Video Article

Constitutive and Inducible Systems for Genetic *In Vivo* Modification of Mouse Hepatocytes Using Hydrodynamic Tail Vein Injection

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Abstract

In research models of liver cancer, regeneration, inflammation, and fibrosis, flexible systems for *in vivo* gene expression and silencing are highly useful. Hydrodynamic tail vein injection of transposon-based constructs is an efficient method for genetic manipulation of hepatocytes in adult mice. In addition to constitutive transgene expression, this system can be used for more advanced applications, such as shRNA-mediated gene knock-down, implication of the CRISPR/Cas9 system to induce gene mutations, or inducible systems. Here, the combination of constitutive CreER expression together with inducible expression of a transgene or miR-shRNA of choice is presented as an example of this technique. We cover the multi-step procedure starting from the preparation of *sleeping beauty*-transposon constructs, to the injection and treatment of mice, and the preparation of liver tissue for analysis by immunostaining. The system presented is a reliable and efficient approach to achieve complex genetic manipulations in hepatocytes. It is specifically useful in combination with Cre/loxP-based mouse strains and can be applied to a variety of models in the research of liver disease.

Video Link

The video component of this article can be found at https://www.jove.com/video/56613/

Introduction

Chronic liver disease presents a major health burden worldwide[1]. Animal research models are essential tools in the study of liver disease and have helped to answer complex questions in liver regeneration, hepatic inflammation, and steatosis as well as liver cancer[2]. A substantial number of these animal models rely on the genetic modification of liver cells. Therefore, efficient tools to manipulate gene expression in hepatocytes are helpful[3]. Established methods such as the breeding of genetically engineered mouse strains or the generation of viral vectors for hepatocyte infection are either time consuming, harbor safety concerns, or yield poor transgene expression in hepatocytes *in vivo*[4,5]. Hydrodynamic tail vein injection (HTVI) is an alternative method for *in vivo* transfection of hepatocytes allowing for easy, fast, and cost-efficient interrogation of gene function in the liver. For HTVI, a vector carrying the desired DNA sequence is dissolved in a volume of saline corresponding to 10% of the body weight of the injected animal. The solution is then injected into the tail vein within 5-10 s[6]. Exceeding cardiac output, the saline flows from the inferior vena cava into the liver veins, leading to expansion of the liver and hydrodynamic transfection of hepatocytes[7]. To achieve stable genomic integration, the method has been combined with transposon-based vectors, such as the *sleeping beauty*-transposon system. This systems mediates the recombination of target vectors with genomic recombination sites catalyzed by a *sleeping beauty*-transposase[8,9]. For models of liver fibrosis or carcinogenesis, it is often desirable to overexpress or silence genes at certain time points of the disease model. For this purpose, tools for inducible gene expression such as the Cre/LoxP-system or the tetracycline-inducible gene expression system (Tet-On) may be used[10].

Here, we describe a protocol for *in vivo* transfection of murine hepatocytes using HTVI of a *sleeping beauty* transposon-based system. In addition to a protocol for stable, constitutive expression of a transgene under the control of a liver-specific promoter, we describe a more advanced vector system that combines constitutive tamoxifen-dependent Cre recombinase (CreER) expression with the inducible expression of a transgene or microRNA-adapted shRNA (miR-shRNA), called the pTC TET-system[11]. In this vector system, inducible transgenes or miR-shRNAs for tetracycline-dependent expression are cloned into the backbone vector with a recombinational cloning system, allowing the fast and easy generation of new vectors[12]. This video-based guide covers the preparation of suitable vectors, injection and treatment of mice to achieve inducible transgene/miR-shRNA expression, and finally preparation of liver tissue for analysis. The method described in this protocol was designed to enable the combination of any Cre/loxP mediated mouse system with the expression or knock-down of any gene of choice, making it a widely applicable system in research of liver disease.

Protocol

All animal experiments were performed according to the guidelines for the care and use of laboratory animals and were approved by responsible authorities (Regierung von Oberbayern, Munich, Germany and Stanford Institutional Animal Care and Use Committee, Stanford, CA, USA). A list of all plasmids for cloning (step 1 through 4) is provided in supplementary table S1.

