13th International Conference on
Structural and Molecular Biology: Techniques & Market Analysis
October 22-23, 2018 | Ottawa, Canada

In vitro synthesised functioning reporter mRNA as a method for evaluating the Kozak sequence function directly in the mRNA strand

Gustavo Torres de Souza
Brazilian Agricultural Research Corporation, Brazil

The importance of RNA-base techniques has been in great prominence over the last few years due to their frequent utilization in the most recent molecular biology tool for genome editing, CRISPR/Cas9, as well as for RNA interferences. That has emphasized the necessity of standardization of experiments with RNA and studying the function of signal sequences in the RNA strand. Thus, here, we aimed at standardizing the production of a eGFP mRNA with the fully known sequence.

***In vitro* synthesized four mRNA molecules:**

1. eGFP mRNA (non-polyadenylated);
2. Kozak-eGFP mRNA (non-polyadenylated, produced from the Kozak-containing amplicon);
3. eGFP-PolyA mRNA (polyadenylated);
4. Kozak-eGFP-PolyA mRNA (containing both signals, Kozak sequence, and poly(A) tail), utilizing a common eGFP plasmid as the reference.

mRNA molecules were transfected into two cell lineages:

1. HEK-293-FT, which is easily transfectable and expresses well both plasmid and mRNA transfected; and
2. MDBK, observed, in our previous experiments, as hard-to-transfect and/or express DNA. We detected eGFP expression by flow cytometric analysis and fluorescent microscopy (utilizing lentivirus-transformed cell lines as positive controls). Flow cytometry allowed us to notice a marked difference among the mRNA groups ($p < 0.01$), both in fluorescent population percentage and inflorescence intensity. Therefore, we could analyze the necessary mRNA elements in order to achieve translation directly by studying the mRNA strand and not by the DNA sequence prior to transcription. Furthermore, the results showed that the presence of the Kozak sequence impacts translation directly regardless of transcription. The method may be useful in the study of other RNA signaling sequences.

Biography

Gustavo was an undergraduate student at Juiz of Fora Federal University and intern in the human genetics and cell therapy laboratory (GENETEC) where had to experience in the culture of mesenchymal and the cancer cell and molecular biology techniques, aiming the use of mesenchymal stem cells as study models. An international student in the year of 2012 at Colorado College where he worked with computational chemistry. He has been developing his graduate research in Genetics and Biotechnology in the Laboratory of Animal Reproduction at EMBRAPA in collaboration with Recombinetics Inc. His aims are the optimization of production of genetically modified animals for various purposes.

gustavotsouza@hotmail.com

Notes: