# Developing a predictive method for tunable control over gene expression based on CRISPR interference technology.

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**Project Goals: This project focuses on the development of new methods for controlling cellular gene expression in a multiplex, tunable and predictable manner. The goal is to develop a tool that enables to obtain a specified level of gene expression based on transcriptional control. This method would allow to fine-tune the level of transcription and to investigate the dynamic range of cellular functions. The advantage of this system is that it does not require making chromosomal modifications, as the regulation occurs at the level of transcription. Moreover, it offers a temporal control over gene expression, making it suitable for studying dynamic cellular responses. Such a system can be used, for instance, for metabolic flux optimization of industrial strains, as well as for investigating the mechanism of complex traits that rely upon the coordinated action of combinations of genes and regulators. Applications for these technologies lie within the general areas of sustainable fuels and chemicals production.**

Precise control over gene expression is essential for advancing metabolic engineering, as well as general understanding of the global context of cellular regulatory networks. Engineering and optimizing metabolic pathways requires being able to fine-tune expression of multiple genes simultaneously in a precise manner, which is restricted by time-consuming traditional strain engineering methods and a limited number of dose-response promoters. These limitations can be overcome by using CRISPR-based gene repression and activation [1]. In addition to offering multiplex gene regulation, CRISPR interference allows to control the degree of gene expression at the level of transcription. Transcriptional control can be achieved by adjusting the strength of interaction between the guide RNA and target gene through incorporating mismatches into the guide RNA sequence. The number, type and position of mismatches can affect the strength of gRNA binding and consequently, target gene expression. We seek to describe the relationship between gRNA sequence and its effective strength by using an adjustable parameter Markov model. The model can be trained on real expression data and be used as a tool for predicting and precisely controlling the level of transcription for a given gene. The application of this method has the potential to greatly simplify and reduce the cost of strain engineering, as well as provide insight into fundamental properties of metabolic networks.

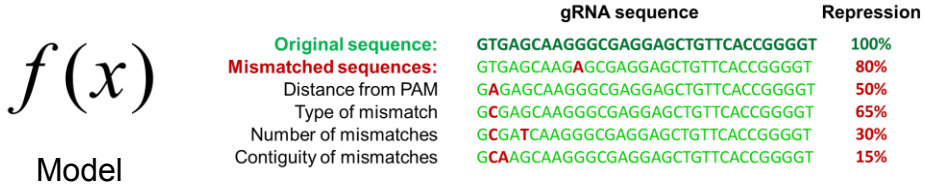


Figure 1: Example of the parameters that go into a machine-learning algorithm to train the model.

**References**

[1] Qi, Lei S., et al. "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression." Cell 152.5 (2013): 1173-1183.

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