1. Cloning of a Transgene for Constitutive Gene Expression

1. Design primers for transgene amplification[13,14].
2. Add restriction sites for PacI (TTAATTAA) to the 5' end of the forward primer. Add restriction sites for AscI (GGCGCGCC) or FseI (GGCCGGCC) to the 5' end of the reverse primer.
3. Amplify transgene by PCR using conditions optimized for the desired transgene[14]. Purify using a commercially available DNA purification kit.
   NOTE: Annealing time depends on the primers designed in step 1.1., elongation time depends on the length of the construct. For example, for a construct of 1,000 bp length use 60 s of elongation time.
4. Digest transgene and vector for constitutive gene expression[15] with respective restriction nucleases (see step 1.2) and buffer in a total volume of 50 µL at 37 °C overnight. For example, digest construct with 5 units PacI and 5 units FseI if appropriate (see step 1.2).
5. Gel purify digested vector and insert using a commercial gel extraction kit according to the manufacturer's instructions. Perform a standard ligation of 100 ng of vector and 100–1,000 ng of insert using 400 U T4 ligase at 14 °C for 16 h. Heat inactivate at 65 °C for 10 min.
6. Transform competent bacteria using a standard heat-shock-protocol[16].
7. Plate on agar plates containing 100 µg/mL ampicillin. Incubate at 30 °C for 24 h.
8. Pick single colonies, purify plasmid DNA using a commercial miniprep kit according to the manufacturer's instructions and verify the sequence by Sanger sequencing[17] (sequencing primer: 5' TGCTGGAGTTCTTCGCC 3').
9. Use positive colonies for maxi scale amplification of plasmid DNA and purify using an endotoxin-free plasmid preparation kit[18] according to the manufacturer's instructions.
10. Construct is ready for injection, thus continue with step 5.

2. Cloning of a Transgene for Inducible Gene Expression

1. Design primers for transgene amplification[13,14].
2. Add restriction sites for SacI (GAGCTC), SpeI (ACTAGT), or KpnI (GGTACC) to the 5' end of the forward primer. Add restriction sites for NotI (GCGGCCGC) or XhoI (CTCGAG) to the 5' end of the reverse primer.
3. Amplify transgene by PCR using conditions optimized for the desired transgene[14]. Purify using a commercial DNA purification kit.
4. Digest the purified transgene and 4 µg of Entry vector (pEN_TTmcs-Vector, see **Table of Materials**)[19] separately with appropriate restriction nucleases (see step 2.2) and buffer in a total volume of 50 µL at 37 °C overnight.
5. Gel purify digested vector and insert using a commercial gel extraction kit according to the manufacturer's instructions. Perform standard ligation with 100 ng of vector and 100–1,000 ng of insert using 400 U T4 ligase at 14 °C for 16 h. Heat inactivate at 65 °C for 10 min.
6. Transform competent bacteria using a standard heat-shock-protocol[16].
7. Plate on agar plates containing 15 µg/mL gentamicin. Incubate at 37 °C for 16 h.
8. Pick single colonies, purify plasmid DNA, and verify insert by sequencing[17] using the pCEP forward primer: 5' AGAGCTCGTTTAGTGAACCG 3'.
   NOTE: PCR on single colonies can be performed without prior purification with primers pCEP forward and pCEP reverse (5' AGA AAG CTG GGT CTA GAT ATC TCG 3'). This step can useful for pre-selection of positive colonies.
9. Proceed to step 4.

3. Cloning of a miR-shRNA for Inducible Gene Knock-down

1. Design miR-shRNA oligonucleotides according to the pSLIK cloning protocol[19]. Anneal and purify oligonucleotides and dilute 1 : 20 in ddH_2O.
2. Digest 3 µg of Entry vector with 5 U BfuAI at 50 °C for 3 h, then inactivate the reaction at 65 °C for 20 min.
   NOTE: If co-expression of green fluorescent protein (GFP) is desired, use pEN_TTGmiRc[19] as an entry vector, otherwise use pEN_TTmiRc2[19].
3. Gel purify digested vector as in step 2.5. Perform standard ligation of 100 ng of vector and 1 µL of purified and diluted shRNA oligonucleotides (step 3.1) using 400 U T4 ligase at room temperature for 1 h. Heat inactivate at 65 °C for 10 min.
4. Transform competent bacteria using a standard heat-shock-protocol[16].
5. Plate on agar plates containing 15 µg/mL gentamicin. Incubate at 37 °C for 16 h.
6. Pick single colonies, purify plasmid DNA, and verify insert by Sanger sequencing[17] (sequencing primer: 5' TAGTCGACTAGGGATAACAG 3').
7. Proceed to step 4.

