Cell Metabolism
Perspective

Pancreas-Specific Cre Driver Lines and Considerations for Their Prudent Use

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Cre/LoxP has broad utility for studying the function, development, and oncogenic transformation of pancreatic cells in mice. Here we provide an overview of the Cre driver lines that are available for such studies. We discuss how variegated expression, transgene silencing, and recombination in undesired cell types have conspired to limit the performance of these lines, sometimes leading to serious experimental concerns. We also discuss preferred strategies for achieving high-fidelity driver lines and remind investigators of the continuing need for caution when interpreting results obtained from any Cre/LoxP-based experiment performed in mice.

Cre/LoxP and Its Use in Mice

Cre/LoxP is a site-specific recombinase (SSR) system of proven utility. As for many tissues, Cre/LoxP is frequently used to study the function, development, and neoplasia of exocrine and endocrine cells in the pancreas. The widespread use of Cre/LoxP arises from its ability to conditionally eliminate or activate expression of genes in a cell-type- and/or temporal-specific manner, thereby enabling the cell-, tissue-, and/or developmental-stage-specific functions of genes to be explored within animal models. While Cre/LoxP is most commonly used in mice, it and two other SSR systems, Flp/FRT and Dre/Rox, also have utility in other model organisms (Hoess et al., 1982; McLeod et al., 1986; Sauer and McDermott, 2004).

Cre, Flp, and Dre, members of the λ integrase superfamily of site-specific recombinases, were cloned from different organisms. Cre is encoded by bacteriophage P1, Flp by the budding yeast *Saccharomyces cerevisiae*, and Dre, the most recently described SSR, by bacteriophage D6. All three recombinases function as homotetramers and have 34 bp DNA recognition sequences called LoxP, FRT, and Rox, respectively. The small size of these recombination recognition sites enables them to be readily placed within genes where they, in combination with Cre, Flp, or Dre, enable gene deletions, insertions, inversions, or translocations.

Over the past two decades, several useful Cre and Flp derivatives have been described. Undoubtedly, the most useful variant for Cre has been Cre^{ER}, which prevents Cre from entering the nucleus in the absence of tamoxifen due to the addition of a mutated version of the estrogen receptor (ER) hormone-binding domain (Feil et al., 1996). Cre^{ER} enables temporal control of Cre recombination. However, some investigators have found that efficient tamoxifen-induced recombination is not always achieved, thereby requiring that multiple doses of tamoxifen be administered, and that recombination by Cre^{ER} may occur weeks after tamoxifen dosing (Reinert et al., 2012). Another useful derivative of Cre is GFPCre, which is a fusion with green fluorescent protein (GFP) that makes it easy to directly identify cells that express Cre (Gagneten et al., 1997). For Flp, the most useful derivatives are enhanced Flp (FlpE), which improves thermostabilty, and Flpo, a codon-optimized variant that improves expression (Buchholz et al., 1998; Raymond and Soriano, 2007). Although both Flp and Dre also have utility in the mouse, particularly when used in combination with Cre, hereafter we focus our comments on Cre.

As illustrated in Figure 1, the conditional inactivation of a gene using Cre/LoxP requires two different genetic components: (1) a Cre driver line and (2) a target allele in which a gene segment, usually containing one or more exons, has been flanked with tandemly oriented LoxP sites (a so-called floxed allele) (Gu et al., 1994; Orban et al., 1992). Similarly, the required components to conditionally activate gene expression are (1) a Cre driver line and (2) an allele that has been engineered to contain a lox-stop-lox (LSL) sequence upstream of the coding sequences to be expressed. The ubiquitously expressed ROSA26 gene locus has been used extensively for this purpose (Soriano, 1999). Indeed, Cre-dependent activation of ROSA26 alleles containing an LSL upstream of β-galactosidase or different fluorescent proteins (e.g., Gt[ROSA]26Sor^{tm1Sor}, Gt[ROSA]26Sor$^{tm1[EYFP]Cos}$, and Gt[ROSA]26Sor^{tm2Sho}; Mao et al., 2001; Soriano, 1999; Srinivas et al., 2001) has become a standard tool in the Cre/LoxP tool chest. Not only do these alleles enable cell lineage tracing, which is fundamentally important in studies of developmental biology, they can also be used to readily assess both the sites and efficiency of Cre-mediated recombination (Sato et al., 2000).

For many years, the development of new floxed alleles was the limiting factor in using Cre/LoxP to perform a cell- or tissue-specific gene knockout study. This was due to the need to perform gene targeting in mouse embryonic stem cells (mESCs) and then to introduce the mutant allele into the germline of mice. However, as a result of the combined efforts of many individual laboratories and the large-scale Knockout Mouse Project (KOMP) (Austin et al., 2004), the number of floxed alleles available to investigators has skyrocketed. In contrast, there are fewer truly accurate and reliable Cre driver lines, as we discuss in detail below.

Optimal use of the Cre/LoxP depends greatly on the functional precision of the Cre driver line, which is determined in large part

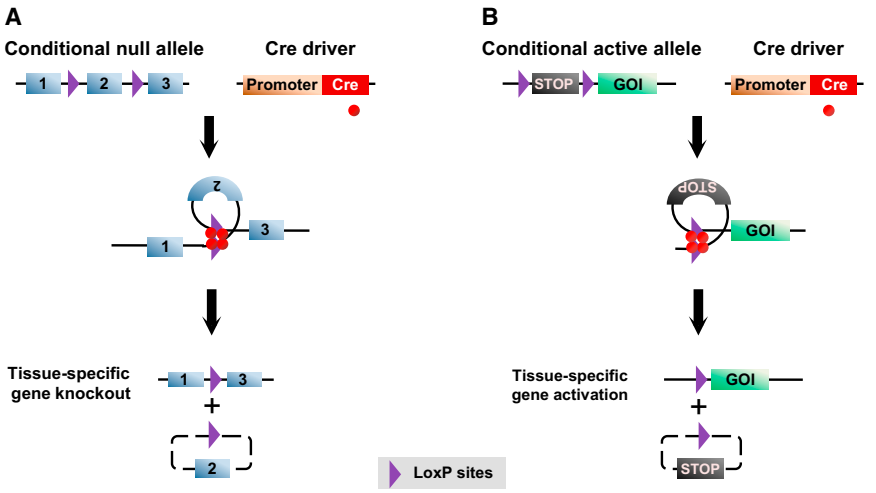

Figure 1. Cre-Mediated Recombination in Mouse Tissues

(A and B) Cre/LoxP can be used to conditionally eliminate or activate expression of genes. (A) Conditional gene knockout. A so-called floxed allele is generated by using gene targeting to flank a coding exon of a gene of interest with two tandemly oriented LoxP sites. Ideally, the codon that is floxed should be of a length that is not divisible by three since this will cause a frameshift in the protein being encoded. Intercrossing of a Cre driver mouse with a floxed-allele mouse will lead to the excision of the flanked exon and loss of a functional protein. (B) Conditional gene activation. A lox-stop-lox (LSL) allele is also generated by gene targeting. In this case, a gene of interest (GOI) is engineered to lie downstream of an LSL cassette containing tandem LoxP sites flanking a selectable marker, usually neomycin, and multiple poly(A) signal sequences. Interbreeding of the LSL-GOI allele to a Cre driver mouse leads to activation of the GOI in a tissue-specific manner. This strategy is frequently used to derive reporter lines whose expression can be activated by Cre.

