Lcn5 Promoter Directs the Region-Specific Expression of Cre Recombinase in Caput Epididymidis of Transgenic Mice

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ABSTRACT

Epididymis plays a crucial role in regulating the development of sperm motility and fertilizing capacity. To study the function of genes in the caput epididymidis using the Cre/loxP system, we generated Lcn5-Cre transgenic mice in which the expression of Cre recombinase is driven by the 1.8-kb Lcn5 promoter. A total of 11 founder mice carrying the Lcn5-Cre transgenic mice were identified by PCR from 38 offspring, and the integration efficiency was 28.9%. However, only 1 of the 11 transgenic mouse lines were revealed with the Cre recombinase expressed specifically in caput epididymidis. Furthermore, expression of Cre protein distribution was assessed by crossing Lcn5-Cre transgenic mice with Aip1flox/+ mice. In summary, a transgenic mouse line expressing Cre recombinase in principal cells of the caput epididymidis was further confirmed using Lcn5-Cre mice crossed with a mouse strain carrying Aip1 conditional alleles (Aip1flox/+). In summary, a transgenic mouse line expressing Cre recombinase in principal cells of caput epididymidis was established. This transgenic mouse line can be used to generate conditional, caput epididymidis-specific knock-out mouse models by crossing with mice harboring floxed (LoxP flanked) genes.

caput, Cre recombinase, epididymis, Lcn5 promoter, male reproductive tract, transgenic/knockout model

INTRODUCTION

The epididymis is a tubular organ that plays an important role in sperm concentration, maturation, transport, and storage. Based on histological and ultrastructural differences, the mouse epididymis can be grossly divided into three regions, each with distinct functions: the initial segment, the caput (head), corpus (body), and cauda (tail). Each epididymal region carries out distinctive functions, with the caput and corpus carrying out early and late sperm maturation events, respectively, while the cauda epididymis primarily serves as a storage site for functionally mature spermatozoa [1, 2]. In some species, such as the mouse, the most proximal epididymal region is also known as the initial segment, which is important for sperm function [1, 2]. The mouse epididymis can be further divided into 10 intraregional segments. After analysis of gene expression data obtained using microarray experiments, researchers found 2168 genes that are up-regulated or down-regulated by greater than 4-fold between at least two different segments [3]. The importance of understanding epididymal functions and sperm maturation is emphasized by the fact that up to 40% of infertile men exhibit idiopathic infertility that may reflect sperm maturation or epididymal disorders [1]. Despite the considerable research effort, the molecular and biochemical events that are integral for epididymal sperm maturation are largely unknown.

An effective way to annotate epididymal gene functions is to knock out (KO) epididymis-specific genes in mice and to assess the consequences on male fertility. Using conventional KO strategies, targeted mutations of some genes that were expressed in the mouse epididymis can reveal the link between epididymis abnormality and infertility. For instance, after deleting the receptor tyrosine kinase c-ros gene, the only abnormalities in these homozygous mutant males is developmental failure of initial segment and infertility. Thus, these mice can be used as a model for the study of epididymal regulation of sperm function [4]. Deletion of other genes, such as Bmp8a [5], HE6 [6], ER-α [7], Foxl1 [8], Inha [9], Dusp6 [10], etc., also caused sperm maturation or epididymal disorders. Although these conventional KO models have provided clear answers to physiologic questions, it should be noted that most mice with deletion of epididymis-specific genes generated by conventional KO approaches do not show fertility failure. The possible reason is that the more important a gene is, the more compensatory mechanisms will have been created during evolution [11]. In this regard, our laboratory used the RNA interference (RNAi) approach to repress epididymis-specific target gene expression in vivo as a transient conditional gene knockdown tool [12, 13]. However,
**Table 1.** Primers used in this study.

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<th>Size (bp)</th>
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</table>

*Note: Multiple PCR primer pairs for given target genes.

RNAl strategies that depend on delivery strategies and only knock down gene expression may not work effectively. Moreover, there are many ubiquitous genes that play important roles in epididymis; deleting those genes through conventional gene targeting strategies may face complex problems, such as embryo lethality. Thus, the spatially and temporally conditional KO approach is the only ideal approach.

