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Essentials of Recombinase-Based Genetic Fate Mapping in Mice

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Abstract

Fate maps, by defining the relationship between embryonic tissue organization and postnatal tissue structure, are one of the most important tools on hand to developmental biologists. In the past, generating such maps in mice was hindered by their in utero development limiting the physical access required for traditional methods involving tracer injection or cell transplantation. No longer is physical access a requirement. Innovations over the past decade have led to genetic techniques that offer means to “deliver” cell lineage tracers noninvasively. Such “genetic fate mapping” approaches employ transgenic strategies to express genetically encoded site-specific recombinases in a cell type-specific manner to switch on expression of a cell-heritable reporter transgene as lineage tracer. The behaviors and fate of marked cells and their progeny can then be explored and their contributions to different tissues examined. Here, we review the basic concepts of genetic fate mapping and consider the strengths and limitations for their application. We also explore two refinements of this approach that lend improved spatial and temporal resolution: (1) Intersectional and subtractive genetic fate mapping and (2) Genetic inducible fate mapping.

Keywords

Fate map; Cre; Flp; Subtractive genetic fate mapping; Intersectional genetic fate mapping; Genetic inducible fate mapping

1 Introduction

Knowing how embryonic tissue organization relates to postnatal tissue structure and function is fundamental for understanding development and disease. In studying the etiology of human disorders, especially informative are tissue fate maps generated in mammalian models. Yet mammalian models such as the mouse are poorly suited to traditional fate mapping techniques (reviewed in ref. 1). For example, in utero development makes it difficult to physically access embryonic cells for labeling by tracer injection. But no longer is physical access a fate mapping requirement. In 1998, technology was reported [2, 3] that allows, in effect, cell lineage tracers to be “delivered” to mouse embryonic cells in utero via noninvasive transgenic, rather than physical, means. The approach, called “genetic fate mapping,” exploits a type of molecule referred to as a site-specific recombinase (SSR), which, being genetically encoded, is amenable to in vivo delivery by transgenesis. The two recombinases most commonly used are Cre (named because it causes *re*combination of the bacteriophage P1 genome) and Flp (named for its ability to invert, or “flip,” a DNA segment

in *Saccharomyces cerevisiae*). Through their capacity to produce precise DNA excisions, Cre and Flp each can be engineered to act as genetic on-switches, able to convert a silenced reporter transgene into a constitutively expressed one that has lineage tracing capability (Fig. 1).

In this fate mapping strategy, there are two basic elements, a recombinase-expressing transgene and a silenced reporter transgene that can be activated by recombination. For the first element, recombinase expression is directed in utero to a desired embryonic cell population. This is achieved by using gene regulatory elements, either incorporated into a transgene that drives recombinase expression selectively in certain cells or in the form of a gene knockin where recombinase-encoding sequence is inserted into a capable endogenous locus. Only in the recombinase-expressing cells is the second element, the silenced reporter transgene, switched, by recombinase action, to the “on,” reporter-expressing configuration. This is achieved through SSR-mediated excision of an “off” or “stop” portion of the reporter transgene (Fig. 1). The consequence is that SSR-expressing embryonic cells are marked by reporter expression. Importantly, their descendant cells are also marked. This is because they inherit the “on” form of the reporter transgene and because the transgene has been engineered to remain on, that is to sustain constitutive reporter expression, regardless of subsequent cell differentiation and independent of any further recombinase expression. Thus, the reporter transgene has, in effect, been transformed into an indelible cell lineage tracer. The non-SSR-expressing embryonic cells (which typically is most of the embryo) are reporter-negative because they continue to harbor the unrecombined “silenced” form of the reporter transgene. So in this way, fate mapping no longer requires physical access to embryonic cells but rather genetic access via gene enhancer and promoter elements. Thanks to the extensive gene-expression data being generated presently and over the past decade [5–10] such driver elements are available and can be exploited to drive recombinase expression in most any embryonic cell type. In short, fate maps now can be generated in mice with relative ease.

