mRNA approach for transient expression in dinoflagellates

Claudio Slamovits

Abstract

!!! ~~~~~ IN DEVELOPMENT ~~~~~ !!!

This approach uses in-vitro transcription to generate a dinoflagellate-type mRNA (i.e. capped with the dino-SL leader), which is introduced into cells using an RNA transformation reagent. Screening for expression of the protein encoded in the mRNA follows.

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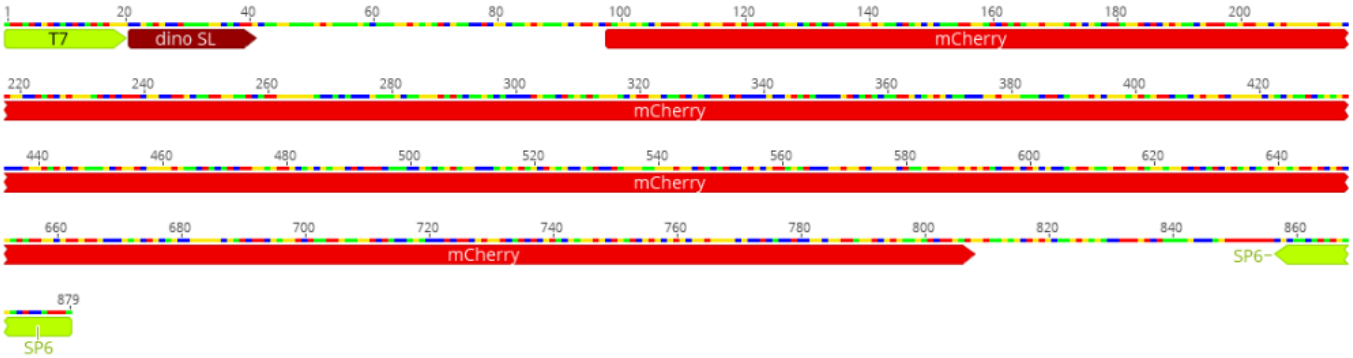
Guidelines



Protocol

Design of the artificial gene

Step 1.

A gene with the following structure is generated, either by subcloning or gene synthesis: 5’-SP6-dinoSL-UTR-ORF-UTR-T7-3'

SP6: promoter for SP6 RNA polymerase for in-vitro transcription (e.g. MEGA Script by Ambion)

DinoSL: 22-bp spliced leader present in all dinoflagellate mRNAs : CCGTAGCCATTTTGGCTCAAG

UTRs are from the O. marina HSP90 gene

ORF: encodes the protein of interest. In our case we use mCherry with codon usage optimized for Oxyrrhis marina

T7: promoter for T7 RNA polymerase: it can be used to generate an antisense RNA to be used as a control

For a fairly short gene (e.g. 1Kb) gene synthesis is (presumably) preferably to subcloning, especially if custom codon usage is desirable

In vitro transcription

Step 2.

1) Linearize vector using a restriction enzyme with a single site away from the insert

2) Perform in-vitro transcription (e.g. MEGA Script by Ambion)

3) Perform CAPping reaction (Vaccinia Capping System by NEB)

4) Perform polyadenylation (e.g. E. coli poly (A) polymerase by NEB)

Delivery of mRNA to cells

Step 3.

Introduce the synthetic mRNA into cells (e.g. TransIT mRNA transfection system)

Expression screening

Step 4.