Thermoswitch
A Computer-aided Temperature-response Regulatory RNA Design

**Contribution**
- RNAThermo is a software written to design RNAT according to their regulation temperature.
- Designed RNATs were proved to be structurally and functionally correct.
- Applications of RNAThermo were discussed.

**Introduction**
RNA thermometer (RNAT) is a kind of temperature-sensing regulatory mRNA sequence.

**Algorithm**
- As the environmental temperature changes, the RNAT can fold into a series of different secondary structure, such as stem loops.
- Some of the structures can block ribosomes’ access to the mRNA thus hinder translation.
- DNA → RNA → Protein
- Rational design of RNATs provides a new way of constructing metabolic pathways in synthetic biology.

**Software**
RNAThermo is composed of a graphical user interface for setting parameters and a core program for designing desired RNATs.

- **Physical Principle**
  Secondary structure prediction is the central issue in RNAT design. According to algorithm requirement, two physical principles were taken to solve such problem:
  - Free energy minimization (MFE) - regards structure with minimal free energy as optimal solution.
  - Partition function - gives each structures’ probability of appearance.

**Verification**
- **Structural Verification**
  The first step of verification is testing the temperature-response structure of RNAT in vitro, with inline probing.
  - Single-stranded RNA is susceptible to spontaneous cleavage through in-line attack. Thus, the number of unpaired nucleotides is indicated by the number of bands.
  - At high temperature, sections marked by red boxes melted thus bands appeared. The result indicated the designed RNAT folded into desired structure.

- **Functional Verification**
  The second step of verification is testing the temperature-response regulatory function in vivo, with eGFP as the reporter gene.
  - The eGFP gene is extended with designed RNAT sequence by PCR.
  - E.coli with and without RNAT were cultured until they reached stationary phase.
  - E.Coli from the same tube was divided into two groups, cultivated at 30°C and 45°C respectively for 2 hours. Each group’s fluorescence was measured.

**Fluorescence Result**
RNATs are tested in a double-control method and here is one of them. Left shows at low temperature, RNAT switches off and inhibit expression. At high temperature, RNAT switches on and the inhibition relieved. Right shows the quantitative result.

**Application**
RNAThermo has a wide-scale application in industry and scientific research.
- In industry
  - Lysate engineered microorganism to extract the products. (RNAT + Lysozyme)
  - Avoid toxic products expression during cell accumulation. (RNAT + Toxic product)

- In scientific research
  - In developmental biology, switch-on or switch-off the gene to be studied. (RNAT + gene to be studied)
  - Align several RNATs to accomplish fine regulation. (RNAT-1+RNAT-2+pathways)

---Switch on the world!