Microarray Analysis: Single Cell Gene Expression by GeneChip Protocol

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Abstract: The microarray technology is a new, powerful and useful tool for gene expression, clinical diagnosis, food safety control and other the biochemical researches. Using the microarray technology, more than 10,000 genes or proteins can be printed on one location. The supports can be silicon chips, nylon membranes or glass slides, etc. This article is giving a brief description of microarray protocol of GeneChip in the single cell gene expression as an example. [Life Science Journal. 2006;3(2):45-49] (ISSN: 1097-8135).

Keywords: DNA; GeneChip; microarrays; protein

1 Introduction

Microarrays are new methods. Using microarray technology more than 10,000 genes or proteins can be printed on a glass slide (MacBeath, 2000) and thousands of genes can be detected and analyzed in an array simultaneously by the microarray analysis. Microarray could be named as biochip, DNA chip, DNA microarray, gene array, gene chip, and protein array, etc. Microarrays have become a crucial component of gene expression and genotype research recently. Microarray technologies are powerful tools to measure the expression of many genes simultaneously.

The most important microarray is DNA microarray. For the DNA microarray, thousands of different DNA molecules (genes) are fixed on a support. The supports can be silicon chips, nylon membranes or glass slides. The DNA is printed, spotted, or actually synthesized directly onto the support. Each single-stranded DNA fragment is made up from four different nucleotides, adenine (A), thymine (T), guanine (G), and cytosine (C). During DNA molecule synthesis, A is the complement of T, and G is the complement of C. Therefore, the complementary sequence of A-C-G-T-T-G-C-A will be T-G-C-A-A-C-G-T. When two complementary sequences match to each other, such as the target DNA (immobile DNA) and the sample DNA (mobile DNA), cDNA, or mRNA, they will combine together (hybridize). The mobile DNA can be labeled with fluorescence as the mobile probe to detect gene expression level if there are complementary molecule sequences existing in the immobile slides.

Right ventricular hypertrophy and failure are prominent features in cyanotic congenital heart disease, tetralogy of Fallot. To detect the molecular mechanisms of right ventricular hypertrophy and to identify gene(s) involved in tetralogy of Fallot, Sharma and colleagues measured the differential gene expression using expression-based microarray technology on right ventricular biopsies from young tetralogy of Fallot patients who underwent primary correction. By using quantitative immuno histochemistry, expression of vascular endothelial growth factor, flk-1, and extracellular matrix proteins (collagens and fibronectin) as well as vessel counts and myocyte cell size was evaluated in TF patients in relation to age-matched controls. From these studies, they concluded that the upregulation of genes encoding vascular endothelial growth factor and extracellular matrix proteins were the key events contributing to right ventricular hypertrophy and stunted angiogenesis in patients with tetralogy of Fallot (Sharma, 2006).

Cross-validation represents a tool for reducing the set of initially selected genes to those with a sufficiently high selection frequency. Using cross-validation it is also possible to assess variability of different performance indicators (Qiu, 2006).

Cytoplasmic control of the adenylation state of mRNAs is a critical post-transcriptional process involved in the regulation of mRNAs stability and translational efficiency. The early development of Xenopus laevis is a major model to study this regulation. Graindorge et al used microarray method to identify mRNAs that were regulated by changes in their adenylation state during oogenesis and early development of the diploid frog Xenopus tropicalis.

The microarray data were validated using qRT-PCR and direct analysis of the adenylation state of endogenous maternal mRNAs during the period studied. They successfully identified more than 500 mRNAs regulated at the post-transcriptional level among the 3000 mRNAs potentially detected by the microarray (Graindorge, 2006).

Pterygium is an ocular-surface lesion that can decrease vision. In order to detect the genes that may play roles in pterygium pathogenesis, John-Aryankalayil et al analyzed the global gene expressions of pterygium. In John-Aryankalayil's studies, oligonucleotide microarray hybridization was used and the selected genes were further characterized by RT-PCR, Western blot, and immunohistochemistry, and comparisons were made with limbal and corneal tissues. Their results showed both novel and previously identified extracellular-matrix-related, proinflammatory, angiogenic, fibrogenic, and oncogenic genes expressed in human pterygium (John-Aryankalayil, 2006).

Using the tissue microarray technology with highly reliable method of fluorescent in situ hybridization, Dimova et al showed similar frequencies of epidermal growth factor receptor gains in different grade tumors, while EGFR amplification increased from grades 1 to 2 to 3 (Dimova, 2006).

Enzyme-linked immunosorbent assay (ELISA) microarray technology can simultaneously quantify levels of multiple proteins, which has the potential to accelerate validation of protein biomarkers for clinical use (Zangar, 2006).

As the microarray technology development, massive amounts of microarray images are produced. The storage and the transmission of the microarray images are significant important for the research and application of microarray technology. Lonardi and Luo proposed lossless and lossy compression algorithms for microarray images originally digitized at 16 bpp (bits per pixels) that achieve an average of 9.5 − 11.5 bpp (lossless) and 4.6 − 6.7 bpp (lossy, with a PSNR of 63 dB). The lossy compression was applied only on the background of the image, thereby preserving the regions of interest. The methods were based on a completely automatic gridding procedure of the image (Lonardi, 2006).

