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Spotlight

A new system for multiplexed mosaic analysis of gene function in the mouse

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In a recent issue of *Cell*, Liu et al. present an innovative mouse model system in which Cre/lox stochastically turns on transgenic expression of one out of up to 100 sgRNAs in somatic cells, creating genetic mosaicism that enables the multiplexed assessment of gene function *in vivo*.

Genetic analysis within model organisms, including the mouse, have been instrumental in uncovering genotype-phenotype relationships. Genetically engineered mouse models have been widely used to explore gene function across mammalian development, homeostasis, and disease. Individual efforts to assess the functions of genes of interest have been complemented by broad international efforts like the International Mouse Phenotyping Consortium. However, the throughput of allele generation has been a major bottleneck for conventional mouse genetic approaches, and thus many genes remain unstudied or understudied, especially across different tissues, cell types, and developmental stages.

Beyond simple germline gene inactivation and modification, the Cre/lox system has been widely used to induce genetic alterations in defined cell types and/or at defined times. Variant loxP sites have increased the versatility of genetic modifications enabling reversible gene inactivation and even the stochastic expression of a small number of fluorescent proteins or cDNAs from a single transgene (Livet et al., 2007; Pontes-Quero et al., 2017; Robles-Oteiza et al., 2015). Despite being a powerful tool for spatiotemporal control of *in vivo* genomic alterations, the Cre/lox system remains limited in scale.

Conversely, CRISPR/Cas9-based genome editing has enabled extensive high-throughput genetic screening. Within cancer cell lines and primary cultured cells *in vitro*, these screens are highly scalable and have been most commonly used to identify clonal expansion-related genes. However, not all cell and tissue types can be cultured *ex vivo*, and tissue culture systems fail to recapitulate many of the important interactions that occur within the physiological environment. Alternatively, gene function can be perturbed *ex vivo* followed by transplantation. However, these approaches are only optimized for a small number of cell types and run the risk of inadvertently changing cellular phenotypes. Multiplexed CRISPR/Cas9-based approaches, often relying on viral vectors to deliver single guide RNAs (sgRNAs), have also been employed to directly assess gene function in normal somatic cells and autochthonous cancer models (Cai et al., 2021; Jin et al., 2020). However, many cell types, tissue types, and developmental stages are either not amenable to viral approaches or require delicate surgical procedures (Beronja et al., 2013). These technical obstacles highlight the value of developing better non-invasive approaches to alter panels of genes within somatic cells *in situ.*

To address this need, Liu et al. developed a seemingly versatile technology called inducible mosaic animal for perturbation (iMAP) that integrates Cre/lox-based recombination with CRISPR/Cas9-mediated gene inactivation (Liu et al., 2022). They first developed a new *loxP* variant pair (Lox71-LoxTC9) that undergoes only one Cre-mediated recombination event (Figure 1). They used Golden Gate assembly to generate large arrays of up to 100 sgRNA cassettes that are preceded by a U6 protomer with an integrated Lox71 site and flanked by LoxTC9 sites. After transposon-mediated integration into zygotes, the transgenic mice were interbred with constitutive Cas9 and CreER mice. Notably, despite their repetitive nature, these sgRNA transgenic cassettes remained stable over many generations. Tamoxifen treatment led to transgene recombination, expression of one of the downstream sgRNA in each cell, and target gene inactivation (Figure 1). While not perfectly even, the representation of sgRNAs appeared sufficient to generate cells with all of the target genotypes. Analogous to the analysis of most *in vitro* CRISPR screens, subsequent amplification and next-generation sequencing of the U6-proximal sgRNA quantified overall enrichment and depletion of cells expressing each sgRNA. Enrichment of certain expected sgRNAs and depletion of sgRNAs targeting essential genes confirmed the effectiveness of the iMAP system. Their initial analysis identified many novel genes that regulate homeostasis across multiple tissue types.

At the genomic analysis level, iMAP has several key unique features. iMAP enables the parallel investigation of large number of genotypes within a limited number of mice. The internally controlled nature of iMAP should minimize the influence of mouse-to-mouse variability, thereby increasing precision relative to experimental methods that compare across mouse groups. Importantly, iMAP creates mosaic genetic inactivation across tissues. Such mosaics allow for the study of cell-autonomous effects, as many genotypes of cells exist within the same context. Finally, iMAP can efficiently inactivate genes across many different cell types and tissues in parallel, including organs that are difficult to access by viral methods. Thus, iMAP is uniquely high