Using a knock-in strategy, in which Cre recombinase coding complementary DNA (cDNA) was inserted into the epididymis-specific gene locus (e.g., Crisp [14], Defb41 [15], and Rnase10 [16]), epididymis-specific Cre recombinase transgenic mouse models have been established. However, these lines of transgenic mice were characterized with the predominant Cre mRNA in the initial segment. As described above, epididymis is composed of four main anatomical regions, which possess distinct gene expression profiles, ensuring different epididymal functions essential for the different steps of sperm maturation [17, 18]. The caput epididymis is a region in which sperm begin acquiring forward motility. Therefore, to clarify gene-specific roles in caput epididymis, a caput epididymis-specific Cre mouse line is needed.

The murine epididymal retinoic acid-binding protein, also known as Lcn5, is specifically synthesized and secreted by the principal cells of the middle/distal caput epididymis. In addition, the expression of Lcn5 gene was detected from 30 days and progressively increased until 60 days of age [19]. In previous studies, it was demonstrated that the promoter fragment of the Lcn5 gene can dictate androgen-dependent and epididymis region-specific gene expression in vivo [20, 21]. The 5-kb promoter fragment of the Lcn5 gene was also successfully used to establish transgenic mice overexpressing mBin1b in caput epididymis [22]. Here, we describe the generation of the caput epididymis-specific Cre transgenic mouse by using Lcn5 promoter, as well as the temporal and spatial patterns of Cre expression in these mice.

**Materials and Methods**

**Chimeric Constructs**

The transgenic vector pUBC was double digested with the restriction enzymes Mlu I and Avr II to excise a 167-bp fragment consisting of the mammalian ubiquitin C (UBC) promoter sequence. Additionally, the multiple cloning sites in pUBC vector expression were modified by replacing Avr II site with Kpn I. The 1.8-kb promoter of mouse Lcn5 was amplified from pBLCAT-5m-RABP plasmid [20] with introduction of Mlu I and Kpn I sites at each end using primers lcn5-pro-S and lcn5-pro-A (listed in Table 1). The PCR product was digested with Mlu I and Kpn I. Subsequently, the 1.8-kb Lcn5 promoter was ligated into the digested promoterless pUBC plasmid using standard methods. The coding sequence of Cre was excised from the expression vector pCAg-Cre [23] with Not I and EcoR I, and the coding sequence of Cre was ligated into the modified pUBC plasmid. The new construct was named Lcn5-Cre. All restriction endonucleases were purchased from New England Biolabs (Ipswich, MA).

**Cell Culture and Transfection Assays**

Human embryonic kidney cell line 293T (HEK 293T) cells were maintained in Dulbecco modified Eagle medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco). Cells were inoculated the day before transfection, which was performed at ~60% cell confluence using FuGENE HD Transfection Reagent (Roche, Switzerland) based on the manufacturer’s instructions. Because the promoter of Lcn5 gene was regulated by androgens, pcDNA3.1-AR expression vector coding for full-length human androgen receptor (AR) was cotransfected and dihydrotestosterone (Sigma-Aldrich) was added [20]. To detect the activity of Cre recombinase driven by Lcn5 promoter in vitro, the Cre activity reporter vector UBb-DsRed-emGFP was used (a kind gift from Dr. Si-Tse Jiang from the National Laboratory Animal Center, Taiwan). A total of 2 μg of UbB-DsRed-emGFP, 5 μg of Lcn5-Cre, and 1 μg of pcDNA3.1-AR plasmids were cotransfected for each well in a six-well plate. At 48 hours after transfection, dihydrotestosterone (1000 x stock solutions in dimethyl sulfoxide) was added to a final concentration of 1 nM. After 12 additional hours of incubation, fluorescence images of the cells were taken with a CKX41 microscope (Olympus, Japan) equipped with appropriate filters and processed by DP (Digital Performer) controller software. In all experiments where multiple plasmids were used, the total plasmid amount was kept constant by supplementing with the empty vector.

