Published in final edited form as:
Mamm Genome. 2012 October ; 23(0): 587–599. doi:10.1007/s00335-012-9430-2.

Beyond knockouts: cre resources for conditional mutagenesis

Stephen A. Murray[1,6], **Janan T. Eppig**[1], **Damian Smedley**[2], **Elizabeth M. Simpson**[3], and **Nadia Rosenthal**[4,5]

[1]The Jackson Laboratory, Bar Harbor, Maine 04609, USA

[2]The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK

[3]Centre for Molecular Medicine and Therapeutics at the Child & Family Research Institute, Departments of Medical Genetics and Psychiatry, University of British Columbia, Vancouver, BC, V5Z 4H4, Canada

[4]National Heart and Lung Institute, Imperial College London, London, W12 0NN, UK

[5]EMBL Australia; Australian Regenerative Medicine Institute, Monash University, Clayton, Vic 3800, Australia

Abstract

With the effort of the International Phenotyping Consortium (IMPC) to produce thousands of strains with conditional potential gathering steam, there is growing recognition that it must be supported by a rich toolbox of cre driver strains. The approaches to build cre strains have evolved in both sophistication and reliability, replacing first generation strains with tools that can target individual cell populations with incredible precision and specificity. The modest set of cre drivers generated by individual labs over the past 15+ years is now growing rapidly, thanks to a number of large-scale projects to produce new cre strains for the community. The power of this growing resource, however, depends upon the proper deep characterization of strain function, as even the best designed strain can display a variety of undesirable features that must be considered in experimental design. This must be coupled with the parallel development of informatics tools to provide functional data to the user, and facilitated access to the strains through public repositories. We will discuss the current progress on all of these fronts and the challenges that remain to ensure the scientific community can capitalize on the tremendous number of mouse resources at their disposal.

Introduction

By awarding the 2007 Nobel Prize for Medicine to Oliver Smithies, Mario Cappecci, and Martin Evans, the enormous impact of the ability to genetically manipulate the mouse was officially recognized by the scientific community. Since these first experiments were performed, however, it has become clear that there are limitations to the technology, particularly if deletion of the gene of interest led to a lethal phenotype. Development of the Cre/loxP system of conditional mutagenesis in the mid-1990s allowed investigators to specifically delete a gene of interest in a specific tissue or cell type in a time-controlled manner, dramatically increasing the number of questions that could be asked and answered with targeted mutagenesis. In addition to simple gene ablation, cre/loxP technology provides a means for tissue-specific and temporally restricted expression of transgenes and to engineer large chromosomal alterations. Since that time, the popularity of this approach has

[6]Corresponding Author: 600 Main Street, Bar Harbor, ME 04609, USA, steve.murray@jax.org, Phone: (207) 288-6857Fax (207) 288-6149.

increased tremendously, becoming an essentially routine approach to gene targeting experiments and spurring the development of new cre driver mouse lines to manipulate the ever-increasing number of loxP-flanked ("floxed") alleles. The size and sophistication of the genetic toolbox available to the scientific community continues to expand rapidly, providing users with unprecedented power to manipulate the mouse genome. In this review, we will describe the current state of the art in recombinase driver line development, highlight several large programs that are building new sets of cre driver lines, and provide the reader with a comprehensive overview of information and animal resources to enable their research.

IKMC and cre resources: next era of functional genomics

The international program to generate targeted mutations for all genes in the mouse genome (the International Knockout Mouse Consortium, IKMC; http://www.knockoutmouse.org/), complemented by the current effort to turn these ES cell resources into mice for broad-based phenotyping (the International Mouse Phenotyping Consortium; IMPC; http://www.mousephenotype.org/), promise to deliver massively parallel functional annotation of the mouse genome over the next 10 years. In addition, the long-term legacy of these programs will include archived mouse strains available to the scientific community. Notably, a majority of these resources will include conditional potential, allowing individual investigators enormous power to engage in more detailed analysis of phenotypes relevant to their individual research programs. For an extensive description of the IKMC resources available, please see the accompanying review in the issue from Bradley, et. al.)

In most cases, to produce these conditional mutants, mouse ES cell lines are engineered with an altered or "floxed" allele of a single gene in which an essential sequence (usually a critical exon) is flanked by short 34 bp recognition sites (loxP) for bacterial cre recombinase. These floxed alleles are incorporated into mice by injecting the corresponding ES cell into a host mouse blastocyst and screening for germline integration in the resulting progeny bearing the floxed allele. Deletion of the targeted sequence is activated by crossing with a mouse expressing cre recombinase, which mediates recombination between the sites. Restricting the cre expression to a desired cell or tissue type results in conditional mutagenesis. Additional temporal mutagenesis is typically achieved via tamoxifen induction of recombinase-estrogen receptor fusion proteins (see Section Inducible cre drivers: timing is everything below), thereby targeting the mutation to the desired tissue (spatial control) and/or life stage (temporal control). Thus, the ability to leverage the potential of conditional mutagenesis is necessarily limited by the availability of a diverse, well-characterized set of cre driver strains.

Although much progress has been made over the past decade in expanding our international resources of cre-bearing strains, many challenges remain in terms of design and construction, quality characterization, systematic collection of information about these strains, and public access to these strains through repositories.

