(05-1-100)

Normalized full-length cDNA library construction and its quality estimation in *Brassica rapa* L.

Da-Yeong Seo[1], Hyo-Jin Jung[1], Kwon-Kyu Kang[2], and Ill-Sup Nou[1]*

[1]Faculty of plant Science, Sunchon National University, 315 Maegok-Dong, Sunchon 540-742, Korea
[2]Department of Horticulture Hankyong National University, 67 Seokjeong-dong Anseong city Kyunggi-Do 456-749, Korea

Objectives

To cloning of full-length cDNA from *Brassica rapa* L, normalized full-length cDNA library was constructed into lambda FLC vector, And the cDNAs were introduced *Agrobacterium tumefaciens* strain GV3101 carrying a binary T-DNA over expression vector pBigs.

Materials and Methods

1. Material

Plant Bud, leaf and stem tissue of *Brassica lapa* L. (cv. Osome), *E. coli* strain DH10B/Lambda FLCIII, *Agrobacterium* strain GV3101/pBigs

2. Methods: Biotin technology, PCR, Cloning, Transformation, Electroporation, Cap-trapping, Sequence

Results and Discussion

Isolation of full-length cDNAs is a time-consuming task, and is the rate-limitting step in gene cloning and large-scale cDNA sequencing studies. With oligo dT column it purify the each mRNA from the flower organs, leaf tissue and stem tissue of the *Brassica rapa* L. About 1-2μgof mRNA was denatured at 65℃ for 10 min, together with 1μg of the flist-strand restriction enzyme primer-adapter. To follow the yield of the first-strand synthesis, 0.5$\mu\ell$ of the radiolavelled reaction aliquot was spotted on DE-81 paper and the incorporation was calculated by measuring the radioactity before and after three washing with phosphate buffer. The biotinylation reaction was performed either before or after the cDNA synthesis. When performes after the first-strand synthesis to the sample 0.8$\mu\ell$ 0.5 M EDTA and 1.3 $\mu\ell$ 5 M NaCl were added. The sample was extracted once with phenol chloroform and chloroform, followed by ethanol precipitation. First-strand full-length cDNA/mRNA hybrid was captured on magnetic porous glass particles coated with streptavidin. Before binding of the nucleic acids, 100$\mu\ell$ of beads (1% suspension; 1 mg of beads can bind 800 pmol of a biotinylated 25-meroligonucleotide) were blocked by adding 10 $\mu\ell$ of 40μg/$\mu\ell$ DNA-free total RNA and incubating on ice for 1 hr with occasional gentle vortexing. Just before nucleic acid capture, the beads were separated using a magnetic stand and the supernatant was removed by pipetting. This cDNA libraries had a two characteristics: (i) it was contained a very high proportion of full-length cDNAs and (ii) the frequency of individual will reflect the original frequency of the corresponding mRNAs into the cell without any bias or less of specific sequences caused by the cloning system. Also, to remove a incomplete-length cDNA, cap/trapping method was used. After cap/trapping in the single strand cDNA combination, two kinds of adaptor (GN5, N6) were used for second cDNA synthesis. To estimation of library quality, 5′ frame sequencing was performed in 300 clones. And about 115,000 cDNAs from the library were transformed into T-DNA binary vector, pBigs.

Acknowledgements: This work was supported by a grantfrom BioGreen 21 Program, Rural Development Administration, Republic of Korea.

* *Corresponding author* : Ill-Sup Nou, TEL: 061-750-3249, E-mail: nis@sunchon.ac.kr