

Transcriptome analysis

mRNA from hypocotyl and root was isolated from three independent experiments, mixed, and analyzed twice using the same procedure for high-coverage gene expression profiling. Samples were rapidly frozen in liquid nitrogen and ground to a powder using a mortar and pestle. Total RNA was extracted using the RNeasy plant mini kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's protocol, and treated with 1.5 U/mL DNase I at 37°C for 10 min.

The high-coverage gene expression profiling analysis reaction was performed according to a previous report [1]. Briefly, total RNA was converted to single-stranded cDNA by reverse transcriptase with 5' biotinylated oligo-d(T) primer and the second strand was then synthesized. The double-stranded cDNA was cut with *MspI* and ligated with an *MspI* adapter using T4 DNA ligase. The ligated products bearing biotin at the 3' terminus were collected by streptavidin-coated magnetic beads and washed twice with 1.0 ml of washing buffer containing 5 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 1.0 M NaCl. The cDNA fragments on the magnetic beads were digested with *MesI* and the supernatants were collected. Ligation was performed with a *MesI* adapter using T4 DNA ligase in the presence of *MesI* in the reaction mixture. The resulting solution was used as a template for selective PCR and then the PCR products were analyzed by electrophoresis on an ABI PRISM 3100 (Applied Biosystems, Foster City, CA, USA). Electropherograms of PCR products were analyzed with the MS-3000 Analyzer (Maze, Inc., Tokyo, Japan). The peaks of interest were fractionated with a standard slab gel (20 cm x 40 cm x 4 mm). The gel slices corresponding to the peak of interest were excised and used for sequencing by the dye terminator method or after cloning with a TA cloning kit (pGEM-T Easy, Promega, Madison, WI, USA).

- [1] Fukumura, R., Takahashi, H., Saito, T., Tsutsumi, Y., *et al.*, A sensitive transcriptome analysis method that can detect unknown transcripts, *Nucl. Acids Res.* 2003, *31*, e94.