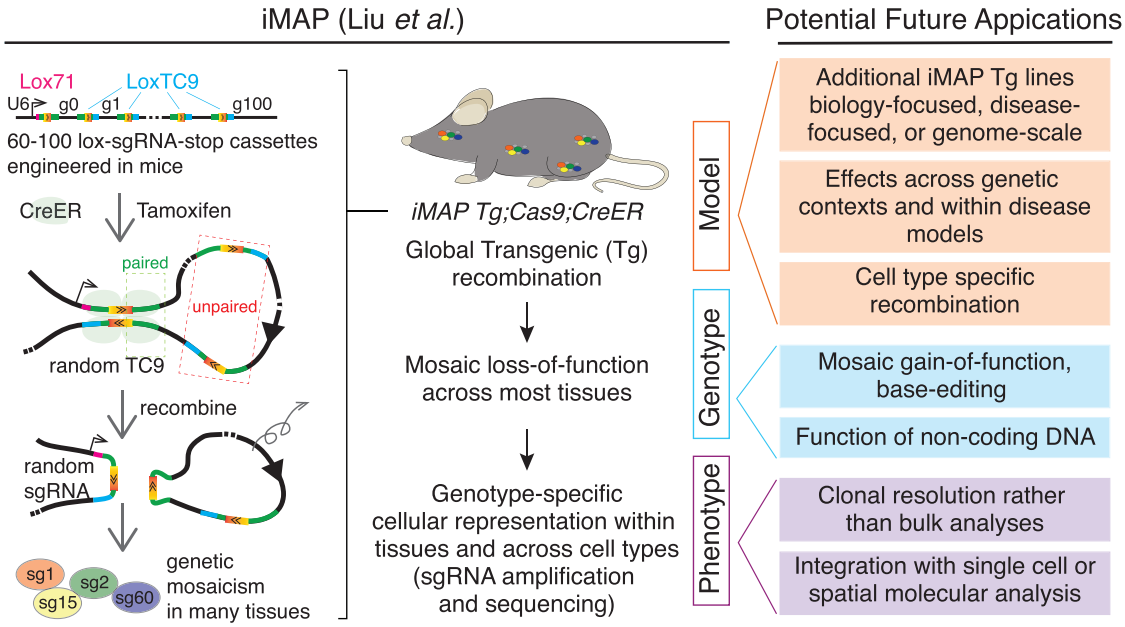

Figure 1. Cre/lox-regulated sgRNA expression to create genetic mosaics *in vivo*

Liu et al. (2022) developed iMAP in which CreER recombines the upstream Lox71 and one of the many LoxTC9 sites, resulting in a mosaic of cells expressing different sgRNAs across various tissues and organs. The highlights of this mouse model system and potential future applications are indicated.

throughput, not only regarding the number of genetic alterations that are created but also regarding the extent of different organs that can be investigated.

Given the value of quantitative multiplexed *in vivo* analyses, there are many potential future applications of this technology. Greater application of iMAP could enable the analysis of a large number of genes during development and homeostasis. Applications to cell types and tissues that are not readily interrogated using viral-based approaches could have the highest value. Perhaps a consortium-style project (a Cell Perturb atlas) could be complemented by the generation of more bespoke iMAP lines targeting genes involved in specific biological processes. Lineage-specific CreER alleles could be employed in conjunction with iMAP to determine the function of genes in specific cell types without potentially complicating phenotypes in other tissues.

To date, an understanding of the differential impact of diverse genes and pathways during homeostasis and within disease states is still mostly lacking. Therefore, the application of iMAP within different disease models could illuminate genes that contribute to or suppress disease phenotypes. Since the first sgRNA (g0) is expressed prior to Cre/lox-mediated recombination, all Cas9-expressing cells are edited by this sgRNA in addition to the recombination-activated sgRNA (Figure 1). Thus, genetic epistasis could also be assessed by either customizing g0 or integrating floxed alleles. While gene inactivation studies are a cornerstone of functional genomics, coupling iMAP-style transgenic mice with CRISPRa could enable analogous studies on spatiotemporal activation of genes. Moreover, the integration with other types of programmable nucleases could potentially provide insights into the effects of disease-associated alleles in a multiplex manner.

In future iterations, the resolution and dimensionality of the data generated by iMAP could be further enhanced. The current metric of overall sgRNA representation is an aggregate of the expansion of all cells with each sgRNA in a tissue. CRISPR-UMI-based analyses *in vitro* and similar approaches *in vivo* provide resolution on the number of cells in individually tagged clones of each genotype (Cai et al., 2021; Michlits et al., 2017). Integration of methods for clonal tagging that could be compatible with iMAP analysis would increase the resolution and provide insights into heterogeneity of responses (Pei et al., 2017; Yang et al., 2022). Finally, there are obvious opportunities to integrate approaches to provide molecular information on the cells of different genotypes. Incorporating single-cell or spatial omics approaches, such as integration of sgRNA capturing methods with scRNA-seq, would allow genotype to be linked with gene expression state (Jin et al., 2020; Yang et al., 2022). This would provide additional information on cellular heterogeneity and the linkage of genotype to molecular outputs.

Identifying the genetic drivers of cellular behavior *in vivo* remains a critical goal that will ultimately contribute to our ability to prevent, treat, and revert human disease states. The ability to assess gene function in a multiplexed manner will accelerate these studies. Liu et al. unveil a valuable addition to the functional genomic toolbox, which should complement existing approaches and has the potential to provide crucial new insights into the mechanisms of development, homeostasis, and disease.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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