**Transgenic Mice**

The protocol conformed to internationally accepted guidelines for the care and use of laboratory animals. All research involving animals was conducted according to the approval of the Institutional Animal Care Committee of Shanghai Institute of Biochemistry and Cell Biology (permit no. SYXK2007-0017). The Lcn5-Cre transgenic construct was excised from the expression vector backbone by SgrD I and Fsp I digestion and purified using QIAquick Gel Extraction Kit (Qiagen, Germany). Transgenic mice (C57BL/6 background) were generated by microinjection of the DNA into the male pronucleus of fertilized oocytes using standard techniques [24]. Transgenic animals were then identified by PCR-based screening assay using genomic DNA isolated from mouse tail. For PCR analysis, DNA fragments were amplified for 32 cycles (94°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec). The Cre mRNA was detected by PCR amplification using primer pairs Tg-Cre-S and Tg-Cre-A (listed in Table 1), and 481-bp PCR products were analyzed on a 2% (w/v) agarose gel. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a genomic DNA quality control (primers listed in Table 1).

**Hematoxylin and Eosin and Immunofluorescence Staining**

Tissues collected from Postnatal Day 10 (P10) to 6-month-old wild-type (WT) and Lcn5-Cre transgenic mice were fixed in the Bouin solution (Sigma-
Aldrich) and kept at room temperature for more than 4 h were then embedded in paraffin and prepared for histological analyses. Hematoxylin and eosin staining was performed by standard procedures. To visualize the enhanced green fluorescence protein (EGFP) and red fluorescence protein signals in the epididymis, immunofluorescence assay was performed. Samples collected from Lcn5-Cre; mt/mG double transgenic mice [25] were embedded in Tissue-TekOCT (Sakura, USA), snap frozen in liquid nitrogen, frozen sections (6-μm thick) cut using a CM1950 (Leica, Germany) on “double plus” slides, and air dried at room temperature. The frozen sections were blocked with 10% normal horse serum for 20 min at room temperature. The slides were then incubated with the chick anti-water channel protein 9 (AQP9) 1:100 (AIP1flox/+; Abcam) antibody overnight at 4°C. The slides were rinsed thoroughly with PBS. DiLight 405 AffiniPure Donkey Anti-Chicken IgY (700-475-155; Jackson Immunoresearch Laboratory) secondary antibody was added to be added at room temperature for 30 min. Nuclei were visualized using ToPro3 I:1000 (T3605; Molecular Probes Inc.), then mounted with Permount Mounting Medium and analyzed by SZX10 microscope (Olympus, Japan). Confocal images were captured with an Olympus FV1000 laser confocal microscope (Olympus, Japan) using a UPLFLN 40×, numerical aperture, 1.30 objective. Images were deconvoluted with DP controller software. Tissues collected from at least three different animals were analyzed and the results were consistent.

Sample Collection, RNA Extraction, and RT-PCR

Cotransfection of Lcn5-Cre and pcDNA3.1-AR plasmids into HEK 293T cells was performed as described above. At 48 hours after transfection, the total RNA was extracted from the cells using TRinzol (Life Technologies). The cDNA was synthesized using the ReverTra Ace-α (FSK-100; ToyoBo, Japan) according to the manufacturer’s instructions. For PCR analysis, cDNA was amplified for 32 cycles (94°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec). The Cre mRNA was detected by using the same primers (Tg-Cre-S and Tg-Cre-A) as described above, which yielded a fragment of 481 bp. The pCAG-Cre expression vector was used as a positive control.

To screen Lcn5-Cre transgenic mouse lines that specifically expressed Cre mRNA in the caput epididymidis, multiple tissues were collected from 60-day-old WT and transgenic mice. Samples were immersed in liquid nitrogen immediately after collection and then stored at −80°C. Total RNA was isolated from different tissues using the TRIzol. RT-PCR was conducted as described above. Mouse endogenous Lcn5 gene, which was specifically expressed in the caput epididymidis, was used as a positive control. The primers were Lcn5-CDS-S and Lcn5-CDS-A (listed in Table 1). For PCR analysis, cDNA was amplified for 25 cycles (94°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec). PCR products were analyzed on a 2% (w/v) agarose gel.

Real-Time PCR Assays

For further detection of Cre mRNA expression in mouse caput epididymidis during postnatal development, the caput epididymidis from the transgenic mice at 10, 20, 30, 40, 50, 60, and 70 days were collected. RNA extraction and cDNA synthesis was performed as described above. Real-time PCR was performed using a standard SYBR Green PCR kit (Toyobo, Japan) on the Rotor-Gene 3000 Real Time Thermal Cycler (Corbett Life Science). The Cre mRNA was detected using another set of primers (Ecre-S and Ecre-A; listed in Table 1) that amplified a fragment of 155 bp, and β-actin gene was used as the internal control (primers listed in Table 1). All reactions were performed in triplicate. The 2−ΔΔCt method was used to determine differences in the expression levels of the tissues examined [26].

