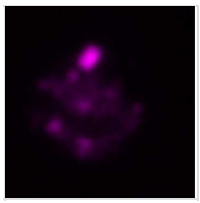

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Electroporation of fluorescent antisense molecules into *Bodo saltans* and live imaging

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Symbiosis Model Systems Bodo protocols

Ewa Chrostek

This is the protocol used in our Laboratory at the University of Liverpool to electroporate molecules into Bodo saltans.

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Overview

1 Antisense peptide nucleic acids (PNAs, synthetised by Panagene, South Korea) were electroporated into *Bodo slatans* cells using Neon® Transfection System MPK1025 (Invitrogen).

Bodo culture conditions

2 *Bodo saltans* was cultured in a cerophyl-based medium enriched with 3.5 mM sodium phosphate dibasic (Na_2HPO_4)[1]. Cultures were incubated at 22 °C in T25 tissue culture flasks containing 20 ml of media bacterized with *Klebsiella pneumoniae subsp. Pneumoniae* (ATCC® 700831). 3 to 4

day old cultures were used for electroporation.

Preparing cells for electroporation

3 The steps below describe how to prepare *Bodo* cells for electroporation.

3.1 1-Filter the culture through 100 and 8 µm filter.

3.2 2-Harvest the cells by centrifugation at 1200 × g for 12 mins at 19 °C.

3.3 3-Wash the cells with 10 ml PBS and centrifuge as above.

3.4 4-Re-suspend the cells in 5 ml PBS, count the cells using hemocytometer and take the volume of cells which contains 5×10^5 cells, as recommended for Neon transfection kit for 10 µl tip.

3.5 5-Centrifuge at 1200 × g for 12 mins at 19 °C.

3.6 6-Remove the PBS and resuspend the cells in resuspension buffer for electroporation.

3.7 7-Add the antisense molecule at the final concentration of 50 µM to the *Bodo* cells and mix well by pipetting.

3.8 8-Aspirate the mix into Neon pipette and electroporate using 1800 V, 10 ms pulse width and 1 pulse.

Preparing electroporated cells for imaging

4 The steps below describe how to prepare electroporated *Bodo* cells for imaging.

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4.1 1-Empty the pipette of Neon system in a well of a 96-well plate after electroporation, add 5 µl of PBS, mix, add 15 µl of warm low melting temperature agarose (Thermo Fisher Scientific), and mix with the cells. Let is set for a few seconds.

4.2 2-Add 200 µl of Hochest 33342 (Thermo Fisher Scientific)diluted 1:2000 in PBSto the agarose-embedded *Bodo* and incubate 10 mins at room temperature (RT).

4.3 3-Wash the agarose with PBS (2×5mins) at RT.

4.4 4-Remove the agarose from the well with clean forceps and place it on a microscope slide.

4.5 5-Add a drop of a mounting medium (eg. Vectashield, Vector Laboratories), and flatten the agarose as much as you can using the coverslip.

4.6 6-Proceed with either fluorescence or confocal imaging.

4.7

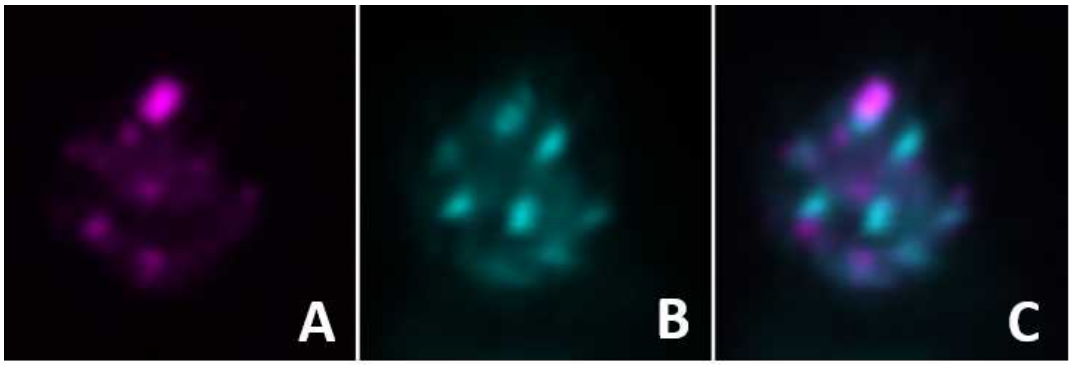

Figure 1. Confocal image of live*Bodo* cell electroporated with antisense peptide nucleic acid (PNA). A) TAMRA-labelled antisense molecules inside *Bodo* cell. B) DNA of bacterial cells inside *Bodo* stained with Hoechst 33342.C) Overlay of two channels.

References

5 Gomaa Fatma, Li ZuHong, Docampo Roberto, Girguis Peter, E. V. Bodo saltans culture protocol V.2. *Protocols.io* (2018). doi:10.17504/protocols.io.sh6eb9e

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