AtMap1 DNA microarray experiment

1. > 100 seedlings are collected and lyophilized.

2. Genomic DNA is extracted from the lyophilized materials with a DNeasy Plant Mini Kit (Qiagen, Valencia, CA).

3. Double-stranded DNA is quantified with a Quant-iT dsDNA Broad-Range Assay Kit (Invitrogen, Carlsbad, CA).

4. The genomic DNA (1 µg) is used for labeling reaction with a Genomic DNA Labeling Kit (Agilent technology) according to direct labeling protocol, except following points.

- **The fragmentation of genomic DNA is performed with two sets of restriction enzymes instead of the sonication.**
- **A half of the genomic DNA (0.5 µg) is digested with RsaI/AluI and the rest (0.5 µg) is digested with DraI.**

5. Hybridization is performed with an Oligo aCGH/ChIP-on-Chip Hybridization Kit (Agilent Technology) according to the standard protocol, except that **Cot-1 DNA is not added to the hybridization solution.**

6. After hybridization, the microarray is washed in Oligo aCGH/ChIP-on-Chip Wash Buffer 1 and 2 (Agilent Technology). Immediately after washing the microarray slideis scanned by a DNA microarray scanner (Agilent Technologies). The signals are quantified and normalized with Feature Extraction Software ver. 9.1 (Agilent Technologies) using the software's default parameters.

7. The design file of AtMap1 for the Feature Extraction is available from http://podb.nibb.ac.jp/Organellome/FunctionalPDF/MAP1/ExFullGEML.xml.

8. The annotation file for AtMap1 is available from http://podb.nibb.ac.jp/Organellome/FunctionalPDF/MAP1/ExFullGEML.xml. The file contains information about the features of each probe (gene, intergenic region and transposable element), the AGI code of the nearest gene and the annotation about the gene (TAIR8).

9. The .BED design file of AtMap1 for the UCSC Genome Browser is available from
http://podb.nibb.ac.jp/Organellome/FunctionalPDF/MAP1/015638_BED.bed.

Reference

Nagano, A.J., Fukazawa, M., Hayashi, M., Ikeuchi, M., Tsukaya, H., Nishimura, M., and Hara-Nishimura, I. (2008). AtMap1: a DNA microarray for genomic deletion mapping in *Arabidopsis thaliana*. Plant J. 47, in press. (doi: 10.1111/j.1365-313X.2008.03656.x)