Cloning and characterization of novel promoter using thermal asymmetric interlaced (TAIL)–PCR based method from T–DNA tagged *Brassica napus cv westar*

Hee Duck Yang¹, Dong Wook Kwak², Ho Youn Song, Sun Kyo Lin², Hye Min Kim², Je Geun Yoo¹, and Young Min Kim¹

Department of Biological Sciences, Hannam University, 133 Ojeong-dong, Daedeuk-ku, Daejeon 306-791, South Korea

**Objectives**

To obtain unknown sequence (novel promoter) of 5’–and 3’ ends of T–DNA from *Brassica napus cv westar*, Thermal Asymmetric Interlaced PCR (TAIL–PCR) were performed. To identify β–glucuronidase (GUS) expression of novel promoter acquired from TAIL–PCR procedure, several target cells, were bombarded by particles coated with bombarding vector which include novel promoter and gus genes, and then transformed *Nicotiana tabacum* (Xanthi) plants were developed from embryogenic callus following *Agrobacterium tumefaciens* mediated transformation.

**Materials and Methods**

2. Methods : TAIL–PCR (Liu et al. 1995). Callus were obtained from leaf cuttings of *Brassica napus cv westar*. Culture was maintained by MES–α media containing phytohormone (0.1mg/L α-Naphthaleneacetic acid and 1.0mg/L 6-benzyladenine), 15g/L Sucrose, 0.5g/L Carbenicillin, 0.1g/L Kanamycin

**Results and Discussion**

Fig. 1. Agarose gel analysis of TAIL–PCR product amplified from T–DNA insertion lines. The product specificity was confirmed by the size shift between lanes II and III. Multiple product bands observed in some samples were nested–fragment derived from annealing of the AD primer at more than one site along the target sequence molecules, as described previously (Liu and Whittier, 1995).

Fig. 2. Identification of Gus activity by histochemical staining in target cells. Expressing the novel promoter – gus chimeric gene : A : *Cucumis melo var. makuwa*, B : *Nicotiana tabacum* (Xanthi), C : *Nicotiana benthamiana*, D : *Brassica napus cv westar*. GUS activity in various tissues of the primary promoter tagged transgenic plant (*Nicotiana tabacum* (Xanthi)): E: GUS activity is exhibited in stems. F: GUS activity is exhibited leaves. G: GUS activity is exhibited predominantly in the root meristem and root hairs.

*Corresponding author : Je Geun Yoo, Young Min Kim*, TEL: 042-629-7481, E-mail: nonono68@daum.net