Fluorescence activated cell sorting (FACS) of Perkinsus marinus transformants

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Abstract

Citation: Imen Lassadi Fluorescence activated cell sorting (FACS) of Perkinsus marinus transformants. **protocols.io** dx.doi.org/10.17504/protocols.io.hh2b38e
Published: 03 Apr 2017

Protocol

Cell Culture and Electroporation

Step 1.

See protocol "Oyster parasite *Perkinsus marinus* transformation using Amaxa electroporator and non-proprietary electroporation buffer"
Monitor transfection efficiency, by testing for the presence of fluorescent cells 5 to 6 days post transfection.

Cell recovery

Step 2.

Once the transfection success is confirmed, transfer the cells to a larger volume in a T75 flask (or equivalent) to allow cell number to increase (up to one week).

Fluorescence activated cell sorting protocol

Step 3.

The experiment should be undertaken in sterile conditions

Use *Perkinsus marinus* wild type cells as a control to set gating for non-fluorescent, single, live cells.

Set up a template that includes a bivariate plot to display forward scatter (FSC) and side scatter (SSC), and one histogram for each fluorophore that will be used (e.g. eGFP, mCherry)

Gate cells for fluorescence above that seen for the untransformed control cells, and sort these cells either as population or as single cells to a 96 well plate.

Sorted single cells in 96 well plates can then be cultured for 2 months at 25°C in the dark.

Inspect cells by microscopy to confirm fluorescence status.