Approaches to building cre drivers

The variety and sophistication of approaches to generate cre driver strains continues to evolve, and are highlighted in Table 1. All approaches have potential advantages and disadvantages, but the ultimate choice of approach will depend on the goals of the project as there is no "one size fits all" all solution for engineering cre strains. Traditionally, cre strains were created by random transgenic insertion of a short, promoter-driven cre cDNA into the mouse genome. Due to limitations in the size of the promoter, only a subset of the regulatory elements required for expression of the native gene can be incorporated into the transgene, which, coupled with insertion site and copy number variation, means many individual founders must be screened and that the ultimate functionality of the strain is determined

empirically. Not surprisingly, many transgenic cre strains exhibit “off-target” activity due to these and other caveats commonly observed in transgenic strains (described in detail below). For well-characterized promoters, however, traditional transgenesis might be an ideal option, as it allows the flexibility to incorporate a fragment of promoter sequence with known functionality.

Targeted knock in of cre into the endogenous locus of a driver gene is an alternative approach that potentially provides greater fidelity of activity. However, this presumes complete knowledge of the expression of the native gene; unanticipated activity due to previously unappreciated gene expression, particularly early in development or in the maternal germline, is captured by the lineage marking nature of the cre recombinase. As described below, this can be circumvented through the use of an inducible version of cre. Furthermore, many knockin cre strains will target genes central to developmental or disease pathways of interest, haploinsufficiency at the driver locus has the potential to impact the phenotype under investigation, complicating the analysis. For example, a commonly used knock-in cre line, *Foxg1*$^{tm1(cre)Skm}$, displays defects in forebrain development (Shen et al., 2006; Eagleson et al., 2007; Siegenthaler et al., 2008), the region targeted by this driver strain. Furthermore, the Mouse Genome Informatics (MGI) phenotype database contains nearly 40 examples of cre strains that, in isolation, display phenotypes as heterozygotes (MGI custom query; www.informatics.jax.org).

BAC transgenes provide a hybrid approach that allows for incorporation of regulatory elements without generating haploinsufficiency at the gene driver locus (Gong et al., 2007). Because of their large size, BACs are less susceptible to position effects and are likely to contain many of the critical regulatory elements that define gene expression. Founders still frequently display variable activity, suggesting BAC transgenesis does not solve all of the caveats of traditional transgenics, such as local the effect of chromatin structure at the integration site, and the very real risk of insertional mutagenesis. Moreover increased copy number of other “tag along” genes resident on the BAC clone could confound the phenotype under investigation, and is occasionally unavoidable for drivers that reside in gene dense loci. Despite these downsides, BAC transgenics offer clear advantages over traditional small promoter driven constructs.

A more recent strategy that seeks to circumvent the issue of random insertion employs the use of a defined “neutral” locus for insertion of cre drivers. This can include the use of a recombinase mediate cassette exchange (RMCE) docking site, and has been successfully employed for loci including *Gt(ROSA)26Sor* (Zambrowicz et al., 1997; Badea et al., 2003; Jullien et al., 2007; Raymond and Soriano, 2007; Ventura et al., 2007), *Hprt* (Yang et al., 2009), and *Hipp11*(Tasic et al., 2011). The former is well-suited to ubiquitous gene expression, while the latter two have been shown to be permissive for tissue specific expression. Notably, a recent publication describes a modification to the Gt(ROSA)26Sor locus that permits use of exogenous, tissue-specific promoters (Tchorz et al., 2012). This thus allows for transgenic cre driver insertion without positional effects and insertional mutagenesis risk. Additionally, entire BACs can be inserted into docking sites via RMCE, taking advantage of the loxP and lox511 sites in BAC backbone vectors, while incorporating a greater range of regulatory elements to direct the desired expression pattern. These approaches combine the flexibility of promoter design (BAC or small promoter), the advantage of a defined locus and copy number, without generating haploinsufficiency at the driver locus, and thus represents a popular strategy for future cre generation.

A alternative knock-in approach is to introduce the cre construct into the 3′ UTR with an IRES element, or to replace the stop codon of the native gene with a Picornaviridae virus 2A “self-cleaving” peptide (Szymczak et al., 2004) linked to the cre recombinase, a strategy that

appears to provide more reliably equal levels of expression of the two linked ORFs (Hennecke et al., 2001; Douin et al., 2004; Szymczak and Vignali, 2005). Both approaches have the distinct advantage of functioning as a knockin allele without generating haploinsufficiency at the target locus. Caveats remain, including the introduction of a heterologous neo cassette into the 3′ UTR (partially mitigated if flanked by FRT sites and subsequently removed), and in the case of the 2A peptide, a 17-21 amino acid tag remains attached to the c-terminus of the native protein, potentially altering its function. In addition, new data suggests different 2A flavors (P2A, T2A, E2A, F2A) have different cleavage efficiencies in mammalian cells(Kim et al., 2011). Despite these concerns, the use of a 3′ targeting approach is gaining popularity, and is one strategy employed by the EUCOMMTOOLS project (see discussion of large-scale programs below)(Kim et al., 2011).