by the method used to derive the line. Moreover, the functionality of some lines, particularly those made by pronuclear DNA microinjection of short transgenes, can be impaired or destroyed due to transgene silencing as the lines are passaged. All of these factors argue for caution when acquiring and using lines, especially those that do not have a proven history of use. Indeed, when using any line, investigators need to remain keenly aware of the limitations of Cre/LoxP itself, in addition to the known deficiencies of a given line, before drawing scientific conclusions from any experiments that utilize this method.

Pancreas-Specific Cre Driver Lines

An almost certainly incomplete list of Cre driver lines that have been used in studies of pancreas development and/or function is shown in Table 1. These 79 driver lines, which were identified based on either published descriptions or the Beta Cell Biology Consortium website (www.betacell.org), have been arbitrarily subdivided into four partially overlapping categories based on the cell types in which Cre is expressed. Together, these lines have utilized the promoters of 32 different genes to direct the expression of Cre.

The first three categories of pancreas-specific Cre driver genes, endocrine, exocrine, and ductal, reflect the three main epithelial-derived cell types of the pancreas. These cell types are easily distinguished by the genes they express. For instance, peptide hormones have long been used to define individual cells of the pancreatic islet and digestive enzymes have been used to mark pancreatic exocrine cells. The fourth category, pancreatic progenitor cells, is the most diverse since it contains Cre driver lines that, while they may have been derived for studies of pancreas development, may also be of utility for studies of pancreatic function. The utility of this latter group partially stems from the identification of a series of transcription factors that are temporally expressed in the pancreatic or prepancreatic endoderm during embryogenesis but that later become restricted to specific adult cell lineages, as illustrated in Figure 2.

Endocrine Cell-Type-Specific Driver Lines

Pancreas-specific endocrine cells have long been defined by the expression of five different hormones: glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide (PP), which mark α, β, δ, ε, and PP cells, respectively. Thus, genes for each of these hormones have been used to generate many different Cre driver lines. Not surprisingly, of the 21 lines that use endocrine genes to drive Cre expression, 14 utilized an insulin gene to direct expression to pancreatic β cells. Interestingly, most of the insulin-Cre (Ins-Cre) driver lines have utilized promoter sequences from species other than mouse. Indeed, the majority of the reported lines employed the rat *Ins2* gene (often referred to as RIP, rat insulin promoter) (Ahlgren et al., 1998; Crabtree et al., 2003; Dor et al., 2004; Herrera, 2000; Leiter et al., 2007; Postic et al., 1999; Ray et al., 1999), but pig (Dahlhoff et al., 2012) and human (Hamilton-Williams et al., 2003) DNA has also been used.

The most widely used Ins-Cre driver lines have been Tg(Ins2-cre)^{25Mgn}, Tg(Ins2-cre)^{23Herr}, and Tg(Ins2-cre/ERT)^{1Dam}. These lines, all of which are based on the use of short insulin gene promoter fragments (typically about 0.6 kb), are expressed in 80% or more of β cells (Dor et al., 2004; Herrera, 2000; Postic et al., 1999). However, a significant shortcoming of these lines is that they exhibit leaky expression in the brain and other neuroendocrine cell types such as the pituitary gland (Song et al., 2010; Wicksteed et al., 2010). It is not entirely clear whether the leaky transgene expression reflects the absence of an essential regulatory element or is gene specific, given that *Ins2* expression has been reported in certain types of neurons (Madadi et al., 2008). In either case, in an effort to increase β cell specificity, several lines have been derived that utilize longer fragments of DNA containing the insulin promoter, including Tg(Ins2-cre)^{1Heed} (Ahlgren et al., 1998) and Tg(Ins2-cre)^{1Dh}(Crabtree et al., 2003). Interestingly, the Tg(Ins2-cre)^{1Dh} line, despite the use of a longer promoter, appears to be expressed in only about 10%–20% of β cells based on recombination of the Tg(ACTB-Bgeo/ALPP)^{1Lbe} reporter allele (Crabtree et al., 2003). However, the Tg(Ins1-cre/ERT)^{1Lphi} line, which utilizes a fragment of the mouse insulin 1 gene, is active in most β cells and does not exhibit expression in the brain (Wicksteed et al., 2010). Thus, it appears to be the best cell line available at present for achieving β cell-specific recombination in a tamoxifen-inducible manner. Because