4. Recombinational Cloning to Generate Ready-for-Injection Clones

1. Mix 150 ng of Entry vector (from step 2 or step 3) and 150 ng of pTC TET-vector[20].
2. Add TE buffer to a total volume of 8 µL (pH=8).
3. Transfer the LR-clonase enzyme mix II (see **Table of Materials**) to ice, incubate for 2 min. Vortex twice.
4. Add 2 µL of LR-clonase enzyme mix II to the reaction and incubate at 25 °C for 1 h.
5. Stop the reaction by adding 1 µL of Proteinase K-solution. Incubate at 37 °C for 10 min.
6. Transform Stbl3 competent bacteria with 2 µL of recombinational cloning mix using a standard heat-shock-protocol[16].

7. Plate on agar plates containing 100 µg/mL ampicillin. Incubate at 30 °C for 24 h.
8. Pick single colonies, purify plasmid DNA using an endotoxin-free plasmid preparation kit[18] according to the manufacturer's instructions, and confirm vector integrity by Sanger sequencing[17] (sequencing primer 5' AGGGACAGCAGAGATCCAGTTTGG 3').
9. Construct is ready for injection, continue with step 5.

5. Preparing Solution for Hydrodynamic Tail Vein Injection

NOTE: Preparation of constructs for constitutive and inducible gene expression are described in Step 1, 2, 3, and 4.

1. Prepare sterile 0.9% saline for injection (do not use PBS). Use volume corresponding to about 10% of mouse body weight. Example: for a mouse weighing 20 g, prepare 2 mL of solution.
2. Prepare injection vectors that were purified using an endotoxin-free plasmid purification kit[18] (see step 1.8 or 4.8, respectively).
3. Add 10 µg or 15 µg of endotoxin-free *sleeping beauty* vector construct (from step 1 use 10 µg, from step 4 use 15 µg, respectively) and 1 µg of endotoxin-free pc-HSB5[21] per mL of sterile saline.
4. Store solution for up to 4 h at 4 °C. Do not freeze.

6. Performing Hydrodynamic Tail Vein Injection

1. Use a restrainer for tail vein injection (commercial or prepared from a 50 mL conical tube with holes for breathing and for the tail). Fill bottom of the tube with tissue paper.
2. For injection, use mice of about 8–10 weeks of age with weights of 20–25 g.
3. Weigh mice before injection and prepare injection volume according to body weight (corresponding to 10% of body weight, see step 5.1). Prepare a sterile 3 mL-syringe with a 27 G-needle for injection and fill with the required volume.
4. Place the mouse into the restrainer. Adjust the amount of tissue paper (see step 6.1) to leave only minimal space for movement but enough space for breathing.
5. Ensure that the mouse is breathing regularly.
6. Warm the tail using an infrared lamp for 30-60 s. Carefully watch for signs of overheating.
7. Clean the tail with an alcohol swab.
8. Insert the needle almost horizontally into either one of the two lateral tail veins close to the base of the tail.
   NOTE: If placed successfully, a small amount of blood might flow back into the cone of the needle. It is not recommended to actively aspirate as any additional movement of the needle can result in its displacement and/or injury of the vein.
9. Inject the total volume into the tail vein within 8-10 s.
10. Immediately remove the mouse from the restrainer. Compress injection wound for at least 30 s or until any bleeding subsides.
11. Place the mouse into a separate cage. Once the mouse has recovered from the procedure (about 30–60 min), transfer the mouse back to its original cage. Check on the mouse regularly for the next 24 h.
    NOTE: Mild sedation of the mouse is routinely observed for up to 2 h after injection.
12. Before proceeding with further experiments (*i.e.*, step 7), wait 10–15 days for clearance of non-integrated vectors.

7. Induction of Transfected CreER with Tamoxifen

CAUTION: Tamoxifen is harmful, may be cancerous or damage fertility. Please refer to the safety data sheet.

