Practical activity (6)

Simulated DNA Microarray Analysis

(Teacher's Guide)

I. Objectives of the experiment

1. To carry out a simulated DNA microarray experiment; and
2. To differentiate the gene expression patterns in normal and in cancerous cells.

II. Expected Learning Outcomes

Upon the completion of the activities, students should be able to:

1. recognise the basic principle of DNA microarray;
2. briefly describe the differences in gene expression in normal and in cancerous cells; and
3. explain the results observed from the simulated microarray experiment.

III. Teaching notes:

1. Introduce the tasks by going through the "Background" with the students or have the students read through this part and the "Guiding questions about the design of the experiment" as a pre-class activity.
2. Discuss the design of the experiment with the students using the "Guiding questions about the design of the experiment".
3. Plan the laboratory work with reference to the "Time allocation for the experimental activities" in section IV according to the lesson time of your schools.
4. Always remind the students about the safety precautions of each part of the experiment.
5. Go through the "Results" and "Discussion" with the students.

IV. Time allocation for the experimental activities:

Experimental activities		Duration
		In class
Pre-lab session		
1	Introduction of microarray	30 min
Lab session: The experiment		
1	Loading of simulated gene probes onto microarray slide	10 min
2	The hybridisation of simulated cDNA mixture with simulated gene probes	10 min
3	Observation of colour changes and analysis of experimental results	10 min
Total lesson time for the experimental activities		Pre-lab session: 30 min Lab session: 30 min

V. Equipment, materials, and preparatory work for the experiments

A commercial education kit "DNA Chips: Genes to Disease Kit; Carolina # 211520" will be needed for this experiment.

This kit has enough materials and reagents for 20 students (5 groups of 4 students). Some additional equipment or materials may be required.

Website: https://www.carolina.com/gene-expression-advanced-topics/dna-chips-genes-to-disease-kit/211520.pr#

a) Equipment (per group)

- Hot water bath (70°C) × 1 (per class)
- Float rack × 1
- Micropipettes (P20) and sterile tips

b) Materials (per group)

- Simulated gene probe 1 (25 µl, tube 1) × 1
- Simulated gene probe 2 (25 µl, tube 2) × 1
- Simulated gene probe 3 (25 µl, tube 3) × 1
- Simulated gene probe 4 (25 µl, tube 4) × 1
- Simulated gene probe 5 (25 µl, tube 5) × 1
- Simulated gene probe 6 (25 µl, tube 6) × 1
- Simulated cDNA mixture (150 µl) × 1
- Special glass slide × 1

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

c) Preparatory work

Melt and aliquot of experimental reagents **(to be done by teacher/laboratory technicians before the lab session)**

1. Warm all simulated gene probe bottles (6 probes) in the hot water bath (70°C) for 15−45 minutes to completely melt the simulated gene probes.

2. Aliquot each of the 6 simulated gene probes into micro-centrifuge tubes (25 µl per tube). Prepare 1 set of 6 probes per group.

3. Keep all the aliquots warm at 70°C until use.

4. Aliquot the simulated cDNA mixture into micro-centrifuge tubes (150 µl per tube). Prepare 1 tube per group. Keep the mixture at room temperature until use.

d) Other teaching kits suitable for this experiment

1. TaqMan® Array Human Lung Cancer lncRNA; ThermoFisher #4413255

2. Gene Expression Hybridization Kit; Agilent #5188-5242

e) Cleaning of slides

Immerse the special glass slides in 10% chlorine bleach for 2 hours. Rinse the slides thoroughly with distilled water, and allow them to air dry before storage.

VI. Suggested answers to the "guiding questions about the design of the experiment"

1. When a normal cell develops into a cancerous cell, the expression level of

   (a) the tumor suppressor gene (cancer-preventing gene) will ___*decrease*___.

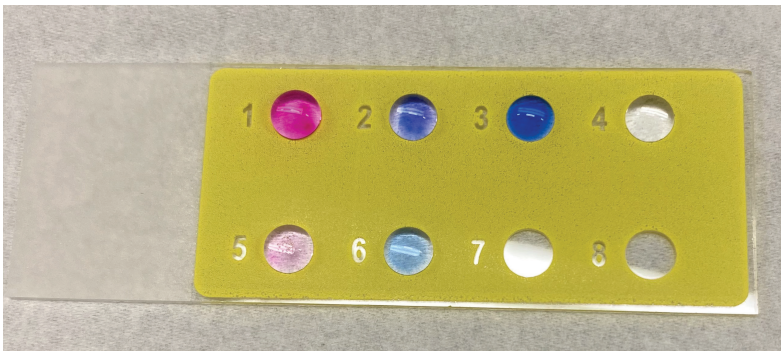
   (b) the oncogene (cancer-promoting gene) will ___*increase*___.

2. How can the differences in gene expression patterns in normal and cancerous cells be reflected in a DNA microarray experiment?

   If the cDNA of normal tissue is tagged with a green fluorescent dye and the cDNA of cancerous tissue is tagged with a red fluorescent dye, then the normal genes should appear green and the cancerous genes should appear red on the microarray chip.

VII. Results

1. Paste the photo or draw a picture of the microarray glass slide in the box below.



2. Record the results of your experiment in the following table.

Spot	Gene probe	Original colour	Colour after simulated cDNA mixture added
1	*1*	*Colourless*	*Deep pink*
2	*2*	*Colourless*	*Purple*
3	*3*	*Colourless*	*Deep blue*
4	*4*	*Colourless*	*Colourless*
5	*5*	*Colourless*	*Light pink*
6	*6*	*Colourless*	*Light blue*

VIII. Discussion

1. Describe the development of fluorescent signals from normal and cancerous cells in the authentic DNA microarray experiments.

   In DNA microarray experiments, the cDNAs derived from tumor tissues can be tagged with a red fluorescent dye, and those derived from normal tissues can be tagged with a green fluorescent dye. The two groups of tagged cDNA samples are then mixed and loaded onto a DNA chip. The cDNA samples from normal and cancerous cells interact with different DNA probes on the DNA microarray via nucleic acid hybridization. If the gene probes hybridise with cDNA from cancerous cells, the spots may appear red on the microarray slide. If the gene probes hybridise with cDNA from normal cells, the spots may appear green on the microarray slide. If there is equivalent hybridisation with cDNAs of both types of cells, the spots may appear yellow. If no hybridisation occurs, no dye stays at the spots.

2. In the current simulated DNA microarray experiment, the coloured signals are different from those seen in the authentic DNA microarray experiments. Here, the genes expressed in cancerous cells appeared pink, and the genes expressed in normal cells appeared blue. Interpret your results using the following table.

Gene probe	Colour after simulated cDNA mixture added	The implicated expression level of the corresponding gene probe in normal cells and cancerous cells
1	*Deep pink*	*This gene is mainly expressed in cancerous cells but not in normal cells.*
2	*Purple*	*This gene is expressed in both cancerous cells and normal cells.*
3	*Deep blue*	*This gene is mainly expressed in normal cells but not in cancerous cells.*
4	*Colourless*	*This gene is expressed in neither cancerous cells nor in normal cells*
5	*Light pink*	*This gene is slightly expressed in cancerous cells but not in normal cells.*
6	*Light blue*	*This gene is slightly expressed in normal cells but not in cancerous cells.*

IX. References

1. Govindarajan, R., Duraiyan, J., Kaliyappan, K., & Palanisamy, M (2012). Microarray and its applications. *Journal of Pharmacy & Bioallied Sciences 4* (Suppl 2), S310–312.