Identification of Recombinant Alleles Using PCR Amplification

The Lcn5-Cre transgenic mice were crossed with “Floxed” Aip1 allele (Aip1lox/lox). Genomic DNA were isolated from multiple tissues of Lcn5-Cre; Aip1lox/lox mice. The recombinated alleles were detected by using primers AIP1-KO-F and AIP1-KO-R (listed in Table 1), which flank both the 5’ and 3’ loxP sites, resulting in one 3.4-kb product for the WT allele and a 348-bp band for the recombinant allele. 5S RNA was used as genomic DNA quality control (primers listed in Table 1).

![FIG. 1. The Cre recombinase activity examination in vitro. a) A schematic representation of the Lcn5-Cre vector. The 1.8-kb promoter from the mouse Lcn5 (also named E-RABP) was cloned upstream of the Cre cDNA, and a polyadenylation signaling sequence (bGH-pA) was added to generate the transgenic vector. b) Cre mRNA expression in HEK 293T cells under control of a caput epididymidis-specific promoter from Lcn5. AR, pcDNA3.1-AR plasmid; NTC, nontemplate control. Positive control, the pCAG-Cre plasmid, was used as template. c) Cre recombinase activity analysis in HEK 293T cells transfected with Lcn5-Cre vector.](Image 144x525 to 468x738)

![FIG. 2. Identification of Lcn5-Cre transgenic founders. A total of 11 transgenic founders from 38 total offspring were identified through Cre gene amplification by PCR.](Image 315x80 to 566x176)
Statistical Analyses

All experiments were conducted at least three times. Differences between groups were considered significant at $P < 0.05$, as analyzed by a Student t-test. Error bars presented in figures represent standard deviations.

RESULTS

Generation of Lcn5-Cre Transgenic Mouse Lines

The Lcn5-Cre transgenic vector mainly contains three elements: the 1.8-kb promoter fragment of the Lcn5 gene, the 1032-bp cDNA fragment of Cre recombinase coding sequence, and bovine growth hormone polyadenylation fragment (bGH-pA), which was constructed as described in the Materials and Methods section (Fig. 1a). This expression vector was transfected into HEK 293T cells to check whether Lcn5 promoter in the construct could drive the Cre gene transcription. The results showed that Cre mRNA could be detected at high levels in HEK 293T cells by RT-PCR assay (Fig. 1b). To examine further the Cre recombinase activity in vitro, a construct with fluorescent indicators (UBb-DsRed-emGFP) was cotransfected with Lcn5-Cre and pcDNA3.1-AR plasmid into HEK 293T cells, which expressed red fluorescence prior to and green fluorescence following Cre-mediated recombination in widespread cell types. This feature allows convenient determination of whether the Cre recombinase is active. The results showed that when transfected UBb-DsRed-emGFP plasmid alone, only red fluorescence was detected, but after cotransfected UBb-DsRed-emGFP Lcn5-Cre and pcDNA3.1-AR plasmids, red fluorescence was reduced remarkably, while green fluorescence appeared (Fig. 1c), indicating that Lcn5-Cre transgene could drive the expression of the active Cre recombinase and eliminate the DsRed fragment.

Subsequently, the 9783-bp DNA fragment of Lcn5-Cre transgene was introduced into fertilized zygotes by microinjection. The injected eggs were implanted into the oviducts of female mice. Among 38 offspring, 11 transgenic founders with positive insertion were detected by PCR (Fig. 2), indicating that the integration efficiency of Cre gene was 28.9%.

