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FUNCTIONAL ANALYSIS OF CRISPR-CAS TYPE I-D SYSTEM AND WYL DOMAIN-CONTAINING PROTEIN

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The research of arms race between viruses and bacteria resulted in many molecular tools that are now successfully used in genetic engineering. One of the most popular examples is the CRISPR-Cas system - an adaptive immunity found in bacteria and archaea. It can be easily reprogrammed to target different DNA sequences and therefore used to regulate gene expression or edit genomes [1].

The most widespread class 1 type I systems consist of multiprotein crRNA-guided effector (Cascade). It binds dsDNA target distinguished by a short sequence (PAM) only found in the foreign DNA. Once R-loop is formed the Cas3 helicase-nuclease degrades the target DNA [1]. The subtype I-D carries a type III like large subunit Cas10d which encodes a HD-nuclease domain and is considered an evolutionary intermediate between type I and type III systems [2]. This complex itself might perform the initial target nicking, while Cas3 acts as a helicase. Currently published results about type I-D CRISPR-Cas system are contradictory, one side claims the discovery PAM-dependent dsDNA and PAM-independent ssDNA cleavage [2], while the other denies the ssDNA cleavage and observes a ssRNA binding activity [3].

WYL domain-containing proteins can interact with DNA and are often described as transcription factors, but their function is not yet fully understood. Many of them are linked with antiviral defense systems and their genes are often found near CRISPR-Cas, BREX and other systems [4]. In our case, the WYL gene is in the same operon as type I-D system and could possibly regulate its transcription or cleavage activity. Therefore, we aim to determine the mechanism of type I-D CRISPR-Cas system and the possible interaction with WYL protein by utilizing *in vivo* phage and plasmid interference assays and *in vitro* DNA cleavage methods.

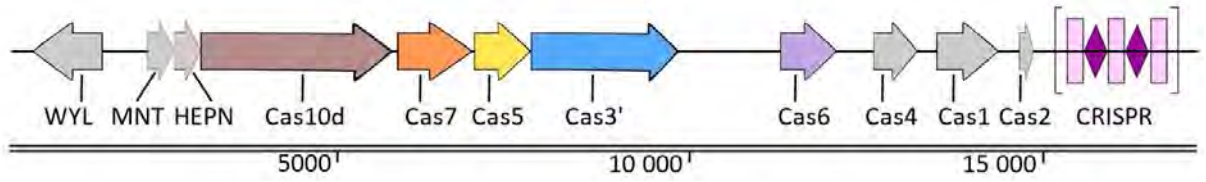

Fig. 1. *A. flos-aquae* genome operon carrying WYL and type I-D CRISPR-Cas.

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