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 ≥ 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 ≥ 1,2 (determination by running the *Eukaryote Total RNA Nano* assay on an Agilent 2100 Bioanalyzer).

  OD 260/280 should be ≥ 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 µg.

   mRNA input: 0,2 – 2 µg.

   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).

   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 ≥ 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 ≤ 3.

Background should be below 100 (signal value).

Relative ratios of scaling factors should be ≤ 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 ≤ 3.

Background should be below 100 (signal value).

Relative ratios of scaling factors should be ≤ 3.

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