研究简报

以精子为载体把外源 DNA 导入中华绒螯蟹的初步研究

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PRELIMINARY STUDY ON SPERM AS VECTOR FOR INTRODUCING FOREIGN DNA INTO CHINESE MITTEN CRAB (*ERIOCHEIR SINENSIS*)

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The study of crustacean genetics and breeding has been received more and more attention due to the rapid development of the shrimp and crab aquaculture. Chromosome set manipulation on shrimp and crab[1][2], and the work on transgenic shrimp[3][4] have got some progress. However, there is no transgenic research reported on crab to our knowledge. The purpose of this paper is to study the feasibility of using sperm as vector to introduce foreign DNA into Chinese mitten crab (*Eriocheir sinensis H. milne-Edwards*), an economically important crab in China.

1 MATERIALS AND METHODS

1.1 Foreign DNA The ninth part of cDNA of GCHV (hemorrhagic virus of grass carp) was cloned in the SamI site of pUC19 and named pUCV9 as the foreign DNA used

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in this study.

1.2 Procedures 60μL mature sperm obtained from seminal receptacle of spawning female Chinese mitten crab (*E. sinensis*) was mixed with 200μL Ca^{2+}, Mg^{2+} free D-Hanks solution (NaCl 8.0g, KCl 0.4g, $Na_2HPO_4 \cdot 12H_2O$ 0.12g, glucose 1.0g, KH_2PO_4 0.06g/L), 40μL 2.0mol/L glucose and 20μL plasmid DNA (3.2μg/μL). DMSO was added to the final concentration 3%. After 0℃ water bath for a bout 30 minutes, the sperm solution was fertilized with the Chinese mitten crab eggs which were sucked out directly from the ovary of the female beginning to spawn. The fertilized eggs were hatched at 20 ℃ for about 20 days. Then the DNA of 3 reduplicative groups (8 foreign DNA introduced embryos in each group) was phenol-isolated respectively, following the procedure described by Sambrook et al[5] and was finally dissolved in 10μL water. The control DNA was isolated from 8 normal embryos.

1.3 PCR detection PCR was used to screen for the presence of pUC-V9 DNA in the 3 groups of embryos. The primers were (1) 5′-ACATCTACTGTGCTTCACCT-3′, (2) 5′-TAGTGTGTCAATAGCGTCCA-3′. The target DNA fragment was 223 bp. Each amplification mixture (25μl) contained 1 μL the embryo DNA. The PCR program consisted of predenaturation for 1 min at 94℃, 35 cycles of 1 min at 94℃, 45s at 56.5℃, and 45s at 72℃. A final extension of 7 min at 72℃ was included. The PCR products were analyzed by 1.4% agarose gel electrophoresis with EB staining.

2 RESULTS AND DISCUSSIONS

The electrophoretogram of the PCR products was shown in Fig. 1.

The control (normal *E. sinensis* DNA) did not have the 223 bp PCR product, and there was no visible positive signal in group 1. A weak PCR product (around 220 bp) appeared in group 2 and 3, which is probably because only part of embryos in the groups is positive. Besides, even the embryo was positive, it could be mosaic.

This result represents that at least 2 out of the 24 embryos (3 groups) after 20 days hatching still contain pUC-V9 DNA and the transferring rate is more than 8.3%.

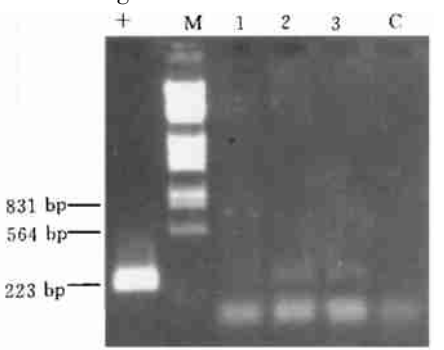


Fig. 1 Analysis of the *E. Sinensis* embryos DNA introduced pUCV9 DNA
C: control, amplification of DNA from the normal *E. Sinensis* embryos; M: λ DNA (EcoR1/Hind Ⅲ); +: positive control, amplification of pUCV9 DNA; 1, 2, 3. amplification of the embryos DNA introduced pUCV9 DNA

The use of sperm for gene transfer was firstly reported by Lavitrano *et al* in mice ova in 1988[6]. This simple method is particularly appealing in the field of crustacean

transgenics since microinjection, the more current and effective transgenic method in fish, is more difficult to operate due to the hard chorion of the crustacean eggs. In this experiment, we have showed that sperm of *E. sinensis* could introduce foreign DNA into the eggs, but whether the foreign DNA has entered the sperm or just sticks to the surface of sperm still needs further study.

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