1. Plan intraperitoneal tamoxifen injections on three consecutive days.
2. On day 1, dissolve 10 mg of tamoxifen in 40 µL ethanol. Incubate at 55 °C for 10 min. Vortex several times until the tamoxifen has dissolved.
3. Add 960 µL corn oil. Incubate for 5 mins at 55 °C. Vortex several times to get a clear solution.
4. Prepare the solution in a 1-mL insulin syringe with a 27 G needle.
5. Scruff the mouse by grabbing the neck of the mouse carefully with the thumb and the second finger, fixing the tail between the base of the hand and the fourth and fifth finger.
6. Inject 0.1 mL (= 1 mg of tamoxifen) of the solution intraperitoneally into the left lower quadrant of the abdomen.
7. Repeat the injections on days two and three.

8. Induction of Tetracycline-dependent Gene or shRNA Expression

CAUTION: Doxycycline may be harmful. Please refer to the safety data sheet.

NOTE: Depending on the type and duration of the experiment, doxycycline can be supplied in drinking water (step 8.1) or chow (step 8.2)

1. **For short term experiments (<10 days) administer doxycycline via drinking water using the following protocol.**
   1. Dissolve 5 g sucrose in 100 mL of tap water. Autoclave.
   2. Dissolve 100 mg of doxycycline-hyclate in 5 mL of sucrose solution (step 8.1.1) in a 15-mL conical tube.
   3. Using a 10-mL syringe, sterile filter the solution through a 0.2 µm filter. Add to the sucrose solution prepared in step 8.1.1.
   4. Supply doxycycline-sucrose solution as drinking water to the mouse. Check daily and replace when the solution becomes cloudy indicating bacterial overgrowth (replace clear solution after three days at the latest).

2. For long term experiments (>10 days) or experiments sensitive to metabolic changes, use commercial doxycycline-chow (*e.g.*, Doxycycline Hyclate Chow 0.625 g/kg) to avoid dehydration and/or sucrose-induced changes in the livers of treated animals.

9. Preparation of Mouse Liver for Analysis by Immunostaining

CAUTION: Paraformaldehyde may be harmful. Please refer to the safety data sheet.

NOTE: The timepoint when mice will be analyzed depends on the experiment. It is recommended to analyze liver tissue after no less than three days of doxycycline treatment to ensure sufficient induction of transgene or shRNA expression.

1. Prepare a 1 mL syringe with a 27 G needle with 1 mL of 4% paraformaldehyde solution (PFA).
2. Euthanize the mouse by an appropriate method according to an approved animal protocol.
   NOTE: Guidelines for appropriate methods of euthanasia may vary depending on the institution.
3. Using dissecting scissors and anatomical forceps, carefully open the abdominal cavity with a median laparotomy to expose the liver. Move the small intestine to the right to expose the portal vein and the inferior vena cava (IVC).
4. Insert the needle of the prepared syringe (see step 9.1) into the IVC and cut the portal vein. Inject 1 mL of PFA slowly into the IVC to perfuse the liver tissue and remove auto-fluorescent red blood cells (desirable if immunofluorescent staining will be performed).
5. Remove the liver. Rinse in water and transfer to 5–10 mL of 4% PFA solution.
6. For paraffin sections, fix tissue for 36–48 h. Tissue is ready for dehydration and paraffin embedding.
7. **For frozen sections, fix tissue in 4% PFA for 1 h.**
   1. For cryoprotection, transfer to 10% sucrose solution. Incubate 60 min.
   2. Transfer to 20% sucrose solution. Incubate 60 min.
   3. Transfer to 30% sucrose solution. Incubate 12–16 h. Embed in embedding compound for frozen sections and freeze at -20 °C.

Representative Results

Transfection efficacy by hydrodynamic tail vein injection: The percentage of murine hepatocytes that are transfected hydrodynamically by a single injection is variable and depends on multiple parameters such as injection volume, injection time, amount of injected DNA, and size of the injected construct[6,22,23]. Additionally, the transfection efficiency is generally lower in larger animals, where a larger vascular diameter as well as larger sinusoidal area leads to a decrease in overall pressure. To visualize transfection efficiency, HTVI of a CreER transposon construct was performed in mice harboring a *Rosa26*^{mTmG} reporter gene followed by tamoxifen-mediated activation of the CreER construct. Low injection volume or prolonged injection time for technical reasons results in reduced transfection efficiency with only a few transfected hepatocytes detectable in the whole liver, while optimal injection conditions result in higher transfection efficiencies as depicted by reporter staining after HTVI of a CreER transposon (**Figure 1A**). To assess transfection efficacy, immunostaining of the transfected transgene or – as in the case of CreER - of a suitable reporter gene is highly recommended. Alternatively, transfection efficiency might be estimated from mRNA expression analysis of the transfected transgene.

