

Office of Research and Innovation spotlights...

Biological Macromolecule Core Facility

The mission of the Macromolecule Core is to provide investigators with validated molecular reagents, vectors, viruses, proteins and cell lines, to enable rapid progress towards individual research goals.

INTRODUCING CRISPR/CAS9 FOR GENOME EDITING!

CRISPR/Cas genome editing in cultured cells is a new service to assist investigators in generating cell lines with targeted mutations. CRISPR/Cas9 is a highly efficient bacterial surveillance system evolved to protect against the DNA of invading bacteriophages and other species, which has been adapted for use in mammalian cultured cells, and in animals of virtually any species.

The Cas9 endonuclease is a dual active site enzyme that makes a double stranded cut in target DNA adjacent to a 3-base recognition site (PAM motif, NGG). The targeting guide RNA is a gene specific sequence adjacent to any PAM motif, linked to a scaffold to which the endonuclease binds. Double-strand breaks in the genome of any cell is repaired by error-prone non-homologous end joining (NHEJ), which frequently results in small insertions or deletions (INDELS) at the site of the break. Placing the guide RNA sequence within the coding sequence of a target genes results in frame-shift mutations, deleting the function of the gene.

Specific mutations within Cas9 convert the enzyme into a single-strand nickase; two guide RNAs directed against each strand along with the mutant enzyme generates staggered nicks in the target DNA (Figure 1). This promotes homology-dependent repair (HDR), allowing site-specific insertion of mutations, reporter tags, or loxP sites for conditional deletion of genes or gene segments. A homolog of Cas9, Cpf1 (Fig 2), provides expanded targeting capability with the added advantage that this enzyme makes staggered single strand breaks in the target DNA, minimizing the potential off-target mutations.

The Biological Macromolecule Core Facility offers a range of applications, which have been successfully applied to multiple target genes in different cell line backgrounds. Each of these applications can be generated as mono-allelic or biallelic changes, depending on the needs of the investigator.

- · INDEL KO frameshift mutations
- Insertion of a variety of tags including affinity purification tags and fluorescent reporters
- Insertion of loxP sites for conditional deletion of specific, alternatively spliced exons or entire genes
- Insertion of site-specific mutations
- · Combinations of the above

Please visit our web site at

www.massey.vcu.edu/research/cores/biological-macromolecule/services/ for more information about this exciting new technology.

Contact Shirley Taylor
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editing project.



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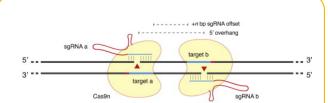


Figure 1: Double Nicking Facilitates Efficient Genome Editing in Human Cells (A) Schematic illustrating DNA double-stranded breaks using a pair of sgRNAs guiding Cas9 D10A nickases (Cas9n). The D10A mutation renders Cas9 able to cleave only the strand complementary to the guide RNA.

Ran et al, Cell, 154, 2013, 1380 – 1389. Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity

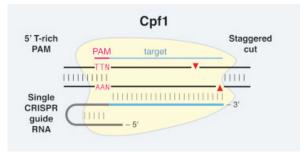


Figure 2: Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System that generates staggered single strand nicks

Zetsche et al, Cell 2015 (doi:10.1016/j.cell.2015.09.038)