Basic Study

Construction of Gpm6a/ReelinGFPCreERT2 by BAC recombination using a specific gene in hepatic mesothelial or stellate cells

Hong-Bo Shi, Jin-Li Lou, Hong-Lin Shi, Feng Ren, Yu Chen, Zhong-Ping Duan

Hong-Bo Shi, Hong-Lin Shi, Feng Ren, Zhong-Ping Duan, Beijing Institute of Hepatology, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

Jin-Li Lou, Clinical Laboratory Center, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

Yu Chen, Zhong-Ping Duan, Artificial Liver Center, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

Author contributions: Shi HB and Lou JL contributed equally to this work; Shi HB and Shi HL carried out most of the experiments; Ren F purified the strain and performed PCR; Chen Y analyzed the pattern of restriction enzyme digestion; Shi HB drafted the manuscript, and Lou JL analyzed the experimental data; Duan ZP and Lou JL conceived and supervised the study; Duan ZP was involved in editing the manuscript; all authors read and approved the final manuscript.

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Abstract

AIM

To prepare a Gpm6a/ReelinGFPCreERT2 construct with a rapid and reliable strategy using a bacterial artificial chromosome (BAC).

METHODS

Gpm6a and Reelin BACs were purified and transformed into SW102 E. coli by electroporation. The GFPCreERT2 fragment was prepared from a shuttle vector and transformed into SW102 E. coli carrying a BAC. Homologous recombination was induced in SW102 E. coli. Recombinant clones were screened and confirmed by PCR and restriction enzyme digestion. Recombinant clones were transformed into SW102 E. coli to remove the kanamycin unit.
RESULTS
A complete BAC was successfully transformed into SW102 E. coli by electroporation because BAC purified from SW102 E. coli showed the same pattern as the original BAC with BamHI digestion. The GFPCreERT2 fragment was deemed to have been prepared successfully because we obtained the same size fragment as expected. Homologous recombination was induced, and GFPCreERT2 was deemed to have been inserted into the correct site of the BAC because we found the band change was the same as the expected pattern after restriction enzyme digestion. The kanamycin unit was deemed to have been removed successfully because we obtained different sizes of bands that were consistent with the results expected by PCR with different primers.

CONCLUSION
The construct of Gpm6aGFPCreERT2 or ReelinGFPCreERT2 was prepared successfully, which will establish a foundation for tracing the hepatic stellate cell lineage and studying its function.

Key words: Bacterial artificial chromosome; Homologous recombination; Glycoprotein M6a; Reelin

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Core tip: Until now, there have been few specific mouse lines that allowed recombination for tracing hepatic mesothelial cells or hepatic stellate cells. Here, we describe a rapid and reliable strategy for construct preparation using a bacterial artificial chromosome. This study prepared a Gpm6a/ReelinGFPCreERT2 construct for the first time, which is the first step for the preparation of a Gpm6aGFPCreERT2 or ReelinGFPCreERT2 mouse line.

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INTRODUCTION
Excessive extracellular matrix (ECM) of the liver results in cirrhosis, which is an end stage liver disease with high mortality and for which efficacious medical treatments are not currently available, except for liver transplantation. Hepatic stellate cell (HSC) activation is considered a major mechanism in the formation of fibrosis and cirrhosis. However, fundamental questions concerning the cell fate regulation of HSCs remain largely underexplored. A recent study reported that hepatic mesothelial cells are the potential precursors for HSCs in the development of liver disease and can transdifferentiate into myofibroblast cells in mouse liver fibrosis[1-3].

