Docket #: S23-209

Cas12a Mouse Models: Multiplex Somatic Genome Editing - Jax Stock Nos. 038388 and 038389

Stanford researchers have developed novel AsCas12a-expressing mouse models for simultaneous editing of multiple genomic loci in vivo with unique targeting capabilities relative to traditional Cas9 models, enabling the rapid creation of complex genotypes in somatic cells and cancer models.

To accelerate the generation of complex genotypes in somatic cells, Stanford researchers have generated a novel transgenic mouse models with Cre-regulated and constitutive expression of enhanced Acidaminococcus sp. Cas12a knockin to the H11 locus (H11LSL-enAsCas12a and H11enAsCas12a alleles). These mice enable in vivo multiplex genome editing and the simultaneous inactivation of multiple genomic loci using a simple targeting vector. Unlike Cas9, Cas12a offers increased accuracy and broader targeting capabilities using a single programmable transcript containing multiple spacers. These mice allow for streamlined editing, facilitating the investigation of concurrent somatic genomic alterations in vivo. This invention represents a significant advancement, paving the way for deeper insights into the impact of combinatorial gene inactivation.

The inventors' published work documented the unique value of these Cas12a mouse models in generating complex genotypes in somatic cells in order to better model human cancer. Cancer is an important outcome of somatic alterations, with tumors being genetically complex and driven by multiple genomic changes. While existing mouse models have been instrumental in studying single cancer genes, creating new models to model complex cancers is prohibitively time-consuming and expensive. Although in vivo somatic genome editing methods using Cas9-expressing mice are widely used, challenges persist in effecting multiple targeted edits simultaneously. The generation of targeting vectors is complex and time-consuming,

limiting the total number of concurrent alterations. Using these mice they generate compound genotypes, including diverse cancers driven by inactivation of trios of tumor suppressor genes or an oncogenic translocation. They generate tumous with inactivation of all combinations of nine tumor suppressor genes and find that the fitness of triple-knockout genotypes is largely explainable by one- and two-gene effects. These data suggest that Cas12a alleles will enable further rapid creation of disease models and high-throughput investigation of coincident genomic alterations in vivo.

Stage of Development

Research - in vivo. Mice homozygous for the Cas12a transgenes have been generated and are viable. Cas12a transgene functionality has been validated across multiple tissues (Hebert et al., Nature Biomedical Engineering, 2025).

Applications

- Rapid generation of complex genotypes in somatic cells
- Analysis of the in vivo effects of multiple genomic alterations in oncology and other fields
- Facilitates the discovery of targets for precision oncology through multiplexed genome editing
- Generation of pairwise and higher-order combinations of genetic alterations for various cell types to map phenotypes to complex genotypes
- Rapid generation of cancer models

Advantages

- Rapid and efficient multiplex genome editing
- Simultaneous targeting of multiple genomic loci
- Simple and easy to use
- Precise multiplexed editing using a single programmable transcript
- Unique targeting capabilities compared to Cas9
- Less complex patent landscape than Cas9
- Mice can be shipped directly from Jackson Laboratory into any vivarium (JAX# 038388 and 038389)

Publications

- Hebert JD, Xu H, Tang YJ, Ruiz PA, Detrick CR, Wang J, Hughes NW, Donosa O, Siah VP, Andrejka L, Karmakar S, Aboiralor I, Tang R, Sotillo R, Sage J, Cong L, Petrov DA, Winslow MM. Efficient and multiplexed somatic genome editing with Cas12a mice. *Nature Biomedical Engineering*. 2025 Nov;9(11):1982-1997. doi: 10.1038/s41551-025-01407-7. Epub 2025 May 30.PMID: 40447760

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