Table 1. Pancreas-Specific Cre Driver Lines

MGI Name	Common Name	Type	Driver Gene (Size if Applicable or Known)/ Expression Site(s) in Pancreas	Reference
Endocrine Cell Specific				
Tg(Ins2-cre)^{1Heed}	RIP1-Cre	transgene	rat *Ins2* (0.7 kb)/β cells	Ahlgren et al., 1998
Tg(Ins2-cre)^{6Fcb}	RIP-Cre	transgene	rat *Ins2* (0.45 kb)/β cells	Ray et al., 1999
Tg(Ins2-cre)^{7Fcb}	RIP-Cre	transgene	rat *Ins2* (0.45 kb)/β cells	Ray et al., 1999
Tg(Ins2-cre)^{23Herr}	RIP-Cre	transgene	rat *Ins2* (0.60 kb)/β cells	Herrera, 2000
Tg(Ins2-cre)^{25Mgn}	RIP-Cre	transgene	rat *Ins2* (0.66 kb)/β cells	Postic et al., 1999
Tg(Ins2-cre/ERT)^{1Dam}	RIP-CreER	transgene	rat *Ins2* (0.66 kb)/β cells	Dor et al., 2004
Tg(Ins2-cre)^{1Dh}	RIP7-Cre	transgene	rat *Ins2* (10 kb)/β cells	Crabtree et al., 2003
Tg(INS-cre)^{2Rms}	HIP-Cre	transgene	human *INS* (1.9 kb)/β cells	Hamilton-Williams et al., 2003
Tg(Ins2-cre)^{3Lt}	RIP-Cre3	transgene	rat *Ins2* (0.7 kb)/β cells	Leiter et al., 2007
Tg(Ins2-cre)^{5Lt}	RIP-Cre5	transgene	rat *Ins2* (0.7 kb)/β cells	Leiter et al., 2007
Tg(Ins2-cre)^{6Lt}	RIP-Cre6	transgene	rat *Ins2* (0.7 kb)/β cells	Leiter et al., 2007
Tg(INS-icre)^{18Msdr}	PIP-iCre	transgene	porcine *INS* (1.5 kb)/β cells	Dahlhoff et al., 2012
Ins2$^{tm1(cre/ERT2)Kcmm}$	Ins2^{CreERT2}	knock-in	mouse *Ins2*/β cells	Nakamura et al., 2011
Tg(Ins1-cre/ERT)^{1Lphi}	MIP-CreER	transgene	mouse *Ins1* (8.5 kb)/β cells	Wicksteed et al., 2010
Tg(Slc2a2-cre)	pGlut2-Cre	BAC transgene	mouse *Slc2a2*/β cells	Mounien et al., 2010
Tg(Gck-cre)^{TG7Gsat}	Gck-Cre	BAC transgene	mouse *Gck*/β cells	Gong et al., 2003
Tg(Gcg-cre)^{1Herr}	GLUC-Cre	transgene	rat *Gcg* (1.6 kb)/α cells	Herrera, 2000
Tg(Gcg-cre)^{1Slib}	Glu-Cre	transgene	rat *Gcg* (2.3 kb)/α cells	Shen et al., 2009
Tg(Ppy-cre)^{1Herr}	PP-Cre	transgene	rat *Ppy* (0.6 kb)/PP cells	Herrera, 2000
Sst$^{tm1(cre/ERT2)Zjh}$	Sst-CreER	knock-in	mouse *Sst*/δ cells	Taniguchi et al., 2011
Sst$^{tm2.1(cre)Zjh}$	Sst-Cre	knock-in	mouse *Sst*/δ cells	Taniguchi et al., 2011
Ghrl$^{tm2.1(cre/EGFP)Suss}$	Ghrl$^{Cre-GFP}$	knockin/RMCE	mouse *Ghrl*/ε cells	Arnes et al., 2012
Acinar Cell Specific				
Tg(Amy2-cre)^{1Herr}	SV40/Amy-Cre	transgene	mouse *Amy2a* (0.9 kb)/acinar cells	Kockel et al., 2006
Tg(Cela1-cre/ERT)^{1Lgdn}	BAC-Ela-CreErT	BAC transgene	mouse *Cela1*/acinar cells	Ji et al., 2008
Tg(Ela1-cre/ERT2)^{1Stof}	Ela-CreERT	transgene	rat *Cela1* (0.5 kb)/acinar cells	Desai et al., 2007
Tg(Ela1-cre/ERT)^{1Dam}	Ela-CreERT	transgene	rat *Cela1* enhancer (0.15 kb)-hsp68 promoter/acinar cells	Murtaugh et al., 2005
Tg(Vil-cre)^{20Syr}	Vil-Cre	transgene	mouse *Vil1* (9 kb)/acinar cells	el Marjou et al., 2004
Tg(Vil-cre/ERT2)^{23Syr}	Vil-CreER	transgene	mouse *Vil1* (9 kb)/acinar cells	el Marjou et al., 2004
Tg(Vil-cre)^{1Mka}	Vil-Cre	transgene	mouse *Vil1* (9 kb)/acinar cells	Chen et al., 2003
Tg(Vil-cre)^{997Gum}	Vil-Cre	transgene	mouse *Vil1* (12.4 kb)/acinar cells	Madison et al., 2002
Cpa1$^{tm1(cre/ERT2)Dam}$	Cpa1^{CreERT}	knockin	mouse *Cpa1*/prepancreatic endoderm, acinar cells	Zhou et al., 2007
Ductal Cell Specific				
Krt19$^{tm1(cre/ERT)Ggu}$	CK19^{CreERT}	knockin	mouse *Krt19*/ductal cells	Means et al., 2008
Tg(CA2-cre)^{1Subw}	CAII-Cre	transgene	human *CA2* (1.6 kb)/ductal cells	Inada et al., 2008
Tg(CA2-cre/Esr1*)^{1Subw}	CAII-CreER	transgene	human *CA2* (1.6 kb)/ductal cells	Inada et al., 2008
Muc1$^{tm1.1(cre/ERT2)Lcm}$	Muc1$^{IRES-CreERT2}$	knockin	mouse *Muc1*/acinar, ductal cells	Kopinke and Murtaugh, 2010
Progenitor Cell Specific				
Foxa2$^{tm1(cre)Heli}$	Foxa2^{Cre}	knockin	mouse *Foxa2*/endoderm	Uetzmann et al., 2008
Foxa2$^{tm1.1(icre)Hri}$	Foxa2^{T2AiCre}	knockin	mouse *Foxa2*/endoderm	Horn et al., 2012
Foxa2$^{tm2.1(cre/Esr1*)Moon}$	Foxa2^{Cre-ER}	knockin	mouse *Foxa2*/endoderm	Park et al., 2008
Tg(Foxa3-cre)^{1Khk}	Foxa3-cre	YAC transgene	mouse *Foxa3*/endoderm	Lee et al., 2005
Sox17$^{tm1(icre)Heli}$	Sox17$^{2A-iCre}$	knockin	mouse *Sox17*/endoderm	Engert et al., 2009
Sox17$^{tm2(EGFP/cre)Mgn}$	Sox17^{GFPCre}	knockin/RMCE	mouse *Sox17*/endoderm	Choi et al., 2012
Cldn6$^{tm1(cre/ERT2)Dam}$	Cldn6^{CreER}	knockin	mouse *Cldn6*/endoderm	Anderson et al., 2008