Until now, there have been few specific mouse lines that cause recombination (Cre recombinase, Cre) for tracing hepatic mesothelial cells or HSCs. The Wt1CreERT2 mice are useful to trace hepatic mesothelial cells, but the labeling efficiency and specificity is low[4-6]. Specific genes have been identified in hepatic mesothelial cells and HSCs by microarray[7,8]. We plan to develop Cre mouse lines with specific markers for the study of HSCs or hepatic mesothelial cells. Based on a previous study, glycoprotein M6a (Gpm6a) has been identified as a specific surface marker of hepatic mesothelial cells. It covers the surface of the liver and migrates from the surface into the center[4]. Reelin is an extracellular matrix glycoprotein, which is a specific HSC marker in the mouse liver and has similar amounts in resting and activated HSCs[9].

The Gpm6aGFPCreERT2 or ReelinGFPCreERT2 mouse line will express a fusion protein of green fluorescent protein (GFP), Cre, and estrogen receptor induced by tamoxifen (ERT2) under the control of the Gpm6a or Reelin promoter. GFP is used to track the labeled protein as a marker. Cre recombinase is used to delete a segment of DNA flanked by LoxP sites (floX). The ERT2 system is used to activate Cre activity by tamoxifen treatment[10,11].

Cre mouse lines are very useful tools that can generate knockout mice through the cross breeding of Cre and floX mouse lines. A mouse line is needed to trace HSC lineages and study their function through the knockout of specific genes in specific cells. It is, therefore, necessary to prepare a Gpm6aGFPCreERT2 or ReelinGFPCreERT2 mouse line. In this study, we investigated the preparation and identification of a Gpm6a/ReelinGFPCreERT2 construct, which is the first step for the preparation of a Gpm6aGFPCreERT2 or ReelinGFPCreERT2 mouse line.

MATERIALS AND METHODS
Bacterial artificial chromosome DNA clones and a shuttle vector
Gpm6a Bacterial artificial chromosome (BAC) (RP23-410D17) and Reelin BAC (RP23-143M9) were purchased from the BACPAC Resource Center (BPRC) located at the Children’s Hospital Oakland Research Institute in Oakland, California, United States. The female (C57BL/6J) mouse BAC library was made from kidney and brain DNA cloned into the pBACe3.6 vector at the EcoRI site and transformed into DH10B E. coli. The reporter gene GFPCreERT2 is located in the shuttle vector that was developed by the Biomed Company. The 5arm and 3arm were inserted into both sites of the reporter gene. The arm sequences were amplified according to the BAC template by polymerase chain reaction (PCR).

Purification of BAC DNA
DH10B E. coli were streaked onto LB plates with 25
**Table 1** Primer sequences of Gpm6a and Reelin

|         | Forward                      | Reverse                      |
|---------|------------------------------|------------------------------|
| Gpm6a 3arm | CCG TAC CTT TCA             | AGG TAC CCG CCG GCC         |
|         | TGT TTT CAT GGT             | ATG ACA GCA AAC ACT         |
|         | TGT CA                      | GCC TCT A                   |
| Gpm6a 5arm | ACC CAA TCT CCC             | TTA CAG                      |
|         | TTT CAG                      | TGA ACT TGT GGC TTT AGA     |
| Gpm6a Cre | ACC TGA AGA TGT             | ACC GTC AGT AGC TGA         |
| Reelin 3arm | ACG CTC CAC GCC             | AGG TAC CCG CCG GCC         |
|         | ATC CCT ACC CCG C           | ACA GCC GCT CTG TTT CTT     |
| Reelin 5arm | ACC CAA TCT CCC             | TGA ACT TGT GGC TTT AGC     |
|         | TTT CAG                      | TC                          |
| Reelin Cre | ACT TAA CCT CTT             | GAG G                       |
|         | TCG GCC AGC G               | TCA AGT CGA CGC GCC         |
|         | TGG CGC AGC G               | GCT CCG T                   |

mg/mL chloramphenicol (Cmr; Sigma, St Louis, MO, United States) at 32 ℃. A single colony was picked and cultured in 25 mL of LB medium with Cmr. BAC DNA was purified with a large construct kit (Qiagen, Hilden, Germany). BAC DNA was digested with the restriction enzyme BamHI (New England Biolabs, Ipswich, MA, United States).

