

## General:

Recommended operating procedures (ROC) for Affimetrix GeneChip Expression Array Analysis

## Procedure:

### 1. RNA quality control

The following parameters are important for assessment of RNA quality:

- For mammalian total RNA, **28S/18S rRNA ratio** should be  $\geq 1,5$  (determination by running the *Eukaryote Total RNA Nano* assay on an Agilent 2100 Bioanalyzer).
- For Arabidosis total RNA, **25S/15S rRNA ratio** should be  $\geq 1,2$  (determination by running the *Eukaryote Total RNA Nano* assay on an Agilent 2100 Bioanalyzer).

**OD 260/280** should be  $\geq 1,5$  (determination by spectrophotometer).

Alternatively, a denaturing formaldehyde-agarose gel may be run for assessment of S rRNA ratios.

### 2. Determination of RNA concentration

RNA concentration should be determined by measuring OD 260 with a spectrophotometer.

### 3. cDNA synthesis

Total RNA input: 3 – 15  $\mu\text{g}$ .

mRNA input: 0,2 – 2  $\mu\text{g}$ .

Double-stranded cDNA is synthesized following the manufacturer's (= Affymetrix) instructions (see *GeneChip Expression Analysis Technical Manual*;

[http://www.affymetrix.com/support/technical/manual/expression\\_manual.affx](http://www.affymetrix.com/support/technical/manual/expression_manual.affx) ).

As an alternative, Roche's *cDNA Synthesis Kit* (Order-No. 1117831) may be used.

### 4. Purification of cDNA

Purification of cDNA is performed with the *GeneChip Sample Cleanup Module* (Affymetrix, Order-No. 900 371) according to the manufacturer's instructions.

### 5. In vitro transcription

Antisense-cRNA is produced following the manufacturer's (= Affymetrix) instructions. As an alternative, Ambion's *T7 Megascript Kit* (Order-No. 1334) may be used.

### 6. Purification of cRNA

Purification of cRNA is performed with the *GeneChip Sample Cleanup Module* (Affymetrix, Order-No. 900 371) according to the manufacturer's instructions.

### 7. Quality control of cRNA

Before hybridization of cRNA to a test array or expression array, the **mean fragment length** of the cRNA should be determined by running the *mRNA Smear Nano* assay on an Agilent 2100 Bioanalyzer. **Mean fragment length should be  $\geq 1,000$  nt.**

### 8. Determination of cRNA concentration

It is essential to determine cRNA concentration to hybridize each type of expression array with an equal and appropriate amount of cRNA (values for each chip type are given in the *GeneChip Expression Analysis Technical Manual*). RNA concentration should be determined by measuring OD 260 with a spectrophotometer.

**9. Production of cRNA hybridization solution**

Production of cRNA hybridization solution should be performed according to the manufacturer's (= Affymetrix) instructions.

**10. Hybridization of arrays**

Hybridization of arrays must be performed for 16-18 hours at 45°C and 60 rpm in the *Hybridization Oven 640* (Affymetrix, Order-No. 800 139).

**11. Washing and staining of arrays**

Washing and staining of arrays must be performed in the *Fluidics Station 400/450* (Affymetrix) by using the appropriate fluidics protocols, following the manufacturer's instructions.

**12. Scanning of arrays**

Scanning of arrays must be performed with the *GeneChip Scanner 3000*, following the manufacturer's instructions.

**13. Hybridization of cRNA to a test array**

Before hybridization of cRNA to an expression array, it is strongly recommended to hybridize it to a test array as an additional quality control.

The following parameters are useful for assessment of cRNA and chip data quality:

**3'5'-ratios** of housekeeping control genes should be  $\leq 3$ .

**Background** should be below 100 (signal value).

**Relative ratios of scaling factors** should be  $\leq 3$ .

**Spike-in controls** *bioC*, *BioD* and *cre* must be present. Spike-in control *bioB* must be present in at least 50% of hybridizations.

**14. Parameters for assessment of expression array data quality**

The following parameters are useful for assessment of chip data quality:

**3'5'-ratios** of housekeeping control genes should be  $\leq 3$ .

**Background** should be below 100 (signal value).

**Relative ratios of scaling factors** should be  $\leq 3$ .

**Spike-in controls** *bioC*, *BioD* and *cre* must be present. Spike-in control *bioB* must be present in at least 50% of hybridizations.

Version	Tracking of changes	Name	Date