Transfection of pericentral hepatocytes: After hydrodynamic injection, a pressure gradient along the sinusoidal space - where the pressure is highest close to the central vein and lowest in the periportal areal - might lead to preferred transfection in the pericentral area. We analyzed livers with a low percentage of transgene-expressing hepatocytes and assessed their relative position in the liver lobule by co-immunostaining for glutamine synthetase (GS), a marker for pericentral hepatocytes. Interestingly, the majority of hepatocytes that showed reporter gene expression after HTVI of a CreER construct clustered around the central vein but not the portal area (**Figure 1B**) indicating higher transfection efficiency around the central vein.

***In vivo* imaging after HTVI of a luciferase transposon:** To assess if the single copy integration observed after HTVI of transposon constructs[24] is sufficient for *in vivo* imaging, we injected a transposon construct harboring a luciferase expression cassette under the control of a liver specific promoter construct. Mice were injected with luciferin and imaged using an *in vivo* imaging system two weeks after HTVI (**Figure 2A**). Imaging showed robust and stable luciferase bioluminescence in the liver 15 days after injection and at later time points (**Figure 2B** and data not shown). These results show that the system can be successfully used to follow the presence of transfected cells *in vivo* by bioluminescence imaging.

Combined CreER and inducible transgene or shRNA expression: We previously generated a transposon construct that combines the constitutive expression of an inducible Cre recombinase (CreER) with inducible expression of a transgene or an shRNA of choice[20] (**Figure 3A**). Injection of this construct into *Rosa26*^{mTmG}-reporter mice and CreER activation by tamoxifen showed robust expression of the reporter gene comparable to the results obtained with a transposon construct for single transgene expression (**Figure 3B**). After doxycycline treatment, inducible transgene expression can be visualized by suitable antibodies in transfected hepatocytes (**Figure 3C**). For inducible shRNA expression, the use of a GFP-shRNA construct is recommended as cytoplasmic GFP expression detected by immunostaining can be used as a surrogate marker for shRNA expression (**Figure 3D**). Of note, transgene or shRNA expression, respectively, is only detectable in a subset of hepatocytes, which might be due to the relatively high level of protein expression required for detection by immunostaining[20].

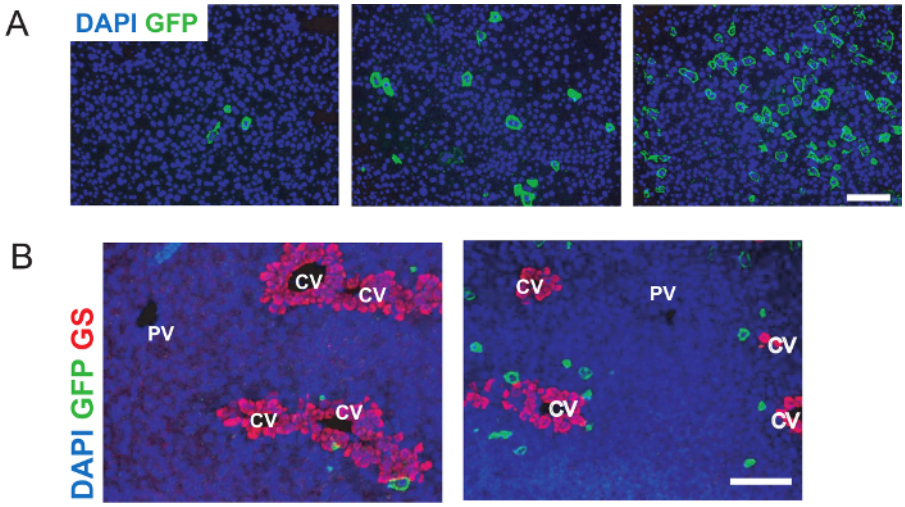

Figure 1: Transfection of hepatocytes by HTVI. (A) Variable transfection efficiency after HTVI of a CreER-transposon into mice harboring a *Rosa26*$^{mTmG/+}$ reporter and followed by treatment with tamoxifen. Transfected hepatocytes are positive for membrane-bound green fluorescent protein (GFP, green). **(B)** Co-staining for CreER activated hepatocytes (green) in mice harboring a *Rosa26*^{mTmG} reporter with the central vein marker glutamine synthetase (GS, red). Here PV: portal vein, CV: central vein. Scale bars represent 100 µm. Please click here to view a larger version of this figure.

