II. Purposes

In our lab, we have recently cloned and characterized three muscle-specific sub-isoforms of CK from carp muscle tissue, designated M1-, M2-, and M3-CK (Sun *et al.*, 1998) and have demonstrated that the M3-CK isoenzyme remains stable and maintains its enzyme activity even at lower temperatures (Sun *et al.*, 2002). These results indicate that the M3-CK isoenzyme might function to allow the organism to adapt to low temperatures *in vivo*. Aside from these, we also cloned and characterized two different promoters, one is cold inducible from HSC70 (CIP) (Fig.1 and Fig. 2) (Chang TL, 2006) of zebrafish and the other is from M3-CK of carp.

We hypothesized that the carp M3-CK isoenzyme might substitute for the other isoforms at the lower temperatures to maintain its swimming ability, enhancing its cold tolerance *in vivo* and otherwise in non-acclimated fish.

For this reason, we decide to link the two genes (M1-CK and M3-CK) up the three kinds of promoters (CMV, CIP, and M3CKpro) and ligating with pEGFPC1 individually and to establish an in vivo system that is a tissue-specific inducible system in zebrafish (*Danio rerio*).