Genetic fate mapping offers additional benefits. Perhaps most importantly, mouse genetics can be exploited concomitantly. Cells marked by genetic fate mapping can be studied in the context of mutant genes and other genetic alterations to reveal effects, for example, on their contributions to different tissues. Another benefit of genetic fate mapping is that the embryonic cells under study are selected based on their expression of a particular gene or by the activity of a particular enhancer element. As a consequence, the cell marking achievable from animal-to-animal is exceptionally precise and reproducible. Further, this molecular signature—used to select the embryonic cell population for labeling—can provide important clues about signaling pathways possibly relevant for acquiring particular fates. Further, it can reveal homologies in development among other cell populations that are otherwise disparate anatomically but at some point earlier during their development shared similar gene-expression signatures.

In this chapter, we review the basic concepts of recombinase-based genetic fate mapping and considerations for their application—as with all methods, there are strengths and limitations. We then describe two strategy variations that lend improved spatial and temporal resolution to genetic fate maps: the first variation is called intersectional and

subtractive genetic fate mapping [11, 12] and the second, genetic inducible fate mapping [13]. Early applications of these tools centered on studies of nervous system development (among examples are refs. 2, 3, 11, 12, 14–22) but today are being applied to study numerous cell types in a wide range of tissues and biological processes in the mouse.

2 Recombinase-Based Genetic Fate Mapping

Cre and Flp are the hinge-pins of genetic fate mapping technologies because of their capacity to produce precise, predetermined rearrangements of chromosomal DNA. DNA deletions, insertions, inversions, or exchanges are possible. Specifically which type of rearrangement depends on orientation of the DNA recognition sites (reviewed in ref. 23). Cre recombinase recognizes what are called *loxP* sites (locus of crossover (x) in P1) [24], and Flp, *FRT* sites (Flp recombinase recognition target) [25]. Both type of sites, while comprised of different DNA sequences, are 34-bp in length and not normally found in the mouse (or fly) genome, allowing for their creative insertion to engineer predetermined modifications. For example, when target sites are positioned on the same DNA molecule and oriented in the same direction, SSR action catalyzes deletion of the sequence lying in between, leaving behind a single target site. It is this DNA deletion reaction that has been exploited most for mouse fate mapping (Fig. 1). Of note, part of the foundation for SSR use in higher eukaryotes stems from work in *drosophila*, where SSRs are commonly used to catalyze exchange of chromosomal DNA that lies distal to target sites positioned on homologous chromosomes during the G2 phase of the cell cycle, with X segregation during mitosis allowing for the generation of mutant cell clones in an otherwise wild-type fly [26–29]. Thus, whether in mice or flies, once introduced into a cell, Cre or Flp can modify a *loxP*- or *FRT*-containing target locus, and depending on the configuration, turn it on, off (Fig. 1), or exchange distal sequence [26–29]. Turning on permanent expression of a reporter molecule, like β -galactosidase (β -gal) or green fluorescent protein (GFP), in select embryonic cells and their descendants is the basis of genetic fate mapping (Fig. 1).

As briefly introduced above, genetic fate mapping requires two types of transgenes or modified loci: (1) a recombinase “driver” transgene expressing the particular SSR in a gene- or enhancer- specific fashion to achieve specificity in the initially labeled cell type and (2) an “indicator” transgene designed to permanently express a reporter molecule following site-specific excisional recombination. When *SSR driver* and *indicator* transgenes are partnered in a double transgenic (*SSR driver, indicator*) animal, SSR-mediated recombination of the indicator transgene leads to constitutive reporter expression (Fig. 1). It is worth noting here that we use the term “indicator,” as opposed to “reporter,” to describe the target (reporter-encoding) transgene because it serves to indicate (by reporter expression) not only cells that are currently under the influence of SSR action but also cells that previously underwent SSR-recombination but are no longer expressing the recombinase. In other words, the presence of a recombined (reporter-expressing) target transgene indicates those cells with a history of recombinase expression, which means a history of expression of the particular driver gene or enhancer element. This feature is central to genetic fate mapping and worth emphasizing: once an indicator transgene has been “activated” by SSR-mediated recombination, the encoded reporter molecule is expressed constitutively by that lineage from that point onward, independent of any further recombinase expression. This feature

ensures robust marking of descendant cells regardless of cell type or developmental stage. In most cases, it is possible to visualize lineage contributions to adult structures even though much time may elapse between the initial (embryonic) recombination event and the actual (adult) tissue analysis. Thus, long-term cell lineage tracing is enabled.

