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**Technology Offer**

## *Genome-Editing*-Technologies

# Cas9 double mutant with improved specificity compared to wild-type

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**The RNA-programmable DNA-endonuclease Cas9 is widely used for genome engineering, where a high degree of specificity is required. Though this is a mechanism well controlled by canonical base-pairing, there is evidence that Cas9 does accept mismatches under certain circumstances.**
**This might be seen as a threat to precision needed in genome engineering at least in environments where such high precision is ultimately needed and acceptance of mismatches needs to be avoided, e.g.: cellular gene therapy.**

## Technology

Scientists from Emmanuelle Charpentier´s laboratory at the Max Planck Unit for the Science of Pathogens in Berlin elaborated on Cas9 properties linked to binding and cleavage. They demonstrated that arginines in the Cas9 bridge helix critically influence guide RNA, and target DNA binding and cleavage. These arginines cluster in two groups that either increase or decrease the Cas9 sensitivity to mismatches.
Aiming for increasing the sensitivity of Cas9 to mismatches, hence making Cas9 and its guide RNA more specific for an intended target sequence, they identified a double mutant, **Cas9_R63A/Q768A**, that in most cases showed increased specificity compared to the wild type protein in human cells. And this gain in precision did come with no or only a slight, but still tolerable, reduction in activity in a target dependent fashion.
Therefor it might be beneficial to use such variant over the wild type in critical human genome editing approaches, e.g.: genome editing in human primary cell cultures *in vitro*, whenever precision is regarded as of utmost importance.

## Patent Information

A priority application has been filed in 2019 and is pursued under WO20/182941

## Literature

(1) Bridge helix arginines play a critical role in Cas9 sensitivity to mismatches, Bratovic et al. Nat Chem Biol 2020 May;16(5):587-595



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