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PLASMIDS WITH GENETIC VARIANTS OF CEL

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These plasmids encode carboxyl ester lipase (CEL) with and without genetic variants that are associated with chronic pancreatitis.

To make the plasmids, the researchers used a full-length human CEL cDNA clone. The cDNA encoded for a 14-repeat VNTR, the variant present in the CEL MODY family. The EcoRI/XhoI fragment containing the entire CEL cDNA coding region was subcloned into yeast (pPICZA) and mammalian (pcDNA3) protein expression vectors. The desired mutations were introduced by using customized primers and a QuikChange II XL site-directed mutagenesis kit. If stated, a FLAG tag was inserted in between human CEL secretory signal peptide and its mature polypeptide by overlap PCR to facilitate protein detection, and the insertion had no detectable effect on the properties of the CEL variant proteins. Dideoxynucleotide sequencing validated all plasmid DNA constructs.

Publication: A Carboxyl Ester Lipase (CEL) Mutant Causes Chronic Pancreatitis by Forming Intracellular Aggregates That Activate Apoptosis