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Table 1. ***Continued***

MGI Name	Common Name	Type	Driver Gene (Size if Applicable or Known)/ Expression Site(s) in Pancreas	Reference
Tg(Ipf1-cre)1^{Tuv}	Pdx1-Cre	transgene	mouse *Pdx1* (4.5 kb)/prepancreatic endoderm	Hingorani et al., 2003
Tg(Pdx1-cre)6^{Cvw}	Pdx1-Cre	transgene	mouse *Pdx1* (4.5 kb)/prepancreatic endoderm	Gannon et al., 2000
Tg(Pdx1-cre)1^{Herr}	Pdx1-Cre^{Late}	transgene	mouse *Pdx1* (4.5 kb)/prepancreatic endoderm	Herrera, 2000
Tg(Pdx1-cre)^{1Heed}	Ipf1-Cre	transgene	mouse *Pdx1* (4.5 kb)/prepancreatic endoderm	Steneberg et al., 2005
Tg(Pdx1-cre)^{PBMga}	Pdx1^{PB}-Cre	transgene	mouse *Pdx1* enhancer (1 kb PstI-BstbII fragment, areas I and II)-Hsp68 promoter/ embryonic and adult endocrine cells	Wiebe et al., 2007
Tg(Pdx1-cre)^{XBMga}	Pdx1^{XB}-Cre	transgene	mouse *Pdx1* enhancer (1.1 kb XhoI-BglII fragment, area III)-Hsp68 promoter fusion/ prepancreatic endoderm, endocrine cells	Wiebe et al., 2007
Tg(Pdx1-cre/Esr1*)^{1Mga}	Pdx1^{PB}-CreER^{TM}	transgene	mouse *Pdx1* enhancer (1 kb PstII-BstEI fragment, areas I and II)-Hsp68 promoter/ embryonic and adult endocrine cells	Zhang et al., 2005
Tg(Pdx1-cre)$^{89.1Dam}$	Pdx1-Cre^{Early}	transgene	mouse *Pdx1* (5.5 kb)/prepancreatic endoderm	Gu et al., 2002
Tg(Pdx1-cre/Esr1*)$^{35.6Dam}$	Pdx1-CreER	transgene	mouse *Pdx1* (5.5 kb)/prepancreatic endoderm	Gu et al., 2002
Tg(Pdx1-cre/Esr1*)$^{35.10Dam}$	Pdx1-CreER	transgene	mouse *Pdx1* (5.5 kb)/prepancreatic endoderm	Gu et al., 2002
Tg(Pdx1-cre/Esr1*)$^{\#Dam}$	Pdx1-CreER	transgene	mouse *Pdx1* (5.5 kb)/prepancreatic endoderm	Gu et al., 2002
Ptf1a$^{tm1(cre)Hnak}$	Ptf1a$^{Cre(ex1)}$	knockin	mouse *Ptf1a*/prepancreatic endoderm, acinar cells	Nakhai et al., 2007
Ptf1a$^{tm1.1(cre)Cvw}$	Ptf1a^{Cre}	knockin/RMCE	mouse *Ptf1a*/prepancreatic endoderm, acinar cells	Kawaguchi et al., 2002
Ptf1a$^{tm2(cre/ESR1)Cvw}$	Ptf1a$^{Cre-ERTM}$	knockin/RMCE	mouse *Ptf1a*/prepancreatic endoderm, acinar cells	Kopinke et al., 2012
Tg(Sox9-cre/ERT2)^{1Msan}	Sox9-CreER	BAC transgene	mouse *Sox9*/prepancreatic endoderm, ductal cells	Kopp et al., 2011
Sox9$^{tm1(cre/ERT2)Haak}$	Sox9^{CreERT2}	knockin	mouse *Sox9*/prepancreatic endoderm, ductal cells	Soeda et al., 2010
Sox9$^{tm3(cre)Crm}$	Sox9-Cre	knockin	mouse *Sox9*/prepancreatic endoderm, ductal cells	Akiyama et al., 2005
Tg(Hnf1b-cre/ERT2)^{1Jfer}	Hnf1b-CreER	transgene	mouse *Hnf1b*/prepancreatic endoderm, ductal cells	Solar et al., 2009
Tg(Neurog3-cre)^{1Dam}	Ngn3-Cre	transgene	mouse *Neurog3* (6.5 kb)/pre-endocrine cells	Gu et al., 2002
Tg(NEUROG3-cre)^{1Herr}	NGN3-Cre	transgene	human *NEUROG3* (5.7 kb)/pre-endocrine cells	Desgraz and Herrera, 2009
Tg(Neurog3-cre)^{24Syos}	Ngn3-Cre	transgene	mouse *Neurog3* (23 kb)/pre-endocrine cells	Yoshida et al., 2004
Tg(Neurog3-cre)^{C1Able}	Ngn3-Cre	BAC transgene	mouse *Neurog3*/pre-endocrine cells	Schonhoff et al., 2004
Tg(Neurog3-cre/Esr1*)^{1Dam}	Ngn3-CreER	transgene	mouse *Neurog3* (6.5 kb)/pre-endocrine cells	Gu et al., 2002
Neurog3$^{tm1.1(cre/ERT)Ggu}$	Neurog3^{CreERT}	knockin	mouse *Neurog3*/pre-endocrine cells	Wang et al., 2008b
Tg(Nkx2-2*-cre)^{1Mtse}	Nkx2.2-Cre	transgene	mouse *Nkx2.2* (1.2 kb)/prepancreatic endoderm, β cells	Wang et al., 2011
Tg(Nkx2-2-cre/ERT2)^{1Wdr}	Nkx2.2-CreER	BAC transgene	mouse *Nkx2.2*/prepancreatic endoderm, β cells	Tsai et al., 2012

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Table 1. *Continued*

MGI Name	Common Name	Type	Driver Gene (Size if Applicable or Known)/ Expression Site(s) in Pancreas	Reference
Mnx1$^{tm4(cre)Tmj}$	Hb9-Cre	knockin	mouse *Mnx1*/prepancreatic endoderm, endocrine cells	Yang et al., 2001
Myt1$^{tm1(EGFP/icre)Ldh}$	Myt1$^{GFP-Cre}$	knockin	mouse *Mnx1*/prepancreatic endoderm, endocrine cells	Hudson et al., 2011
Tg(Neurod1-cre)^{RZ24Gsat}	Neurod1-Cre	BAC transgene	mouse *Neurod1*/endocrine cells	Gong et al., 2003
Tg(Pax4-cre)^{1Dam}	Pax4-Cre	transgene	mouse *Pax4*/endocrine cells	Greenwood et al., 2007
Tg(Pax4-cre,GFP)^{1Pgr}	Pax4-Cre	transgene	mouse *Pax4* (0.4 kb)/endocrine cells	Brink and Gruss, 2003
Pax4$^{tm1(cre/ERT2)Sosa}$	Pax4^{CreER}	knockin	mouse *Pax4*/endocrine cells	Wang et al., 2008a
Tg(Pax6-cre,GFP)^{1Pgr}	Pax6-Cre	transgene	mouse *Pax6* (6.5 kb)/endocrine cells	Ashery-Padan et al., 2000
Rfx6$^{tm1.1(EGFP/cre)Mger}$	Rfx6^{eGFPcre}	knockin	mouse *Rfx6*/endoderm, endocrine cells	Smith et al., 2010
Isl1$^{tm1(cre)Sev}$	Isl1^{Cre}	knockin	mouse *Isl1*/endocrine cells	Yang et al., 2006
Isl1$^{tm1(cre)Tmj}$	Isl1^{Cre}	knockin	mouse *Isl1*/endocrine cells	Srinivas et al., 2001
Isl1$^{tm1(cre/Esr1*)Krc}$	Isl1$^{Cre-ERT}$	knockin	mouse *Isl1*/endocrine cells	Laugwitz et al., 2005

many of the most efficient Ins-Cre driver lines exhibit leaky expression in neural tissues, some investigators have turned to the use of Pdx1-Cre, as discussed below, in lieu of an Ins-Cre driver. However, this can only be done when the gene being knocked-out exhibits β cell-specific expression since the *Pdx1* promoter also drives expression in some regions of the brain, though to a lesser extent than the insulin promoter.