Inducible cre drivers: timing is everything

The power of cre and other recombinase-expressing strains is limited by the lineage marking nature of the tool. That is, recombination occurs following the first instance of expression of the driver promoter, which can result in more widespread and early recombination than is ideal for the experiment. Temporal control of cre function is most commonly achieved through fusion protein of the cre enzyme with the ligand-binding domain of the estrogen receptor (ER), which is mutated to be only responsive to synthetic ER ligands such as 4-hydroxytamoxifen, the active metabolite of the drug tamoxifen (Feil et al., 1997; Metzger and Chambon, 2001). In the absence of ligand, the cre-ER fusion is sequestered in the cytoplasm and is thus inactive. Upon ligand binding, the fusion is translocated to the nucleus where it is free to excise loxP-flanked regions. Second generation versions of this fusion protein, such as cre-ER^{T2}, are more sensitive and responsive to tamoxifen (Indra et al., 1999). Clearly, the ability to induce recombination at a particular time and place greatly increases experimental flexibility, but also adds new challenges that the user needs to consider carefully, including leakiness (activity prior to induction) (Kemp et al., 2004; Liu et al., 2010) and toxicity (described below).

Employing the well-established Tet system in a biallelic manner is another strategy for temporal control of gene ablation (St-Onge et al., 1996; Schonig et al., 2002; Belteki et al., 2005). Generally, a tissue-specific tet transactivator allele (tTA or rtTA, for Tet-off and Tet-on systems, respectively) is combined with a tet-responsive cre allele (tetO-cre). This approach can take advantage of specific rtTA or tTA alleles that exist and have been validated, an important consideration in past years when the availability of cre strains was fairly limited. The disadvantages are the intrinsic, ligand-independent basal level of activity (leakiness) of the Tet system (Corbel and Rossi, 2002) and the complexity of the breeding scheme required to get both alleles combined with the conditional gain or loss of function allele of interest. Despite this downside, a tissue-specific Tet biallelic system could be very useful in combination with other recombinase systems, such as Flp/FRT (Dymecki, 1996; Branda and Dymecki, 2004), PhiC31/AttP (Belteki et al., 2003; Monetti et al., 2011) and Dre/ROX (Anastassiadis et al., 2009), for which large resources of recombinase drivers do not currently exist.

Although not strictly inducible, another approach to further refine cell targets in both time and space is to use an intersectional approach, where two independent gene drivers define a cell type via overlapping expression. Generally, this approach uses a combination of different recombinases (e.g. CRE and FLP) driven by separate promoters in combination with reporter genes to mark cells (Dymecki et al., 2010). However, a new approach to target loxP-flanked alleles employs a system where separate fragments of the CRE recombinase are expressed from distinct drivers, resulting in excision in cells where both are expressed (known as “split cre”) (Hirrlinger et al., 2009b). Although not is widespread use, the

approach has the potential to greatly increase the precision of cre activity. Inducible flavors of this approach involving both tamoxifen (Hirrlinger et al., 2009a) and rapamycin (Jullien et al., 2003; Jullien et al., 2007) induction systems are in development.

Large-scale cre driver generation programs

GENSAT cre project

The Gene Expression Nervous System Atlas (GENSAT) cre project at Rockefeller University uses the high-throughput BAC engineering platform built to interrogate neuronal gene expression patterns using BAC-eGFP transgenics(Gong et al., 2007; Gong et al., 2010). This project generated a wealth of gene expression data that were leveraged to rapidly produce cre driver strains targeting a diverse array of structures and cell types, with the goal of producing cre lines that target a diverse set of neuronal cell types in a highly specific manner. Updates are available at http://www.gensat.org/CrePipeline.jsp, where currently 140 unique drivers (gene targets) are currently publically available out of the total of 258 unique drivers for which founders exist and are being characterized. For this and other projects described below, “unique driver” refers to unique gene targets chosen to drive cre expression. This is distinct from total strains, which could include multiple founder lines for a given driver. A majority of these strains are of constitutive cre design, although a few creER^{T2} drivers populate the collection. Characterization builds upon the GENSAT expression analysis pipeline, providing detailed annotation of function in the brain and spinal cord (see http://www.gensat.org/cre.jsp for details). Notably, the GENSAT program uses a GFP reporter strain (B6;129-*Gt(ROSA)26*^{tm2Sho}/J), followed by anti-GFP immunohistochemistry to visualize targeted cell populations. Validated strains are publicly available through the Mutant Mouse Regional Resource Center (MMRRC) and University of California, Davis (UCDavis) has initiated a program to extend characterization of these strains to additional tissues.

Pleiades/CanEuCre

An extension of the Genome Canada Project and the Atlas of Gene Expression in Mouse Development, the goal of the Pleiades Promoter Project is to identify and validate promoters of genes expressed in specific regions and cell types in the brain. Validation includes the generation of a large set of eGFP reporter strains generated via targeting of the promoter-reporter construct to the HPRT locus (Yang et al., 2009; Portales-Casamar et al., 2010) (http://pleiades.org/). This effort included the creation of six cre driver lines, and has served as a template for the much larger CanEUCre effort (http://www.caneucre.org/). Leveraging the findings and strain generation pipeline of the Pleiades Project, the goal of this program is to build 30 novel drivers targeting the brain an nervous system, including a number of adeno-associated viral cre vectors. All strains and viral vector resources will be made available through public repositories.

