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Expression of ß-galactosidase in *Meretrix* Clam by Electroporation

Wen-Lung Wu[1*] and Chyi-Lih Tsai[1]

ABSTRACT

The generation of transgenic meretrix clam (*Meretrix lusoria* Röding) by using a square pulse electroporator. The plasmid pmiwZ was linearized at *Kpn*I site and electroporated into the meretrix clam embryos at the two- and four-cell stages. The activity of β -galactosidase was detected in digestive duct of veliger larvae which are not processed with plasmid transfer. The β -galactosidase report system functioned well in veliger larvae of meretrix clam by electroporation.

Key Words : Mollusca, Gene transfer, Veliger.

INTRODUCTION

The production of transgenic animals has proven to be a powerful tool in the study of developmentally regulated gene. The traditional method of microinjecting foreign DNA into fertilized eggs is tedious and time comsiming (McMahon, et al., 1985). We report here the generation of transgenic meretrix clam (*Meretrix lusoria* Röding) by using a square pulse electroporator (Baekon 2000, USA). The plasmid pmiwZ (Suemori, et al., 1990) was linearized at *Kpn*I site and electroporated into the meretrix clam embryos at the two- and four-cell stages.

MATERIALS AND METHODS

The electroporation condition is designed at voltage, 4-10KV; pulse time, 160μs; number of pulses, 1024; burst time, 0.8s; cycle, 5-10; dial, 220; sample volume 100μl (500 embryos); plasmid concentration, 50μg/ml; electroporation buffer, autoclaved seawater. After electroporation, the embryos were transfered to petri dishes with 5ml autoclaved seawater and hatched two days until developing veliger stage.

[1]Institute of Zoology, Academia Sinica, Taipei, Taiwan.

* To whom correspondence should be addressed.

RESULTS AND DISCUSSIONS

Veliger larvae were fixed in 2.0% glutaraldehyde for 30mins and washed several times in PBS. β-galactosidase activity was made visible by incubation in X-Gal solution (0.05% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside in dimethylformamide 6mM $K_3Fe(CN)_6$; 6mM $K_4Fe(CN)_6$; 0.1% TritonX-100; 1mM $MgCl_2$ in PBS, pH 8.0 overnight at 37℃. The activity of β-galactosidase was detected in digestive duct of veliger larvae which are not processed with plasmid transfer (Fig. 1A, B ; p.201).

The resultant enzyme activity may originate from engulfed symbiotic bacteria, cells or endogenous β-galactosidase by oneself because this stage already start to feed phytoplankton or bacteria. The result was similar to rainbow trout (Inoue, et al., 1991) In trangenic veliger larvae, the activity of β-galactosidase was detected ubiquitously in the whole body of larvae (Fig. 1C, D ; p.201) and partially mosaical on velum or rudiment of foot. The percentage of positive blue stain was 5-30%.

From this result, the plasmid pmiwZ used to drive the β-galactosidase gene is an avian chimeric promoter, miw (consist of RSV LTR sequence and β-actin promoter) Thus, it is expected to have a strong and stable activity regardless of host cell types. In fact, nearly ubiquitous expression had been achieved in mouse ES cells and chimeric embryos (Suemori, et al., 1990), and it was used in cell lineage analysis in chimeric mice (Kadokawa, et al., 1990). But mosaical expression occurred in embryos and fry of rainbow trout (Inoue, et al., 1991). Hence, it is interesting to see whether miw has a strong promoter activity in mollusks. In meretrix clam, expression of the miw was ubiquitous that was similar to mouse but not to rainbow trout. Thus, β-galactosidase report system functioned well in veliger larvae of meretrix clam by electroporation.