Diaz-Uriarte and Alvarez de Andres investigated the use of random forest for classification of microarray data using simulated and nine microarray data sets they showed that random forest has comparable performance to other classification methods, including DLDA, KNN, and SVM. Because of its performance and features, random forest and gene selection using random forest should probably become part of the standard method for class prediction and gene selection with microarray data (Diaz-Uriarte, 2006).

Theoretical considerations of protein microarrays were done in the 1980's by Roger Ekins and colleagues (Ekins, 1989;1991;1994;1999).

Oligo GEArray is a new oligonucleotide-based gene expression array from SuperArray Bioscience Corporation. It combines current oligo-based array design with SupperArray's proven nylon membrane based array technology.

Using microarray analysis, a typical experimental protocol could be:

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Isolating RNA

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Converting the RNA samples to labeled cDNA via reverse transcription

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Hybridizing the labeled cDNA to identical membrane or glass slide arrays

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Removing the unhybridized cDNA

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Detecting and quantitating the hybridized cDNA

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Data analysis with/without software

2 Gene Blots (96-well size) Making Protocol in the Single Cell Gene Expression

The following gives the brief steps for the gene plots making protocol in the single cell gene expression experiment, as the reference for the researchers.

I. Making DNA blots

1. Add 1 μl plasmid with gene into 1.5 ml eppendorf tube
2. Add 10 μl DEPC H_2O
3. On ice 5 min
4. Add 20 μl competent cells
5. On ice 30 min
6. 42 ℃ 50 sec
7. On ice 3 min
8. Add 1 ml LB medium
9. 37 ℃ 1 h
10. Add 100 μl of above cell suspension to agar plate with ampicillin
11. Spread
12. 37 ℃ over night
13. Pick one colony
14. Grow in 3 ml LB medium with ampicillin over night

15. Spin 10 min at 10,000 rpm
16. Remove supernatant away
17. Suspend in 250 μl P1 buffer (Invitrogen DNA mini-purification kit)
18. Vortex
19. Add 250 μl P2 buffer (Invitrogen DNA mini-purification kit)
20. Add 350 μl N3 buffer (Invitrogen DNA mini-purification kit)
21. Spin 1 min
22. Pour to column (Invitrogen DNA mini-purification kit)
23. Spin 1 min
24. Wash with 750 μl PE buffer (Invitrogen DNA mini-purification kit)
25. Spin 1 min
26. Through away pass through
27. Spin another 1 min and transfer column onto another new eppendorf tube
28. Add 50 μl DEPC H_2O
29. Spin 1 min
30. Take 1 μl, and add 100 μl DEPC H_2O
31. Read O D 260 nm
32. Calculate volume for digestion
33. Add purified P-DNA and endonuclease
34. 37℃ over night
    1) Prepare 0.8% agrose gel
    2) Run gel for 1.5 h 100 v
    3) Ethydine bromide stain
    4) Take picture to check the purity of the gene
35. Wet filter paper
36. Wet N-bond membrane
37. Lay the membrane on the blot machine
38. Add DNA clones (genes)
39. Suck 10 min
40. Cross linking the membrane

II. Making immuno-staining

41. Stepwise treat the tissue slides with xylene, ethanol, methanol, H_2O
42. Wash 10 min with running water
43. Dip in 0.1 M Tris-HCl for 5 min
44. Add 2% FBS on the slide and keep for 5 min
45. Add primary antibody and keep at 4 ℃ over night
46. Wash with 0.1 M Tris-HCl for 5 min
47. Keep in 2% FBS for 5 min
48. Add secondary antibody and keep for 1 h
49. Wash with 0.1 M Tris-HCl for 5 min
50. Keep in 2% FBS for 5 min
51. Keep in A/B reagent for 1 h
52. Add DAB reagent on the slide and keep for 10 min at room temperature
53. Wash with DEPC H_2O for 5 min
54. Soak in DEPC H_2O

III. Single cell gene expression

55. Take the slide out from DEPC H_2O
56. Add 100 μl proteinase K
57. Keep at 42℃ for 30 min
58. Rinse with DEPC H_2O
59. Make oligo-dT mix
60. Add 100 μl oligo-dT mix onto slide
61. Keep at room temperature over night
62. Wash off oligo-dT with 2 × SSC buffer
63. Soak in 2 × SSC buffer for 15 min
64. Dilute 10 × first buffer to 1 × first buffer
65. Add 100 μl of the 1 × first buffer onto the slide
66. Keep at room temperature for 30 min
67. Remove the 1 × first buffer
68. Add the 1 × first buffer reaction mixture 100 μl on slide
69. Keep at 37 ℃ for 90 min
70. Remove the 1 × first buffer reaction mixture
71. Soak in 2 × SSC buffer