This “long-term” cell lineage tracing offered by SSR-based genetic fate mapping (with its recombinase and indicator transgenes) is worth contrasting with the often “short-term,” more ephemeral, cell marking achievable when a nonconditional (non-SSR) *progenitor cell-specific promoter::reporter* transgene or knockin allele is used. In this case, the term “reporter transgene,” rather than indicator transgene, is used because reporter expression directly reflects the transient nature of the upstream cell type-specific driver sequences. Marked by the reporter molecule, as a consequence, are cells in which the driver gene enhancer elements are active at the time of tissue harvest. In some cases, later time points can be analyzed for marked descendant cells because of persistence of the reporter molecule itself beyond its immediate window of transcription, allowing for short-term lineage tracing. The “fate maps” resulting from this surrogate type of approach, though, may suffer loss in accuracy because some cell lineages may be missed because of their more rapid elimination of reporter molecules and/or because of lowering starting level of reporter expression. Erroneous exclusion of certain cells from a fate map may result.

The long-term cell lineage tracing enabled by SSR-based genetic fate mapping methods relies on constitutive reporter expression driven by the recombined indicator allele—that is, constitutive reporter expression in descendant cells regardless of the differentiated descendant cell type. This means that the indicator transgene must have the potential to be able to sustain robust reporter expression in virtually every cell type at all developmental and adult stages, or at least in every cell type of the tissue or organ under study, in order to ensure completeness of the fate map. This is a tall order. Various promoter/enhancer elements are being tested and used for this purpose. One that has been met with success in many tissue types is use of the endogenous mouse *Gt(ROSA)26Sor (R26)* locus [30], with [12, 31] or without augmentation by enhancer elements from the chicken β -actin gene and cytomegalovirus genome (CAG sequences) [32]. Other exploited loci include *tau* [33], for nervous system studies, and *colAI* [34]. The actual range of cell types that can be marked by a given indicator transgene must be determined empirically. For example, an approximation of scope can be ascertained by analyzing tissue from an animal in which the target indicator transgene had been partnered with a transgene that drives broad SSR expression early in the development of the tissue of interest such that most constituent cells will harbor the recombined “active” form of the indicator allele. Cells can then be assessed for robustness of reporter expression. Any unmarked populations should be noted, and caution taken when interpreting the actual experimental (progenitor-specific) fate mapping results—exclusion of such cells from the fate map should not be concluded as they simply may not be capable of being marked by the indicator allele.

As introduced above, the choice of embryonic cell type for genetic fate mapping is determined by the cell type- or tissue- specific enhancer elements employed to drive SSR expression. Such elements can be engineered to drive SSR expression by one of multiple ways: by conventional transgenic methods (*cell type-specific promoter::SSR* transgene of

less than ~20 kb or so), bacterial artificial chromosome (BAC) transgenic strategies (constructs typically around ~200 kb), or by targeted gene knockin approach. The choice of method is determined by experimental need and availability of cell type- or tissue-specific enhancer elements.

