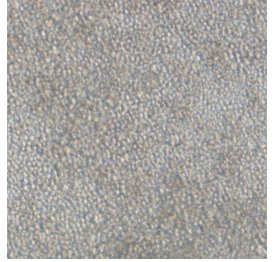


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## In vitro synthesized mRNA transfection to *Perkinsus marinus*

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**Protocol status:** Working

**We use this protocol and it's working**


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## 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<sup>7</sup>G 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 *PvuII*). 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<sup>7</sup> 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.