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Modified protocol to improve Bodo saltans yield in culture.

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ABSTRACT

This protocol is a modified version to improve the yield of Bodo saltans cell density in culture. The original protocol is: https://www.protocols.io/view/bodo-saltans-culture-protocol-sh6eb9e

ATTACHMENTS

Modified protocol to improve Bodo Saltans yield in culture.docx

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EXTERNAL LINK

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1 Prepare the bacteria-Bodo saltans medium as described in the protocol （https://www.protocols.io/view/bodo-saltans-culture-protocol-sh6eb9e）.

2 Collect Bodo cells from a T25 tissue culture flask by centrifugation at 1200 x g for 6 minutes. After removing the supernatant, add deionized water to resuspend the pelleted cells. After two washes with water, resuspend the cells in culture medium. Usually the Bodo saltans/bacteria ratio is >1 after two washes, as determined by FACS analysis.

3 Inoculate a T25 tissue culture flask (50 ml) containing 10 to 15 ml of fresh medium with 10 mg/ml puromycin. Puromycin at this concentration has no inhibitory effect on the growth of Bodo saltans but it can slow down bacterial growth. Transfer 100 ml-0.5 ml cells from step 2 into the flask. Incubate horizontally at 18C with loosely adjusted cap.

4 After 2-4 days, when the density of Bodo cells reaches to ~3 X 106/ml, collect and wash the cells for downstream applications.

5 The advantages of this protocol are improved yield and purity. 10 mg/ml puromycin can efficiently prevent bacterial population overgrowth in the flask. The medium is much cleaner and there are no aggregates formed by overgrown bacteria. Usually, we can only get 1 X 106/ml of Bodo cells using conventional culture conditions, and need filtration and 4-5 washes to remove all bacteria from the culture for other applications, such as electroporation or DNA extraction. After these modifications Bodo cells can reach 3 X 106/ml in the culture resulting in a purer population of cells after only 2 washes.

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