Fewer lines exist for other islet endocrine cell types. For α cells, the rat glucagon promoter-driven transgenes have been used to derive two lines, Tg(Gcg-cre)^{1Herr} and Tg(Gcg-cre)^{1Slib}, both of which have been reported to efficiently cause recombination in glucagon-positive cells (Herrera, 2000; Kawamori et al., 2009; Shen et al., 2009). However, some investigators have noted lower efficiencies of recombination than reported, perhaps suggesting transgene silencing. For PP cells, a rat PP-driven transgene has been used in Tg(Ppy-cre)^{1Herr}. Lineage tracing with this PP driver and reporter lines driven by insulin, glucagon, and PP genes indicate that this driver line causes recombination in both PP and β cells (Herrera, 2000). Driver lines for δ and ε cells have been generated by knocking Cre into the *Sst* or *Grhl* genes, respectively (Arnes et al., 2012; Taniguchi et al., 2011). Interestingly, Ghrl^{GFPCre} marks a large proportion of ε and α cells, and 5% of PP cells, but none of the β or δ cells in the adult islet, suggesting that ghrelin-expressing pancreatic progenitor cells contribute to several cell lineages during development (Arnes et al., 2012). Also, it should be kept in mind that glucagon, somatostatin, PP, and ghrelin are all normally expressed in non-pancreatic enteroendocrine cells, and some of these genes are also expressed in the brain, so Cre driver lines using these genes should be expected to exhibit multiple sites of recombination.

Acinar Cell Driver Lines

Cre driver lines for pancreatic acinar cells have generally used transgenes containing the promoter regions of genes for digestive enzymes such as elastase and amylase, but the performance of these lines has frequently been less than optimal. For instance, the Tg(Ela1-cre/ERT2)^{1Stof} line made using the rat elastase promoter to express Cre^{ER} is reported to achieve only about 30%–40% recombination after administration of tamoxifen (Desai et al., 2007; Means et al., 2005). Use of a transgene containing a fusion of elastase 1 enhancer to the *Hsp68* promoter has yielded the somewhat better-performing Tg(Ela1-cre/ERT)^{1Dam} line, which results in at least 50% of cells undergoing recombination after tamoxifen induction (Murtaugh et al., 2005). The highest performing line in this group appears to be that made using a bacterial artificial chromosome (BAC)-based Cela1-Cre^{ER} transgene, since nearly 100% of acini exhibits recombination of a ROSA26 reporter allele after tamoxifen treatment (Ji et al., 2008). The Tg(Amy2-cre)^{1Herr} line, which utilized a fragment from the *Amy2a* gene, also efficiently marks acinar cells. However, due to the expression of *Amy2a* at early developmental stages, this driver line also results in recombination in many ductal and islet cells (Kockel et al., 2006).

The Tg(Vil-cre/ERT2)^{23Syr} line, made using the intestinal-specific villin gene, has also been shown to mark acinar cells. This appears to be due to recombination in visceral endoderm, which results in sufficient residual Cre activity to cause scattered recombination in the intestine, kidney, and pancreas; however, in pancreatic tissues, recombination is seen only in acinar cells and not in endocrine or ductal cells (el Marjou et al., 2004; Means et al., 2005). In addition, both the *Ptf1a*- and *Cpa1*-driven Cre^{ER} lines can be used to target adult acinar cells. Although both of these genes are expressed in pancreatic multipotent progenitor cells (MPCs) during development, they exhibit expression that is restricted to acinar cells within the pancreas of adult animals (Kopinke et al., 2012; Zhou et al., 2007).

Ductal Cell Driver Lines

In contrast to pancreatic endocrine and exocrine cells, the identification of genes that can be used to derive ductal cell-specific Cre driver lines has been more of a challenge. The *Krt19* (CK19), *Muc1*, and *Car2* (CA2) genes, which have all been used in an attempt to derive ductal-specific Cre driver lines, exhibit recombination in other pancreatic cell types besides ductal cells. For instance, Tg(CA2-cre)^{1Subw} causes recombination in adult ductal, acinar, and a small percentage of endocrine cells (5%) (Inada et al., 2008). Similarly, a Muc1^{CreER} knockin allele (Muc1$^{tm1.1(cre/ERT2)Lcm}$) causes recombination in ductal as well as acinar cells in adult tissue (Kopinke and Murtaugh, 2010). The CK19^{CreERT} knockin allele exhibits recombination that is

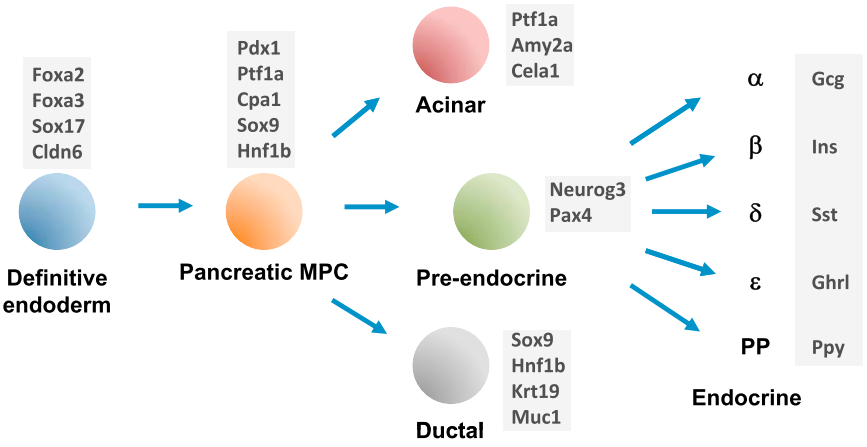

Figure 2. Highly Simplified Scheme of Pancreas Development

The pancreas is an endodermally derived organ that arises in a stepwise, progressive manner that involves the specification and differentiation of definitive endoderm (DE) into specific cell types. First, DE is specified into prepancreatic multipotent progenitor cells (MPCs). Second, the pancreatic MPCs are specified into either an acinar, ductal, or endocrine cell lineage. Third, the endocrine cell lineage is further specified into five different endocrine cell types. On account of this progressive cellular differentiation, Cre driver lines that are expressed early in development will result in recombination across multiple cell lineages. In addition, genes that have been used to drive Cre expression in specific lineages and/or cell types are indicated in the boxes. For example, the expression of Ptf1a-Cre, since it is expressed in pancreatic MPCs during development, will result in a recombined allele in all three main pancreatic cell types in adult animals. Similarly, the expression of Ngn3-Cre in pre-endocrine progenitor cells will result in recombined allele being present in all five endocrine cell types.

