A computational approach to building gene silencing modules

Gene silencing by antisense RNA

The past decades pointed out RNA has a lot of functions besides being an information carrier (mRNA). Small RNAs (e.g. antisense RNA) form an essential part of different prokaryotic regulatory mechanisms by, for example, blocking ribosome binding sites (RBS). As such sRNA can be used in synthetically constructed biological devices to silence a gene on demand. Tools and know how for model-based design of RNA molecules that efficiently block the RBS of a specific gene are however still limited. Here we present a method to design silencing modules that can efficiently block translation. This approach uses knowledge on antisense RNA to model the physical nature of the biological interactions involved.

Identifying important features

Based on a literature review 11 features involved in sRNA silencing were identified[2],[3]. These features determine key steps in the process of gene silencing (see figure below).

Using a fixed 5’UTR and and RBS 500,000 candidate antisense sequences with a length of 20, 30 and 40 bp were generated based on the 5’UTR. For all sequences these features were determined in silico using several dynamic programming algorithms, which allow to accurately predict RNA-RNA interactions[11],[12],

Reducing redundant information

To increase the understanding of these features and to reduce the enormous search space an in silico evaluation was performed. Herein, redundant information is eliminated by verifying whether some of these features are inherently correlated. After a Pearson correlation analysis, using the 500,000 candidate antisense sequences, only 7 independent features, i.e., FAA, FAB, EIS, RBS11, PT, PAU, and L, were retained of the initial 11 (-0.9 < PCC < 0.9).

In vivo analysis of sRNA

An initial experimental design was generated for the 7 selected features and the corresponding candidate antisense sequences were selected from the pool of 500,000 in silico generated sequences. To evaluate their silencing efficiency in vivo, the fluorescent protein RFP was used as reporter gene and the maximal RFP expression rate (dRFP/dt/OD600) as indicator.

In vivo screening of important features

The in vivo screening was performed to determine the importance of all 6 features (lengths are treated separately). The features are rescaled and from the 500,000 generated sequences the most suitable sequences were selected that contain 5 out of 6 features. The maximal RFP expression rate (dRFP/dt/OD600) of these tested sequences is depicted in Figure 2.

From Figure 2, it is clear that the majority of designed antisense sequences resulted in a decreased RFP expression of more than 50%. To identify the most determinative features a preliminary linear regression was performed (see Figure 3). This revealed that the formation energy of the antisense-UTR complex (FAB) and the estimated seed energy (EIS) are of utmost importance for silencing.

Conclusions

A computational approach to unravel the physical nature of gene silencing was developed. Initial tests with different designed antisense sequences show that more than 50% of RFP expression was inhibited by RBS blockage. Preliminary analysis shows that the formation energy of the antisense-UTR complex (FAB) and the estimated seed energy (EIS) are of great importance though further research is needed to fully evaluate the importance of the various features. This should ultimately lead to the development of an efficient and reliable tool in silico design gene silencing modules.

References


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