VIRGINIA COMMONWEALTH UNIVERSITY

VCU

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Office of Research and Innovation spotlights...

September 2015

VCU Transgenic/Knockout Mouse Core

The mission of the VCU Transgenic/Knockout (TG/KO) Mouse Core is to provide an efficient and economical means for producing genetically modified mice, and to provide support services to facilitate the use of these models by VCU researchers.

Genetically modified mice include:

- Transgenic mice, in which customized genes are introduced into the mouse genome (either by random integration or by targeted insertion into a pre-determinded locus)
- Knockout mice, in which an endogenous mouse gene has been inactivated
- Knock-in mice, in which an endogenous mouse gene has been modified (e.g., "floxed", introduction of point mutation, etc)

Additional TG/KO Core services include:

- Breeding colony management
- Genotyping
- Mouse line rederivation
- Sperm and embryo cryopreservation
- MMRB IVIS imager management
- Consulting on project design and all things mouse-related

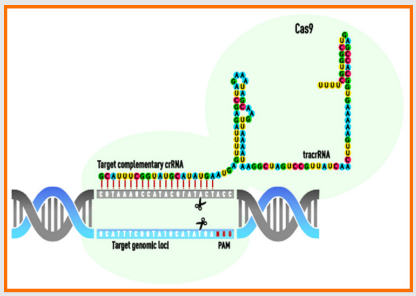
INTRODUCING CRISPR/CAS9 FOR GENOME EDITING!

Until recently, most knockout and knock-in mice were made by the same laborious methods originally developed in the mid-1980s. In late 2013, all that changed with the advent of CRISPR/Cas9, a system for creating RNA-guided DNA double strand breaks at precise genomic locations (repurposed from a bacterial adaptive immune defense against phage infection). The use of CRISPR/Cas9 technology eliminates the need for targeting in ES cells and generating chimeras – and makes KO mouse production as easy as generating transgenic mice!

The TG/KO is now offering CRISPR/Cas9 services, including:

- Design and synthesis of sgRNA and design of DNA repair templates (ssODNs)
- Microinjection of fertilized mouse eggs and screening of resulting pups for presence of the desired allele
- Screening for off-target mutation events

Contact Jolene Windle to discuss your CRISPR/Cas9 project!



Courtesy of Life Technologies™

Minimal requirements:

- a custom "sgRNA" consisting of both guide RNA (homologous to desired cleavage site) and scaffold RNA sequences
- Cas9 enzyme
- Optional: a single-strand or double-strand DNA repair template

These are co-injected into fertilized mouse eggs.

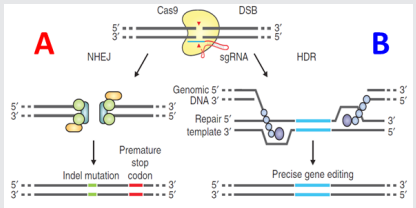


Figure 2 | DSB repair promotes gene editing. DSBs induced by Cas9 (yellow) can be repaired in one of two ways. In the error-prone NHEJ pathway, the ends of a DSB are processed by endogenous DNA repair machinery and rejoined, which can result in random indel mutations at the site of junction. Indel mutations occurring within the coding region of a gene can result in frameshifts and the creation of a premature stop codon, resulting in gene knockout. Alternatively, a repair template in the form of a plasmid or ssODN can be supplied to leverage the HDR pathway, which allows high fidelity and precise editing. Single-stranded nicks to the DNA can also induce HDR.

Nature Protocols 8:2281-2308, 2013

A Following creation of a double-strand break, the cell can rejoin the two ends by Non-Homologous End Joining, often creating small insertions or deletions. If this is in the coding region, it may create a frameshift that results in a KO allele.

– OR –

B If a DNA template homologous to the cleavage site but containing a desired modification (e.g., point mutation, loxP site, etc) is included, Homology-Directed Repair may create a modified allele containing the desired modification.

BOTH PROCESSES ARE HIGHLY EFFICIENT!

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