Specific and Elevated Expression of Cre mRNA in the Caput Epididymidis During Postnatal Development

To check the spatial and temporal expression patterns of Cre recombinase in the Lcn5-Cre transgenic mice, different types of tissues were collected from 60-day-old WT and transgenic mice. Fortunately, one of the 11 transgenic mouse lines revealed the specific expression of Cre recombinase in the caput epididymidis, according to RT-PCR analysis (Fig. 3a). Next, the time course change of the Cre mRNA expression in transgenic males was observed. As shown in Figure 3b, the mRNA expression of Cre recombinase in caput epididymidis started at Day 30, and was significantly up-regulated during the first 2 mo of epididymis development. In the following days, the expression level of mRNA declined, but was still maintained at a relatively high level. These results were further confirmed by real-time PCR assay (Fig. 3c).
on 10- and 30-day-old transgenic males, respectively. As shown in Supplemental Figure S1 (all Supplemental Figures are online at www.biolreprod.org), no Cre expression was detected in any selected tissues at Day 10. Cre was expressed specifically in the caput epididymidis at Day 30. The spatial and temporal expression patterns of Cre mRNA exactly mimicked that of the Lcn5 (see Supplemental Fig. S1). Subsequently, the expression level of Lcn5 gene on caput epididymidis in 70-day-old males was compared with that of WT males. The results showed that there was no significant difference between transgenic and WT males ($P = 0.38$), indicating that Cre recombinase itself did not affect endogenous Lcn5 expression (see Supplemental Fig. S2).

**Monitoring Cre-Mediated Excision In Vivo by Using a Cre-Reporter Mouse Strain**

To screen for Cre activity, Lcn5-Cre mice were crossed to the reporter strain, mT/mG. These mice which possess loxP sites on either side of a membrane-targeted tdTomato (mT) cassette, could express strong red fluorescence in all tissues and cell types examined [25]. When mT/mG transgenic reporter mice bred with Lcn5-Cre mice, resulting offspring had the mT cassette deleted, allowing expression of the membrane-targeted EGFP cassette in the Cre-expressing tissue(s). As shown in Figure 4, the expression of GFP in the Lcn5-Cre; mT/mG double transgenic mice were strictly restricted to the caput epididymidis, while no GFP signals observed in the other selected tissues. Moreover, no GFP signals were visualized in the initial segment.

**FIG. 4. Tissue-specific distribution of active Cre recombinase in Lcn5-Cre; mT/mG double transgenic mice.** The mT/mG, a double-fluorescent Cre reporter mouse, was used in this study. The GFP signals were detected in tissues with recombination. Tissues were collected from 50-day-old Lcn5-Cre; mT/mG mice.

<table>
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*CAPUT EPIDIDYMIDIS-SPECIFIC Cre TRANSGENIC MOUSE*

*Downloaded from www.biolreprod.org.*
corpus, or cauda epididymides, indicating that no recombination events occurred (Fig. 5). Time course analysis of the excision activity of Cre recombinase was further performed by detecting the GFP signals in postnatal development of caput epididymidis from Lcn5-Cre; mT/mG males. The results showed that the GFP signals in caput epididymidis were first observed on P30, and became stronger on Day 60, consistent with the expression pattern of Cre mRNA, which was elevated during postnatal development (see Supplemental Fig. S3).

Previous studies demonstrated that the Lcn5 protein was located in principal cells [19]. To check whether the cellular location of Cre recombinase was exactly the same as endogenous Lcn5 protein, immunofluorescence assay was performed. AQP9, which is expressed specifically in the apical brush border membrane of principal cells [8], was used as a marker for principal cells. The results showed that the GFP signals were colocalized with that of AQP9 (Fig. 5c). Because the GFP protein in the mT/mG mice was fused to a membrane-targeted signal sequence that can make GFP protein move to the cell membrane surfaces (Fig. 5c), the cellular location of GFP signals did not directly represent the location of Cre recombinase protein, which only showed that Cre existed and possessed a recombination activity in the GFP-positive cells. Taken together, the above results indicate that Cre activity was only present in principal cells of mid/distal caput epididymidis.