IV. cDNA synthesis

72. Prepare electrode buffer
73. Add 20 μl electrode buffer into eppendorf tube
74. Pick positive standard cell
75. Add the positive standard cell into tube
76. Keep at 37 ℃ for 1 h
77. Add 50 μl phenol-chloform
78. Vortex
79. Spin at 14,000 rpm for 20 min
80. Transfer top layer to a new tube
81. Add 100 μl ethanol (100%) and 10 μl 3M NaAc and 0.5 μl tRNA
82. Keep at −80℃ over night

V. Loop expression

83. Spin at 14,000 rpm at 4 ℃ for 15 min
84. Remove ethanol and dry pellet for 20 min
85. Suspend pellet in 20 μl DEPC H_2O
86. Keep at 95℃ for 15 min
87. Add 22 μl 2nd strand DNA synthesizing mixture
88. Incubate at 14℃ for 4 h or over night
89. Make loop excision mixture
90. Add 350 μl for the above mixture into each 42 μl sample
91. Keep at 37℃ for 5 min
92. Extract with 400 μl phenol-chloroform
93. Spin at 14,000 rpm for 15 min

94. Remove top layer to a new tube
95. Add 1 ml ethanol (100%)
96. Keep at −80℃ over night
97. Spin at 14,000 rpm for 15 min

VI. Blunt ending

98. Remove ethanol and dry pellet
99. Suspend pellet in 17.5 μl TE buffer
100. Make blunt mixture
101. Add 7.5 μl of the above mixture into each tube
102. Keep at 37℃ for 15 min
103. Add 25 μl phenol-chloroform
104. Vortex
105. Spin at 14,000 rpm for 15 min
106. Remove top layer to a new tube
107. Add 55 μl ethanol and 7.7 μl 3 M NaAc
108. Keep at −80℃ over night

VII. RNA analysis and labeling

109. Spin at 14,000 rpm for 15 min
110. Remove ethanol and dry the pellet for 20 min
111. Suspend the pellet in 20 μl DEPC H_2O
112. Prepare RNA amplification buffer with 32P-UTP
113. Add 8.5 μl of the RNA amplification buffer into 2 μl cDNA
114. Keep at 37℃ for 4 h
115. Prepare denature gel
116. Run gel for 1.5 h at 100 v
117. Wash gel with cold 10% TCA for 4 times
118. Press dry gel by paper towel for 4 h or over night
119. Expose gel to X-ray film for 1-3 days
120. Develop X-ray film

VIII. Hybridization

121. Wet DNA blot with DEPC H_2O and 2 × SSC buffer
122. Prepare hybridization buffer
123. Prehybridize blots in 20 ml hybridization buffer at 43 ℃ for 2 h
124. Keep 32P-cRNA (from step 114) at 95 ℃ for 5 min to denature
125. Keep on ice quickly
126. Add the denatured 32P-cRNA into hybridigization tube
127. Hybridigize at 43℃ for 72 h
128. Wash blots with 2 × SSC buffer 4 times
129. Semi dry blots
130. Bleach phospho-image screen for 30 min
131. Expose the labeled DNA blot to phospho-image screen for 1 − 3 days
132. Run phospho-image in image machine
133. Down load to computer
134. Analyze image with image software

3 Discussion

The microarray technology is a useful tool for gene expression, clinical diagnosis, food safety control and other the biochemical researches. In a review article, Roy and Sen pointed that the cDNA microarray approach is an emergent technology in diagnostics and food safety test (Roy, 2006). Its values lie in being able to provide complimentary molecular insight when employed in addition to traditional tests for food safety, as part of a more comprehensive battery of tests. Gene-expression biomarkers measured by microarray method can be used to identify promising candidate caloric restriction mimetics that may be involved in determining human longevity (Spindler, 2006).

Gene expression is the essential characterization for organisms to adapt to changes in the external environment. The measurements of gene expression supply the information about the mechanism of organisms' living activities. The development of high-quality microarrays has allowed this technology to become a standard tool in molecular detection including cell toxicology. Several national and international initiatives have provided the proof-of-principle tests for the application of gene expression for the study of the toxicity of new and existing chemical compounds. In the last few years the field has progressed from evaluating the potential of the technology to illustrating the practical use of gene expression profiling in toxicology. The application of gene expression profiling to ecotoxicology is at an earlier stage, mainly because of the many variables involved in analyzing the status of natural populations. Nevertheless, significant studies have been carried out on the response to environmental stressors both in model and in non-model organisms. It can be easily predicted that the development of stressor-specific signatures in gene expression profiling in ecotoxicology will have a major impact on the ecotoxicology field in the near future. International collaborations could play an important role in accelerating the application of genomic approaches in ecotoxicology (Lettieri, 2006).

In the past decade, microarray technology has become a major tool for high-throughput comprehensive analysis of gene expression, genotyping and re-sequencing applications (Scaruffim, 2006). The industrial era of microarray will come soon. It will enhance the molecular biology development to a new level.

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