Just as it is critical to determine the scope of cell types an indicator allele is capable of mapping, it is equally critical to determine the extent to which SSR expression matches that of the endogenous driver element profile. Ectopic SSR expression (expression outside the endogenous driver profile) would confound fate mapping studies by switching on the lineage tracer in cells outside of the lineage being studied. This would result in overestimation of the descendant population by erroneously including in the map unrelated cells. Reciprocally, if SSR levels permit recombination in only a subset of those cells in which the driver element is actually active, the resultant fate map will underestimate the descendant population. Thus, it is important to establish the extent to which initial expression of the activated reporter matches the initial SSR driver gene-expression profile. Later, expression of the activated reporter will diverge from that of the driver because the reporter is cumulatively and permanently tracking all cells that ever expressed the driver gene, whereas driver gene expression is transient (Fig. 1b). In other words, it is critical to determine whether all or only part of an initial gene-expression domain is being fate mapped. This is of particular concern if the driver gene exhibits a gradient of expression. It is possible that the lowest expressers in the gradient may be missed because levels of recombinase are inadequate to activate reporter expression. Such discrepancies are critical to determine.

Worth noting are the different variants of Flp recombinase that can be used in mice. They are referred to as wild-type Flp (Flp-wt), low-activity Flp (FlpL), enhanced Flp (Flpe), and optimized Flpe (referred to as Flpo). The enhanced Flpe contains four point mutations that together confer increased protein thermostability resulting in up to a tenfold increase in recombinase activity compared to Flp-wt [35, 36]. Recently, Flpe has been codon-optimized [37] with the goal of boosting Flp activity in mouse embryonic stem (ES) cells. By contrast to Flpe and Flpo, FlpL contains a single amino acid substitution that renders the recombinase thermolabile [38] resulting in at least a fivefold reduction in recombinase activity from Flp-wt. When expressed in mice, Flpe and Flpo have been shown to function with similar efficacy as Cre [11, 12, 20, 22, 39–44]. By contrast, FlpL achieves more modest recombination efficiency [16, 20]. In mouse embryonic stem cells, Flpo shows the greatest activity [37].

3 Dual Recombinase-Mediated Intersectional and Subtractive Genetic Fate Mapping

Despite the molecular precision of recombinase-based genetic fate mapping, many biological questions remain unanswerable because of the broad extent of cell types marked by the expression of a single gene. For example, embryonic gene-expression domains are often restricted along the dorsoventral (DV) or anteroposterior (AP) axis of a tissue or germinal zone while extending the length of the orthogonal axes (Fig. 2a). In this case, the gene-expression domain may actually contain multiple subpopulations of cells each

distinguished by their expression of a second gene whose expression overlaps along this orthogonal axis (Fig. 2a) [11, 12, 39, 44]. The ability to fate map these subpopulations and their descendent lineages is not possible using single recombinase-based genetic fate mapping. To overcome this limitation, gene combinations need to be employed. Towards this goal, a method called “intersectional” genetic fate mapping in which the initial population to be traced is selected based on its expression of two genes (and consequently two recombinases) rather than one (Fig. 2) was developed [11]. The ability to resolve highly selective cell populations for fate mapping can be substantial, depending on the extent of overlap between the two chosen driver genes.

For intersectional genetic fate mapping, two SSRs, Cre and Flp, are needed to activate expression of a reporter molecule—the “intersectional reporter” [11, 12, 31, 39, 45, 46]; reviewed in refs. 4, 23, 47 (Fig. 2b). In other words, two excisional recombination events are required, one mediated by Cre (driven by *gene A*) and the other by Flp (driven by *gene B*) (Fig. 2). Only those cells lying at the intersection of the two gene-expression domains (*A* and *B*) will therefore activate reporter expression (Fig. 2) and their descendant cells marked. This intersectional approach can indeed be highly efficient for marking progenitor cells lying at the intersection of two gene-expression domains, as demonstrated in recent developmental studies of the brainstem [11, 12, 39, 44].

Of note, in this approach, the expression of the two genes (e.g., driver *gene A* and driver *gene B*) does not have to coincide temporally. They may be expressed at different times in a cell’s developmental history with activation of the intersectional reporter occurring only after the second recombination event has been completed. This means that temporal as well as spatial resolution in lineage tracing can be improved.