**Cre Activity Was Further Confirmed by Crossing with Aip1 Floxed Alleles**

To double check the tissue distribution and the excision activity of Cre recombinase in vivo, the Lcn5-Cre transgenic line was crossed with a mouse strain carrying Aip1 conditional alleles (Aip1^{lox/lox}; data not shown). Through Cre-mediated recombination, the DNA fragment between the loxP sites is deleted, resulting in a small PCR product. Thus, a 348-bp fragment corresponding to the recombined allele was detected in the DNA from Lcn5-Cre; Aip1^{lox/lox} double transgenic males, but was absent in that from Lcn5-Cre or Aip1^{lox/lox} mice. Because the WT band is 3.4 kb, when amplifying the gene under the same condition, the larger fragment is much less efficient than the short one; only the short band was detected by PCR amplification. The PCR analysis on a panel of tissues revealed that the Cre-mediated recombination occurred specifically in the caput epididymidis, indicating that the Lcn5-Cre transgenic mice generated in this study expressed the Cre recombinase that can disrupt the floxed gene in vivo (Fig. 6a). We further performed time course analysis of the excision activity of Cre recombinase by detecting the Aip1 deletion at caput epididymidis from 10-, 30-, and 50-day-old Lcn5-Cre; Aip1^{lox/lox} males. The PCR results showed that the deletion occurred in the same patterns as Cre mRNA and protein expression, further confirming that the Cre-mediated recombination event started at Day 30 (Fig. 6b).

**Lcn5-Cre Transgenic Mice Show Normal Development**

Finally, we determined whether this Lcn5-Cre transgenic line itself induced abnormal phenotype in the mouse. Through examining the appearance and behavior of Lcn5-Cre transgenic males and females, no apparent abnormalities were observed. The gross morphology of different tissues from postnatal development transgenic males was then compared between transgenic males and their WT controls. We found that the size...
of selected tissues, such as heart, kidney, and testis, from transgenic mice were normal on P10, P30, P90, and P180 (data not shown). Furthermore, testis and epididymis were chosen for histological analysis to check whether Cre recombinase causes toxicity in these organs. The histological structure of testis and caput, corpus, and cauda epididymides from transgenic mice was apparently normal compared with their WT controls (see Supplemental Fig. S4). The epididymal lumen from Day 90 transgenic males was filled with a large quantity of sperm (see Supplemental Fig. S4). The fertility of both Lcn5-Cre males and females were normal (data not shown), indicating that the sperm and oocyte maturation of transgenic animals are normal. Taken together, these results show that the transgenic mice containing exogenous Cre gene did not affect the development or function of organs.

**DISCUSSION**

In this study, we generated an Lcn5-Cre transgenic mouse strain expressing the Cre recombinase under the control of the 1.8-kb promoter of mouse Lcn5 gene. The spatial and temporal expression patterns of Lcn5-Cre transgene exactly mimicked that previously reported for the endogenous Lcn5 gene [27]. These observations imply that the 1.8-kb Lcn5 promoter contains all the information necessary for this highly cell-specific gene expression and successfully drives the expression of Cre gene.

Based on the feature of the Lcn5-Cre transgenic mouse described above, the Cre recombinase transgenic mice can be used to direct specific gene targeting in principal cells of mid/distal caput during postnatal epididymis development. Because the adult epididymis not only contains principal cells, but also includes basal, clear, narrow, apical, and halo cells [28], the Lcn5-Cre transgenic mice cannot be used to specifically delete genes in the caput epididymidis that were not expressed in principal cells. Considering this limitation, to satisfy the requests for the study of the functions of genes in the epididymis for different purposes, transgenic mice in which expression of Cre recombinase in different epididymal cell types or different epididymal regions, such as the corpus or cauda region, or the whole epididymal region, need to be established in the future.

Consistent with the Lcn5, Cre recombinase expression of the Lcn5-Cre transgenic mice started at Day 30 and became significantly higher on P40 and P50, which suggest it could be used in studying the functions of the target genes in mice in the adult stage. If we want to study further the mechanisms of cellular differentiation of epididymal epithelial cells, the expression of Cre should work in the early stages of postnatal epididymis development because the differentiation of epididymal epithelial cells may finish before Day 49 [29]. In addition, to control Cre activity precisely in the epididymis during development, transgenic mice expressing CreER T2 that is driven by epididymis-specific promoter should be established. The activity of Cre recombinase can then be controlled by tamoxifen drug.

In summary, this study shows that the Cre recombinase in an Lcn5-Cre transgenic mouse model is not only expressed, but also possesses enzymatic activity, as it caused recombination between loxP sites in two floxed lines, mT/mG and Aip1lox/+.

This transgenic mouse line, with caput epididymidis-limited expression of Cre beginning in the late stages of postnatal epididymis development, was established. It should be a useful tool for future functional studies of genes involved in the posttesticular sperm maturation in the epididymis.

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**REFERENCES**