considerably more specific for ductal cells (Means et al., 2008). However, this line also marks hepatic ducts, stomach, and intestine when tamoxifen is administered after birth. Nonetheless, nearly 45% of pancreatic ducts are recombined with the labeling of only a few (<1%) acinar and endocrine cells. Cre^{ER} lines driven by the *Sox9* and *Hnf1b* genes can also be used to achieve ductal cell-specific recombination in adult animals. In both cases, up to 40%–70% of adult ductal cells have been shown to undergo recombination with only an occasional labeling of acinar and endocrine cells after activation of Cre^{ER} by tamoxifen (Kopp et al., 2011; Solar et al., 2009).

Driver Lines Based on Genes Expressed in Progenitor Cells

Genes encoding transcription factors that play critical roles in the development of pancreatic cells, and that are expressed in specific progenitor cell populations, have been used to derive 44 different Cre driver lines. However, only a few can be put to practical use in mature animals due to the highly varied nature of the timing and cell-type specificity of these genes.

Global Endoderm Deleters

During embryogenesis, the pancreas arises from the definitive endoderm, which is marked by the expression of the Foxa genes (*Foxa1*, *Foxa2*, and *Foxa3*) and *Sox17*. Of the three Foxa genes, *Foxa3* is the most endoderm specific and, unlike *Foxa1* and *Foxa2*, is not expressed in the notochord, floorplate, or ventral forebrain (Monaghan et al., 1993). For this reason, the $Tg(Foxa3\text{-}cre)^{1Khk}$ line, made using a yeast artificial chromosome (YAC)-derived transgene, causes recombination throughout the entire gut endoderm, including in the prepancreatic region (Lee et al., 2005; Xuan et al., 2012). $Foxa2^{Cre}$ and $Foxa2^{CreER}$ knockin alleles have also been used to achieve Cre recombination in the endoderm as well as in other sites of *Foxa2* expression such as the notochord and floorplate (Horn et al., 2012; Park et al., 2008; Uetzmann et al., 2008). A $Sox17^{GFPCre}$ knockin allele can be used to achieve recombination throughout the definitive endoderm. However, Sox17 is also expressed in hemogenic endothelial cells beginning around embryonic day 9.5 (E9.5), so it too is not entirely endodermal specific (Choi et al., 2012). A $Cldn6^{CreER}$ knockin allele is also available for achieving recombination in definitive endoderm (Anderson et al., 2008).

Pancreas-wide Deleters

The *Pdx1* gene is expressed in prepancreatic endoderm starting at E8.5. As development proceeds, it becomes more abundant in β cells, with lower levels in acinar and other endocrine cells (Jonsson et al., 1994; Offield et al., 1996). Accordingly, Pdx1-Cre driver lines, such as $Tg(Pdx1\text{-}cre)^{89.1Dam}$, mark all pancreatic cell types during lineage tracing. However, when the same *Pdx1* promoter fragment is used with Cre^{ER}, such as in $Tg(Pdx1\text{-}cre/Esr1^{*})^{\#Dam}$ in adult animals, recombination is observed only in the islet and acinar cells (Gu et al., 2002). Most Pdx1-Cre driver lines, including $Tg(Pdx1\text{-}cre)6^{Cvw}$/$Tg(Ipf1\text{-}cre)1^{Tuv}$, $Tg(Pdx1\text{-}cre)1^{Herr}$, and $Tg(Pdx1\text{-}cre)^{1Heed}$, have utilized an ~4.5 kb *Pdx1* promoter fragment and have been found to exhibit mosaic expression within the *Pdx1* expression domain (Gannon et al., 2000; Herrera, 2000; Steneberg et al., 2005). Interestingly, some of these Cre drivers exhibit significant differences in terms of their temporal and spatial activity. $Tg(Pdx1\text{-}cre)^{89.1Dam}$, termed $Pdx\text{-}Cre^{Early}$, displays early and robust Cre recombinase activity, while the other $Tg(Pdx1\text{-}cre)1^{Herr}$, termed $Pdx\text{-}Cre^{Late}$, has slightly delayed and more mosaic Cre expression (Heiser et al., 2006). It is worth noting that besides marking pancreas, *Pdx1* driver alleles also cause recombination in the duodenum, antral stomach, bile duct, and, as recently shown, hypothalamus and inner ear (Honig et al., 2010; Schonhoff et al., 2004; Song et al., 2010; Wicksteed et al., 2010; Yoshida et al., 2004).

By utilizing different enhancer fragments derived from regulatory regions located upstream of the *Pdx1* promoter, additional lines have been derived that express Cre in a more restricted, but still pancreas-specific, manner. The $Tg(Pdx1\text{-}cre)^{PBMga}$ line, which fuses a 1 kb DNA fragment ($Pdx1^{PB}$) containing regulatory areas I and II to the *Hsp68* promoter, causes Cre expression not only in β cells, but also to some extent in all endocrine cells (Wiebe et al., 2007). Conversely, $Tg(Pdx1\text{-}cre)^{XBMga}$, a Cre driver transgene containing regulatory area III ($Pdx1^{XB}$) mediates recombination throughout the developing pancreas similar to the 4.5 kb *Pdx1* promoter (Wiebe et al., 2007). The

tamoxifen-inducible Tg(Pdx1-cre/Esr1*)^{1Mga} line (also known as *Pdx1*^{PB}-CreER) allows spatial and temporal control of gene manipulation specifically in pancreatic islets (Zhang et al., 2005).