FaceBase cre project

This program is part of the FaceBase Consortium, the goal of which is to support research into the eitology of orofacial clefting by providing resources to the craniofacial research community (Hochheiser et al., 2011; Murray, 2011)(www.facebase.org). Employing a combination of BAC transgenics, 3′ 2A peptide-mediated knock-in alleles and cre strains driven by highly conserved enhancer sequences, the project will deliver at least 15 new cre driver strains to the community. The latter approach combines forces with another Consortium project to identify enhancers active during craniofacial development. Both constitutive cre and inducible cre strains are being generated, depending on their predicted utility, and all will be characterized for intended function and evaluated using the JAX cre strain general characterization scheme (cre.jax.org).

Neuroscience Blueprint cre project

The overall goal of the Neuroscience Blueprint Cre Driver Network is to equip the neuroscience community with a large array of genetic tools that provide cell type and temporal control of gene deletion (Taniguchi et al., 2011). The network comprised three pipelines that employed a variety of approaches to achieve this goal (Table 2). For example, the program at Baylor University used a gene trap strategy to generate a library of targeted ES cell lines, which were subsequently screened for integration within genes known to be expressed in the brain. The Scripps and CSHL programs used more traditional knockin and transgenic approaches to target specific gene expression patterns. A clear emphasis was placed on creER^{T2} inducible lines, representing 51 out of the 95 total lines (84 unique promoter drivers). The Scripps program also included a number of tetracycline-inducible strains. Each group performed significant characterization of each strain for brain-specific functionality (www.credriverline.org) and this effort is being complemented by more extensive characterization using the high-throughput system and a standardized induction protocol developed at the Allen Institute for Brain Science (http://connectivity.brain-map.org/). All strains are currently deposited in public repositories (JAX and MMRRC) providing easy access for the scientific community to the strains.

Allen Institute for Brain Science cre project

The Allen Institute for Brain Science has also taken a proactive role in expanding the neuro-specific cre toolbox (Madisen et al., 2010). In addition to providing a robust, high-throughput brain characterization pipeline for cre lines from a number of sources, AIBS has produced a number of cre lines using several approaches including BAC and traditional transgenesis and 3′ knockin. In addition to 11 unique drivers (17 total lines) and tTA line, they have produced a number of very popular, bright fluorescent cre reporter lines (Madisen et al., 2010) all targeted to the *Gt(ROSA)26Sor* locus. These reporters provide the user with a variety of color options, plus several sophisticated cre-inducible fluorescent tools (e.g. tdTomato fused to channelrhodopsin) that will be useful for neurobiology applications (Madisen et al., 2012). As with the NIH Blueprint program, these strains have been deposited in a public repository (JAX) to facilitate access for the user community.

CREATE and EUCOMMTOOLS

The **CREATE** Consortium was initially founded as an internationally based Coordination Action funded by the EU, and represents a core of eight major European and international mouse database holders and research groups involved in conditional mutagenesis. Together with an International Advisory Group, these Partners have been enlisted to address inaccessibility to existing cre driver strains, their incomplete characterization, and an inadequate coverage of cell and tissue types in which they are active. To provide the necessary structure for worldwide access to these critical resources, CREATE established a web portal representing the majority of cre driver databases worldwide (www.creline.org, see cre Database Resources below). To build on this initiative, CREATE is directly involved in a recent EU-funded project, EUCOMMTOOLS, (www.eucommtools.org) to provide the scientific community with new conditional and inducible cre driver transgenic mouse lines. Specifically, the project will identify 500 genes and their promoters with specific tissue and cell type expression profiles suitable as cre drivers covering all organs and the major cell types of the body. The first 250 of these new alleles will be used to generate mice and characterize cre expression patterns, both at embryonic and adult stages, thereby extending the IKMC resources for worldwide distribution.

ICS cre zoo

The Institut Clinique de la Souris (ICS) CreER^{T2} zoo was developed in the early 2000 (http://www.ics-mci.fr/mousecre/) to propose inducible Cre lines to the scientific community in a panel of tissues targets. All the lines were produced by pronuclear injection of BAC modified with a creER^{T2} cassette or of standard transgenes. The details concerning the type of construct and the genetic background (BAC reference, creER^{T2} cassette, etc.) can be found on the web site. Thirty-five lines are described and have been analyzed following a well-defined characterization flow scheme(Smedley et al., 2011). Lines are selected based on first pass expression analysis of the *creER^{T2}* transgene by qRT-PCR. Those lines that express the *creER^{T2}* transgene at sufficient levels and in tissues consistent with the endogenous promoter are then characterized in detail for excision activity using the *Gt(ROSA)26Sor^{tm1Sor}/J* reporter (R26R) (Soriano, 1999). The level of characterization of each line is updated regularly with the ultimate goal of integration with other databases to serve the scientific community. Thirty-six lines are currently distributed to the academic community.

