Vanderbilt Genome Editing Resource

VGER

Summer 2021 Newsletter

VGER continues to efficiently use CRISPR/Cas9 to derive new mouse models, and is pleased to announce progress related to the tagging of proteins and use of CRISPR interference in mice. We are currently optimizing our protocols for producing targeted mutations in iPSCs as a service, and remain happy to help you design gene editing strategies in immortalized cell lines.

Tagging proteins in mice. Fusion proteins have wide utility ranging from live cell or whole animal imaging to ChIP-seq. We have produced mouse lines that express a diversity of protein tags including fluorescent proteins, the HaloTag system, which is useful for cell imaging and protein analysis (Los et al., 2008), and smaller epitope tags, such as the ALFA-tag for nanobody-based applications (Gotzke et al., 2019). In designing a fusion protein, it is important to consider its intended use, which tag to use and its placement, and whether the tag could interfere with proper cellular localization or function of the fusion protein. We can help you work though these considerations, assist in choosing optimal guide RNAs, and maximize your chances of obtaining a fusion protein model that achieves your research needs.

CRISPR interference. VGER, working in collaboration with the Magnuson Lab, has successfully used *piggyBac* transgenesis to heritably express guide RNAs targeted to the promoter of a gene. When these mice are interbred with a mouse that expresses a dCas9-KRAB fusion protein (Yoneshiro et al., 2019), a 97% reduction in targeted gene expression is observed. If you are interested in using CRISPR interference or CRISPR activation technologies to modulate the expression of a gene or genes of interest to you, please directly contact Dr. Magnuson to obtain additional details and to weigh the pros and cons of this approach with Cre/LoxP.

Alternative genome editing strategies. Most of the projects that VGER performs are accomplished by injecting a ribonucleoprotein, consisting of SpCas9 and a guide RNA, along with a single stranded DNA donor into single cell mouse embryos. While this approach works well for DNA deletions and incorporation of DNA sequences under 2 kb, other approaches and strategies can be used to produce genome edited models, including base-editing, prime-editing, double-stranded DNA donors, Cas variants, and alternative CRISPR delivery methods (Clark et al., 2020). We are happy to collaborate with you to implement a custom-tailored genome editing strategy.

Search for a new staff member. In order to keep up with the increasing demand for services, and to continue to develop new services, we opened a search for a Research Assistant to join our team. If you know of anyone who may be interested, additional details are available at https://ecsr.fa.us2.oraclecloud.com/hcmUI/CandidateExperience/en/sites/CX.

As always, please contact Leesa Sampson at leesa.sampson@vanderbilt.edu or Jennifer Skelton at jennifer.skelton@vanderbilt.edu to discuss and initiate a project.

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