Ptf1a is another gene that is crucial for pancreatic organogenesis. Unlike *Pdx1*, the expression of *Ptf1a* within the developing gut is restricted to cells that only give rise to the pancreas, so it does not cause recombination in the distal stomach or proximal gut. Lineage tracing experiments using a Ptf1a^{Cre} knockin allele have demonstrated recombination in all three main pancreatic cell types (Kawaguchi et al., 2002), consistent with the expression of *Ptf1a* in pancreatic MPCs. In addition to being expressed in the pancreas, *Ptf1a* is also expressed in the developing cerebellum and retina (Nakhai et al., 2007).

Highly specific, pancreas-wide gene knockouts have been achieved using driver mice derived using the *Cpa1*, *Sox9*, and *Hnf1b* genes. For instance, the Cpa1-Cre^{ER} allele (Cpa1$^{tm1[cre/ERT2]Dam}$), due to the expression of *Cpa1* in pancreatic MPCs early during pancreas development, results in recombination in all three pancreatic lineages. However, tamoxifen must be administered before E14.5 for this outcome to be achieved (Zhou et al., 2007). Similarly, *Sox9* and *Hnf1b*, which are expressed in the pancreatic epithelium beginning at E10.5, cause recombination in endocrine, acinar, and duct cells (Kopp et al., 2011; Solar et al., 2009). Thus, provided that tamoxifen is administered after E13.5, the *Hnf1b*-Cre^{ER} driver line will cause recombination only in ductal and endocrine cells, not in acinar cells (Solar et al., 2009).

Endocrine Lineage-Specific Deleters

The emergence of pancreatic endocrine cells from pancreatic endoderm is triggered by the expression of *Neurog3* (*Ngn3*) beginning around E13.5 (Gradwohl et al., 2000). Several Ngn3-Cre driver lines have been described, some of which utilize Cre^{ER}. Lineage tracing studies using Tg(Neurog3-cre)^{1Dam} and Tg(Neurog3-cre/Esr1*)^{1Dam} have shown high-efficiency recombination in all endocrine cell types in the islet (Gu et al., 2002). In addition, several other Ngn3-Cre driver lines have also been used to mark endocrine cells in the pancreas. These include Tg(NEUROG3-cre)^{1Herr}, which utilizes a 5.7 kb fragment of the human *NEUROG3* gene (Desgraz and Herrera, 2009), Tg(Neurog3-cre)^{24Syos}, which is driven by a 23 kb fragment of the Ngn3 gene (Yoshida et al., 2004), the BAC transgenic line Tg(Neurog3-cre)^{C1Able} (Schonhoff et al., 2004), and a Ngn3^{CreER} knockin allele (Neurog3$^{tm1.1(cre/ERT)Ggu}$; Wang et al., 2008b). Since expression of *Neurog3* is not restricted to pancreatic endocrine cells, these driver lines also cause recombination in gastrointestinal cells, the neural system, and testis (Schonhoff et al., 2004; Song et al., 2010; Yoshida et al., 2004). Prudent use of *Neurog3*-Cre driver lines requires consideration of the fact that *Neurog3* is expressed in a narrow developmental time window, and recombination may occur in cells that no longer express *Neurog3* due to the perdurance of Cre. Indeed, this consideration should be applied to all Cre lines driven by transiently expressed genes.

Other proendocrine transcription factor genes that have been used to drive Cre expression include *Neurod1*, *Pax4*, *Myt1*, *Rfx6*, *Isl1*, *Nkx2.2*, *Pax6*, and *Mnx1*. All are characterized by recombination in other sites besides the pancreatic endocrine cells as well as major differences in their timing of expression. For instance, Pax4-Cre efficiently labels all four pancreatic endocrine cell lineages, whereas Rfx6-Cre causes recombination in all tissues derived from endoderm, presumably due to expression at an earlier time during development (Smith et al., 2010). Moreover, many proendocrine factors are also expressed in neural and other organ systems, making it necessary to consider whether nonpancreatic sites of expression will impact experimental design and interpretation. *Pax6*, for instance, is expressed in the eye lens, and *Isl1* is expressed in the mesoderm and plays an important role in heart development (Ashery-Padan et al., 2000; Gong et al., 2003; Hudson et al., 2011; Wang et al., 2011; Yang et al., 2001, 2006). Because of their more complex expression patterns, these lines are not widely used in metabolism-oriented studies in adult animals.

Limitations of Short Promoter Fragment Transgenes

Of the driver genes listed in Table 1, 45 (well over half) were made using relatively short driver gene (promoter) fragments, and the other 34 drivers were made by BAC transgenesis (8) or gene targeting (26). This is a vitally important consideration since the pronuclear microinjection of DNA results in both randomly integrated DNA fragments and variable transgene copy numbers, both of which can negatively impact the accuracy and duration of Cre expression. Moreover, short driver DNA fragments may lack key *cis*-regulatory elements important to obtain precise cell- or tissue-specific gene expression.

Randomly inserted transgenes are characterized not only by inexact expression patterns, but sometimes also by the silencing of gene transcription. These expression artifacts are due either to position effect variegation (PEV) or position effect silencing, a phenomenon that was first described in studies using *Drosophila.* PEV was first described as variable gene silencing of the *white* gene (which is responsible for red eye color) when it was translocated into a heterochromatic region of DNA. Silencing of the *white* gene resulted in easily discernible red and white patches in the mature *Drosophila* eye due to subpopulations of cells that exhibited a mosaic pattern of gene expression (Henikoff, 1992). Studies of this phenomenon led to the conclusion that alterations in chromatin-associated proteins can have a dramatic effect on the expression of genes, both positively and negatively, whether they are endogenous or a randomly inserted transgene (Ebert et al., 2006; Karpen, 1994; Reuter and Spierer, 1992).

Gene silencing, which is analogous to PEV, has since been observed in yeast, plants, and mammals (Fischer et al., 2006; Tham and Zakian, 2002). Factors governing the silencing of transgenes include the integration site, the number of copies of transgene in an integrated array, and the components of a transgene (Martin and Whitelaw, 1996). Furthermore, both DNA methylation and epigenetic modifications are known to silence gene expression, with the expression of transgenes integrated near heterochromatic regions being inhibited (Law and Jacobsen, 2010; Meyer, 2000). Similarly, the integration of transgenes near telomeres may also affect the extent of variegation. Tissues with more heterochromatin also exhibit a higher degree of transgene silencing.

