Poster

BiFC and CRISPR-dCpf1, a new tool to study DNA-proteins interactions

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ABSTRACT

Motivation: CRISPR based systems allow directing a protein (Cpf1) to specific genome regions guided by a small RNA molecule (known as sgRNA), which leads Cpf1 to a specific DNA sequence. We are using this system in combination with the Bimolecular fluorescence complementation (BiFC) to monitor DNA-Protein interactions. This new system consists in tagging a fragment of YFP to the endonuclease dead dCpf1 which, by the action of the sgRNA previously designed, will still be guided to the specific DNA sequence without making the dsb. On the other hand, another native know to interact with the same region is tagged to the other YFP fragment. The interaction between the protein and that specific sequence would allow the association of the fragments produce a bimolecular fluorescent complex. This proof-of-principle to monitor DNA-Protein (and eventually DNA-DNA) interactions will allow genome dinamics studies in vivo.

Methods: We will use a dead version of the CRISPR based Cpf1 nuclease in combination with BiFC. We will obtain dCpf1 using a directed mutagenesis mechanism by PCR from the pMZCpf1. To validate the system we will first direct the dCpf1-YFP fusion to the centromeres, as a control region of the genome. This fusion will be created by overlapping PCR and also we will put it under the regulation of a medium strength promotor (nmt41), flanked by both halves of the Hygromycin resistance gene. We will integrate this cassette into a Hygromicin resistant strain by homologous recombination. We will obtain the specific sgRNA, which will guide Cpf1-YFP to the centromeres. To show proper localization we will use a cnp1-mCherry (centromeric protein) to assess co-localization. Once validated that level, we will split YFP by tagging the two proteins (dCpf1 and Cnp1) to validate biFC system.

Conclusions: The development of this system provides a great tool that would enable observing DNA- proteins interactions with a huge precision. With regards to the future, several sgRNAs could be generated to study interactions of proteins with multiple sequences of a genome.This would allow to study genomic loci dynamics and nuclear topology in vivo.

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