In vitro synthesized mRNA transfection to Perkinsus marinus

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Protist Research to Optimize Tools in Genetics (PROT-G)

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Plasmid preparation

1 We created the plasmid vector (T7-SL-eGFP) that the *egfp* gene with a 5′ UTR of the *P. marinus* TPT2 gene and a 3′ UTR of the *P. marinus* MOE gene was inserted in pSP72 (Promega). The 5′ UTR contains the spliced leader sequence (ACCGTAGCCATCTTGGCTCAAG) found in *P. marinus* nuclear genes. The construct of T7-SL-eGFP can be obtained from T7-SL-eGFP.pdf

In vivo mRNA synthesis

2 mRNA with a 5′ m^7G cap and 3′ poly(A)-tail was synthesized by mMESSAGE mMACHINE T7 Transcription Kit (Ambion) with the linearized plasmid of T7-SL-eGFP (it was digested by *Pvu*II). The synthesized mRNA was treated by TURBO DNase, and collected by ethanol precipitation according to the manufacturer's instructions. The mRNA concentration was calculated by Qubit 3.0 (Thermo Life Technologies).

Transfection by electroporation

3 *P. marinus* cells (5×10^7 log-phase trophozoites) were collected by a brief centrifugation (3,000 g × 5 min) and the pellet was resuspended by 100 µL solution 2 supplied by Basic Parasite Nucleofector Kit 2 (Lonza). Synthesized mRNA was added to the solution in different concentration (1, 5, 10, or 20 µg). Electroporation was performed by Amaxa Nucleofector II (Lonza) with the D-023 program, and then the cells were transferred to 2 mL fresh medium (ATCC medium 1886) in a 6-well plate.

GFP observation

4 After 24 to 48 h, transfected cells were observed under an inverted fluorescence microscope with a GFP filter.