NorCOMM2ls

NorCOMM2LS will use an enhancer-trap approach to produce up to 50 new cre driver mouse lines. A Sleeping Beauty transposon with a human beta globin minimal promoter-cre transgene will be inserted in a single copy into the C57BL/6N genome. Mobilization of the transposon in the germline of mice will result in insertion in genomic locations and “test driver” cre lines will be selected when insertion occurs in so-called “gene deserts”. Distal enhancers can act on promoters in these gene deserts to drive expression from the minimal promoter in distinct and reproducible domains. New cre driver lines will be selected based on domains of cre activity determined by crossing the test driver cre lines with cre reporter lines (e.g. *Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$* (Ai14-TdTomato) (Madisen et al., 2010) and/or *Gt(ROSA)26Sor^{tm1Sor}* reporter (R26R) (Soriano, 1999)) and analyzing reporter activation in E10.5 embryos. More details can be found at www.norcomm2.org (this site scheduled to go live by July 2012).

Repositories and Resources: Where are the cres?

While many investigators have created cre-bearing mice for their own laboratory's projects, these are not always deposited in repositories where other researchers can obtain these resources. As the scientific grant funding agencies have developed data and resources sharing plans in recent years, the disparity between what resources have been created and what resources are available is improving. Currently about 45% of cre lines that have been made can be found in public repositories (Table 3). This percentage will continue to increase as new large-scale projects to develop new cre lines contribute a larger share of the cre pool (e.g., see descriptions of new cre development projects such as CanEuCre, EUCOMMTOOLS, and Nor2ls elsewhere in this review.) Large programs come with their own challenges for repositories, however. In the past, cre strains donated for distribution were well-used and published in the community, and therefore were more or less a “known quantity”. This provides reassurance for risk-averse users, who tend to choose strains that have been successfully employed in other labs. The flood of new strains from large-scale programs lack this history of use, and therefore face significant barriers to widespread adoption. Moreover, many of the new strains are quite sophisticated and target a specific structure or cell type. While this provides new power for genetic analysis, it also practically divides the user base into smaller groups, compounding the issue of establishing a track record of successful application in published work. For example, despite the large number of cre strains (over 100) arriving from the NIH Blueprint project (Taniguchi et al., 2011), the Pleiades project (Yang et al., 2009; Portales-Casamar et al., 2010) and the AIBS cre effort

(Madisen et al., 2010), the classic neuro-specific strain Nes-cre (B6.Cg-Tg(Nes-cre)1Kln/J) is by far the most widely distributed neuro-specific strain at JAX. In addition, the high-throughput nature of these projects precludes extensive characterization beyond the target tissue. Given the potential caveats known to afflict cre strains in general (described below), repositories have recognized that end users rely on them for the functionality of distributed strains, and have taken steps to improve quality control through broad characterization of new strains. Moving forward, as more projects produce resources in a high-throughput manner (cre driver networks, IKMC projects, Collaborative Cross), repositories will need to continue to evolve their distribution strategy to ensure adoption and use of resources by the scientific community.

Cre Database Resources

As stated earlier, the choice of cre-bearing strains for conditional mutagenesis experiments is critical to the downstream analysis of results and interpretation of biological significance. Thus, knowing the existence and availability of cre lines, and their expression and specificity characteristics are key to experimental design. A number of databases hold cre strain information and vary in their intended purpose and data coverage. Table 4 provides URLs for publicly available cre database resources and outline of their contents. Here we briefly discuss the two cre data resources containing the most comprehensive information, CrePortal.org and Creline.org.

CrePortal.org

The crePortal (www.creportal.org, (Blake et al., 2011)), a free publicly accessible site, was developed to provide critical data about recombinase constructs, the driver/promoter contained, whether they are inducible (and by what), accessibility in public repositories, and publications describing the construct and conditional mutagenesis work done using them. Annotations and images (in-situ, immunohistochemistry) of tissues, anatomical structures, and ages assayed define the specificity of the cre allele and aid in visualizing cre driver expression. Through links to Mouse Genome Informatics resources, MGI (www.informatics.jax.org, (Eppig et al., 2012)), phenotype information for conditional genotypes that have been studied using each cre construct is also available.

At the cre Portal (www.creportal.org), searches can be done by anatomical system where cre is active, by specific driver, or by symbol, synonym or ID of the cre-containing transgene or knock-in allele. New features being developed include the ability to search with fine-grained anatomical terms (e.g., oocyte vs. reproductive system) and the ability to view comparisons of cre driver strain specificities based on common drivers or on common anatomical sites of specificity. Data from groups systematically producing cre driver strains at large-scale have been imported (Pleiades project, (www.pleiades.org, (Yang et al., 2009)) and GENSAT (www.gensat.org, (Gong et al., 2007)). Additional cre data from projects that are slated for inclusion in 2012 are the Allen Brain Atlas (http://connectivity.brain-map.org/transgenic/, (Madisen et al., 2010)), EUCOMMTOOLS (http://lib.bioinfo.pl/projects/view/20794), the NIH Neuro Blueprint project (www.credrivermice.org), the ICS MouseCre data (www.ics-mci.fr/mousecre/) and the CanEuCre project (www.caneucre.org). Major efforts at full characterization of existing cre strains is being carried out at the Jackson Laboratory (http://cre.jax.org/) and Allen Brain Institute as described elsewhere in this review. These data will be integrated into crePortal as they become available.

