Transformtion of Perkinsus marinus by Amaxa and Bio-Rad

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Abstract

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Protocol

Step 1.

A genetic transformation method has been established in *Perkinsus marinus* using the Amaxa Nucleofector system (Fernández-Robledo et al. 2008, Sakamoto et al. 2016). However, all electroporation conditions (voltage, resistance, capacity as well as transformation buffer components) are trade secrets. We tried to optimize the transformation conditions for *P. marinus* using the Gene Pulser Xcell (Bio-Rad) system.

We determined an optimal condition using FITC-dextran introduction tests: 200 V, 15 ms square wave in Bio-Rad 0.2 cm cuvette with 100 µL modified cytomix solution (70 mM KCl, 5.8 mM potassium phosphate buffer, 2.9 mM MgCl2, 14.5 mM HEPES, 0.09 mM $CaCl_2$, 1.2 mM EGTA, 0.2 M mannitol, pH 7.4). We compared the efficiency of FITC-dextran introduction between two different systems of Amaxa (parasite nucleofector solution 2 with the program D-023) and Bio-Rad (described above). We detected that more than 50% of cells showed positive fluorescence in the both cases (Fig. 1 and 2).

Fig. 1: FITC fluorescence in the Amaxa condition

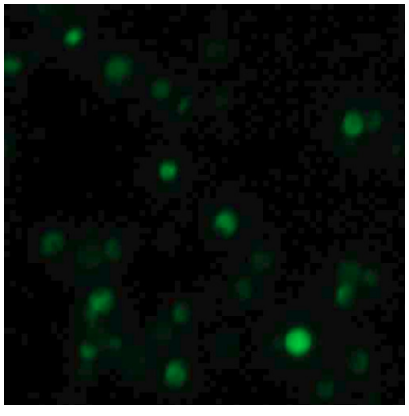
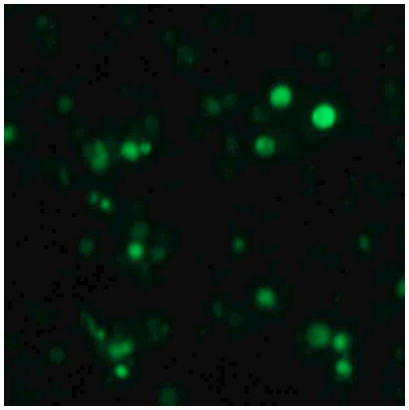


Fig. 2: FITC fluoresncence in the Bio-Rad condition



P. marinus cells were transiently transformed with pMOE-GFP by the both conditions, and we observed GFP fluorescence 48 h after transformation. Transformation efficiency of the Amaxa condition was more than 100 times higher than the Bio-Rad condition, although the FITC-dextran tests showed the similar efficiency (Fig. 3 and 4). I guess FITC-dextran introduction efficiency would not be correlated with actual transformation efficiency.

Fig. 3: GFP expressing cells transformed by the Amaxa condition

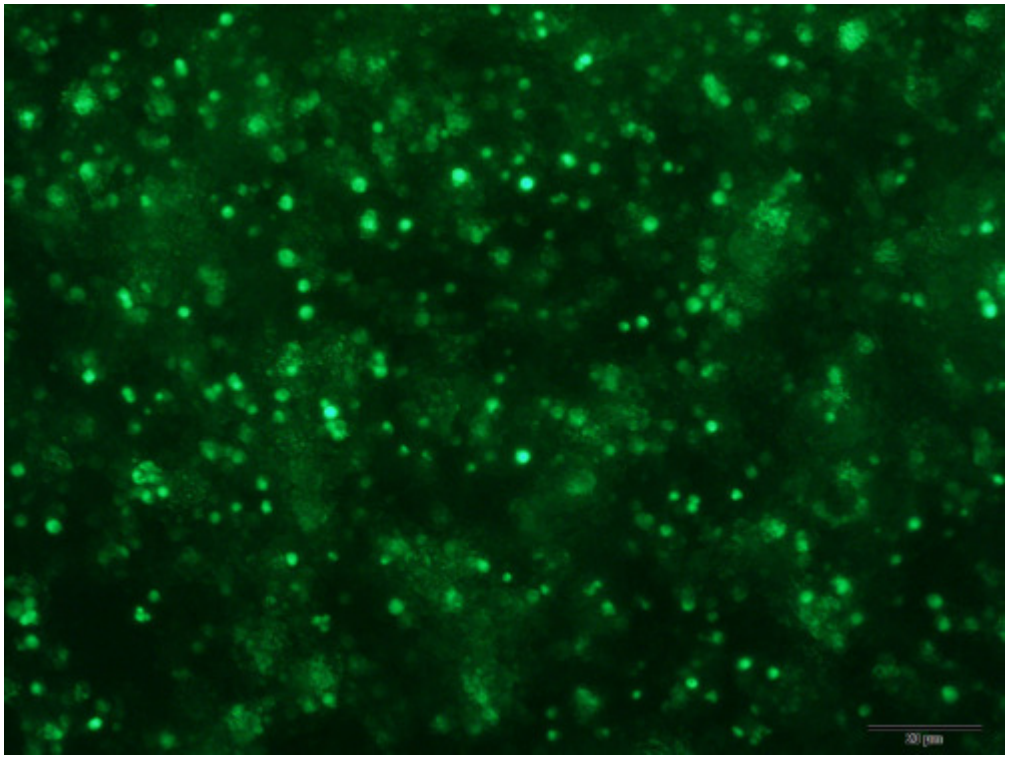


Fig. 4: GFP expressing cells transformed by the Bio-Rad condition