**Modified protocol to improve *Bodo saltans* yield in culture.**

**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>

1. Prepare the bacteria-*Bodo* *saltans* medium as described in the above protocol.
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 g/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 l-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 g/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.