Creline.org

The CREATE (coordination of resources for conditional expression of mutated mouse alleles) portal (www.creline.org, (Smedley et al., 2011)) was developed under EU

framework 7, with one of its specific goals to provide a BioMart-based (Smedley et al., 2009) and web-based interface to selected cre data acquired from the CrePortal (Blake et al., 2011), Cre-X (Nagy et al., 2009), and CreZoo databases (see Table 4). Ten key fields that were common among these data sources were selected for this integrated portal. These included: Driver, MGI_ID, Allele Symbol, Allele Name, Strains, original Pubmed ID, Inducible (Y/N), IMSR (Y/N), Repository strain ID, and Anatomical site(s) of expression. Links to each respective database source are provided to give users maximal information.

The web interface to the CREATE database is viewable as a BioMart instance, by using a simple gene search (for cre Driver), or by navigating the mouse adult or embryonic anatomy ontologies for sites of cre expression. The ontology interface is particularly useful for visualizing whether cre lines already exist for a particular anatomical structure (or sub-parts of it) and navigating to the cre lines in question. Once a specific Driver is chosen, users may open the data further to reveal specific alleles and strains, expression/specificity data, and links to the data resource from which the data were derived and links to IMSR, if the line is available from a repository.

Expect the unexpected: cre caveats and the importance of detailed characterization

As discussed earlier, the design of the cre driver can introduce a number of caveats to the functionality of a given cre driver mouse line. Although these issues were certainly appreciated anecdotally in individual laboratories, several publications have highlighted some of these issues in general (Matthaei, 2007; Schmidt-Supprian and Rajewsky, 2007). Specific examples include mosaic and/or inconsistent deletion activity, which are seen in Tg(MMTV-cre)4Mam (Lu et al., 2008), *Krt19*$^{tm1(cre)Mmt}$ (Means et al., 2005), *Gata4*$^{tm1(cre)Svs}$ (Pilon et al., 2008) and Tg(Nes-cre/ERT2)1Fsh (Balordi and Fishell, 2007), which in particular shows variable leakiness. The consistency of a particular cre line is critical for interpretation of experimental results, and will have a major impact on the number of samples (animals) that need to be examined before a conclusion is drawn.

Frequently, unexpected/undesired/ectopic expression is observed for a given line. This could pose serious problems if the targeted gene is essential for viability or can lead to misinterpretation of a resulting phenotype, particularly in cases where questions of cell autonomy are being asked. Examples of this issue can be found for several cre lines including Tg(Myh6-cre)2182Mds (Eckardt et al., 2004), *Osr2*$^{tm2(cre)Jian}$ (Lan et al., 2007), Tg(Vil-cre)20Syr (Kucherlapati et al., 2006) and Tg(Ddx4-cre)1Dcas (Gallardo et al., 2007), Tg(Ins2-cre)25Mgn (Fex et al., 2007), Tg(Pdx1-cre)89.1Dam (Wicksteed et al., 2010), although it is clear this is only a sample. Additionally, several cre lines have been shown to exhibit inconsistent, promiscuous activity in the germline, leading to widespread cre activation (Weng et al., 2008).

Perhaps less well appreciated is the effect of genetic background, which is known to have a major impact on many different phenotypes, and can lead to dramatic differences in the outcome of a gene targeting experiment (Threadgill et al., 1995). *Foxg1*$^{tm1(cre)Skm}$ (Hebert and McConnell, 2000) and Tg(Ins2-cre)25Mgn (Fex et al., 2007) are two examples, but it seems likely that the limited number of reported instances is the result of limited examination. Interestingly, the sex of the animal delivering the cre line appears to affect the deletion phenotype, likely due to cre expression in the female germline that persists in the early embryo (Lomeli et al., 2000; Gallardo et al., 2007). Although many of the new large-scale cre driver projects, in addition to the IKMC, use a standardized genetic background (C57BL/6), many existing cre strains in repositories and individual labs are maintained and

used on segregating, mixed backgrounds. Thus, the potential confounding influence of genetic background remains a real concern that users should control for in their experiments.

Finally, there is a growing awareness of the potential for cre toxicity (Forni et al., 2006; Jimeno et al., 2006; Naiche and Papaioannou, 2007; Takebayashi et al., 2008; Higashi et al., 2009; Huh et al., 2010). This is thought to be the result of both legitimate (via induced loxP sites) recombination and cre mediated double strand breaks at cryptic loxP elements inducing a DNA damage response. Interestingly, one recent report shows that the level of apoptosis correlates with the number of loxP sites introduced, and that cell death is p53-dependent (Zhu et al., 2012). With ubiquitous creER strains, there appears to be both line-dependent and dose-dependent toxicity, independent of excision function (Higashi et al., 2009; Huh et al., 2010), S.Murray, N. Rosenthal, unpublished results), indicating the need for careful examination of lines and titration of ligand dosing in individual labs.

Whether the result of cre-mediated toxicity, insertional mutagenesis, or haploinsufficiency at the driver locus, the potential for the presence of the cre allele to generate an observable phenotype needs to be carefully considered in the experimental design and choice of controls. There are a number of examples of this effect in highly used cre strains, including hypopituitarism in Tg(Nes-cre)1Atp (Galichet et al., 2010), microcephaly and hydrocephaly in a distinct Tg(Nes-cre)1Wme line (Forni et al., 2006), and germ cell defects in Tg(Tek-cre)12Flv (Leu et al., 2009), and hypoglycemia in Tg(Ins2-cre)25Mgn (also known as RIP-cre) (Lee et al., 2006), to name a few. Many of these phenotytpes are likely to be directly related to the hypothesis being tested, and thus have to the potential to confound interpretation of results without the proper controls.