**Electroporation of BAC DNA into SW102 E. coli**

SW102 E. coli (NCI, Frederick, MD, United States) was streaked onto plates with 50 mg/mL tetracycline (Tc; Sigma) at 32 ℃. A single colony was picked and inoculated in LB medium with Tc and incubated for 4-6 h. After placing on ice, competent cells were made from SW102 E. coli. The cells were washed with ice-cold water, and 5 mL of BAC DNA was added to 85 mL of competent cells. A Gene Pulser Xcell (Bio-Rad, Hercules, CA, United States) was used for electroporation at 1.75 KV for 25 mF (time constant: 4.5-5.0). A total of 0.6 mL of LB was added and cultured at 32 ℃ for 1 h. The sample was then plated onto LB plates with 25 mg/mL Cmr and 12.5 mg/mL Kam. The recombinant BAC DNA was confirmed by PCR with platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, United States) and restriction enzyme digestion.

**Removal of the Kam unit and selection of the deleted Kam clone**

SW105 E. coli (NCI) was streaked without antibiotics. A single colony was picked and competent cells were made. Recombinant BAC DNA was transformed into competent cells with electroporation and inoculated in LB medium with L-arabinose (Sigma) for removal of the Kam unit. They were then plated onto LB plates with Cmr only. We confirmed that no colonies were on the LB plate with Cmr and Kam. Sixteen colonies were picked and checked by PCR using the Sarm primer (Table 1). One colony was confirmed by PCR using different primers (Table 1). The PCR product was digested with BamHI and AflII (New England Biolabs) for further confirmation.

**RESULTS**

**Conformation of BAC by restriction enzyme BamHI digestion**

As mentioned previously, we purchased BAC from BPRC and confirmed the BAC sequence. From Figure 1, it can be observed that, after digestion with BamHI, Gpm6a BAC had 11 bands, which was the expected pattern. For Reelin BAC, the same results were obtained. Therefore, we confirmed that we received the BAC clone.

**Confirmation of BAC DNA transformed into SW102 E. coli**

BAC DNA is so long that it is impossible to transform BAC into SW102 E. coli by chemical transduction. We transformed purified BAC into SW102 E. coli by electroporation. After electroporation, we purified BAC from SW102 E. coli and identified BAC with restriction enzyme BamHI digestion. For Gpm6a BAC, after BamHI digestion, selected clones did not show the same digestion pattern with original BAC, as shown in Figure 2, which suggested the original BAC DNA may have been fragmented during the purification step. For Reelin BAC, we obtained two positive clones that showed the same pattern as the original BAC after BamHI digestion. We improved the purification methods, and we finally succeeded in transforming the complete Gpm6a BAC into SW102 E. coli.
BamHI digestion

Gpm6a BAC                          Reelin BAC                                    Gpm6a BAC with BamHI digestion

M     1     2      3      4      5      6      1      2      3     4      5     6    M     1     2     3     4     5     6      7     8      9     10    11    12

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Preparation of the reporter gene GFPCreERT2
The reporter gene GFPCreERT2 was in the shuttle vector that contained some genes encoding the 5arm, flp recombinase target (FRT), Kam, FRT, GFPCreERT2, 3arm and ampicillin. We used NotI and FseI to digest the shuttle vector and obtained the fragment of GFPCreERT2 flanked by the 5arm and 3arm. As shown in Figure 3, after digestion with NotI and FseI, we obtained two bands; one was approximately 7600 bp, and the other was approximately 2800 bp. We recovered the long band containing GFPCreERT2 from the agarose gels for transformation.

