Synthetic Promoters

One aim of mammalian synthetic biology is to construct promoters which are responsive to defined signaling pathways. In our project, we have developed a novel method using PCR to randomly assemble certain transcription factor binding sites to the ε promoter. Then we were able to characterize the expression levels of this library using flow cytometry and microscopy (Fig. 2). Furthermore, we have generated a NF-κB responsive promoter (clone 31) with approx. 100% upregulation upon induction with TNF-α (Fig. 3).

Measurement

Comparison of prokaryotic promoters is commonly carried out by using Relative Promoter Units (RPUs). So far, due to the higher level of complexity, no such unit has been reported for mammalian cells. In our project, we aimed to establish a methodology that would allow easy-to-use comparison of promoter strength in mammalian cells. To this end, we have defined the following units to describe promoter strength:

1. Relative Expression Units (REU): The amount of total folded protein generated by a promoter relative to the amount of folded protein generated by a reference promoter (JeT) under the same cellular conditions (measured protein levels, analyzed by fluorescence intensity with flow cytometry and microscopy (Fig. 3)).

2. Relative Mammalian Promoter Units (RMPU): The ratio of mRNA generated by the promoter of interest and JeT (measured mRNA level, analyzed by real-time PCR (Fig. 4)).

HEARTBEAT

(Heidelberg Artificial Transcription factor Binding site Engineering and Assembly Tool)

HEARTBEAT is a model to predict functional synthetic promoter sequences. In contrast to existing biological models, we are modeling the function of single parts. We describe promoters as a module of functional subunits which is influenced by 4 parameters (Fig. 8):

- The distance of each TFBS to the transcription start site; synregistic effects
- The quality of the binding motive; the purity of the spacer sequences connecting the TFBS. We scored over 4000 human promoter sequences for these information, enabling us to create synthetic promoters responsive to Sterol Regulatory Element Binding Protein (SREBP).

Two sequences designed by HEARTBEAT revealed a significant response upon induction (Fig. 7). This shows that HEARTBEAT helps to minimize the efforts for the generation of synthetic promoters.

Standards in Mammalian Synthetic Biology

The Heidelberg iGEM Team

HEARTBEAT FN modeling can be used to describe dynamic gene regulatory networks as well as for characterizing newly designed synthetic promoters.

Outlook

The aim of this year’s Heidelberg iGEM project was to lay the foundations for standardized synthetic mammalian biology (SMB). We have created a new cloning standard (SBB) as well as standardized measurement methods and units (REU and RMPU) in order to add to this we provide an in silico guided method to construct new parts. The next steps include producing a cell line with a stable integration site as chassis with a multi-color and multi-compartment output to simultaneously monitor several user-defined pathways at the same time. We are already on our way to making this a reality and the future. Possibilities of controllable and targeted gene expression for medical applications are overwhelming – for example, selective targeting of cancer cells in vitro. There is great potential in this new field of synthetic biology and we hope our project will accelerate the progress and enable other iGEM teams to enrich the eukaryotic section of the registry.