In addition to fate mapping intersecting Cre/Flp cell subpopulations, this technology can be engineered to allow simultaneous tracing of Cre/non-Flp lineages, referred to as “subtractive” populations. The subtractive populations are what remain after Cre/Flp-intersecting cells are subtracted from the Cre-only expressing domain [12] (Fig. 2c). In order to visualize the subtractive population, the intersectional indicator allele needs to be configured such that a second reporter molecule is encoded (the “subtractive reporter”) and its expression is dependent only upon excision by Cre of the *loxP*-flanked (floxed) stop cassette (Fig. 2c). Thus, two genetically distinct lineages can be tracked simultaneously, one by the intersectional reporter and the other by the subtractive reporter. The subtractive population can also be engineered to be the reciprocal Flp/non-Cre population; this is achieved simply by changing the order of the floxed and flrtd (*FRT*-flanked) stop cassettes in the intersectional indicator allele (Fig. 2c) [11, 31, 39]. It is worth noting that use of a reporter molecule with a relatively short half-life is helpful as the subtractive reporter. This ensures that the subtractive reporter will not persist substantially and therefore be detected in intersectional descendents despite its coding sequence having been excised from the indicator allele. Of course, this can be rigorously discerned by reporter co-detection experiments, with the intersectional population being defined by constitutive expression of the intersectional reporter possibly along with (unwanted) persistence of the subtractive reporter; the subtractive population would be rigorously identified as harboring only the subtractive reporter molecule and not both.

Beyond improving fate map resolution, intersectional alleles offer another practical advantage. Three different mouse lines can be generated from one initial transgene construction and strain generation: one dual recombinase-responsive indicator allele and two derivative strains that are either responsive to Cre only or to Flp only. The latter are derived through germline deletion of either the floxed or flrtd cassettes [12].

4 Genetic Inducible Fate Mapping

To better resolve temporal aspects of lineage allocation during mouse development, a method coined GIFM [48, 49], for genetic inducible fate mapping, has been developed. This approach relies on a driver transgene expressing a ligand-regulated form of Cre or Flp to temporally control SSR activity (Fig. 3a). In GIFM, the SSR is fused to an estrogen receptor (ER) ligand-binding domain (LBD) that has been mutated, rendering it insensitive to the natural ligand 17 β -estradiol at physiological concentrations but responsive to the synthetic ligand 4-hydroxytamoxifen (4-OHT) [50–55] (Fig. 3a). Temporal control of recombinase activity relies on the ability of the ER-LBD to sequester the SSR into a cytoplasmic heat shock protein 90 (Hsp90) complex in the absence of 4-OHT. Upon 4-OHT binding, the ER-LBD undergoes a conformational change that frees the fused SSR, allowing it to enter the nucleus where it can mediate recombination of a target site-containing locus (Fig. 3b) (For a detailed review *see* ref. 49). There are at least three different ER-LBDs available—two human ER variants, ER^T [50–53, 55, 56] and ER^{T2} [13, 57–60], and one from the mouse, ERTM [17, 54, 61, 62]. ER^{T2} appears to be the most sensitive form for both nuclear [13, 57–60] translocation and recombinase activity [13, 57–60]. CreER^{T2} [13, 58–60], FlpeER^{T2} [63], and FlpoER^{T2} [42] fusions have been generated, and all are effective *in vivo*. Worth noting, CreER^{T2} may outperform FLPeER^{T2} in certain cell types when expressed at low levels (N.L. Hunter and S.M.D., unpublished observations), despite the fact that the constitutive forms, Cre and Flpe, show comparably robust activity *in vivo*.

