General remarks

The procedure describes the preparation of fluorescent DNA probes from human mRNA or total RNA.

Wear laboratory gloves. All laboratory material such as disposables and solutions which come in contact with RNA have to be RNase-free. Usually, autoclaving is sufficient. The working place should be clean and not be used for experiments with RNase.

Use of aerosol-barrier pipet tips is recommended.

All centrifugations are performed in a microfuge with 12,000-14,000 rpm.

Material

1. RNAse-free H_2O (e.g. autoclaved in the presence of 0.1% Diethylpyrocarbonat, DEPC)
2. Superscript III kit (200 u/µl; Invitrogen, cat. no. 18080-044) contains Superscript III,
3. 5x First strand buffer and 0.1M DTT)
4. dAGT (5mM each; e.g. Roche, cat. nos. 1934-511, -538, -546)
5. dCTP (3mM; e.g., Roche, cat. no. 1934-520)
6. Cy^3-dCTP and Cy^5-dCTP (2mM; e.g. Amersham, cat. nos. PA53021 and PA55021)
7. Random Hexamers (e.g., Roche, 10x mix, cat. no. 1277081)
8. RNase Inhibitor (e.g., Fermentas, 40 u/µl, cat. no. EO0311)
9. 50mM EDTA, pH 8
10. 1M NaOH
11. 5M acetic acid
12. Microcon YM-30 columns (Millipore, cat. no. 42410)
13. variable pipettes: 10, 200 µl

Procedure

(for 2 microarray hybridizations)

Methods

A. Labeling (Steps 1-8: 2h)

1. to 9.5 µl amplified RNA (4 µg)
2. add 2 µl Random Hexamers and incubate for 10 min at 70°C.
3. place on ice for 2 min.
4. add :
    a. 5 µl 5x First strand buffer
    b. 2.0µl 0.1M DTT
    c. 2 µl dGAT (5mM each)
    d. 1 µl dCTP

e. 1 µl Cy3-dCTP or Cy5-dCTP
f. 1 µl RNase Inhibitor

5. leave at room temperature (RT) for 5 min, then add 1 µl SuperScript III.
6. incubate for 60 min. at 52°C.
7. add 2.5 µl 50mM EDTA, pH8, and 10 µl 1M NaOH; mix, centrifuge briefly and incubate at 65°C for 10 min.
8. add 2 µl 5M acetic acid, mix and centrifuge briefly.

B. Probe purification with Microcon YM-30 column(Steps 9-17: ~30 min)

9. calibrate the column with 120 µl RNase-free H2O
10. centrifuge for 2 min at 12,000 rpm
11. apply labeled probe solution to column (max. 500 µl)
12. centrifuge for 8 min at 12,000 rpm
13. apply 15 µl RNase-free H2O to the column and incubate for 5 min
14. turn column upside down into a new Eppi
15. centrifuge for 5 min at 12,000 rpm
16. adjust the volume of the eluate (labeled probe) to 16 µl with RNase-free H2O
17. store probe at –20°C

Version	Tracking of changes	Name	Date