Screening and confirmation of recombinant clones
We induced the SW102 E. coli carrying BAC to become competent cells and transformed the fragment of GFPCreERT2 into the SW102 E. coli carrying the BAC. After homologous recombination between BAC and GFPCreERT2, GFPCreERT2 replaced the first exon in the BAC vector. Then, we purified the recombinant BAC for screening and confirmation. As shown in Figure 4B, we obtained 14 positive clones for Gpm6a (14/25, 56%), as evidenced by PCR screening. For Reelin, we obtained 22 positive clones (22/25, 88%). We further confirmed the positive clones by restriction enzyme digestion. For Gpm6a, we found that the band change was the same as the expected pattern after BamHI or KpnI digestion (Figure 4C). Reelin had the same results (Figure 4D). The results suggested that GFPCreERT2 was inserted into the correct site in the BAC vector and that we acquired the recombinant BAC.
Screening and confirmation of recombinant clones without the Kam gene

Finally, we removed the Kam unit that could interfere with the expression of the reporter gene. We purified the recombinant BAC carrying GFPCreERT2 and transformed it into SW105 E. coli. The Kam unit was removed by homologous recombination. As shown in Figure 5B, we obtained 16 positive clones for Gpm6a (16/16, 100%), as evidenced by PCR screening. We further confirmed the positive clones by PCR with different primers. We obtained different sizes of bands that were consistent with what was expected (Figure 5C). We digested the PCR product of 4527 bp with BamH1 or AflI, and the bands obtained were the same as expected (Figure 5D). This suggested that the Kam unit was removed and that we obtained BAC carrying GFPCreERT2 without Kam.

DISCUSSION

First of all, we needed to choose a vector to prepare the Gpm6a/ReelinGFPCreERT2 construct. As a conventional vector, its advantage was that it is easy to handle, but it had low expression levels of the reporter genes and low specificity because the DNA size is approximately 3-5 kb and contains only a promoter. For the 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 approximately 200 kb and contains all the regulatory sequences upstream and downstream of the encoding sequence. Thus, we chose a BAC vector to prepare the constructs of Gpm6aGFPCreERT2 and ReelinGFPCreERT2.

Second, how to insert reporter genes into BAC DNA was a question. In 200 kb of BAC DNA, there would be 49 recognition sites for 6 nucleotide cutters such as EcoRI and BamHI, so it was impossible to use restriction enzyme digestion and ligation. We, therefore, used homologous DNA recombination for gene insertion. In BAC DNA, we designed the primers upstream or downstream of the first exon and obtained the 5arm and 3arm fragment by PCR amplification. In SW102 E. coli, the reporter gene that is GFPCreERT2 replaced the first exon in the BAC vector by homologous recombination. The expression of GFPCreERT2 is regulated by the Gpm6a or Reelin promoter.

Finally, we removed the Kam unit by homologous recombination of the FRT using SW105 E. coli. The FRT is similar to LoxP, which is used to delete a segment of DNA flanked by LoxP sites. The FRT cassette was excised with high frequency, which was close to 100%. Then, we obtained the constructs of Gpm6aGFPCreERT2 and ReelinGFPCreERT2 successfully. After microinjection, we can develop the mouse line with Gpm6aGFPCreERT2 and ReelinGFPCreERT2.

Homologous recombination was performed with modified E. coli bacteria strains: SW102 and SW105. SW102 E. coli carries genes such as exo, bet and gam.
Exo encodes 5’-3’ exonuclease, and Bet encodes the overhang binding protein, which enables annealing and recombination with complementary DNA; Gam encodes the inhibitor of *E. coli* exonuclease to protect the introduced DNA. SW105 *E. coli* carries the L-arabinose-inducible *flp* gene, which encodes recombinase, allowing DNA modification without restriction enzymes and DNA ligase[21].

Cre-Lox recombination is a site-specific recombinase technology that is widely used to carry out deletions, insertions, translocations and inversions in the DNA of cells. It allows the DNA modification to be targeted to a specific cell type or to be triggered by a specific external stimulus[22,23]. For a mouse line with Gpm6a<sup>GFPCreERT2</sup> and Reelin<sup>GFPCreERT2</sup>, once the specific genes, such as Gpm6a or Reelin, begin to express, the GFPCreERT2 will express in the specific cell. Using GFP as a marker, we can trace the specific cells. Using the CreERT2 system, we can knock out the specific gene in specific cells, which is a conditional knockout[24].