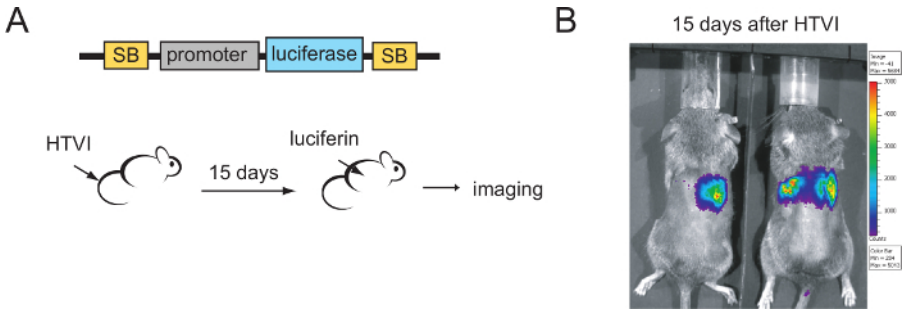

Figure 2: *In vivo* imaging of luciferase. (A) Transposon construct containing a luciferase expression cassette (not drawn to scale). Injection and treatment scheme for visualization of hepatic luciferase expression. SB: sleeping beauty recognition sites, HTVI: hydrodynamic tail vein injection. **(B)** Representative image of hepatic luciferase bioluminescence 15 days after HTVI of a transposon-luciferase construct together with pHSB5 using an *in vivo* imaging system. Please click here to view a larger version of this figure.

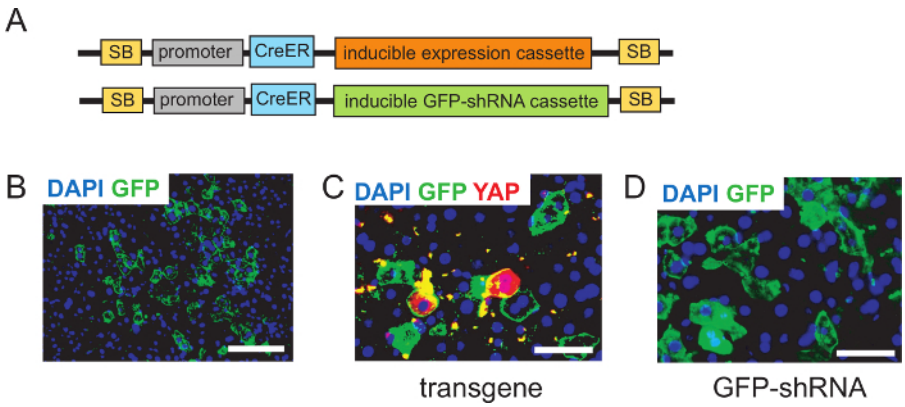

Figure 3: Combined CreER and inducible gene/shRNA expression. (A) Transposon construct for expression of inducible Cre recombinase together with expression of a tetracycline-inducible transgene or shRNA (pTC Tet), not drawn to scale. **(B)** Green membrane staining indicates transfected hepatocytes after injection of the pTC Tet construct into *Rosa26*$^{mTmG/+}$ reporter mice and CreER activation with tamoxifen. Scale bar represents 100 µm. **(C)** Inducible gene expression 5 days after doxycycline treatment can be visualized by immunostaining for the transgene (YAP, red) in transfected hepatocytes. **(D)** Inducible miR-shRNA expression 5 days after doxycycline treatment indicated by cytoplasmic green fluorescent protein (GFP) staining of the GFP-shRNA construct. Transfected hepatocytes are depicted by membrane-bound GFP. Scale bars in C) and D) represent 50 µm. Please click here to view a larger version of this figure.