Tamoxifen, the precursor to 4-OHT, is typically the reagent used in GIFM. It is both easier to work with (it is more soluble) and less costly than 4-OHT. Conversion of tamoxifen to 4-OHT takes approximately 6–12 h *in vivo*, resulting in an initial lag between administration of tamoxifen and onset of recombinase-mediated target gene recombination. The 6–12 h lag is followed by an approximately 24 h window during which recombination is catalyzed and the initial cell population is marked [13, 17, 18, 61, 63]. Given such kinetics, it is important to establish the extent of recombinase activity following tamoxifen administration; in other words, it is critical to establish the extent to which expression of the indicator transgene-encoded reporter molecule matches the expected driver gene-expression profile between 24 and 48 h after tamoxifen administration. The degree of concordance indicates whether most or only a subset of the highest driver gene expressors can be tracked. Once these parameters are set, it should be possible to visualize the fate of these cells at any later time point. The resulting fate map will mark just those cells that have emerged from a gene-expression domain during a particular 24 h window. Duration of the recombination window will of course vary depending on the tamoxifen dosing regimen, with higher amounts and repeated administrations lengthening that window.

It is important to note, however, that tamoxifen, when administered at high or repeated doses, can be lethal to the developing embryo. Therefore, it is critical to establish the administration regimen of tamoxifen that maximizes recombination at the desired embryonic stage but minimizes unwanted side effects. It appears that inbred mouse strains are more sensitive to higher doses of tamoxifen than outbred strains such as Swiss Webster [48, 49]. The coadministration of progesterone with high tamoxifen doses also may improve litter viability [48, 49]. Another consideration is gestational age. Later-stage embryos are better able to tolerate higher doses of tamoxifen. The level of expressed SSR-ER^{T2} protein must also be considered. While higher levels of SSR-ER^{T2} protein are better able to induce recombination at lower tamoxifen levels, if too high, the capacity for tight induction may be compromised resulting in unwanted recombination even in the absence of tamoxifen.

By contrast to both standard and intersectional genetic fate mapping where populations of progenitor cells and their descendants are marked, GIFM has the potential to allow for clonal analyses because low doses of tamoxifen can permit, in certain cases, the marking of a single progenitor cell in a region and therefore just its daughter cells [64]; reviewed in ref. 48. Another SSR-based approach, distinct from GIFM, that permits clonal analyses and is therefore important to include in this review is a technique called mosaic analysis with double markers (MADM). This approach, like the FRT-mediated clonal analyses in *drosophila* mentioned above, relies on rare SSR-mediated translocation events between two homologous chromosomes during the G2 phase of the cell cycle; X segregation of the recombined chromosomes during mitosis then results in two daughter cells each expressing one or the other marker [65]; reviewed in ref. 66.

While the majority of Cre and CreER transgenics function well with few side effects, it is important to note that toxicity, seen as upregulated apoptosis and cell cycle arrest for example, has been reported in a number of cases [67]. DNA damage secondary to abortive recombination attempts on endogenous noncanonical *loxP* sites may be the cause [67]—a situation most likely to occur when Cre activity is quite high. Especially susceptible may be CreER transgenics because there is no selection against extremely high CreER expressors. This is because Cre activity is held in check by heat shock complex sequestration, allowing founder lines of a range of expression levels to be generated and propagated, even when expression levels are high such that noncanonical recombination might occur under tamoxifen induction. By contrast, for constitutive *cre* lines, there may be selection against high expressors in the founder generation, thus weeding out the most problematic lines. Thus critical for sussing out Cre toxicity is a comparison between tamoxifen-induced and uninduced CreER-positive animals, in the absence of any target transgene [67]. Toxicity associated with Flp recombinase (and its Flpe, Flpo, and ER variants) has not been reported, but may be lurking and simply not observed yet. Thus, single recombinase controls, regardless of which recombinase, are essential.

5 Conclusion

Genetic fate mapping, and its variations, have enabled powerful science: (1) it is now possible to more easily generate fate maps in a mammalian system; (2) using mice, with its genetics, the behaviors of marked cells and their contributions to different tissues can now

be studied in the context of mutant genes and other genetic alterations, thereby informing on how gene products affect development or lead to disease; and (3) progenitor cells can be selected for tracking based on an expressed gene—a feature that enables exceptionally precise and reproducible cell marking from animal to animal. Moreover, this latter feature can serve to illuminate potential roles of gene products in development, as well as developmental homologies between unexpected and otherwise disparate anatomical structures.