In the next stage, we will label hepatic mesothelial and HSCs in the Gpm6a/Reelin<sup>GFPCreERT2</sup> transgenic mouse to trace the lineage of HSCs. We will also make a conditional TGFβ<sup>−/−</sup>-knockout mouse through cross breeding of the TGFβ<sup>−/−</sup> mouse and the Gpm6a/Reelin<sup>GFPCreERT2</sup> mouse to explore the function of HSCs.

**Figure 4** Screening and confirmation of recombinant clones. A: Diagram of homologous recombination between BAC and GFPCreERT2; B: Electrophoretogram of the PCR product according to the recombinant BAC template with the 3Arm primer; C: Electrophoretogram of recombinant Gpm6a BAC digested by BamHI and KpnI; D: Electrophoretogram of recombinant Reelin BAC digested by BamHI and Smal. M: Marker; OB: Original BAC; RB: Recombinant BAC. BAC: Bacterial artificial chromosome.
Hepatic stellate cell (HSC) activation is considered a major mechanism in the formation of fibrosis and cirrhosis. However, fundamental questions concerning the cell fate regulation of HSCs remain largely underexplored. A recent study reported that hepatic mesothelial cells are the potential precursors of HSCs in the development of the liver and can transdifferentiate into myofibroblast cells in mouse liver fibrosis. Until now, there have been few specific mouse lines that could cause recombination (Cre) for tracing hepatic mesothelial cells or HSCs. Here, we describe a rapid and reliable strategy for construct preparation using the bacterial artificial chromosome (BAC).

**Research frontiers**
A recent study reported that hepatic mesothelial cells are the potential precursors for HSCs in the development of the liver and can transdifferentiate into myofibroblast cells in mouse liver fibrosis. Glycoprotein M6a (Gpm6a) has been identified as a specific surface marker of hepatic mesothelial cells, which cover the surface of the liver and migrate from the surface into the center. Reelin is an extracellular matrix glycoprotein, which is a specific HSC marker in the mouse liver and has similar amounts in resting and activated HSCs.

**Innovations and breakthroughs**
Until now, there have been few specific mouse lines that could Cre for tracing hepatic mesothelial cells or HSCs. Here, the authors describe a rapid and reliable strategy for construct preparation using a BAC. This study prepared a Gpm6a\textsuperscript{GFPCreERT2} construct for the first time, which is the first step in the preparation of a Gpm6a\textsuperscript{GFPCreERT2} or Reelin\textsuperscript{GFPCreERT2} mouse line.

**Applications**
In this study, a construct of Gpm6a\textsuperscript{GFPCreERT2} or Reelin\textsuperscript{GFPCreERT2} was prepared successfully, which will establish the foundation for tracing the HSC lineage and studying its function.

**Terminology**
Cre-Lox recombination is a site-specific recombinase technology widely used to create deletions, insertions, translocations and inversions in the DNA of cells. It allows the DNA modification to be targeted to a specific cell type or

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**Figure 5** Screening and confirmation of recombinant clones without the kanamycin unit. A: Diagram of the removal of the Kam unit in SW105 \textit{E. coli}; B: Electrophoreogram of the PCR product according to the recombinant BAC template with the 5arm primer; C: Electrophoreogram of the PCR product according to the recombinant BAC template with different primers; D: Electrophoreogram of the PCR product with BamH1 or AflI digestion. M: Marker; OB: Original BAC; RB: Recombinant BAC; DkB: Deleted kanamycin BAC; 5F: Forward primer of the 5arm; 5R: Reverse primer of the 5arm; 3F: Forward primer of the 3arm; 3R: Reverse primer of the 3arm; Cre: Cre primer.
triggered by a specific external stimulus. In a mouse line with Gpm6aGFPCreERT2 and ReelinGFPCreERT2, once the specific genes such as Gpm6a or Reelin begin to express, the GFPCreERT2 will express in the specific cell. Using GFP as a marker, we can track the specific cells. Using the CreERT2 system, we can knock out the specific gene in a specific cell, which is a conditional knockout.