Discussion

Transfection of hepatocytes with hydrodynamic tail vein injection has become an established method since its introduction more than 15 years ago[6]. The injected volume exceeds cardiac output and flows from the inferior vena cava into the sinusoids of the liver[7], leading to transfection of about 10-20%, in some cases up to 40% of hepatocytes[25,26]. Predictors of a successful transfection are the injected volume per injected time[22,23]. Hence, a low transfection efficiency (**Figure 1A**, left panel) is usually due to the failure to maintain the injection speed during the procedure[7]. However, even with an optimal technique, the transfection efficiency that can be achieved by HTVI will remain below the rate obtained by viral infection with adenoviruses or adeno-associated viruses that can reach almost 100%[5,27]. To achieve successful tail vein injections, it is critical to ensure a stable positioning of the needle in the blood vessel. This is easily achieved in veins with larger diameter closer to the base of the tail. Additionally, dilation of the vein by warming up the tail is highly recommended. The best results are achieved using an infrared lamp, but a non-infrared heat lamp or immersion of the tail in warm water may also be used. In some cases, supplementing up to 200 µL of saline by intraperitoneal injection around 30 minutes before HTVI will improve the hydration status of the animals resulting in dilation of blood vessels. To maintain a constant injection speed, the tail of the mouse should be thoroughly restrained to avoid any movement of the tail, which can result in displacement of the needle. For validation purposes, we suggest using a construct that can be detected by immunostaining. Alternatively, transfection efficacy can be estimated by mRNA expression analysis or sequencing of integrated DNA[28].

Our data indicate that transposon integration is preferably observed in the pericentral area of the liver lobule. The predominant transfection of hepatocytes around the central vein is likely due to the unique hemodynamics of HTVI as it is also observed in non-transposon based transfection[29]. This finding might be of relevance for some applications, such as induction of acute liver damage by CCl_4, which primarily affects pericentral hepatocytes. In the context of low transfection efficiency, CCl_4 treatment could therefore lead to significant reduction of the number of transfected hepatocytes. Additionally, HTVI is of limited use to target periportal cells including bile duct cells[15].

In addition to systems that utilize HTVI of transposon constructs to achieve stable expression of a single transgene, we recently presented a system that allows the co-expression of CreER and inducible expression of a gene or a miR-shRNA from a single vector[11]. This system is especially useful to interrogate specific genes in Cre/LoxP-based mouse strains. As transgenes or shRNA constructs are introduced by a fast and reliable recombinational cloning procedure, the system can easily be adapted for screening approaches. The vector system mediates reliable inducible expression *in vivo* that is entirely dependent on the delivery of doxycycline[11,30]. This practical video-based guide provides step-by-step instructions from cloning of suitable vectors over induction of gene expression to analysis of liver tissue.

However, to ensure efficiency of the system, several aspects should be kept in mind: To maintain long term expression, genomic integration is mediated at TA-sites by the *sleeping beauty* transposase[8,11]. Since integration efficiency is dependent on transposon size, it is important to keep the size of the transgene construct in mind when designing the vector[31]. Furthermore, expression efficiency of the inducible gene product in the liver is dependent on the rtTA3-promoter[11]. For optimal expression results, use of a vector construct with a liver specific ApoE.HCR.hAAT-promoter is recommended (Addgene #85578), as it shows the highest efficiency in a comparison of three promotors[11]. With an optimized promoter construct, inducible transgene/shRNA expression can be detected by immunostaining in up to 30% of transfected cells[20]. If inducible protein levels exist below a certain threshold that is required for detection by immunostaining, this needs to be determined. Importantly, no transgene/shRNA expression can be detected by immunostaining in mice that were not treated with doxycycline. Lastly, expression of genes under control of the tetracycline response element (TRE) is dependent on the dose of doxycycline[32,33]. For short term experiments, doxycycline administration via drinking water is well established[34,35]. Sucrose is usually added to give a better taste. However, this may lead to polydipsia and dehydration, and the use of doxycycline chow is highly recommended for long-term experiments[36,37].

In summary, hydrodynamic tail vein injection is a widely-established method in liver research. Its application ranges from studies of hepatitis B to liver fibrosis or hepatocellular carcinoma models[38,39,40,41]. The system described in this manuscript is especially useful in the interrogation of specific target genes in Cre/LoxP-based models of liver disease. Additionally, overexpression in the liver may also be used for research of hematologic diseases[42,43] or to tackle immunologic questions[44,45]. Beyond the analysis of specific genes of interest, the presented system can also be easily adapted to screening or multiplexing approaches. This video-based guide will therefore be helpful for a large community of researchers.

Disclosures

The authors have nothing to disclose

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