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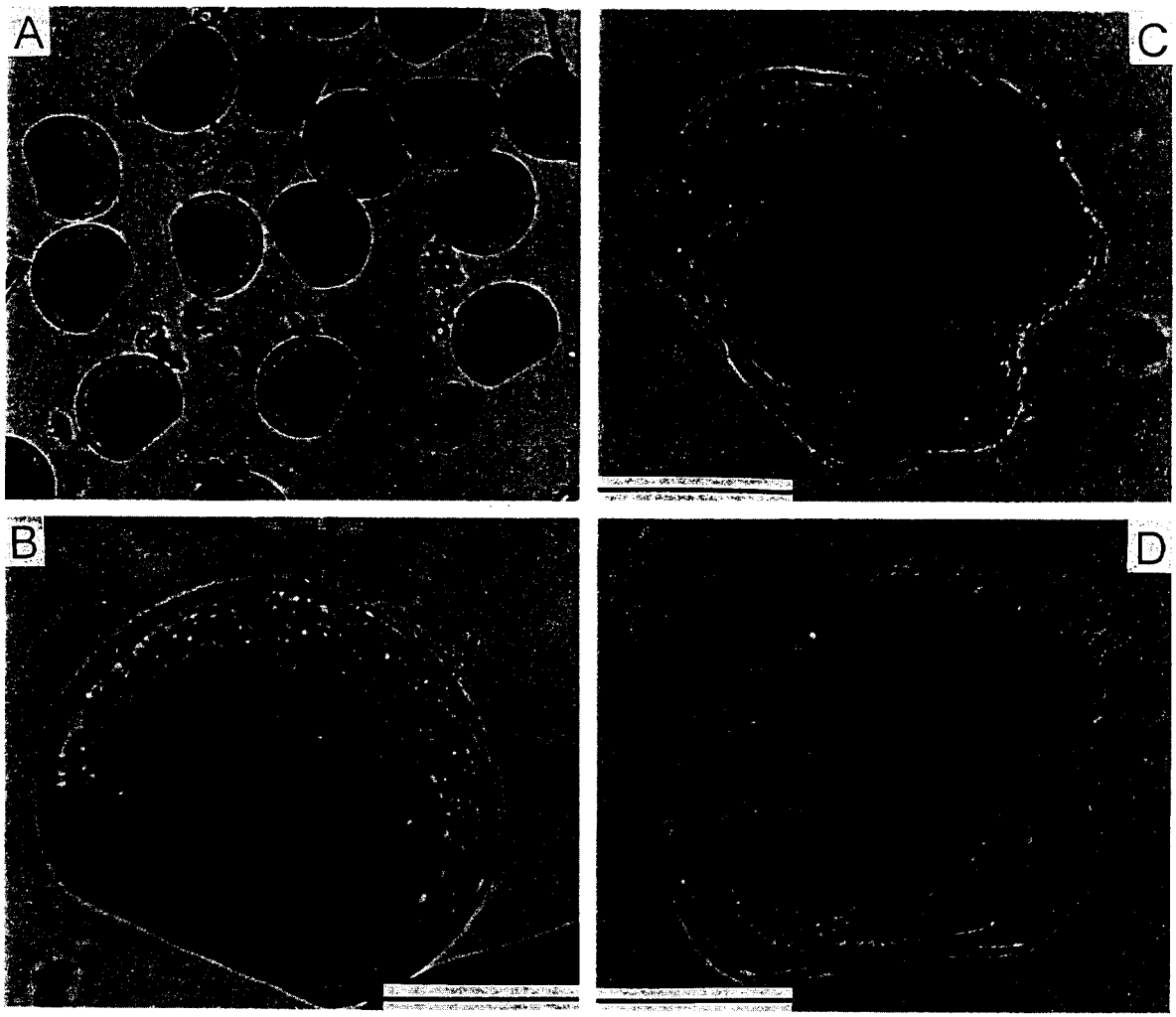

Fig 1. Expression of pmiwZ in veliger of *Meretrix lusoria* Röding. Larvae were fixed and stained histochemically. (A) and (B) were electroporated without pmiwZ. (C) and (D) were electroporated with 50μg/ml pmiwZ, the activity of β-galactosidase was detected ubiquitous in veliger larvae. Bar is 50μm.

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文蛤幼生基因轉殖的表現

巫文隆[1]・蔡奇立[1]

文蛤受精卵經由電破法(最適條件為4-10 kv電壓、脈衝時間為160μs、 脈衝數目為1024、反應時間為0.8s、反應周期為5-10、作用為220)，電破法所用的反應體積為100μl（內含500個受精胚體），基因轉殖的質體濃度為50μg/μl。文蛤受精胚體經電破法處理之後，以滅菌海水清洗，並經兩天的孵化培養後，發育到面盤幼生，即可檢測pmiwZ在文蛤面盤幼生體內的表現程度。本研究所得到的結果是：文蛤受精卵發育到2-16細胞期可進行基因轉殖實驗，處理後，發育到面盤幼生時，全身可出現藍色反應或鑲嵌狀分佈。整體而言，文蛤胚體經電破法的基因轉殖後，有5-30%具有顯著的藍色反應。本研究同時亦獲得以下結論：外來基因與細胞內染色體結合機率，就遠較在以單細胞胚胎基因轉殖時，外來基因須擴散入細胞核內之機率為大。因此2-16細胞胚體是較適合以電破法進行基因轉殖。

關鍵詞：軟體動物、基因轉殖、面盤幼生。

[1] 中央研究院動物研究所，台北市，台灣。