**Peer-review**

This is a well-designed study in which the author prepared a construct of Gpm6aGFPCreERT2 or ReelinGFPCreERT2 successfully, which is the first step for the preparation of a Gpm6aGFPCreERT2 or ReelinGFPCreERT2 mouse line. Cre mouse lines are very useful tools that can generate knockout mice through the cross breeding of Cre and flox mouse lines.

**REFERENCES**

1. Lua I, Ashahina K. The Role of Mesothelial Cells in Liver Development, Injury, and Regeneration. Gut Liver 2016; 10: 166-176 [PMID: 26394883 DOI: 10.5009/gnl15226]
2. Lua I, James D, Wang J, Wang KS, Ashahina K. Mesodermal mesenchymal cells give rise to myofibroblasts, but not epithelial cells, in mouse liver injury. *Hepatology* 2014; 60: 311-322 [PMID: 24488807 DOI: 10.1002/hep.27035]
3. Li Y, Lua I, French SW, Ashahina K. Role of TGF-β signaling in differentiation of mesothelial cells to vitamin A-poor hepatic stellate cells in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2016; 310: G262-G272 [PMID: 26702136 DOI: 10.1152/ajpgi.00257.2015]
4. Vicente-Stejn R, Schertpong RW, Kruthof BP, Duint SN, Goumans MJ, Wisse LJ, Zhou B, Pu WT, Poelmann RE, Schalij-Mink WJG. Regional differences in WT-1 and Tcf21 expression during ventricular development: implications for myocardial compaction. *PLoS One* 2015; 10: e0136025 [PMID: 26390289 DOI: 10.1371/journal.pone.0136025]
5. Li Y, Wang J, Ashahina K. Mesothelial cells give rise to hepatic stellate cells and myofibroblasts via mesothelial-mesenchymal transition in liver injury. *Proc Natl Acad Sci USA* 2013; 110: 2324-2329 [PMID: 23345421 DOI: 10.1073/pnas.1214136110]
6. Ashahina K, Zhou B, Pu WT, Tsukamoto H. Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. *Hepatology* 2011; 53: 983-995 [PMID: 21294146 DOI: 10.1002/hep.24119]
7. Lua I, Li Y, Zagory JA, Wang KS, French SW, Sénévéry J, Ashahina K. Characterization of hepatic stellate cells, portal fibroblasts, and mesothelial cells in normal and fibrotic livers. *J Hepatol* 2016; 64: 1137-1146 [PMID: 26806818 DOI: 10.1016/j.jhep.2016.01.010]
8. Many S, Nasseir MM, Zohary MA, Hassanein MH, Guda MF, Othman MM, Abulatalab H. Value of reelin for assessing hepatic fibrogenesis in a group of Egyptian HCV infected patients. *Clin Chem Lab Med* 2014; 52: 1319-1328 [PMID: 24803609 DOI: 10.1515/ccm-2014-0030]
9. Kobold D, Grundmann A, Piscaglia F, Eisenbach C, Neuhauser K, Stellfgen I, Ramadori G, Kiehl T. Expression of reelin in hepatic stellate cells and during hepatic tissue repair: a novel marker for the differentiation of HSC from other liver myofibroblasts. *J Hepatol* 2002; 36: 607-613 [PMID: 11983443]
10. Lizen B, Claus M, Jeanotte L, Rijli FM, Golfft F. Perinatal induction of Cre recombination with tamoxifen. *Transgenic Res* 2015; 24: 1065-1077 [PMID: 26393570 DOI: 10.1007/s11248-015-9905-5]
11. Miwa H, Era T. Generation and characterization of PGDFRα-GFPCreERT2 knock-In mouse line. *Genesis* 2015; 53: 329-336 [PMID: 25884589 DOI: 10.1002/dvg.22853]
