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High Through Put Screening of Tissue Specific Genes in *Magnolia kobus*(목련)

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Objectives

To isolate the potential candidate genes which is likely to be incorporated in medicinal compounds metabolism in Magnolia kobus

Materials and Methods

1. Plant materials: Flower bud, flower, bark, fruit, and leaves. of *Magnolia kobus*(목련)

2. Methods: Hetero-sourced cDNA library construction : mRNA extracted from 7 different tissues were used as sources for hetero-sourced cDNA library construction. The original tissue source of each cDNA clone in the library is recognizable by tissue-specific tag sequence which had been incorporated into the vector in the first strand cDNA synthesis step(Table 1). ~106pfu library phage were excised and converted into plasmid form, then transformed into *E.coli.* host. Total of 46,080 clones were spotted into 386 well micro-titer plates, and high density colony array(HDR) filters were prepared with them in a density of 9,216clones/22 x 22cm^2 . Averaged insert size is 1.1kb(Figure 1).

2-Step screening : Base HDR filters were first screened out with subtracted cDNAs as probes. This could discriminate the qualitatively expressed genes(on/off) in each tissues. Resulted clones were arrayed again to new HDR filter for second step-quantitative expression screening. Suppression-subtractive hybridization(SSH) techniques was used to generate this first step subtracted probes. PCR-Select cDNA subtraction kit from ClonTech was used. 4 Testers were flower bud, flower, bark, and fruit, and driver was, all, leaves. For second step-quantitative screening, Amersham's HotScribe first-strand cDNA labeling with both oligo-dT primer and random hexamer primer was used to label the normalized mRNA from various tissues..

Results and Discussion

A total of 2,409 clones were screened out from 1st stage qualitative screening(Figure 2, Table 2). Hybridization with flower and flower bud showed most of the signals. But bark and fruit showed very little number of expressed signals. 36% of positive signals were common between probes and total number of unique positive signaled clones was 1,536 and rearrayed. Total of ~200 clones were screened out from 2nd stage quantitative screening(Figure 3) and sequenced. Among them 20 clones were confirmed to be tissue-specific to bark and flower bud, and 5 clones were estimated to have functions related to secondary metabolism - 4 were related to terpenoid biosynthesis(Table 3).

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