Elaborations on these SSR basics are ongoing in many laboratories, with the field advancing in numerous directions. One example is to use these paradigms of conditional gene “delivery” to express various genetically encoded effector molecules capable of revealing functional properties of a genetic lineage or of the processes it participates in refs. 68, 69. The possibilities indeed seem endless; even further so when combined with classical and complementary methodologies, such as the clonal information afforded by retroviral lineage tracing or the neuroanatomical information provided upon combining axon tract tracing techniques with genetic fate mapping, just to name a few exciting examples.

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Fig. 1.

Genetic fate mapping relies on site-specific recombinase-mediated DNA excision to “switch on” reporter expression. **(a)** Structure of a generic SSR- responsive transgene inserted as a single copy into the mouse genome. SSR- mediated recombination between two directly oriented SSR recognition sites (*triangles*) results in deletion of intervening transcriptional stop sequences (*red octagonal stop sign*) and consequent expression of a reporter molecule. **(b)** Illustration of how site-specific recombination can be used for genetic fate mapping. In the *top panel* the generic SSR-responsive transgene is modified by incorporation of a broadly active promoter (BAP) ideally capable of driving transgene expression in any cell type at any stage in development, such that after a recombination event in a given cell, that cell and all its progeny cells should be marked by reporter expression regardless of subsequent cell differentiation. The *lower panel* illustrates the strategy for SSR-based genetic fate mapping. *Cylinders* represent the neural tube at early (*left*) to late (*right*) developmental stages. *Top row* illustrates the transient expression of hypothetical *gene A* by progenitor cells located in the dorsal neural tube (*yellow domain*) at an early developmental stage. *Middle row* illustrates SSR-expressing transgene utilizing enhancer elements from *gene A*. *Bottom row* illustrates coupling of *gene A::SSR* with an Genetic indicator transgene.

Cells expressing the SSR will activate production of the reporter molecule (for example, β -gal). Activation of reporter molecule expression is permanent, and all cells descended from the SSR expressing (*gene A*-expressing) progenitors will continue expressing the reporter. Descendant cells are depicted here as *blue circles*. (Reproduced from ref. 4 with permission from Elsevier Science) (Color figure online)

a Multiple uniquely coded molecular subdomains may comprise a single gene expression domain

b Dual recombinase-responsive indicator allele for accessing gene expression subdomains

c Stop-cassette order determines the subtractive population relative to the intersection

d Intersectional and subtractive genetic fate mapping

Fig. 2.

Intersectional and subtractive genetic fate mapping strategy. **(a)** Multiple uniquely coded molecular subdomains may comprise a single gene-expression domain. Shown are schematics of the neural tube (*gray cylinder*), with different gene-expression domains depicted in different colors. The expression domain for hypothetical gene A (*yellow*) restricts along the dorsoventral (DV) axis but extends along the anteroposterior (AP) axis; by contrast, the expression domains for genes B (*pink*) and C (*red*) restrict along the AP axis but extend along the DV axis. Thus, the gene A expression domain (*yellow*) is subdivided into three molecularly distinct subdomains: one in which genes A and B are coexpressed

