can use “left” and “right”.  
**Answer:** I inserted “A” and “B” in Figure.1.