An Easy and Efficient Method to Detect Concatemers in rAAV-Mediated Knock-In Projects Using Stuffer DNA

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Résumé

Introduction:
Genome editing with the CRISPR/Cas9 system is a powerful tool for creating animal models. However, achieving precise knock-ins often faces inefficiencies, particularly due to concatemerization of donor DNA during integration. Recombinant adeno-associated viruses (rAAV) have emerged as a promising solution for efficient donor DNA delivery, offering improvements in knock-in workflows.

Aims:
This study aimed to optimize an rAAV-based knock-in protocol for rodents and establish a robust method to detect and characterize concatemers using PCR targeting "stuffer" DNA sequences.

Methods:
We developed 10 new animal lines (4 rat and 6 mouse) using an optimized protocol inspired by Mizuno et al. (2018). Zygotes were electroporated with CRISPR/Cas9 ribonucleoproteins and incubated for 5-6 hours in an rAAV donor DNA preparation. Embryos were then washed and re-implanted into foster females on the same day. To detect concatemers, we included a "stuffer" sequence -non-essential 'junk' DNA- at one end of the donor DNA, positioned between ITRs. Detection of this sequence via PCR indicates that homologous recombination did not occur correctly, suggesting the presence of concatemes at the target locus. Comprehensive allele characterization was performed using 5' and 3' PCR, Sanger sequencing, and droplet digital PCR copy counting (ddPCR).

Results:

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At least two positive founders per project were obtained with fewer than 80 embryos, demonstrating high efficiency. The inclusion of stuffer DNA enabled effective detection of concatemerized donor DNA through PCR. In one example, a 3764 bp knock-in in Sprague Dawley rats achieved 100% knock-in efficiency, with concatemerization easily identified using this approach. Other examples will be shown to highlight the success and potential of this screening approach.

Conclusions:

The use of stuffer DNA in rAAV constructs simplifies concatemer detection, improving the reliability of knock-in generation. This optimized workflow aligns with the 3Rs principles by reducing animal use and refining experimental methods.

Mots-Clés: targeted transgenesis, AAV, CRISPR/Cas9, mouse, rat, concatemers