(*tan domain*), another in which genes A and C are coexpressed (*orange domain*), and finally, that territory (*yellow*) marked by gene A expression, but not B or C. Similarly, both the gene B and C expression domains are each subdivided. **(b)** Structure of a prototypical dual recombinase (Cre and Flpe)-responsive indicator allele. In contrast to a single recombinase-responsive indicator allele (Fig. 1b), a dual recombinase-responsive indicator allele has two stop cassettes, one flanked by directly oriented loxP sites (*triangles*) and the other by FRT sites (*vertically oriented rectangles*). Cre-mediated stop cassette removal results in expression of n β -gal, while the remaining FRT-flanked stop cassette prevents GFP expression. Following removal of both stop cassettes, requiring Cre- and Flpe-mediated excisions, GFP expression is turned on and n β -gal expression off. **(c)** Illustration of intersectional and subtractive populations and the latter dependency on stop-cassette order. In the “PF” configured allele, the loxP-flanked stop cassette precedes the FRT-flanked cassette (*left panel*), while the reciprocal order characterizes the “FP” configuration (*right panel*). Shown are schematics of the neural tube (*gray cylinder*), with the expression domain for hypothetical gene A and Flpe recombinase (*yellow*) restricting along the DV axis but extending along the AP axis (*top row*); in contrast, the expression domain for gene B (*pink*) restricts along the AP axis but extends along the DV axis (*middle row*). When gene A::Flpe and gene B::cre are coupled with a PF dual recombinase-responsive indicator allele (*bottom row, left*), cells expressing cre and Flpe activate production of GFP (*green domain*, intersectional population), while cells expressing only cre activate production of n β -gal (*blue domain*, subtractive population). When gene A::Flpe and gene B::cre are coupled with an FP-configured allele (*bottom row, right*), cells expressing cre and Flpe still activate production of GFP in the same intersectional population (*green domain*), but now cells expressing only Flpe (rather than cre) activate production of n β -gal (*blue domain*, new subtractive population). **(d)** Illustration of the selective fate mapping achievable using an intersectional and subtractive approach. *Cylinders* represent the neural tube at early (*left*) to late (*right*) developmental stages. *Top row* illustrates transient Flpe expression, driven by *gene A*, in progenitor cells located in the dorsal neural tube (*yellow domain*) at an early developmental stage. *Middle row* illustrates transient Cre expression, driven by *gene B*, in progenitor cells located at a particular anteroposterior level of the neural tube at an early developmental stage (*pink domain*). *Bottom row* illustrates coupling *gene A::Flpe* and *gene B::cre* are coupled with a dual recombinase-responsive indicator allele (FP configuration), cells expressing Flpe and Cre activate production of GFP, while cells expressing only Flpe activate production of n β -gal. Activation of reporter molecule expression is permanent, and all cells descended from Flpe-expressing or Flpe- and cre-expressing progenitors will continue expressing the blue or green marker, respectively. Descendant cells from the intersectional domain are denoted by *green circles*, those from the subtractive population by *blue circles*. (Reproduced from ref. 4 with permission from Elsevier Science) (Color figure online)

Fig. 3.

Genetic inducible fate mapping strategy. **(a)** Schematic of a transgene encoding the site-specific recombinase-steroid fusion protein, SSR-ERT2, whose activity is regulated posttranslationally by the ligand 4-hydroxy tamoxifen (4-OHT, orange circle). **(b)** Inducible recombination and cell marking using SSR-ERT2. In the absence of 4-OHT, SSR-ERT2 is inactive due to sequestration of the fusion protein into an Hsp90 complex. Binding of 4-OHT to SSR-ERT2 results in a conformational change that disrupts the Hsp90 interaction, freeing the recombinase to enter the cell nucleus and mediate recombination at its target sites (triangles) positioned within an indicator transgene. Excisional recombination renders cells positive for reporter expression (for example, cytoplasmic β -gal as indicated in dark blue). **(c)** Cumulative versus inducible genetic fate mapping. Development of the neural tube is again rendered as simple cylinders progressing left to right in each row. Cumulative genetic fate mapping is schematized in the top two rows, much as done previously in Fig. 1b. *Top row* illustrates transient, midgestation cre recombinase expression, driven by *gene*

A, in progenitor cells of the dorsal neural tube. *Second row* illustrates activation of n β -gal, for example, as a lineage tracer in all cells that ever in their history expressed *gene A::cre*. Inducible genetic fate mapping is schematized in the bottom two rows. *Third row* illustrates transient, midgestation cre recombinase expression, driven by *gene A*, in progenitor cells of the dorsal neural tube. *Bottom row* illustrates selective activation of n β -gal in late-emerging cohorts (*blue triangles*) following administration of 4-OHT and subsequent induction of recombinase-mediated recombination of the indicator transgene. (Reproduced from ref. 4 with permission from Elsevier Science) (Color figure online)