12. Cui B, Smoother PM, Rouch DA, Deighton MA. Enhancing DNA electro-transformation efficiency on a clinical Staphylococcus capitis isolate. *J Microbiol Methods* 2015; 109: 25-30 [PMID: 25477024 DOI: 10.1016/j.mimet.2014.11.012]
13. Hasse S, Hyman AA, Sarov M. TransgeneOmics—A transgenic platform for protein localization based function exploration. *Methods* 2016; 96: 69-74 [PMID: 26475212 DOI: 10.1016/j.ymeth.2015.10.005]
14. Hall RN, Meers J, Fowler E, Mahony T. Back to BAC: the use of infectious clone technologies for viral mutagenesis. *Viruses* 2012; 4: 211-235 [PMID: 22470833 DOI: 10.3390/v4020211]
15. Zhou F, Li Q, Wong SW, Gao SJ. Autoexcision of bacterial artificial chromosome facilitated by terminal repeat-mediated homologous recombination: a novel approach for generating traceless genetic mutants of herpesviruses. *J Virol* 2010; 84: 2871-2880 [PMID: 20071577 DOI: 10.1128/JVI.01734-09]
16. Narayanan K, Williamson R, Zhang Y, Stewart AF, Ioannou PA. Efficient and precise engineering of a 200 kb beta-globin human/bacterial artificial chromosome in E. coli DH10B using an inducible homologous recombination system. *Gene Ther* 1999; 6: 442-447 [PMID: 10435094 DOI: 10.1038/sj.gt.3300901]
17. Yang XW, Model P, Heintz N. Homologous recombination based modification in Esherichia coli and germine transmission in transgenic mice of a bacterial artificial chromosome. *Nat Biotechnol* 1997; 15: 859-865 [PMID: 9360600 DOI: 10.1038/mbi0997-859]
18. Wang S, Zhao Y, Leiby M, Zhu J. A new positive/negative selection scheme for precise BAC recombineering. *Mol Biotechnol* 2009; 42: 110-116 [PMID: 19160076 DOI: 10.1007/s12033-009-9142-3]
19. Shah R, Li F, Vozyanova E, Vozyanov Y. Target-specific variants of Flp recombinase mediate genome engineering reactions in mammalian cells. *FEBS J* 2015; 282: 3323-3333 [PMID: 26077105 DOI: 10.1111/febs.13345]
20. Fan HF, Cheng YS, Ma CH, Jayaram M. Single molecule TPM analysis of the catalytic pentad mutants of Cre and Flp site-specific recombinases: contributions of the pentad residues to the pre-chemical steps of recombination. *Nucleic Acids Res* 2015; 43: 3237-3255 [PMID: 25765648 DOI: 10.1093/nar/gkv114]
21. Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG. Simple and highly efficient BAC recombineering using galK selection. *Nucleic Acids Res* 2005; 33: e36 [PMID: 15733329 DOI: 10.1093/nar/gni035]
22. Hubbard EJ. FLP/FRT and Cre/lox recombination technology in C. elegans. *Methods* 2014; 68: 417-424 [PMID: 24874786 DOI: 10.1016/j.ymeth.2014.04.007]
23. Yarmolinovsky M, Hoess R. The Legacy of Nat Sternberg: The Genesis of Cre-lex Technology. *Annu Rev Virol* 2015; 2: 25-40 [PMID: 26958905 DOI: 10.1146/annurev-virology-100114-054930]
24. Hu MW, Wang ZB, Schatten H, Sun QY. New understandings on folliculogenesis/oogenesis regulation in mouse as revealed by conditional knockout. *J Genet Genomics* 2012; 39: 61-68 [PMID: 22361505 DOI: 10.1016/j.jgg.2012.01.004]

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