Transformtion of Perkinsus marinus by Amaxa and Bio-Rad

Version 3

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Abstract

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Guidelines

A genetic transformation method has been established in *Perkinsus marinus* using the Amaxa Nucleofector system (Fernández-Robledo et al. 2008, Sakamoto et al. 2016). 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

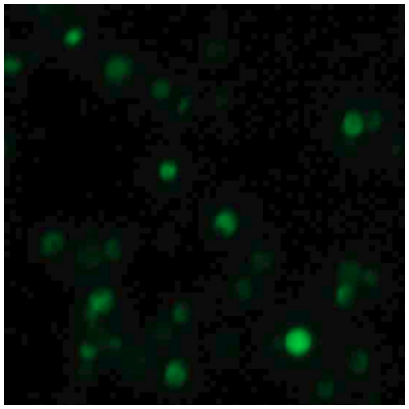
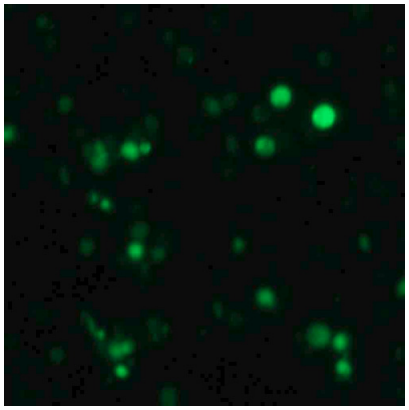


Fig. 2: FITC fluoresncence in the Bio-Rad condition



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

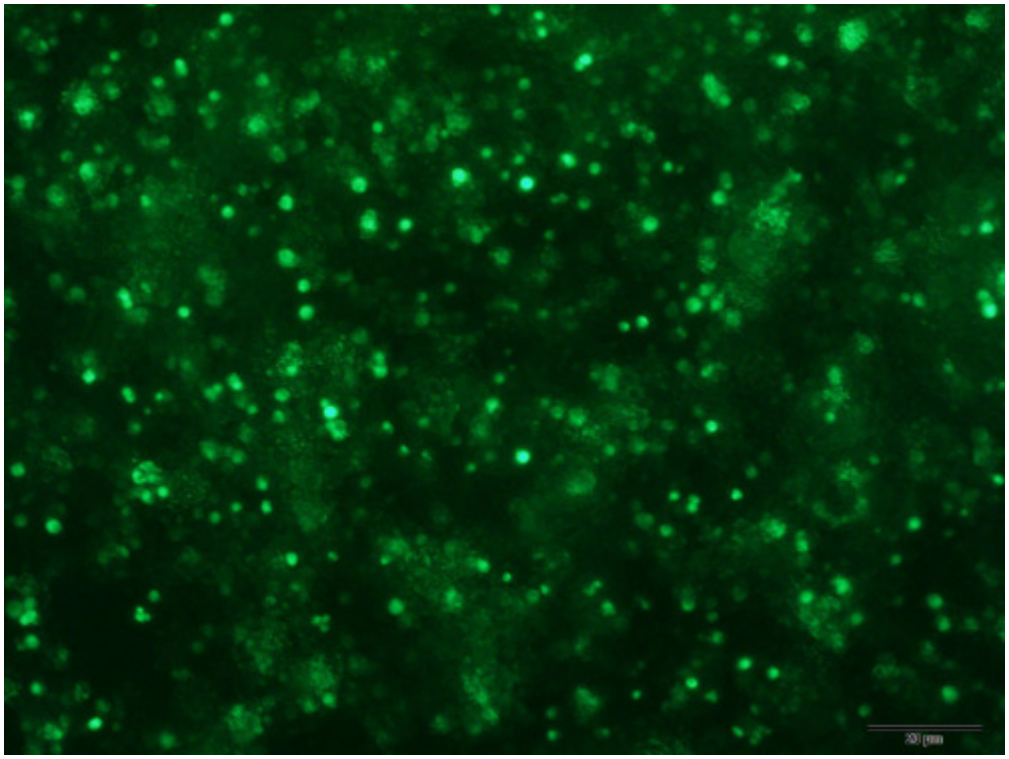
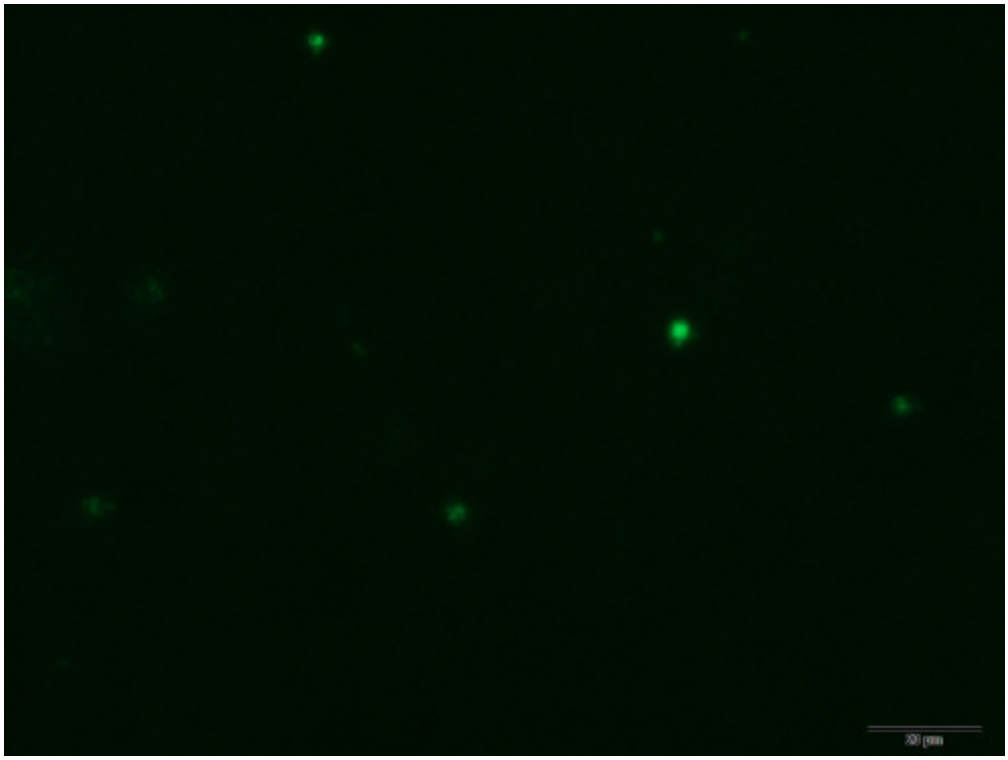


Fig. 4: GFP expressing cells transformed by the Bio-Rad condition



Protocol