Together, these examples paint a clear picture that despite the power and versatility of cre driver lines, all of these potential caveats need to be explored systematically for these tools to reach their full potential. The common thread in all these caveats is ascertainment bias. Unexpected cre driver activity is primarily due to incomplete characterization of the strain, as most investigators have very specific goals in mind when they generate a new driver. However, as these strains become a public resource, their uses diversify and therefore require more rigorous examination of excision activity in both the intended target tissue as well as a broad range of tissues and cell types at multiple time points. Unfortunately, this approach is inconsistently applied. While, for example, the ICS Cre Zoo program has consistently engaged in characterization of multiple tissues for each strain (Smedley et al., 2011) and a similar comprehensive plan is in place for EUCOMMTOOLS, many of the large-scale cre production programs lack the resources to characterize more then the target tissue (e.g. brain and spinal cord), potentially limiting the broad utility of the resources. This is problematic because of the potential for confounding off-target activity, which could result in lethality or non-autonomous phenotypes depending on the floxed gene of interest, but also limits any potential emergent use for the line, as much “off target” activity represents true expression of the driver gene. Thus, a cre strain targeting the brain may also have use for targeting the kidney, but only if this information is available.

Some of the responsibility for extending characterization of strains beyond the target tissue has been taken on by Repositories, who are charged with providing the resource to the scientific community. Both JAX (all cre strains) and the MMRRC at UCDavis (GENSAT cre strains) have programs to extend the characterization of cre strains as both a quality control measure for the community and to potentially uncover new uses for a given strain based on previously unrecognized excision activity. For example, the JAX program characterizes cre activity using the B6.129S4-*Gt(ROSA)26Sor*^{tm1Sor}/J reporter strain at four time points (adult, P7, E15.5 and E11.5) using a standard annotation scheme that captures 11 organ systems, 28 organs/structures and 78 substructures for postnatal animals (male and

female). A similar standard annotation scheme is used for embryos. All results are published on a dedicated web page (cre.jax.org) and submitted to creportal.org, combining it with published data to create a central resource for cre activity information, as described above.

Summary

Although the IKMC currently dwarfs the scale of the efforts to generate new cre driver strains, clearly the collective resource of tool strains available to the scientific community is growing rapidly. Apart from the valuable and successful work of the CREATE program, there has been little international coordination of cre programs in general, leading to duplication of effort and a lack of cross-program standardization. An effort similar to that of the IKMC and the affiliated International Mouse Phenotyping Consortium (IMPC) would be extremely helpful in this regard as the ultimate value of these resources is limited by the quality of the characterization of the strains, easy and centralized access to functional data, and perhaps most importantly access to the strains themselves through public repositories. It is these practical considerations, as much as the generation of the strains, that will allow the scientific community to properly leverage and add value to the impending tidal wave of conditional strains produced by the IMPC over the next several years.

Acknowledgments

The authors would like to thank Laura Kus, Hongkui Zeng, Marie-Christine Burling and Lauryl Nutter for information about individual cre driver programs. Special thanks to Caleb Heffner for his help with details and references for the JAX cre characterization program. This work is supported by NIH grants HG000330 (J.T.E), HD062499 (J.T.E.), RR032656 (J.T.E. and S.A.M.), DE020052 (S.A.M), RR026117 (S.A.M) and EU grant HEALTH-F4-2009-223487 (J.T.E., D.S., and N.R.).

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Table 1

Cre driver strategies

Approach	Advantages	Disadvantages	Projects
Small-Insert-Random Insertion (plasmid)	Simple, rapid Partial, empirically defined promoters No haploinsufficiency at endogenous locus	Position effects Insertional mutagenesis Copy number effects Need to screen founders	AIBS Cre Neuroscience Blueprint Cre
Small-Insert Knock-in at Docking Site (plasmid) (*Hprt*, *Gt(ROSA)26Sor*)	Defined locus Partial, empirically defined promoters No haploinsufficiency at endogenous locus	Intermediate effort gene targeting Single copy, potentially weak expression	CanEuCre Pleiades
5′ Knock-in at Endogenous Locus	Defined locus All promoter/ enhancer elements	Labor intensive gene targeting Haploinsufficiency at endogenous locus Single copy, potentially weak expression	AIBS Cre Neuroscience Blueprint Cre
Large-Insert-Random Insertion (BAC, YAC)	Intermediate effort Greater complement of promoter/enhancer elements No haploinsufficiency at endogenous locus	Intermediate level position effects Insertional mutagenesis Copy number effects Need to screen founders “Tag along” gene risk	AIBS Cre GENSAT
Large-Insert Knock-in at Docking Site (BAC) *(Hprt)*	Defined locus Greater complement of promoter/enhancer elements No haploinsufficiency at endogenous locus	Intermediate effort gene targeting Single copy, potentially weak expression “Tag along” gene risk	CanEuCre EUCOMMTOOLS
3′ Knock-in at Endogenous Locus	Defined locus All promoter/ enhancer elements	Labor intensive gene targeting Some haploinsufficiency, or hypomorphic risk at endogenous locus Single copy, potentially weak expression	EUCOMMTOOLS AIBS Cre

Mamm Genome. Author manuscript; available in PMC 2013 October 01.