Transgene copy number is well known to contribute to transgene silencing, with an inverse relationship between copy number and expression level often observed. The fact that high-copy number lines are often transcriptionally inactive is thought to be

due to the presence of repetitive sequences in such arrays. However, the existence of some transgenes with copy-number-dependent expression argue for the existence of regulatory elements, e.g., locus control regions (LCRs), that may prevent this repeat-induced silencing. Other components of transgenes, such as enhancers and matrix attachment regions (MARs), can positively influence expression (Harraghy et al., 2008; Kioussis and Festenstein, 1997). Conversely, the presence of plasmid sequences, as well as bacterial genes such as LacZ (which are CpG-rich and prone to methylation), have long been known to impair transgene expression, as have viral DNA sequences such as long terminal repeats (LTRs). In addition, patterns of transgene expression are sometimes affected by age and genetic background of the mice. Silencing of transgene expression can occur within a single generation or more gradually over several generations. Finally, the genetic background may also influence the extent of variegation, perhaps due to chromosomal differences between inbred strains of mice (Allen et al., 1990).

Approaches for Obtaining High-Fidelity Cre Driver Line Expression

Due to the deficiencies of transgenes containing short driver DNA fragments, two other strategies have gained favor for deriving Cre driver lines that exhibit expression patterns that closely match that of the driver gene. The first strategy involves the use of BACs (Wang et al., 2009). The advantage of using BAC-derived transgenes to make tissue- or cell-type-specific Cre driver lines lies in the large size of DNA fragments that BAC clones contain: typically over 100 kb and sometimes even more than 200 kb. As the amount of flanking DNA increases so does the prospect for faithful reproduction of endogenous expression patterns (Giraldo and Montoliu, 2001). Even so, some BAC-derived Cre transgenes may lack key regulatory elements and may fail to fully mimic expression of the endogenous gene. Methods for manipulating BAC DNA, referred to as BAC recombineering, have been described that are simple and reliable to perform. In addition, indexed BAC libraries from the genomic DNA of several commonly used strains of mice have been available for years (Osoegawa et al., 2000; Sharan et al., 2009). While BAC-derived transgenes may be less susceptible to position effects than more conventional transgenes, variegated expression and silencing of BAC-derived transgenes has nonetheless been reported (Alami et al., 2000). This suggests that some BAC transgenes, despite their long length, may still fall under the influence of the surrounding chromatin environment.

The second method for deriving new Cre driver lines, and the one which we generally prefer, is to perform gene targeting in mouse ESCs to place Cre in a fully native genetic context (e.g., a gene knockin). This strategy results in Cre being expressed in a manner that reflects all endogenous regulatory elements, even those that are located a long distance from the transcriptional start site. However, while this method is almost certain to result in a high-fidelity expression pattern, it has three drawbacks. First, it is time consuming to perform gene targeting in mESCs. Second, unless the gene is engineered to maintain expression of the gene that is targeted, haploinsufficiency will occur. Third, breeding a Cre driver allele to homozygosity causes the knockout of genes that are often essential for the development or life of the animal. While these issues can be overcome by the careful engineering of a Cre-expressing allele to retain expression of the endogenous gene, it adds additional technical complexity when designing a targeting vector. Nonetheless, we believe that the high-fidelity expression of Cre that is nearly always achieved outweighs these limitations.

The derivation of new Cre knockin mouse lines can be facilitated by two other technologies. The first is recombinase-mediated cassette exchange (RMCE), which allows Cre-mediated insertion of a target cassette into a predefined genomic locus, or a loxed cassette acceptor (LCA) allele, that contains inverted (Feng et al., 1999) or heterotypic LoxP sites (Araki et al., 2002). The principal advantage of this method is that it allows multiple allelic variants to be made at a defined genetic location with greater ease than can be achieved by repetitive gene targeting. Thus, once a genomic locus has been converted into a LCA allele, it becomes possible to readily generate lines of mice that express, for instance, Cre, Cre^{ER}, or GFPCre. As an effort within the Beta Cell Biology Consortium, we (and others) have generated mESCs containing LCAs for over a dozen genes, many of which are useful for deriving driver alleles for expressing SSRs, the reverse tetracycline transactivator (rtTA), or various fluorescent protein (FP) reporters. The genes for which LCA alleles have been derived include *Pdx1* (Potter et al., 2012), *Ptf1a* (Burlison et al., 2008), *Nkx2.2* (Papizan et al., 2011), *Sox17* (Choi et al., 2012), *Neurog3*, *Insm1*, *Ghrl* (Arnes et al., 2012), *Sst*, and *Ins2*, and several of these LCA alleles have already been used to derive new Cre or Cre^{ER} drivers (Arnes et al., 2012; Choi et al., 2012; Kopinke et al., 2012).

While RMCE can facilitate the generation of new Cre driver alleles, this technology may soon be superseded by the use of zinc finger nucleases (Urnov et al., 2010), transcription activator-like effector nucleases (TALENs) (Joung and Sander, 2013), and the clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) system (Cong et al., 2013). These emerging technologies greatly improve the efficiency of gene targeting, thereby making it possible to more readily engineer loci to express any of the SSRs or their derivatives (Cui et al., 2011).

Despite the existence of a seemingly large number of Cre driver lines, there remains a need for more high-fidelity driver lines, not only for Cre, but also for other SSRs. Indeed, given that some mouse models require the simultaneous activation and/or knockout of two different genes, reliable Flpo and Dre drivers will likely also be of value. Thus, we encourage efforts directed at deriving new SSR driver lines whose expression patterns are accurately defined.

A Continuing Need for Caution

While Cre/LoxP has become an indispensible tool for performing genetic manipulations in the mouse, the experience of many investigators has also taught us about some of the limitations of the system. For instance, it is important to not extrapolate from one recombination event to another since recombination at one floxed allele in a cell does not always mean that a second floxed allele in the same cell will have also recombined (Liu et al., 2013). This is due to apparent differences in the susceptibility of alleles to Cre-mediated recombination (Vooijs

et al., 2001). Indeed, the efficiency of deletion of a floxed allele in given cell type may differ from that in a second cell type, even when the amount of Cre in the cell is the same (Long and Rossi, 2009). Also, even when a driver line is of high efficiency and specificity, recombination at a given floxed allele may vary due to differences in the background strains used.

Both the timing and efficiency of Cre recombination can also greatly influence an experimental outcome. For example, the use of different Pdx1-Cre deleter mice in studies of Wnt signaling during early pancreas development led to markedly different experimental conclusions (Dessimoz et al., 2005; Heiser et al., 2006; Murtaugh et al., 2005), an outcome that emphasizes the continuing need for caution, particularly with respect to data interpretation. Indeed, we believe it is prudent to maintain some skepticism about all published descriptions of Cre reporter lines and to occasionally reassess both the efficiency and specificity of Cre recombination. Finally, it may be vital to use a Cre-only control to exclude any unexpected phenotypes caused by Cre itself, as described in the accompanying perspective by Harno et al. (2013).

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