Table 2

Large-scale Cre-Driver Projects

Projects	Approaches	Cre-Drivers planned/produced	Availability
AIBS Cre (http://connectivity.brain-map.org/)	Small-Insert-Random Insertion 5′ Knock-in at Endogenous Locus 3′ Knock-in at Endogenous Locus	17	JAX
CanEuCre (http://www.caneucre.org/)	Small-Insert Knock-in at Docking Site (5′ of *Hprt)* Large-Insert Knock-in at Docking Site (5′ of *Hprt)*	30 planned/8 produced so far	TBD
EUCOMMTOOLS (http://www.knockoutmouse.org/about/eucommtools)	Large-Insert knock-in at Docking Site *(Hprt)* 3′ Knock-in at Endogenous Locus	250 strains/500 ES lines	EMMA
GENSAT (http://www.gensat.org/CrePipeline.jsp)	Large-Insert-Random Insertion (BAC)	256/124 available	MMRRC
Neuroscience Blueprint Cre (www.credrivermice.org)	Small-Insert-Random Insertion 5′ Knock-in at EndogenousLocus Enhancer trap	95	MMRRC JAX
NorCOMM2ls	Transposon-based enhancer trap	50 planned	CMMR
ICS Cre Zoo (http://www.ics-mci.fr/mousecre/)	Large-Insert-Random Insertion (BAC) 5′ Knock-in at endogenous locus Small-Insert-Random Insertion	50 produced	ICS
FaceBase Cre (www.facebase.org)	Large-Insert-Random Insertion (BAC) 3′ Knock-in at Endogenous Locus Enhancer Knock-in at Docking Site	15 planned	JAX
Pleiades (http://pleiades.org/)	Small-Insert Knock-in at Docking Site (5′ of *Hprt*)	6 planned/6 produced	JAX

TBD, to be determined

Table 3

Repositories holding cre-bearing mice (as live or cryopreserved embryos or sperm) As of April 24, 2012

Repository	No. of cre Strains	URL
IMSR (International Mouse Strain Resource), catalog of *all* holdings *	791	www.findmice.org
APB (Australian Phenomics Bank, Australia)	4	pb.apf.edu.au/phenbank
CMMR (Canadian Mouse Mutant Resource, Canada)	1	www.cmmr.ca
EMMA (European Mouse Mutant Archive, Italy)	75	www.emmanet.org
Harwell (Mammalian Genetics Unit, U.K.)	11	www.har.mrc.ac.uk
JAX (The Jackson Laboratory, U.S.A.)	368	cre.jax.org/data.html
MMRRC (Mouse Mutant Regional Resource Centers, U.S.A.)	242	www.mmrrc.org
MUGEN (Mutant Mouse Models of Human immunological Disease Network, Greece)	13	www.mugen-noe.org
NCIMR (National Cancer Institute Mutant Resource, U.S.A.)	7	mouse.ncifcrf.gov
RBRC (RIKEN Biology Resource Center, Japan)	68	www.brc.riken.jp/lab/animal/en
RMRC-NLAC (National Applied Research Laboratories, Taiwan, R.O.C.)	2	www.nlac.org.tw/RMRC/en1/page/01_aboutrmrc/01/rmrc.html

* The IMSR is a global catalog of available mouse resource. It contains records for 791 cre lines distributed across many repositories in many countries. Note that other repositories also participate in the IMSR, but many hold no cre lines, and thus do not appear on this list.

Table 4
Database Resources for Cre Driver Data

Database Name	URL	Number of cre records (19 April 2012)	Content
CrePortal	www.creportal.org	1,725	Symbol, synonym, name of the cre transgene or knock-in, MGI_ID, strain background, molecular construct, cre driver, inducibility, availability, sex, publications, anatomical structure/age specific expression, expression level/pattern, expression images (e.g. in-situ, immunohistochemistry), reporter
Cre-X	nagy.mshri.on.ca/cre_new	672	Strain background, cre driver, MGI_ID, inducibility, availability, initial publication, anatomical structure specific expression, expression pattern
CreZoo	bioit.fleming.gr/crezoo	174	Symbol, synonym, name of the cre transgene or knock-in, MGI_ID, strain background, cre driver, inducibility, availability, initial publication, anatomical structure/age specific expression
MouseCre	www.ics-mci.fr/mousecre/	38	Symbol, name of the cre transgene or knock-in, MGI_ID, strain background, molecular construct, cre driver, inducibility, availability, sex, publication, anatomical structure/age specific expression, expression images (e.g. in-situ, immunohistochemistry)
Create	www.creline.org	1,761*	Symbol, name of the cre transgene or knock-in, MGI_ID, strain background, cre driver, inducibility, IMSR, publication, anatomical structure for expression (system level and detail)
International Mouse Strain Resource	www.findmice.org	1,043	International catalog of mouse holdings at repositories worldwide; provides ‘index’ to all available cre lines with links to repository and MGI data on these cre lines.

*This number represents a Biomart instance of data downloaded from CrePortal, Cre-X and CreZoo that has been analyzed for UNIQUE entries.